Three-photon Absorption Process In Organic Dyes Enhanced By Surface Plasmon Resonance

2006

Ion Cohanoschi

University of Central Florida

Find similar works at: http://stars.library.ucf.edu/etd

University of Central Florida Libraries http://library.ucf.edu

Part of the Electromagnetics and Photonics Commons, and the Optics Commons

STARS Citation

http://stars.library.ucf.edu/etd/1022

This Doctoral Dissertation (Open Access) is brought to you for free and open access by STARS. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of STARS. For more information, please contact lee.dotson@ucf.edu.
THREE-PHOTON ABSORPTION PROCESS IN ORGANIC DYSES ENHANCED BY SURFACE PLASMON RESONANCE

by

ION COHANOSCHI

B.S. Physics, University of Bucharest, 1995
M.S. Optics, University of Central Florida, 2002

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Optics & Photonics/CREOL & FPCE at the University of Central Florida Orlando, Florida

Fall Term
2006

Major Professor: Florencio E. Hernandez
© 2006 Ion Cohanosci
Multi-photon absorption processes have received significant attention from the scientific community during the last decade, mainly because of their potential applications in optical limiting, data storage and biomedical fields. Perhaps, one of the most investigated processes studied so far has been two-photon absorption (2PA). These investigations have resulted in successful applications in all the fields mentioned above. However, 2PA present some limitations in the biomedical field when pumping at typical 2PA wavelengths. In order to overcome these limitations, three-photon absorption (3PA) process has been proposed. However, 3PA in organic molecules has a disadvantage, typical values of $\sigma_3'$ are small ($10^{-81}$ cm$^6$s$^2$/photon$^2$), therefore, 3PA excitation requires high irradiances to induce the promotion of electrons from the ground state to the final excited state. To overcome this obstacle, specific molecules that exhibit large 3PA cross-section must be designed. Thus far, there is a lack of systematic studies that correlate 3PA processes with the molecular structure of organic compounds. In order to fill the existent gap in 3PA molecular engineering, in this dissertation we have investigated the structure/property relationship for a new family of fluorene derivatives with very high three-photon absorption cross-sections. We demonstrated that the symmetric intramolecular charge transfer as well as the $\pi$-electron conjugation length enhances the 3PA cross-section of fluorene derivatives. In addition, we showed that the withdrawing electron character of the attractor groups in a pull-pull geometry proved greater 3PA cross-section.

After looking for alternative ways to enhance the effective $\sigma_3'$ of organic molecules, we investigated the enhancement of two- and three-photon absorption processes by means of Surface Plasmon. We demonstrated an enhancement of the effective two- and three-photon
absorption cross-section of an organic compound of 480 and 30 folds, respectively. We proved that the enhancement is a direct consequence of the electric field enhancement at a metal/buffer interface.

Next, motivated by the demands for new materials with enhanced nonlinear optical properties, we studied the 3PA of Hematoporphyrin IX and J-aggregate supramolecular systems. As a result, we were able to propose the use of 3PA in photodynamic therapy using Photofrin, the only drug approved by the FDA for PDT.
ACKNOWLEDGMENTS

I would like to acknowledge the professors, staff and students of College of Optics & Photonics/CREOL who gave me a great support and friendship during the years. The last years have been the most rewarding years of my life. Many thanks and gratitude go to the professors David J. Hagan, Patrick Likamwa, Aristide Dogariu and Kevin D. Belfield for agreeing to serve on my committee and for their support.

A very special Thanks goes to my advisor, Dr. Florencio Eloy Hernandez, for his professional suggestions and great help for this work. The modest success I have had as a graduate student is due to the training I have received from him during all this time. Without his help this dissertation would not be possible.

The members of Dr. Hernandez group, Marisol Garcia and Carlos Toro have inspired me and provided a great assistance towards this work. They are great colleagues. I also wish to sincerely thank the members of Dr. Kevin Belfield’s group, Sheng Yao, Alma Morales, Katherine Schafer and Mike Bondar for synthesizing the fluorene derivatives.

Finally, I am very thankful to my wonderful wife, Mihaela, who was besides me all the time, encouraging and inspiring me. Her love, patience, understanding and support made this work an enjoyable part of my life and guided me from the beginning to the end of this journey.


**TABLE OF CONTENTS**

LIST OF FIGURES .......................................................................................................................... viii

LIST OF TABLES ............................................................................................................................ xii

LIST OF ACRONYMS ...................................................................................................................... xiii

CHAPTER 1: INTRODUCTION ........................................................................................................ 1

1.1. Multiphoton Absorption Processes ...................................................................................... 1

1.2. Introduction to Nonlinear Polarization and Susceptibility ................................................ 3

1.3. Two- and Three-Photon Absorption Effects ....................................................................... 5

1.3.1. Applications of Two-Photon Absorption Effect ........................................................... 7

1.3.2. Applications of Three-Photon Absorption Effect ......................................................... 8

1.4. Introduction to Surface Plasmons ........................................................................................ 9

1.4.1. Electric Field Enhancement Due to Surface Plasmons ................................................ 13

1.5. Dissertation Statement and Overview ................................................................................. 17

CHAPTER 2: STUDY OF THE STRUCTURE-PROPERTY RELATIONSHIP .................................... 20

2.1. The Molecular Structure and Linear Optical Properties of Fluorene Derivatives .............. 20

2.2. Multiphoton Induced Fluorescence Measurements ........................................................... 25

2.3. Computer Simulation of Multiphoton Absorption Processes ............................................ 38

2.4. The Effect of Molecular Symmetry on Three-photon Absorption Cross-Section ................ 42

2.5. The Effect of $\pi$-Electron Conjugation Length on 3PA Cross-Section ............................... 47

CHAPTER 3: ENHANCEMENT OF NONLINEAR ABSORPTION USING SURFACE PLASMON RESONANCE EFFECTS........................................................................................................... 58

3.1. Surface Plasmon Resonance Effect in Metallic Films: Kretschmann-Raether Configuration ........................................................................................................................................ 58

3.2. Surface Plasmon Enhanced Two- and Three-photon Absorption Using Metallic Nanoparticles ....................................................................................................................................... 74

3.2.1. Nanoparticles Synthesis and Gold Colloid Preparation ............................................... 77

3.2.2. Two- and Three-photon Absorption Measurements of Hoechst 33258 ...................... 81

3.2.3. Enhancement of Two- and Three-photon Absorption Cross-Sections of Hoechst 33258 in the Presence of Activated Gold Colloid ......................................................... 84

CHAPTER 4: ENHANCEMENT OF THREE-PHOTON ABSORPTION CROSS-SECTION IN J-AGGREGATES .......................................................................................................................... 89

4.1. Description of J-Aggregates ................................................................................................ 89

4.2. Linear Optical Properties of Pseudoisocyanine J-Aggregate ............................................ 90

4.3. Three-Photon Absorption Enhancement in Pseudoisocyanine J-Aggregates .................... 92

CHAPTER 5: APPLICATION OF THREE-PHOTON ABSORPTION IN PHOTODYNAMIC THERAPY (PDT) .......................................................................................................................... 95
LIST OF FIGURES

Figure 1: The evanescent field at the interface between medium $\varepsilon_1$ and medium $\varepsilon_2$.......................... 11

Figure 2: The ATR method: dispersion relation (DR) for a quartz/metal/air system, $\varepsilon_2 = 1$, c: light line in vacuum, $c/\varepsilon_0^{1/2}$: light line in the medium $\varepsilon_0$................................................................. 12

Figure 3: Calculated enhancing factor of electric-field intensity............................................................ 15

Figure 4: Maximum electric-field intensity enhancement (MEFIE) vs. $\lambda$ for Au nanospheres ... 16

Figure 5: Molecular structure of all fluorene derivative compounds .................................................... 20

Figure 6: (a) Linear absorption and emission; (b) d band structure for the asymmetric compound 1 (D-$\pi$-A) .............................................................................................................................. 22

Figure 7: (a) Linear absorption and emission spectra for 2 in hexane; (b) band structure for the symmetric compound 2 (D-$\pi$-D)............................................................................................................................... 23

Figure 8: (a) Linear absorption and emission of A-$\pi$-A in hexane ($10^{-5}$ M); (b) Band structure of the symmetric compound A-$\pi$-A. The inset of 5 (a) represents the molecular structure of A-$\pi$-A fluorene derivative ........................................................................................................................................... 24

Figure 9: Experimental setup for multiphoton absorption induced fluorescence. The excitation wavelength was changed between 300 nm and 1200 nm to allow both processes linear and nonlinear absorption induced fluorescence to be studied. ......................................................... 26

Figure 10: One- two- and three-photon absorption induced fluorescence spectra for the A-$\pi$-A compound. The pumping wavelength was 365 nm (1PA), 600 nm (2PA) and 1095 nm (3PA)........................................................................................................................................................................ 27

Figure 11: Order of the absorption process vs. input wavelength of A-$\pi$-A in hexane solution at a concentration [A-$\pi$-A] = $1 \times 10^{-2}$ M................................................................. 28

Figure 12: One- two- and three-photon induced fluorescence spectra of 9,9-di-decyl-2,7-bis-(N,N- diphenylamino)fluorene (D-$\pi$-D). The excitation wavelengths were 370 nm, 640 nm and, respectively, 1110 nm ........................................................................................................................................................................ 31

Figure 13: Order of the absorption process for the D-$\pi$-D molecule................................................. 32

Figure 14: One- two- and three-photon absorption induced fluorescence spectra for the D-$\pi$-A compound........................................................................................................................................... 33
Figure 15: Order of absorption process versus excitation wavelength for D-\pi-A compound. The solid line makes separation between different multiphoton absorption processes. ............... 34

Figure 16: Fluorescence Intensity versus Input Intensity for each compound. The solid lines represent the theoretical fittings using the function $y=a \times x^n$. For the asymmetric compound the best-fit parameter $n$ was 2.85, and for the symmetric 2.92, and 2.95, respectively........ 35

Figure 17: Fluorescence Intensity versus Input Intensity induced at 1200 and 1600 nm (a) and open aperture Z-Scan curves for D-\pi-A/hexane pumping at 1600 nm (b) ......................... 37

Figure 18: Simulation of two-, three- and four-photon absorption using a laser beam with Gaussian temporal and spatial profiles. .............................................................. 42

Figure 19: Experimental setup for 3PA measurements. The sample is translated along $z$ direction over an 80 mm interval with a step of 150 microns................................................................. 43

Figure 20: Open aperture Z-scan for D-\pi-A (A), D-\pi-D (B) and A-\pi-A (C) in hexane solution at 0.0098 M. The solid lines are the theoretical best-fitting curves using three-photon absorption equations for change in transmittance................................................................. 45

Figure 21: Molecular structure of A-\pi-\pi-\pi-A (1'), D-\pi-\pi-\pi-D (2') and D-\pi-\pi-\pi-A (3') compounds. ................................................................. 48

Figure 22: Linear absorption (triangles) and emission (squares) spectra. The linear absorption spectra were measured in dye/THF solution at a concentration of $10^{-5}$ M......................... 50

Figure 23: Three-photon absorption induced fluorescence spectra of A-\pi-\pi-\pi-A (open squares), D-\pi-\pi-\pi-D (filled squares) and D-\pi-\pi-\pi-A (semi-open squares) pumping at 1230 nm, 1280 nm and 1260 nm, respectively. ......................................................... 51

Figure 24: Open-aperture Z-scan curves of D-\pi-\pi-\pi-A, D-\pi-\pi-\pi-D and A-\pi-\pi-\pi-A in THF solution ($10^{-3}$ M), taken at three different energies for each compound. Solid curves are the best theoretical fittings using Gaussian beam equations for 3PA......................... 53

Figure 25: Transmitted vs. Input Intensity curves of compound 3’ (a), 2’ (b), and 1’ (c)........... 56

Figure 26: Experimental setup for Surface Plasmon Resonance measurements. The SPs are excited by light using Kretschmann-Raether configuration. ......................................................... 60

Figure 27: Reflectivity curves for a BK7/Silver/Air system. The silver film has a thickness of 40 nm and the excitation wavelength was tuned between 633 nm and 1200 nm. .................... 61

Figure 28: Experimental and theoretical dispersion curves for Surface Plasmons. The silver layer has thickness of 40 nm and the prism material is BK7 (ATR setup)................. 63
Figure 29: Calculated wavelength-dependent reflectivity for a 40-nm-thick silver film coated with a 15-nm-thick MgF$_2$ spacer........................................................................................................ 65

Figure 30: Experimental curves of Reflectivity for glass/silver/MgF$_2$ system, a) coated with dye, and b) without dye coating. The excitation wavelength was 1200nm. ...................... 67

Figure 31: SF-11 prism coated with gold film, SiO$_2$ spacer and asymmetric dye. The red arrows represent the incident and reflected beams (1200nm). .................................................. 68

Figure 32: Reflectivity curves for SF-11/Au/SiO$_2$/dye structure. The excitation wavelength was 1200 nm, which corresponds to 3PA of asymmetric compound ......................... 70

Figure 33: Calculated reflectivity curves for a 55 nm gold film on SF-11 using D-π-A dye layers with thickness between 0 and 60 nm. The excitation wavelength was 1200 nm........ 72

Figure 34: Reflectivity curves for SF-11/Ag/MgF$_2$/dye structure, pumping at 1200 nm........ 73

Figure 35: Molecular structure of Hoechst 33258 (top). Normalized absorption (solid line) and emission (dotted line) spectra of 10$^{-5}$ M Hoechst 33258/water solution. Anisotropy curve (Δ) of a 5×10$^{-6}$ M Hoechst/glycerol solution................................................................. 76

Figure 36: Absorption spectra for gold nanospheres of 3.5 nm (open squares), 16 nm (open triangles) and 35 nm diameter (open circles)................................................................. 78

Figure 37: TEM images of gold nanospheres a) and gold nanorods b)........................................ 79

Figure 38: Absorbance vs. wavelength of 16 nm diameter gold nanospheres (dashed line), gold activated colloid (dotted line), and Hoechst 33258/H$_2$O solution (2.3×10$^{-3}$ M) in the presence of activated gold colloid (solid line). The inset shows a closer view of the low absorbance region of the spectra................................................................. 80

Figure 39: Open aperture Z-scan curves of Hoechst 33258/H$_2$O solution (2.3×10$^{-3}$ M) pumping at 550 nm (a) and 1064 nm (b). Solid lines are the best theoretical fittings using the equations for 2PA and 3PA................................................................. 82

Figure 40: Open aperture Z-scan curves of Hoechst 33258/H$_2$O solution in the presence of activated gold colloid, pumping at 550 nm (a) (8.3×10$^{-4}$ M) and 1064 nm (b) (1.4×10$^{-3}$ M). Solid lines are the best theoretical fittings using the equations for nonlinear transmittance derived for 2PA and 3PA................................................................. 85

Figure 41: Experimental normalized transmittance vs. input irradiance of pure Hoechst/H$_2$O for 2PA (O) and 3PA (□), and their corresponding theoretical curves................................. 87

Figure 42: Splitting of the excitation energy of one molecule during the dimmer formation..... 90
Figure 43: Linear absorption of the monomer (...) and J-aggregate (—) in aqueous solution at a concentration of $2.5 \times 10^{-4}$ M and $2 \times 10^{-3}$ M, respectively. The molecular structure of 1,1'-diethyl-2,2’-cyanine iodide is presented in the inset.

Figure 44: Open aperture Z-scan curves of PIC J-aggregate taken at three different pump energies (symbols) and corresponding theoretical fittings for 3PA (—).

Figure 45: Molecular structure and absorption spectrum of Hematoporphyrin IX in DMSO solution at a concentration of 0.1 M.

Figure 46: Measured transmitted intensity versus input intensity (open squares). The solid line represents the theoretical fitting using Equation 22 for nonlinear transmittance and the dotted line is the fitting obtained with parameter $\alpha_3 = 0$.

Figure 47: Z-Scan curves of Hematoporphyrin IX / DMSO solution at a concentration of 0.1 M. The solid curves are the theoretical fittings using Eq. 23 for transmittance.

Figure 48: Autocorrelation interferometer for measuring the pulsewidth of the laser.

Figure 49: Autocorrelation curves for the 1064 nm laser. The solid line represents the theoretical fitting using a Gaussian function.

Figure 50: Determination of the beam waist by scanning a razor blade across the beam.

Figure 51: Intensity versus distance across the beam. The solid line is the best-fit using the complementary error-function.
LIST OF TABLES

Table 1: Three-photon absorption cross-sections and the corresponding excitation wavelengths for all compounds. ................................................................. 54

Table 2: Two- and three-photon absorption cross-sections in the absence and in the presence of gold hot nanoparticles. ................................................................. 86
LIST OF ACRONYMS

1PA------------------------ One-Photon Absorption
2PA------------------------ Two-Photon Absorption
3PA------------------------ Three-Photon Absorption
4PA------------------------ Four-Photon Absorption
3D ------------------------ Three Dimensional
SHG------------------------ Second Harmonic Generation
THG------------------------ Third Harmonic Generation
SFG------------------------ Sum Frequency Generation
DFG------------------------ Difference Frequency Generation
IDRI------------------------ Intensity Dependent Refractive Index
PDT------------------------ Photodynamic Therapy
NIR------------------------ Near Infra Red
SPR------------------------ Surface Plasmon Resonance
OR ------------------------ Optical Rectification
mm ------------------------ Milimeter (10^{-3} m)
nm ------------------------ Nanometer (10^{-9} m)
µm  ----------------------- Micrometer (10^{-6} m)
GM ------------------------ Goppert-Mayer unit for 2PA cross-section (10^{-50} cm^4 s photon^{-1})
ps  ----------------------- Picosecond (10^{-12} s)
FDA------------------------ Food and Drug Administration
CHAPTER 1: INTRODUCTION

1.1. Multiphoton Absorption Processes

Multiphoton Absorption is a nonlinear process based on the simultaneous absorption of two or more photons in a single event. The most common multiphoton absorption processes are degenerate two- and three-photon absorption effects. The scientific interest for multiphoton absorption processes has been growing dramatically, especially during the last decade. Multiphoton absorbers have been applied to three-dimensional data storage, [1] frequency up-converted fluorescence microscopy, [2] optical limiting, [3] biophotonics and photodynamic therapy treatments.[4] Two-photon absorption (2PA) has found multiple applications in fluorescence spectroscopy and 3D imaging because of its large effective Stoke’s shift and high spatial resolution [2,5]. In addition, this process has been recently tested in photodynamic therapy (PDT) [6,7] as a consequence of its greater penetration depth. Nevertheless, in biological and medical applications, 2PA is still showing a limited penetration depth.[4] The wavelengths typically used for 2PA excitation, 700-800 nm, are still too short to even further reduce the Rayleigh scattering. To overcome this limitation, higher order nonlinear processes, such as three-photon absorption (3PA) can be employed. Because three-photon absorption typically takes place at longer wavelengths, the Rayleigh scattering losses and linear absorption through biological tissues in the near infrared (NIR) region can be minimized. Consequently, 3PA can penetrate to unprecedented depth.[8] Therefore, a great impact in the fields of biological imaging and photodynamic therapy can be expected.
The application of 3PA resides on several key advantages: a) improvement in penetration depth when working in the living organism transparency windows in the NIR [9]; b) drastic reduction of scattering losses when longer wavelengths are used [10]; and c) higher image contrast and spatial resolution [11]. However, 3PA in organic molecules has a disadvantage: it requires high irradiances to induce the promotion of electrons from the ground state to the final excited state. In order to surmount this main obstacle, nanoscience and nanotechnology offer some possibilities.

The fast development of nano- and biophotonics has motivated the search for new materials and hybrid systems with enhanced nonlinear optical properties. The new potential candidates should possess very large two- or higher order photon absorption cross-section, a good thermal and photochemical stability, and high fluorescence or singlet oxygen quantum yields. One of the purposes of these materials is to avoid using very sophisticated and costly femtosecond lasers, which are now-a-day necessary to induce three- or higher order photon excitations.

The high irradiance demand for three-photon excitation is determined by the fact that the transition probability for three-photon absorption is directly proportional to the 3PA cross-section and cubic irradiance of the incident radiation. Typical values of $\sigma_3$ in organic molecules are very small, i.e. $10^{-81} \text{cm}^6\text{s}^2/\text{photon}^2$. Therefore, only specifically designed molecules that exhibit a larger 3PA cross-section make possible a lower irradiance required to induce 3PA. In addition, new strategies have to be developed to enhance the electric-field intensity. A complementary approach to enhance multi-photon absorption processes without changing the nonlinear optical properties of the material by molecular engineering is through the use of Surface Plasmon Resonance. By enhancing the electric field at a metal-buffer-interface one can
expect to amplify the effective three-photon absorption cross-section and perhaps higher order absorption processes in organic compounds.

Two of the main goals of this research were: 1\textsuperscript{st}) to identify specific molecules that exhibit large three-photon absorption cross-sections and understand the relationship between their structural configurations and their nonlinear optical properties of those molecules, 2\textsuperscript{nd}) to demonstrate that using metal nanoparticles, the nonlinear absorption of organic molecules can be increased via Surface Plasmon Enhancement (SPE).

1.2. Introduction to Nonlinear Polarization and Susceptibility

The interaction of intense laser radiation with matter can lead to different nonlinear effects such as second and third-harmonic generation, frequency conversion, parametric amplification and oscillation, two- and three-photon absorption, intensity-dependent refractive index, among others.[10] The predominant effect depends on the material properties.[10] All nonlinear phenomena are accompanied by a change in polarization.

In linear optics, the presence of the electric field, inside of a given material, will induce a polarization \( \mathbf{P}^L(r, t) \) with a linear dependence on the applied field:

\[
\bar{P}^L(r, t) = \varepsilon_0 \chi^{(1)} \bar{E}(r, t) \quad (1)
\]
The macroscopic polarization \( \vec{P}(r,t) \) is given by the summation over the dipole moments induced in atoms and molecules by the applied electric-field \( \vec{E}(r,t) \). The linear susceptibility, \( \chi^{(1)} \), is a complex quantity given by:

\[
\chi^{(1)}(-\omega,\omega) = \frac{Ne^2(\omega_i^2 - \omega^2)}{m\epsilon_0\left[(\omega_i^2 - \omega^2)^2 + \omega^2 \Gamma^2\right]} + i\frac{Ne^2\omega \Gamma}{m\epsilon_0\left[(\omega_i^2 - \omega^2)^2 + \omega^2 \Gamma^2\right]} \tag{2}
\]

where the real part of the susceptibility represents the dispersion in the refractive index of the medium and the imaginary part signifies the dispersion in loss.

In nonlinear optics, the nonlinear optical response of the medium can be described by expanding the material polarization as a power series in the optical field \( E(r,t) \):

\[
\vec{P}(r,t) = \epsilon_0\chi^{(1)}\vec{E}(r,t) + \epsilon_0\chi^{(2)}\vec{E}(r,t)\vec{E}(r,t) + \epsilon_0\chi^{(3)}\vec{E}(r,t)\vec{E}(r,t)\vec{E}(r,t) + \ldots \tag{1a}
\]

One can distinguish two parts in the right term of the last relation: one linear part represented by the right term of equation (1) and another part given by the nonlinear polarization:

\[
\vec{P}^{NL}(r,t) = \epsilon_0\chi^{(2)}\vec{E}(r,t)\vec{E}(r,t) + \epsilon_0\chi^{(3)}\vec{E}(r,t)\vec{E}(r,t)\vec{E}(r,t) + \ldots \tag{2}
\]

Nonlinear effects related to the second-order susceptibility, \( \chi^{(2)} \), are SHG, SFG, DFG and OR. On the other hand, for the third-order susceptibility, \( \chi^{(3)} \), we have THG, IDRI, SFG. All of
these effects are parametric processes characterized by the time-invariance of populations of the quantum mechanical states. It means that the initial and final quantum-mechanical states of the system are identical. Consequently, in a parametric process population can be removed from the ground state only for those brief intervals of time when it resides in a virtual state. On the contrary, the non-parametric processes such as two- and three-photon absorption, which are third and fifth order nonlinearities respectively, are accompanied by a transfer of populations to higher excited states.[10]

1.3. Two- and Three-Photon Absorption Effects

Two- and three-photon absorption processes are based on the simultaneous absorption of two and three photons, respectively, in a single event. Considering a plane wave, $E(\mathbf{r}, t) = 0.5[E(\mathbf{k}, \omega)e^{i(kr - \omega t)} + c.c.]$, propagating into a nonlinear medium, and using Maxwell equations, the wave equation can be deduced. This equation can be written in the following form [12]:

$$\nabla^2 \mathbf{E} - \frac{n^2}{c^2} \frac{\partial^2 \mathbf{E}}{\partial t^2} = \mu_0 \frac{\partial^3 \mathbf{P}^{NL}}{\partial t^2}$$  \hspace{1cm} (3)

Making the slowly-varying-amplitude approximation $\left(\frac{\partial^2}{\partial z^2} \mathbf{E}_0(z) \ll 2k \frac{\partial}{\partial z} \mathbf{E}_0(z)\right)$ and considering the nonlinear polarization $\mathbf{P}^{NL}$ as a third-order nonlinear polarization given below:[13]
\[ P^{(3)}(\omega) = \frac{3}{4} \varepsilon_0 \chi^{(3)}_{xxx} (-\omega, -\omega, -\omega) |E_x(z)|^2 E_x(z) \]  

(4)

equation (3) becomes:

\[ \frac{d}{dz} E_x(z) = \frac{3i\mu_0 \omega c}{8n} \varepsilon_0 \chi^{(3)}_{xxx} (-\omega, -\omega, -\omega) |E_x(z)|^2 E_x(z) \]  

(5)

The relation between the electric field and intensity is given by the following formula:

\[ I(z) = \frac{1}{2} n_0 c \varepsilon_0 |E_{0x}(z)|^2 \]  

(6)

Substituting Eq. (6) into Eq. (5) one can obtain the beam attenuation due to two-photon absorption within an isotropic medium for the degenerate case of a single beam input:

\[ \frac{dI(z)}{dz} = -\frac{3\omega}{2n_0^2 c^2 \varepsilon_0} \chi^{(3)}_i (-\omega, -\omega, -\omega) I(z)^2 \]  

(7)

In the right term of equation (7) we can identify the two-photon absorption term, which characterizes macroscopically the isotropic medium:

\[ \alpha_2 = \frac{3\omega}{2n_0^2 c^2 \varepsilon_0} \chi^{(3)}_i (-\omega, -\omega, -\omega) \]  

(8)

Substituting eqn. (8) into eqn. (7) we get:

\[ \frac{dI(z)}{dz} = -\alpha_2 I(z)^2 \]  

(9)

Similarly, for three-photon absorption, one can get the change of irradiance with depth into the sample as below:

\[ \frac{dI(z)}{dz} = -\frac{5\omega}{2n_0^3 c^3 \varepsilon_0} \chi^{(5)}_i (-\omega, -\omega, -\omega, -\omega, -\omega) I(z)^3 = -\alpha_3 I(z)^3 \]  

(10)
where the three-photon absorption coefficient is defined as:

\[
\alpha_3 = \frac{5\omega}{2n_0^3 c \varepsilon_0^2} \chi^{(5)}(-\omega, \omega, -\omega, \omega, -\omega, \omega)
\]  

(11)

1.3.1. Applications of Two-Photon Absorption Effect

Over the last ten years a huge number of studies related to two-photon absorption have been reported.[14-18] Many of these studies have resulted in biomedical application as shown in this section. However, not much has been reported in the literature on 3PA, and only few applications are known until now, as stated in section 1.3.2. Two-photon absorption has been used in biological applications since the pioneering work of Webb and co-workers on two-photon confocal imaging [2]. In 1999, Schilders and Gu studied three-dimensional autofluorescence of rat skeletal muscle tissue under two-photon excitation, achieving good resolution and image contrast [19]. This can be explained if one look at equation (8) which shows that absorption due to 2PA is spatially confined to a small volume around the focal point of a laser beam focused into a sample. Recently, Webb’s group synthesized and characterized new semiconductor nanocrystals with the largest 2PA cross-section ever reported,[20]. These nanocrystals can be potential candidates for applications in multicolor imaging systems and living tissues. Nevertheless, for more practical applications in biochemistry, biophysics and
medicine, there is still a great need for superior molecules with strong higher order nonlinear absorption response. Three-photon absorbers should result the best candidate.

1.3.2. Applications of Three-Photon Absorption Effect

In 1995, three-photon-excitation-induced fluorescence was observed for 2,5-benzothiazole 3,4-didecyloxy thiophene (BBTDOT) using focused nanosecond lasers [21]. Also, it was demonstrated that for a given illumination wavelength, the resolution for imaging a thick object using three-photon induced fluorescence can be improved by 50% comparatively to that of 2PA [11]. This is suggested also by the relation (9), which proves that for 3PA the excitation presents a higher spatial confinement since it shows a cubic power dependence with the incident irradiance. In 1996, Hell et al. demonstrated for the first time the possibility of using three-photon excitation in fluorescence microscopy [22]. Additionally, 3PA process has been employed to image labeled DNA with DAIP [23] and induce fluorescence in Indo-1 [24]. In 1997, Webb et al. measured the serotonin distribution in living cells using 3PA imaging [25]. Recently, the 3PA cross-section of water, methanol and n-heptane were measured using femtosecond laser pulses [26]. Upconversion fluorescence and optical limiting effects based on 3PA have been also studied in an organic dye known as BPAS [27]. In 2002, the observation of stimulated emission by three-photon excitation was reported [28].

Although, 3PA has been used in many applications as illustrated before, only few systematic studies related to 3PA have been reported in the literature [29-34]. This situation has
allowed a gap between 3PA and its relationship with the molecular structure. Filling this gap will pave a new road in the design of more powerful three-photon absorbers through molecular engineering. However, further approaches to enhance even more 3PA are going to be required because of the cubic dependence this process have with the irradiance (eq. 9). One possible way to achieve such an enhancement is through the use of surface plasmons generated at the surface of metal nanoparticles. The outcome of this new approach will be of great importance in multiphoton enhanced absorption in organics.

1.4. Introduction to Surface Plasmons

The concept of surface plasmon was introduced for the first time by R. H. Ritchie in June 1957.[35] Since then, our understanding of the Surface Plasmon has been improved continuously. Between 1957 and 1970 the theoretical advances of Surface Plasmons relied on classical approximations, while the SP studies after 1970 included quantum mechanical response of the electrons at the metallic surface. Ritchie and Marusak derived in 1966 the equation for the Surface Plasmon dispersion [36]:

\[
\omega_{sp}(q_{II}) = \omega_R(1 + \alpha_s q_{II})
\]  

(12)

where \( \omega_R = \omega_p/(2+\epsilon_b)^{1/2} \) is called Ritchie frequency and \( \alpha_s \) is a constant depending on the electron gas density, given by:
In the 1970s and 1980s a remarkable theoretical progress was achieved, setting the foundation for understanding of the electromagnetic field at a surface [37,38]. Also, many experimental observations made during this period of time improved our understanding of the collective modes at the vacuum-solid interface.

Surface Plasmons can be defined as quantized oscillations of the collective electrons in a metallic interface. They are transverse electromagnetic waves with magnetic vector perpendicular to the direction of propagation of the surface-plasmon wave and parallel to the plane of interface. This is why only TM-polarized light can be used to satisfy the boundary conditions necessary to excite the Surface Plasmons. Using Maxwell’s equations and the continuity relations, one can get the dispersion relation of the SP propagating along the interface between two media with dielectric constant $\varepsilon_1$ and $\varepsilon_2$:

$$\frac{k_{z1}}{\varepsilon_1} + \frac{k_{z2}}{\varepsilon_2} = 0$$  \hspace{1cm} (14)

In each medium the equation for the wavevector is given by

$$\varepsilon_i\left(\frac{\omega}{c}\right)^2 = k_z^2 + k_{z1}^2, \ i=1,2$$  \hspace{1cm} (15)

From relations (14) and (15) one can obtain the wavevector of the SP which represents the dispersion relation of the Surface Plasmons in the system:

$$k_z = \frac{\omega}{c}\left(\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1 + \varepsilon_2}\right)^{1/2}$$  \hspace{1cm} (16)

Because $k_z > \omega/c$ (we assume that medium with $\varepsilon_1$ is a metal), $k_{z1}$ from Eq. (15) becomes
an imaginary quantity. Therefore, the optical field associated with the surface plasmon has a maximum value at the surface and decays exponentially along the normal to the surface as shown in the Figure 1.

\[ \varepsilon_1 \]

\[ \varepsilon_2 \]

\[ z \]

\[ E_z \propto e^{-k_z z} \]

Figure 1: The evanescent field at the interface between medium \( \varepsilon_1 \) and medium \( \varepsilon_2 \)

For example, at \( \lambda = 600 \text{ nm} \) one can obtain for surface plasmons on silver interface, a skin depth \( z = 1/|k_{Bz}B| \) (depth at which the field falls to \( 1/e \) of its maximum) of 390 nm within the medium \( \varepsilon_2 \) (which is air). The propagation length \( L_i = (2k_x) \)\(^{-1} \), after which the intensity of the surface plasmon decreases to \( 1/e \), would be 22 microns [39] along the interface, assuming a wavelength of 514 nm.

There are two ways to excite the Surface Plasmons: excitation by electrons and excitation by light. The first method is more suitable for studying the dispersion relation of Surface Plasmons at larger \( k_x \). The second method allows probing the SP dispersion relation down to very low \( k_x \) values. The Surface Plasmons can be excited either by using a Grating Coupler or using the ATR Coupler.
The Surface Plasmons can not be excited by shining directly the metal with a laser beam, because their dispersion relation lies right from the light line \( (k_x > \omega/c) \) as shown in Figure 2.[39]

![Figure 2: The ATR method: dispersion relation (DR) for a quartz/metal/air system, \( \varepsilon_2 = 1 \), c: light line in vacuum, \( c/\varepsilon_0^{1/2} \): light line in the medium \( \varepsilon_0 \).](image)

To couple the SP by light, the wave vector \( \hbar\omega/c \) has to be increased by a \( \Delta k_x \) value in order to “transform” the photons into SPs. One way to do this is by using a right angle quartz prism coated with a noble metal such as silver on its largest face. If the light is reflected at a metal surface covered with quartz, its momentum increases to \( (\hbar\omega/c)\sqrt{\varepsilon_0} \) and the DR of Surface Plasmons, up to the intersection point P (see Figure 2), lies to the left of the light line in quartz, \( c/\sqrt{\varepsilon_0} \), so they can be excited by light. The excitation of SP is recognized as a minimum in the
totally reflected intensity. This is why the method is called attenuated total reflection (ATR
method). The resonance condition for SPs is given by:

\[
\frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}} = \frac{\omega}{c} \sqrt{\varepsilon_0} \sin(\theta_0)
\]  \hspace{1cm} (17)

When this condition is fulfilled, the phase matching between the incident light and the Surface
Plasmon which propagates in the interface metal/air is achieved, leading to a minimum in the
reflectivity curve.[39]

1.4.1. Electric Field Enhancement Due to Surface Plasmons

A strong enhancement of the electromagnetic field at the metallic interface can be
achieved when the Surface Plasmons are excited by light. When the reflectivity reaches its
lowest value, the electromagnetic field has its maximum value in the surface. The value of the
enhancement is given by the ratio of the field intensity on the interface metal/air and the
incoming field intensity for p-polarized light [39]:

\[
\left| \frac{H_y(2/1)}{H_y(0/1)} \right|^2 = \left| t_{01}^p \right|^2 = \frac{t_{01}^p t_{12}^p \exp(ik_1d_1)}{1 + r_{01} r_{12} \exp(2ik_1d_1)}
\]  \hspace{1cm} (18)
where $r_{01}$, $r_{12}$, and $t_{01}^p$, $t_{12}^p$ represent Fresnel’s reflection, and transmission coefficients, respectively, for one-boundary system and $d_1$ is the metallic film thickness.

Perhaps, the most interesting quantity is the maximum enhancement of the electric-field intensity ($T_{\text{max}}^{el}$), which is obtained from

$$
\left( \frac{|H_{y}(2/1)|^2}{|H_{y}(0/1)|^2} \right)_{\text{max}} = \varepsilon_2 \left( \frac{|E(2/1)|^2}{|E_0(0/1)|^2} \right)_{\text{max}} = \frac{\varepsilon_2}{\varepsilon_1} T_{\text{max}}^{el}
$$

(19)

as

$$
T_{\text{max}}^{el} = \frac{1}{\varepsilon_2} \frac{2|\varepsilon_1|^2}{\varepsilon_1} \frac{a}{1 + |\varepsilon_1|}
$$

(20)

with

$$
a^2 = |\varepsilon_1| (\varepsilon_0 - 1) - \varepsilon_0
$$

(21)

In figure 3, the theoretical enhancement factor calculated with the Fresnel formula is shown for different excitation wavelengths in silver and gold layers on BK7 [40]. The enhancement factor was determined by using equation 20 and taking into account the dispersion of the refractive index in glass and metals. Figure 3 shows the maximum values of the enhancement factor, which are obtained for specific thicknesses of the metallic films (silver and gold) and angles of incidence. On the figure one can see that for a two-photon excitation wavelength of 700 nm, which corresponds to a 350 nm peak fluorophore such as DAPI, Indo1 or Fura2, the enhancement factor is 80 for gold and 270 for silver film.
On the other hand, theoretical calculations carried out in metal nanospheres at 1100 nm show a maximum enhancement of the electric-field intensity of $1.1 \times 10^5$ in Ag and $2.0 \times 10^3$ in Au.[39] In Fig. 4 we show theoretical calculation of the maximum electric-field intensity enhancement (MEFIE = $|3\varepsilon_c/\varepsilon_{im} P|^2$) vs. wavelength, calculated on Au nanospheres. At 1100 and 1300 nm, the more attractive wavelengths for 3PA, the MEFIE is approximately 1800 and 1600, respectively.
Because of its tremendous electric-field enhancement, the SPR has been applied in nonlinear optics to second-harmonic generation (SHG),[41,42] Raman scattering,[43,44] amplification of light, [45] and more recently to two-photon absorption processes [46-48]. The two main advantages of using metal nanoparticles are the possibility to work in solutions and the tremendous concentration of the electric-field (local-field enhancement).[39]

A few years ago, Kano et al. reported for the first time fluorescence enhancement when two-photon excitation was assisted by surface plasmon using the Kretschmann geometry [40]. They showed a fluorescence enhancement of 90 times due to excitation of the surface plasmon comparative to the case without silver film deposited on the surface. More recently near-field fluorescence microscopy based on two-photon excitation with metal tips was also demonstrated [49].
1.5. Dissertation Statement and Overview

Our research addressed a significant challenge in bioimaging and photodynamic therapy (PDT): the enhancement of the activation penetration depth of organics using three-photon absorption (3PA). The main objectives of this work were to perform a systematic study of the structure/property relationship of three-photon absorbers and, to demonstrate surface plasmon enhanced 3PA using gold nanoparticles in solution. The secondary aims of our investigation were to validate 3PA enhancement in supramolecular systems and to measure the 3PA cross-section of the only drug approved by the FDA for photodynamic therapy, Photofrin®. Our original approach was based on two main aspects: a) the selection of a specific series of organics and b) the employment of the electric-field enhancement achieved via surface plasmon resonance on noble metal nanoparticles to assist three-photon excitation. One of the key issues in the development of biomedical applications (PDT) of nonlinear absorption effects is very high irradiance required to induce three- or higher-order absorption effects. To overcome this issue, this dissertation proposed the use of surface plasmon resonance effect combined with nonlinear absorption effects. This combination of processes allowed an enhancement of “the effective cross-section” of nonlinear absorption processes. Therefore, these new approaches will allow for an unprecedented radiation penetration depth that will result in practical application in cancer treatment and imaging. Our expectation is to fulfill the existent gap in bioimaging and PDT by combining nonlinear optics with nanoscience.

The second chapter of this dissertation presents the study of the structure-property relation for a whole family of fluorene derivatives. The linear optical properties are described for each compound and the three-photon absorption measurements are presented in details. Higher
order absorption processes such as pure four-photon absorption, observed in these molecules are shown. Multiphoton induced upconverted fluorescence was demonstrated for each fluorene derivative. The determined order of the absorption process for symmetric compounds A-π-A, A-π-π-A, D-π-D, D-π-π-D and the asymmetric ones, D-π-A, and D-π-π-A are shown, as well as the study of the effect of structural motif and the π-electron conjugation length on three-photon absorption cross-section.

The third chapter describes the enhancement of the effective two- and three-photon absorption cross-section using Surface Plasmon Resonance effect. The measurements were done using Kretschmann-Raether geometry described in details in this chapter and also using activated gold colloids, which takes advantage of the electric field enhancement produced at the surface of metallic nanoparticles, via Surface Plasmon Resonance. Also, the gold nanoparticles preparation method is presented here.

In chapter four the study of the three-photon absorption effect in supramolecular assemblies such J-aggregates is illustrated. J-aggregates are molecular aggregates of cyanine dye molecules in water characterized by a strong coupling of transition dipole moments. The huge enhancement of the three-photon absorption cross-section in 1,1’-diethyl-2,2’-cyanine iodide (better known as pseudoisocyanine, PIC) J-aggregate assembly is discussed in this chapter.

The fifth chapter shows the results of three-photon absorption measurements in Hematoporphyrin IX, which is one of the main constituent of Photofrin® II, the only approved photodynamic therapy drug by the Food and Drug Association. This fundamental result is expected to take PDT to an advanced level of sophisticated, highly efficient and localized cancer treatment that will afford an unprecedented penetration depth avoiding complicated and high risk surgery.
Finally, the last chapter of this dissertation, chapter 6, gives a summary of the experiments performed and presents the conclusions, based on the experimental results. For the future work, new experiments and directions are suggested, which can improve further our understanding in this topic. Additional details of the work presented in this dissertation are described in the appendixes.
CHAPTER 2: STUDY OF THE STRUCTURE-PROPERTY RELATIONSHIP

2.1. The Molecular Structure and Linear Optical Properties of Fluorene Derivatives

For our study, we have selected six fluorene derivatives which were synthesized by Dr. Kevin D. Belfield, Professor of Chemistry and Chairman, University of Central Florida. They are divided in two categories: the first three with short $\pi$-conjugation length (1, 2, 3) and the last three with long $\pi$-conjugation length (1’, 2’, 3’). Their molecular structures are displayed in Figure 5.

Figure 5: Molecular structure of all six fluorene derivative compounds
The first compound (1) is an asymmetric molecule which consists of a fluorenyl core (the \( \pi \)-conjugated bridge) that links together a donor group D (diphenylamine) and an acceptor group A (benzothiazole). Compounds 2 and 3 are symmetric molecules characterized by the same fluorenyl core linking together two donor groups (diphenylamine) or, two acceptor groups (benzothiazole), respectively. The last three compounds (1’, 2’, 3’) are described in detail in the section 2.5.

We have chosen these molecular structures for three main reasons: a) the large polarizability via electron delocalization through the \( \pi \)-conjugated aromatic core which promises a strong nonlinear interaction upon excitation, b) the rigid ring system (fluorenyl core) gives high thermal and photochemical stabilities, and c) the highly functionality of the fluorene aromatic ring which allows more core ability to introduce particular functionality through molecular engineering on the positions 2 and 7 [49-53]. The latter gives the ability to generate different chemical archetypes by affecting the electron accepting and donating properties of the system as well as the possibility of extending the \( \pi \)-electron conjugation length. In addition, these chromophores present large two-photon absorption cross-section and high fluorescence quantum yield [52-54].

We started this research with compound 1 (7-benzothiazol-2-yl-9,-didecylfluoren-2-yl)-diphenylamine), which has the molecular and band structure displayed in Figure 6. Allowed transitions from ground state \( S_0 \) to the excited states \( S_1 \) and \( S_2 \) are shown by using arrows of different colors. The number of arrows represents the number of photons involved in each particular transition.
Figure 6 also displays the linear absorption and emission spectra (excitation at 400 nm) of the D-\(\pi\)-A molecule in hexane solution at a concentration of 10\(^{-5}\) M. The measurements were carried out using an Agilent 8453 diode array UV-vis spectrometer and PTI Quanta-Master spectrophotometer, respectively (cell effects and solvent contribution were properly subtracted). From the absorption spectrum one can see two absorption peaks at 300 nm and 400 nm. With this information and the fluorescence anisotropy [51] of this molecule we were able to identify the wavelengths corresponding to the \(S_0 \rightarrow S_1\) and the \(S_0 \rightarrow S_2\) transitions, as indicated in Figure 6 (dotted arrows). The linear emission spectrum shows a maximum value of fluorescence intensity at 445 nm, and emission light is visible to the naked eye.
Figure 7: (a) Linear absorption and emission spectra for 2 in hexane; (b) band structure for the symmetric compound 2 (D-\(\pi\)-D)

In a similar way we measured the linear absorption and emission spectra and proposed a band structure for the symmetric compound D-\(\pi\)-D (9,9-di-decyl-2,7-bis-(N,N-diphenylamino)fluorene) as shown in the Figure 7. It was found that the transition of this compound to the first excited state \(S_1\) of this compound corresponds to a wavelength of 370 nm and the second excited state corresponds to 320 nm. The maximum of fluorescence intensity was centered on 412 nm.

In the Figure 8, it is shown the linear absorption and emission spectra of the A-\(\pi\)-A compound (2,7-bisbenzothiazole-9,9-didecylfluorene), as well as its molecular and band structure.
Figure 8: (a) Linear absorption and emission of A-π-A in hexane (10^{-5} M); (b) Band structure of the symmetric compound A-π-A. The inset of 5 (a) represents the molecular structure of A-π-A fluorene derivative.

The symmetric compound A-π-A is a fluorescent probe which exhibits a high molar extinction coefficient at 365 nm (Figure 8 (a)), fluorescence with emission maximum at 417 nm (Figure 5 (b)), high quantum yield (Q_f = 0.91 ±0.05) and a maximum degenerate two-photon absorption cross-section σ_2' = 420 GM at 600 nm for the S_0 → S_2 transition [50]. For the asymmetric compound D-π-A, σ_2' = 72 GM at 770 nm and for the symmetric molecule D-π-D, σ_2' = 89 GM at 620 nm.[16] The positions of the S_0 → S_1 and S_0 → S_2 transitions were determined in all cases by fluorescence anisotropy [51,52]. The highly molecular symmetry of 2,7-bisbenzothiazole-9,9-didecylfluorene decreases the probability of transitions forbidden by quantum mechanics selection rules. However, because the benzothiazole groups are in the
positions 2 and 7, this molecule do not posses a center of symmetry, improving its excitation tunability from 300 nm to 1200 nm as shown later.

2.2. Multiphoton Induced Fluorescence Measurements

The multiphoton excitation of compounds 1, 2 and 3 in hexane solution, at a concentration of $10^{-2}$ M, was induced with a tunable OPG pumped by the third harmonic of a Mode-Locked, 25 ps full-width at half-maximum (FWHM), Nd-YAG laser (EKSPLA), operating at 10 Hz repetition rate. The experimental setup for measuring the multiphoton absorption-induced upconverted fluorescence is presented in Figure 9. The excitation beam was spatially filtered using two lenses of 250 mm EFL (L1 and L2) and a diamond pinhole (PH). The 100 mm focal length lens L3 was focusing the beam into the 10 mm path length quartz cell containing the dye solution. The fluorescence spectra were measured with a high performance JY-TRIAX 550 spectrophotometer. The fluorescence light was collected with an aspheric lens L4, having a 2 inch diameter and 5 cm focal length, and focused into the entrance slit of the spectrometer. The TRIAX spectrometer is equipped with a Spectrum One CCD array head, which uses liquid Nitrogen for cooling system to reduce temperature and therefore, dark current (< 1 electron/pixel/hour). The Spectrum One CCD system allows detecting very low signal levels such as Raman, fluorescence, and absorption spectroscopy. The Quantum efficiency of this system is 85% at 550 nm. Also, TRIAX 550 has a very high resolution, which depends on the grating selected. For example, if the grating has 1200 grooves/mm, the resolution is 0.025 nm for the scan parameters of 253.65 nm (wavelength) and 100 ms (integration time). The spectrometer
and CCD system are controlled by computer using the SpectraMax for Windows data acquisition and data manipulation program.

Figure 9: Experimental setup for multiphoton absorption induced fluorescence. The excitation wavelength was changed between 300 nm and 1200 nm to allow both processes linear and nonlinear absorption induced fluorescence to be studied.

One- two- and three-photon absorption induced fluorescence spectra were measured for each fluorene derivative. Figure 10 shows the fluorescence spectra of 2,7-bisbenzothiazole-9,9-didecylfluorene (A-π-A) in hexane solution at a concentration of $1 \times 10^{-2}$ M, pumping at 365, 600 and 1095 nm. These wavelengths correspond to the maximum of the emission band for one-, two- and three-photon excitation [51].

The shapes of the three emission spectra are nearly identical, with a maximum at approximately 417 nm. This result demonstrated that the radiative relaxation processes take
place from the same final excited state ($S_1$), independent of the excitation wavelength.[55] Because of the high concentration needed to induce three-photon absorption, an apparent red-shift was observed. This is a consequence of the re-absorption due to the small Stoke’s shift of the A-π-A molecule in hexane solution (see Figure 8).

Figure 10: One- two- and three-photon absorption induced fluorescence spectra for the A-π-A compound. The pumping wavelength was 365 nm (1PA), 600 nm (2PA) and 1095 nm (3PA).
The fluorescence emission spectrum was also measured, tuning the pumping wavelength from 300 nm to 1200 nm, changing the wavelength by ca. 30 nm per step. The shape of all spectra was similar to those displayed in Figure 10.

In order to corroborate the order of the absorption process (OAP) at different excitation wavelengths from 300 nm to 1200 nm, the emission intensity versus incident intensity was measured. Figure 11 shows the order of the absorption process versus wavelength.

![Graph showing the order of the absorption process vs. input wavelength of A-π-A in hexane solution at a concentration [A-π-A] = 1×10^{-2} M.](image)

Figure 11: Order of the absorption process vs. input wavelength of A-π-A in hexane solution at a concentration [A-π-A] = 1×10^{-2} M.
The plot displayed in Figure 11 reveals five different spectral regions that should be analyzed separately. Also, a Jablonski diagram depicting the different transitions for 2,7-bisbenzothiazol-9,9-didecyfluorene is shown in the top-left corner of Figure 11. The number of arrows represents the number of photons involved in each transition. In the region from 300 nm to 420 nm, a first-order dependence of emission intensity versus incident intensity was observed. All transitions in this region correspond to one-photon absorption (1PA) between the ground state $S_0$ and the first two excited states, $S_1$ and $S_2$.

The quadratic dependence from 600 nm to 800 nm validated two-photon absorption (2PA) for the $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_1$ transitions previously reported by Belfield et al. [51]. They demonstrated that the maximum 2PA cross-section for this compound is found at 600 nm, corresponding to the highest 2PA probability for the $S_0 \rightarrow S_2$ transition. An intermediate order was observed between ca. 460 nm and 560 nm, a region where 1PA between $S_0$ and $S_1$ can compete with 2PA between $S_0$ and $S_2$, and $S_0$ and higher excited states $S_n$. According to quantum mechanics rules, 1PA has a greater probability to occur between states with different parities while in 2PA the probability is greater when the transition takes place between states that possess equal parities. Therefore, at shorter wavelengths, where the probability of the $S_0 \rightarrow S_1$ transition via single photon is much greater than 2PA between $S_0 \rightarrow S_n$, the OAP is closer to one. At longer wavelengths where the linear absorption of the chromophore is very weak and 2PA between $S_0 \rightarrow S_2$ is significantly high, 2PA dominates and the OAP becomes closer to the second-order.

From 1030 to 1200 nm the best fit predicts a cubic dependence, a characteristic of 3PA process that falls within the $S_0 \rightarrow S_1$ transition band. A maximum of the fluorescence emission intensity at 1095 nm was observed, indicating a greater probability of 3PA at this wavelength. Since picosecond pulses were employed, and there was no intermediate state between ground
state $S_0$, and the first excited state $S_1$, one can be certain that this excitation process was induced purely by the simultaneous absorption of three photons.

Finally, in the spectral region from ca. 860 nm to 1010 nm one can see again the intermediate orders of absorption. Near 860 nm 2PA between $S_0$ and $S_1$ dominates over 3PA for the $S_0 \rightarrow S_2$ transition. Although both transitions are, in principle, forbidden by selection rules, a lower intensity to induce 2PA is required, favoring it. At 900 nm one can see a maximum value of OAP within this spectral region. At this wavelength 3PA between $S_0$ and $S_2$ and 2PA for the $S_0 \rightarrow S_1$ transition compete with each other. Again a similar situation was observed where both transitions are, in principle, forbidden by the selection rules and the demands of intensity favor 2PA. At 1010 nm a drop in OAP is observed as a consequence of the competition between weak 2PA at the longer wavelength side of the absorption spectrum and weak 3PA on the shorter wavelength side within the electronic transition band $S_0 \rightarrow S_1$. Though 3PA should dominate according to the selection rules, 2PA has a more significant contribution due to its modest demand of intensity, thus OAP $\approx$ 2.3.

In a similar way, multiphoton induced fluorescence measurements were done for the symmetric D-$\pi$-D compound. Figure 12 shows the fluorescence spectra of 9,9-di-decyl-2,7-bis-(N,N-diphenylamino)fluorene, using 370 nm, 640 nm and 1110 nm excitation wavelengths which correspond to one-, two- and, three-photon absorption, respectively. The solvent used to dissolve this fluorene derivative was hexane and the concentration of solution was $10^{-2}$ M. The fluorescence spectra of D-$\pi$-D have an identical shape and the emission peak is located at 412 nm. One can observe that the noise level is increased in the last two spectra. This can be explain by the fact that two- and three-photon induced fluorescence signal is much weaker than linear
fluorescence signal, therefore, we have to make the spectrometer entrance wider to increase the signal, which leads to a lower signal to noise ratio (SNR).

Figure 12: One- two- and three-photon induced fluorescence spectra of 9,9-di-decyl-2,7-bis-(N,N- diphenylamino)fluorene (D-π-D). The excitation wavelengths were 370 nm, 640 nm and, respectively, 1110 nm.

For different excitation wavelengths, between 300 nm and 1600 nm, we determined the order of absorption process. Figure 13 displays the order of the absorption process versus wavelength for D-π-D molecule. It is important to note that we were able to observe even four-photon induced fluorescence using an excitation wavelength of 1280 nm and 1480 nm. For an
excitation wavelength of 1600 nm, there is a competition between four and five-photon absorption. Therefore, the order of absorption process is between 4 and 5, more exactly 4.5. In the spectral range 100-1200 nm we observed experimentally three-photon absorption for the transitions $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$. This was confirmed by the order of absorption process, which was close to 3, as shown in Figure 13.

![Figure 13: Order of the absorption process for the D-\pi-D molecule](image)

Quantum mechanics dictates that in centrosymmetric molecules, multiphoton absorption processes respect an even-parity selection rule for two (2PA) and four-photon absorption (4PA)
and an odd-parity for one- (1PA) and three-photon absorption (3PA). In asymmetric molecules more relaxed selection rules allow forbidden transitions to occur with higher probability. This effect could introduce multiphoton absorption tunability over a wide range of wavelengths. For the asymmetric dye with D-\(\pi\)-A structural motif, the one- two- and three-photon induced fluorescence spectra are shown in Figure 14.

![Figure 14: One- two- and three-photon absorption induced fluorescence spectra for the D-\(\pi\)-A compound](image-url)
The pumping wavelength was 400 nm (1PA), 600 nm (2PA) and, 1200 nm (3PA), respectively. The emission peak corresponds to a wavelength of 445 nm. We did the same measurements to determine the order of absorption versus wavelength for D-π-A molecule. Figure 15 shows the OAP. A remarkable feature of 7-benzothiazol-2-yl-9,9-didecylfluoren-2-yl)diphenylamine is the fact that fluorescence emission can be induced at any wavelength from at least 300 nm to 1600 nm through different absorption processes. This is the result of the overlapping between $S_1$ and $S_2$, and the lack of molecular symmetry of D-π-A compound, which imposes less severe selection rules.

Figure 15: Order of absorption process versus excitation wavelength for D-π-A compound. The solid line makes separation between different multiphoton absorption processes.
The strong emission observed provides compelling evidence of high absorption cross-sections for different multiphoton excitation processes in all three molecules as a direct consequence of the strong intramolecular charge transfer and the \( \pi \)-conjugation length.[55-57] The shape of all spectra were nearly identical in each compound, indicating that the emission process takes place from the same first excited state (\( S_1 \)), independent of the excitation wavelength for all three compounds.

Figure 16: Fluorescence Intensity versus Input Intensity for each compound. The solid lines represents the theoretical fittings using the function \( y=a*x^n \). For the asymmetric compound the best-fit parameter \( n \) was 2.85, and for the symmetric 2.92, and 2.95, respectively
Measurements of the fluorescence intensity versus input intensity in compound D-\(\pi\)-A demonstrated 3PA at 1200 nm corresponding to the \(S_0 \rightarrow S_1\) transition, \([56]\) while in compound D-\(\pi\)-D and A-\(\pi\)-A similar measurements were performed, providing an order three for the transition \(S_0 \rightarrow S_1\) at 1110 nm and 1095 nm, respectively. In Figure 16 are displayed both the experimental curves and the theoretical fittings for each of the three compounds. The function \(f(x) = a \cdot x^n\) used to fit the experimental curves provided the best-fit parameters \(n = 2.85\) (D-\(\pi\)-A compound), \(n = 2.92\) (A-\(\pi\)-A), and \(n = 2.95\) (D-\(\pi\)-D), which indicates a three-photon absorption for the \(S_0 \rightarrow S_1\) transition. Higher order nonlinear absorption processes such as four-photon absorption were also observed for these fluorene derivatives.

Figure 17 (a) shows the experimental curves for three- and four-photon induced fluorescence as function of input energy, represented in log-log scale. The solid lines represent the best-fit and for an excitation wavelength of 1600 nm the slope is 4.15.\([56]\) This result indicates the simultaneous absorption of four photons for the excitation of D-\(\pi\)-A in hexane, from its ground state \(S_0\) to its first excited state \(S_1\). To determine the 4PA cross-section for this transition, open aperture Z-Scan curves were performed at two different excitation energies, 380 nJ and 400 nJ, with OPG-OPA-generated femtosecond pulses, pumped by a Clark-MXR-2001. After fitting the experimental curves \([56]\), the corresponding 4PA coefficient \(\alpha_4 = (2.5 \pm 0.3) \cdot 10^{-33}\) \(\text{cm}^5 \cdot \text{GW}^{-3}\) was obtained. Relating this coefficient with the solute concentration \(d_0\) (\(\text{mol} \cdot \text{L}^{-1}\)) and the photon energy \(\hbar(c/\lambda)\), the 4PA cross-section of NPH2Bz was estimated to be \(\sigma_4 = 8.1 \cdot 10^{-109}\) \(\text{cm}^8 \cdot \text{s}^3 \cdot \text{photon}^{-3}\).\([56]\) We thank Dr. Van Stryland and Dr. Hagan for kindly providing the femtosecond laser for the Z-Scan experiments.
Figure 17: Fluorescence Intensity versus Input Intensity induced at 1200 and 1600 nm (a) and open aperture Z-Scan curves for D-π-A/hexane pumping at 1600 nm (b)
2.3. Computer Simulation of Multiphoton Absorption Processes

To investigate the absorption of third- and higher-order nonlinearity, one of the most used methods is the well-known open-aperture Z-Scan technique [58]. In this method, the transmittance of a nonlinear sample is measured in the far-field as the sample is moved through the focus along the z-axis of a focused Gaussian beam. To simulate on computer the nonlinear absorption processes, we derived the transmittance equations for three- and four-photon absorption. In order to obtain the three-photon absorption coefficient \( a_3 \) we first derived the equation for nonlinear transmittance that allows us to fit the experimental Z-scan curves for 3PA. Equation (10) from Chapter 1 gives the change in irradiance with depth in the sample for three-photon absorption. Neglecting the linear absorption, equation (10) has the following solution:

\[
I(L,r,t) = \frac{I(z = 0,r,t)}{\sqrt{1 + 2a_3LI^2(z = 0,r,t)}} \tag{22}
\]

where \( L \) is the sample thickness, \( I(z=0,r,t) \) is the irradiance at the front side of the sample and \( I(L,r,t) \) is the irradiance at the back surface of the sample. To take into account the temporal and spatial Gaussian profile of our laser beam, equation (22) has to be integrated over time and space and rearranged to obtain the normalized energy transmittance. The final equation for the normalized transmittance is expressed in terms of the functions \( X_3(x,Z) \) and \( c_3(Z) \): [55]

\[
T_{3PA}(Z) = \frac{1}{\sqrt{\pi} \cdot c_3(Z)} \int_0^1 \frac{dx}{x \cdot \sqrt{-\ln(x)}} X_3(x,Z) \tag{23}
\]
where

\[ X_3(x, Z) = \ln \left\{ c_3(Z) \cdot x + \sqrt{1 + c_3(Z)^2 x^2} \right\} \]  \hspace{1cm} (24)

\[ c_3(Z) = \sqrt{2\alpha_4 I_0(Z)^2 L} \]  \hspace{1cm} (25)

In equation (20), L is the distance traveled by the beam through the sample. The peak value of the incident irradiance \( I_0(Z) \) at \( z=0 \) can be estimated, for different cell positions, using

\[ w(Z) = w_0\left[1 + \left(Z^2 / Z_0^2\right)\right]^{1/2}, \]

which yields \( I_0(Z) = 2E_0 / \pi \sqrt{\pi} t_p w(Z)^2 \) [10]. \( E_0 \) is the pulse energy, \( t_p \) is the laser pulsewidth (FWHM), \( w_0 \) is the beam waist (HW1/e^2M), \( w(Z) \) is the beam size at any position, \( Z_0 \) is the confocal parameter and \( Z \) is the cell position with respect to the focal plane.

Considering pure four-photon absorption (4PA), the beam attenuation due to 4PA, neglecting the linear absorption is given by:[56]

\[ \frac{dI(z, r, t)}{dz} = -\alpha_4 I(z, r, t)^4 \]  \hspace{1cm} (26)

where \( \alpha_4 \) is the 4PA coefficient of the sample at the wavelength of the incident radiation. The solution of Equation (21) is given by:

\[ I(z, r, t) = \frac{I(z = 0, r, t)}{\left[1 + 3\alpha_4 z I^3(z = 0, r, t)\right]^5} \]  \hspace{1cm} (27)
To take into account the temporal and spatial Gaussian profile of our laser beam, one needs to integrate Eq. (27) over radial and temporal variables \(r\) and \(t\). After integration and rearrangement of terms, one can obtain the normalized energy transmittance as function of \(X_4(x)\) and \(R(\Delta)\) [56]:

\[
T_{4PA}(L) = \frac{1}{\sqrt{\pi} \sqrt{3 \alpha_4 LI^3 (z = 0)}} \int_0^1 \frac{1}{x \sqrt{-\ln(x)}} \left[ \ln[X_4(x)] + R(\Delta) \right] dx \tag{28}
\]

where

\[
X_4(x) = \ln \left\{ x \left[ 3 \alpha_4 LI^3 (z = 0) \right]^{\frac{1}{3}} + \left[ 3 \alpha_4 LI^3 (z = 0) x^3 + 1 \right]^{\frac{1}{3}} \right\} \tag{28a}
\]

\[
R(\Delta) = \ln \left\{ \frac{1 + \Delta(x) + \Delta(x)^2} {1 + \Delta(x)} \right\} + \frac{1}{\sqrt{3}} \arctan \left[ \frac{2 \Delta(x) + 1} {\sqrt{3}} \right] - \frac{\pi} {6\sqrt{3}} \tag{28b}
\]

\[
\Delta(x) = \frac{1} {1 + \left[ 3 \alpha_4 LI^3 (z = 0) x^3 \right]^{\frac{1}{3}}} \tag{28c}
\]

Using equations (18) and (23) for the nonlinear transmittance due to three- and four-photon absorption, and also equations given in [58] for two-photon absorption we were able to simulate the theoretical curves for those nonlinear process. Figure 18 shows the nonlinear transmittance versus distance (in centimeters) along \(z\) axis for each of the three nonlinear absorption processes (2PA, 3PA, and 4PA).

The values of multi-photon absorption coefficients used for simulation were: \(\alpha_2 = 0.285\) cm/GW, \(\alpha_3 = 0.042\) cm\(^3\)/GW\(^2\), and respectively \(\alpha_4 = 0.0057\) cm\(^5\)/GW\(^3\).
In the simulation shown in the Figure 18, we assumed a nonlinear sample of 1 mm thickness, a pulsewidth of 25 ps (FWHM) and 1200 nm (wavelength) laser beam focused by a lens of 10 cm EFL. The beam waist was 13 microns and its irradiance at the focal plane was 17 GW/cm$^2$. As the order of multi-photon absorption increases, the absorption curves become narrower and steeper, thus the excitation is more confined around the focal point. This characteristic is very useful for bioimaging and medical applications such as photodynamic therapy because the spatial resolution is increased and the absorption can be confined in a small volume.

As we can see from the plot in Figure 18, one can easily distinguish between the 2PA (red line) and 3PA (blue line) curves, the former being much wider. However, it is more difficult to distinguish between three- and four-photon absorption because the difference between the two of them is not so obvious. To differentiate them, one should measure the fluorescence emission intensity versus input intensity and determine what kind of dependence exists between one and another. If one finds a cubic dependence, then the process is three-photon absorption, and for a fourth order dependence the process is 4PA.
2.4. The Effect of Molecular Symmetry on Three-photon Absorption Cross-Section

We measured the three-photon absorption coefficients of D-π-A, D-π-D, and A-π-A compounds in hexane, at an excitation wavelength of 1200 nm, 1110 nm, and 1095 nm, respectively, which corresponded to the 3PA peak. The experimental setup is based on open-aperture Z-scan technique [58] and is depicted in Figure 19.
Degenerate three-photon absorption measurements in solution were performed for each dye/solvent solution using the output beam of the optical parametric generator (OPG) system. The fluorene derivative/hexane solution at a concentration of ca. $10^{-2}$ M was contained in a 1-mm-path cell which was placed on a 3-axis micrometer stage controlled by a stepper motor. The transmittance was recorded using a data acquisition system controlled by computer. Because the excitation wavelength was within Near-IR spectral domain, two germanium detectors were employed: D1-as signal detector and D2-as reference detector. After spatial filtering [lenses L1, L2, and the pinhole (PH)], the laser beam was directed toward the sample by the silver mirrors M1 and M2. Lenses L3 and L4 form together a beam expander, which doubles the beam size from 5 to 10 mm (FW1/e²M). A beamsplitter BS was used to separate the laser beam into two.
beams, a weak beam for the reference and a strong focused beam for the signal. Lenses L7 and L8 with an effective focal length of 10 cm were employed to focus the laser beams into detectors D1 and, respectively D2.

Using the setup shown in Figure 19 we measured the nonlinear transmittance of each fluorene derivative in hexane solution at a concentration ca. $9.8 \times 10^{-3}$ M. To estimate the three-photon absorption coefficients, each Z-scan curve was fitted using the basic theoretical considerations for three-photon absorption equations for changes in transmittance, explained in section 2.3. (Equation 23). Figure 20 shows both, the experimental and theoretical Z-scan curves. The solid lines are the best theoretical fitting with the parameters given by the three-photon absorption coefficients. The corresponding coefficients $\alpha_3 = (8.9\pm0.8) \times 10^{-21}$ cm$^3$W$^{-2}$ for D-$\pi$-A, $\alpha_3 = (15.2\pm2.3) \times 10^{-21}$ cm$^3$W$^{-2}$ for D-$\pi$-D, and $\alpha_3 = (5.2\pm0.3) \times 10^{-20}$ cm$^3$W$^{-2}$ for A-$\pi$-A molecule. The measurements were done using the excitation wavelength corresponding to the peak of three-photon absorption and also three different energies for each compound.
Then, we correlated the three-photon absorption coefficient $\alpha_3$ with the three-photon absorption cross-section $\sigma_3'$ by using the following formula [59]:

Figure 20: Open aperture Z-scan for D-\(\pi\)-A (A), D-\(\pi\)-D (B) and A-\(\pi\)-A (C) in hexane solution at 0.0098 M. The solid lines are the theoretical best-fitting curves using three-photon absorption equations for change in transmittance.
\[
\sigma_3' = \frac{\alpha_3}{N_A d_0} \frac{(hc)^2}{10^{-3}}
\] (29)

Here \(N_A\) is the Avogadro’s number, \(d_0\) (mol/L) is the solution concentration and \(hc/\lambda\) is the energy of the incident photons. Using equation (29), we obtained the following values for the three-photon absorption cross-section: \(\sigma_3' = 37 \cdot 10^{-78} \text{cm}^6\cdot\text{s}^2\cdot\text{photon}^{-2}\) for \(D-\pi-A\) pumping at 1200 nm [55], \(\sigma_3' = 82 \cdot 10^{-78} \text{cm}^6\cdot\text{s}^2\cdot\text{photon}^{-2}\) for \(D-\pi-D\) pumping at 1110 nm [55], and \(\sigma_3' = 335 \cdot 10^{-78} \text{cm}^6\cdot\text{s}^2\cdot\text{photon}^{-2}\) for \(A-\pi-A\) compound pumping at 1095 nm [57]. The large \(\sigma_3'\) for each fluorene derivatives is the outcome of the large \(\pi\)-conjugation and electron delocalization along the molecular systems. The \(\sigma_3'\) for the symmetric molecule \(D-\pi-D\) is 2.2 times greater than \(\sigma_3'\) for the asymmetric homologous. This result indicates that symmetric intramolecular charge transfer from the amino groups to the conjugated core in the \(D-\pi-D\) molecule enhances 3PA in these molecules. Therefore, the charge redistribution, and consequently the magnitude of the transition dipole moment in the bis-donor conjugated compound is more important than in the asymmetric fluorene derivative with moderately strong donor-acceptor groups. This is in agreement with Bublitz and co-workers [60], where they demonstrated, that in push-pull molecules, the change in dipole moment between the ground and first electronic excited state decreases with the strength of the donor and acceptor groups. Furthermore, the fact that the 3PA cross-section of \(A-\pi-A\) is the highest demonstrate that symmetrical charge transfer from the center to the periphery in \(A-\pi-A\) fluorene derivative enhances the three-photon absorption cross-section of this family of molecules. Semi-empirical calculations of the transition dipole moment between the ground state and the first excited state
using ZINDO/S support our experimental results.[53] These extraordinary results show that intramolecular charge transfer plays a crucial role on three-photon absorption cross-section enhancement in this class of organic molecules. On the other hand, molecular asymmetry contributes positively to the tunability of multiphoton absorption since it increases the probability of forbidden transitions to take place.

2.5. The Effect of $\pi$-Electron Conjugation Length on 3PA Cross-Section

The highly functionality of the fluorene aromatic ring allows more core ability to introduce particular functionality through molecular engineering on positions 2 and 7. As a result, using fluorene core one has the ability to generate different chemical archetypes by affecting the electron accepting and donating properties of the system as well as the possibility of extending the $\pi$-electron conjugation length. In this subchapter we present the second part of our structure-property systematic study given by the effect of the $\pi$-electron conjugation length on the three-photon absorption cross-section of this family of fluorine derivatives. The $\pi$-electron conjugation length of each compound was extended by inserting two phenylene-vinylene groups, one on each side of the fluorenyl core in positions 2 and 7 [51]. The three-photon absorption cross-sections of these three different fluorene derivatives, with extended $\pi$-electron conjugation lengths was experimentally measured and compared with their shorter $\pi$-electron conjugation lengths matching part. The three fluorene derivatives, which have three different structural motifs, D-$\pi$-$\pi$-$\pi$-A (4-((E)-2-(7-(4-(benzo[d]thiazol-2-yl)styryl)-9,9-didecyl-9H-fluoren-2-yl)vinyl)-N,N-dibutylaniline, D-$\pi$-$\pi$-$\pi$-D (7-diyl)bis(ethene-2,1-diyl)bis(4,1-phenylene))dibenzo[d]thiazole) and
A-\pi-\pi-A \quad (2,2'-(4,4'-(1E,1'E)-2,2'-(9,9-didecyl-9H-fluorene-2,7-diyl)bis(ethene-2,1-diyl)bis(4,1-phenylene))dibenzo [d]thiazole) are characterized by a longer distance over which the electric charge can be transferred between donor and acceptor groups via a conjugated bridge or, from the ends of the molecule to the conjugated core and vice versa. Their molecular structure is presented in Figure 21. The increase in the \pi-electron conjugation length leads to a stronger \pi-electron delocalization. Therefore, an enhancement of the imaginary part of the fifth-order hyperpolarizability $\chi^5(-\omega;\omega,-\omega,\omega,-\omega,\omega)$, i.e. the three-photon absorption cross-section, was anticipated.

![Molecular structure of A-\pi-\pi-A (1'), D-\pi-\pi-D (2') and D-\pi-\pi-A (3') compounds.](image)

Figure 21: Molecular structure of A-\pi-\pi-A (1'), D-\pi-\pi-D (2') and D-\pi-\pi-A (3') compounds.

The first compound, A-\pi-\pi-A (1'), is symmetric and, consists of a fluorinyl core, two phenylene-vinylene groups attached on it and one acceptor group (benzothiazole) at each of the
two extremities. The second compound, D-\(\pi-\pi-\pi\)-D (2’), is also symmetric, but the end groups are electron donors (NC\(_8\)H\(_{18}\)). The last compound, D-\(\pi-\pi-\pi\)-A (3’), is an asymmetric molecule with a benzothiazole group on one side and a NC\(_8\)H\(_{18}\) group on the other. The detailed synthesis of the symmetric compound A-\(\pi-\pi-\pi\)-A (1’) is described in the reference [51].

The absorption spectra of the organic dyes were measured using an Agilent 8453 diode array UV-vis spectrometer (cell effects and solvent contributions were properly subtracted). To carry out these measurements, a 1-mm-path quartz cell filled with a THF solution, at a concentration of 10\(^{-5}\) M on the targeted compound, was employed. The linear emission spectral measurements were carried out using a JY-TRIAX 550 Spectrophotometer exciting at 420 nm.

Figure 22 displays the linear absorption and emission spectra of each fluorene derivative studied. Each compound shows strong linear absorption in the spectral range of 300-500 nm, with absorption maximum at 410 nm, 415 nm and 425 nm, for A-\(\pi-\pi-\pi\)-A, D-\(\pi-\pi-\pi\)-D and D-\(\pi-\pi-\pi\)-A, respectively. These wavelengths corresponds to the transition between ground state and first excited state \(S_0 \rightarrow S_1\).[51] Also, one can observe that above 500 nm there is no linear absorption for any of these three molecules. The one-photon fluorescence spectrum of each compound is shown in Figure 22 by square symbols. The fluorescence band shows a peak wavelength at 489 nm, 500 nm and 596 nm, for the A-\(\pi-\pi-\pi\)-A, D-\(\pi-\pi-\pi\)-D and D-\(\pi-\pi-\pi\)-A, respectively.
Figure 22: Linear absorption (triangles) and emission (squares) spectra. The linear absorption spectra were measured in dye/THF solution at a concentration of $10^{-5} \text{ M}$. 
The linear absorption spectra were measured in dye/THF solution at a concentration of $10^{-5}$ M using an Agilent 8453 diode array spectrometer. Linear emission was recorded with a high performance JY-TRIAX 550 spectrometer using a more concentrated solution ($10^{-3}$ M). The symmetric compounds present a Stoke’s shift smaller than that of the asymmetric, as expected, due to only a small change in the molecular dipole moment upon electronic excitation. [51]

![Normalized Fluorescence Intensity vs Wavelength](image)

**Figure 23:** Three-photon absorption induced fluorescence spectra of $A-\pi-\pi-\pi-A$ (open squares), $D-\pi-\pi-\pi-D$ (filled squares) and $D-\pi-\pi-\pi-A$ (semi-open squares) pumping at 1230 nm, 1280 nm and 1260 nm, respectively.
Using the same experimental setup described in section 2.2., we measured the three-photon induced fluorescence in all three fluorene derivatives with the extended $\pi$-electron conjugation length (1’, 2’, and 3’). Figure 23 shows the 3PA induced fluorescence spectra of A-$\pi$-$\pi$-$\pi$-A, D-$\pi$-$\pi$-$\pi$-D and D-$\pi$-$\pi$-$\pi$-A, pumping at excitation wavelengths of 1230 nm, 1280 nm and 1260 nm, respectively. The results indicate maximum emission intensity at 489 nm, 502 nm and 596 nm corresponding to A-$\pi$-$\pi$-$\pi$-A, D-$\pi$-$\pi$-$\pi$-D and D-$\pi$-$\pi$-$\pi$-A, in that order. Virtually identical spectra were obtained for all three compounds (see Fig. 22) using single-photon excitation. This indicates that the final excited state is independent of the excitation wavelength, and the excitation takes place between the ground state $S_0$ and first excited state $S_1$. Because, there is no intermediate real state between $S_0$ and $S_1$ and, these compounds do not present linear absorption at excitation wavelengths above 500 nm, the measured nonlinear process can be certainly attributed to pure three-photon absorption.

Using the well known open-aperture Z-scan technique [58], we measured the three-photon absorption coefficient of each compound at different wavelengths, tuning the excitation wavelength within the $S_0 \rightarrow S_1$ band, over a range of 100 nm. We obtained a peak of 3PA coefficient for the A-$\pi$-$\pi$-$\pi$-A, D-$\pi$-$\pi$-$\pi$-D and D-$\pi$-$\pi$-$\pi$-A molecules pumping at 1230 nm, 1280 nm and 1260 nm, respectively. For all Z-scan measurements we used a 1-mm-pathlength cell, filled with the corresponding organic dye in a THF solution at a concentration of $10^{-3}$ M. Typical irradiiances above 45 GW/cm$^2$ were employed for 3PA measurements. Figure 24 shows both, the experimental Z-scan curves given by points and the theoretical fitting curves represented by solid lines. To fit the experimental curves we employed the equations for three-photon absorption process for a temporal and spatial Gaussian profile, which give the nonlinear transmittance relation expressed in Equation (23). For each of the three compounds, the three-photon
absorption coefficient was measured employing three different input energies. The relative error of the absorption coefficient measurements was approximately 14%. The corresponding 3PA coefficients were $\alpha_3 = (5.5\pm0.8)\times10^{-21}$ cm$^3$W$^{-2}$ for D-π-π-A pumping at 1260nm, $\alpha_3 = (3.9\pm0.5)\times10^{-21}$ cm$^3$W$^{-2}$ for D-π-π-D pumping at 1280 nm and $\alpha_3 = (1.18\pm0.16)\times10^{-20}$ cm$^3$W$^{-2}$ for A-π-π-π-A molecule pumping at 1230 nm.

Figure 24: Open-aperture Z-scan curves of D-π-π-π-A, D-π-π-π-D and A-π-π-π-A in THF solution (10^{-3} M), taken at three different energies for each compound. Solid curves are the best theoretical fittings using Gaussian beam equations for 3PA.
Measurements were done using a laser beam with the size at the focal plane (beam waist) of ca. $14.25 \, \mu\text{m}$, $14.55 \, \mu\text{m}$ and $13.75 \, \mu\text{m}$ (HW1/e²M) at $\lambda_p = 1260\text{nm}$, $1280\text{nm}$, and $1230\text{nm}$, respectively. Using equation (24), we obtained the following values for the three-photon absorption cross-sections: $511 \times 10^{-78} \, \text{cm}^6\text{s}^2/\text{photon}^2$, $145 \times 10^{-78} \, \text{cm}^6\text{s}^2/\text{photon}^2$ and $245 \times 10^{-78} \, \text{cm}^6\text{s}^2/\text{photon}^2$ for $\text{A-}\pi\text{-}\pi\text{-}\pi\text{-A}$, $\text{D-}\pi\text{-}\pi\text{-}\pi\text{-D}$ and $\text{D-}\pi\text{-}\pi\text{-}\pi\text{-A}$, respectively. These values indicate that $\sigma_3'$ is enhanced by a factor of 1.6, 1.8 and 6.6 with respect to the corresponding shorter $\pi$–electron conjugation length molecules $\text{A-}\pi\text{-A}$, $\text{D-}\pi\text{-D}$ and $\text{D-}\pi\text{-A}$, respectively (see Table 1).

In Table 1 we present the 3PA cross-sections corresponding to both longer and shorter $\pi$–electron conjugation length compounds and the excitation wavelengths.

Table 1: Three-photon absorption cross-sections and the corresponding excitation wavelengths for all compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>D-\pi-A</th>
<th>D-\pi-D</th>
<th>A-\pi-A</th>
<th>D-\pi-\pi-A</th>
<th>D-\pi-\pi-D</th>
<th>A-\pi-\pi-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation wavelength</td>
<td>1200nm</td>
<td>1110nm</td>
<td>1095nm</td>
<td>1260nm</td>
<td>1280nm</td>
<td>1230nm</td>
</tr>
<tr>
<td>$\sigma_3'$ [\text{cm}^6\text{s}^2/\text{photon}^2]</td>
<td>37$^a$</td>
<td>82$^a$</td>
<td>335$^b$</td>
<td>245</td>
<td>145</td>
<td>511</td>
</tr>
</tbody>
</table>

The increase on $\pi$–electron conjugated bridge length by inserting phenylene-vinylene groups, leads to an extension of the distance over which the electric charge can be transferred and, therefore, an increase of the $\pi$-electron delocalization in the first excited state $S_1$. This electronic delocalization enhances the $S_0 \rightarrow S_1$ transition dipole moment, which makes an important contribution to the 3PA cross-section.[57]
In order to confirm the three-photon absorption process, we used an alternative method to measure the three-photon absorption cross-section, so called the intensity-dependent transmittance measurements. For this, we measured the transmitted intensity versus incident intensity holding the sample at the focal point of the focusing lens L5 (see Figure 18). The laser beam was separated into two beams (using a beam splitter), one weak for the reference and a strong focused beam for the signal. Because NIR radiation was employed, Germanium Photodiode detectors were used. The incident intensity was gradually increased from 5 GW/cm² up to 90 GW/cm² using neutral density filters. The excitation wavelength was tuned from 1230 nm to 1260 nm and, 1280 nm, corresponding to the peak of 3PA cross-section of each compound, as determined by Z-scan measurements. Figure 25 shows the transmitted intensity versus input intensity curves for each of the three fluorene derivatives molecules. Equation (22) can be numerically solved for $\alpha_3$, thus the cross-section can be determined. The excitation wavelengths in each case were: 1260 nm (a), 1280 nm (b) and 1230 nm (c). The dashed lines are the fitting curves with parameter $\alpha_3 = 0$. The solid lines represent the theoretical fittings with the best-fit parameter $\alpha_3 = 2.5 \times 10^{-21}$ cm³W⁻², $\alpha_3 = 1.32 \times 10^{-21}$ cm³W⁻², and $\alpha_3 = 1.18 \times 10^{-21}$ cm³W⁻² for A-π-π-π-A, D-π-π-π-D and D-π-π-π-A, respectively. These values are of the same order of magnitude as the values obtained using Z-scan. However, they are 3 to 4 times smaller. The discrepancy between the two methods is given by the fact that the theoretical fitting for the second method assume a square temporal profile instead of Gaussian profile.
Figure 25: Transmitted Intensity vs. Input Intensity curves of compound 3’ (a), 2’ (b), and 1’ (c).
In summary, we have demonstrated an enhancement of the three-photon absorption cross-section of both symmetric and asymmetric fluorene derivatives by extending the $\pi$-electron conjugation length of this family of molecules. The asymmetric compound D-$\pi$-$\pi$-$\pi$-A presented the largest enhancement (7-fold) comparative to the shorter $\pi$–electron conjugation length fluorene derivative D-$\pi$-A. The symmetric compounds gave an enhancement of 2-fold. In terms of molecular design, our results suggest that the 3PA cross-section of this family of molecules can be enhanced by increasing the distance over which the electric charge can be transferred.
CHAPTER 3: ENHANCEMENT OF NONLINEAR ABSORPTION USING SURFACE PLASMON RESONANCE EFFECTS

3.1. Surface Plasmon Resonance Effect in Metallic Films: Kretschmann-Raether Configuration

Kano and Kawata showed for the first time the possibility to enhance two-photon induced fluorescence by Surface Plasmons using the Kretschmann geometry [36]. By pumping at 800 nm, the authors were able to demonstrate a fluorescence enhancement factor (FEF) $\approx 90$ on LD490 dye-doped PMMA film deposited on a silver nanolayer. Later, Sanchez and coworkers [43] demonstrated near-field fluorescence microscopy based on two-photon excitation with metal tips with an FEF 10 times greater than one reported by Kano and Kawata. Prasad’s group [58] reported a two-photon emission enhancement of ca. 750 on doped organic molecules adsorbed onto silver surface, using near-field probing surface plasmon.

More recently, we have investigated the Surface Plasmon resonance effect using metallic interfaces such as silver and gold. Figure 26 shows the experimental setup for Surface Plasmon measurements, in a Kretschmann configuration. The pair of lenses L1 and L2 forms an afocal system which reduces the size of the laser beam to about 1.5mm ($HW_1/e^2M$). A horizontal polarizer was used to allow only TM component passing through the BK7 prism. The largest face of the prism was coated with a thin silver film of 40 nm thickness. There are two common methods used to deposit thin films on the substrate (glass or quartz). One method is given by thermal evaporation, which involves an evaporation source providing the evaporants atoms which are adsorbed on the substrate surface. Another method for physically depositing films is
sputtering, which is described later in this chapter. Over the silver film it was deposited a 10 nm layer of MgF$_2$ which acts as a spacer and on top of this film will be the organic dye. The role of this spacer was to avoid the fluorescence quenching by resonance transfer energy from the excited molecules to the metallic surface. A Silicon photodetector D1 was used to measure the reflected beam from the silver interface and another photodetector D2 worked as reference.

Using a Kretschmann-Raether configuration we were able to excite the Surface Plasmons for different excitation wavelengths. To do this we needed to achieve the wavevectors matching condition. When the tangential component of the incident light wavevector matched the wavevector of Surface Plasmon, the reflectivity curve reached its minimum point and the light is coupled to SP. This condition, known as surface plasmon resonance (SPR), can only be achieved with p-polarized light at a specific incident angle $\theta_0$, and is presented by relation (30):

$$k_{\text{light}} = k_{\text{SP}} \Rightarrow \frac{\omega}{c} n_p \sin(\theta_0) = \frac{\omega_{\text{SP}}}{c} \sqrt{\frac{\epsilon_m \epsilon_s}{\epsilon_m + \epsilon_s}}$$  \hspace{1cm} (30)$$

where $\epsilon_m$ and $\epsilon_s$ are the dielectric constants of the metal, and respectively, the sample, and $n_p$ is the refractive index of the prism.
Figure 26: Experimental setup for Surface Plasmon Resonance measurements. The SPs are excited by light using Kretschmann-Raether configuration.

We measured the light reflected at the prism face coated with the metallic film, using a photodector (D1). Reflectivity was given by the ratio between the values indicated by D1 and D2. With this experimental setup we were able to change the incident angle by rotating the BK7 prism, which was mounted on the rotation stage controlled by a stepper motor connected to the computer. The incident angle was changed in small steps of 0.02 degrees and the scanning range for each curve was about 3 degrees. The data acquisition system is based on LabView software, which controls the stepper motor and detectors.
Figure 27: Reflectivity curves for a BK7/Silver/Air system. The silver film has a thickness of 40 nm and the excitation wavelength was tuned between 633 nm and 1200 nm.

When the incident angle was equal to the plasmon coupling angle, the reflectivity presented a minimum value. At this point the Surface Plasmon Resonance (SPR) was achieved. Figure 27 shows the reflectivity curves obtained for different excitation wavelengths.

As the excitation wavelength was increased, the surface plasmon coupling angle was decreased and the reflectivity curves became narrower and deeper as shown in Figure 27. The dielectric constants of the prism and silver depend on wavelength, and this can explain the
wavelength-dependence of the surface plasmon angle. As we go to longer wavelengths, the real part of the dielectric constant $\varepsilon_m$ becomes more negative, reflecting more complete response of the free electrons of the metal to the lower frequency. Because the real part of $\varepsilon_m$ is much larger in absolute value than the imaginary part of $\varepsilon_m$, we can approximate the propagation constant of surface plasmons by:

$$k_{SP} = \frac{\omega}{c} \sqrt{\varepsilon_r \varepsilon_i}$$

(31)

From Equation (26) one can see that for longer wavelengths, the surface plasmon wavevector $k_{SP}$ is decreasing, thus the coupling angle given by Equation 25 is decreasing too, as shown in Figure 27. Theoretical calculations gave us a value of 42.2° for the coupling angle at 1200 nm excitation wavelength, knowing the refractive index of the prism ($n_p = 1.505$ at 1200 nm) and the optical constants of silver ($n = 0.085$ and $k=8.75$ at 1200 nm). There was a good agreement between our experimental results (42.7° coupling angle at 1200 nm) and the theoretical value (42.2° at 1200 nm). Measuring the coupling angle of Surface Plasmons for different wavelengths we can determine the dispersion relation of Surface Plasmons for a quartz/metal/air system.

Figure 28 shows both the experimental curve of the wavevector of Surface Plasmons versus incident light frequency and theoretical dispersion curve. As we can see in the Figure 28, the dispersion curve of the surface plasmon (red line) lies on the right side of the light line (black line), which represents the propagation of light in air. That means that we can not excite the surface plasmons directly from air, so we need a different medium (BK7 prism) in contact with the metallic film to excite the collective charge oscillations on the interface.
Figure 28: Experimental and theoretical dispersion curves for Surface Plasmons. The silver layer has thickness of 40 nm and the prism material is BK7 (ATR setup)

The blue line represents the light speed inside the prism ($c/\sqrt{\varepsilon_0}$) and its intersection point with the SP dispersion line will give the highest light frequency which still can excite the Surface Plasmons. Above this point, the SPs cannot be excited, because their dispersion curve lies to the right of $c/\sqrt{\varepsilon_0}$ line.

While the theoretical value of plasmon coupling angle ($\theta_0$) can be easily deduced from equation (25), it is more difficult to calculate the reflectivity curves for our BK7/Silver/Air system. The reflectivity (R) can be calculated using Fresnel’s reflection coefficients for the three-layer system.
0/1/2 which consists of a substrate with dielectric constant \( \varepsilon_0 \) (BK7 or Quartz), metallic film with \( \varepsilon_1 \) and thickness \( d_1 \), and dielectric medium \( \varepsilon_2 \), which can be air or some organic dye: [38]

\[
R = \left| r_{012}^p \right|^2 = \left| \frac{E_r^p}{E_0^p} \right|^2 = \left| r_{01}^p + r_{12}^p \exp(2ik_{x1}d_1) \right|^2 \left| 1 + r_{01}^p r_{12}^p \exp(2ik_{x1}d_1) \right| (32)
\]

with
\[
r_{ik}^p = \left( \frac{k_{z2}}{\varepsilon_i} - \frac{k_{z1}}{\varepsilon_k} \right) \left( \frac{k_{z2}}{\varepsilon_i} + \frac{k_{z1}}{\varepsilon_k} \right) (33)
\]

Using some approximations determined by the special conditions of our three-layer system, \( |\varepsilon_i| >> 1 \) and \( |\varepsilon_i| << |\varepsilon_j| \), the reflectivity can be approximated by the following relation:

\[
R = 1 - \frac{4\Gamma_i\Gamma_{rad}}{\left( k_x - (k_x^0 + \Delta k_x) \right)^2 + \left( \Gamma_i + \Gamma_{rad} \right)^2} (34)
\]

where \( \Gamma_i \) and \( \Gamma_{rad} \) are internal, and respectively, additional damping given by the imaginary parts of \( k_x^0 \) and \( \Delta k_x \). Equation (26) gives the SP wavevector \( k_x^0 \) and \( \Delta k_x \) can be approximated by the relation:

\[
\Delta k_x = \left[ \frac{\omega}{c} \frac{2}{1 + \frac{|\varepsilon_i|}{|\varepsilon_i| - 1}} \right] \frac{3}{2} \exp(-2|k_x^0|d) \left| r_{01}^p(k_x^0) \right| (35)
\]

From equation (29) one can see that reflectivity reaches its minimum, which is zero, when \( \Gamma_i \) and \( \Gamma_{rad} \) become equals. This condition gives us the thickness value \( d_{min} \) for which the reflectivity drops to its minimum value.
Figure 29: Calculated wavelength-dependent reflectivity for a 40-nm-thick silver film coated with a 15-nm-thick MgF₂ spacer.

An alternative way to calculate the reflectivity curves is to use commercial software or free software available at [http://corninfo.ps.uci.edu](http://corninfo.ps.uci.edu). There one can find a web-based version of four-phase Fresnel calculation program, developed by the Corn research group at University of California, Irvine. This program plots s and p reflectivity plus the electric fields (Eₓ, Eᵧ, and Eｚ) as function of incident angle, for a three- and four-phase system.

Figure 29 shows the calculated reflectivity curves for a 40-nm-thick silver film coated with a 15-nm-thick Magnesium Fluoride layer, using the web-based calculation program. The
silver and MgF$_2$ films were deposited on a BK7 prism by thermal evaporation. The MgF$_2$ layer acts as a spacer between the metallic interface and organic dye to avoid quenching of fluorescence due to energy transfer (ET) from excited dye molecules to the metallic surface. Our experimental results show a coupling angle between 42.7° and 46.4° (see Figure 28), which is in good agreement with the theoretical evaluations (42.2° ÷ 44.7°, see Figure 29).

The next step in our experiments was to deposit a thin layer of organic dye D-$\pi$-A over the spacer layer (MgF$_2$). To do this we used a spin-coating device, and an Aluminum prism holder designed to hold the right angle prism (21-mm-length of hypotenuse) during the spin-coating process. The asymmetric dye D-$\pi$-A was dissolved in Dichloromethane at a concentration of 10$^{-2}$ M and then a polymer (PMMA) was added to this solution (10$^{-3}$ M). The new layer deposited should have a thickness of less than one micron. Reflectivity measurements were done and they did not show any Surface Plasmon resonance. The Reflectivity of glass/silver/dielectric is very sensitive to the properties of the silver/dielectric interface such as refractive index and thickness of dielectric medium. The plasmon resonance angle was shifted to higher values as the refractive index or thickness is increased.

To reduce the thickness of the organic dye layer, we deposited the D-$\pi$-A dye directly on the MgF$_2$ layer, without polymer (PMMA). We used this time a BK7 prism with a 35-mm-length hypotenuse, coated with a silver film and a 10-mm-thick MgF$_2$ layer on top of silver. The organic dye/hexane solution at a concentration of 10$^{-3}$ M was applied on top of the Magnesium Fluoride using spin-coating technique. The rotation speed of the spin-coating device was about 3000 rot/min and the spinning time was 2-3 minutes.
Figure 30: Experimental curves of Reflectivity for glass/silver/MgF$_2$ system, a) coated with dye, and b) without dye coating. The excitation wavelength was 1200nm.

Figure 30 shows the experimental curves of reflectance for both cases with and without the organic dye layer applied on the interface. The experimental results show that the reflectivity reaches the minimum value (0.26) when the incidence angle becomes 42.58° (before applying the dye layer). Theoretical calculations gave us an angle of 42.24° corresponding to a reflectivity of 0.23 for the structure BK7/60nmSilver/15nmMgF$_2$/air. After applying the D-π-A compound on top of our system, the plasmon coupling angle was shifted to 70.5° as shown in Figure 29.
Also, the depth of the ATR minimum was drastically reduced to 0.9. No three-photon induced fluorescence was observed during experiments for an excitation wavelength of 1200 nm.

As the incidence angle increases, the loss due to reflection increases too. To reduce the plasmon coupling angle we used an equilateral prism made of high refractive index material (SF-11). Figure 31 shows the equilateral prism having the bottom face coated with gold, then SiO$_2$ spacer and asymmetric dye D-$\pi$-A. Each edge of the equilateral prism has a length of 25 mm.

![Figure 31: SF-11 prism coated with gold film, SiO$_2$ spacer and asymmetric dye. The red arrows represent the incident and reflected beams (1200nm).](image)

To prepare the sample illustrated in Figure 31, we used a sputtering device, from Electrical Engineering department of UCF. One face of the SF-11 prism, which is presented as the bottom side in Figure 30, was coated with a gold film of 55-60 nm thickness, using the RF Sputter Deposition technique. This process took place inside of a vacuum chamber containing two electrodes connected to a RF power supply. An Ar gas was maintained in the chamber at a...
low pressure (100 mTorr). The prism was fixed on the Anode, located on the top, and the silver (gold) target was mounted on the Cathode, which is on the bottom side. Upon bombarding the target surface with Ar ions, the surface atoms were sputtered away and then deposited on the substrate (prism). In a similar way we deposited 15nm of SiO$_2$ (spacer) over the metallic film. Finally, using a spin-coating and/or dip coating device we covered the spacer layer with the organic dye doped polymer.

The incidence angle inside the prism ($\theta_i$) can be changed by rotating the prism, and plasmon coupling angle corresponds to the minimum value of reflectivity. In Figure 32 are displayed the reflectivity curves as functions of incident angle $\theta_i$ shown in Figure 31. On the top of bottom face of the prism (see Figure 31) was deposited the asymmetric dye D-$\pi$-A by spin-coating. For this purpose we used a dye/hexane solution at different concentrations. For small concentrations such as $10^{-6}$ M and $10^{-5}$ M, no shift in Surface Plasmon coupling angle was observed.
Figure 32: Reflectivity curves for SF-11/Au/SiO$_2$/dye structure. The excitation wavelength was 1200 nm, which corresponds to 3PA of asymmetric compound

When concentration reached the value $10^{-4}$ M, we observed a small shift in the plasmon resonance angle. As one can see in Figure 32, the coupling angle in the case of no dye on the surface was $35.7^\circ$, while its value after coating the prism with $10^{-4}$ M dye/hexane solution increased to $35.8^\circ$. Thus, the angular shift was $0.1^\circ$.

For 8 drops of solution at higher concentration ($4\times10^{-4}$ M), the coupling angle became $36.2^\circ$ and adding 8 more drops of solution at the same concentration ($4\times10^{-4}$ M) led to a resonance angle of $36.8^\circ$. Also, besides the displacement of the ATR minimum with increasing thickness of dye layer, the depth and width (FWHM) of the reflectivity curves are changing with thickness. The
three-photon induced fluorescence in D-π-A fluorene derivative was too weak to be observed by naked eye or detected by Silicon detector. One possible reason for this could be the low concentration of dye/hexane solution. Increasing more the concentration of solution led to a large shift of the resonance angle and also a drastic reduction of the depth of the ATR minimum. Theoretical calculations confirmed our experimental results. Figure 33 shows the Reflectivity curves calculated for a 55 nm gold layer on SF-11 prism. The refractive index of gold and SF-11 material was 0.334 + 8.017i and 1.75, respectively, at an excitation wavelength of 1200 nm. The thickness of the asymmetric dye layer deposited on top of the spacer (15 nm SiO₂) had values between 0 and 60 nm. The Surface Plasmon coupling angle was shifted to the larger values as the thickness was increased and also the width of the curves became larger.
Figure 33: Calculated reflectivity curves for a 55 nm gold film on SF-11 using D-π-A dye layers with thickness between 0 and 60 nm. The excitation wavelength was 1200 nm.

Using the same SF-11 prism as shown in Figure 31, but coated with Silver instead of Gold, we have measured the reflectivity for the two cases: with and without the asymmetric dye D-π-A deposited on top. The excitation wavelength was again 1200 nm, which corresponded to three-photon absorption process in D-π-A.
In figure 34 we present the reflectivity curves for the SF-11 prism coated with 60nm-thick silver and 10nm-thick MgF$_2$, then with fluorene derivative D-π-A (second curve) and without dye coating (first curve). We believe, three-photon induced fluorescence couldn’t be observed because the quenching of fluorescence due to nonradiative energy transfer from excited dye molecules to the metallic surface.
3.2. Surface Plasmon Enhanced Two- and Three-photon Absorption Using Metallic Nanoparticles

To overcome the difficulties we encountered before, we decided to use spherical metallic surfaces, i.e. gold nanospheres, to enhance further the electric field, thus, the three-photon absorption. In addition, there are some other advantages of using metallic nanoparticles instead of metallic films to enhance the nonlinear absorption of organic dyes. First, their practical applicability in biomedical fields, second, their low cytotoxic effects in medical applications such as \textit{in vivo} bioimaging and photodynamic therapy (PDT). Because the Surface Plasmons on metallic films require a complicated geometry (see Kretschmann configuration in Figure 26), it is not suitable for practical applications. Thus, metallic nanoparticles in solution are the best candidate for medical applications. It has been proven already that gold nanoparticles can be injected in human body without any negative post treatment effects. So far, there was no experimental evidence of three-photon excitation process assisted by surface plasmon resonance. Also, most of the studies previously described have been done in doped polymeric films on metallic nanolayers or nanoparticles and measurements of fluorescence do not give a good indication of the electric-field enhancement by itself.

More attractive biological and medical applications require multiphoton excitation enhancement via Surface Plasmon Resonance in colloidal aqueous suspensions since water soluble molecules are more compatible with living organisms.

We studied the enhancement of the two- and three-photon absorption on a specific molecule, HOECHST 33258 pentahydrate (bis-benzimide) in solution. To show this effect, we took advantage of the electric-field enhancement produced at the surface of metal nanoparticles.
via surface plasmon resonance. To decide which compound could allow us to demonstrate two- and three-photon absorption enhancement in aqueous solution using surface plasmon resonance we considered three main features: a) extended π–conjugation length to improve the nonlinear absorption, b) strong linear absorption between 250 and 400 nm to be able to induce 2PA and 3PA within the water non-absorbing window, c) water solubility. Hoechst 33258 (2′-(4-hydrophenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bis-(1H-benzimidazole) pentahydrate), a commercial dye from Molecular Probes Inc., routinely used in common practice of visualization of DNA in gels [61], fulfilled all these requirements. Before proceeding to the two- and three-photon enhanced absorption via SPR in solution we fully characterized the dye selected for our study (Hoechst 33258).

The molecular structure of this dye is showed in the top of Figure 35. Figure 35 also displays the linear absorption and emission spectra (excitation at 350 nm) of Hoechst 33258 in aqueous solution at a concentration of $1 \times 10^{-5}$ M. The measurements were carried out using an Agilent 8453 Diode Array UV-VIS spectrophotometer and PTI Quanta-Master Spectrofluorimeter, respectively (cell effects and solvent contribution were properly subtracted). Hoechst 33258 presents high extinction coefficient (46,000 cm$^{-1}$) at 345.5 nm, fluorescence emission with maximum at 510 nm in water, and quantum yield $Q_f = 0.034$. The dye solution has no measurable absorption above 450 nm. Excitation beyond this wavelength can only occur through nonlinear processes. Also, the measured fluorescence anisotropy given by the formula:

$$r' = \frac{I_{\|} - I_{\perp}}{I_{\|} + 2I_{\perp}}$$  \hspace{1cm} (31)
for the Hoechst dye/glycerol solution at a concentration of $0.5 \times 10^{-5}$ M is shown. The fluorescence anisotropy was obtained with the PTI Quanta-Master spectrofluorimeter upon excitation at 350 nm with linearly polarized light.

Figure 35: Molecular structure of Hoechst 33258 (top). Normalized absorption (solid line) and emission (dotted line) spectra of $10^{-5}$ M Hoechst 33258/water solution. Anisotropy curve ($\Delta$) of a $5 \times 10^{-6}$ M Hoechst/glycerol solution.

The emission intensity was measured through a polarizer oriented parallel ($I_\parallel$) and perpendicular ($I_\perp$) to the direction of the polarized excitation. This curve reveals the spectral
regions for the $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions with maximum excitation at ca. 275 and 345 nm, respectively (see Figure 34).

3.2.1. Nanoparticles Synthesis and Gold Colloid Preparation

Gold nanospheres of 16 nm average diameter ($<$16% size distribution) were synthesized using the wet chemical method of Turkevich et. al. [63]. Briefly, 50 mL of preboiled $2.5 \times 10^{-4}$ M aqueous solution of AuHCl$_4$ was mixed with 1.0 mL of $3.4 \times 10^{-3}$ M solution of sodium citrate. Then, this mixture was heated and stirred for 5 min. This method has proven to be an efficient method that affords narrow size distribution and give absolute control on the average diameter.[64,65] The nanoparticles were characterized by UV-vis absorption, AFM, SEM-EDX, TEM, and XPS. All the instrumentation was available in our laboratory or at the material characterization facility (MCF), UCF. The position of the SPR bands in UV-vis spectroscopy is very useful to determine the shape of the nanoparticles [64] and have a good idea of their average size (See Figure 36). The nanoparticles size, size distribution and shape were always determined by transmission electron microscopy (TEM) using an FEI Tecnai F30 TEM available at the Material Characterization Facility (MCF) on campus (see Figure 37). We have chosen Au nanoparticles for two main reasons: a) their stability in the presence of water and oxygen, and b) their excellent biocompatibility. [66,67]
Silver and gold spherical nanoparticles posses a very strong absorption band at 420 nm and 530 nm, respectively. These bands correspond to their surface plasmon resonance (SPR). Figure 36 shows this band for the gold nanoparticles with diameter of 3.5 nm (open squares), 16 nm (open triangles) and 35 nm (open circles). As the nanoparticles size increases, their absorption peak is slightly shifted to longer wavelengths. Theoretical calculations carried out in gold nanospheres at 1100 nm show a maximum enhancement of the electric-field intensity of
2000. In Figure 37 are presented two pictures of gold nanospheres (a) and gold nanorods (b) taken by using the TEM technique.

Figure 37: TEM images of gold nanospheres a) and gold nanorods b)

In order to ensure 3PA enhancement using Au nanospheres at longer excitation wavelengths in the NIR region, we employed a method commonly used in bulk surface enhanced Raman scattering (SERS), which consist on activating the metal colloid by electrolyte-induced aggregation [68]. Activated gold colloid (hot particles) was be prepared by adding 0.5 ml of 1 M NaCl(aq) to 5.0 ml of the original gold nanoparticles solution. To determine whether the optically active hot particles were generated, we recorded a UV-Vis absorption spectrum of the final solution 20 minutes after its preparation. In Figure 38 we show the typical broad absorption spectrum of activated gold colloid that extends to at least 1100 nm. This effect is induced by the
coupling of the individual dipole oscillators of the small isolated particles. As a result normal modes of plasmon excitation that hold the cluster and cover a wide frequency region extended from visible to the near-IR are generated [69]. Activated samples were prepared by mixing 0.5 mL of $10^{-2}$ M Hoechst 33258/H$_2$O with 5.5 mL of activated gold colloidal solution. The final concentration of Hoechst 33258 in the activated solution was $8.3 \times 10^{-4}$ M for 3PA and $1.4 \times 10^{-3}$ M for 2PA. The absorption spectrum of the final mixture is shown in Figure 37. The presence of hot particles did not change the dye absorption spectrum. However, a background signal due to presence of hot particles was observed.

Figure 38: Absorbance vs. wavelength of 16 nm diameter gold nanospheres (dashed line), gold activated colloid (dotted line), and Hoechst 33258/H$_2$O solution ($2.3 \times 10^{-3}$ M) in the presence of activated gold colloid (solid line). The inset shows a closer view of the low absorbance region of the spectra.
The inset in Figure 38 shows a closer view of the lower absorption region of the spectra. The SPR band of hot particles in Hoechst 33258 solution was shifted to approximately 580 nm. This is an indication of dye molecules partially covering the hot particles. It is well-known that the position of SPR band is very sensitive to the dielectric constant of the surrounding medium.[38] The two bands with maxima at 620 nm and 980 nm suggest the generation of different shape clusters in the presence of the dye.

Measurements of pure dye were done on a Hoechst 33258/H$_2$O solution at a final concentration of $2.3 \times 10^{-3}$ M. NaCl(aq) was also added to this pure dye solution to avoid matrix effects. The concentration of this solution was slightly greater than that with hot particles to ensure three-photon absorption of the dye in the absence of activated gold colloid (see Figure 39).

3.2.2. Two- and Three-photon Absorption Measurements of Hoechst 33258

Two- and three-photon absorption excitations were induced with a tunable OPG pumped by third harmonic of a mode-locked, 25 ps FWHM, Nd:YAG laser operating at 10 Hz repetition rate as shown in Figure 18. The beam was focused to 10.0 µm (HW1/e^2M) at 550 nm and 14.5 µm (HW1/e^2M) at 1064 nm with a 15 cm focal distance achromatic lens. The sample was contained in a 1 mm path length quartz cell placed on a translation stage to scan it around the focal plane on the z direction. Using the well-known open aperture Z-scan technique [56], we measured the two- and three-photon absorption coefficients of Hoechst 33258/H$_2$O in the absence and the
presence of hot particles [70]. The experimental setup for these measurements is presented in
Figure 18.

![Graphs showing normalized transmittance vs. Z (cm) for different input energies with theoretical fittings.](image)

Figure 39: Open aperture Z-scan curves of Hoechst 33258/H₂O solution (2.3 × 10⁻³ M) pumping at 550 nm (a) and 1064 nm (b). Solid lines are the best theoretical fittings using the equations for 2PA and 3PA.
Based on the information contained in absorption and anisotropy spectra of Hoechst dye presented in Figure 35, 2PA was excited at 550 nm ($S_0 \rightarrow S_2$) and 3PA at 1064 nm ($S_0 \rightarrow S_1$). These wavelengths correspond to two- and three-photon absorption transitions with the highest probability. Figure 38 shows the open aperture Z-scan curves for 2PA (Figure 39a) and 3PA (Figure 39b) and their theoretical fittings. 2PA and 3PA measurements were done at three different input energies: 3.0 µJ (□), 4.0 µJ (△), and 4.5 µJ (Δ) and 18.4 µJ (□), 19.5 µJ (○), and 20.4 µJ (Δ), respectively. The average values of the 2PA and 3PA coefficients were $\alpha_2 = (9.0 \pm 0.2) \times 10^{-11}$ cm$^2$W$^{-1}$, pumping at 550 nm, and $\alpha_3 = (3.3 \pm 0.3) \times 10^{-22}$ cm$^3$W$^{-2}$, pumping at 1064 nm. Relating the 2PA absorption coefficient $\alpha_2$ to the solute concentration $d_0$ (molarity) in solution and the photon energy ($hc/\lambda$) of the incident radiation, we can estimate the two-photon absorption cross-section $\sigma_2'$:

$$\sigma_2' = \frac{\alpha_2}{10^{-3} N_A d_0} \left(\frac{hc}{\lambda}\right)$$  \hspace{1cm} (36)

For the case of three-photon absorption, the relation between $\alpha_3$ and $\sigma_3'$ is given by equation (29). The absorption coefficients presented before, yielded two- and three-photon absorption cross-sections $\sigma_2' = (2.4 \pm 0.2) \times 10^{-47}$ cm$^4$•s•photon$^{-1}$ and $\sigma_3' = (8.3 \pm 0.8) \times 10^{-78}$ cm$^6$•s$^2$•photon$^{-2}$.

The high values of $\sigma_2'$ and $\sigma_3'$ of Hoechst 33258 are attributed to the extended $\pi$-electron conjugation in the molecule [55]. The comparison of our results regarding $\sigma_2'$ and $\sigma_3'$ of pure Hoechst 33258 with our literature search [33,51,55,56] shows that this dye is a better two-photon absorber than a three-photon absorber. It has been demonstrated that while asymmetric charge transfer can confer high two-photon absorption cross-section to organic molecules with push-pull
3.2.3. Enhancement of Two- and Three-photon Absorption Cross-Sections of Hoechst 33258 in the Presence of Activated Gold Colloid

After the pure dye was characterized, we measured the 2PA and 3PA coefficients of the Hoechst 33258/H$_2$O solution in the presence of activated gold colloid. The excitation wavelengths for 2PA and 3PA were again 550 nm and 1064 nm, respectively. Figure 40 displays the open aperture Z-scan curves pumping at 550 nm (Figure 39a), and 1064 nm (Figure 39b) and their theoretical fittings. The measured 2PA and 3PA coefficients $\alpha_2(+Au) = (2.7 \pm 0.2) \times 10^{-8}$ cm$^2$W$^{-1}$ and $\alpha_3(+Au) = (3.5 \pm 0.2) \times 10^{-21}$ cm$^3$W$^{-2}$ yielded the effective cross-sections $\sigma_2'(+Au) = (1160 \pm 20) \times 10^{-47}$ cm$^4$ s$^{-1}$ photon$^{-1}$ and $\sigma_3'(+Au) = (244 \pm 10) \times 10^{-78}$ cm$^6$ s$^{-2}$ photon$^{-2}$, respectively. $\alpha_n(+Au)$ and $\sigma_n'(+Au)$ refer to the $n^{th}$ order absorption coefficients and cross-sections in the presence of activated gold colloid. We used three different input energies for 2PA as shown in Figure 39: 3.0 ($\Delta$) 3.8 ($\Box$), and 4.3 nJ ($\Diamond$), and 18.4 ($\square$), 19.5 ($\bigcirc$), and 20.4 $\mu$J ($\Delta$) for 3PA, respectively. The observed enhancement of the 2PA and 3PA of Hoechst 33258 in the presence of gold hot particles is revealed through absorption cross-section ratio, $\sigma_n'(+Au)/\sigma_n'$. Table 2 summarizes the absorption cross-sections for two- and three-photon absorption of Hoechst 33258 in the absence and presence of gold hot particles, and also displays their ratio $\sigma_n'(+Au)/\sigma_n'$. Using open aperture Z-scan, we measured pure nonlinear absorption, due to two- and, three-photon absorption, respectively.
Figure 40: Open aperture Z-scan curves of Hoechst 33258/H$_2$O solution in the presence of activated gold colloid, pumping at 550 nm (a) (8.3×10$^{-4}$ M) and 1064 nm (b) (1.4×10$^{-3}$ M). Solid lines are the best theoretical fittings using the equations for nonlinear transmittance derived for 2PA and 3PA.
Therefore, the two- and three-photon absorption enhancement achieved, is mainly attributed to the electric-field enhancement produced by the surface plasmon resonance. A contribution of additional relaxation mechanisms (radiative or nonradiative) to the total enhancement is not totally discarded. Molecules that can return faster to their ground state can experience more excitation-relaxation cycles in time [45].

Table 2: Two- and three-photon absorption cross-sections in the absence and in the presence of gold hot nanoparticles.

<table>
<thead>
<tr>
<th>λ(nm)</th>
<th>OAP</th>
<th>( \sigma_2' ) (cm(^4)s(^{-1})•photon(^{-1}))</th>
<th>( \sigma_2'(+Au) ) (cm(^4)s(^{-1})•photon(^{-1}))</th>
<th>( \sigma_2'(+Au)/\sigma_2' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>550</td>
<td>2</td>
<td>((2.4±0.2)\times10^{-37})</td>
<td>((1160±20)\times10^{-47})</td>
<td>\approx480</td>
</tr>
<tr>
<td>1064</td>
<td>3</td>
<td>((8.3±0.8)\times10^{-78})</td>
<td>((244±10)\times10^{-78})</td>
<td>\approx30</td>
</tr>
</tbody>
</table>

To reject any contribution to the nonlinear effect from hot particles, we did measurements on pure activated gold colloid at 550 nm and 1064 nm, at higher energies than that used with the dye. No change in transmission versus position at both excitation wavelengths was observed as shown in Figure 39. Therefore, changes in transmission curves are merely produced by the organic dye. 2PA and 3PA enhancement was also attempted in Hoechst 33258 in the presence of 16 nm diameter gold nanospheres that were not previously activated. No enhancement was observed. Stellacci and co-workers observed a similar effect on a self-assembly of two-photon chromophores on metal nanoparticles [74]. Theoretical calculations of the maximum electric-
field intensity enhancement (MEFIE) of gold nanospheres displayed in Figure 4 indicate a factor of 100 at 550nm and, respectively of 2100 at 1064 nm. Because the shape and the size of the activated gold nanoparticles are not precisely known, and the theoretical model only applies to spherical nanoparticles, the calculated MEFIE is not definitive. However, a greater enhancement for the longer wavelengths should be expected because the imaginary part of the permittivity of gold is lower at 1064 nm than that at 550 nm, while its real part is higher. It is known that the transition probability of two- and three-photon processes presents a quadratic and, cubic dependence with irradiance, respectively. Therefore, higher irradiance is required for 3PA excitation than 2PA excitation, since $\sigma_2'$ is always much larger than $\sigma_3'$. Thus, 3PA could be considered less sensitive to the electric-field enhancement via SPR than 2PA.

Figure 41 shows the experimental and calculated transmittance vs. input irradiance of Hoechst 33258. We used our experimental parameters and the measured two- and three-photon

![Figure 41: Experimental normalized transmittance vs. input irradiance of pure Hoechst/H$_2$O for 2PA (○) and 3PA (□), and their corresponding theoretical curves](image)

87
absorption coefficients of the organic dye. Comparing the normalized transmittance vs. irradiance of 2PA and 3PA, one can notice that the irradiance corresponding to the starting point of 2PA of Hoechst 33258 is at least 10 times lower than that of 3PA. For this reason, 2PA is more sensitive to electric field enhancement than 3PA in the lower irradiance region, i.e. below \(10^{11}\) W/cm\(^2\). At higher irradiance, i.e. above \(10^{11}\) W/cm\(^2\), the normalized transmittance of 3PA decreases faster than that of 2PA as expected. Consequently, 3PA is more sensitive to electric-field intensity enhancement once it gets initiated. To preserve the integrity of our cell, we avoided measurements at higher irradiance values.

In summary, Hoechst 33258 presents high two- and three-photon absorption cross-sections. In the presence of activated gold colloid, the two- and three-photon absorptions of Hoechst 33258 are enhanced by 480- and 30-fold, respectively. This is a direct consequence of the electric-field enhancement generated by the Surface Plasmons on activated gold colloid. Activated gold colloid is essential for 2PA and 3PA enhancement as it is for SERS. The efficiency of multiphoton absorption enhancement via surface plasmon resonance strongly depends on the multiphoton absorption cross-sections of the chromophores. This observation has to be carefully considered when reporting surface plasmon enhanced multiphoton absorption in molecular probes. The disparity observed among literature values of surface plasmon enhancement of 2PA has long been attributed to the experimental geometry, nanostructure composition, size, and shape. Our results demonstrate that the existent discrepancy might have been also originated by the differences in the multiphoton absorption cross-section of the different dyes.
CHAPTER 4: ENHANCEMENT OF THREE-PHOTON ABSORPTION CROSS-SECTION IN J-AGGREGATES

4.1. Description of J-Aggregates

New strategies to understand the structure-property relationship for three-photon absorbing materials have been proposed in chapter 2 to increase $\sigma_3'$ in conjugated organic systems [55] and new methods have been anticipated in chapter 3 to enhance the electric-field intensity via surface plasmon resonance to facilitate the 3PA. However, novel channels to further enhance $\sigma_3'$ are still necessary for more practical applications. Supramolecular assemblies such as J-aggregates [75] opens many possibilities in the field of nonlinear optics.[76, 77]

J-aggregates are molecular aggregates formed by cyanine dye molecules in a polar solvent, water being the most popular. Cyanine dye molecules are synthesized in a large variety and usually they are utilized for sensitizing the photographic films. They are characterized by a linearly extended $\pi$-electron system that leads to a large polarizability. The transition dipole moments of the molecules are strongly coupled, whereas the geometrical arrangement of the molecules determines the coupling energy. Let’s consider the simplest case of dimmer formation. As Figure 42 shows, the dipole coupling leads to splitting of excitation energy of one molecule (M) into two new states; however, in the case of a parallel alignment only the transition to the upper state is allowed (H), and in the case of an oblique alignment only the transition to the lower state (J) becomes allowed. In the later case the structure is called J-Aggregate, while the
first case is called H-Aggregate [78]. A new band in the absorption spectrum that is red-shifted compared to monomer absorption is the most pronounced feature of J-Aggregates.

![Figure 42: Splitting of the excitation energy of one molecule during the dimmer formation.](image)

4.2. Linear Optical Properties of Pseudoisocyanine J-Aggregate

We investigated 1,1’-diethyl-2,2’-cyanine iodide (better known as pseudoisocyanine, PIC) J-aggregate assembly. The formation of such supramolecular aggregate was observed at relatively high chromophore concentrations ($4 \times 10^{-3}$M) and are characterized by a sharp absorption band that is red-shifted relative to the monomer [75] The unusual sharpness of the J-band (see Figure 43) is the result of the collective effects in the optical response that are caused
by the strong interaction between the molecules within the aggregate [80]. On the other hand, the delocalization of the electronic states over many molecules results in the so called exciton states. These states can be envisaged as a coherent movement of an excitation over the chain of molecules that can result in unprecedented optical nonlinearities in J-aggregates [80]. Theoretical predictions and experimental evidence of strong two-photon absorption (2PA) in J-aggregates have been reported in the literature [81]. However, heretofore, there was no experimental evidence of higher order nonlinear absorption in supramolecular systems.

Figure 43: Linear absorption of the monomer (…) and J-aggregate (—) in aqueous solution at a concentration of $2.5 \times 10^{-4}$ M and $2 \times 10^{-3}$ M, respectively. The molecular structure of 1,1’-diethyl-2,2’-cyanine iodide is presented in the inset.
Spectral measurements were done using a UV-vis CARY-3 spectrophotometer. The J-aggregate preparation is well described in reference [78]. Briefly, PIC monomer from Across organics, used without further purification was dissolved in DI water to a concentration of $2.5 \times 10^{-4}$ and $2.0 \times 10^{-3}$ M. Then, NaCl 0.2M was added to the more concentrated solution and heated at $60^\circ C$ for 30min to form the J-aggregate. Figure 43 shows the linear absorption of the monomer (dotted line) and the polymer (solid line) of 1,1'-diethyl-2,2'-cyanine iodide in aqueous solution at a concentration of $2.5 \times 10^{-4}$ M and $2 \times 10^{-3}$ M, respectively. An interesting feature in Figure 43 is the absence of linear absorption at wavelengths greater than ca. 600 nm. This indicates that excitation beyond 600 nm can only occur through nonlinear (multiphoton absorption) processes. In addition, since the J-band corresponds to an enhanced one-photon allowed transition, it should also be allowed for three-photon excitation. Thus, a strong 3PA in the supramolecular assembly was predicted. Because there is no significant linear absorption in the monomer solution at 573 nm, we should not expect any significant 3PA in this solution at the equivalent energy.

4.3. Three-Photon Absorption Enhancement in Pseudoisocyanine J-Aggregates

The measurements of the three-photon absorption coefficient of PIC monomer and J-aggregate in aqueous solution at a concentration of $2.5 \times 10^{-4}$ and $2.0 \times 10^{-3}$ M, respectively, were done at 1720nm (3×573nm) using the open aperture Z-scan technique.[58] The PIC solutions were placed in a 0.1mm path cell and pumped with the OPG output beam. Typical low intensities for 3PA of approximately 0.2 GW/cm$^2$ were employed. This irradiance value was below the
photon fluence for irreversible changes in J-aggregate optical properties.[83] The beam waist at the focal plane was ca. 28 µm. According to anisotropy studies of PIC-J-aggregates reported in the literature,[82] the induced transition at 573 nm correspond to the lowest excitonic state $S_1$ with transition dipole moment orientation nearly parallel to the chain direction.

![Open aperture Z-scan curves of PIC J-aggregate taken at three different pump energies (symbols) and corresponding theoretical fittings for 3PA (—)](image)

Figure 44: Open aperture Z-scan curves of PIC J-aggregate taken at three different pump energies (symbols) and corresponding theoretical fittings for 3PA (—)

In Figure 44, we show the normalized measured transmittance vs. sample position $Z$ pumping at 1720 nm on the J-aggregates, at three different energies. The solid lines are the
corresponding theoretical fittings (solid lines) using basic theoretical considerations for three-photon absorption. Employing equation 23 derived in chapter 2, which gives us the normalized energy transmittance for pulses with temporal and spatial Gaussian profile, and assuming negligible linear absorption at the pump wavelength, the average value of the 3PA coefficient \( \alpha_3 = (2.1 \pm 0.4) \cdot 10^{-15} \text{ cm}^3 \text{W}^{-2} \) was obtained. Then relating \( \alpha_3 \) with the PIC concentration \( d_0 \) (M) in the J-aggregate and the photon energy \( [\hbar(c/\lambda)] \), this coefficient yielded a pure 3PA cross-section for the supramolecular assembly, \( \sigma_3' = 2.5 \cdot 10^{-71} \text{ cm}^6 \text{s}^2\text{-photon}^2 \). Similar measurements were performed in the monomer solution at higher irradiances and no measurable 3PA was observed. On the other hand, two-photon absorption was not present when pumping at 1720nm because there is no absorption at 860 nm as can be witnessed in Figure 43.

According to Luo and co-workers theoretical calculations for 3PA in symmetric molecules using truncated sum-over-states (SOS) and response theory,[61] the only parameter that contributes to the final 3PA cross-section is the transition dipole moment between the ground state and the final state, \( \mu_{0f}^0 \). Therefore, the determined high \( \sigma_3' \) is the result of the strong transition dipole moment coupling.

We have demonstrated 3PA enhancement in a supramolecular J-aggregate assembly, formed by molecules of 1,1’-diethyl-2,2'-cyanine iodide (PIC). The origin of the 3PA enhancement is the strong transition dipole moment coupling of monomer molecules in the J-aggregate supramolecular assembly. No experimental evidence of 3PA in the monomer was observed. This encouraging result will pave a new road for 3PA in supermolecules.
CHAPTER 5: APPLICATION OF THREE-PHOTON ABSORPTION IN PHOTODYNAMIC THERAPY (PDT)

The last part of our research addressed a significant challenge in photodynamic therapy (PDT): the enhancement of the activation penetration depth of photosensitizers (PS). Although two-photon absorption (2PA) have shown some improvement on the radiation penetration depth in PDT, typical wavelengths used in two-photon excitation can only penetrate tissue to a maximum depth of approximately one centimeter. Penetration beyond this limit, which is crucial for medical applications, requires the study of materials and processes that can overcome this restriction. Three-photon excitation induced at longer wavelengths is an alternative powerful process that has not been explored yet in PDT because of the lack of information on the 3PA structure-property relationship and the required high pump intensities.

Photodynamic therapy (PDT) has been extensively investigated over the past three decades and is currently an approved therapeutic modality for skin cancer, the treatment of superficial bladder cancer, early lung cancer, and advanced esophageal cancer. Several advantages make PDT the preferred treatment. PDT can successfully treats cancer in organs where open surgery is not feasible, such as the trachea. In PDT, the patient receives an intravenous injection or a topical application of a photosensitizer. Then, the photosensitizer is selectively localized in rapidly growing cells in the tumor tissue by modulating the lipophilic character of the sensitizing agent [84] and by selecting the appropriate delivery system.[85] Next, the absorption of light by a chromophore, i.e. the photosensitizer, generates cytotoxic species such as singlet oxygen or free radicals, leading to irreversible destruction of the treated tissue.[86]
Current efforts in the field of PDT are focused on the development of second generation (SG) photosensitizers, such as porphyrins,[87] meso-tetrahydroxymethylchlorin,[88] bacteriochlorins, hypocrellins, texaphyrins,[88] tin etiopurpurin,[88] and 5-aminolevulinic acid. Their common goal is to solve the main drawbacks of the first generation PS, i.e. complex composition, weak red absorption, slow clearance from normal tissues and significant side effects. The SG photosensitizers have an appreciable quantum yield for singlet oxygen production and strong absorption in the red region (600 – 700 nm) where linear absorption and light scattering is small. The latter improves the radiation penetration depth through tissue. Unfortunately, the light activation of most PS at present is based on linear optical excitation. As a consequence, two important limitations appear when using single-photon photodynamic therapy: a) linear optical excitation with UV and visible light is intrinsically cytotoxic and mutagenic and has a limited penetration depth due to optical scattering and absorption by blood and tissue, and b) 1PA is a non-localized process, thus significant absorption occurs all along the path of a focused beam of suitable wavelength light, requiring the light source to be in the immediate proximity of the cancerous cells. In order to surmount these limitations, the scientific community has been looking fervently for alternative light activating methods in the last few years. Because the production of singlet oxygen is independent of the PS excitation process, two-photon absorption has been proposed,[55,57] and to some extent it has overcame these limitations.
5.1. Linear Optical Properties of Hematoporphyrin IX

Our goal was to measure the linear and nonlinear optical properties of Hematoporphyrin IX in DMSO solution and demonstrate its potential application in 3PA-PDT. Hematoporphyrin IX was chosen because it is one of the main components of Photofrin II®, the only drug approved by the FDA for PDT. In addition, it presents a) high extended π−conjugation to enrich the nonlinear absorption, b) strong linear absorption at 400 nm required to induce 3PA at the tissue non-absorbing window, c) high solubility in DMSO, and d) it is commercially available from Sigma-Aldrich.

![Molecular structure and absorption spectrum of Hematoporphyrin IX in DMSO solution at a concentration of 0.1 M](image)

Figure 45: Molecular structure and absorption spectrum of Hematoporphyrin IX in DMSO solution at a concentration of 0.1 M
In Figure 45 we show the molecular structure of HpD-IX and its linear absorption spectrum from 250 to 1400nm taken in an HpD-IX/DMSO solution 0.1M, placed in a 10µm thickness cell using an UV-Vis-NIR CARY-500 SCAN spectrophotometer (cell effects and solvent contribution were properly subtracted). HpD-IX was used with no further purification. From Figure 45 one can see that the maximum intensity linear absorption band is centered at 400nm. Since 1PA and 3PA respect virtually the same quantum mechanics selection rules, by pumping at 1200nm 3PA in HpD-IX should be achievable. On the other hand, at 600nm HpD-IX does not present any linear absorption. The Q-Band of free-base porphyrin expected at 600nm is so weak in HpD-IX because the transition is pseudoparity-forbidden due to the alternacy symmetry.[80] This indicates that 2PA is negligible when pumping at 1200nm and no assisted 3PA will be present throughout the measurements.

5.2. Three-Photon Absorption Cross-Section of Hematoporphyrin

The three-photon excitation in HpD-IX was induced with a tunable OPG pumped by the third harmonic of a Mode-Locked, 25 ps full-width at half-maximum (FWHM), single-longitudinal mode, Nd-YAG laser (EKSPLA model PL2143B/TH), operating at a 10 Hz repetition rate. The beam was focused into a 0.1 mm path length quartz cell with a 15 cm focal distance achromatic lens, as shown in Figure 18. Typical intensities above 50 GW/cm$^2$ were employed for 3PA. We prepared HpD-IX/DMSO solution at a concentration 0.1 M in order to ensure a relative high 3PA.

Before determining the 3PA cross-section of HpD-IX we measured the power dependence of the absorption process. Typically, the order of the absorption process was determined by the
emission intensity dependence with the input irradiance.[56] Because the fluorescence quantum yield of HpD-IX was extremely low we measured the transmitted Intensity vs. input Intensity, using a 10-mm-thick quartz cell placed at the focus of the lens L5. Figure 46 shows this plot for HpD-IX/DMSO solution at a concentration 0.1 M. The solid line is the theoretical fitting using a cubic dependence law (see Equation 10). The result confirmed the presence of pure 3PA in hematoporphyrin IX at 1200nm.

![Graph](image_url)

Figure 46: Measured transmitted intensity versus input intensity (open squares). The solid line represents the theoretical fitting using Equation 22 for nonlinear transmittance and the dotted line is the fitting obtained with parameter $\alpha_3 = 0$
To fit the experimental curve, we solved numerically Equation 22, and the best-fit parameter was \( \alpha_3 = 3 \times 10^{-21} \text{ cm}^2 \text{ W}^{-2} \).

Afterward, using open aperture Z-scan [58] we measured the three-photon absorption coefficients of HpD-IX at 1200nm and calculated its 3PA cross-section. Figure 47 shows the experimental curves taken at three different energies and their corresponding theoretical fittings using basic theoretical considerations for three-photon absorption (Equation 23). After relating \( \alpha_3 \) with the solute concentration \( d_0 \) (Molar) and the photon energy \( \left[ \hbar c / \lambda \right] \), using Equation 29, the calculated 3PA coefficient yielded a three-photon absorption cross-section \( \sigma_3' = 1.2 \times 10^{-78} \text{ cm}^6 \text{s}^2 \text{ph}^{-2} \). The 3PA cross-section is significantly important and reveals that Photofrin® II is a potential candidate for deep PDT via 3PA.

In summary, we have measured the three-photon absorption in HpD-IX/DMSO solution at 1200nm. Its three-photon absorption cross section \( \sigma_3' \) was determined using open aperture Z-scan technique. Pure 3PA in HpD-IX was confirmed by the cubic dependence law obtained from the transmitted intensity vs. input intensity. These results open a new road in PDT that can afford an unprecedented penetration depth, pumping at wavelengths between 1100nm and 1300nm, where the tissue transparency window is positioned.
Figure 47: Z-Scan curves of Hematoporphyrin IX / DMSO solution at a concentration of 0.1 M. The solid curves are the theoretical fittings using Eq. 23 for transmittance.
6.1. Dissertation Summary

The structure/property relationship for a whole family of fluorene derivatives was determined. The three-photon absorption cross-section of fluorene derivatives can be optimized through molecular engineering. The most effective structure turned out to be the symmetric A−π−π−π−A, indicating that intramolecular charge transfer enhances the 3PA cross-section of this family of molecules and, that the withdrawing electron character of the attractor groups and the π-electron conjugation length increases $\sigma_3$. Pure three-photon absorption was corroborated after obtaining a cubic dependence on the three-photon induced fluorescence and transmittance vs. input measurements. Based on our results, fluorene derivatives can be considered potential candidates for medical applications such as fluorescence imaging and photodynamic therapy (PDT).

It was shown surface plasmon assisted two- and three-photon absorption enhancement in a Hoechst 33258 pentahydrate (bis-benzimide) aqueous solution containing gold colloid. The measured enhancement is a consequence of the electric-field enhancement via surface plasmon resonance.

It was experimentally showed an exceptional enhancement of 3PA cross-section in J-aggregates, result of the strong transition dipole moment coupling of monomer molecules in the J-aggregate supramolecular assembly.
Linear and nonlinear properties of Hematoporphyrin IX in DMSO solution were experimentally determined. The measured three-photon absorption cross-section $\sigma_3$ of Hematoporphyrin IX in DMSO solution is a good evidence of the potential practical applications of Photofrin<sup>®</sup> in 3PA-PDT.

6.2. Future Work

There are few directions this research may follow. Regarding the possibility of enhancement of the effective 2PA and 3PA cross-section using Surface Plasmon Resonance effects, one can study the effect of gold and silver nanorods in solution on cross-sections. It is expected an enhancement factor higher than that of metallic nanospheres. Therefore, more experiments should be done using gold nanorods instead of gold nanospheres. Another way is to prepare the nanoparticles arrays that display localized surface plasmon resonance, using nanospheres lithography. The advantage of this method is the huge tunability of SPR throughout visible, near-IR and mid-IR spectral domain.

The surrounding medium has an important influence on the three-photon absorption cross-section. It is important to understand how the environmental parameters such as solvent molecular orientation, reorientation of the electrons in the solvent, dielectric constant and polarity of the solvent contribute to the 3PA cross-section of organic compounds, especially fluorene derivatives. This subject is currently under investigation in our group.
APPENDIX A: SECOND HARMONIC GENERATION

AUTOCORRELATOR
An autocorrelator was built to measure the laser pulsewidth. Figure 48 shows the experimental setup for this autocorrelator, which in fact is a Michelson interferometer with a movable mirror and has the output directed into a second harmonic generation crystal.

Figure 48: Autocorrelation interferometer for measuring the pulsewidth of the laser

The beam splitter divides the input beam into two beams which travel separate paths and are reflected back by the two mirrors. After reflection, the two pulses are recombined and directed to the second harmonic generator crystal (SHG). One pulse is offset from the other by a time $T = 2d/c$, where $d$ is the movable mirror displacement and $c$ is the speed of light. The second harmonic signal is proportional to the square of the irradiance of the fundamental beam.
Therefore, the SH signal is a minimum when the pulses are completely separated and a maximum when they are overlapped in time. The electric field of the fundamental beam is proportional to:

\[ e^{-\frac{1}{2}\left(\frac{t}{\tau}\right)^2} + e^{-\frac{1}{2}\left(\frac{t-T}{\tau}\right)^2} \]

and its irradiance is proportional to:

\[ e^{-\left(\frac{t}{\tau}\right)^2} + e^{-\left(\frac{t-T}{\tau}\right)^2} + 2e^{-\frac{1}{2}\left(\frac{t}{\tau}\right)^2} \cdot e^{-\frac{1}{2}\left(\frac{t-T}{\tau}\right)^2} \]

The second harmonic generated will be proportional to the integral of the sum of each irradiance term squared separately:

\[
\int_{-\infty}^{+\infty} \left( e^{-2\left(\frac{t}{\tau}\right)^2} + e^{-2\left(\frac{t-T}{\tau}\right)^2} \right) dt + 4 \int_{-\infty}^{+\infty} e^{-2\left(\frac{t}{\tau}\right)^2} e^{-2\left(\frac{t-T}{\tau}\right)^2} dt
\]

\[
= \sqrt{2\pi}\tau \left( 1 + 2e^{-\frac{1}{2}\left(\frac{T}{\tau}\right)^2} \right) \]

\[
= ct_1 + ct_2 e^{-\frac{1}{2}\left(\frac{T}{\tau}\right)^2}
\]

(A.1)

In relation (A.1), \( \sqrt{2}\tau \) is the HW1/eM of the autocorrelation signal versus the time delay T. In Figure 49 are displayed the experimental and theoretical fitting curves of the SHG signal versus mirror displacement. The pulsewidth obtained was 25 ps (HW1/eM) for the pump beam.
Figure 49: Autocorrelation curves for the 1064 nm laser. The solid line represents the theoretical fitting using a Gaussian function.
APPENDIX B: SPATIAL BEAM PROFILE MEASUREMENTS
We measured the beam size using the well-known knife-edge technique. This method allows determination of the beam waist by scanning a razor blade across the beam and measuring the total integrated intensity of the unblocked beam (see Figure 50). The spatial intensity profile of a Gaussian laser beam can be described by the following relation:

\[
I(x, y) = I_0 \exp\left(-\frac{2(x^2 + y^2)}{w^2}\right)
\]  

(B.1)

where \(I_0\) is the maximum intensity and \(w\) is the beam radius defined as the radius at which the intensity falls to \(1/e^2\) of the peak intensity.

Figure 50: Determination of the beam waist by scanning a razor blade across the beam

By chopping the beam with a knife-edge the transmitted intensity follows a complementary error-function curve:
By fitting the experimental curve of transmitted intensity versus horizontal distance (x) with the equation (B.2) we obtained a beam waist of 15 microns for the excitation laser beam at 1064 µm wavelength. Figure 51 shows the experimental and theoretical curves for irradiance versus x distance.

Figure 51: Intensity versus distance across the beam. The solid line is the best-fit using the complementary error-function.
REFERENCES


