Transient Fluorescence Spectroscopy And Laser Induced Fluorescence Lifetimes
Of Terbium Doped Dipicolinic Acid

by

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DEDICATIONS

To my parents

To my brother

To those whom I love and love me back
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TRANSIENT FLUORESCENCE SPECTROSCOPY AND LASER INDUCED FLUORESCENCE LIFETIMES OF TERBIUM DOPED DIPICOLINIC ACID

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ABSTRACT

We have investigated the use of deep UV laser induced fluorescence for the sensitive detection and spectroscopic lifetime studies of terbium doped dipicolinic acid (DPA-Tb) and used this to study the optical characteristics of DPA which is a chemical surrounding most bacterial spores. Background absorption spectra, fluorescence spectra, and Excitation Emission Matrix (EEM) spectra were made of the DPA-Tb complex, using both fixed 266 nm wavelength and tunable (220 nm – 280 nm) UV laser excitations. Of importance, the fluorescence lifetimes of the four main fluorescence peaks (488 nm, 543 nm, 581 nm, and 618 nm) of the DPA-Tb complex have been measured for the first time to our knowledge. The lifetimes of all the fluorescing lines have been measured as a function of DPA-Tb concentration, solvent pH, and solvent composition, including that for the weakest fluorescing line of DPA-Tb at 618 nm. In addition, a new spectroscopic lifetime measurement technique, which we call “Transient Fluorescence Spectroscopy”,
was developed. In this technique, a weak, quasi-CW, amplitude modulated UV laser (8.5 kHz) was used to measure the lifetimes of the fluorescence lines, and yields insight into energy transfer and excitation lifetimes within the system. This technique is especially useful when a high power laser is not either available or not suitable. In the latter case, this would be when a high power pulsed deep-UV laser could produce bleaching or destruction of the biological specimen.

In addition, this technique simulated the excitation and fluorescence emission of the DPA-Tb using a 4-level energy model, and solved the dynamic transient rate equations to predict the temporal behavior of the DPA-Tb emitted fluorescence. Excellent agreement between the experiments and the simulation were found. This technique has the potential to provide a more accurate value for the fluorescence lifetime values. In addition, with the use of asymmetric excitation waveforms, the dynamic transient rate equation analysis may allow for detailed studies of selected transfer mechanisms in a wide range of other spectroscopic applications including rare-earth solid-state lasing materials and biological samples.
CHAPTER 1. INTRODUCTION

Optical fluorescence is a sensitive technique that is often used for the detection and identification of a wide range of chemical compounds and biological species. The technique is often used for the detection of biological and chemical species, both in the laboratory and in the field, and offers enhanced sensitivity over other analytical techniques. Often, these measurements are conducted using a commercial spectrofluorometer instrument that utilizes a conventional xenon light source and a scanning spectrometer or optical fluorescence emission channels.\textsuperscript{1-4} Studies have shown, however, that considerable improvement in sensitivity may be achieved through the use of a UV laser for the excitation source.\textsuperscript{1} The studies presented in this dissertation will quantify such an improvement for the detection of bacteria endospores and the development of a new spectroscopic technique for the determination of the lifetime of the fluorescence emission lines.

Previously, a considerable amount of work has been conducted for the use of laser induced fluorescence for the detection of biological specimens.\textsuperscript{1-4} For example, the sensitive detection of several chemical and biological species using dye based reagents that attach to the bacterium or virus has been studied and reported.\textsuperscript{4} Fluorescence of the dye was used for tracking and detecting the specimen. Also, time-resolved fluorescence has been used to detect and discriminate between bioaerosol particles.\textsuperscript{5} Along these lines, the pioneering work of Rosen indicated that although the direct or natural
fluorescence of dipicolinic acid (DPA) contained within a bacteria spore was weak, the rare-earth ion terbium (III) (Tb$^{3+}$) could be introduced into the specimen’s DPA complex, enhancing the fluorescence by several orders of magnitude.\(^6\)

Based upon these earlier results with Tb doped DPA, also referred to as Tb-DPA or DPA-Tb interchangeably from here on, we have investigated the use of deep-UV excitation for the laser-induced-fluorescence of the DPA-Tb complex, and the use of several new and unique spectroscopic techniques for the determination of the fluorescence lifetime of the DPA-Tb complex. In particular, our measurements yield, for the first time to our knowledge, the fluorescence lifetimes of the principal four emission lines of Tb doped DPA, and also show the variation of these lifetimes as a function of solvent concentration and pH. In addition, we developed a new spectroscopic lifetime measurement technique, which we call “Transient Fluorescence Spectroscopy”. In this technique a detailed dynamic transient solution to the rate equation model for Tb doped DPA was made, and excellent agreement between the experiments and theory were found. This transient fluorescence technique allows for the use of a weak quasi-CW laser (8 KHz pulse repetition frequency) that is also amplitude modulated to measure the lifetimes of selected fluorescence emission lines when a high power laser is not either available or suitable. In the latter case, this would be when a high power pulsed deep-UV laser could produce bleaching or destruction of the biological specimen.

This dissertation research is reported in the following Chapters. Chapter 2 provides background information on basic absorption and fluorescence optical processes related to laser-induced-fluorescence, and basic biological information on bacteria spores, such as Bacillus globigii, an Anthrax “imitator”, which produces a protective sheath of
DPA when the anthrax goes into a dormant spore state. In addition, the absorption and fluorescence properties of DPA and Tb-DPA as measured previously by others are presented. Chapter 3 presents our initial experimental work on the enhancement of the Tb-DPA fluorescence using a pulsed UV laser compared to that using a more conventional CW Xenon flash lamp for excitation as in a conventional spectrofluorometer. In Chapter 4 the detailed absorbance spectra and absorbance values of the DPA molecule in different solutions is presented as measured by using a UV-visible lamp and a conventional spectrometer for detection. The absorption spectra of the TbCl$_3$ molecule in different pH and buffer solutions and of the mixture of the two (DPA and TbCl$_3$) in different solutions is also presented.

Chapter 5 presents Excitation Emission Matrix (EEM) spectra for mixtures of DPA and TbCl$_3$ in different solution backgrounds using a tunable (220 nm – 280 nm) UV frequency-doubled dye laser for excitation. Some EEM spectra are also presented for DPA and TbCl$_3$ by themselves in solution and show the optimal excitation wavelengths for fluorescence emission of these mixtures. In Chapter 6 we present and discuss initial fluorescence lifetime measurements of mixtures of DPA and TbCl$_3$ in different solutions when excited with a low pulse-repetition-frequency (PRF) UV dye laser. The lifetimes of some of the stronger fluorescence lines of the terbium ion by itself in solution are presented in this chapter but also the limitations due to the weak fluorescence of some of the lines in the low concentration solutions are shown. Chapter 7 presents a new experimental technique that is used to measure the fluorescence lifetime of mixtures of DPA and TbCl$_3$ in different solutions. In this technique a high PRF (8 KHz) microchip laser in combination with a slow speed (50 Hz) optical chopper is used in the lifetime
experiments. In this chapter we analyze the same samples as were used in Chapter 6, and compare the fluorescence lifetime results. The fluorescence lifetimes as a function of concentration of DPA and TbCl$_3$ are also presented. In Chapter 8 a new technique for measuring the lifetime and transition rates of the DPA-Tb fluorescing complex is presented which involves the effect of the chopper modulation on the approach to saturation of the fluorescence emission. These experimental results are then compared to a transient numerical solution of the rate-equations for a model of the DPA-Tb energy-levels and the appropriate fluorescence and energy transfer rates. This new technique shows excellent agreement between experimental measurements and that predicted from the rate-equation model. We call this technique Transient Fluorescence Spectroscopy (TFS). Chapter 9 presents further analysis of and comparison between the experimental fluorescence signals as a function of time and the approach to saturation of the fluorescence emission. In Chapter 10 the maximum fluorescence intensities for different solutions of DPA and TbCl$_3$ are presented. In Chapter 11 we present conclusions and possible future studies. Finally, the appendices show detailed absorption spectra, examples of lifetime calculations, effects of asymmetrical excitation intensity waveforms, and the Matlab© computer program written by the author for the solution of the dynamic rate equation model of the DPA-Tb complex.
CHAPTER 2. BACKGROUND INFORMATION ON OPTICAL ABSORPTION AND FLUORESCENCE OF BACTERIA SPORES (DPA) AND TERBIUM IONS

This chapter contains background information regarding the absorption and emission of light by matter as reported by others. In particular, absorption and fluorescence spectra from bacteria spores, dipicolinic acid (DPA), and terbium chloride.

2.1 Absorption, Scattering, and Fluorescence of Optical and UV Light

When light interacts with matter, it can be absorbed, scattered, and/or transmitted. The percent to which each of these phenomena occurs is dependent on the optical properties of the material being illuminated, the frequency of light being used, and the overlap of the excitation light with the absorption spectrum of the material.

In the case where the excitation light is absorbed by the sample, some the molecules or ions in the sample (the ones that absorbed the energy) will become more energetic. That increase in energy is displayed in different ways depending on the frequency of the excitation light. For example, nuclear spins will be flipped in a magnetic field if radio frequencies (3 MHz-3 GHz) are used, rotational transitions in molecules and electron spin flips occur if microwave frequencies (3 GHz - 3,000 GHz) are used, frequencies in the infrared region (100 cm$^{-1}$ – 13,000 cm$^{-1}$) will induce vibrational transitions, and valence electron rearrangements in molecules will be stimulated if visible and ultraviolet (UV) frequencies (1000 nm-10 nm) are used.
2.1.1 Rayleigh and Mie Scattering of Light

The deviation of light by fine particles from the main direction of a beam is a form of scattering.\(^8\) Depending upon the size of the particle with respect to that of the light’s wavelength, we can have different types of scatterings.\(^9,10\) Rayleigh scattering refers to when the diameter of the particle, \(d\), is much smaller than the wavelength, \(\lambda\), of the incident light. This approximation assumes that the entire molecule is in a nearly constant or homogeneous electric field. Mie Scattering refers to the scattering of light by spherical particles whose size is comparable to the wavelength of the incident light. The angular distribution of the scattered light intensity often exhibits strong scattering lobes or beams due to interference effects within the scattered beam, which depends upon the ratio of the diameter of the particle and wavelength of the light.\(^9,10\)

2.1.2 Raman Scattering

Raman scattering involves a type of scattering in which the scattered light has a different wavelength to that of the incident light. The frequency difference corresponds to the energy difference between the rotational or vibrational energy levels of the molecule. If the scattering is of a longer wavelength than that of the incident light it is referred to as Stoke’s Raman scattering, and if it is a shorter wavelength then it is referred to as Anti-Stoke’s Raman scattering.\(^9,10\) This effect is different from fluorescence in that the incident light does not coincide with the sample’s absorption band and is usually not a resonance effect.
2.1.3 Absorption of Light

In optics, the process by which the energy of a photon is taken up by another entity is known as absorption. The photon is destroyed in the process. The absorbed energy can be lost by heat and (or) radiation. As an example, the energy of an incident beam can be absorbed by an atom, causing its electrons to make a transition between two electronic energy levels. A measure of the strength of the absorption process is the absorption cross section, $\sigma$, with units of cm$^2$/molecule. It is a measure of the probability of a collision between the incident photon and a number of the sample’s molecules, and represents the effective area, in units of cm$^2$, of the absorbing particle present to the incident beam.

The attenuation of the optical beam due to the absorption process is given by the Beer-Lambert law,

$$I = I_o e^{-\sigma N l},$$

where $N$ is the concentration of the molecules in molecules/cm$^3$, $I_o$ is the incident optical intensity (photons/cm$^2$ s), $l$ is the optical path length in cm, and $I$ is the optical intensity after traveling a distance $l$. The transmission, $T$, of the beam is given by $I/I_o$, the absorption is $1-T$, and the absorbance, $A$, is given by $-\log_{10}(T)$. Other useful optical measures of absorption often used by different scientific communities include the optical density, OD, or absorbance, $A$, which is given by

$$A = \log_{10}(I_o/I) = \epsilon c l,$$

and

$$OD = -\ln(T) = \sigma N l,$$
where \( c \) is the concentration in moles per liter (i.e. molar concentration, M), and \( \varepsilon \) is the molar extinction coefficient \( (\text{M}^{-1} \text{cm}^{-1}) \), also known as the molar absorptivity. It is common to drop the 10 subscript in \( \log_{10} \) and write this as \( \log \).

In the biological community it is also common to express concentration in units of Molar, M, which is defined as the number of moles per liter of a substance.

Using \( \ln(x) = 2.303 \log(x) \), and combining Eqs. (2.2) and (2.3), one can relate \( \varepsilon \) to \( \sigma \) by

\[
-\log(\frac{1}{I_0}) = \sigma \left( \frac{6.023 \times 10^{20}}{2.303} \right) c \ l ,
\]

(2.4)

where

\[
N \frac{\text{molecules}}{\text{cm}^3} \times \frac{1\text{mol}}{6.023 \times 10^{23} \text{molecules}} \times 1000 \frac{\text{cm}^3}{\text{liter}} = c \frac{\text{moles}}{\text{liter}} ,
\]

(2.5)

\[
-\log(\frac{1}{I_0}) = \varepsilon \ c \ l ,
\]

(2.6)

and, therefore

\[
\varepsilon = \sigma \frac{6.023 \times 10^{20}}{2.303} = \sigma \times 2.61 \times 10^{20} .
\]

(2.7)

### 2.1.4 Emission of Optical Radiation and Energy Relaxation

Once energy has been absorbed, there are several ways the molecule (or atom) can return to its ground state. Figure 2.1 shows a schematic of the absorption and emission of radiation for a large molecule which also exhibits different spin states of the
Where
S₀ is the ground electronic singlet state
S₁ is the first excited electronic singlet state
S₂ is the second excited electronic singlet state
T₁ is the first excited electronic triplet state
T₂ is the second excited electronic triplet state

Figure 2.1 Jablonski energy level diagram of competing excitation and relaxation processes in a large molecule (adapted from Bernarth⁷).
molecule. As can be seen from this figure, there are often competing processes. Some of these relaxation paths are listed and discussed in the following sections.

2.1.4.1 Vibrational Relaxation

Molecules have energy states that may not involve electronic transitions. These states are related to the rotational and vibrational motions of the molecules and lead to energy levels that are much more closely spaced than electronic transitions, which are seen in atoms. These vibrational levels present a pathway of nonradiative relaxation for an excited molecule to return to the lowest vibrational level within the same excited state. This energy is given up in the form of near-infrared quanta and/or kinetic energy imparted to surrounding molecules in the form of collisions. This process typically has a lifetime of about $10^{-14}$ s to $10^{-12}$ s.

2.1.4.2 Internal Conversion

An excited molecule can non-radiatively decay down to a lower electronic state of the same spin multiplicity, via vibrational relaxation. This process is known as internal conversion. Internal conversion may occur when, in thermal equilibrium, the lowest lying vibrational levels of the excited state overlap with the highest lying vibrational levels of the ground state.

2.1.4.3 Fluorescence

The term fluorescence is used within the atomic spectroscopy field to describe an allowed electric dipole transition between energy levels of the atom which results in the
emission of optical radiation. The term fluorescence is often used within the molecular spectroscopy field to designate spin allowed radiative transitions between the first excited singlet state and the ground state, where the electron in the excited orbital is paired (of opposite spin) to the second electron in the ground state orbital. The emitted radiation is usually in the UV or visible part of the spectrum. Fluorescence emission rates are on the order of $10^8$ per second, which gives a typical fluorescence lifetime of around 10 ns.

If the emitted photon has the same energy as the absorbed photon, it is known as resonance fluorescence. This type of fluorescence usually occurs in small, gaseous molecules. However, if the emitted photon has less energy than the absorbed photon, it is known as relaxed fluorescence; this type of fluorescence usually occurs in large, condensed phase molecules.

The molecule can decay down to its ground state via internal conversion or fluorescence depending upon the number of vibrational levels present, the energy gaps, and the relative strength of the optical transitions and relaxation transitions. A molecule with a high degree of freedom, and hence a high number of vibrational levels, has a higher chance of de-exciting by internal conversion, whereas more rigid molecules tend to decay through optical emission.

Under certain assumptions, i.e. where the fluorescence transition is produced by single frequency absorption, the fluorescence intensity may be given by

$$I_F = I_0 A \eta \frac{\Omega}{4\pi} ,$$

(2.8)
where \( I_F \) is the emitted fluorescence intensity in \( \text{W/cm}^2\text{-ster} \), \( \Omega/4\pi \) is the fraction of the solid angle (\( \Omega \)) seen by the collection system, \( I_o \) is the incident intensity of the exciting source, \( A \) is the absorbance, and \( \eta \) is the fluorescence quantum yield. The quantum yield is defined as the ratio of the number of photons emitted as fluorescence compared to the number of photons absorbed, as given by\(^{15}\)

\[
\eta = \frac{\gamma_f}{\gamma_f + \sum \gamma_d + \sum \gamma_q \frac{[Q]}{[O]}} ,
\]

where \( \gamma_f \) is the rate of fluorescence emission, \( \Sigma \gamma_d \) is the sum of rate constants for the various de-excitation processes, and \( \Sigma \gamma_q \) is the sum of the product of rate coefficient, \( \gamma_q \), and the concentration of the various quenchers present, \([Q]\).

Substituting Eq. (2.2) and Eq. (2.9) into Eq. (2.8), the fluorescence equation can be written as

\[
I_F = \frac{\Omega}{4\pi} I_o e c l \frac{\gamma_f}{\gamma_f + \sum \gamma_d + \sum \gamma_q \frac{[Q]}{[O]}} .
\]

Fluorescence intensity can also be defined in terms of the number of atoms per unit volume, i.e. the population density, of the fluorescing or excited state energy level, \( N_u \), as given by\(^{14}\)

\[
I_F = h\nu A_{ul} N_u .
\]

where \( h \) is Planck’s constant, \( \nu \) is the radiation frequency (\( \text{s}^{-1} \)), \( A_{ul} \) is Einstein’s coefficient for spontaneous emission (\( \text{cm g}^{-1} \)), and \( N_u \) is the number of atoms in the upper state per unit volume (\( \text{cm}^{-3} \)).
2.1.4.3.1 Fluorescence Lifetime

When a sample is excited, a number of the molecules, \( N_u \), are left in an excited electronic state. The average time these molecules remain in the excited state is known as the fluorescence lifetime, \( \tau \).\(^4\) The population density of the excited state decays exponentially according to

\[
N_u = N_u^0 e^{-A_{ul}t},
\]

(2.12)

where \( N_u^0 \) is the initial population density in the upper level at the time the excitation pulse occurs and \( A_{ul} \) is the rate at which the population is being transferred from the upper to the lower level in units of 1/sec.\(^11\) The inverse of the sum of all the possible radiative decay rates, \( \gamma_u \), to lower-lying levels is equal to the lifetime of the upper energy level, \( u \), as given by

\[
\tau_u = \frac{1}{\sum_i \gamma_{ui}} = \frac{1}{\gamma_u}.
\]

(2.13)

In the absence of nonradiative processes this is called the intrinsic or natural lifetime, \( \tau_n \).\(^4\) The quantum yield, \( \eta \), can be obtained by dividing the measured fluorescence lifetime, \( \tau \), by the intrinsic lifetime, \( \tau_n \),\(^4\)

\[
\eta = \frac{\tau}{\tau_n}.
\]

(2.14)

The total width of the spectral line emitted from a transition from an upper level, \( u \), to a lower level, \( l \), known as the natural line width, is equal to the total decay rate of the decaying system, \( \gamma_{ul} \).\(^16\) In turn, the total decay rate of a decaying system is equal to the addition of the individual decay rates of the upper level, \( u \), and the lower level, \( l \), as given by\(^11,17,18\)
\[ \gamma_{ul} = \gamma_u + \gamma_l = \frac{1}{\tau_u} + \frac{1}{\tau_l} \]  \hspace{1cm} (2.15)

From Eq. (2.15) it can be noted that the total decay rate depends on the lifetimes of both the initial and final states. In other words, the fluorescence lifetime is determined classically as the inverse of the normalized linewidth. Normally, the lifetime of the lower state is infinite, so that \(1/\tau_l = 0\). However, when \(\tau_l \neq \infty\), then \(\gamma_{ul}\) is given as in Eq. (2.15).

2.1.4.4 **Intersystem Crossing**

When an electron in an excited singlet state flips its spin, it leaves the molecule in an excited triplet state. An example of such a transfer from the excited singlet state to the excited triplet state can occur, for example, via a molecular collision. The probability of intersystem crossing occurring in a molecule is proportional to the number of vibrational states that overlap between the excited singlet state and the triplet state. The terms singlet and triplet states are the two superposition wavefunctions of the spin for two electrons. Such states are common for molecules and multi-electron atoms.

2.1.4.5 **Phosphorescence**

An optical transition or transfer from a higher lying energy level to a lower lying level of a different multiplicity is called phosphorescence. Because such transitions are spin forbidden, these types of radiative transitions have relatively long lifetimes, i.e. milliseconds to minutes. Phosphorescence transitions may be forbidden due to not being allowed for electric dipole transitions, but may be allowed for electric quadrupole
transitions with a much lower probability of transition. Hence, the long lifetime of the radiative transition.\(^4\) It should be noted that often the term phosphorescence is not used within the optical physics community, but is assumed to be contained within the term “fluorescence”, although with a longer lifetime.

2.2 Background on Bacterial Cells and Spores

Interest in bacterial spore detection is present in a number of different fields, the most common of which are the agricultural sector, and the military and defense sector.\(^21\) The agricultural sector’s interest in detection of bacterial spores is mainly due to the possibility of naturally infected bovine entering the food chain. The military and defense sector’s interest in bacterial spore detection is mainly related to threat of the use of biological agents in an attack.

The bacterial spore threat most wildly recognized by the public sector is, arguably, that of anthrax (i.e. Bacillus anthracis). The disease caused by this spore is known by the same name. Anthrax can be caught in two ways: through the skin (i.e. cutaneous anthrax) and inhalation (i.e. inhalation anthrax). A biological attack of aerosol anthrax would cause inhalation anthrax, which has a 90%-100% mortality rate. A lethal dose of anthrax is considered to be about 10,000 spores.\(^22\)

Under nutrient limiting conditions, certain types of bacterial cells have the ability to salvage the most critical components of their structure and store it in a resistant, dormant cell type called an endospore, also known as a bacteria spore, or simply spore. These endospores have the ability to then return to their original, active state, as a bacterial cell, also known as vegetative cell, when, and if, they encounter favorable
growth conditions. Figure 2.2 shows a diagram of the typical life cycle of bacteria and stages of spore formation. Bacterial spores vary slightly in their size depending on their taxonomic grouping, but are mostly in the order of one micrometer and are composed of different coatings and sections as depicted in Fig.2.3 (a). For comparison purposes, the active bacteria of the genus Bacilli, which are rod shaped with dimensions of about 1 µm by 3-5 µm, are shown in Fig. 2.3 (b). The Bacillus subtilis type of bacteria tends to form chains or aggregates of about 10 to 40 bacteria.

Usually, the active bacterial cell is made up of about 70% water. However, when the cell goes to an endospore state, water makes up approximately 15% of the bacterial spore. Endospores do not metabolize or reproduce and can withstand drying. In fact they are 10,000 times more resistant to heat and 100 times more resistant to UV rays than are vegetative cells, and can last for centuries in an outside dry environment.22,23

As a point of reference, the extinction coefficient of the Bacillus subtilis at an excitation wavelength of approximately 266 nm has been reported as 4.1 x 10^{-8} cm²/spore.24

2.2.1 DPA Coating Surrounding Endospores

It is believed that the toughness of bacterial spores is due, at least in part, to the chemical dipicolinic acid (also known as DPA, or pyridine-2,6-dicarboxylic acid), found in its cortex. This chemical can constitute from 5% up to 15% of the dry weight of a bacterial spore.22,23

In the cortex of a bacterial spore, DPA is found to be ionically bound to calcium, forming thus, calcium dipicolinate25 as shown in Fig. 2.4.
Figure 2.2  Stages of spore formation (from D. Lim	extsuperscript{22}).
Figure 2.3  a) Electron microscope cross-section of a spore of Bacillus subtilis. The spore is 1.2 µm across. (from http://www.berkeley.edu/news/media/releases/2003/02/10_spores.shtml); b) Scanning electron microscope image of Bacillus subtilis bacteria cells. The cell is about 2.1 µm in length (from D. Lim\textsuperscript{23}).
Figure 2.4  Structure of dipicolinic ion (DPA$^{2-}$), dipicolinic acid (H$_2$DPA), and calcium dipicolinate (Ca(DPA)) (from D. L. Rosen$^{25}$; reprinted with permission from Applied Spectroscopy).
bacteria spore detection systems it is common to extract the dipicolinic acid from the sample for better sensitivity.\textsuperscript{26} Dipicolinic acid is present naturally only in bacterial spores.\textsuperscript{27}

2.2.2 Absorption and Fluorescence Spectra of DPA

There has been some previous but limited work on the optical spectra of DPA.\textsuperscript{28-34} A UV absorption spectrum from an aqueous suspension of Bacillus subtilis is shown\textsuperscript{28} in Fig. 2.5. For comparison purposes, a graph of the absolute absorbance of [100\(\mu\)M] DPA in aqueous solution as a function of wavelength is also shown\textsuperscript{34} in Fig. 2.6. As can be seen in these figures, DPA has an absorption peak near 250 to 280 nm.

Dipicolinic acid’s intrinsic fluorescence is very weak and it has been believed to be masked by the fluorescence of other components of the bacteria spores. However, some authors have detected, after minutes of UV irradiation, fluorescence from what is believed to be DPA which may have been radiationally changed or damaged as the UV intensity is increased in time.\textsuperscript{29,33} This is shown in Fig 2.7 which shows measured changes in the DPA fluorescence near 400 nm being increased as the exposure to 300 nm UV excitation is increased.\textsuperscript{33} The energy level for the lowest lying triplet state of the DPA molecule has been published in several articles\textsuperscript{35,36} as ranging between 24,272 cm\(^{-1}\) and 27,050 cm\(^{-1}\).

The molar absorptivity, \(\varepsilon\), of the DPA molecule, has also been previously published. In the UV Atlas of Organic Compounds\textsuperscript{37} it has been reported as 36,500 (M\(^{-1}\) cm\(^{-1}\)) for \(\lambda_{\text{ext}} = 193\) nm, as 6,400 (M\(^{-1}\) cm\(^{-1}\)) for \(\lambda_{\text{ext}} = 217\) nm, and as 7,100 (M\(^{-1}\) cm\(^{-1}\)) \(\lambda_{\text{ext}} = 274\) nm.
Figure 2.5  UV absorption spectrum of an aqueous suspension of intact Bacillus subtilis spores (from E. Ghiamati\textsuperscript{28}, reprinted with permission from Applied Spectroscopy).
Figure 2.6  Absorption spectrum of [100µM] DPA in aqueous solution (from F. Peral\textsuperscript{14}, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Copyright 2000, with permission from Elsevier).
Figure 2.7  Fluorescence emission spectra of 10 µM DPA in water at pH of 2.0 after 0, 5, 15, 30, and 60 minutes of UV irradiation ($\lambda_{ex}= 300$ nm), corresponding respectively from the lowest fluorescent signal to the highest fluorescing signal. (from R. Nudelman$^{33}$; reprinted with permission from Applied Spectroscopy).
The fluorescence strength of pure DPA is very weak, thus not lending itself to in-depth determination of energy levels and excitation transfer rates within the DPA energy levels or those associated with collisional de-excitation with other substances. However, in 1997 a method was presented and patented by D. Rosen in which it was determined that the addition of the terbium ion (Tb$^{3+}$) to the DPA can significantly enhance the fluorescence emission from the DPA-Tb complex, thus allowing for easier detection of the presence or absence of bacteria endospores via fluorescence. Characteristics of the terbium ion as well as those of the DPA-Tb complex are covered in the following sections.

2.3 Use of Rare Earth Ions (Tb) to Enhance Fluorescence Emission

Terbium III (Tb$^{3+}$) is a rare earth ion which retains its luminescent properties both under biological conditions and in solution media when acting as a dopant for biomolecular systems. Its placement in the periodic table of elements is shown in Fig. 2.8.

The involvement of the $f$-orbitals in bonding in lanthanides has been a controversial issue. This is due to the belief that the regions of greatest $4f$ electron concentration in lanthanides does not extend far enough out from the nucleus as shown in Fig. 2.9. The $4f$ and $5d$ electrons are of comparable energies.

The terbium atom has the [Xe].$4f^96s^2$ electronic configuration as shown in Fig. 2.10. Upon excitation, which can be either direct (in the near UV or visible region) or indirect (via a resonant coupling between a donor molecule, e.g. DPA, and the Tb$^{3+}$), the terbium atom will lose electrons and will have the [Xe].$4f^8$ configuration.
<p>| | | | | | | |</p>
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Figure 2.8  Periodic Table: Lanthanides (from Solid State Physics\textsuperscript{39} 1\textsuperscript{st} edition by Aschcroft/Mermin. 1976. Reprinted with permission of Brooks/Cole, a division of Thomson Learning: www.thompsonrights.com, Fax 800 730-2215).
Figure 2.9  Radial part of the hydrogenic wave functions for the 4f, 5d, and 6s orbitals of cerium (from *Lanthanides and Actinides*\(^4\), used with permission from the Journal of Chemical Education, vol. 41, Iss. 7, p. 354 (1964)).
Figure 2.10  Electronic configuration of the terbium atom (adapted from WebElements™, http://www.webelements.com/) Mark Winter, The University of Sheffield and WebElements Ltd, UK).
The energy level diagram for the lanthanides is shown\(^{41}\) in Fig. 2.11 and that of the terbium ion (Tb\(^{3+}\)) by itself\(^{42}\) in Fig. 2.12. The numerical values for the terbium ion’s energy levels are listed in Table 2.1.

The absorption spectrum as a function of wavenumber of an aqueous solution of Tb\(^{3+}\) in either HClO\(_4\) or DClO\(_4\) is shown\(^{43}\) in Fig. 2.13. The molar absorptivity of the terbium ion in solution has been reported as 0.25 for \(\lambda_{\text{exct}} = 284\) nm, as 0.31 for \(\lambda_{\text{exct}} = 350\) nm, as 0.34 for \(\lambda_{\text{exct}} = 368\) nm, and as 0.21 for \(\lambda_{\text{exct}} = 377\) nm.\(^{43}\) The terbium ion can substitute the calcium ion in the calcium dipicolinate molecule partly due to their similar size (radius of Tb\(^{3+}\) is approximately 1.12 Å and radius of Ca\(^{2+}\) is approximately 0.99 Å) and terbium’s strong affinity towards negatively charged donor groups. When the terbium ion substitutes the calcium ion, the terbium dipicolinate complex is formed. This DPA complex is shown\(^{25}\) in Fig. 2.14.

It is interesting to note that there are different complexes that can be formed between the terbium ion and the dipicolinate ion. Although this is more relevant to the chemical properties of the DPA-Tb complex, some initial studies were done by D. Rosen where he was able to determine that the lifetime of the DPA-Tb complex changes depending on which type of complex has formed.\(^{25}\) The terbium dipicolinate complex can form \(\text{Tb(dpa)}_{n}^{3-2n}\), where \(n = 1, 2,\) or 3. When DPA is in great excess, i.e. \(n = 3\), \(\text{Tb(dpa)}^{3-}\) tends to form. When Tb\(^{3+}\) is in great excess, i.e. \(n = 1\), \(\text{Tb(dpa)}^{+}\) tends to form.\(^{25}\) The terbium ion has a total of 9 coordination sites, i.e. sites of possible bond formation, whereas each dipicolinate anion has three. In situations where the DPA is not in excess, i.e. \(n < 3\), the remaining coordination sites of the terbium ion are occupied by
Figure 2.11  Energy levels of the trivalent terbium ion (Tb$^{3+}$) (from G. H. Dieke$^4$; reprinted with permission from Optical Society of America).
Figure 2.12 Detailed diagram of selected energy levels of the trivalent terbium ion (Tb$^{3+}$) (Data points obtained from W.T. Carnall$^{42}$).
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<td>$^5K_9$, $^5D_2$</td>
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Table 2.1  Experimental energy levels of Tb$^{3+}$:LaF$_3$ (adapted from W.T. Carnall$^{42}$).
Figure 2.13  Optical absorption of a solution of Tb$^{3+}$ (aq) as a function of wavenumber (bottom axis) and wavelength (nm) (top axis) (from Carnall$^{43}$).
Figure 2.14  Structure of Tb(DPA)$^+$ and Tb(dpa)$_2^-$ complexes with water molecules (from D. L. Rosen$^{25}$; reprinted with permission from Applied Spectroscopy.)
water molecules, whose OH vibrational modes interact with the terbium ion.\textsuperscript{25,38,44-50} The OH oscillators dominate the relaxation of energy in the cation and shorten the lifetime of the excited state.\textsuperscript{25}

The photoluminescence lifetime of the DPA-Tb complex varies greatly with chelation number, i.e. the number of DPA molecules attached to the terbium ion. For example, the photoluminescence lifetime of Tb(DPA)$^+$ = 0.66 ms, of Tb(DPA)$_3^{3-}$ = 2.0 ms, and of Tb(DPA)$^{2-}$ = 1.4 ms.\textsuperscript{25} However, ligand exchange between the three chelation states results in their mixture having a monoexponential lifetime that is a weighed average of the lifetimes of the three states of the DPA-Tb complex.\textsuperscript{25}

Although the terbium ion fluoresces in solution, this fluorescence is not very strong. However, when terbium is bound to DPA, its fluorescence enhances significantly due to the nonradiative transfer of energy that occurs between the DPA molecule (energy acceptor) and the terbium ion (the fluorescing species).\textsuperscript{25} This is seen in initial work\textsuperscript{51} by Rosen who measured the fluorescence spectrum of a solution of [200\textmu M] TbCl$_3$ blank, a solution of 50 nM [Tb(DPA)$_3$]$^{3-}$, and a suspension of 0.08 mL B. Subtilis in 200 \textmu M TbCl$_3$ all in a [50mM] TRIS buffer solution. These results are shown in Fig. 2.15. In this work Rosen investigated the effect of the different structures the DPA-Tb complex formed and their effect on the combined fluorescence lifetime of the complex. The fluorescence lines were not resolved. The fluorescence intensity of this complex is highly sensitive to the chemical make up of its surrounding environment.\textsuperscript{52-55}

The molar absorptivity of the DPA-Tb complex has been previously reported by Latva et al.\textsuperscript{35} as 12,800 (M$^{-1}$ cm$^{-1}$) for an excitation wavelength of 275 nm.
Figure 2.15  Fluorescence emission spectrum of 0.08-mL B. subtilis suspension in 200 $\mu$M TbCl$_3$ both before (- -) and after (s) filtering, for 200 $\mu$M TbCl$_3$ blank (- - -), and for scaled 50 nM [Tb(DPA)$_3$]$^{3+}$ (.-.), in [50mM] TRIS buffer solution ($\lambda_{exc} = 270$nm) (from D.L. Rosen$^{31}$; reprinted with permission from Analytical Chemistry, Copyright 1996 American Chemical Society.)
CHAPTER 3. INITIAL ENHANCEMENT OF Tb-DPA FLUORESCENCE USING A PULSED UV LASER

In this chapter the initial measurements of the fluorescence from the DPA-Tb complex are discussed. Spectral fluorescence measurements were made using a conventional optical excitation source (Xenon lamp) and then the fluorescence was measured using a UV pulsed laser. An enhancement was observed using the pulsed UV laser for excitation.

3.1 Tb-DPA Spectrofluorometer System Using Conventional Lamp Source

Fluorescence spectroscopy has been used in the past to detect various concentrations of biological endospores, such as bacillus globigii spores. In traditional fluorescence based detection systems, a xenon flash lamp is usually used as the excitation source. The flash lamp can be used in combination with a monochromator and/or optical filters for narrow-band excitation, or by itself for broad-band excitation. To measure the fluorescence spectrum of Tb-DPA, we used a conventional spectrofluorometer system (Ocean Optics, Inc.) as shown in Fig. 3.1. It consisted of a pulsed xenon flash lamp (with a pulse width of 5 μs) which was used to excite the contents of a quartz cuvette and a spectrometer to measure the fluorescence emission. The fluorescence was collected 90° to the excitation source beam. In addition, a
Figure 3.1 Schematic of commercial xenon flash lamp excitation fluorometer used to detect bacteria spores (Endospore Detection System, Ocean Optics, Inc.).
multipass mirror was used in the excitation beam to increase the excitation and a mirror was used in the fluorescence collection geometry. The fluorescence was collected via an end-of-fiber collection lens. The collected light then passed through a VIS/NIR fiber which was attached to a spectrometer (Ocean Optics Inc Model USB 2000-FLG.) The spectrometer’s entrance slit was 200 µm wide. The spectrometer had a 600 lines per millimeter grating, blazed at 500 nm, a 2048-element linear silicon CCD array with a mounted collection lens and a wavelength range of 380nm – 1050nm.

### 3.1.1 Modified Spectrofluorometer System Using Pulsed UV Laser

To increase the detection sensitivity, the pulsed xenon flash lamp was replaced by either a pulsed 4th harmonic Nd:YAG laser with a 266nm output or a 3rd harmonic Nd:YAG laser with a 355nm output. A schematic diagram for this setup is shown in Fig. 3.2. Both lasers were high-PRF (Pulse-repetition-frequency) diode-laser pumped Nd:YAG microchip lasers (JDS Uniphase, Model NU-10110-100). Figure 3.3 shows a photograph of the conventional set up along with the 4th harmonic Nd:YAG laser, which could be substituted for the xenon flash lamp.

#### 3.1.1.1 4th Harmonic Nd:YAG Microchip Laser

The microchip laser was a 4th harmonic of a 1064 µm Nd:YAG laser, with an output wavelength of 266 nm, an output pulse energy of 0.25 µJ/pulse at a pulse-repetition-rate of 8.5 kHz, and a pulse width of 0.4 ns. The output of the laser was vertically polarized (100:1). The average power of this laser was 2 mW. The specifications for this laser are listed in Table 3.1.
Figure 3.2  Schematic of laser fluorometer system using a high PRF 266nm (or 355nm) laser for excitation.
Figure 3.3 Photograph of fluorometer system that could use either the 4th harmonic Nd:YAG laser or the pulsed xenon flash lamp for excitation.
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<tr>
<td>Polarization (H:V)</td>
</tr>
<tr>
<td>Beam Size</td>
</tr>
</tbody>
</table>

Table 3.1  Laser parameters of the 266 nm high PRF microchip laser (JDS Uniphase, Model NU-10110-100).
This laser consisted of Nd\textsuperscript{3+}:YAG crystal bonded to a thin layer of Cr:YAG saturable absorber (passive Q-switching), which was coated with a thin film mirror to form the laser cavity. The crystal was end pumped by a CW diode laser. The Cr:YAG was opaque to 1064 nm and prevented lasing until a critical amount of energy had been absorbed. When the absorber reached its threshold, it saturated and lasing began. The pulse repetition frequency was determined by the time constant of the absorber and the amount of energy being input to the gain medium. Pulse energy was independent of the pump power as the Q-switching was always at the same intracavity energy, and was directly proportional to the thickness of the absorber medium. The pulse width is partially determined by the cavity’s roundtrip time, so that the compactness of the cavity resulted in pulse widths shorter than a nanosecond, about 0.4 ns (Uniphase manual, NU-10110-100).

The output of the laser was frequency doubled by using a KTP (Potassium Titanyl Phosphate) crystal. The doubled output at 532 nm was frequency doubled again by a BBO crystal to 266 nm. The residual light at 1064 nm and 532 nm were reduced by using optical blocking filters.\textsuperscript{61} A schematic of the microchip laser is shown in Fig. 3.4.

3.2 Initial Fluorescence Measurements

In this section we present some initial fluorescence measurements that will serve as an introduction to the fluorescence properties and behavior of the terbium ion and the Tb-DPA complex.
Figure 3.4  Schematic of 4th harmonic Nd:YAG microchip laser (JDS Uniphase, Model NU-10110-100).
3.2.1 Fluorescence of Terbium Ion

The laser induced fluorescence system shown in Fig. 3.2 was used to measure the fluorescence spectrum from TbCl$_3$ alone. Figure 3.5 shows the fluorescence intensity as a function of wavelength for a solution of [100 µM] TbCl$_3$ in distilled water when excited with the 4$^{th}$ harmonic Nd:YAG laser at 266 nm. The fluorescence spectrum of the terbium ion has four clearly visible peaks in the visible, which arise from the following electronic transitions, starting with the strongest fluorescence intensity: $^5$D$_4$ to $^7$F$_5$ ($\lambda_{\text{emission}} \approx 543$ nm), $^5$D$_4$ to $^7$F$_6$ ($\lambda_{\text{emission}} \approx 488$ nm), $^5$D$_4$ to $^7$F$_4$ ($\lambda_{\text{emission}} \approx 581$ nm), and $^5$D$_4$ to $^7$F$_3$ ($\lambda_{\text{emission}} \approx 618$ nm). The spectrum was measured over a time period of 100 ms. Note the weakness of the intensity and the low S/N ratio of the data. This shows that the terbium ion by itself in water has a relatively weak fluorescence.

3.2.2 Fluorescence of the DPA-Tb Complex

As indicated earlier in Chapter 2 (Fig. 2.15), the fluorescence of the terbium ion can be greatly enhanced with the addition of the DPA molecule. As such, a measurement of the fluorescence emission from the DPA-Tb using the laser source was made. Figure 3.6 shows a comparison of the measured 100 ms integrated fluorescence intensities as a function of wavelength of a mixture of [50 µM] DPA with [50 µM] TbCl$_3$ in [0.01M] HCl, when excited with the three different excitation sources. From this figure it can be seen that the excitation source that induced the highest fluorescence emission was the 266 nm 4$^{th}$ harmonic Nd:YAG laser, followed by the pulsed xenon flash lamp used in the commercial setups, and the 355 nm 3$^{rd}$ harmonic Nd:YAG laser. It is
Figure 3.5  LIF emission spectrum of [100µM] TbCl₃ in water, $\lambda_{\text{exc}} = 266$nm. (Tb³⁺'s electronic transitions labeled to corresponding spectral peaks.)
Figure 3.6  Fluorescence intensity as a function of wavelength of a solution of [50μM] DPA with [50μM] TbCl₃ in [0.01M] HCl, when excited with three different sources: 266nm laser, 355nm laser and a conventional pulsed xenon flash lamp.
also quite evident that the latter excitation source at 355 nm fails to produce any considerable amount of fluorescence from this mixture of pure dipicolinic acid and terbium in weak hydrochloric acid.

Also, it is important to note that the shape of the fluorescence of the DPA-Tb complex (Fig. 3.6) is the same as the shape of the fluorescence of the terbium ion alone (Fig. 3.5), which is consistent with earlier reports that the terbium ion is the fluorescing species.

3.2.3 Fluorescence of the Mixtures of Bacteria Doped Tb

For comparison purposes, a solution of Bacillus globigii spores with [50 µM] TbCl$_3$ in [0.01M] HCl was also excited with the same three different excitation sources. The results are shown in Fig. 3.7 and indicate similar enhancement of the fluorescence emission using the 266nm laser source compared to the xenon flash lamp excitation. However, as can be seen, the overall fluorescence intensity has decreased significantly, possibly due to the lack of readily available DPA, which is now deep in the cortex of the spores. Also, the 355 nm 3$^{rd}$ harmonic Nd:YAG laser seems to have produced a fluorescence emission from the sample. This emission is believed to be mostly due to the nicotinamide adenine dinucleotide (NADH) which is associated with cell metabolism, although this remains a cause of debate since by their nature bacteria spores are in a dormant state.$^1$
Figure 3.7  Fluorescence intensity as a function of wavelength of a solution of Bacillus globigii spores with [50µM] TbCl$_3$ in [0.01M] HCl, when excited with three different sources: 266nm laser, 355nm laser and a conventional pulsed xenon flash lamp.
CHAPTER 4. DETAILED ABSORBANCE MEASUREMENTS OF DPA, TbCl₃, AND THE DPA-Tb COMPLEX

Previous absorption measurements of DPA and DPA-TbCl₃ were made under a limited number of conditions. As such, to expand upon these past studies, an investigation was made to determine the detailed absorption spectrum under a controlled set of background conditions. This chapter describes our experimental absorbance measurements and the measured absorption cross sections obtained when terbium chloride and dipicolinic acid, as well as the mixtures of the two in different proportions, were examined in a number of different solvents.

4.1 Experimental Setup for Detailed Absorbance Measurements

This section presents the experimental setup used to measure the absorbance spectrum of the different DPA and TbCl₃ solutions. The general setup for the absorbance experiments is shown in Fig. 4.1. This setup consisted of a stable, continuous wave, deuterium tungsten halogen UV-VIS lamp (CHEM 2000, Ocean Optics, Inc) that had output from 200 nm to 2062 nm. The output intensity as a function of wavelength of this light source was measured using a grating spectrometer (with range of 200 nm to 900 nm) and the results are shown in Fig. 4.2 to indicate the relative UV-visible portion of the output. A cuvette holder was attached to the aperture of the light
Figure 4.1  Schematic of experimental set-up for absorbance measurements.
Figure 4.2 CHEM-2000’s CW light source output spectrum over the range of 200 nm to 900 nm; drop-off near 900 nm due to drop-off in measuring grating efficiency.
source and the signal was collected via a fiber coupled collection lens positioned at 180° of the said aperture.

The signal was analyzed by the first channel (Channel #1) of an ST-2000 spectrometer (Ocean Optics, Inc.), and was sent to a desktop computer for storage. The spectrometer had a crossed Czerny-Turner design, as shown in Fig. 4.3. The entrance to the channel used in the spectrometer was a 25 µm slit. This channel had a grating with 600 lines/mm, which was blazed at 400 nm. The relative efficiency as a function of wavelength for this grating is shown in Fig. 4.4. The 2048-element linear silicon CCD array detector was equipped with a collection lens. The spectrometer’s specifications are listed in Table 4.1.

4.1.1 Quartz and Plastic Cuvettes

It was possible to use both quartz and plastic cuvettes for our experiments. The transmission spectra of a “UV transparent” plastic cuvette (Brandtech Scientific, Inc, Model UV Plastic Cuvette) was measured and compared with that of a Suprasil quartz cuvette (Starna Cells, Inc, Model # 23-Q-10,). As can be seen from the results which are shown in Fig. 4.5, the percent transmission of the quartz cuvette far surpasses that of the plastic cuvette across all wavelengths, especially the UV region. Due to this, the quartz (fused silica) cuvettes were used in order to maximize the incident intensity and to minimize any extraneous fluorescence. The dimensions of the quartz cuvette were 12.5mm × 12.5mm × 45mm, with an inner path length of 10mm. These cuvettes were used throughout all the experiments presented in this dissertation.
Figure 4.3  ST-2000 spectrometer’s internal schematic for one optical channel.
Figure 4.4  Relative efficiency as a function of wavelength of the grating used in the ST-2000 spectrometer.
<table>
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<tr>
<td>Integration Time</td>
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</table>

Table 4.1  ST-2000 spectrometer’s parameters.
Figure 4.5  Measured transmission spectra of a quartz cuvette and a "UV transparent" plastic cuvette.
4.2 Absorbance Measurement Procedure

This section describes in detail the procedure that was followed in order to obtain the absorbance spectra, as well as the theory behind the calculations necessary to calculate the absorption cross sections of the different compounds in solution.

Before spectral data was taken, the CHEM 2000 CW light source was warmed up for 15 minutes. In a darkened room, and after the output spectra of the lamp had stabilized, the output light was blocked and a dark or background spectrum, D, was taken. The light was then unblocked and its spectra was saved as a reference spectrum, R. Next, the sample was placed in the cuvette holder, i.e. in the path of the light, and the sample’s spectrum, S, was taken. All of these spectra were normalized to the grating efficiency, and detector responsivity. The absorbance, $A$, of the sample was then obtained by applying

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

at each wavelength $\lambda$.

Unless stated otherwise, all the spectra presented are the average of 100 runs. All the absorption spectra have been normalized to the fiber attenuation, the spectrometer’s grating efficiency, and the CCD’s sensitivity.

The absorption cross sections, $\sigma$, were calculated from the absorbance measurements using

$$\sigma_\lambda = \frac{A_\lambda}{N \log_{10}(e) I}$$
4.3 TbCl$_3$ and DPA Solution Preparation

It was determined that a wide range of DPA and TbCl$_3$ concentrations were required for our studies, including the use of different solvents. The different solvents were guided by past studies into the chemical and spectral nature of DPA. Toward this end, four different basic solvent concentrations were prepared in order to produce different proportions of terbium and dipicolinic acid. [0.01M] HCl and [1M] HCl were prepared by diluting [17M] HCl (Fisher Scientific) with distilled water. Solutions of [1M] sodium acetate buffer (SAB), pH 5.6, were created by mixing [1M] sodium hydroxide with [1M] acetic acid until a pH of approximately 5.6 was obtained. Distilled water by itself was also used as a solvent. It should be noted that one of the reasons we used SAB pH 5.6 as a solvent was because previous work$^{52}$ had indicated that this buffer yielded an optimum environment for maximum fluorescence.

Once the solvents were prepared, terbium chloride hexahydrate (TbCl$_3$ . 6H$_2$O) (Sigma-Aldrich, 99.9% purity), was added to each of the solvents in order to produce the following concentrations: [5μM], [10μM], [15μM], [20μM], and [25μM]. Also, DPA (99.5%, Sigma Aldrich, Inc.) was added separately to each of the solvents to form the following concentrations: [5μM], [10μM], [15μM], [20μM], and [25μM]. These solutions were used to obtain absorbance data for each compound by itself, without the interaction effect between the two. The resultant list of different starter solutions are given in Table 4.2.

In order to see the effect of the concentration on the absorbance spectra once the two compounds were mixed, terbium chloride hexahydrate (TbCl$_3$ . 6H$_2$O) (Sigma-Aldrich, 99.9% purity) and pyridine-2,6-dicarboxylic acid, or DPA, (Sigma Aldrich, Inc.)
Table 4.2  List of different concentration of starting solutions used in the experiments where the absorbances and the absorption cross sections of DPA and TbCl$_3$ were measured separately.
were mixed in the same solution in order to produce a matrix of different concentrations in each of the solvents described above. For each of the solvents, a total of 25 mixed solutions were formed and analyzed.

4.4 General Absorbance Spectra of DPA and TbCl₃, and Their Mixtures

In this section we present the experimental results of the absorbance measurements that were conducted on solutions of different concentrations of DPA by itself in different solvents, of TbCl₃ by itself in different solvents, and of mixtures of DPA and TbCl₃ in different solvents. The absorption cross sections were also calculated using the absorbance values at 266 nm obtained for each compound and their mixtures.

4.4.1 Measurements of Absorbances and Absorption Cross Sections for Individual DPA and TbCl₃ Solutions

Absorbance spectra were taken from solutions of [5µM] DPA, [10µM] DPA, [15µM] DPA, [20µM] DPA, and [25µM] DPA, each in distilled water (DW), [0.01M] HCl, and in [1M] sodium acetate buffer (SAB) pH 5.6. The same was done with solutions of TbCl₃.

Figure 4.6 shows the measured absorbance values as a function of wavelength of [15µM] DPA in distilled water (DW) (Fig. 4.6 (a)), in [0.01M] HCl (Fig. 4.6 (b)), and in [1M] sodium acetate buffer (SAB), pH 5.6 (Fig. 4.6 (c)). Figure 4.7 shows the absorbance as a function of wavelength of [15µM] TbCl₃ in distilled water (DW) in Fig. 4.7 (a), [0.01M] HCl in Fig. 4.7 (b), and [1M] sodium acetate buffer (SAB), pH 5.6 in Fig. 4.7 (c). The background of the solvents, i.e. distilled water, [0.01M] HCl, and [1M]
Figure 4.6  Absorbance spectra of [15µM] DPA measured in different solutions. Note: The background spectrum of each solvent, which was not subtracted, has been plotted along in a fainter dotted line.
Figure 4.7 Absorbance spectra of [15µM] TbCl₃ measured in different solutions. Note: The background spectrum of each solvent, which was not subtracted, has been plotted along in a fainter dotted line.
SAB, have not been subtracted from these spectra, but rather, they are shown along side of them, in a fainter dotted line. The spectra for the remaining concentrations as given in Table 4.2 are presented in Appendix A.

Upon visual evaluation of the solvents in the graphs, it is noticeable that sodium acetate buffer has a distinct absorption band in the 440 nm to 487 nm region, with the peak absorbance occurring near 469 nm. The absorption for distilled water and [0.01M] HCl is uniformly low across the shown spectral region.

As can be seen in Fig. 4.6 (a), (b), and (c), dipicolinic acid has an absorption band from 218 nm to 296 nm, with three distinct peaks near 221 nm, 231 nm, and 271 nm. There is a much weaker absorption band from around 430 nm to 532 nm. This is in agreement with measurements done by previous investigators.

A magnified region of the absorption graph of DPA in distilled water (with the water background already subtracted) is shown in Fig. 4.8 where the individual absorption peaks are seen more easily. The absorbance spectrum of TbCl₃ in Fig. 4.7 shows negligible absorption for the terbium ion in solution in this wavelength region. This is in agreement with previously published measurements.

Figure 4.9 shows the absorbance values at 266 nm as a function of the DPA molecule’s concentration in distilled water, in [0.01M] HCl, and in [1M] SAB. The background absorbance values for each of the solvents have been subtracted, and hence, the absorbance values shown in this graph are, in theory, independent of the effect of the solvent. For the sake of completeness, however, it has been indicated in the graph which points belong to which solvent. From this figure it can be seen that the absorption of
Figure 4.8  Enlarged view of the absorbance spectra of [15µM] DPA in distilled water; water background has been subtracted.
Figure 4.9  Measured absorbance of DPA at 266 nm as a function of DPA concentration. The vertical line inside the markers at a concentration of around $15 \times 10^{15}$ molecules per cm$^3$ corresponds to an error of about 0.01 in the absorbance scale. Each data point is the average of 100 runs.
DPA at 266nm increases as the concentration increases. This is consistent with Eq. (2.2) where A is proportional to the concentration.

However, some chemical products such as clusters or other dimers or molecules may produce a non-linear effect.

Figure 4.10 shows the absorbance values at 266 nm as a function of the terbium ion’s concentration in distilled water, in [0.01M] HCl, and in [1M] SAB. The absorbance values for each of the solvents have also been subtracted in this instance. The absorbance of the terbium ion seems negligible at 266 nm.

4.4.2 Absorption Cross Section of DPA and TbCl₃ Individually

Using Eq.(4.2), and the absorbance values presented in Figs. 4.9 and 4.10, the absorption cross section values for the DPA molecule and for the Tb³⁺ ion at 266 nm were calculated. A graphical presentation of the absorption cross section values for the DPA molecule and the terbium ion is shown in Figure 4.11. The averages of these values are $(2.10 \pm 0.38) \times 10^{-17}$ cm²/molecule for the DPA molecule and $(0.18 \pm 0.22) \times 10^{-17}$ cm²/molecule for the Tb³⁺ ion. It is interesting to note that the terbium ion’s absorption cross section in solution at 266nm is about twelve times smaller than that of the DPA molecule.

The value obtained for the absorption cross section of the DPA molecule is very close to data obtained by the UV Atlas of Organic Compounds,³⁷ which found the absorption cross section of the DPA molecule at 274nm to be around $2.7 \times 10^{-17}$ cm²/molecule.
Figure 4.10  Measured absorbance of TbCl$_3$ at 266nm as a function of TbCl$_3$ concentration. The vertical line inside the markers at a concentration of around $15 \times 10^{15}$ molecules per cm$^3$ corresponds to an error of about 0.01 in the absorbance scale. Each data point is the average of 100 runs.
Figure 4.11  Absorption cross section of DPA and TbCl$_3$ at 266nm. The vertical line inside the markers at a concentration of around $15 \times 10^{15}$ molecules per cm$^3$ corresponds to an error of about $0.15 \times 10^{-17}$ cm$^2$/Molecule in the absorption cross section scale (~5% error).
The molar absorptivity of the terbium ion in solution has been reported as $9.6 \times 10^{-22}$ cm$^2$/molecule for $\lambda_{\text{exc}} = 284$ nm.\textsuperscript{43} It must be noted that even though the numerical values of this published data and our findings are quite far from each other, they are both much smaller than the absorption cross section value obtained for the DPA molecule. Also, it should be noted that there is an almost 20 nm difference between the excitation wavelength used in our experiment and that used in this reference.

### 4.4.3 Absorbances of Mixtures of DPA and TbCl$_3$

As stated earlier, the absorbance values for a mixture of DPA and TbCl$_3$ is much greater than that for TbCl$_3$ alone or for DPA alone. As such, absorbances were also measured for solutions that contained varying concentrations of mixtures of DPA and TbCl$_3$ in different solvents, i.e. distilled water, HCl, and SAB. Our results are shown in the following figures.

Figure 4.12 shows the measured absorbance spectra of solutions of varying TbCl$_3$ concentrations, i.e. [5\,\mu M], [10\,\mu M], [15\,\mu M], [20\,\mu M], and [25\,\mu M], mixed with different DPA concentrations. Figure 4.12 (a) shows the absorbance spectra as a function of wavelength of mixtures of the various TbCl$_3$ concentrations with [5\,\mu M] DPA in distilled water. Figure 4.12 (b) shows the absorbance spectra of mixtures of the various TbCl$_3$ concentrations with [10\,\mu M] DPA in distilled water. Figure 4.12 (c) shows the absorbance spectra of mixtures of the various TbCl$_3$ concentrations with [15\,\mu M] DPA in distilled water. Figure 4.12 (d) shows the absorbance spectra of mixtures of the various TbCl$_3$ concentrations with [20\,\mu M] DPA in distilled water. In Fig. 4.12 (e) one can see
Figure 4.12  Absolute absorbance spectra of solutions of [5µM] DPA measured with varying [TbCl₃] (i.e. [5µM], [10µM], [15µM], [20µM], and [25µM]), (a), of [10µM] DPA with varying [TbCl₃], (b), of [15µM] DPA with varying [TbCl₃], (c), of [20µM] DPA with varying [TbCl₃], (d), and of [25µM] DPA with varying [TbCl₃], (e), in distilled water.
the absorbance spectra of mixtures of the various TbCl₃ concentrations with [25µM] DPA in distilled water. The distilled water background has been subtracted from each one of these spectra.

For completeness, the detailed spectra of mixtures of TbCl₃ and DPA in solutions of [0.01M] HCl, and [1M] SAB (pH 5.6) were also measured. These results are presented in Appendix A.

As can be seen in Fig. 4.12, the absorbances of the solutions increase in their peak values with an increasing DPA concentration. It is also interesting to note from these figures that as the DPA concentration increases a slight splitting in the main absorption peak near 271 nm becomes more prominent. This effect can be seen better in the enlarged graphs of Fig. 4.13. This phenomenon has been observed before by Cable and seems to be due to the effect of the π* – π orbital interaction in the DPA.⁵⁸ Also, a weaker secondary absorption band (~ 430 nm to 532 nm), that was also visible in the spectra of the DPA by itself (Figs. 4.6(a) - 4.6(c)), becomes more visible.

Figure 4.14 shows an enlarged view of the absorbance of mixtures of [15µM] DPA with [5µM], [10µM], [15µM], [20µM], and [25µM] TbCl₃ in distilled water, as a function of wavelength. It is clear from this figure that the absorbance spectra of the mixtures do not seem to be affected by the concentration of the TbCl₃ in any systematic way.

Next, the absorbance value for each different solution at 266 nm was extracted, and a graph of absorbance as a function of varying DPA and TbCl₃ concentrations was plotted for each solvent. The corresponding solvent’s background absorbance value has been subtracted from each data point.
Figure 4.13 Enlarged view of the absolute absorbance spectra of solutions shown in Fig. 4.12.
Figure 4.14  Enlarged view of absorbance spectra of [15µM] DPA with different concentrations of TbCl₃ in distilled water; water background has been subtracted. This graph corresponds to Figure 4.12 (c).
Figure 4.15 shows the measured absorbance values at 266 nm as a function of concentration for mixtures of DPA and TbCl$_3$ in distilled water. When there is no DPA present, the absorbance of TbCl$_3$ at 266 nm is essentially zero. As noted before, the absorbance of TbCl$_3$ does not seem to change considerably for a fixed concentration of DPA, regardless of the TbCl$_3$ concentration. This suggests an almost linear relationship between the absorbance of light at 266nm and the concentration of the DPA molecules, for the DPA-TbCl$_3$ complex, indicating that DPA is the main, and possibly only, absorbing species in the DPA-TbCl$_3$ complex. The same pattern seems to hold true for mixtures of DPA and TbCl$_3$ in [0.01M] HCl and in [1M] SAB (pH 5.6), within experimental uncertainty, as shown in Fig. 4.16 and Fig. 4.17. The indentations in the graphs are thought to be due to experimental error.

4.4.4 Absorption Cross Section of the DPA-TbCl$_3$ Complex

Next, the absorption cross section values for the DPA-Tb complex were calculated. The absorbance values presented in Figs. 4.15 - 4.17, were used in these calculations. Since the DPA molecules were shown to be the main absorbers of the incident light at 266 nm, their concentration alone was used for the concentration variable, N, in Eq. (4.3). In other words:

$$\sigma_{DPA-Tb} = \frac{A_{DPA-TbCl_3}}{N_{DPA} \log_{10}(e) l}.$$  \hspace{1cm} (4.4)

The graphical presentation of the calculated absorption cross section values are presented in Fig. 4.18 for the DPA-Tb complex in distilled water, in Fig. 4.19 for the DPA-Tb complex in [0.01M] HCl, and in Fig. 4.20 for the DPA-Tb complex in [1M] SAB.
Figure 4.15  Measured absorbance as a function of DPA and TbCl$_3$ concentration in distilled water at a wavelength of 266 nm. Water background spectra subtracted.
Figure 4.16  Measured absorbance as a function of DPA and \( \text{TbCl}_3 \) concentration in [0.01M] HCl at a wavelength of 266 nm. HCl background spectra subtracted.
Figure 4.17  Measured absorbance as a function of DPA and TbCl$_3$ concentration in [1M] SAB at a wavelength of 266 nm. SAB background spectra subtracted.
Figure 4.18   Absorption cross section values at 266 nm of mixtures of DPA and TbCl$_3$ at varying concentrations, in distilled water.
Figure 4.19  Absorption cross section values at 266 nm of mixtures of DPA and TbCl$_3$ at varying concentrations, in [0.01M] HCl.
Figure 4.20  Absorption cross section values at 266 nm of mixtures of DPA and TbCl₃ at varying concentrations, in [1M] SAB.
(pH 5.6). Except for small discrepancies in the data, it is clear that the absorption cross section of the terbium doped dipicolinate molecule is the same as that for the DPA alone, within experimental error, which is significantly larger than that for the terbium ion alone.

The absorption cross section value for the DPA-Tb complex was calculated to be about \((1.9 \pm 0.46) \times 10^{-17}\) cm\(^2\)/molecule. This value falls within the uncertainty of the absorption cross section value for the DPA molecule, which was \((2.1 \pm 0.38) \times 10^{-17}\) cm\(^2\)/molecule.

The obtained value for the absorption cross section of the DPA-Tb complex is within a factor of 2 of similar data obtained by Latva\(^{35}\), which was around \(4.9 \times 10^{-17}\) cm\(^2\)/molecule at 275 nm.

To better see the dependence of the absorption cross section of the DPA-Tb complex on the concentration of the individual components, two graphs were plotted. A plot of the average absorption cross section as a function of TbCl\(_3\) concentration, shown in Fig. 4.21 (a), shows a relatively invariant dependence of \(\sigma_{\text{DPA-Tb}}\) with respect to the terbium chloride concentration. A plot of the average \(\sigma_{\text{DPA-Tb}}\) as a function of DPA concentration shows a stronger dependence of the absorption cross section on the amount of DPA present in the mixture, with the value dramatically decreasing (to \(\sigma_{\text{Tb}}\)) when there is no DPA present, Fig. 4.21 (b).
Figure 4.21  Average absorption cross section of the terbium doped dipicolinic acid as a function of the terbium ions’ concentration, (a), and as a function of DPA’s concentration, (b), in different solvents at $\lambda_{\text{exct}} = 266$ nm.
4.5 Comparison of Absorption and Fluorescence Spectrum of DPA, Tb, and the DPA-Tb Complex

It is important to compare the absorption and fluorescence spectrum of DPA, Tb, and the DPA-Tb complex.

For absorption, the DPA absorption spectrum was given in Fig. 4.6, and showed almost the same absorption spectrum as that for the DPA-Tb complex (Fig. 4.12). Note that the absorption spectrum of terbium alone given in Fig. 4.7 was essentially zero. As a result, the absorption spectrum of DPA-Tb is essentially the same as that for the DPA.

For fluorescence, Fig. 3.5 shows the fluorescence spectrum emitted by terbium alone and Fig. 3.6 shows the spectrum emitted by the DPA-Tb complex. Figure 2.7 shows negligible fluorescence emission from DPA alone for the case of no UV irradiation; these results are also confirmed later in Chapter 5 of this thesis which shows the negligible fluorescence emission from DPA alone (see Fig. 5.14). As a result, the fluorescence emission spectrum from the DPA-Tb complex is essentially the same as that for terbium alone.

Of importance, though, is that while the above absorption and fluorescence spectrum are similar in shape, they differ in magnitude and intensity.

Based upon this, we conclude that the absorption of light by the DPA-Tb complex is mostly due to the DPA, and the fluorescence emission of the DPA-Tb complex is mostly associated with Tb emission. As such, for simplicity, we can use the same descriptions of the energy levels for DPA and Tb$^{3+}$ as that for the DPA-Tb complex.
CHAPTER 5. UV EXCITATION EMISSION MATRIX (EEM) SPECTRA OF TERBIUM CHLORIDE AND DIPICOLINIC ACID

In order to study the absorption spectrum of DPA and DPA-TbCl$_3$, an investigation was made to determine the optimized laser wavelength to be used for excitation. Toward this end, an Excitation Emission Matrix (EEM) spectral study was made to vary the laser excitation wavelength and detect the emitted fluorescence light.

This chapter describes the experimental setup and the spectral results obtained when different proportions of terbium chloride and dipicolinic acid were mixed in a number of different solvents, and their fluorescence spectra were recorded in the form of excitation emission matrices (EEM), after being excited with a low pulse repetition frequency (PRF) UV tunable dye laser.

5.1 Experimental Setup for the Excitation Emission Matrix Measurements

This section presents the experimental setup used to obtain the EEM spectra of DPA and TbCl$_3$ in different solutions. The overall EEM setup is shown in Fig. 5.1, and consisted of a pulsed nitrogen pumped, frequency doubled tunable dye laser whose UV output was focused into the center of a quartz cell containing about 3 ml of each solution. With the help of an end-of-fiber lens, and a UV/VIS fiber, the fluorescence spectra were collected at right angles to the quartz cuvette containing the samples. The fluorescence was then analyzed using a USB-2000 spectrometer (Ocean Optics Inc.).
Figure 5.1 Schematic of set up used for laboratory excitation emission (EEM) spectroscopy measurements.
The incident laser light was partially deviated to two UV enhanced silicon detectors, before and after the cuvette, by using two beam splitters. These detected laser intensities were used to normalization the spectra with respect to the incident laser power.

5.1.1 Tunable Laser Source

A schematic of the tunable dye laser is shown in Fig. 5.2. This laser consisted of three separate modules. The first module was a pulsed nitrogen laser (Laser Science Inc., Model VSL-337ND-S), with an output wavelength of 337 nm, output pulse energy of 300\(\mu\)J/pulse, a repetition rate of 10Hz, and a pulse width of 4 ns.\(^{62}\)

The output of the nitrogen laser pumped the second module, which consisted of the dye laser (Laser Science Inc., Model DLMS-220). The nitrogen laser’s output was focused onto a quartz cuvette containing an organic dye by means of a cylindrical lens. This dye laser gain medium in the dye cuvette was inside a linear mirror to mirror cavity, with the end mirror being a 2400 lines/mm grating arranged in a Littrow configuration. The dye laser was tunable from 360 nm to 700 nm, depending on the emission band of the dye used. For each dye used, different output wavelengths could be chosen by changing the grating angle, which was calibrated to read the emission wavelength. The line width of the output was about 0.3 nm. The energy of the outgoing laser pulse was about 50 \(\mu\)J/pulse at 500nm.\(^{63}\) The output of the dye laser was polarized 8:1, vertical to horizontal, respectively.

The dyes that were used in these experiments were Coumarin 440, Coumarin 481, and Coumarin 500 (Exciton Inc.). The relative spectral outputs of these dyes are shown in Fig. 5.3. The lifetime of the dyes were prolonged by using a magnetic stirrer to
Figure 5.2  Nitrogen pumped, frequency doubled UV tunable dye laser (Laser Science; Models VSL-337ND, DLMS-220 and UVS-300).
Figure 5.3  Tuning curves for Coumarin 440, Coumarin 481, and Coumarin 500 dyes that were used for the EEM experiments. (Graph obtained from http://www.exciton.com/).
circulate the dyes and, therefore, minimize photo-bleaching. Coumarin 440 was used for the 440-470 nm output range. Coumarin 481 was used for the 470-490 nm range. Coumarin 500 was used to obtain an output wavelength range of 490-555 nm.

The output of the dye laser was sent into a frequency doubling module (Laser Science Inc., Model UVS-300). The vertically and horizontally polarized parts of the dye laser’s output were separated by a polarizing beam splitter, which directly transmitted the horizontally polarized part of the beam out of the module, and it reflected the vertically polarized component of the beam into the remainder of the module. This reflected part of the beam was focused into the center of a non-linear frequency doubling β-barium borate (BBO) crystal, which was oriented to frequency double vertically, polarized light. The frequency doubled light was then separated from the fundamental beam by using a Pellin-Broca prism.

Two separate BBO crystals were used in the doubling module for the EEM measurements, and were cut and polished to cover the wavelength ranges between 220 nm to 250 nm and from 240 nm to 285 nm, respectively.

The laser output from the doubling module had a pulse width of 3 ns and a line width of 0.2 nm. At a repetition rate of 10 Hz, the pulse energy varied from about 0.5 µJ/pulse to 5 µJ/pulse, depending on the wavelength of the laser. The parameters of this laser are listed in Table 5.1.

5.1.2 Spectrometer

The fluorescence spectra of the samples was detected by a spectrometer (Ocean Optics USB-2000) with an entrance slit of 200 µm and a 2048-element silicon CCD
### Tunable Laser Parameters

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Table 5.1 Specifications for the tunable laser (Laser Science Inc.) tuned to 266nm.
detector. The design of the optical layout was a crossed Czerny-Turner, as shown in the previous chapter (Fig. 4.3). The spectrometer’s effective detection range was 380 nm-1050 nm.

The grating used in the spectrometer had 600 lines/mm and was blazed at 500 nm. Details about the spectrometer’s specifications are listed in Table 5.2. The grating efficiency as a function of wavelength is shown in Fig. 5.4.

A UV-VIS optical fiber, whose attenuation as a function of wavelength is shown in Fig. 5.5, was attached to the entrance slit, and at the other end of the fiber, i.e. the collection side, there was a fused silica collection lens suitable for the 200 nm - 2000 nm wavelength range.

The spectrometer had a Sony ILX511 linear silicon CCD array detector with 2048 pixels and a 250:1 signal-to-noise ratio at full signal. The detector’s sensitivity as a function of wavelength is shown in Fig. 5.6, and its specifications are given in Table 5.3.

5.2 Background Subtraction and Normalization Procedures

Before fluorescence spectra were obtained, a background or dark spectrum was obtained while the illumination source was blocked. This background spectrum was then subtracted from each fluorescence spectral data. A sample background spectrum is shown in Fig. 5.7.

All the obtained data that are presented in this chapter were normalized to the incident laser’s power, shown in Fig. 5.8, the spectrometer’s grating efficiency (Fig. 5.4) and to the spectrometer’s detector’s sensitivity (Fig. 5.6) for the corresponding wavelengths. Specifically, the normalization process for the spectral data used
Table 5.2  USB-2000 spectrometer’s specifications (Ocean Optics Inc.).

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<td>380 nm – 1050 nm</td>
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<tr>
<td>Grating Spacing</td>
<td>600 lines/mm</td>
</tr>
<tr>
<td>Grating Blazing</td>
<td>500 nm</td>
</tr>
<tr>
<td>Grating Efficiency</td>
<td>350 nm – 850 nm</td>
</tr>
<tr>
<td>Optical Resolution</td>
<td>10 nm FWHM</td>
</tr>
<tr>
<td>Integration Time</td>
<td>3 ms to 65 s</td>
</tr>
<tr>
<td>Fiber Diameter</td>
<td>600 µm (VIS/NIR)</td>
</tr>
</tbody>
</table>
Figure 5.4  USB-2000 grating’s relative efficiency as a function of wavelength (600 lines/mm blazed at 500 nm).
Figure 5.5  Attenuation curve for 600µm diameter UV-VIS fiber.
Figure 5.6  Relative sensitivity of 2048-element Sony silicon CCD array (Sony ILX511).
Note: The relative sensitivity data for wavelengths below 400 nm was not available.
Detector: Sony ILX511 linear silicon CCD array

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of elements:</td>
<td>2048 pixels</td>
</tr>
<tr>
<td>Pixel size:</td>
<td>14 µm x 200 µm</td>
</tr>
<tr>
<td>Pixel well depth:</td>
<td>62,500 electrons</td>
</tr>
<tr>
<td>Signal-to-noise ratio:</td>
<td>250:1 (at full signal)</td>
</tr>
<tr>
<td>A/D resolution:</td>
<td>12 bit</td>
</tr>
<tr>
<td>Dark noise:</td>
<td>2.5 RMS counts</td>
</tr>
<tr>
<td>Corrected linearity:</td>
<td>&gt;99.8%</td>
</tr>
</tbody>
</table>

Table 5.3  CCD detector’s specifications.
Figure 5.7  Background spectra (noise) of the USB-2000 spectrometer with the excitation source blocked.
Figure 5.8  Dye laser power output normalized to the detector’s responsivity.
where $F_{\text{Normalized}}$ represents the normalized fluorescence spectrum, $F_i$ represents the (raw) fluorescence spectrum corresponding to an incident wavelength $i$, $D$ represents the dark spectra, $P_i$ is the dye laser’s power at wavelength $i$, $E_{\text{Grating}}^\lambda$ is the efficiency of the grating at wavelength $\lambda$, and $S_{\text{CCD}}^\lambda$ is the sensitivity of the silicon CCD detector at wavelength $\lambda$.

5.3 Solution Preparation

Four different solvents were prepared in order to dilute different proportions of terbium and dipicolinic acid. [0.01M] HCl and [1M] HCl were prepared by diluting [17M] HCl (Fisher Scientific) with distilled water. Solutions of [1M] sodium acetate buffer (SAB), pH 5.6, were created by mixing [1M] sodium hydroxide with [1M] acetic acid until the desired pH was obtained. Distilled water by itself was also used as a solvent.

Once the solvents were prepared, terbium chloride hexahydrate (TbCl₃ . 6H₂O) (Sigma-Aldrich, 99.9% purity) was added to each solvent in order to produce [100µM] TbCl₃. Next, dipicolinic acid (Sigma Aldrich, Inc.) was added to each solvent in order to make [25 µM] DPA.
The final set of solutions consisted of mixtures of DPA with TbCl₃ in each solvent, to form [25μM] DPA with [100μM] TbCl₃ mixed in each of the four different solvents described above.

5.4 Experimental Results: Measured EEM Spectra for DPA, TbCl₃ and Mixed Solutions of Both

EEM spectra were obtained from a variety of solutions and background solvents. The laser wavelength was tuned over 6 different wavelengths from 225 nm to 275 nm, and the fluorescence spectrum recorded for each sample. As an example, Fig. 5.9 shows the normalized fluorescence intensity of a sample of distilled water as function of the emission wavelength, \( \lambda_{\text{em}} \), with respect to the incident excitation wavelength, \( \lambda_{\text{exc}} \). As can be seen, for the emission wavelength range of 470 nm-650 nm distilled water’s fluorescence is undetectable for excitation wavelengths of 225 nm to 275 nm. Additional EEM spectra were obtained for a series of solutions of TbCl₃ in distilled water, TbCl₃ in [0.01M] HCl, TbCl₃ in [1M] HCl, and TbCl₃ in [1M] SAB (pH 5.6). In addition, EEM spectra were obtained for DPA in distilled water; and mixtures of DPA with TbCl₃ in distilled water, in [0.01M] HCl, in [1M] HCl, and in [1M] SAB (pH 5.6). These EEM results are discussed in the following sections.

5.4.1 EEM of TbCl₃ in Solutions

The normalized fluorescence spectrum of a solution of [100μM] TbCl₃ in distilled water was measured for excitation wavelengths of 225 nm to 275 nm in increments of 10 nm, and the data is shown in Fig. 5.10. As can be seen in this and most of the EEM
Figure 5.9  EEM spectra of distilled water.
Figure 5.10  EEM spectra of [100µM] TbCl\textsubscript{3} in distilled water.
graphs, there is more noise present in the spectra corresponding to excitation wavelengths 225 nm, 235 nm, and 275 nm; we attribute this to the low laser power at these excitation wavelengths.

In Fig. 5.10, there is a very weak fluorescence peak at around 540 nm for the 235 nm, 255 nm, and 265 nm excitation wavelengths. The strongest fluorescence signal, however, occurred when the sample was excited with 225 nm light. At this excitation wavelength, there are four fluorescence peaks corresponding to the four strongest (electronic) transitions in the terbium ion ($\text{Tb}^{3+}$), as described in chapter 2.

No fluorescence was detected when measuring the EEM spectra of [100 µM] TbCl$_3$ in [1 M] HCl, and this is shown in Fig. 5.11. Although the same is true for the fluorescence from [100 µM] TbCl$_3$ in [0.01 M] HCl (Fig. 5.12), a small but negligible peak was consistently present at around 540 nm. In the case of the [100 µM] TbCl$_3$ in [1 M] SAB (pH 5.6) solution, no fluorescence was observed (Fig. 5.13).

5.4.2 EEM of DPA in Solutions

Figure 5.14 shows the measured EEM spectra of [100 µM] DPA in distilled water, when excited from 225 nm to 275 nm in increments of 10 nm. As can be seen, the fluorescence emission for this chemical is negligible. These results are consistent with previous data. Dipicolinic acid, which is thought to offer protection from UV radiation to bacteria spores, has a very weak fluorescence.$^{29,33}$ The data presented in this section is in accordance with these studies, in that the fluorescence of DPA in solution is negligible, when UV-irradiated for less than at least 1 minute.
Figure 5.11  EEM spectra of [100µM] TbCl₃ in [1M] HCl.
Figure 5.12  EEM Spectra of [100μM] TbCl₃ in [0.01 M] HCl.
Figure 5.13  EEM spectra of [100μM] TbCl₃ in [1M] SAB pH 5.6.
Figure 5.14  EEM spectra of [100µM] DPA in distilled water.
5.4.3 EEM of Mixtures of DPA and TbCl$_3$ in Solution

Figure 5.15 shows the measured EEM spectra of [50µM] DPA mixed with [50µM] TbCl$_3$ in distilled water. As can be seen, all four fluorescence peaks are present in all of the emission spectra, regardless of the excitation wavelength, although the fourth peak (i.e. 618 nm peak) is less pronounced. There does not seem to be a strong dependence between the excitation wavelength and the emission intensity for this solution.

Figure 5.16 shows the EEM spectra of [25µM] DPA mixed with [100µM] TbCl$_3$ in [0.01M] HCl, for excitation wavelengths of 225 nm to 275 nm. For this case of DPA-Tb in a weak acid solution, all four fluorescence peaks are clearly present in each emission spectrum. The strongest emission at 543 nm occurs with excitation at 225 nm. Figure 5.17 shows the EEM spectra of [25 µM] DPA mixed with [100 µM] TbCl$_3$ in [1M] HCl, for excitation wavelengths of 225 nm to 275 nm. In the case of DPA-Tb in a strong acid solution, no fluorescence is detected for any of the excitation wavelengths. This, we believe, is due to the high acidity of the solvent, i.e. [1M] HCl, and is consistent with previous pH dependent fluorescence quantum yield experimental data.$^{65}$

Figure 5.18 shows the EEM spectra of [25 µM] DPA mixed with [100µM] TbCl$_3$ in [1M] SAB, pH ~ 5.6, for excitation wavelengths of 225 nm to 275 nm. All four fluorescence peaks are clearly present in each emission spectrum, and once again, no strong or direct relation is evident between the excitation wavelength and the emission intensity near 543 nm.
Figure 5.15 EEM spectra of [50μM] DPA [50μM] TbCl₃ in distilled water.
Figure 5.16  EEM spectra of [25μM] DPA in [100μM] TbCl₃ in [0.01 M] HCl.
Figure 5.17   EEM spectra of [25µM] DPA in [100µM] TbCl₃ in [1M] HCl.
Figure 5.18  EEM Spectra of [25μM] DPA in [100μM] TbCl₃ in [1M] SAB.
5.5 EEM Spectra Conclusions

The above results indicate that the wavelength for excitation can be within the range of 225 nm to 270 nm for the mixture of DPA and TbCl$_3$. The EEMs did not follow a pattern between the excitation wavelengths and the emission fluorescence intensities for any of the solutions. As a whole, 235 nm excitation wavelength seemed to produce some of the strongest fluorescence of all of the wavelengths in most of the solutions, i.e. in [0.01M] HCl, in distilled water, and in [1M] SAB, for the DPA-Tb complex.

The solvent that seemed to provide the most fluorescence-inducing environment with most of the excitation wavelengths seemed to be the [0.01M] HCl, followed by distilled water, which was slightly better than [1M] SAB pH 5.6. [1M] HCl did not enable the generation of fluorescence in the solutions.

The EEMs of terbium ion in solution do not show a detectable fluorescence for most excitation wavelengths in most of the solvents, except for $\lambda_{\text{exc}} = 225$ nm in distilled water, where terbium’s four distinct fluorescence peaks are clearly visible. Despite the fact that the OH vibrational modes in water tend to bond to the terbium ion and are effective quenchers, the deep UV excitation wavelength is able to excite the ion. This is in agreement with previously published absorption data for the terbium ion in solution where the ion has a relatively high absorbance in the deep UV region as was shown in Fig. 2.13.
CHAPTER 6.  FLUORESCENCE LIFETIMES OF SOLUTIONS OF
DPA AND TbCl₃, WHEN EXCITED WITH A LOW
PRF PULSED TUNABLE UV DYE LASER

This chapter describes the experimental setup and the fluorescence lifetimes obtained when mixtures of DPA and TbCl₃ in different solutions were excited with a low PRF tunable UV dye laser (266 nm). Using a monochromator, the fluorescence signal was resolved into its four different lines or components. The lifetimes of each of these lines were measured separately, and to the best of our knowledge, this is the first time that these measurements have been reported. Previous measurements had determined the lifetime of the combined fluorescence signal, but not of the individual lines. However, while some of the stronger fluorescence lines were measured using the low PRF tunable dye laser, several of the weaker lines could not be measured with higher accuracy.

6.1 Experimental Set-Up for the Lifetime Measurements Using the Dye Laser

The initial fluorescence lifetime of the DPA-TbCl₃ complex was measured using a low PRF UV laser as described earlier. Figure 6.1 shows the schematic diagram of the setup used to measure the fluorescence lifetimes of the DPA samples, and Fig. 6.2 shows a photograph of the system. A pulsed nitrogen pumped, frequency doubled tunable dye
Figure 6.1  Schematic of the lifetime measurement experimental apparatus using the low PRF tunable dye laser.
Figure 6.2 Photograph of the “complete” lifetime measurement experimental setup. Note: the dye laser and the 4\textsuperscript{th} Harmonic Nd:YAG laser were setup such that either experiment could be carried out without needing major rearrangements. This photograph shows the “complete” set up, the second half of which (i.e. the one that includes the 4\textsuperscript{th} Harmonic Nd:YAG) is described in the following chapter.
laser (Chapter 5, Section 5.1.1) was used as the excitation source, at a frequency of approximately 13 Hz and an output wavelength of 266 nm. The UV output from the dye laser was focused into the center of a quartz cell, using a quartz lens, which contained about 3 ml of the sample. Perpendicular to the incident beam side of the cuvette, a pair of collection lenses gathered the fluorescence signal from the sample and focused it onto the entrance slit of a grating spectrometer (CVI Digikrom 240 Monochromator). A PMT (Photo Multiplier Tube) detector module (Hamamatsu, Model # H6780-03) was placed at the exit slit of the monochromator. The signal from the PMT was then sent to a 500 MHz oscilloscope (Lecroy, Waverunner LT 344), whose specifications are in Table 6.1. The trigger signal for the oscilloscope was the fundamental beam from the dye laser, which was focused onto a fast silicon photo-detector (New Focus, Model # 1621). The data was then transferred to a PC for further analysis and storage via a GPIB interface.

It is important to note that the dye laser was tuned so that the frequency doubled beam had a wavelength of 266 nm, in order to later compare these lifetimes with those obtained when using the high PRF 4th harmonic (266 nm) Nd:YAG laser (Chapter 7).

6.1.1 Monochromator

The monochromator that was used to resolve the fluorescence signal of the sample into its different components was a CVI Digikrom 240 Monochromator. This monochromator could be used in a manual or a computer-controlled mode. The control electronics were internal and calibration was automatic. The monochromator had a modified Czerny-Turner configuration, with three different gratings mounted on a rotating platform. The efficiency of the grating that was used in our experiments is
Table 6.1 Specifications for the Lecroy oscilloscope (Waverunner LT344).

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bandwidth (-3dB)</td>
<td>500 MHz</td>
</tr>
<tr>
<td>Sampling Rate</td>
<td>500 MS/s</td>
</tr>
<tr>
<td>Vertical Resolution</td>
<td>8 bits</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>2 mV – 10 V/div</td>
</tr>
<tr>
<td>Processor</td>
<td>160 MHz PowerPC</td>
</tr>
<tr>
<td>Memory</td>
<td>16 Mbytes</td>
</tr>
</tbody>
</table>

Figure 6.3 Grating spectrometer’s (CVI Digikrom 240) grating efficiency.
shown in Fig. 6.3. Although it is not shown in the graph, the range of wavelengths that was covered by this grating was about 300 nm to 900 nm. Since spatial resolution was not a priority in this experiment, and the signal from some of the transitions were quite weak, the entrance slit and the exit slits were set to a width of 2 mm each to increase the transmitted light.

6.1.2 PMT Module

To collect and temporally resolve the fluorescence signals, a PMT module (Hamamatsu, Model # H6780-03) was positioned flat against the exit slit of the monochromator. The module consisted of a PMT detector and a high voltage power supply, both enclosed in a small package (5cm × 2cm × 2cm). A supply voltage of 12 V was provided to the module. A control voltage (0-1 V) was applied to the module which varied the output of the high voltage of the power supply, thus controlling the gain of the detector. Fig. 6.4 (a) and (b) show the gain curve and the spectral response curve for this PMT module, respectively. The typical control voltage used was around 0.8 V (0.75 V to 0.85 V), which corresponds to an applied high voltage of about 800 V and a PMT gain of approximately $7 \times 10^5$. Table 6.2 lists the specifications for this PMT detector.

For a maximum anode current of 100 µA and using the PMT gain value, the current at the photocathode can be calculated to be $0.14 \text{ nA} (100 \mu \text{A}/7 \times 10^5)$ or $8.9 \times 10^8$ electrons/s. At 266 nm the energy per photon can be calculated to be $7.47 \times 10^{-19}$ J. The maximum average power that can impinge on the PMT photocathode can be calculated to be $6.65 \times 10^{-10}$ W ($7.47 \times 10^{-19}$ J × $8.9 \times 10^8$ electrons/s). For 10 Hz repetition rate of the
Figure 6.4 Gain as a function of control voltage (a) and sensitivity as a function of wavelength (b) for the Hamamatsu PMT (Model # H6780-03). Note: the curves marked as -03 correspond to the module used in our setup.
Table 6.2  Specifications for the Hamamatsu PMT.

<table>
<thead>
<tr>
<th>Effective Area</th>
<th>8mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supply Voltage</td>
<td>11.5 V - 15.5 V</td>
</tr>
<tr>
<td>Control Voltage</td>
<td>0.25 V - 0.95 V</td>
</tr>
<tr>
<td>Min Wavelength</td>
<td>185 nm</td>
</tr>
<tr>
<td>Max Wavelength</td>
<td>650 nm</td>
</tr>
<tr>
<td>Rise Time</td>
<td>0.78 ns</td>
</tr>
</tbody>
</table>
dye laser, the maximum signal energy that can be detected by the photocathode safely was about 66.5 pJ, i.e. $6.65 \times 10^{-10}$ W/10 Hz. For the 4th harmonic Nd:YAG laser, with an output of 266 nm and a repetition rate of 8.5 KHz, which was used in a similar setup (see Chapter 7) the maximum signal energy per excitation pulse that could be safely detected by the photocathode was about 0.08 pJ.

### 6.1.3 Fast Silicon Detector

A fast silicon photodetector (New Focus, Model # 1621) was used to detect the laser pulse and was used as a trigger signal. The detector’s diameter was 0.8 mm. The typical rise time for this detector was $\leq$ 1 nanosecond (for 50 Ω load resistance setting). A plot of responsivity for this detector is shown in Fig. 6.5.

### 6.2 Experimental Procedure

The fluorescence lifetime, $\tau$, of the emission lines was measured by recording the fluorescence intensity as a function of time and determining the decay time to the 1/e intensity point. To determine the value of $\tau$, the natural logarithm of the fluorescence intensity as a function of time was plotted using an Excel or Matlab program. The fluorescence lifetime of the sample was then calculated by taking the negative inverse of the slope of the semi-log plot, or,

$$
\tau = -\frac{1}{\text{Slope of semi-log plot}}
$$

(6.1)
Figure 6.5  Plot of responsivity as a function of wavelength of the fast silicon photodetector (New Focus, Model # 1621) used as a trigger.
The process of lifetime calculations is explained in more detail in Appendix B. In order to find the fluorescence lifetime of the samples, \( \tau \), the average of 100 runs of the fluorescence intensity as a function of time was often used.

All the data presented here have been normalized to the laser’s power, the monochromator’s grating efficiency at the relevant wavelengths, and the PMT’s sensitivity at the relevant wavelengths. The measurements were also performed in a darkened room in order to minimize background noise from ambient light.

### 6.3 Solution Preparation

Three different solvents were used in order to dilute different proportions of terbium and dipicolinic acid. [0.01M] HCl was prepared by diluting [12N] HCl (Fisher Scientific, 34%-37%) with distilled water. A solution of [1M] sodium acetate buffer (SAB), pH 5.6, was created by mixing [1M] sodium hydroxide with [1M] acetic acid until a pH of approximately 5.6 was obtained. Distilled water by itself was also used as a solvent.

Once the solvents were prepared, terbium chloride hexahydrate (TbCl\(_3\).6H\(_2\)O) (Sigma-Aldrich, 99.9% purity), was added to each of the solvents in order to make a solution with a final concentration of [50 \( \mu \)M] TbCl\(_3\) in it. Next, DPA (Sigma Aldrich, Inc.) was added to each of the previous solutions to form final solutions that consisted of [50\( \mu \)M] DPA with [50\( \mu \)M] TbCl\(_3\) in distilled water; [50\( \mu \)M] DPA with [50\( \mu \)M] TbCl\(_3\) in [0.01M] HCl; and [50\( \mu \)M] DPA with [50\( \mu \)M] TbCl\(_3\) in [1M] SAB (pH 5.6). These solutions were then evaluated in order to obtain their fluorescence lifetime. The pHs of
the solvents that were used were as follows: distilled water pH ~ 7, [1M] SAB pH ~ 5.6, and [0.01M] HCl pH ~ 2.

6.4 Lifetime Data Using Low PRF Dye Laser

The experimental apparatus given in the previous section was used to measure the fluorescence lifetime of DPA and TbCl$_3$ in different solutions. It is important to note that the pulse width of the dye laser used in this experiment, which was about 3 ns, was negligible in comparison with the time scale of the fluorescence decay of the samples, which was on the order of milliseconds. In addition, the speed of the oscilloscope and PMT detector response is also much faster than the fluorescence decay time.

6.4.1 Measured Fluorescence Lifetime of Mixtures of DPA with TbCl$_3$ in Different Solvents

The first sample that was analyzed was a solution of [50 µM] DPA with [50 µM] TbCl$_3$ in distilled water. The measured fluorescence intensity as a function of time is presented in Fig. 6.6 (a). As can be seen from the figure, all four individual lines and the combined fluorescence signal show lifetimes of about 1 ms. The 618 nm fluorescence line had the weakest intensity. The lifetime is more easily seen as in Fig. 6.6 (b), which shows a plot of the natural logarithm of the fluorescence intensity as a function of time for the same solution. The lifetime for the strongest lines is easily seen, but digitization noise (finite resolution) of the oscilloscope A/D converter and low signal-to-noise is evident for the weakest lines or at the end of the fluorescence tail.
Figure 6.6  (a) Plot of fluorescence intensity as a function of time, and (b) semi-log plot of the fluorescence intensity as a function of time of a solution of [50 µM] DPA with [50 µM] TbCl₃ in distilled water.
Similar measurements for the [50 µM] DPA with [50 µM] TbCl₃ in [0.01M] HCl are presented in Fig. 6.7, and that for [50 µM] DPA with [50 µM] TbCl₃ in [1M] SAB are shown in Fig. 6.8.

As seen in the figures, the 543 nm line and the 488 nm lines are the strongest lines and exhibit a decay close to that of a “straight” line, indicative of a single exponential decay in the semi-log plots. The two weaker fluorescence signals, i.e. those corresponding to the 581 nm and the 618 nm lines, have significantly more noise and exhibit less of a straight line in their semi log plots. This can be indicative of a multi-exponential decay, but in this case it is most probably due to the low S/N ratio which in turn is due to the relatively low average laser power. The semi-log plot from all the fluorescence signals mixed together also has a slight curvature; this, however, is probably due to the additive influence of the two weakest fluorescence lines.

The fluorescence lifetimes of all of the lines mixed together, as well as for each fluorescence line by itself, were calculated and are listed in Table 6.3; a curve fitting routine in the Matlab© software was used to estimate the slope and uncertainty value in the middle of the curve. For each of the samples, the lifetimes of the first three individual fluorescence lines are within uncertainty of each other. This is consistent with these electronic transitions emanating from the same upper level, i.e. \(^5\!D_4\). The lifetime measured for the weakest line is within this value considering the larger uncertainty in the measurement.

It is interesting to compare the magnitude of the lifetimes of these samples with respect to the solvent’s properties. The lifetimes for these samples have the following trend: \(\tau_{SAB} > \tau_{DW} > \tau_{HCl}\), with the numerical value for all of the fluorescence lines mixed
Figure 6.7  (a) Plot of fluorescence intensity as a function of time, and (b) semi-log plot of the fluorescence intensity as a function of time, of a solution of [50 μM] DPA with [50 μM] TbCl₃ in [0.01M] HCl.
Figure 6.8  (a) Plot of fluorescence intensity as a function of time, and (b) semi-log plot of the fluorescence intensity as a function of time, of a solution of [50 µM] DPA with [50 µM] TbCl₃ in [1M] SAB (pH 5.6).
<table>
<thead>
<tr>
<th></th>
<th>Distilled Water</th>
<th>[0.01M] HCl</th>
<th>[1M] SAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>0.89 ± 0.12</td>
<td>0.75 ± 0.01</td>
<td>1.11 ± 0.01</td>
</tr>
<tr>
<td>488 nm</td>
<td>0.88 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>1.14 ± 0.01</td>
</tr>
<tr>
<td>543 nm</td>
<td>0.89 ± 0.01</td>
<td>0.76 ± 0.01</td>
<td>1.08 ± 0.01</td>
</tr>
<tr>
<td>581 nm</td>
<td>0.87 ± 0.01</td>
<td>0.70 ± 0.01</td>
<td>1.16 ± 0.01</td>
</tr>
<tr>
<td>618 nm</td>
<td>1.43 ± 0.08</td>
<td>1.09 ± 0.13</td>
<td>5.37 ± 0.57</td>
</tr>
</tbody>
</table>

Table 6.3  Measured Fluorescence lifetimes of solutions of [50 µM] DPA with [50 µM] TbCl₃ in the indicated solvents when excited with the dye laser, λ_{exct} = 266 nm.
together for each of these solution being 1.11 ms for the solution in [1M] SAB, 0.89 ms for the solution in distilled water, and 0.75 ms for the solution in [0.01M] HCl.

As stated earlier, lifetimes of terbium doped DPA complexes are very sensitive to the nature of their environment, especially to the number of water molecules occupying the inner coordination sites.\textsuperscript{38,66}

Based on this, we would expect to see the lowest lifetime values, as well as the lowest fluorescence intensities, for the solutions mixed in distilled water. In these experiments the lowest lifetime values belong to the solutions mixed in [0.01M] HCl. This could be an effect of the low pH of the [0.01M] HCl, in addition to that of the OH vibrational oscillators.

According to previously published results,\textsuperscript{65} the quantum yield of \([\text{Tb(DPA)}_3]^{3-}\) in water, which is proportional to the fluorescence lifetime, steadily increases as a function of pH until it reaches a maximum around pH \(\sim 7.5\). In this case, the order of the lifetimes’ magnitudes does not follow that of the solvent’s pHs, which is pH\(_{\text{DW}}\) > pH\(_{\text{SAB}}\) > pH\(_{\text{HCl}}\). This could be due to the fact that the DPA to TbCl\(_3\) ratio is 1:1 in these solutions of [50\(\mu\)M] DPA with [50\(\mu\)M] TbCl\(_3\), and therefore, the tris- species, i.e. [Tb(DPA)\(_3\)]\(^{3-}\), which tends to form in the excess of DPA, as described earlier in Chapter 2, was not dominantly present.

Lastly, as can be seen from all of the figures, there exist a distinctive “build up” time before the start of the fluorescence decay. This may be due to the transfer of energy from the DPA to the Tb\(^{3+}\) ion. A visual inspection of Fig. 6.6 yields an estimate of about 0.4 ms for this transfer time. This transfer time has been previously suggested to be on the order of 2.5 ms, but these results were obtained under different solvent conditions.\textsuperscript{25}
6.4.2 Measured Fluorescence Lifetime of TbCl$_3$ in Distilled Water

A sample of [50 µM] TbCl$_3$ in distilled water was excited using the low PRF 266 nm laser pulse from the dye laser. The fluorescence intensity as a function of time for this sample is plotted in Fig. 6.9 (a), and its semi-logarithmic plot is shown in Fig. 6.9 (b).

As can be seen in the figures, the 488 nm fluorescence line was extremely weak and was not able to be measured. In addition, the 618 nm line was also very weak, and the PMT voltage was increased to help increase this signal. This resulted in an increase in the detected fluorescence signal of the 618 nm line which was not accounted for, i.e. normalized, in the data presented here. While this influenced the relative strength of the 618 line in the Fig 6.9 (a), it does not affect the slope of the curve in Fig. 6.9 (b) which is the logarithmic plot of the intensity.

The lifetimes of each of these lines were calculated using the inverse slope of the semi-log plot; the lifetime of all the lines mixed together was found to be (0.36 ± 0.02) ms. The lifetimes of the weaker 488 nm fluorescent line in distilled water could not be detected. Due to the weak signals with TbCl$_3$ in HCl and in SAB, further experiments to measure the lifetimes of TbCl$_3$ in the other solvents were not carried out.

6.5 Lifetime Analysis

Table 6.4 shows the compilation of lifetimes measured using the tunable UV dye laser system. As can be seen only lifetimes of TbCl$_3$ in distilled water are presented.
Figure 6.9  (a) Fluorescence intensity as a function of time, and (b) semi-log plot of fluorescence intensity as a function of time of a solution of [50\mu M] TbCl$_3$ in distilled water.
Similar measurements were initially tried for TbCl$_3$ in HCl and SAB solutions, but experimental difficulties precluded obtaining these results.

However, later experiments presented in this thesis yielded good value for τ for the mixture of DPA and TbCl$_3$ in all of these solutions. As mentioned previously, the fluorescence signal from aqueous terbium was very difficult to obtain. This is because of the relatively good radiationless energy transfer from the terbium ion to its aqueous environment, i.e. the OH oscillators, and its low absorption cross section.

The fluorescent lines’ lifetimes that we were able to detect do fall within uncertainty of each other (this is consistent with all of the transitions in the Tb$^{3+}$ ion starting at the same upper ‘excited’ level). These lifetime values are within the ranges of previously published data for which the lifetime of the $^5$D$_4$ emitting state of the terbium (III) ion falls in the 0.4 ms to 5.0 ms range.$^{44,67,68}$

Figure 6.9 for TbCl$_3$ alone shows almost zero buildup time for the fluorescence to start. This is different than the lifetime data for the DPA-TbCl$_3$ complex (Fig. 6.6 (a)), due to this being a direct excitation of the Tb$^{3+}$ ion’s $^5$F$_1$ level.

It may be added that the above measured lifetimes were probably not modified due to radiation trapping. For example, all of the DPA-Tb complex absorption values near the fluorescence emission wavelengths had absorbance (A) values of less than 0.02 for the 1 cm path length (see Fig. 4.12, and Fig. A.7). A value of A = 0.02 yields a transmission of 96% or absorption of 4%; Fig. A.8 for SAB yields values about 2 to 3 times greater. Radiation trapping only occurs when the absorption is much stronger, approaching 90% or more for the optical path.
It is important to mention that the slight variations in the fluorescence lifetimes of the four different fluorescence lines from the DPA-Tb complex, as well as from the terbium ion by itself, could be due to slight variations in the lifetime of the termination level (see Section 2.1.4.3.1) and the possibility that the different lines originate or terminate on similar levels but which may have different degeneracy or parity which could then influence the overall transition rate or lifetime of the transitions.

In these data we averaged 100 runs and then obtained the lifetime. The results maybe slightly different if the reverse order was made, but this would depend upon the correlation properties of the noise.
Table 6.4  Lifetimes of TbCl₃ in distilled water. The lifetimes that we were unable to obtain are indicated with a dashed entry.
CHAPTER 7. FLUORESCENCE LIFETIMES OF SOLUTIONS OF DPA AND TbCl₃, WHEN EXCITED WITH A HIGH PRF AND MODULATED PULSED UV LASER

In order to detect the weaker fluorescence lines, especially the 618 nm line, we needed a higher average power UV laser with which to excite the sample. As such, a high-PRF UV 266 nm microchip laser was used which had about several orders of magnitude greater average power than that of the pulsed low-PRF dye laser. This chapter describes the experimental setup and the measured fluorescence lifetimes obtained when mixtures of DPA and TbCl₃ in different solutions were excited with a high PRF microchip 4th harmonic Nd:YAG laser (266 nm). However, because of the quasi-CW nature of the high-PRF (8.5 kHz) 266 nm laser, additional slower modulation of the laser excitation pulse train was required to allow detection of the 1 ms fluorescence decay times. These experiments and results are presented in the following sections.

7.1 Experimental Setup for the Lifetime Measurements Using the Microchip Laser

A high pulse repetition frequency, 4th harmonic Nd:YAG microchip laser (JDS Uniphase) with an output wavelength of 266 nm, in combination with an optical chopper, replaced the nitrogen pumped dye laser as the excitation source in the setup described in Chapter 6, and is shown in Fig. 7.1.
Figure 7.1  Schematic of lifetime measurement’s experimental setup using the high PRF pulsed laser and the optical chopper.
The laser beam was routed using two alignment mirrors, passed through an optical chopper’s blades, and then focused into the center of the sample cuvette using a fused silica lens. Part of the beam was split off to trigger the fast silicon detector. Except for the excitation source and chopper, the rest of the setup remained as described in Section 6.1.

7.1.1 Optical Chopper

The optical chopper (Stanford Research Systems, Model# SR-540) was used to modulate the excitation beam and used one of the two following chopper blades: one with 6 outer slits (slit width of approximately 2 cm) for frequencies up to 400 Hz, and one with 30 outer slits (slit width of approximately 4 mm), for frequencies of up to 3.7 kHz (Fig. 7.2). For the lifetime experiments described in this chapter, the blade with 30 outer slits was used at the indicated frequencies. The laser beam passed through the center of the outer slits.

The optical chopper also produces an optically generated TTL reference signal which was used for triggering the oscilloscope; the fast silicon detector was also used to trigger the oscilloscope.

7.2 Effect of Optical Chopper on Excitation and Fluorescence

The laser beam was modulated using the optical chopper at different frequencies in order to modulate the optical excitation at a rate closer to that of the expected 1 ms fluorescence decay time. However, the physical act of chopping has an effect on the
Figure 7.2  Optical chopper’s blades used to modulate the laser beam. The blade on the left hand side of the picture was used for the experiment described in this chapter. (From Stanford Research System’s website at http://www.thinksrs.com/products/SR540.htm).
geometry of the incident beam, which consequently affects the fluorescence signal. This geometrical effect is explained in following section.

7.2.1 Finite Beam Size and Geometrical Effect of Chopper Wheel on Incident Beam

The geometrical effect of the finite size of the laser beam and the speed of the chopper wheel was modeled to determine its effect upon the fluorescence intensity time dependence. The beam was usually focused down to a small circular spot size (≤ 1 mm), before going through the chopper. While the exact beam distribution was not measured, it appeared to be approximately close to a TEM$_{00}$ Gaussian mode with little if any side lobes within the pattern. As such, we modeled the laser beam before passing through the chopper by a Gaussian distribution as shown in Fig. 7.3. In order to explore this effect, different parameters were chosen to describe the Gaussian beam size and placement using a variance, $\sigma^2$, and a possible offset, $\mu$. Figure 7.4 shows different Gaussian distributions in terms of the size compared to 1 mm and a possible offset. For our experiments, the beam’s diameter was approximately 1 mm, and the widths of each of the chopper blade’s slits were approximately 4 mm. The beam’s geometrical distribution after passing through the chopper’s blades was graphed by taking into account the interaction of the Gaussian distribution of the original beam with the effect that the blade’s slits had on it by integrating the spatial Gaussian beam profile with that of the moving slit function. The resultant function, called the Cumulative Distribution Function (CDF),$^{69}$ has been calculated for Gaussian beams before, and is shown in Fig. 7.5. The resultant effect of using the CDF function is to modify the rising and fall-off
Figure 7.3 Diagram of Gaussian laser beam (TEM$_{00}$) passing through a moving chopper blade.
Figure 7.4  Plot of Gaussian distributions with different variances. We have used the plot with a variance of 1 to represent the TEM$_{00}$ laser beam from microchip laser before going through the chopper’s blade, i.e. the original beam (from http://en.wikipedia.org/wiki/Normal_distribution).

Figure 7.5  Cumulative distribution function (CDF) for a Gaussian distribution (from http://en.wikipedia.org/wiki/Normal_distribution).
patterns of the beam after passing through the blades. This effect is shown in Fig. 7.6 for our experimental case of 1 mm beam size ($\sigma$ of 1 mm) and slit width of 4 mm.

In the experiments presented in this chapter, the chopper’s frequency was kept at 50 Hz. At this frequency, it took approximately 20 ms for one period of the chopper, defined as the start of one open slit to the end of next closed slit (8 mm), to pass an infinitesimally small point. This means that every millimeter of the chopper blade’s width takes about 2.5 ms to pass an infinitesimally small point at 50 Hz.

From Fig. 7.6 it can be seen that from the time the chopper allows the beam to first pass through until the time the full intensity of the beam is able to go through the slit, it takes approximately 2.5 ms. Since this is a symmetric effect, it also takes approximately 2.5 ms from the time the chopper first blocks off the beam until the time when the intensity of the beam is zero after the blade. This analysis deals only with the spatial geometry of the beam and the blade, as well as the times involved in this geometric interaction which are much longer than the pulse width or the PRF period of the laser.

7.2.2 Effect of Chopper Speed and Laser PRF on the DPA-TbCl$_3$ Fluorescence Signal

When the laser excitation was turned on (without the optical chopper) the fluorescence emission was steady, as seen in Fig. 7.7. As can be seen, the fluorescence signal did not increase or decay over this 45 ms time period. In this data, the sample used was a solution of [50 $\mu$M] DPA with [50 $\mu$M] TbCl$_3$ in SAB.
Figure 7.6  Plot of the 1 mm diameter laser beam’s intensity after interacting with the chopper’s slits. Also shown in the upper part is the turning on and off of the chopper blade’s window. Note the similarity between the build up and the fall down of this graph as compared to the CDF in Figure 7.5.
Figure 7.7  Fluorescence intensity as a function of time when using the high PRF microchip laser with the chopper turned off.
However, when the chopper was turned on and was operating at a frequency of 50 Hz, the incident beam was blocked every 10 ms for a duration of 10 ms. This can be seen in Figure 7.8 which shows two periods of the fluorescence signal, i.e. two periods of the chopper blade, when the sample had been excited with the chopped incident laser beam.

A graphical summary of this process is shown in the experimental data shown in Fig. 7.9, where in part (a) the pulses from the microchip laser before they have passed through the chopper are shown, in part (b) a TTL reference signal from the 50 Hz chopper driver is shown, and in part (c) the measured fluorescence signal obtained from the excitation of the sample is shown.

### 7.3 Experimental Measurements of DPA-TbCl<sub>3</sub> Lifetimes Using Chopper Technique

The optical chopper UV laser excitation just described was used to stimulate the fluorescence emission from different solutions containing DPA-TbCl<sub>3</sub>. Figure 7.10 (a) shows the fluorescence intensity signal and Fig. 7.10 (b) shows the semi-log plot as a function of time for a sample of [50 µM] DPA with [50 µM] TbCl<sub>3</sub> in distilled water for all the fluorescence lines mixed together and for each of the fluorescence lines resolved separately. As can be seen, after the chopper slits are completely closed then the fluorescence decay appears to be linear. For clarity, this figure shows marks indicating the corresponding positions on the signals where the chopper’s blade was “open”, i.e. letting the beam through, and where the chopper’s blade was “closed”, i.e. when the
Figure 7.8  Fluorescence signal of sample when excited with a modulated incident beam, i.e. laser beam that has passed through the optical chopper’s blade.
Figure 7.9  Plot of amplitude as a function of time of the high PRF 266nm 4th harmonic Nd:YAG laser’s output (a), as compared to the chopper’s TTL signal at 50 Hz (b), and the sample’s fluorescence (c).
Figure 7.10  (a) Fluorescence intensity as a function of time, and (b) semi-log plot of the normalized fluorescence decay lines from [50μM] DPA with [50μM] TbCl$_3$ in distilled water ($f_{\text{chopper}} = 50$Hz).
blade was blocking the beam. Each graph has also been marked with the time that it takes for the beam to be completely blocked off.

It should be noted that the intensity of the weakest fluorescence line in this figure, which is approximately 50 a.u., is about 100 times stronger than compared to the fluorescence intensity of the same sample when excited with the low PRF dye laser, shown in Fig. 6.6 (a), which was about 0.5 a.u. This increase in the fluorescence intensity is reflected in the greater accuracy of the lifetime measurement for the weaker lines.

Similar fluorescence intensity measurements as a function of time are shown in Figs. 7.11 and 7.12 for a sample of [50μM] DPA with [50μM] TbCl$_3$ in [0.01M] HCl for and for [50μM] DPA with [50μM] TbCl$_3$ in [1M] SAB, respectively.

The measured lifetime values of these samples, for each of the fluorescent lines and all of them mixed together are listed in Table 7.1. The uncertainties shown in Table 7.1 were calculated using variations of the slope due to a 2-point analysis of the data (see Appendix A). A more accurate technique using a curve fitting routine in Matlab© yielded uncertainties about a factor of 3 lower for the strongest lines. These values are similar to those obtained earlier using the low-PRF pulsed dye laser as given in Table 6.3. As was the case for the lifetime of the samples when excited with the dye laser (Chapter 6), the magnitudes of the lifetimes when excited with the microchip laser (in combination with the chopper) have the same order, i.e. $\tau_{SAB} > \tau_{DW} > \tau_{HCl}$. This will be discussed in Chapter 10.
Figure 7.11  (a) Fluorescence intensity as a function of time, and (b) semi-log plot of the normalized fluorescence decay lines from [50µM] DPA with [50µM] TbCl$_3$ in [0.01M] HCl ($f_{chopper}$ = 50Hz).
Figure 7.12  (a) Fluorescence intensity as a function of time, and (b) semi-log plot of the normalized fluorescence decay lines from [50µM] DPA with [50µM] TbCl₃ in [1M] SAB (f_chopper = 50Hz).
Table 7.1  Table of lifetimes of solutions of [50µM] DPA with [50µM] TbCl₃ in the indicated solvents when excited with the modulated microchip laser, λₑₓᶜₑᵗ = 266 nm.

<table>
<thead>
<tr>
<th></th>
<th>Distilled Water</th>
<th>[0.01M] HCl</th>
<th>[1M] SAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>0.93 ± 0.04</td>
<td>0.77 ± 0.05</td>
<td>1.07 ± 0.03</td>
</tr>
<tr>
<td>488 nm</td>
<td>0.89 ± 0.04</td>
<td>0.75 ± 0.05</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>543 nm</td>
<td>0.95 ± 0.04</td>
<td>0.78 ± 0.05</td>
<td>1.05 ± 0.04</td>
</tr>
<tr>
<td>581 nm</td>
<td>0.91 ± 0.05</td>
<td>0.77 ± 0.05</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>618 nm</td>
<td>1.04 ± 0.05</td>
<td>0.83 ± 0.06</td>
<td>1.04 ± 0.03</td>
</tr>
</tbody>
</table>
7.3.1 Lifetime Dependence on DPA and TbCl$_3$ Concentrations

In order to better view any possible trend in the lifetime values, a three-dimensional plot of the lifetimes of the fluorescence of the DPA-Tb complex was made for each of the lines for different concentrations of [DPA] and [TbCl$_3$] in distilled water. This data is shown in Fig. 7.13. Figure 7.13 (a) through (e) shows the lifetimes from all of the samples’ fluorescence signals mixed together, from the samples’ 488 nm fluorescence line, the 543 nm fluorescence line, the 581 nm fluorescence line, and the 618 nm fluorescence line, respectively. As can be seen, a general upwards trend in $\tau$ seems to exist as we approach an increasing DPA concentration and a decreasing TbCl$_3$ concentration at the same time.

Similar plots were generated for the DPA-TbCl$_3$ samples in the low concentration [0.01M] HCl solvent (Fig. 7.14), and for those in the [1M] SAB solvent (Fig. 7.15). In these solvents the values of the lifetimes do not seem to be strongly dependent upon the DPA and the TbCl$_3$ concentrations. This seems to be different than that observed for the DPA-TbCl$_3$ solution in distilled water shown in Fig. 7.13. However, further work would be required to better quantify this effect and to reduce the measurement uncertainty.
Figure 7.13  Plots of lifetime as a function of DPA and TbCl$_3$ concentration in distilled water. A sample error bar is shown in each of the figures at around the [25µM] DPA with [5µM] TbCl$_3$.
Figure 7.14  Plots of lifetime as a function of DPA and TbCl$_3$ concentration in [0.01M] HCl. A sample error bar is shown in each of the figures at around the [25µM] DPA with [5µM] TbCl$_3$. 

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Figure 7.15  Plots of lifetime as a function of DPA and TbCl$_3$ concentration in [1M] SAB. A sample error bar is shown in each of the figures at around the [25μM] DPA with [5μM] TbCl$_3$.
CHAPTER 8. NEW FLUORESCENCE LIFETIME MEASUREMENT TECHNIQUE USING TRANSIENT FLUORESCENCE SPECTROSCOPY (TFS)

As explained in the previous chapter, the fluorescence signals that were obtained when using the microchip laser as the excitation source were modulated due to the optical chopper. During these studies, it was noticed that the fluorescence intensity and modulation depth varied with the speed of the chopper. An in-depth analysis of the fluorescence energy levels and excitation transitions led us to investigate this effect more. As such, we investigated the relation between the chopper’s frequency and the lifetime signal’s behavior experimentally and theoretically by numerically computing a simple energy level model of the DPA-Tb complex. We have found excellent agreement between our numerical solutions of the transient energy rate equations for the DPA-Tb energy levels and experimental measurements. In addition, these numerical simulations have provided curve fitting to the experimental data which yields a fitted fluorescence lifetime that is very close to that measured in the previous chapter. This new lifetime technique is called Transient Fluorescence Spectroscopy (TFS), which is the dynamic, time dependent solution of the transient rate equations, and may provide some new advantages over other lifetime techniques or provide another way to measure the lifetime. Details of this are presented in the following sections.
8.1 Experimental Observation of the Effect of Chopper Modulation on Approach to Fluorescence Saturation

When a sample containing a mixture of DPA and TbCl$_3$ was excited with the modulated microchip laser, the fluorescence signal was also modulated. For the purpose of lifetime measurements, when the frequency of the chopper was maintained at around 50 Hz the signal was able to fully decay to zero intensity. However, as the chopper’s frequency was increased, the amplitude of the fluorescence signal decreased significantly. This is shown in Fig. 8.1 where the fluorescence intensity as a function of time is shown for several different chopper speeds. As can be seen, as the chopper speed increased, the maximum extent of the modulated fluorescence decreased.

One explanation was that due to the relatively long fluorescence lifetime of the samples, which are in the millisecond range, the higher chopper frequencies were not allowing enough time for the fluorescence to build up to its maximum or saturated intensity or to fully decay. The time available for the laser beam to reach the sample, i.e. when the chopper’s blade was in an “open” position, varied depending on the chopper’s frequency of rotation. Due to the symmetric pattern of the blade, this time was the same as when the chopper’s window was “closed”. The open/close (or on/off) times for the frequencies shown in Fig. 8.1 are as follows: in Fig. 8.1 (a) the blade was “open” for 10 ms for $f_{\text{chopper}} = 50$ Hz (also, the blade was “closed” for 10 ms); this left enough time for the signal to fully decay to the zero intensity level. In Fig. 8.1 (b) the blade was open and closed for 5 ms in each case, for $f_{\text{chopper}} = 100$ Hz; in this case the signal was able to decay down almost completely. In Fig. 8.1 (c) the blade was open and closed for 1 ms in each case, for $f_{\text{chopper}} = 500$ Hz; and for $f_{\text{chopper}} = 1000$ Hz, the blade was open and closed.
Figure 8.1  Experimentally measured fluorescence intensity as a function of time of a sample of [50 µM] DPA with [50 µM] TbCl₃ in distilled water when excited with the chopper-modulated-microchip laser, for the chopper operating at four different frequencies.
for 0.5 ms in each case. The signal appears to be reduced symmetrically, possibly due to the symmetry of the excitation waveform. However, this will be discussed more fully later for the asymmetrical excitation case.

To better understand the behavior of the maximum fluorescence level and the minimum fluorescence level observed, the chopper’s blades were modified to be non-symmetrical with regard to the on-time and off-time. In one case every other slit in the 30-slit blade was taped off, thus cutting the number of open slits by half to 15, and, therefore, tripling the available decay time by blocking off the beam. This modification is shown in Fig. 8.2 (a). In another instance the 30-slit and 6-slit blades were physically superimposed so that the blade pattern shown in Fig. 8.2 (b) was created. The resulting modified 15-slit blade was open for 1 mm and closed for 3 mm, Fig. 8.2 (a). In the case of the superimposed blades the blades were open for 1 mm, closed for 1 mm, open for 1 mm, closed for 1 mm, open for 1 mm, and closed for 5 mm, Fig. 8.2 (b). The graphical representations of these new modified blades for the intensity as a function of time for 1 period of each blade are shown in Fig. 8.3 (a) and (b).

By using these modified blades we were able to control or influence the peak and the trough of the fluorescence signal, as shown in Figs. 8.4 and 8.5.

Figure 8.4 shows the resulting fluorescence signals obtained at 50 Hz and 500 Hz using the modified blade with 15 slits. One can see that even at 500 Hz, the beam was blocked off for long enough and thus the signal was able to decay to the same level as when the chopper’s frequency was at 50 Hz, while the build up’s maximum still remained at a lower level at 500 Hz than when the chopper was at 50 Hz.
Figure 8.2  (a) Original 30-slit chopper blade on the left, and modified 15 slit blade on the right; (b) original 6-slit chopper blade on the left, and 30-slit and 6-slit blades superimposed on the right. Note: only the outer slits in each blade were used for modulation purposes in these experiments.
Figure 8.3  Chopper periods, or TTL signals, for the 15-slit modified blade showing 1-on and 3-off (a), and for the superimposed 30-slit and 6-slit blades showing a group of 3 on-off followed by 5-off (b); these correspond to Fig. 8.2 (a), and (b), respectively.
Figure 8.4  Fluorescence signal as a function of time for a DPA-Tb solution when excited with the chopper-modulated-microchip laser, for the chopper operating at two different frequencies, with a modified blade with 15 slits (1 run).
Figure 8.5  Fluorescence signal as a function of time for a DPA-Tb solution when excited with the chopper-modulated-microchip laser, for the chopper operating at a frequency of 50 Hz (average of 100 runs).
As a second test, the 30-slit blade was superimposed on the 6-slit blade, as in Fig. 8.2 (b), and the combination of the two was used to modulate the excitation beam. The resulting fluorescence signal is shown in Fig. 8.5. The fluorescence signal using the 15-slit modified blade is also overplotted in the same graph. It may be noted that the solution used for Fig. 8.4 was a mixture of low DPA-Tb concentration in water, while the solution used for Fig. 8.5 was [250µM] DPA with [250µM] TbCl₃ in SAB.

The intensity of the fluorescence signal whose excitation beam was modulated with the 15-slit modulated blade is slightly higher than the intensity of the fluorescence signal whose excitation beam was modulated with the superimposed 6-slit and 30-slit blades. At 50 Hz each slit in the 15-slit modulated blade takes about 4.7 ms to pass an infinitesimally small point, while it takes one slit in the superimposed blade combination about 1.9 ms to pass an infinitesimally small point, for the slits’ width of 4 mm. This means that the sample whose excitation is being modulated with the superimposed modified blade has less than half the “build up” time as compared to the one whose excitation is modulated using the 15-slit modified blade. Further measurements using the asymmetrical chopper patterns at different frequencies are presented in Appendix C.

8.2 Theoretical Modeling of DPA and TbCl₃ Energy Level Rate Equations and Influence on Fluorescence Intensity

Several previous studies have been made regarding the saturation effects of laser induced fluorescence using pulsed laser excitation. However, nothing has been reported, as far as we know, on the use of a simulation as a possible means of predicting the lifetime of fluorescing samples. With the ultimate goal of predicting the lifetime of
the samples, we set out to try to model the fluorescence behavior of the samples when they were excited with a modulated laser beam. To this end a theoretical model made of a simple energy level diagram representing the DPA molecule, the Tb\(^{3+}\) ion and the energy transfer between them was developed, and the transient rate equations for this model were solved.

### 8.2.1 Energy Level Model for DPA-TbCl\(_3\) Fluorescence

As it was shown in Chapter 4, the DPA molecule in solution has a strong absorption peak in the UV, while the Tb\(^{3+}\) in solution is practically lacking the presence of any absorption bands. It was also shown in the same chapter that the absorption cross section for the DPA-Tb complex in solution is within experimental uncertainty of the value for the absorption cross section of the DPA molecule, providing further proof that in this complex the DPA molecule seems to be the energy donor and the Tb\(^{3+}\) ion the energy acceptor, as well as the fluorescing species, as shown by its signature fluorescence spectrum.

As stated in Chapter 2, the DPA molecule’s lowest lying triplet state has been reported as 24,272 cm\(^{-1}\) by Lima and at 27,050 cm\(^{-1}\) by Latva.\(^{35,36}\) Figure 8.6 shows the energy levels for the triplet state of the DPA molecule and for the Tb\(^{3+}\) ion.

As can be seen from this figure, depending on the true location of the DPA’s triplet state, the transfer of energy between the DPA molecule and the terbium ion may take different paths.

If DPA’s triplet state is indeed at around 27,000 cm\(^{-1}\), then this transfer most probably takes place via the \(^5L_{10}\) or \(^5G_6\) terbium levels, which sit at 26,946 cm\(^{-1}\) and at
Figure 8.6  Comparison of the energy levels of terbium ion $^{42}$ with the lowest lying triplet state of the DPA molecule, as measured by Latva $^{35}$, and by Lima $^{36}$. 
26,405 cm\(^{-1}\), respectively.\(^{42}\) From the \(^5G_6\) level in the terbium ion, the electrons are then transferred to the \(^5D_4\) fluorescing level, which sits at around 20,500 cm\(^{-1}\), from where they decay down to the ground state (the \(^7F\) series, not shown in Fig. 8.6) in the form of fluorescence.

However, if the state is at around 24,000 cm\(^{-1}\) then this could be a direct transfer between the DPA’s triplet state and the terbium ion’s \(^5D_4\). The energy transfer between the DPA molecule and the terbium ion has been usually assumed to be nonradiative.\(^{25}\)

Taking these levels and the assumed transfer processes noted, a simple energy level diagram for the DPA and Tb complex (DPA-Tb, or Tb(dpa)\(_n^{3-2n}\) in Section 2.3) was developed, and is shown in Fig. 8.7.

Figure 8.7 shows a relatively simple energy level model for the DPA-Tb complex and the assumed excitation and de-excitation rates as shown. In this model, the population density of the DPA-Tb levels is given by \(N_0\), \(N_1\), \(N_2\), and \(N_3\). The DPA-Tb molecule is excited (\(I\sigma\)) to its lowest lying triplet level, \(N_1\), with excitation intensity \(I\) and absorption cross section \(\sigma\); stimulated emission \(I\sigma\) back down to the \(N_0\) is also shown. The energy is then transferred nonradiatively to the \(^5G_6\) energy level in the terbium ion, also represented here as \(N_1\), from where it relaxes down to the \(^5D_4\) terbium ion energy level, \(N_2\), with a transfer rate of \(\gamma_{12}\). The electrons then fluoresce from this level to one of the highest energy levels of the terbium ion’s \(^7F\) series, \(N_3\), at a rate of \(\gamma_{23}\). From this level they decay back down to the ground state, \(N_0\), at a rate of \(\gamma_{30}\). The inverse of the \(\gamma_{23}\) transfer rate is equivalent to the fluorescent lifetime, \(\tau\), of the sample. The fluorescence
Figure 8.7  Simple theoretical energy level model representing the energy levels of the DPA-Tb complex.
The intensity of the sample is proportional to the population density of the fluorescing level, \( N_2 \). The rate equations for this system are given by

\[
\frac{dN_0}{dt} = I \cdot \sigma \cdot N_1 - I \cdot \sigma \cdot N_0 + \gamma_{30} \cdot N_3 , \tag{8.1}
\]

\[
\frac{dN_1}{dt} = I \cdot \sigma \cdot N_0 - I \cdot \sigma \cdot N_1 + \gamma_{12} \cdot N_1 , \tag{8.2}
\]

\[
\frac{dN_2}{dt} = \gamma_{12} \cdot N_1 - \gamma_{23} \cdot N_2 , \tag{8.3}
\]

\[
\frac{dN_3}{dt} = \gamma_{23} \cdot N_2 - \gamma_{30} \cdot N_3 , \tag{8.4}
\]

and

\[
N = N_0 + N_1 + N_2 + N_3 . \tag{8.5}
\]

The approximate values for some of these parameters can be obtained from previous experimental measurements. The value used for \( \gamma_{23} \) was 1053/s, which is the inverse of the average of the experimentally obtained lifetime of 0.95 ms for a solution of [50 \( \mu \)M] DPA with [50 \( \mu \)M] TbCl\(_3\) in distilled water, when using the microchip laser and chopper combination. The value used for the number of ground state molecules is \( 3 \times 10^{16} \) per cm\(^3\), which corresponds to a concentration of [50 \( \mu \)M] of DPA since this is the only species that contributes to the absorption of the incident light. For simplicity, we can assume that this also represents the concentration of the DPA-Tb complex molecules; this is valid if there is one DPA molecule bound to one Tb atom (see Section 2.3). The initial value for the remainder of the energy levels was zero, since at room temperature the molecule is in its ground state. The intensity of the microchip laser was experimentally measured to be \( 1.113 \times 10^{22} \) photons/cm\(^2\) sec. The assumed value for the absorption cross section was found experimentally to be \( 1.94 \times 10^{-17} \) cm\(^2\)/molecule, as
explained in Chapter 4, Section 4.4.4. The values for the transfer rates, $\gamma_{12}$ and $\gamma_{30}$ were obtained by trial and error (i.e. curve fitting).

### 8.3 Effect of Varying the Simulation’s Parameters

The rate equations were numerically solved for $dN_0$, $dN_1$, $dN_2$, and $dN_3$ using the initial assumed or “default” values listed in Table 8.1. The transient solution was obtained numerically using a computer program written by the author and explained in more detail in Appendix D. The time increment, $dt$, was set for a value several orders of magnitude smaller than the other temporal processes in the fluorescence model (i.e. $dt \approx 0.04$ ns). The transient solution to the rate equations was solved as a function of time to obtain the population density of each of the levels as a function of time.

Using the numerical simulation, all of the parameters listed in Table 8.1 were kept at their default values except for the transition rate, which is related to the fluorescence lifetime, i.e. $\gamma_{23}$. The resulting population density for the fluorescing level, $N_2$, was calculated and plotted as a function of time (in milliseconds). As explained earlier, $\gamma_{23}$, is inversely proportional to the sample’s fluorescence lifetime.

Figure 8.8 shows the calculated fluorescence signals for a wide range of fluorescence decay rate values obtained as a result of changing $\gamma_{23}$ from its default value of 1053 sec$^{-1}$, to a range of values that span from 500 sec$^{-1}$, which corresponds to a fluorescence lifetime of 2 ms, to 1400 sec$^{-1}$, which corresponds to a fluorescence lifetime of ~0.71 ms, in steps of 100 sec$^{-1}$. These graphs are a plot of the population density of the fluorescing level, $N_2$, as a function of time for the chopper operating at approximately 50 Hz, 500 Hz, and 1000 Hz. These graphs are plotted on the same scale.
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<th>Typical Values</th>
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</thead>
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</tr>
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<td>$\gamma_{23}$ (sec$^{-1}$)</td>
<td>1053</td>
</tr>
<tr>
<td>$\gamma_{30}$ (sec$^{-1}$)</td>
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</tr>
<tr>
<td>$N_0$ (Molecules at $t = 0$)</td>
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</tr>
<tr>
<td>$N_1$ (Molecules at $t = 0$)</td>
<td>0</td>
</tr>
<tr>
<td>$N_2$ (Molecules at $t = 0$)</td>
<td>0</td>
</tr>
<tr>
<td>$N_3$ (Molecules at $t = 0$)</td>
<td>0</td>
</tr>
<tr>
<td>$I_0$ (Photons/cm$^2$.sec)</td>
<td>$1.113 \times 10^{22}$</td>
</tr>
<tr>
<td>$\sigma$ (cm$^2$/Molecule)</td>
<td>$1.94 \times 10^{-17}$</td>
</tr>
</tbody>
</table>

Table 8.1 Typical or “default” values used for the simulation parameters.
Figure 8.8 Simulations with all the parameters' default values, except for $\gamma_{23}$, which is changing as indicated in each graph. Each graph consists of 3 plots for chopper speeds of 50 Hz, 500 Hz, and 1000 Hz.
As $\gamma_{23}$ became larger, i.e. as the fluorescence lifetime became smaller, the fluorescence signals at 50 Hz reached a steady state faster. This clearly influenced the shape of the signal, which was more squared (for higher $\gamma_{23}$) as compared to more pointed (for smaller $\gamma_{23}$) at the top of the signal. The signals maximum peak was also influenced by this change, becoming smaller as $\gamma_{23}$ became larger. At $\gamma_{23} = 500$ the signal was not able to fully decay, because the slow transition rate did not allow the population in $N_2$ to “empty out” fast enough before the chopper’s window opened again allowing the laser to pump the system again. It is interesting to note that the effect of changing $\gamma_{23}$ was not as noticeable in the signals that were excited with the chopper going at 500 Hz and 1000 Hz. Neither their shape nor their amplitudes changed considerably across the simulations in Fig. 8.8.

It is interesting to compare the simulation results to that of our experimental results. In effect, picking the curve in Fig. 8.8 which most nearly agrees with the waveforms measured experimentally. For example, Fig. 8.9 shows a comparison between the measured fluorescence intensity signals for a sample of [50 $\mu$M] DPA with [50 $\mu$M] TbCl$_3$ in distilled water as a function of time, when excited with the chopper modulated microchip laser (chopper frequency ~ 50 Hz, 500 Hz, 1000 Hz), and the population density of the $N_2$ energy level as a function of time obtained using the simulation with the default values listed in Table 8.1 for chopper frequencies of approximately 50Hz, 500Hz, and 1000 Hz. As can be seen, there is excellent agreement between the experimental and simulated waveforms for a set of lifetime parameters chosen judiciously. Details of these simulations are given in the following sections.

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Figure 8.9  Experimentally obtained fluorescence intensity of an aqueous solution containing DPA-Tb complex (a), as compared to the simulation signals obtained using the default values (Table 8.1) (b), for the case where the chopper speed was 50 Hz, 500 Hz, and 1000 Hz.
8.3.1 Detailed Study of Population Levels at Changing Chopper Frequencies

Although the energy level of most interest to us is the fluorescing level, $N_2$, it was important to see the behavior of each of the energy levels of the diagram shown in Fig. 8.7 for a better understanding of the overall system. To this end the population densities of each of the energy levels was plotted as a function of time for three chopper frequencies: 50 Hz, 500 Hz, and 1000 Hz.

Figure 8.10 shows the population densities of each of the levels in the system, i.e. $N_0$, $N_1$, $N_2$, and $N_3$, as a function of time for a chopper frequency of 50 Hz. The plot of $N_0$ as a function of time starts at the maximum population density, $3 \times 10^{16}$, since all of the molecules are in the ground state. As the incident beam pumps the electron population into the next energy level, $N_1$, the population density of $N_0$ starts to decrease; when the chopper blocks the incident laser beam, thus allowing for the electrons to decay back to ground state, the population of $N_0$ starts to build up again. With the exception of the population density of $N_0$, which is opposite to the pattern of the chopper’s TTL signal, the population densities of all the other levels follow the same pattern as the chopper’s TTL signal.

The periodic behavior of the population density of $N_1$ is in phase with that of the chopper’s TTL signal. The population density of $N_1$ mimics the laser pulses strongly in the top part of the peaks in this figure. This is mainly due to the transition rate between the $N_1$ and $N_2$ energy levels, i.e. $\gamma_{12}$, which allows a fast enough transition at its default value of 5,000/sec.
Figure 8.10  Population densities for all of the energy levels as a function of time, as calculated by the simulation, using the default values for the parameters ($f_{\text{chopper}} \approx 50$ Hz).
The graph for the population density of $N_2$ as a function of time, which is in good agreement with the experimentally obtained fluorescence behavior as a function of time, also shows small laser pulse patterns on the peak of the signals. These patterns were also observed in the experimentally obtained fluorescence intensity as a function of time in Fig. 8.1 (a). The population density as function of time of $N_3$ is the only graph that does not show the individual high-PRF laser pulse excitation pattern, unless $\gamma_{12}$ is very large.

As the chopper’s frequency was changed to 500 Hz in the simulation, the amplitudes of the population densities diminished for all of the energy levels, except for $N_1$. This is seen in Fig. 8.11 where the populations are observed to not return to their low intensity or non-saturated levels. As the frequency increased, the signal’s peaks and troughs were decreased relatively symmetrically from both their top half and their bottom half, and as a result, a smaller periodic signal was left.

In accordance to the experimental results, the amplitudes of the signals were reduced even further when the chopper’s frequency was increased to 1000 Hz in the simulation, as shown in Fig. 8.12, for all of the energy levels except $N_1$. When the value of $\gamma_{12}$ was decreased as compared to the default value, however, the amplitude of the population density as a function of time of $N_1$ did decrease.

### 8.3.2 Detailed Study of Population Levels at Changing Incident Intensity

The incident laser intensity, $I$, was varied in the simulations to determine its effect on the population levels, and thus fluorescence intensity from $N_2$. Figure 8.13 shows the effect that different incident intensities have on the population densities of each of the energy levels as a function of time, for a chopper frequency of 50 Hz. In these figures,
Figure 8.11 Population densities for all of the energy levels as a function of time, as calculated by the simulation, using the default values for the parameters ($f_{\text{Chopper}} \approx 500$ Hz).
Figure 8.12  Population densities for all of the energy levels as a function of time, as calculated by the simulation, using the default values for the parameters ($f_{\text{Chopper}} \approx 1000$ Hz).
Figure 8.13  Simulations depicting the population density of each energy level for different intensities, where $I_0$ is the default value $1.113 \times 10^{22}$ photons/cm$^2$ sec for $f_{\text{chopper}} = 50$ Hz. (Section A.)
Figure 8.13  (Continued – Section B)
\[ I = I_0 \times 10^{-2} \]

\[ I = I_0 \times 10^{-3} \]

\[ I = 0.0 \]

Figure 8.13  (Continued – Section C)
the laser intensity was varied by 8 orders of magnitude.

Figure 8.13-A shows this for incident intensities of two, three, and five orders of magnitude higher than the default intensity of $1.1 \times 10^{22}$ photons/cm$^2$ sec. As can be seen from the figure, the intensity that achieved a long term (> 0.1 ms) saturation effect of the fluorescing level, $N_2$, was five orders of magnitude higher than the default value, at which point the population density of the fluorescing level became filled up with almost two thirds of the total number of electrons that the ground level originally had.

Saturation effects in the population levels of $N_0$ and $N_1$ are seen within the 0.4 ns laser pulse at about $10 \times I_0$.

It maybe added that there may be different uses for the term saturation. The term saturation is often used to describe significant deviation in the ground state population which is associated with saturation of the absorption transmission. However, the term saturation can also be applied to population build up within energy levels in a 4-level laser type system, as in the term “saturation of the fluorescence intensity” where the upper population state (and fluorescence signal) has become saturated or no longer linear with excitation intensity.

The shape of the signal for the $N_0$ level did not change considerably throughout the different incident intensities except for when the intensity decreased by three orders of magnitude (Fig. 8.13-C), at which point a strong “step” pattern along with a lack of the laser beam imprint could be detected. Also, as the intensity decreased, the variation in the population density was very small, e.g. for $I = I_0 \times 10^{-3}$ the change in the y-scale is practically negligible.
The maximum number of molecules that $N_1$, $N_2$, and $N_3$ reached as a function of the incident intensity decreased as the intensity decreased. As expected, this maxim for $N_0$ was its ground state population, i.e. $3 \times 10^{16}$ molecules. Except for this, the population densities as a function of time of $N_3$ did not change considerably with changing intensity.

### 8.3.3 Effect of the Transition Rate $\gamma_{30}$

Due to the closeness of the $7F$ energy levels, the rate of transition, $\gamma_{30}$, between the level where the fluorescence terminates, $N_3$, and the ground state, $N_0$, is assumed to be very fast. Figure 8.14 shows the effect that changing this rate had on the signal depicting the population density of the fluorescing level $N_2$ as a function of time for chopper frequencies of 50 Hz, 500 Hz, and 1000 Hz (overplotted on top of each other).

As is shown in this figure, when the default value of 10,000/sec was changed from 0/sec to $2 \times 10^7$/sec no considerable difference can be detected during 40 ms in the shape of these signals with respect to one another. This was not surprising when using higher transition rate values for $\gamma_{30}$, since this facilitates the return of electrons to the ground state.

However, at very low transition rate values of $\gamma_{30}$, the population density of $N_2$ should in theory start to decline, since $N_0$ would not be getting repopulated quickly enough. Figure 8.15 is a plot of the data shown in Fig. 8.14 with $\gamma_{30} = 0$. In this graph where the time axis covers from 0 sec to 1 sec, one can see that indeed the population density of the fluorescing level $N_2$ starts to diminish over time, due to the depletion of
Figure 8.14  Simulations with all the parameters’ default values, except for \( \gamma_{30} \), which is at three different values, two of which are extreme values.
Figure 8.15  Simulation with all the parameters’ default values, except for $\gamma_{30} = 0.0$, over a larger time scale than shown in Fig. 8.14 (a).
electrons in the ground state $N_0$, which in turn is caused by an extremely small transition rate back down to the ground state, $\gamma_{30}$.

### 8.3.4 Effect of the Transition Rate $\gamma_{12}$

The transition rate, $\gamma_{12}$, between the first upper level, $N_1$, and the upper fluorescing level, $N_2$, was changed to a number of extreme values in order to see its effect on the population density of $N_2$ for chopper frequencies of 50 Hz, 500 Hz, and 1000 Hz. Figure 8.16 shows the simulations obtained when changing this transition rate from 0/sec to $2 \times 10^7$/sec over several steps.

For $\gamma_{12}=0$, the population density as a function of time for the fluorescing level is zero. However, for a small transition rate of 0.1/sec, a signal starts to build slowly over the 40 ms time shown in the graph. As the rate increases to 1000/sec the signal resembles the experimental signals obtained at the respective chopper frequencies. However, unlike the case of $\gamma_{30}$, for a very large value of $\gamma_{12} = 2 \times 10^7$/sec the simulation breaks down, leaving a noisy signal. This is because the time increment used in the simulations was not fast enough to capture the correct transient effects within the rate equations. The time increment could have been reduced, but at the expense of significant increased computation time.

Another effect of $\gamma_{12}$ is on the amplitude of the signals at chopper frequencies of 500 Hz and 1000 Hz. In our original trials, the default value that was used for $\gamma_{12}$ was 10,000/sec. After a few trial and errors, we found that as the value of $\gamma_{12}$ decreased from this number, so did the amplitudes of the signal at 500 Hz and 1000 Hz. The amplitude
Figure 8.16  Simulations with all the parameters’ default values, except for \( \gamma_{12} \), which is at four different values, three of which are extreme values.
of the signal at these higher chopper frequencies for the experimental data were about 30%, and about 10% of the of the signal’s amplitude at 50 Hz, respectively. In order to better match the percentage decrease in the amplitude of the signals in the simulation to those found experimentally, $\gamma_{12}$ was set to a new default value 5,000/sec, which brought the amplitudes of the signals at a chopper frequency of 500 Hz and 1000 Hz down to 35% and 12% of the amplitude of the signal at 50 Hz respectively.

8.3.5 Effect of Changing the Absorption Cross Section $\sigma$

The absorption cross section’s value was also varied and its values close to its thresholds were found using the numerical solution to the rate equations. Figure 8.17 shows three plots of the population density of $N_2$ as a function of time for chopper frequencies of 50 Hz, 500 Hz, and 1000 Hz as the value for the absorption cross section was changed. In Fig. 8.17 (a) the population density $N_2$ was plotted as a function of time for the highest value of the absorption cross section, i.e. $\sigma = 2 \times 10^{-12}$ cm$^2$/molecule. At this value, which was five orders of magnitude larger than its default value of $1.94 \times 10^{-17}$ cm$^2$/molecule, the population density of $N_2$ reached saturation at around two thirds of the total number of the electron population. This graph is very similar to the steady state that was reached when the intensity of the incident beam was increased in the simulation by five orders of magnitude as shown in Fig. 8.13. The effect of increasing the intensity of the incident beam by five orders of magnitude is therefore similar to the effect of increasing the absorption cross section by the same amount.

Figure 8.17 (c) shows the signal at an extremely small absorption cross section value, i.e. $\sigma = 1 \times 10^{-320}$ cm$^2$/molecule. Although the behavior of the population density
Figure 8.17  Simulations with all the parameters’ default values, except for \( \sigma \), which is at its largest possible value (a), at its default value (b), and at its smallest value (c).
of N$_2$ is in good agreement with the experimental data, it must be noted that such a decrease in the size of the absorption cross section translated into a maximum population density of approximately $1.3 \times 10^{-29}$ molecules. Figure 8.17 (b) shows the simulations obtained when using the default value of $\sigma$ for comparison purposes.

### 8.4 More Complex 5-Level Rate Equation Model

In order to more closely imitate and control the transition of energy between the DPA molecule’s lowest lying triplet state and the Tb$^{3+}$’s $^5L_{10}$ state, one more level was added to the simple energy level system presented earlier. The transfer of energy between these two levels has been assumed to be a very fast and radiationless process. In order to take into account other possible processes, more transfer routes were added to this new 5-level system.

One of the main processes known to happen in the fluorescence of the DPA-Tb complex in solution is quenching. This quenching tends to occur mainly due to the interaction between the terbium ion and the water molecules in aqueous solutions. In order to take this into account, an energy level model was developed which included quenching rates at every possible level. Figure 8.18 shows a diagram of this new 5-level “quenching model”. The rate equations related to this system are given below.
Figure 8.18  Energy level diagram for the DPA-Tb complex including quenching rates. 
N₁ and N₂ depict the first singlet an triplet states of the DPA molecule, respectively. N₃, 
N₄ depict the ⁵D₄, the ⁷F series of the Tb³⁺ ion, respectively.
\[
\begin{align*}
\frac{dN_0}{dt} &= -I \cdot \sigma \cdot N_0 + I \cdot \sigma \cdot N_1 + \gamma_{10} \cdot N_1 + \gamma_{20} \cdot N_2 + \gamma_{30} \cdot N_3 + \gamma_{40} \cdot N_4, \\
\frac{dN_1}{dt} &= I \cdot \sigma \cdot N_0 - I \cdot \sigma \cdot N_1 - \gamma_{10} \cdot N_1 + \gamma_{12} \cdot N_1, \\
\frac{dN_2}{dt} &= \gamma_{12} \cdot N_1 - \gamma_{20} \cdot N_2 + \gamma_{23} \cdot N_2, \\
\frac{dN_3}{dt} &= \gamma_{23} \cdot N_2 - \gamma_{30} \cdot N_3 - \gamma_{34} \cdot N_3, \\
\frac{dN_4}{dt} &= \gamma_{34} \cdot N_3 - \gamma_{40} \cdot N_4,
\end{align*}
\] (8.6) (8.7) (8.8) (8.9) (8.10)

and

\[
N = N_0 + N_1 + N_2 + N_3 + N_4.
\] (8.11)

The values found that best managed to imitate the experimental results obtained are given in Table 8.2. The values for \( N_0, I_0, \sigma, \gamma_{40}, \) and the fluorescing transfer rate, in this model called \( \gamma_{34}, \) remained the same. The transfer rate between the DPA molecule’s lowest lying triplet and the terbium ion’s \( ^5G_6 \) level, \( \gamma_{12}, \) was chosen to be \( 5 \times 10^5/\text{sec}. \) The transfer rate from the energy level immediately preceding the upper fluorescing level to the upper fluorescing level, \( \gamma_{23}, \) was found to be best kept at the same value as its counterpart in the simple model, i.e. \( 5,000/\text{sec}. \)

It is important to note that using the default values described so far, and setting the quenching rates to zero, the signals that were obtained were identical to those obtained using the simple model described earlier with its default values. At this point the quenching transfer rates were set to 15/\text{sec}. The only effect these quenching rates seemed to have had, was in slightly reducing the maximum peak values reached in the graphs of the population density of the fluorescing level, \( N_3, \) as a function of time. A
<table>
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<td>$\gamma_{20}$ (sec$^{-1}$)</td>
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<tr>
<td>$\gamma_{23}$ (sec$^{-1}$)</td>
<td>$5 \times 10^3$</td>
</tr>
<tr>
<td>$\gamma_{30}$ (sec$^{-1}$)</td>
<td>15</td>
</tr>
<tr>
<td>$\gamma_{34}$ (sec$^{-1}$)</td>
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</tr>
<tr>
<td>$\gamma_{40}$ (sec$^{-1}$)</td>
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</tr>
<tr>
<td>$N_0$ (Molecules) at t = 0</td>
<td>$3 \times 10^{16}$</td>
</tr>
<tr>
<td>$N_1$ (Molecules) at t = 0</td>
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</tr>
<tr>
<td>$N_2$ (Molecules) at t = 0</td>
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</tr>
<tr>
<td>$N_3$ (Molecules) at t = 0</td>
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</tr>
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<td>$N_4$ (Molecules) at t = 0</td>
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<td>$I_0$ (Photons/cm$^2$.sec)</td>
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</tr>
<tr>
<td>$\sigma$ (cm$^2$/Molecule)</td>
<td>$1.94 \times 10^{-17}$</td>
</tr>
</tbody>
</table>

Table 8.2  Typical or “default” values used for the simulation parameters for the 5-level quenching model.
plot of the population density of $N_3$ as a function of time for chopper frequencies of 50 Hz, 500 Hz, and 1000 Hz using the default values given in Table 8.2 is shown in Fig. 8.19.

8.4.1 The Up-conversion Model (Förster Resonance Energy Transfer)

When a distance-related transfer of energy occurs between two molecules nonradiatively due to long-range dipole-dipole coupling mechanisms, an excited molecule (the donor) can transfer its energy to another molecule (the acceptor) leaving the acceptor molecule in an excited state. This phenomenon is commonly known as up-conversion in the optics and laser communities or Förster resonance energy transfer (FRET) in the biology and chemistry community.\(^4\)

Upconversion was added as an additional process to the quenching model described earlier in Section 8.4. The energy level diagram for this model is shown in Fig. 8.20. The rate equations for this system are

\[
\begin{align*}
\frac{dN_0}{dt} &= -I \cdot \sigma \cdot N_0 + I \cdot \sigma \cdot N_1 + \gamma_{10} \cdot N_1 + \gamma_{20} \cdot N_2 + \gamma_{40} \cdot N_4 + \gamma_{up} \cdot N_3^2 + \gamma_{up0} \cdot N_{up}, \\
\frac{dN_1}{dt} &= I \cdot \sigma \cdot N_0 - I \cdot \sigma \cdot N_1 - \gamma_{10} \cdot N_1 + \gamma_{12} \cdot N_1, \\
\frac{dN_2}{dt} &= \gamma_{12} \cdot N_1 - \gamma_{20} \cdot N_2 + \gamma_{23} \cdot N_2, \\
\frac{dN_3}{dt} &= \gamma_{23} \cdot N_2 - 2 \cdot (\gamma_{up} \cdot N_3^2) - \gamma_{34} \cdot N_3, \\
\frac{dN_4}{dt} &= \gamma_{34} \cdot N_3 - \gamma_{40} \cdot N_4, \\
\frac{dN_{up}}{dt} &= \gamma_{up} \cdot N_3^2 - \gamma_{40} \cdot N_4 - \gamma_{up0} \cdot N_{up},
\end{align*}
\]

and
Figure 8.19  Population density of the fluorescing level, N₃, of the 5-level quenching model, as a function of time using the typical values given in table 8.2.
Figure 8.20  Energy level diagram for the DPA-Tb complex including upconversion rates. $N_1$ and $N_2$ depict the first singlet an triplet states of the DPA molecule, respectively. $N_3$, $N_4$, and $N_{up}$ depict the $^5D_4$, the $^7F$ series, and a second excited state of the Tb$^{3+}$ ion, respectively.
The quenching model presented in Fig. 8.18 is retained in this upconversion model, with the exception of the quenching transfer rate, $\gamma_{30}$, which for simplicity was omitted from this system.

The default values used for the parameters in this model remained the same as those that were used for the quenching model, and are listed in Table 8.3. For the two new transfer rates $\gamma_{\text{up}}$ and $\gamma_{\text{up-0}}$ a very small rate value ($10^{-15}$/sec) and larger rate value (250/sec) were chosen respectively, in order to minimize their effect.

A graph of the population density of the fluorescing level, $N_3$, as a function of time is plotted in Fig. 8.21. From this figure we can see that the existence of this new upper level does not significantly affect the shape or peak value of the new signal.

From these different models we can therefore conclude that the simple 4-energy level model is not only suitable but also sufficient for these analyses. However, it is important to note that the 5-level and up-conversion model may be appropriate for other fluorescing systems, such as bio-luminescence cells and even solid-state laser models.\textsuperscript{74-76}

We have also studied the case of a more complex system where $N_0$ of DPA is separate from $N_0$ of Tb\textsuperscript{3+}. In this system the transfer rate between the DPA and the terbium ion has been assumed to be very slow, i.e. in the order of 3 ms. The results, which are not shown here, have not been satisfactory so far, although further modeling is being carried out to improve the outcome.

Finally, we plan to use an asymmetric excitation waveform in our simulation model and then compare it to our experimental data. Some of these initial results are shown in Appendix C, and the initial results are encouraging. The use of asymmetric excitation

\begin{equation}
N = N_0 + N_1 + N_2 + N_3 + N_4 + N_{\text{up}}.
\end{equation}
<table>
<thead>
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<th>Parameters</th>
<th>Typical Values</th>
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</tr>
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<td>$N_1$ (Molecules) at $t = 0$</td>
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<tr>
<td>$N_2$ (Molecules) at $t = 0$</td>
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<td>$N_3$ (Molecules) at $t = 0$</td>
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<td>$I_0$ (Photons/cm$^2$.sec)</td>
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<td>$\sigma$ (cm$^2$/Molecule)</td>
<td>$1.94 \times 10^{-17}$</td>
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Table 8.3  Typical or “default” values used for the simulation parameters for the 5-level upconversion model.
Figure 8.21 Population density of the fluorescing level, \( N_3 \), of the 5-level upconversion model, as a function of time using the typical values given in table 8.3.
excitation waveforms may be important in other spectroscopic fields according to the systems being studied and the complexity of the saturation and decay process.
CHAPTER 9. FLUORESCENCE INTENSITY’S AMPLITUDE AND ITS APPROACH TO SATURATION

In the previous chapter, it was shown that the experimental data could be fitted to a transient solution of the rate equation energy level model of the DPA-Tb complex. The agreement was obtained by varying the assumed fluorescence decay rate (i.e. fluorescence lifetime) to produce the best fit to the experimental data. This procedure yielded results that were within about 5% to 10% of the values obtained by the low-PRF pulsed laser technique used for the strongest of the DPA-Tb emission lines. It should be noted that this technique is essentially a best fit guess by visual observation as to the selection of the fitted fluorescence lifetime value.

A more mathematically rigorous "curve fitting" technique would be to fit all of the varying chopper amplitude data and the change in the amplitudes as the chopper frequency is varied. In this case, the chopper frequency was varied smoothly from 1 Hz to above 1000 Hz, and the simulated results for the predicted fluorescence amplitude was fitted to that of the experimental data. This technique is shown in the following section and yielded a parameter fit to the data with an accuracy on the order of 5%.

9.1 Fluorescence Amplitudes

We define the amplitude of the fluorescence emission as the extent from the lowest intensity measured to the highest intensity measured as shown in Fig. 9.1. Using
Figure 9.1  Fluorescence signals’ amplitudes at 50Hz and 500Hz.
this definition Fig. 9.2 is a plot of the fluorescence signal’s amplitude as a function of the chopper frequency, for the 543 nm line of a solution of [50µM] DPA with [50µM] TbCl₃ in distilled water. We can see that at very low frequencies, the sample was saturated and the fluorescence intensity remained constant. However, as the chopper’s frequency started to increase, the signals’ amplitudes started to decrease in an exponential manner.

Figure 9.3 shows the amplitudes of the signals from the same sample plotted as a function of the cumulative excitation photons. In order to find the number of cumulative photons that were hitting the sample, the following steps were taken: the amount of time it took for an infinitesimally small point to pass an open window of the chopper’s blade was calculated by dividing the inverse of the chopper’s frequency by two. Next, this number was multiplied by the microchip laser’s pulse repetition frequency, i.e. 8,500 Hz. This is the number of laser pulses that made it through one open window of the chopper’s blade at the set frequency. Lastly, we multiplied this number by 1.11 x 10²² photons/cm²sec, which was the intensity of the laser.

9.2 Fluorescence Amplitudes: Comparisons of Simulations and Experiments

As shown in Chapter 8, the signals obtained when using the numerical solutions of the rate equations were in good agreement with those obtained experimentally. In order to test the goodness of fit, the amplitudes of the signals using the numerical simulations were plotted along side the amplitudes of the signals obtained experimentally. From here, the fluorescence lifetime of the simple energy level model presented in Chapter 8, i.e. 1/γ₂₃, was changed to different values in order to compare the behavior of these curves with the experimental one.
Figure 9.2  Fluorescence signal’s amplitude for the 543nm line of a solution of [50µM] DPA with [50µM] TbCl$_3$ in distilled water as a function of the chopper’s frequency.
Figure 9.3  Fluorescence signal’s amplitude for the 543nm line of a solution of [50μM] DPA with [50μM] TbCl$_3$ in distilled water as a function of the number of photons that “passed through” one of the chopper’s open windows. This number is inversely proportional to the chopper’s frequency. Note: the x-axis has been drawn to only 10 x $10^{23}$ for clarity purposes.
Figure 9.4 shows the signals’ amplitudes as a function of the chopper’s frequency for the experimental data as well as several numerically obtained graphs. Since the fluorescence intensity of the experimental signals cannot be numerically compared to the population densities of the graphs obtained using the simulation, all of the graphs were normalized to their individual maxima.

As can be seen from this figure, all of the plots follow the same general pattern for the lower chopper frequencies, followed by an exponential decline in their signals’ amplitudes. The graphs that follow the experimental results closest are those whose $\gamma_{23}$ transition rates were 952.4/sec and 869.6/sec, corresponding to fluorescence lifetimes of 1.05 ms and 1.15 ms, respectively. These lifetime values, however, are slightly further from the experimentally obtained 0.95 ms for the 543 nm fluorescence line for this solution (Table 7.1).

In Fig. 9.5, which shows the same simulations as a function of the cumulative excitation photons that were incident on the sample, the same trend is present.

This method provides an estimate of the fluorescence lifetime to about 5%, and gives a good sense of the accuracy of the chosen numerical values for the different parameters of the energy level system used to mimic the DPA-Tb complex.
Figure 9.4  Fluorescence intensity as a function of chopper frequency, $f_{\text{chopper}}$, for several different lifetime values.
Figure 9.5  Comparison of several numerically obtained fluorescence (i.e. population density) signals' amplitude as a function of the number of cumulative excitation photons that passed through one of the chopper’s open windows with respect to the experimentally obtained fluorescence signals' amplitude. Each graph has been normalized to its maximum. Note: the x-axis has been drawn to only $10 \times 10^{23}$ for clarity purposes.
9.3 Comparison of TFS to Related Fluorescence and Spectroscopy Techniques

A similar term, Transient Saturation Absorption Spectroscopy, has been used in a publication where the differential transmission spectra of GaAs thin films have been obtained. The technique described in their paper, however, appears to be the technique commonly known as “saturation absorption spectroscopy”. Even though “saturation absorption spectroscopy” is a commonly used procedure, it has been suggested that the term used to describe it is a misnomer and the term “pump-probe” spectroscopy would be a more appropriate choice.

Although a number of articles have been published where the saturation behavior of laser induced fluorescence has been studied, including modeling techniques, most of these are studies related to gases or flame spectroscopy. Perhaps the most similar of these, is a study in which the rate equations for a two level system have been solved using an excitation of one square laser pulse. A somewhat conceptually similar study has been published, in which a Laser Induced Fluorescence Transient (LIFT) technique has been developed to remotely measure photosynthetic activity in vegetation.

Laser induced saturation of molecular fluorescence, along with the spontaneous Raman scattering signal of the solvent, has been reported to have been used to measure the fluorescence lifetime of samples, even with laser pulses longer than the fluorescence lifetime of the species.

It should be noted that our TFS looks similar to “phase spectroscopy” or “modulation spectroscopy” where the phase and/or amplitude of the modulated fluorescence emission is compared to that of the modulated excitation in order extract the fluorescence lifetime value. However, the phase or modulation fluorescence technique
was initially developed for use with weak CW (xenon lamp) excitation and later using CW laser excitation. In addition, a simplified two level energy system was used with only excitation and fluorescence emission modeled, sinusoidal excitation assumed, and no population or stimulated emission taken into account.  

Our technique of TFS represents an extension of this technique, and includes the transient excitation of a pulsed laser and modulated amplitude waveform, asymmetric excitation, and transfer rates between other energy levels. Also, the phase and modulation method does not allow for the more detailed study of the complex’s energy levels and transfer rates.

It is interesting to note that our TFS technique is essentially an outgrowth or extension of the simpler “modulation spectroscopy” and a detailed energy-level rate equation analysis, as often used in solid-state laser modeling.
CHAPTER 10. MAXIMUM FLUORESCENCE INTENSITIES FOR DIFFERENT SOLVENTS AND SOLUTIONS OF DPA AND TbCl$_3$

In this chapter we show the relationship between the fluorescence lifetimes of the different samples, and their respective maximum fluorescence intensities as a function of the solvents used. These results show that under some conditions the maximum fluorescence intensity is dependent upon the background solvent and concentration of the DPA and TbCl$_3$. These results are important to those in the biological and chemical fields to help understand the formation of DPA-Tb complexes under different pH and solvent conditions.

10.1 Trends in Fluorescence for [50 μM] Solutions

Figure 10.1 is a three dimensional representation of the maximum fluorescence intensities as a function of the detected fluorescence line(s) and the solvents that were used for samples of [50 μM] DPA with [50 μM] TbCl$_3$ when excited with the dye laser ($\lambda_{\text{exct.}}=266$nm). These intensities have been normalized to the laser’s power, to the grating’s efficiency and to the detector’s sensitivity. As can be seen from the figure, for any particular fluorescence line(s) detected using this technique, there was no strong trend as a function of the different types of solvents, i.e., SAB vs. DW vs. HCl, for the maximum fluorescence intensity the samples. For example, for all the fluorescence signals mixed together, the maximum fluorescence intensity seems to be the same for the
Figure 10.1  Fluorescence maxima from solution of [50 µM] DPA with [50 µM] TbCl$_3$ in different solvents when excited with the dye laser.
sample that was dissolved in distilled water or dissolved in SAB or dissolved in [0.01M] HCl. This trend (or lack thereof) seems to hold true for the 488 nm lines (in the different solvents), and also approximately for the 543 nm line. The maximum intensities in the different solvents for the two weakest lines seem to be roughly the same, but in these cases the fluorescence intensity was very weak and the signal-to-noise was close to 1. In summary, the maximum fluorescence intensities between the different solvents seem to be the same for each of the fluorescence lines for the samples that were excited with the dye laser.

For the same DPA-Tb samples that were measured with the optical chopper/microchip laser combination, in place of the dye laser, there was, in fact, a stronger pattern or trend present in their maximum fluorescence intensities, as shown in Fig. 10.2. Here, it is clear that the fluorescence intensity was at its highest for the samples that were in SAB, followed by those that were in distilled water, and finally by those that were in HCl, i.e. $I_{\text{SAB}} > I_{\text{DW}} > I_{\text{HCl}}$.

The patterns for the lifetime values for [50 µM] DPA with [50 µM] TbCl₃ solutions that were excited with the dye laser and with the microchip laser were both $\tau_{\text{SAB}} > \tau_{\text{DW}} > \tau_{\text{HCl}}$, as discussed in Chapters 6 and 7, respectively. One can conclude that there is a direct proportionality between the order of the lifetimes and their fluorescence intensities, for the [50 µM] DPA with [50 µM] TbCl₃ solutions as a function of their solvents, for those solutions that were excited with the microchip laser, as suggested by Eqs. (2.8) and (2.14).
Figure 10.2  Fluorescence maxima from solution of [50 µM] DPA with [50 µM] TbCl$_3$ in different solvents when excited with the microchip laser.
10.2 Patterns for [5 µM] to [25 µM] Solutions

The normalized maximum fluorescence intensity points for solutions of different DPA and TbCl₃ concentrations in [1M] SAB (pH ~ 5.6) are plotted in Fig. 10.3. Although the fluorescence intensity increases when both the DPA and the TbCl₃ increase, the effect is more pronounced when the DPA concentration is increased (for a fixed TbCl₃ concentration) as compared to when the TbCl₃ concentration is increased for a fixed DPA concentration. According to theory²⁵,⁶⁵ both photoluminescence intensity and lifetime increase as the ratio of DPA to TbCl₃ increases, since this leaves fewer unoccupied binding sites for the terbium ion, hence decreasing the possibility for OH⁻ binding (and for its oscillator’s quenching effect). This pattern holds true for all of the measured fluorescence signals, whether resolved separately, or detected as a combination of all.

The maximum fluorescence intensity points for solutions of different DPA and TbCl₃ concentrations in distilled water and in [0.01M] HCl are plotted in Figs 10.4 and 10.5, respectively. Although the fluorescence intensity seems to also be increasing with an increasing concentration of DPA more so than with an increasing concentration of TbCl₃, the changes seem more sudden for the solutions in distilled water, as compared to the solutions in SAB or HCl. Once again the slope of these graphs may be significantly different because of the effect of their solvents’ pHs.

The patterns for the lifetime values for solutions of changing concentrations of DPA and TbCl₃, which were excited with the microchip laser was $\tau_{DW} \tau_{SAB} \tau_{HCl}$. The pattern for the maximum fluorescence intensity points for these same solutions follows the same order, i.e. $I_{DW} I_{SAB} I_{HCl}$. Again there is a direct proportionality between the
Figure 10.3  Points of maximum fluorescence intensity for solution with different [DPA] and [TbCl$_3$] in [1M] SAB when excited with the combination of the microchip laser and optical chopper ($f = 50$ Hz).
Figure 10.4 Points of maximum fluorescence intensity for solutions with different [DPA] and [TbCl$_3$] in distilled water when excited with the combination of the microchip laser and optical chopper (f = 50 Hz).
Figure 10.5  Points of maximum fluorescence intensity for solution with different [DPA] and [TbCl₃] in [0.01M] HCl when excited with the combination of the microchip laser and optical chopper (f = 50 Hz).
pattern of the lifetime values of these solutions and their maximum fluorescence intensities.

The difference in the patterns of the [50 μM] solutions discussed in section 10.1 and the solutions of varying concentrations, up to [25 μM] solutions, discussed in this section, could be due to concentration effects. At higher concentrations, i.e. the [50 μM] solutions, the [1M] SAB (pH 5.6) seems to induce a higher lifetime and fluorescence intensity, as compared to distilled water. At lower concentrations, however, the opposite is true. In both cases, [0.01M] HCl seems to provide the suitable environment for both the lowest lifetime and the lowest fluorescence intensity.

10.3 Estimate of DPA Concentration in Spores

The above results are dependent upon the exact concentration of DPA, and in the above experiments pure DPA was used. However, if spores are used then an estimate of the concentration of DPA contained in spores is required. As a point of comparison it is important to note that one B. subtilis spore contains approximately $3.65 \times 10^{-16}$ moles of DPA. This translates into a concentration of [0.4 pM] for a suspension of 1 B. subtilis spore in 1mL of solution.
The EEM spectra of the different solutions showed that for the DPA-Tb complex, as well as for the terbium ion by itself in distilled water, the strongest fluorescence signal was obtained using deep UV excitation wavelengths, i.e. 235 nm for the DPA-Tb complex and 225 nm for the Tb$^{3+}$.

Although the optimum conditions for maximum fluorescence intensity depends on the environmental conditions as well as the ratio of DPA to TbCl$_3$, these preliminary data seem to suggest that at lower concentrations, i.e. up to 25µM of each DPA and TbCl$_3$, the most fluorescence inducing solvent for the readily available 266 nm wavelength (i.e. 4$^{th}$ harmonic Nd:YAG laser) may be distilled water, followed by [1M] SAB pH 5.7, and [0.01M] HCl. For higher concentrations, i.e. [50µM] of each DPA and TbCl$_3$, distilled water and [1M] SAB produce equally suitable environments, followed by [0.01M] HCl.

The absorption cross sections for the terbium ion, dipicolinic acid molecule, and the terbium dipicolinate complex were measured at 266 nm. Except for the terbium ion, whose absorption cross section was found to be 3 orders of magnitude larger than a previously published value at a close but not equal excitation wavelength ($\sigma_{284\text{nm}} = 0.96 \times 10^{-21}$ cm$^2$/molecule from Carnall$^{43}$ and $\sigma_{266\text{nm}} = 0.18 \times 10^{-17}$ cm$^2$/molecule from our experiments), the absorption cross sections for DPA and DPA-Tb were found to be very similar to other published values, i.e. for the DPA the UV Atlas of Organic Compounds$^{37}$
found an $\sigma_{274\text{nm}} = 2.7 \times 10^{-17}$ cm$^2$/molecule, while our experimental values were $\sigma_{266\text{nm}} = 2.1 \times 10^{-17}$ cm$^2$/molecule; for the DPA-Tb complex Latva$^{35}$ found $\sigma_{275\text{nm}} = 4.9 \times 10^{-17}$ cm$^2$/molecule, and our experimental values were $\sigma_{266\text{nm}} = 1.9 \times 10^{-17}$ cm$^2$/molecule.

Finally, a new technique was developed to simulate the dynamic behavior of the transient fluorescence signal of the terbium doped dipicolinic acid complex using the numerical solutions to the system’s transition rate equations in conjunction with a modulated pulsed UV excitation source. This technique has the potential to help more accurately measure the fluorescence lifetime. It can also be used as a tool in saturation spectroscopy, as well as the study of solid state lasers. The technique has an accuracy of ±10% in water, but may have larger error in other pH solution. It is felt that this technique may have usefulness in other spectroscopic fields. Material presented in this dissertation has been published and presented in a number of technical conferences.$^{85-91}$

Future plans include further modifying the modeling program in order to control the exposure time of the sample to the incident excitation light, thus simulating asymmetrical excitation sources, and investigating the use of multiple rare earth ions in the DPA complex. The program can also be automated to obtain real time simulations which would closely match the real time fluorescence signal. Finally, these results maybe important for the detection of bacteria spores. It is anticipated that a library of fluorescence lifetimes of the DPA-Tb complex in different solvents in concentrations close to those found in natural occurring contaminations, i.e. less than 10 μM DPA, can be compiled in the aid of such a detection system.
REFERENCES


APPENDIX A

ABSORBANCE SPECTRA

This appendix contains the graphs of the measured absorbance as a function of wavelength for different concentrations of each DPA and TbCl$_3$, separately, in distilled water, in [0.01M] HCl, and in [1M] SAB. The spectra of the absorbance of solutions containing mixtures of different concentrations of DPA and TbCl$_3$, as a function of wavelength, in [0.01M] HCl and in [1M] SAB can also be found here. The last two graphs are an enlarged view, i.e. 200 nm-400 nm, of these same spectra.
Figure A.1  Measured absorbance of different concentrations of DPA in distilled water; water background not subtracted.
Figure A.2  Measured absorbance of different concentrations of DPA in [0.01M] HCl; HCl background not subtracted.
Figure A.3  Measured absorbance of different concentrations of DPA in [1M] SAB; SAB background not subtracted.
Figure A.4  Measured absorbance of different concentrations of TbCl$_3$ in distilled water; water background not subtracted.
Figure A.5  Measured absorbance of different concentrations of TbCl$_3$ in [0.01M] HCl; HCl background not subtracted.
Figure A.6  Measured absorbance of different concentrations of TbCl$_3$ in [1M] SAB; SAB background not subtracted.
Figure A.7  Measured absorbance spectra of solutions of [5µM] DPA with varying [TbCl₃] (i.e. [5µM], [10µM], [15µM], [20µM], and [25µM]), (a), of [10µM] DPA with varying [TbCl₃], (b), of [15µM] DPA with varying [TbCl₃], (c), of [20µM] DPA with varying [TbCl₃], (d), and of [25µM] DPA with varying [TbCl₃], (e), in [0.01M] HCl.
Figure A.8  Measured absorbance spectra of solutions of [5µM] DPA with varying [TbCl₃] (i.e. [5µM], [10µM], [15µM], [20µM], and [25µM]), (a), of [10µM] DPA with varying [TbCl₃], (b), of [15µM] DPA with varying [TbCl₃], (c), of [20µM] DPA with varying [TbCl₃], (d), and of [25µM] DPA with varying [TbCl₃], (e), in [1M] SAB.
Figure A.9  Enlarged view of the measured absorbance spectra of solutions of [5µM] DPA with varying [TbCl₃] (i.e. [5µM], [10µM], [15µM], [20µM], and [25µM]), (a), of [10µM] DPA with varying [TbCl₃], (b), of [15µM] DPA with varying [TbCl₃], (c), of [20µM] DPA with varying [TbCl₃], (d), and of [25µM] DPA with varying [TbCl₃], (e), in [0.01M] HCl.
Figure A.10. Enlarged view of the measured absorbance spectra of solutions of [5μM] DPA with varying [TbCl₃] (i.e. [5μM], [10μM], [15μM], [20μM], and [25μM]), (a), of [10μM] DPA with varying [TbCl₃], (b), of [15μM] DPA with varying [TbCl₃], (c), of [20μM] DPA with varying [TbCl₃], (d), and of [25μM] DPA with varying [TbCl₃], (e), in [1M] SAB.
APPENDIX B
DETAILS OF LIFETIME CALCULATIONS

The method used to obtain the fluorescence lifetimes from the plot of fluorescence intensity as a function of time is presented in this appendix. All of the signals presented here are from a solution of [50µM] DPA with [50µM] TbCl₃ in distilled water.
Figure B.1  Fluorescence intensity as a function of time without any corrections: i.e. the “raw” signal.
Figure B.2. The “raw” signal with the offset (at 6ms) subtracted.
Figure B.3  The “normalized”, i.e. the raw signal with the offset subtracted, and the effects of the laser intensity, the grating’s efficiency, and the PMT’s sensitivity accounted for.
Figure B.4  Natural logarithm of the normalized fluorescence intensity as a function of time for a solution of [50µM] DPA with [50µM] TbCl$_3$ in distilled water.
Figure B.5  Enlarged view of plots of the natural logarithm of the normalized fluorescence intensity as a function of time for a solution of [50µM] DPA with [50µM] TbCl₃ in distilled water.
Figure B.6  Enlarged view of the points used in the calculations to find the slope of the 488 nm line.
Figure B.7 Example of points used to find the slope of the line, the negative inverse of which is equal to the fluorescence lifetime of the sample’s 488 nm line. Note: the y-axis has been labeled arbitrarily, but consistently with other graphs of the same type for this sample.
In order to find the error associated with the slope of the line, i.e. with the lifetime value, alternate lines with the maximum possible slope and the smallest possible slope were also plotted, as they have been shown in the above figure in dotted lines. Next, these slopes were used in order to find the smallest possible and the largest possible lifetime values, using the negative of their inverse, as described previously. From these values the errors for the lifetime were found. It is important to note that the point of reference used to plot the “maximum and minimum” slopes was the upper part of the graph’s slope, since in this area the signal to noise ratio was significantly smaller than that of the bottom part of the slope, which due to digitization had significantly larger S/N ratio. A sample calculation for the most reasonable value of the slope for the data presented in this appendix is given below.

\[
\tau = -\frac{1}{\text{Slope}}
\]

\[
\text{Slope} = \frac{(-0.76) - (-0.03)}{0.0024 - 0.0018} = -1.151 \times 10^3 \text{ / sec}
\]

\[
\tau = -\frac{1}{-1151} = 0.87 \times 10^{-3} \text{ sec}
\]
APPENDIX C

FLUORESCENCE SIGNALS USING ASYMMETRIC EXCITATION WAVEFORMS

The initial study of the effect of asymmetric excitation waveforms on our measurements is shown in this appendix. The asymmetric waveform is when the positive (on) excitation intensity patterns are not equal or symmetric to the off portions of the waveform. Such asymmetries can alter the symmetry of the fluorescence emission, so that the long-term average fluorescence intensity level changes as the chopper speed is varied.

It is anticipated that such asymmetric excitation waveform may be suitable for more complex fluorescing systems, and that the effect of excitation and fluorescence decay processes can be tailored to accentuate selected transfer processes amongst the energy levels.

As can be seen, the interplay of the excitation waveform and the transfer/excitation process within the DPA-Tb complex can be controlled as to the resultant maximum fluorescence peak, the average fluorescence level, and the minimum fluorescence peak.
Figure C.1  Fluorescence signals obtained using the symmetric 30 slit blade at the indicated chopper frequencies.
Figure C.2  Fluorescence signals obtained using the asymmetric 15 slit blade at the indicated chopper frequencies.
Figure C.3 Fluorescence signals obtained using the asymmetric superimposed blades at the indicated chopper frequencies.
APPENDIX D

NUMERICAL EVALUATION OF THE RATE EQUATIONS FOR 4-LEVEL MODEL AND FOR ASYMMETRIC EXCITATION

The following program was written and used in Matlab© to numerically calculate the solution to the set of coupled differential equations which describe the rate equations of the DPA-Tb energy level system. In this program a laser pulse which consists of an “on” time of 0.4 ns (in increments of dt = 0.04ns) and an “off” time of 118 µs (in increments of dt = 1.2 µs), with a PRF of 8.5 KHz, is passed through a “chopper blade” which runs at different frequencies (or range of frequencies based on user input). The new intensity profile of the laser beam, which is very similar to that shown in Fig. 7.6, is then used as the incident intensity in the rate equations of the simple model, i.e. Eq. (8.1-8.5). The population densities of each of the energy levels as a function of time are then obtained numerically by solving for dN₁ at time t = iᵗʰ dt, and calculating N₁[t = (iᵗʰ + 1) dt] = N₁[t = iᵗʰ dt + dN₁(t = iᵗʰ dt), for example]. The resultant values for the population densities were then analyzed and plotted using Matlab©.

Similar programs have been used for the 5-Level (quenching) model and the upconversion model.

Lastly, an initial computer program for asymmetric excitation is also presented, for completeness.
% SIMPLE FOUR LEVEL SYSTEM

clear;

chop_freq = 50; % Operating chopper frequency.
run_time = 0.040; % Time (in seconds) you would like to simulate the
system for.

N0 = 3.0e16; % Population densities at t=0sec. In molecules/cm3.
N1 = 0.0;
N2 = 0.0;
N3 = 0.0;
I = 1.1e22; % Laser intensity in photons/cm2.s
sigma = 1.9e-17; % Experimentally measured absorption cross section of
the DPA-Tb complex. In cm2/molecule.
g12 = 5000; % Transfer rates. In 1/sec.
g23 = 1053;
g30 = 10000;

dt_on = 0.04e-9; % Time step (in sec) when the laser intensity is none-
zero in one period of the laser. This is 1/10 of the pulse width, i.e.
0.4ns/10.
dt_off = 1.18e-6; % Time step (in sec) when the laser intensity is zero
in one period of the laser. This is 1/100 of the laser period, i.e.
118us/100.
num_laser_periods = run_time/118e-6; % Number of laser periods that
"fit" in the specified "run time".
chop_per = 1/chop_freq; % The chopper period (in sec.)
num_chop_per = run_time*chop_freq; % Number of chopper periods that
"fit" in the specified "run time".
chop_pulses = round((0.5*chop_per)/118e-6) %Number of laser pulses that
pass through the open window of the symmetric chopper blade,
% i.e. half period.

n=0; % Initial value of the counter.
t = 0.0; % Initial time.

fid = fopen('E:\Chop_Sim\output.txt', 'a+'); % Opens (or creates) file
and appends data to it. Need to delete old file before Need to delete
old file before running new simulation.

for k=1:num_laser_periods % The program runs for the total number of
laser periods that fit in the "run-time".
    if mod(k,chop_pulses)==0 % Every half chopper period, the counter
        adds one to n.
        n = n+1;
    end
    if mod(n,2)==0 % The chopper window is "on".
        for i=1:10 % The "on" part of one laser period when chopper
            window is open.
            dN0 = (I * sigma * (N1-N0) + g30 * N3) * dt_on;
            dN1 = (I * sigma * (N0-N1) - g12 * N1) * dt_on;
            % Update population densities.
            N0 = N0 + dN0;
            N1 = N1 + dN1;
            N2 = N2 + dt_off * (N1 - N2);
            N3 = N3 + dt_off * (N0 - N3);
        end
    end
end

fid = fclose(fid); % Close the file.
\[ dN2 = (g_{12} \times N1 - g_{23} \times N2) \times dt_{on}; \]
\[ dN3 = (g_{23} \times N2 - g_{30} \times N3) \times dt_{on}; \]
\[ t = t + dt_{on}; \]
\[ N0 = N0 + dN0; \]
\[ N1 = N1 + dN1; \]
\[ N2 = N2 + dN2; \]
\[ N3 = N3 + dN3; \]

fprintf(fid, '%e %e %e %e %e \n', t, N0, N1, N2, N3);
end

for j=1:100 % The "off" part of one laser period when chopper window is open.
\[ dN0 = g_{30} \times N3 \times dt_{off}; \]
\[ dN1 = - g_{12} \times N1 \times dt_{off}; \]
\[ dN2 = (g_{12} \times N1 - g_{23} \times N2) \times dt_{off}; \]
\[ dN3 = (g_{23} \times N2 - g_{30} \times N3) \times dt_{off}; \]
\[ t = t + dt_{off}; \]
\[ N0 = N0 + dN0; \]
\[ N1 = N1 + dN1; \]
\[ N2 = N2 + dN2; \]
\[ N3 = N3 + dN3; \]

fprintf(fid, '%e %e %e %e %e \n', t, N0, N1, N2, N3);
end

else
  if mod(n,2)==1 % The chopper window is "off"
    for i=1:10 % The closed chopper window is killing the "on" part of one laser period...
      \[ dN0 = g_{30} \times N3 \times dt_{on}; \]
      \[ dN1 = - g_{12} \times N1 \times dt_{on}; \]
      \[ dN2 = (g_{12} \times N1 - g_{23} \times N2) \times dt_{on}; \]
      \[ dN3 = (g_{23} \times N2 - g_{30} \times N3) \times dt_{on}; \]
      \[ t = t + dt_{on}; \]
      \[ N0 = N0 + dN0; \]
      \[ N1 = N1 + dN1; \]
      \[ N2 = N2 + dN2; \]
      \[ N3 = N3 + dN3; \]
    fprintf(fid, '%e %e %e %e %e \n', t, N0, N1, N2, N3);
    end
    for j=1:100 % The "off" part of one laser period when the chopper window is closed.
      \[ dN0 = g_{30} \times N3 \times dt_{off}; \]
      \[ dN1 = - g_{12} \times N1 \times dt_{off}; \]
      \[ dN2 = (g_{12} \times N1 - g_{23} \times N2) \times dt_{off}; \]
      \[ dN3 = (g_{23} \times N2 - g_{30} \times N3) \times dt_{off}; \]

\]
t = t + dt_off;
N0 = N0 + dN0;
N1 = N1 + dN1;
N2 = N2 + dN2;
N3 = N3 + dN3;

fprintf(fid, '%e    %e  %e  %e  %e 
', t, N0, N1, N2, N3);
end
end
end

fclose(fid)
% SIMPLE MODEL (ASYMMETRIC CHOPPER)

clear;

chop_freq = 50; % Operating chopper frequency
chop_per_width = 16; % Width of one chopper period. In mm.
chop_open_width = 4; % Width of open window/slits in one chopper period. In mm.
chop_close_width = 12; % Width of closed window/slits in one chopper period. In mm.

run_time = 0.040; % Time (in seconds) you would like to simulate the system for.

N0 = 3.0e16; % Population densities at t=0sec. In molecules/cm3.
N1 = 0.0;
N2 = 0.0;
N3 = 0.0;
I = 1.1e22; % Laser intensity in photons/cm².s
sigma = 1.9e-17; % Experimentally measured absorption cross section of the DPA-Tb complex. In cm²/molecule.
g12 = 5000; % Transfer rates. In 1/sec.
g23 = 1053;
g30 = 10000;

dt_on = 0.04e-9; % Time step (in sec) when the laser intensity is non-zero in one period of the laser. This is 1/10 of the pulse width, i.e. 0.4ns/10.
dt_off = 1.18e-6; % Time step (in sec) when the laser intensity is zero in one period of the laser. This is 1/100 of the laser period, i.e. 118us/100.
num_laser_periods = run_time/118e-6; % Number of laser periods that "fit" in the specified "run_time".
chop_per_t = 1/chop_freq; % Time of one chopper period (in sec).
t_chop_open = (chop_open_width/chop_per_width)*chop_per_t % Time it takes the open window of the chopper to pass a small point.
t_chop_closed = chop_per_t - t_chop_open % Time it takes the closed window of the chopper to pass a small point.
num_chop_per = run_time*chop_freq % Number of chopper periods that fit into the "run-time".
chop_pulses_open = round(t_chop_open/118e-6) % Number of laser pulses that fit in the open window of the chopper (at the specified chop. freq.)
chop_pulses_closed = round(t_chop_closed/118e-6) % Number of laser pulses that fit in the closed window of the chopper (at the specified chop. freq.)
chop_per_pulses = chop_pulses_open + chop_pulses_closed % % Total number of laser pulses that "fit" during one chopper period.

n=0; %Initial value of the counter.
t = 0.0; % Initial time.
fid = fopen('E:\Chop_Sim\output_asymt.txt', 'a+'); % Opens (or creates) file and appends data to it. Need to delete old file before running new simulation.

for k=1:num_laser_periods % The program runs for the total number of laser periods that fit in the "run-time".

    if mod(k,chop_per_pulses)==0 % Keeps track of number of chopper periods.
        n = n+1;
    end

    if (k>=n*chop_per_pulses) && (k<(n*chop_per_pulses+chop_pulses_open)) % Chopper window is open
        for i=1:10 % The "on" part of one laser period when chopper window is open
            dN0 = (I * sigma * (N1-N0) + g30 * N3) * dt_on;
            dN1 = (I * sigma * (N0-N1) - g12 * N1) * dt_on;
            dN2 = (g12 * N1 - g23 * N2) * dt_on;
            dN3 = (g23 * N2 - g30 * N3) * dt_on;
            t = t + dt_on;
            N0 = N0 + dN0;
            N1 = N1 + dN1;
            N2 = N2 + dN2;
            N3 = N3 + dN3;
            fprintf(fid, '%e %e %e %e %e 
', t, N0, N1, N2, N3);
        end
        for j=1:100 % The "off" part of one laser period when chopper window is open
            dN0 = g30 * N3 * dt_off;
            dN1 = - g12 * N1 * dt_off;
            dN2 = (g12 * N1 - g23 * N2) * dt_off;
            dN3 = (g23 * N2 - g30 * N3) * dt_off;
            t = t + dt_off;
            N0 = N0 + dN0;
            N1 = N1 + dN1;
            N2 = N2 + dN2;
            N3 = N3 + dN3;
            fprintf(fid, '%e %e %e %e %e 
', t, N0, N1, N2, N3);
        end
    else % Chopper window is closed
        for i=1:10 % The closed chopper window is killing the "on" part of one laser period...
            dN0 = g30 * N3 * dt_on;
            dN1 = - g12 * N1 * dt_on;
            dN2 = (g12 * N1 - g23 * N2) * dt_on;
            dN3 = (g23 * N2 - g30 * N3) * dt_on;
        end
    end

else % Chopper window is closed
    for i=1:10 % The closed chopper window is killing the "on" part of one laser period...
        dN0 = g30 * N3 * dt_on;
        dN1 = - g12 * N1 * dt_on;
        dN2 = (g12 * N1 - g23 * N2) * dt_on;
        dN3 = (g23 * N2 - g30 * N3) * dt_on;
    end

else % Chopper window is closed
    for i=1:10 % The closed chopper window is killing the "on" part of one laser period...
        dN0 = g30 * N3 * dt_on;
        dN1 = - g12 * N1 * dt_on;
        dN2 = (g12 * N1 - g23 * N2) * dt_on;
        dN3 = (g23 * N2 - g30 * N3) * dt_on;
    end

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t = t + dt_on;
N0 = N0 + dN0;
N1 = N1 + dN1;
N2 = N2 + dN2;
N3 = N3 + dN3;

fprintf(fid, '%e %e %e %e %e
', t, N0, N1, N2, N3);
end

for j=1:100 % The "off" part of one laser period when the chopper window is closed.

dN0 = g30 * N3 * dt_off;
dN1 = - g12 * N1 * dt_off;
dN2 = (g12 * N1 - g23 * N2) * dt_off;
dN3 = (g23 * N2 - g30 * N3) * dt_off;

t = t + dt_off;
N0 = N0 + dN0;
N1 = N1 + dN1;
N2 = N2 + dN2;
N3 = N3 + dN3;

fprintf(fid, '%e %e %e %e %e
', t, N0, N1, N2, N3);
end
end

fclose(fid)
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Anali Makoui Roohy obtained a Bachelor of Science degree in Physics from the University of South Florida, Florida, U.S.A. in 2000, and a Masters of Science degree in Physics from the University of South Florida, Florida, U.S.A. in 2002 under the guidance of Prof. Dennis K. Killinger. She entered the Ph.D. program in Applied Physics at the University of South Florida in Fall 2002.

She completed an industrial practicum at Ocean Optics Inc., Dunedin, Florida as part of the Applied Physics practical training. She has a joint patent in “Differential Spectroscopic Imaging of the Human Retina” (Patent number: US 6,709,109 B1), and has presented papers at several technical conferences including SPIE, OSA, and CLEO.