Two-Photon Single Molecule Spectroscopy

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZÜRICH

for the degree of

DOCTOR OF NATURAL SCIENCES

presented by

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Zürich 1999
Acknowledgements

This thesis has been accomplished through the support of a stimulating research group and the professional services provided by the many people at the Physical Chemistry Laboratory of the ETH Zürich. Here I would like to express my thanks to everybody who has contributed to this work – either scientifically, administratively or psychologically.

In the first place, I would like to thank Prof. Urs P. Wild for initiating this challenging research project and for leaving me with much freedom and confidence in carrying it out. He was also very helpful in allowing me flexibility in my working hours.

Prof. Frédéric Merkt is acknowledged for readily accepting the role of co-examiner. He was a very careful referee of this work and his questions initiated fruitful discussions.

During all this time I have closely collaborated with Dr. Taras Plakhotnik. I owe much of my scientific education to him. He was a great supervisor regarding both experimental and theoretical aspects of this work.

Dr. Alois Renn was the person who has asked the right critical questions in the right moments and who could put things to the point when they became unfocused. He was a great advisor and always took his time to listen to problems when asked.

Part of my work was done in collaboration with Dr. Gert Zumofen. I have enjoyed our discussions and have learned a lot from him. His never-ending curiosity and his interest in my work were very stimulating.

Dr. Marco Pirotta introduced me to the apparatus in the G6 lab and left me with a lot of nice equipment, not to forget his software which is still in use. The last few months of my experimental work I have shared with Thomas Nonn. Although the experiments were not so successful, we had a good time together. Within the whole “Wild-group” I have experienced a very pleasant work climate and a friendly collaboration. Particularly, I want to mention all current and former members of the single-molecule crew: Dr. Hermann Bach, Dr. Mauro Croci, Elizabeth Donley, Dr. Bert Hecht, Dr. Christian Hübner, Dr. Thomas Irngartinger, Tatiana Latychevskaia, Michael Prummer, Jean-Manuel Segura, Beate Sick, Werner Trabesinger and Dr. Udo Wallenborn. I am grateful to Dr. Robin Purchase, our postdoc from “down-under”, for proof-reading part of the manuscript.
The following people are acknowledged for their professional technical support: Bruno Lambilotte, Andreas Hunkeler and Roland Schmidli manufactured masterpieces of mechanics in the work-shop and delivered many hundreds of liters of liquid helium. Peter Nyffeler was always ready to solve electronic problems. Markus Traber was a very patient and helpful system administrator; after his leave, Beate Sick adopted his responsibilities and she is doing a great job. Further technical maintenance was provided by Konrad Boss, Karl Burkhalter and Walter Jäggi. Marie-Therese Werder and Heinrich Willi took care of administrative problems.

Outside the scientific world I have to express my gratitude to Franca Notter and to the people of the child care at Irchel (particularly Luzia, Nadine and Daiana) for looking after my daughter during part of the working days. Indispensable moral support, sharing both the joys and frustrations, was provided by my parents and by many friends. Of particular value were the monthly evenings with Housi, Roger, Stefan, Thomas and Urs, as well as the outdoor adventures with the KTL triple. I want to dedicate this work to my wife Sabine and my daughter Seraina for sharing with me all the ups and downs of life.
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The field of optical probing of individual molecules has been rapidly growing and diversifying during the last decade and has attracted the interest of researchers from various disciplines. Several techniques have been applied to detect single molecules in different environments. Spectroscopic investigations on individual absorbers remove the ensemble averaging that occurs when a whole population is probed simultaneously, and can directly provide the distribution and time trajectory of an observable. Of particular interest in this regard are optical resonance frequency fluctuations of single dopants in a solid environment, which occur due to guest–host interactions.

This thesis is concerned with two-photon excitation spectroscopy of single dopant molecules in a solid matrix. Two-photon absorption is one of the most basic non-linear optical processes and its study on the level of a single quantum system is of fundamental interest. Further, two-photon confocal microscopy is a technique featuring a reduced background if compared with conventional confocal microscopy. Its use for single-molecule imaging opens many possibilities to investigate new materials. One subject of this work is the demonstration of two-photon excitation as an additional technique for single-molecule spectroscopy, and the study of the molecular response to effects which are particularly related to two-photon excitation.

Two-photon spectroscopy of single diphenyloctaetetraene molecules in a n-tetradecane matrix was accomplished at cryogenic temperatures. The experiment was made possible by means of a confocal optical design providing a low background and a high photon collection efficiency. Further, the right choice of chromophore–matrix pair was of crucial importance. By comparing one- and two-photon spectra of the same electronic transition, interactions between the individual molecules and the solid matrix were investigated, with the main focus on IR-induced spectral dynamics in two-photon experiments. Studies of spectral features as a function of temperature and laser power showed the importance of four mechanisms for the analysis of two-photon spectra: (i) the ac-Stark shift at intense laser irradiation, (ii) the activation of local phonons by a laser-induced heating in the excitation volume, (iii) the interaction with non-equilibrium phonons, and (iv) dynamics of tunneling two-level systems that are accelerated by the IR irradiation and the non-equilibrium conditions in the sample.
Another subject of this work is the development of a novel spectroscopic technique that is based on the autocorrelation of fast frequency scans. The method allows for the determination of single-molecule line-shapes at time resolutions down to microseconds. Its application to two-photon excitation of diphenyloctatetraene revealed a line narrowing with decreasing measuring times. This effect was attributed to IR-induced spectral diffusion processes which saturate on a time scale of a second. This kind of spectral diffusion was absent in corresponding one-photon spectra. The new correlation technique appears to be a powerful tool for the study of all kinds of single-molecule time trajectories.
Kurzfassung


welche beschleunigt ist durch die IR-Einstrahlung und die Nicht-Gleichgewichtsbedingungen in der Probe.

# Abbreviations and Symbols

## Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>3PPE</td>
<td>Three-pulse photon echo</td>
</tr>
<tr>
<td>ACF</td>
<td>Autocorrelation function</td>
</tr>
<tr>
<td>a.u.</td>
<td>Arbitrary units</td>
</tr>
<tr>
<td>$A_g, B_u$</td>
<td>Irreducible representations of the $C_{2h}$ symmetry group</td>
</tr>
<tr>
<td>cps</td>
<td>Counts per second</td>
</tr>
<tr>
<td>DPOT</td>
<td>all-trans-1,8-diphenyl-1,3,5,7-octatetraene</td>
</tr>
<tr>
<td>FSR</td>
<td>Free spectral range</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>ITFC</td>
<td>Intensity-Time-Frequency-Correlation</td>
</tr>
<tr>
<td>NQP</td>
<td>Non-equilibrium phonons</td>
</tr>
<tr>
<td>OPE</td>
<td>One-photon excitation</td>
</tr>
<tr>
<td>PM</td>
<td>Photo-multiplier</td>
</tr>
<tr>
<td>PW</td>
<td>Phonon wing</td>
</tr>
<tr>
<td>rms</td>
<td>Root of mean square</td>
</tr>
<tr>
<td>SFS</td>
<td>Statistical Fine Structure</td>
</tr>
<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
</tr>
<tr>
<td>SM</td>
<td>Single molecule</td>
</tr>
<tr>
<td>SMS</td>
<td>Single Molecule Spectroscopy (high-resolution laser spectroscopy in low-temperature solids)</td>
</tr>
<tr>
<td>TD</td>
<td>n-tetradecane</td>
</tr>
<tr>
<td>TLS</td>
<td>Two-level system (of the solid)</td>
</tr>
<tr>
<td>TPE</td>
<td>Two-photon excitation</td>
</tr>
<tr>
<td>ZPL</td>
<td>Zero-phonon line</td>
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</table>

## Symbol

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\hat{A}$</td>
<td>Operator of electro-magnetic vector potential</td>
</tr>
<tr>
<td>$A_{tot}$</td>
<td>Total collection efficiency of the apparatus</td>
</tr>
<tr>
<td>$C_{DW}$</td>
<td>Debye-Waller factor</td>
</tr>
<tr>
<td>E</td>
<td>Local phonon energy (unless noted differently)</td>
</tr>
</tbody>
</table>
\( \hat{\mathbf{E}} \)  
Operator of electric field vector

\( f_{12} \)  
Oscillator strength between states \( |1\rangle \) and \( |2\rangle \)

\( \hat{H} \)  
Vibronic Hamiltonian of a molecule in the force field of a solid environment

\( \hat{H}_I \)  
Interaction Hamiltonian of a two-level molecule and an electro-magnetic field

\( I \)  
Laser intensity

\( I_{\text{sat}} \)  
Saturation intensity of a single molecule

\( I_{\text{ens}} \)  
Average saturation intensity of an ensemble of molecules

\( I(\omega) \)  
Line shape function of an optical transition

\( k_T \)  
Decay rate of triplet state

\( k_{\text{ISC}} \)  
Intersystem crossing rate

\( L \)  
Lorentz local field correction factor

\( N_H \)  
Number of molecules with resonance frequencies within one homogeneous width

\( P \)  
Laser power

\( \phi(i, n) \)  
Poisson distribution function

\( r \)  
Scan rate of laser frequency (unless noted differently)

\( R_{\infty} \)  
Fully saturated count rate (at very high excitation intensity)

\( R_{\text{tot}} \)  
Average number of detected photons

\( R_{\text{ITFC}} \)  
Number of photons contributing to the ITFC signal

\( s \)  
Sound velocity

\( S_{\text{ge}} \)  
Transition polarizability tensor between states \( |g\rangle \) and \( |e\rangle \)

\( S_{\text{ITFC}} \)  
ITFC signal

\( S_{\text{3PPE}} \)  
3PPE signal

\( t_m \)  
Measuring time, time resolution of the measurement

\( T \)  
Temperature

\( T_b \)  
Temperature of the liquid-helium bath

\( T_{\text{eff}} \)  
Effective temperature inside the sample volume probed by laser light

\( T_1 \)  
Population decay time of the excited state

\( T_2 \)  
Total dephasing time of the excited state

\( T_{2^*} \)  
Pure dephasing time

\( V_{\text{BA}} \)  
Vibronic coupling constant between states A and B

\( W_e \)  
Excitation rate of a TLS

\( W_r \)  
Relaxation rate of a TLS
\( W_{ge}^{(2)} \) Rate of two-photon excitation between states \(|g\rangle\) and \(|e\rangle\).

\( \alpha_g(v_L) \) Dynamic polarizability tensor of state \(|g\rangle\) at the laser frequency \(v_L\).

\( \Delta \alpha \) Tensor component of dynamic polarizability difference along the long molecular axis (for DPOT).

\( \Delta \alpha_{dc} \) Difference of static polarizabilities.

\( \Gamma, \Gamma_{\text{hom}} \) Homogeneous linewidth in Hz.

\( \Gamma_0 \) Linewidth at \(T = 0\) K, or linewidth extrapolated to zero excitation intensity.

\( \Gamma_{\text{eff}} \) Effective linewidth.

\( \Gamma_{\text{ens}} \) Average homogeneous linewidth of an ensemble of molecules.

\( \Gamma_{\text{OPE}}^{0,\text{ens}} \) Average linewidth of an ensemble under OPE, extrapolated to zero excitation intensity.

\( \Gamma_{\omega} \) Linewidth in units of angular frequency, \( \Gamma_{\omega} = 2\pi \Gamma \).

\( \Delta \Gamma \) Line broadening in Hz.

\( \delta \) Difference between the local phonon frequency in the molecular ground and excited state.

\( \varepsilon \) Absorption coefficient.

\( \hat{e} \) Unit vector along the laser electric field polarization.

\( \zeta_m(t) \) Stochastic function of time taking values +1 and -1.

\( \lambda \) Wavelength.

\( \Lambda \) Kapitza parameter.

\( \vec{\mu}_{12}, \vec{\mu}_{12} \) Transition dipole moment between states \(|1\rangle\) and \(|2\rangle\).

\( \Delta \mu_{\text{stat}} \) Difference of static dipole moments.

\( \nu \) Frequency in Hz.

\( \nu_0 \) Resonance frequency at \(T = 0\) K.

\( \nu_L \) Laser frequency in Hz.

\( \Delta \nu \) Frequency shift.

\( \sigma_p \) One-photon peak absorption cross section.

\( \sigma_{ge}^{(2)} \) Two-photon absorption cross section.

\( \sigma_{\text{NQP}} \) Cross section of NQP absorption by a local vibration.

\( \tau \) Lifetime of local phonon (unless noted differently).

\( \tau_{\text{NQP}} \) NQP lifetime.

\( \tau_c \) Correlation time of frequency fluctuations.

\( \phi(1) \) Correlation function of the electric dipole moment.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_f$</td>
<td>Fluorescence quantum yield</td>
</tr>
<tr>
<td>$\Psi_k(\Omega')$</td>
<td>Autocorrelation function</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency, for simplicity often denoted as “frequency”; predominantly used for the molecular resonance frequency, $\omega = 2\pi v$</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Static part of the molecular resonance frequency</td>
</tr>
<tr>
<td>$\omega'$</td>
<td>Dynamic part of the molecular resonance frequency</td>
</tr>
<tr>
<td>$\omega_L, \Omega$</td>
<td>Angular laser frequency</td>
</tr>
<tr>
<td>$\omega_{LP}$</td>
<td>Local phonon frequency</td>
</tr>
<tr>
<td>$\omega_{NQP}$</td>
<td>NQP Frequency</td>
</tr>
<tr>
<td>$\Omega'$</td>
<td>Correlation frequency</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

"An atom is a body which cannot be cut in two. A molecule is the smallest possible portion of a particular substance. No one has ever seen or handled a single molecule. Molecular science, therefore, is one of those branches of study which deal with things invisible and imperceptible by our senses, and which cannot be subjected to direct experiment." — J.C. Maxwell in a lecture before the British Association at Bradford (1873).

"Although it is becoming a routine practice in many laboratories, the ability to analyze individual molecules still amazes even the most zealous practitioners of this emerging field." — S. Weiss, in a special issue of "Science" about "Single Molecules" (March 12, 1999).

"Experiments on individual molecules using scanning probe microscopies have demonstrated an exciting diversity of physical, chemical, mechanical and electronic phenomena." — J.K. Gimzewski and C. Joachim, ibid.
Today’s most important fields of technology – communications, computer- and bio-technology – experience a lot of progress through the steady miniaturization of devices. Basic research is particularly challenged by the idea of controlling matter on the level of single electrons, atoms or molecules. Devices on this scale would act according to the laws of quantum physics. The thesis presented here is a contribution to the field of optical spectroscopy of single molecules in a solid. This field has considerably promoted single-molecule techniques in the past 15 years and has exhibited many exciting phenomena.

In contrast to methods investigating ensembles, experiments on single molecules inherently provide information on distributions and time trajectories of observables that would otherwise be hidden. For the study of a time-dependent process, the need of synchronization that is required when a population of molecules is measured simultaneously is removed. Further, the ultimate limit of sensitivity in chemical analysis is reached by the detection of one single entity of a substance, e.g. a single nucleotide of a DNA. In spectroscopic experiments the highest possible resolution is achieved when individual atoms or molecules are measured – particularly when the “material” is cooled to low temperatures and isolated as much as possible from environmental interactions. Quantum mechanical effects become directly observable when studying light-matter interactions on a single quantum system. The extremely high sensitivity of a single-molecule response (e.g. the fluorescence emission properties) to an external perturbation opens a large variety of applications. A single molecule can be viewed as a local reporter of its “nano-environment”, that is the exact arrangement of functional groups, atoms, ions, electrostatic charges or dipole moments in its immediate vicinity. In the following, the motivation to achieve molecular resolution and sensitivity is illustrated by means of several examples ranging from surface science to biophysics to fundamental quantum physics (i).

**Working with single molecules - a brief review**

In 1959 Richard Feynman presented a famous lecture before the American Physical Society, entitled “There’s plenty of room at the bottom” [1]. With his “invitation to enter a new field of physics”, he challenged the listeners to build computers with wires no wider than 100 atoms, a microscope that could view individual atoms, machines that could manipulate atoms one by one, and even circuits involving “quantized energy levels, or the interaction of quantized spins”. Feynman’s vision has become reality with the invention of the scanning probe techniques (STM, AFM and

---

(i) There is no claim of completeness in the choice of the examples.
SNOM (ii) in the 1980s [2-5]. In the final decades of this century, much scientific research has turned towards the manipulation and control of matter on an atomic and molecular scale. Using STM for example, surfaces cannot only be imaged at atomic resolution, they can also be manipulated by re-positioning adsorbed molecules one by one [6], or by moving an individual adsorbate from an immobilized position to a place where it freely rotates [7]. The scanning probe techniques further enable the investigation of novel materials such as carbon nanotubes whose electrical conductance occurs through discrete electron states [8,9]. Thus, such a nanotube behaves as a quantum wire or a single-electron transistor. The importance of the developments in the “nanoscale sciences” and the physical phenomena relevant to the world of single molecules have already been perceived by the public and have attracted the attention of the daily press [10].

In contrast to the scanning probe techniques which are limited to the investigation of surfaces, optical probing of single molecules offers the study of microscopic processes at unprecedented sensitivity in bulk materials. Its “operation at a distance” results in a smaller perturbation of the sample. This operation at a distance is usually accompanied by a loss in spatial resolution, but this can be compensated for by the high spectral resolution which is obtained in solids at temperatures below 4 K. Single-molecule spectroscopy at low temperatures enables studies of light-matter interactions on a single quantum system (iii), and investigations of local dynamics in solids using a single-molecule probe. Since 1989, this field has been rapidly evolving and diversifying (for comprehensive reviews see refs. [11-13]) and has triggered work on single-molecule detection in physiological environments [14]. Low-temperature single-molecule spectroscopy will be reviewed in more detail later in this chapter.

Important early advances in the optical probing of single quantum systems involved the high-resolution spectroscopy of single atoms or ions confined in electromagnetic traps. With those experiments, many fundamental physical predictions were confirmed. Pure quantum effects of both the radiation field and the atom could be directly investigated. Using single Hg⁺ ions in a radio-frequency trap, quantum jumps between fluorescing states and metastable dark states were observed. Atomic transition frequencies were determined at very high resolution by laser-cooling of the trapped ions [15]. In the fluorescent light of individual ions, photon antibunching and sub-Poissonian photon statistics were detected [16]. The development of a single-atom laser enabled the study of the fundamental processes of cavity quantum electrodynamics [17].

(ii) Scanning Tunnelling Microscopy, Atomic Force Microscopy, and Scanning Near-field Optical Microscopy, respectively.

(iii) That is, one or several photons interact with one molecular electron.
In the field of biophysics, recent advances in single-molecule detection by fluorescence imaging offer new techniques for the study of individual macromolecules and their function under physiological conditions (for a review see ref. [14]). For example, single-molecule enzymatic turnovers were monitored in real-time by detecting fluctuations in the native protein fluorescence upon oxidation of the substrate [18]. Site-specifically attached fluorophores and energy transfer techniques were used to study ligand-receptor colocalization [19] or the interaction between single proteins and single-stranded DNA [20]. The coincident determination of the positions of a target DNA fragment and a probe oligonucleotide, each of them binding a specific fluorescent label, seems to be a promising method for single-DNA analysis in genome sequencing or disease detection [21]. Another approach to DNA sequencing is to specifically label the nucleotides of single-stranded DNA immersed in a hydrodynamically focused flow [22]. Single-molecule detection of the fluorescent tags should result in a direct read-out of the sequence. Moreover, functionalized AFM tips and optical tweezers are novel tools for the manipulation of single bio-molecules and the investigation of their mechanical properties. Supercoiling of DNA, reversible protein unfolding and RNA polymerase activity were studied on the single-molecule level [23]. The progress and prospects in the rapidly developing field of single-molecule biophysics have been reviewed in several articles [14,23-24].

**Single-molecule spectroscopy at low temperatures**

In the following, the term single-molecule spectroscopy (SMS) will be used exclusively for high-resolution fluorescence excitation spectroscopy on a single chromophore embedded in a solid host at low temperature. All other techniques resolving individual molecules will be denominated single-molecule detection.

Why is it useful to study individual molecules in a complex condensed matter host? The motivation is threefold. First, a single-molecule optical line is highly responsive to guest–host interactions and to local dynamics in crystalline and amorphous solid matrices. Such dynamics occur on a wide range of time scales and are owing to structural re-arrangements, local dipole moment changes or electronic excitations/relaxations of neighboring absorbers. Since SMS removes ensemble averaging, distributions of the parameters describing such processes are obtained. For example, such distributions have been determined from measurements of single-molecule linewidth distributions [25]. SMS is an appropriate tool to test microscopic theories. Second, once the dynamics in the molecular environment are understood, a sample may be selectively manipulated where the single molecule serves as a sensor of a controlled perturbation [26], or becomes a
switchable light source [27,28] emitting only one photon at a time. Third, a single-molecule measurement is inherently time-resolved. Changes of the quantum state of the molecule itself (e.g. intersystem crossing), or changes in its environment are directly observed as “on-off” processes of the fluorescence intensity. Such processes have been termed “quantum jumping”, “spectral jumping” or “single-molecule blinking”. Fluctuations of the single-molecule optical resonance frequency are another example among the many new phenomena observed in the little explored regime of SMS.

SMS works as follows: the molecular probe, a strongly fluorescing chromophore, is incorporated into a suitable host matrix, making a doped solid (either crystal or glass or polymer). The detection of a single chromophore is achieved by two requirements. First, the sample must be cooled down to liquid helium temperature in order to freeze all rotational and vibrational degrees of freedom. As a consequence, the optical spectrum of the chromophore exhibits a very intense and narrow zero-phonon line (linewidth in the order of 10-100 MHz). Owing to the unique local environment of each chromophore, their transition frequencies show an inhomogeneous distribution that is usually $10^3$ to $10^6$ times broader than the zero-phonon line of a single absorber. In a second step, the high spectral selectivity provided by this frequency distribution is utilized to pick out a specific chromophore by scanning the frequency of a narrow-band laser across the optical resonance while detecting the fluorescence photons. The extreme narrowness of the zero-phonon line makes it a highly sensitive probe to dynamics in the solid matrix.

Subject of this work

This thesis is concerned with two-photon spectroscopy of individual molecules. The general goal is to establish two-photon fluorescence excitation as an additional technique for SMS. On the one hand, two-photon SMS is of fundamental quantum-optical interest. On the other hand, the group of materials accessible for single-molecule investigations is extended by the application of the two-photon method. Further, a new experimental approach is developed to study spectral dynamics at high time resolution and is applied to the investigation of spectral fluctuations in two-photon experiments. The present work includes the study of the specific conditions produced in the material during a two-photon experiment and the resulting response of single-molecule optical lines. This is achieved by a comparison of one- and two-photon single-molecule spectra of the same electronic transition.

Two-photon absorption is one of the most basic non-linear optical processes and should be investigated on the level of a single quantum system. The development of two-photon SMS also opens a way to two-photon confocal [29-31] or two-photon near-field [32] scanning microscopy on
the single-molecule level. These techniques will potentially supplement other methods for single-molecule imaging [11,14,33-35].

The molecular system considered suitable for studies of two-photon excitation is a polyene in a n-alkane matrix. Polyenes have the distinctive photophysical property that the two-photon transition between the ground ($S_0$) and the lowest excited singlet ($S_1$) state is allowed by the selection rules. Moreover, $S_1$ is a mixed parity state in most polyenes, and therefore this state is fluorescent. The parity mixing mainly results from a symmetry breaking induced by the interaction with the surrounding host. These characteristics make polyenes interesting candidates for two-photon excitation studies on the single-molecule level.

This work is structured as follows: In chapter 2, basic knowledge about low temperature spectroscopy of dye doped solids is briefly reviewed. Then, the requirements for single-molecule detection are deduced with regard to the experimental implementation and the choice of the guest-host pair. SMS has demonstrated that the resonance frequency of a dopant molecule fluctuates randomly and the measured line shape thus depends on the time scale of the experiment. The chapter terminates with a discussion of dynamic single-molecule lines, based on Kubo’s line shape theory.

The experimental setup for two-photon SMS is described in chapter 3. Of crucial importance here is a reference cavity which enables the control of the laser frequency at a relative accuracy of $10^{-8}$. Also the preparation procedure and a rough characterization of the samples is presented.

In chapter 4, the spectroscopic properties of diphenyloctatetraene (the polyene under study) are investigated on bulk samples to have a more solid basis for the understanding of single-molecule spectra. The vibronic coupling between the two lowest excited singlet states and the resulting $S_1$ symmetry breaking play an important role. In an n-tetradecane host, two molecular sites are found. One corresponds to an undistorted centrosymmetric conformation and the other to a symmetry-broken conformation.

The first two-photon excitation single-molecule spectra are reported in chapter 5. Optical saturation of the emission rate due to a triplet bottle-neck state, as well as a power-dependent frequency shift are observed.

Chapter 6 deals with a comparison of one- and two-photon single-molecule spectra of the same electronic transition. The light induced frequency shift reported in chapter 5 does not show up in one-photon spectra and is discussed in terms of the following mechanisms: the ac-Stark effect, a laser induced heating and thermal activation of local phonons, the interaction with non-equilibrium phonons. These effects are only revealed by a comparison of the temperature dependence of single-
molecule lines in one-photon spectra with the power dependence under two-photon excitation. Moreover, the single-molecule linewidth is two to three times narrower in one-photon spectra.

In chapter 7, a new technique is introduced that allows the determination of single-molecule line shapes at previously unattainable time resolutions in the milli- to microsecond range. It is based on the autocorrelation of fast frequency scans. Its application to two-photon SMS shows that the broadening in two-photon spectra is caused by an accelerated spectral diffusion induced by the high-power IR irradiation.

The conclusions of this work are drawn in chapter 8.

At the end of this introduction I would like to remind the reader that considerations about the nature of atoms and experiments with single molecules have already been the subject of a lecture by J. C. Maxwell before the British Association at Bradford in 1873 [36]: "An atom is a body which cannot be cut in two. A molecule is the smallest possible portion of a particular substance. No one has ever seen or handled a single molecule. Molecular science, therefore, is one of those branches of study which deal with things invisible and imperceptible by our senses, and which cannot be subjected to direct experiment." The ability of working with single molecules may have changed part of our world outlook at the end of this century. With this perspective however, Maxwell reminds us that human knowledge is transient in many senses and never completed.
Seite Leer / Blank leaf
2. FUNDAMENTALS OF SINGLE MOLECULE SPECTROSCOPY IN SOLIDS

The theoretical framework of low-temperature spectroscopy of dye doped solids is reviewed and the basic concepts of single-molecule spectroscopy are introduced. The requirements on the experimental conditions and the photophysical properties of the chromophore-matrix system, which must be met for single-molecule detection, are outlined. The optical resonance frequency of an individual absorber fluctuates randomly due to the interaction with stochastic processes in its environment. A standard approach based on Kubo’s line shape theory is chosen to theoretically describe such dynamics of single-molecule lines. The approach yields an appropriate treatment of temperature dependences. However, it requires severe assumptions about the time-scales of the frequency fluctuations. The chapter terminates with a discussion of the theory’s limitations.
2.1. INTRODUCTION

Electronic spectra of organic compounds in solutions or in solids usually consist of more or less structure-less bands which are several hundreds or thousands of wavenumbers broad. They contain only little information about the molecules themselves and their environment. To achieve spectra at higher resolution has been the goal of spectroscopists for many decades.

In 1972, Personov et al. [37] found that low-temperature spectra of many organic solutions are mainly inhomogeneously broadened and possess a hidden structure. In fact, the spectra consist of the sum of many zero-phonon lines. This line structure was revealed in emission spectra upon selective narrow-band laser excitation at the frequency of the zero-phonon line. This method was called "fluorescence line narrowing". In 1974, two groups [38,39] discovered that the intensity of the zero-phonon line decreases during selective laser excitation, resulting in a persistent spectral hole in the absorption band. This so-called "spectral hole burning" and the fluorescence line narrowing are techniques of site-selective spectroscopy. They allow for the precise determination of molecular vibronic ground and excited state energies, as well as the investigation of intramolecular relaxation processes. However, site-selective spectroscopy cannot remove all inhomogeneities in the spectra. Individual dopant molecules in a solid which have the same electronic transition energy, still differ from each other with regard to the homogeneous linewidth and the interactions with the matrix. Therefore, even site-selective spectroscopy provides ensemble averaged information. The ultimate limit of spectral selectivity and line-narrowing was achieved in 1989 with the detection of a single guest molecule in a solid. Using a double-modulation absorption technique, Moerner and Kador [40] measured the optical spectrum of single pentacene molecules in a para-terphenyl crystal. One year later, Orrit and Bernard [41] reported about fluorescence excitation spectra of single molecules of the same system. The fluorescence excitation technique has been used in most single-molecule experiments up to date because of its simplicity and low background.

Low-temperature single-molecule spectroscopy (SMS) has revealed that the optical line shape of an individual absorber fluctuates randomly and therefore depends on the measuring time. The problem arises how to define a single-molecule (SM) line shape at all. In this chapter, the characteristics of zero-phonon transitions at low temperatures and the principles of SMS are briefly reviewed in section 2.2, and the photophysical and experimental requirements for SM detection are given in section 2.3. A standard approach to stochastic SM line shapes is presented in 2.4.
2.2. LOW-TEMPERATURE SPECTROSCOPY OF DYE DOPED SOLIDS

2.2.1. Zero-phonon lines and inhomogeneous broadening

Translational and rotational degrees of freedom are completely eliminated in solids at low
temperatures, and matrix isolation of a solute molecule provides a useful technique for obtaining
highly resolved spectra. First we consider transitions between the electronic states of a single dopant
molecule. The optical line shape of such a dopant in an organic mixed crystal is in general the sum of
two components (Fig. 2.1): the zero-phonon line (ZPL) and the phonon sideband, also called phonon
wing (PW) [42,43]. The ZPL arises due to a transition with no net creation or annihilation of
phonons. In the case of a vibronic 0-0 transition (see Fig. 2.2) it is specified as the purely electronic
ZPL. Electronic transitions which are associated with the excitation of intramolecular vibrations but
leave the phonon population of the host unchanged, are called vibronic zero-phonon transitions.
Each vibrational mode produces a copy of the purely electronic ZPL with the intensity determined
by the Franck-Condon factors [42]. At low temperatures (~ 2 K) the width of a purely electronic ZPL
is typically $10^{-4} - 10^{-3}$ cm$^{-1}$, corresponding to an excited-state lifetime in the order of 10 ns.
Vibronic ZPLs are $10^{3}$ to $10^{4}$ time broader because of lifetimes in the picosecond range for
molecular vibrational states. Each ZPL is accompanied by a phonon sideband which results from
electronic transitions that are coupled to lattice vibrations of the host by linear electron-phonon
coupling. Due to the high density of phonon states and the broad spectrum of lattice vibrational
modes, the PW is very broad, usually several tens of wavenumbers.

![Figure 2.1](image)

**Figure 2.1:** Schematic representation of the electronic spectrum of a dopant chromophore in a solid
host. ZPL and PW denote the zero-phonon line and phonon wing, respectively. At low temperature, the
PW arises due to creation of lattice vibrations upon electronic excitation and is thus blue-shifted with
respect to the ZPL.
The relative integrated intensities of the ZPL and the PW, \( S_{ZPL} \) and \( S_{PW} \) respectively, are given by the Debye-Waller factor \( C_{DW} \), which is defined by

\[
C_{DW}(T) = \frac{S_{ZPL}(T)}{S_{ZPL}(T) + S_{PW}(T)}.
\]

In the absence of non-radiative transitions the sum \( S_{ZPL} + S_{PW} \) represents the full intensity \( S \) of the electronic transition. Within this approximation, the sum of the two strongly temperature dependent terms \( S_{ZPL}(T) \) and \( S_{PW}(T) \) is thus temperature independent,

\[
S = S_{ZPL}(T) + S_{PW}(T) = \text{const.}. \tag{2.2}
\]

Correspondingly, an increase of temperature results in a transfer of integrated absorption from the ZPL to the PW. The Debye-Waller factor is large if the electron-phonon coupling is weak. According to the Franck-Condon principle, the transition between two electronic potential surfaces occurs “vertically”, i.e. the electronic excitation is much faster than any rearrangement of the nuclei (see e.g. ref. [44]). As a consequence, the generation of phonon modes associated with an optical transition is weaker the smaller the spatial shift of the excited state potential surface with respect to the ground state potential surface. A small Stokes shift of the fluorescence with respect to the absorption wavelength is often an indication for a weak electron-phonon coupling. Further, \( S_{PW} \) depends on the total phonon density and is thus strongly temperature dependent. The Debye-Waller factor decreases exponentially with temperature. Hence, intensity is transferred from the ZPL to the PW at increasing temperature, and the contribution to the absorption from the ZPL is only important at very low temperatures (\( T < 10 \text{ K} \)). The appearance of intense and narrow zero-phonon transitions forms the basis for high-resolution SMS at high signal-to-background ratio. In the following the term “ZPL” will be used exclusively for the purely electronic 0-0 transition.

The homogeneous shape of a ZPL is to first approximation described by a Lorentzian (a more detailed discussion about line shape functions follows in section 2.4.),

\[
I(v - v_0) = \frac{\Gamma_{\text{hom}}/2\pi}{(v - v_0)^2 + (\Gamma_{\text{hom}}/2)^2}. \tag{2.3}
\]

The homogeneous linewidth \( \Gamma_{\text{hom}} \) (full width at half maximum, FWHM) is related to the total dephasing time \( T_2 \) of the optical transition by

\[
\Gamma_{\text{hom}}(T) = \frac{1}{\pi T_2(T)} = \frac{1}{2\pi T_1} + \frac{1}{\pi T_2^*(T)}. \tag{2.4}
\]
Here $T_1$ corresponds to the lifetime of the excited state. It arises due to population transfer from the electronic excited to the ground state by processes such as fluorescence or internal conversion. $T_2^*$ is the so-called pure dephasing time. It describes the coherence decay of the electronic excitation because of interactions with the environment. At low temperatures, $T_1$ is essentially temperature independent, while $T_2^*$ strongly depends on temperature. The actual value of $T_2^*$ reflects the coupling of the chromophore's electronic excitation to low-frequency modes in the host. These interactions are generally stronger in amorphous compared with crystalline matrices, resulting in correspondingly shorter $T_2^*$ times and thus broader linewidths. Approaching $T = 0$ K, where phonons and local vibrational modes of the host are not activated, $T_2^*$ becomes infinitely long and $\Gamma_{\text{hom}}$ is determined by $T_1$ alone.

The narrow ZPL of each dopant is extremely sensitive to dislocations, point defects, or random internal electric and strain fields and field gradients in the host material. Such imperfections are generally always present, even in single crystals. This leads to a distribution of the center frequencies for the various molecules, and the resulting overall profile is called the inhomogeneously broadened line (see Fig. 2.4) [42,43]. The inhomogeneous broadening reflects the variations in the local environment of each individual chromophore. The spread of center frequencies means that different guest molecules have different resonance frequencies. As a consequence, there is a spectral selection criterion for the isolation of a single molecule. This spectral selectivity is given by the ratio of homogeneous to inhomogeneous linewidth. For the ZPL of an organic mixed crystal this ratio is typically $10^{-3} - 10^{-5}$, and it ranges down to $10^{-7}$ for glassy matrices. A useful quantity for the description of the spectral selectivity is the number $N_H$ of molecules with resonance frequencies within one homogeneous width. The fundamental idea of SMS at low temperature is to select a single absorber in a region of the spectrum where $N_H < 1$, by tuning the frequency of a narrow-band laser in resonance with only that molecule.

### 2.2.2. Peak absorption cross section and optical saturation

Fluorescence excitation techniques work on almost zero background and are thus favorable for SMS at high signal-to-noise ratio. Here we consider the photophysical processes leading to fluorescence of an organic chromophore. The relevant electronic energy levels of a prototypical molecule are shown as a Jablonski diagram in Fig. 2.2. The molecule is excited from the ground state ($S_0$, $v=0$) to the vibrationless level ($v'=0$) of the lowest excited singlet state ($S_1$) using narrow-band laser light (EX). From $S_1$ there are several relaxation paths.
Figure 2.2: Jablonski diagram of an organic dopant molecule in a solid. $S_0$, $S_1$, and $T_1$ are the ground, first excited singlet and the first excited triplet states, respectively. $v$ and $v'$ correspond to the quantum numbers of the vibrational sub-levels. The fine splitting within the vibrational states is due to phonon modes of the host which couple to the dopant molecule. The molecule is excited from $S_0$ to the vibrationless $S_1$ state using a narrow-band laser (EX, 0-0 transition). From $S_1$ there are several relaxation paths: Fluorescence (F) to vibrational states of $S_0$, non-radiative relaxation (also called internal conversion, IC), or intersystem crossing (ISC) from the singlet to the triplet manifold. $k_{21}$ is the total $S_1 \rightarrow S_0$ decay rate to $S_0$, $k_{ISC}$ is the rate of ISC and $k_T$ is the triplet decay rate. The exciton bands of the host are not coupled to the states of the chromophore. For common organic matrices, the exciton absorption bands lie in the ultraviolet spectral range, whereas the absorption of the guest molecule is usually chosen to be in the visible.

First, fluorescence (F) to vibrational sublevels of $S_0$ generates photon emission that is red-shifted compared with the excitation wavelength. After the emission, the molecule quickly relaxes to the ground state by creation of intramolecular or lattice vibrations. The probabilities for the various frequencies of emitted photons (colors) are controlled by the Franck-Condon factors of the corresponding vibronic transitions (see e.g. [44]). In most SMS experiments performed nowadays, the molecule is optically cycled between $S_0$ and $S_1$ and the Stokes-shifted fluorescence photons are detected. Second, $S_1$ may decay non-radiatively to $S_0$ by dissipation of the excitation energy into intramolecular vibrational and phonon modes. This process is called internal conversion (IC). The total $S_1 \rightarrow S_0$ relaxation rate including all processes is $k_{21}$. Third, intersystem crossing (ISC) between isoenergetic levels of the singlet and triplet manifold takes place at a rate $k_{ISC} (= k_{23})$. Since the $T_1$ and $S_1$ energies differ from each other, fast vibrational relaxations are always involved in ISC. Because of the spin-forbidden nature of singlet – triplet transitions, the lifetime of the $T_1$
state, which may range from microseconds to seconds, is orders of magnitude longer than that of $S_1$, which is in the order of $1 - 10$ ns. Therefore, the triplet state is a trap state where the emission of fluorescence photons ceases for a relatively long time. ISC corresponds to a bottleneck in the optical pumping cycle between $S_0$ and $S_1$. The $T_1 \rightarrow S_0$ transition at the rate $k_T$ ($= k_{31}$) is of non-radiative nature in most cases and brings the molecule back to the ground state.

An important quantity that characterizes the interaction between light and molecule in the resonant case is the peak absorption cross section $\sigma_p$. $\sigma_p$ may be regarded as the “effective area” of photon “capture” of the molecule. More precisely spoken, it gives the probability $p_{\text{abs}}$ that the molecule will absorb an incident photon from the monochromatic pumping laser beam of cross sectional area $A$,

$$p_{\text{abs}} = \frac{\sigma_p}{A}. \quad (2.5)$$

The peak absorption cross section for a purely electronic zero-phonon transition of an organic dopant in a solid is given by [45]

$$\sigma_p(v_0, T) = C_{\text{FC}} C_{\text{DW}}(T) \cdot \frac{T_2(T)}{2T_{\text{rad}}} \cdot \frac{3\lambda_0^2}{2\pi} \cdot \beta(\theta). \quad (2.6)$$

In this equation, $C_{\text{FC}}$ is the Franck-Condon factor of the purely electronic ZPL and $C_{\text{DW}}(T)$ the Debye-Waller factor. $\lambda_0 = c/v_0$ is the resonant wavelength of absorption in the peak of the Lorentzian line shape function, and $\beta(\theta) = \cos(\theta)^2$ is a geometry factor accounting for an angle $\beta$ between the molecular transition dipole moment and the electric field vector of the exciting light wave. $T_{\text{rad}}$ is the radiative lifetime defined by $T_{\text{rad}} = T_1/\phi_f$, where $\phi_f$ is the fluorescence quantum yield. In the absence of non-radiative decay of the $S_1$ population, i.e. when $\phi_f = 1$, and in a low-temperature limit where all dephasing processes are quenched ($T_2^* \rightarrow \infty$), we have $2T_{\text{rad}} \approx T_2(T \rightarrow 0)$. Remarkably, $\sigma_p$ depends inversely on $\Gamma_{\text{hom}}$, so that the narrow width of a ZPL translates into a very high absorption probability. At low temperatures where $T_2 = 2T_1$ ($T_1$ in the order of 10 ns), $\sigma_p$ increases up to $10^{-10}$ cm$^2$ for rigid aromatic molecules with a strongly allowed $S_0 \rightarrow S_1$ transition $\dagger$. To visualize the magnitude of this value, this $\sigma_p$ corresponds to approximately $10^4$ times the geometrical area of the molecule. Or, assuming that the molecule is irradiated with a resonant laser beam at $\lambda = 500$ nm and a weak intensity of 1 mW/cm$^2$, the incident photon flux of about $3 \times 10^{15}$ photons s$^{-1}$ cm$^{-2}$ will produce 30000 excitations per second.

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$\dagger$ Typical representatives used in many single-molecule experiments are: Pentacene, Perylene, Terpylene, and derivatives thereof.
The strength of an optical transition may also be described in terms of the oscillator strength $f_{12}$ or in terms of the transition dipole moment $\vec{\mu}_{12}$ (1 and 2 denote the ground and excited state, respectively). Assuming a parallel orientation of the laser electric field polarization and the transition dipole moment, $f_{12}$ and $|\vec{\mu}_{12}|$ are related to the peak absorption cross section as [46]

$$f_{12} = \frac{2\pi \epsilon_0 mc}{e^2} \cdot \Gamma_{\text{hom}} \sigma_\rho = (59.23 \text{ s cm}^{-2}) \cdot \Gamma_{\text{hom}} \sigma_\rho,$$

(2.7)

$$|\vec{\mu}_{12}|^2 = \frac{\epsilon_0 hc}{4\pi} \cdot \Gamma_{\text{hom}} \sigma_\rho = (1.40 \times 10^{-27} \text{ A}^2 \text{s}^2) \cdot \Gamma_{\text{hom}} \sigma_\rho,$$

(2.8)

where $c$ is the speed of light, $m$ the electron mass, $e$ the electronic charge, $\epsilon_0$ the vacuum electric field constant, and $h$ is Planck’s constant. It is sometimes convenient to calculate the peak absorption cross section of a single molecule from an absorption spectrum of a solution at room temperature. The integrated absorption $S$ (in units cm$^{-2}$) that is produced by a number density of absorbers $N_{\text{tot}}$ is independent of temperature and is given by the standard expression [47]

$$\frac{S}{N_{\text{tot}}} = \frac{\pi e^2 \epsilon_0 mc^2}{e_0 mc^2} f_{12} = \frac{8\pi^3 \epsilon_0^2}{e_0 hc^2} |\vec{\mu}_{12}|^2.$$

(2.9)

When the absorption is measured for a solution at room temperature, $S$ must be multiplied by a factor of 3 to account for orientational averaging. Identifying the total integrated absorption per molecule $S/N_{\text{tot}}$ with the peak cross section of a single absorber divided by the peak value of the homogeneous line shape function at low temperature (eq. (2.3)), yields the expression

$$\sigma_p(v_0, T) = \frac{cT_2}{2\pi} C_{\text{FC}} C_{\text{DW}}(T) \left( \frac{S}{N_{\text{tot}}} \right),$$

(2.10)

where $C_{\text{FC}}$ and $C_{\text{DW}}$ account for the fraction of the total oscillator strength in the ZPL.

Until now the interaction between light and molecule has been discussed in the limit of very low laser intensities, when a partial net population of the excited states ($S_1, T_1$) is negligible. High laser excitation rates lead to saturation effects of the optical transition. From the steady-state solution of the optical Bloch equations for a three level system (see e.g. [48,49]), the intensity dependences of the linewidth $\Gamma$ (the index “hom” is omitted in the following) and the fluorescence emission rate $R$ for a single absorber can be derived [50],

$$\Gamma(I) = \Gamma_0 \sqrt{1 + I/I_{\text{sat}}},$$

(2.11)
\[ R = R_\infty \frac{I/I_{\text{sat}}}{1 + 1/I_{\text{sat}}} , \]

where \( I \) is the laser intensity, \( I_{\text{sat}} \) the saturation intensity, \( \Gamma_0 = \Gamma(I \to 0) \) is the linewidth at zero power, and \( R_\infty \) the fully saturated emission rate. \( I_{\text{sat}} \) and \( R_\infty \) are given by

\[ I_{\text{sat}} = \frac{\varepsilon_0 \hbar^2}{8\pi^2 |\mu_{12}|^2 T_2} \cdot K = \frac{\hbar \nu_0}{2\sigma_p} \cdot K , \]

\[ R_\infty = \frac{\phi_f}{2} \cdot K , \]

where the factor \( K \) describes the effect of the triplet bottleneck in the three level system (Fig. 2.2). \( K \) replaces \( T_1^{-1} \) in the expressions describing saturation of a two-level system [49] and reads

\[ K = \frac{k_{21} + k_{\text{ISC}}}{1 + k_{\text{ISC}}/2k_T} = \frac{1}{T_1(1 + k_{\text{ISC}}/2k_T)} . \]

The dependences of the emission rate \( R \) and the linewidth \( \Gamma \) on the normalized laser intensity \( I/I_{\text{sat}} \), as calculated from eqs. (2.12) and (2.11) respectively, are displayed in Fig. 2.3. When the experimentally detected count rate is of interest rather than the molecular emission rate, \( R_\infty \) must be multiplied with the overall photon detection efficiency of the apparatus, \( \Lambda_{\text{tot}} \). An elaborate discussion of \( \Lambda_{\text{tot}} \) including the solid angle of collection and all losses is found in ref. [51].

**Figure 2.3:** Intensity dependencies of the emission rate \( R \) (normalized to \( R_\infty \) at \( I \to \infty \)) and of the homogenous linewidth \( \Gamma \) (normalized to \( \Gamma_0 \) at \( I=0 \)).
2.2.3. From statistical fine structure to single-molecule spectra

The principles of the spectral selectivity provided by the inhomogeneous distribution of narrow ZPLs at low temperature and by the tunability of narrow-band lasers are now discussed in more detail. An inhomogeneously broadened absorption line is schematically shown in Fig. 2.4. Near the inhomogeneous line center, where the number $N_H$ of molecules per linewidth $\Gamma$ is large ($N_H \gg 1$), a high-resolution spectrum is characterized by reproducible fluctuations of the absorption (or: fluorescence excitation) signal (wavelength $\lambda_1$ in Fig. 2.4). These fluctuations are called statistical fine structure (SFS) [52]. The SFS is caused by fluctuations of the number of molecules per frequency interval in the excited volume. The relative rms-amplitude of these fluctuations is approximately equal to $(N_H)^{-1/2}$ (provided $N_H \gg 1$) and thus decreases with an increasing number of absorbers. Hence, SFS measurements allow for the determination of the spectral number density.

**Figure 2.4:** Inhomogeneous broadening and spectral selection of a single molecule. A high-resolution laser scan in the center of the inhomogeneous line shows SFS (wavelength $\lambda_1$). In the wing of the inhomogeneous distribution (wavelength $\lambda_2$), a high-resolution scan reveals single-molecule lines.
The cross correlation of two reproducible frequency scans showing SFS decays with a half-width that corresponds to the average homogeneous linewidth of the underlying ensemble of molecules. Assuming a Lorentzian line shape for each absorber, this cross-correlation is also Lorentzian with an FWHM of $\langle 2\Gamma_{\text{hom}} \rangle$, where the brackets indicate an ensemble average. Investigations of the fine structure can be regarded as a complementary method to spectral hole burning [43], when high-resolution spectroscopic information of an ensemble is required. In contrast to hole burning, the fine structure signal results from the excitation of photo-stable molecules.

In the wings of the inhomogeneous band, the spectral density of absorbers becomes smaller and smaller and eventually reaches $N_H < 1$ (wavelength $\lambda_2$ in Fig. 2.4). In this case, a high-resolution spectrum is characterized by well separated Lorentzian peaks which correspond to absorption lines of individual molecules. From this point of view, a single-molecule spectrum is simply the limit of SFS at very low spectral density. The amplitude of the intensity fluctuations is highest for a single-molecule line.

### 2.3. Requirements for SMS

Based on the knowledge acquired in the preceding section, the requirements for optical isolation of a single impurity molecule in a solid can be formulated. Concisely spoken, SMS is accomplished by selecting experimental conditions such that only one molecule is in resonance in the probed volume at a time. In addition, the guest–host pair and the detection technique must be chosen such that the optical signal from one molecule is observed with a sufficient signal-to-noise within a reasonable time. The requirements that make SMS possible can be divided in an experimental and a photophysical part.

#### 2.3.1. Experimental requirements

The experimental requirements aim in the isolation of one individual dopant in the sample and in the optimization of the collection efficiency for emitted photons. The probed volume is drastically reduced by utilizing the high spatial coherence of laser light to make the excitation spot very small. This requires focusing optics of a high numerical aperture or the use of optical fiber tips as illumination sources. Nowadays, confocal optical setups are often used to achieve a diffraction limited optical resolution, and in parallel a high fluorescence collection efficiency. At room
temperature, confocal techniques yield excitation volumes of around 0.1 $\mu$m$^3$. The low-temperature setup used in this work is based on a microscope objective incorporated into the sample holder and located inside the cooling liquid. The laser spot diameter was about 2 $\mu$m resulting in a probed volume of about 10 $\mu$m$^3$. The emission is collected with the same objective at a high collection angle. The number of chromophores in this volume is further reduced by using diluted samples, concentrations are typically in the range of $10^{-8}$ M to $10^{-5}$ M. The ultimate step of dilution benefits from the spectral selectivity provided by the inhomogeneous broadening and a small ratio of homogeneous to inhomogeneous linewidth. To make use of narrow ZPLs, high-resolution SMS is done at temperatures around 2 K using superfluid liquid helium for cooling. The high spectral selectivity for ZPLs at cryogenic temperatures can only be exploited if the exciting laser is tunable over a frequency range that exceeds by far the inhomogeneous linewidth and if the laser bandwidth is much narrower than the homogeneous linewidth. Fortunately, single-mode laser systems which meet these requirements are commercially available. In this work, a Ti:Sapphire laser with less than 1 MHz bandwidth and a tuning range of more than 100 nm was used.

### 2.3.2. Photophysical requirements

The photophysical requirements aim in the optimization of the fluorescence emission so that it exceeds all background. Two quantities are of crucial importance for a strong single-molecule signal: the rate of fluorescence and the total number of photons that a molecule can emit on average. The total amount of emitted energy is limited by the photo-stability of the guest – host pair. At cryogenic temperatures, a termination of fluorescence emission may occur due to sudden conformational changes of matrix molecules in the local vicinity of the chromophore, which lead to a shift of the absorption line away from resonance with the exciting laser. Such processes may be of various origins and are generally difficult to quantify. The fluorescence rate is determined by three photophysical parameters: the peak absorption cross section $\sigma_p$, the quantum yield of photon emission per absorption event $\phi_f$, and the maximum emission rate $R_{\infty}$ at very high excitation power.

The peak absorption cross section (eq. (2.6)) is high when the linewidth is narrow, i.e. when dephasing of the optical excitation is very slow. As a consequence, the narrowest molecules give rise to the most intense signals and are thus preferentially detected. One must be aware that in general only a particular group of molecules, namely the narrowest ones, are studied in an experiment. When statistical conclusions are drawn from measurements of many single-molecule lines, e.g. when a linewidth distribution is determined, one must assure that the data are representative for a statistical
ensemble. Further, the quantum yield \( \phi_F \) should be as high as possible in the case of fluorescence detection, ideally close to unity. Increasing the laser power generates more signal, but only as long as the optical transition is not saturated. At saturation, a further power increase produces more background rather than signal. The saturation intensity (eq. (2.13)) is governed by bottlenecks in the optical pumping cycle (see Fig. 2.2). In organic dyes, intersystem crossing from the singlet to the triplet manifold is the most important bottleneck. This leads to so-called photon bunching of the single-molecule emission (Fig. 2.5). “Bright” periods of fluorescence emission, which have an average duration of the inverse of the intersystem crossing rate, alternate with “dark” periods enduring the triplet lifetime. Minimization of the triplet bottleneck is accomplished with a minimization of the intersystem crossing rate \( k_{\text{ISC}} \) and a maximization of the total triplet decay rate \( k_T \). Recalling eqs. (2.13) and (2.15), this is equivalent to a high saturation intensity \( I_{\text{sat}} \).

**Figure 2.5:** Schematic representation of the temporal behavior of photon emission from a single quantum system. On the scale of the triplet lifetime photon bunching occurs. On the scale of the inverse of the Rabi frequency there is antibunching.

To date, only a small number of systems has been found that fulfil all these requirements. All guest chromophores stem from the class of rigid, planar aromatic hydrocarbons which have widely delocalized \( \pi \)-electrons. Such molecules in common have strong singlet – singlet absorption, high emission yields and weak triplet bottlenecks. In addition, they feature a high Franck-Condon factor of the 0-0 electronic transition. The choice of the host is generally dictated by the need of a weak electron – phonon coupling and by taking care of a high photo-stability. An overview of the guest – host systems investigated so far is found in refs. [11,12]. Depending on the type of experiment another system is chosen. Quantum optical studies on single molecules have been performed with dibenzanthanthrene in hexadecane [53] where ISC is almost absent, and with terylene doped p-terphenyl crystals [54]. Studies using single molecules as probes of host dynamics have preferably been done with terylene and perylene in Shpol’skii systems [55,56], terylene and di-tertbutyl-terrylene in polymers [25], or with terylene in isotopically mixed naphtalene crystals [26]. Recently,
more sophisticated systems such as bichromophores [26] have been subject to single-molecule investigations at low temperatures.

2.3.3. Proves for the detection of one individual molecule

What kind of experiment can distinguish whether one or several molecules are probed? How does the experimentalist know that he/she looks at only one single molecule? In everyday experiments, the experimentalist just knows by experience if the detected signal originates from a single molecule or from more than one. However, two types of experimental features exist that are unique to single quantum systems.

Photon statistics

On the time scale of the excited state lifetime, the statistics of photon emission from a single quantum system show photon antibunching (see e.g. ch.14 in ref. [48]). Antibunching means that the joint probability for two photons to arrive at the detector at the same time is vanishing. This effect is uniquely quantum-mechanical and is understood as follows. After emission of a photon the molecule is definitely in the ground state and cannot emit a second photon immediately. On average half of a Rabi period \(^{(ii)}\) must pass until the probability for emission of another photon is appreciable. The measurement of photon antibunching provides an unequivocal prove for that only one single molecule is excited. Antibunching of photon emission has been reported for single molecules of pentacene in p-terphenyl [57] and for terrylene in p-terphenyl [58].

On/off fluorescence blinking

Photo-bleaching of a single molecule appears as an instantaneous drop of the fluorescence emission to the zero- (i.e. background-) level. Such a process is often reversible, resulting in a discrete on/off blinking of the single-molecule fluorescence. In contrast, when more than one molecule is probed, several intensity levels are present which are proportional to the number of molecules in resonance. Single-step photo-bleaching and on/off blinking have been observed in many single-molecule experiments. In high-resolution spectroscopy at low temperature, the blinking is caused by discrete frequency jumps of the molecular absorption line in and out of resonance with the exciting laser. If this process is reversibly repeated, the fluorescence signal appear as kind of

\(^{(ii)}\) The Rabi frequency is defined by the scalar product of the transition dipole moment and the exciting electric field amplitude divided by Planck’s constant: \(2\pi\mu_{12}E_{\text{Laser}}/\hbar\), where 1 and 2 denote the ground and excited states, respectively.
"telegraph signal" that jumps between the background level ("0") and a certain number of photo counts ("1"), when the laser is at fixed frequency [59]. At scanning excitation frequency, single-molecule resonance frequency jumps could be traced over many subsequent scans and a "frequency trajectory" could be recorded [55]. Investigations of the blinking behavior of single molecules at room temperature allow for the detailed study of diffusing quenchers in organic crystals [60], of the photophysics in luminescent proteins [28], or the dynamics of enzymatic turnovers [18] – just to mention a few examples.

2.4. Optical line shape of single molecules

In a solid host, the resonance frequency $\omega(t)$ of a SM optical transition can be given as the sum of a static and dynamic component, $\omega_0$ and $\omega'(t)$ respectively. $\omega_0$ includes the molecular vacuum transition frequency and the static solvent shift. Because of lattice inhomogeneities of the host matrix, $\omega_0$ changes from molecule to molecule, giving rise to the inhomogeneous broadening. Line narrowing techniques, such as spectral hole burning, can remove the inhomogeneous broadening. However, the line shape contribution due to $\omega'(t)$ cannot be resolved by such techniques. $\omega'(t)$ results from the dynamic processes in the molecule’s environment and was assumed identical for each chromophore in the past. Accordingly, the effect of broadening due to dynamic frequency components was termed homogeneous line broadening. SMS has established that not only $\omega_0$ but also $\omega'(t)$ changes from molecule to molecule, so that each molecule has its own characteristic dynamic line shape. Moreover, the frequency fluctuations $\omega'(t)$ cause differences between two consecutive spectra of one single molecule, even if the chromophore is in thermodynamic equilibrium with its environment. Thus, the concept of a homogeneous line shape is very troublesome if applied to individual absorbers.

A standard approach to single-molecule spectral fluctuations is based on the fluctuation-dissipation theorem — one of the main results of non-equilibrium statistical mechanics (see ref. [61] for an introduction). This theorem relates the linear response of a driven system (such as the relaxation or the rate of absorption) to the correlations of the equilibrium fluctuations in this

(iii) on the time scale of the experiment
Thus, the fluctuation-dissipation theorem relates macroscopic (ensemble averaged) parameters, which describe the response to an initial disturbance, to the nature of microscopic (single-molecular) dynamics in the equilibrium state. The concepts of linear response theory combined with the fluctuation-dissipation theorem provide the important result that the absorption spectrum of an electric dipole transition is given by the Fourier transform of the electric dipole autocorrelation function [61]. This is used in the optical line shape theory developed by Kubo [62]. We note that the calculation of a Fourier transform or of a correlation function is related with an infinitely long time average or with an ensemble average. However, neither kind of signal averaging is realized with a single-molecule spectrum. Therefore, we are aware of the approximate nature of the following standard approach to single-molecule line shapes. Its weak points and limitations will be discussed in paragraph 2.4.5.

2.4.1. Line shape of a frequency modulated oscillating dipole

This paragraph covers the theoretical background of a dynamic SM line shape, based on the stochastic line shape theory by Kubo [62]. The equation of motion of a dipole oscillating at a randomly modulated frequency $\omega(t)$ is

$$\frac{d\mu}{dt} = i\omega(t)\mu. \quad (2.16)$$

The frequency $\omega(t) = \omega_0 + \omega'(t)$, where $\langle \omega' \rangle_t = 0$, follows a random trajectory which is assumed stationary in time and ergodic. The modulation $\omega'(t)$ has a correlation time $\tau_c$, i.e. the correlation $\langle \omega'(t)\omega'(t+\tau) \rangle$ vanishes for $\tau > \tau_c$. $\tau_c$ thus measures the "speed of modulation". The phase of the dipole moment $\mu$ at time $t$ becomes

$$\mu(t) = \mu(0)e^{i\omega_0 t}\exp\left\{\int_0^t i\omega'(\tau)d\tau\right\}. \quad (2.17)$$

The autocorrelation function of the dipole moment is

(iv) The fluctuation-dissipation theorem was proved by Callen and Welton in 1951. An important consequence of this profound theorem was enunciated already in 1930 by Lars Onsager in his regression hypothesis: "The relaxation of macroscopic non-equilibrium disturbances is governed by the same laws as the regression of spontaneous microscopic fluctuations in an equilibrium system." The "regression of spontaneous microscopic fluctuations" in the words of Onsager corresponds to the decay of the correlation function of a temporally fluctuating property.
\[ \phi(t) = \int_{-\infty}^{\infty} \mu(t+\tau)\mu^*(\tau) d\tau = \langle \mu(t)\mu^*(0) \rangle_t. \] (2.18)

\( \phi(t) \) is also called the relaxation function of the oscillator. By application of the ergodic theorem, the time average \( \langle \cdot \rangle_t \) in eq. (2.18) can be replaced by an ensemble average \( \langle \cdot \rangle_{\text{ens}} \) over all realizations of the random trajectory \( \omega'(\tau) \) during the time interval \([0,t]\). Using eq. (2.17) \( \phi(t) \) can be written as

\[ \phi(t) \sim \langle \exp\left\{ i \int_{0}^{t} \omega'(\tau) d\tau \right\} \rangle_{\text{ens}}. \] (2.19)

As a consequence of the fluctuation-dissipation theorem, the resonant absorption spectrum at the frequency \( \Omega \) is given by the Fourier transform of the electric dipole autocorrelation function \( \phi(t) \) [62-64], and the line shape function \( \text{I}(\Omega) \) reads

\[ \text{I}(\Omega) = \text{Re}\left\{ \int_{0}^{\infty} e^{-i(\Omega - \omega_0)t} \phi(t) dt \right\}. \] (2.20)

**Markovian frequency modulation**

In the following, the random modulation \( \omega'(t) \) is assumed to be a stationary and ergodic Markovian process and the relaxation function \( \phi(t) \) is calculated in the equilibrium state. In this model, the oscillator takes \( r \) discrete states \( S_1, S_2, ..., S_r \) with resonance frequencies \( (\omega_0 + \omega'_j) \) and probabilities \( P_j(t) \) that the oscillator is found in \( S_j \) at time \( t \). The Markovian frequency modulation \( \omega'(t) \) proceeds in instantaneous jumps between values \( \omega'_j \) and \( \omega'_k \). The probability of a jump to a certain state \( S_k \) only depends on the state immediately before the jump. \( P_{jk}(t) \) is the probability that the oscillator is in state \( S_k \) after the time \( t \) when it was in state \( S_j \) at \( t = 0 \). A Markovian process is defined by the Chapman-Kolmogorov equation [63]

\[ P_{jk}(t) = \sum_m P_{jm}(t - \tau)P_{mk}(\tau). \] (2.21)

By letting the jumps become infinitesimally small, the Markovian process can be made a continuous process, e.g. a Gaussian modulation.

Defining the transition matrix \( \gamma \), with \( \gamma_j^{-1} \) being the lifetime of state \( S_j \) and \( -\gamma_{jk} \) corresponding to the transition rate from state \( S_j \) to \( S_k \), eq. (2.21) is transformed into a differential equation for the matrix \( (P_{jk}) \).
The normalized row vector \( \mathbf{W} = \begin{bmatrix} p_{1}^{\text{eq}} & \cdots & p_{n}^{\text{eq}} \end{bmatrix} \) represents the equilibrium distribution of oscillator states. According to the detailed balance condition \( \mathbf{W} \) is the unique solution of the equation 

\[
\mathbf{W} \cdot \mathbf{\gamma} = 0.
\]

The unit column vector \( \mathbf{1} \) correspondingly satisfies \( \mathbf{\gamma} \cdot \mathbf{1} = 0 \). An equation similar to eq. (2.22) can be found for the time evolution of \( \phi(t) \) under the process defined by eq. (2.21). At thermal equilibrium \( \phi(t) \) is given by

\[
\phi(t) = \mathbf{W} \cdot \exp \{ t(i\chi - \gamma) \} \cdot \mathbf{1},
\]

where \( \chi \) is a diagonal matrix with elements \( \omega_j \). After some transformations, the line shape function under Markovian frequency modulation is found [62]:

\[
I(\Omega) \sim \text{Re} \{ \mathbf{W} \cdot (i(\Omega - \chi) + \gamma)^{-1} \cdot \mathbf{1} \},
\]

where \( \Omega - \chi \) denotes the diagonal matrix with elements \( \Omega - \omega_j \).

**The Lorentzian approximation**

We consider a single molecule with dephasing time \( T_2 \). For times \( t \gg \tau_c \), where \( \tau_c \) is the correlation time of the frequency modulation, the dipole moment autocorrelation function \( \phi(t) \) takes its asymptotic form [62]

\[
\phi(t) \to \exp \{- (\frac{1}{T_2} + i\xi) t \} \ (t \gg \tau_c).
\]

The correlation is damped with the time constant \( T_2 \), and \( \xi \) denotes a frequency shift. Fourier transformation of \( \phi(t) \) yields the corresponding spectral line \( I(\Omega - \omega_0) \) which is Lorentzian in the range \( |\Omega - \omega_0| < \tau_c^{-1} \),

\[
I(\Omega) \sim \frac{1/T_2\pi}{(\Omega - \omega_0 - \xi)^2 + T_2^{-2}}.
\]

As long as the interval \( (\omega_0 \pm \tau_c^{-1}) \) covers the important region of the spectral intensity distribution, eq. (2.26) is a useful approximation.

The approximation (2.26) can also be formulated from a more practical point of view. The line shape obtained from eqs. (2.19) and (2.20) is by definition time independent. Therefore, in a real experiment enduring a measuring time \( t_m \), eq. (2.20) is only appropriate if the correlation time \( \tau_c \) of
the frequency modulation is shorter than $t_m$. Under the condition $\tau_c < t_m$, $\phi(t)$ decays exponentially for $t \gg \tau_c$ and $I(\Omega - \omega_0)$ is thus well approximated by a Lorentzian for $|\Omega - \omega_0| < \tau_c^{-1}$.

### 2.4.2. Coupling of a single molecule to random processes

In solids, resonance frequency modulations $\omega'(t)$ of a single-molecule optical transition occur due to coupling of the chromophore to random processes in its environment. Because the dopant chromophore occupies a substitutional position, local vibrations and conformational rearrangements of the lattice play an important role. Of particular interest here is the coupling to tunnelling two-level-systems (TLSs) and to localized lattice modes (local phonons) of the locally disordered host [65,66]. TLSs, unspecified lattice imperfections flipping randomly between two states, induce SM frequency fluctuations on many time scales. They cause the phenomenon of spectral diffusion – a time dependent broadening of the transition. Depending on the host properties, the SM linewidths increase on a logarithmic time scale limited only by the time of the experiment [67]. TLSs may be grouped into two classes: intrinsic TLSs, present without the guest molecule, and TLSs generated by the dopant chromophore. Intrinsic TLSs were postulated to explain the acoustic and thermodynamic properties of amorphous solids and have been adopted later to describe optical phenomena [68,69]. Local lattice vibrations also contribute to the dynamic processes in the molecule's environment. These vibrations are primarily librational modes of the guest molecule within the cage formed by the surrounding matrix molecules. These modes often dominate the line shape at very low temperatures [42,63-64,70-73]. The excited state population of the local phonon mode is usually small, and in such a picture it behaves like a TLS. It thus appears difficult to unambiguously discriminate between local modes and TLSs generated by the dopant.

The TLS model [65-66,68] describes a solid as a set of independent double-well potentials. Each TLS is defined by three parameters: energy splitting, relaxation time and coupling strength with the chromophore, where the TLS – chromophore interaction is assumed to be dipolar. The structural disorder in the host is expressed by distributions of these parameters (v). At low temperatures transitions between the lower and upper state of a TLS are mainly due to phonon-assisted tunnelling. However, the exact nature of the TLSs is basically unknown. According to the sudden jump model

---

(v) The "standard TLS model" which was developed by Anderson et al. [65] and Phillips [66] suggests a constant distribution for all parameters and has been successfully applied to amorphous solids. An ideal crystal does not have any TLSs by definition. In real crystals however, TLSs must be taken into consideration, but their parameters do not necessarily follow a standard model distribution.
Accordingly, $\omega'(t)$ follows a Markovian process [62-64] defined by

$$\omega'(t) = \sum_j \xi_j(t)v_j.$$  

In this equation $\xi_j(t)$ is a stochastic function with correlation time $\tau_{c,j}$ and taking values -1 or +1 for the lower or upper state of the j-th TLS, respectively. The up and down flip rates are governed by the energy splitting and the relaxation rate of the TLS. The sum in eq. (2.27) runs over all TLSs. The phase of the molecular transition dipole moment becomes randomly modulated and its temporal evolution is given by eq. (2.17). An example of TLS–chromophore interaction and the resulting SM frequency trajectory is schematically shown in Fig. 2.6. It must be emphasized that the TLS model and eq. (2.27) are in principle only applicable at thermal equilibrium. In non-equilibrium systems, such as glasses, additional relaxation processes occur which are not described by independently flipping TLSs. In such systems, structural re-orientations occur within a very complicated potential whereof the TLSs are only a particular contribution. However, these relaxation processes usually

![Figure 2.6](image_url)

**Figure 2.6:** (a) Interaction of a chromophore with two TLSs. (b) SM frequency trajectory which, according to the sudden jump model, evolves in discrete steps. In this example, TLS #1 has a high flip rate $\gamma_1$ and a small coupling to the chromophore, thus inducing frequency fluctuations with a small amplitude $v_1$ and a short correlation time $\tau_{c,1}$ $\sim \gamma_1^{-1}$. TLS #2 has a low flip rate $\gamma_2$ and causes a large frequency shift $v_2$. Depending on the time of measurement ($t_1$, $t_2$, $t_3$) different portions of the frequency trajectory are probed in the experiment, resulting in different SM line shapes.
take place on a very long time scale, and for short measuring times the TLS model still represents a reasonable approximation [75].

A spectrum is only correctly described by eq. (2.20) when measured for an infinitely long time. However, any real experiment starts at a certain time $t_0$ and takes a finite measuring time $t_m$. This can be accounted for by multiplying the integrand in eq. (2.20) with the heavyside function $\Theta_{t_m}(t_0,t)$, which takes values 1 for $t_0 \leq t \leq t_0 + t_m$ and 0 for all other times. The line shape function is thus modified to

$$I(Q-\omega_0,t_0,t_m) = \text{Re} \left\{ \frac{1}{2\pi} \int_0^\infty e^{-i(\Omega-\omega_0)t} \exp \left\{ \int_{t_0}^t i\dot{\omega}(\tau) d\tau \right\} \Theta_{t_m}(t_0,t) dt \right\}. \quad (2.28)$$

In contrast to eq. (2.19), the dipole moment autocorrelation is not ensemble averaged here since eq. (2.28) refers to a single-shot SM experiment. In this case, only one specific realization of the stochastic trajectory $\omega'(\tau)$ (eq. (2.27)) contributes to $I(Q-\omega_0,t_0,t_m)$. It must be emphasized that eq. (2.28) represents the SM spectrum accurately only if the correlation time of the frequency fluctuations is much shorter than the duration of the experiment, $\tau_c \ll t_m$. Only under this condition the truncation function $\Theta_{t_m}(t_0,t)$ does not affect the result of the Fourier transformation in eq. (2.19), and the line shape is Lorentzian in the frequency range $|\Omega - \omega_0| < \frac{1}{t_m}$ as already mentioned above.

In general, however, the TLS parameters and thus the correlation times $\tau_{c,j}$ follow broad distributions. As a consequence, $I(Q)$ is in general a time dependent non-Lorentzian function which is not described by a single parameter such as a linewidth. When using the approach based on eq. (2.28), further approximations about TLS relaxation rates are required. However, such approximations may raise serious problems [76]. In order to obtain a proper expression for the SM line shape $I(Q)$ in the case of a scanning excitation source, the optical Bloch equations of the molecule (see refs. [77],[48]) should be solved in the presence of a random modulation of the transition frequency.
2.4.3. Spectral diffusion and spectral jumps

In practical data analysis, the observed time dependent line shape is usually fitted to a Lorentzian. Within this approximation, SM coupling to TLS dynamics is described by a shift of the center frequency and an effective linewidth $\Gamma$ which depends on the measuring time $t_m$. The time dependence of $\Gamma$ is artificially introduced to account for a distribution of TLS relaxation rates over a wide range with respect to $t_m^{-1}$, and to overcome the limitations of the Lorentzian approximation (2.26) which only allows for TLSs flipping much faster than $t_m^{-1}$.

The term spectral diffusion is used to designate line broadening due to fast TLSs. Slow dynamics which move the center frequency of the Lorentzian line by more than $\Gamma$ are termed spectral jumps (for examples see ref. [55]). “Spectral diffusion” and “spectral jumps” have the same origin and are phenomenologically based terms to introduce different time scales into a theoretical treatment which is in principle time independent. $t_m$ acts as a reference time. This phenomenological description of SM spectral dynamics is very perceptual and has been widely applied [11, 55, 69]. However, when investigating SM lines at different time resolutions (see chapter 7), one must be aware that the concept of a time dependent effective linewidth and a time dependent transition frequency maximum is just an approximation which does not yield the correct line shape function.

2.4.4. Temperature dependence

The frequency modulation due to TLSs of any nature is of crucial importance for excited-state relaxations and thus for linewidths at low temperatures. In the following we approximate the SM line by a time independent Lorentzian function with an effective width $\Gamma_{\text{eff}}$. In glassy and polymeric hosts at low temperatures ($0.4 \leq T \leq 4 \text{K}$), the temperature dependence of the linewidth follows a power law $\Gamma_{\text{eff}} \propto T^\alpha$ with $1.2 \leq \alpha \leq 1.7$. This is characteristic of TLS-induced broadening (assuming a distribution of TLS parameters according to the “standard model” [65, 66]). Above $2 - 5 \text{ K}$, depending on the system, an additional exponentially activated term contributes to $\Gamma_{\text{eff}}$ which results from SM coupling to local lattice modes [69]. At even higher temperatures, bulk phonon scattering becomes relevant which causes line broadenings following a $T^7$ law [78]. Thus, the effective linewidth is

$$\Gamma_{\text{eff}}(T) = aT^\alpha + b \exp \left[ -\frac{E}{kT} \right] + cT^7. \quad (2.29)$$
In crystalline systems, the TLS energies are not so widely distributed as in amorphous ones and they are generally much lower than local phonon energies. Hence, only the exponential term in eq. (2.29) is important between ~1 K and ~10 K. In organic mixed crystals the lowest local mode usually dominates below 20 K, and the temperature dependence of $\Gamma_{\text{eff}}$ is described by a single activation energy $E$ which is typically in the range $10 \text{ K} < E/k < 40 \text{ K}$.

In the following, we consider the case of a single TLS with a correlation time much shorter than the lifetime $T_1$ of the electronic excited state. This clearly holds for local phonons. Hence, a time independent Lorentzian is a reasonable approximation for the SM line shape. In case of a single TLS the dynamics of the molecular transition can be described by an effective 4-level system, as shown in Fig. 2.7. At thermal equilibrium the excitation rate $W_e$ and relaxation rate $W_r$ of the TLS state are related as $W_e/W_r = W_e \tau = \exp(-E/kT)$ (detailed balance condition). Because of different frequencies for the molecular transitions $(0,0) \to (1,0)$ and $(0,1) \to (1,1)$, flips between the two TLS states are associated with random frequency jumps of $\pm \delta$. Correspondingly, $\omega'(t)$ follows a kind of telegrapher function. This modulation causes a temperature dependent center frequency shift and broadening of the line shape, which is found by solving eq. (2.24) for the processes illustrated in Fig. 2.7. In this case, eq. (2.24) reads [73]

$$I(\Omega) \sim \text{Re}\{\vec{W} \cdot \mathbf{A}^{-1} \cdot \mathbf{1}\}, \quad (2.30)$$

where the matrix $\mathbf{A}$ and the vector of equilibrium populations, $\vec{W}$, are given by

![Figure 2.7](image-url)

**Figure 2.7:** Effective four-level system of a probe molecule interacting with one TLS. In states $(0,0)$ and $(1,0)$ the TLS is in its ground state. $(0,1)$ and $(1,1)$ correspond to excited TLS states. $E$ and $E+h\delta$ are the TLS energies in the ground and excited molecular state respectively. $W_r = \tau^{-1}$ and $W_e$ denote the relaxation and excitation rates of the TLS.
\[
A = i(\Omega - \chi) + \gamma = \begin{bmatrix} i(\Omega - \omega_0) + W_e & -W_e \\ -W_r & i(\Omega - \omega_0 - \delta) + W_r \end{bmatrix},
\]

(2.31)

\[
\vec{W} = \begin{bmatrix} W_r \\ W_e + W_r \\ W_e + W_r \end{bmatrix}.
\]

(2.32)

Near resonance \((\Omega \approx \omega_0)\) the line shape can be approximated by a Lorentzian by setting \(\Omega - \omega_0 \approx \delta \approx \delta\) [72], resulting in

\[
I(\Omega) = \frac{1}{1 + \left( \frac{W_e \delta \tau}{1 + \delta^2 \tau^2 + 2W_e \tau} + \omega_0 - \Omega \right)^2} \left( \frac{1 + \delta^2 \tau^2 + 2W_e \tau}{(W_e \delta \tau^2)^2 + 2W_e} \right).
\]

(2.33)

In the low temperature approximation \((kT \ll E)\) we have \(W_e \tau \ll 1\), and approximate expressions for the line broadening and frequency shift are [70,73]

\[
\Delta \Gamma = \Gamma - \Gamma_0 = 2\tau \delta \left[ \frac{\delta/2\pi}{1 + \tau^2 \delta^2} \right] \cdot W_e \tau \equiv \beta_\Gamma \cdot W_e \tau,
\]

(2.34)

\[
\Delta \nu = \nu - \nu_0 = \frac{\omega(\mathcal{T}) - \omega_0}{2\pi} = \left[ \frac{\delta/2\pi}{1 + \tau^2 \delta^2} \right] \cdot W_e \tau \equiv \beta_\nu \cdot W_e \tau,
\]

(2.35)

where \(\Gamma_0\) and \(\nu_0\) are the linewidth and frequency at \(T=0\ K\). Note that \(\nu, \Gamma\) are in units of Hz, whereas \(\delta, \omega\) are angular frequencies in rad-s\(^{-1}\). Introducing the detailed balance condition \(W_e \tau = \exp(-E/kT)\) into eqs. (2.34) and (2.35), yields

\[
\Delta \Gamma(\mathcal{T}) = \Gamma(\mathcal{T}) - \Gamma_0 = \beta_\Gamma \cdot \exp\left\{-\frac{E}{kT}\right\},
\]

(2.36)

\[
\Delta \nu(\mathcal{T}) = \nu(\mathcal{T}) - \nu_0 = \beta_\nu \cdot \exp\left\{-\frac{E}{kT}\right\}.
\]

(2.37)

Eqs. (2.36) and (2.37) have been shown to be very useful for the description of the temperature dependent line broadening and line shift of optical transitions at very low temperatures. It is important to notice that eqs. (2.36) and (2.37) are only correct at low temperature where the prefactors \(\beta_\nu\) and \(\beta_\Gamma\) are temperature independent. If \(kT \geq E\), these equations are no longer valid and the full expression (2.33), which includes the temperature dependencies of \(\beta_\nu\) and \(\beta_\Gamma\), must be used.
Regarding the ratio $|\Delta \Gamma / \Delta \nu| (=2|\delta|)$ three regimes can be distinguished \[72,73\]. In the "fast exchange" regime ($|\delta| < 1$) the broadening is reduced and the shift to broadening ratio is maximized, in the "slow exchange" regime ($|\delta| \gg 1$) broadening and shift are negligibly small, while in the "intermediate exchange" regime ($|\delta| \sim 1$) frequency-shift and linewidth changes are of similar magnitude.

De Bree and Wiersma \[79\] examined the limitations of the approach outlined above and pointed out that eqs. (2.36) and (2.37) are only appropriate under the following conditions: (a) The local phonon scattering amplitudes must be identical in the molecular ground and excited state. This leads to equal lifetimes $\tau_{(0,1)} = \tau_{(1,1)} = \tau$ of the (0,1) and (1,1) states (Fig. 2.7) and to a simple interpretation of the shift ($\beta_\nu$) and broadening ($\beta_\Gamma$) factors in terms of $\tau$ and $\delta$. (b) In the limit of intermediate to fast exchange ($|\delta| \leq 1$). (c) In the limit of low temperature ($W_\nu \tau \ll 1$), as already stated above.

2.4.5. Concluding remarks

A theory based on a Fourier transformation is by definition only suitable to describe the stationary properties of a system. With regard to SM line shapes, this means that the spectral dynamics must exclusively occur on time scales either much shorter than the optical transition rate or much longer than the measuring time. The first case applies for example to the phenomenon of optical exchange when the electronic transition is coupled to a low-energy mode. This has been discussed above in the context of the temperature dependence of linewidth and center frequency. In the second case, when the time scale of any spectral dynamics exceeds by far \[vi\] the experimental time scale, these dynamics are just not relevant and the optical line shape is negligibly affected by a truncation of the time domain Fourier transformation. However, for a molecule interacting with a fluctuating environment, the standard approach based on eqs. (2.19) and (2.20) fails in principle – though it is often a useful approximation. SM frequency fluctuations generally occur on a wide time scale, and any approximation to account for finite measuring times leads to discrepancies between experimental results and theory \[76\]. Another claim is related to the irreproducibility of two consecutive spectra of one and the same molecule, even when the molecule is in thermodynamic equilibrium with the environment. A theory based on the fluctuation-dissipation theorem, which includes an averaging of the transition frequency over all stochastic realizations (eq. (2.19)), does not

\[vi\] As a rule of thumb at least by a factor of 10
account for such irreproducibilities. In conclusion, there is a need for a real time-dependent approach to SMS. A new technique for time resolved SMS, called Intensity-Time-Frequency-Correlation, will be introduced in chapter 7.
3. EXPERIMENTAL AND INSTRUMENTATION

The experimental apparatus is introduced, including a description of the optical setup, the laser light sources, the cryostats and the detector. Emphasis is placed on the design and characteristics of a reference cavity, which has been made as a frequency standard for the measurement of single-molecule spectral shifts. By means of a numerical simulation, the photon collection efficiency of the optical setup is examined. Further, a short introduction is given into chromophore-matrix systems of the Shpol’skii type and the system under investigation, DPOT in n-tetradecane, is presented. The preparation of the samples is described and their preliminary characterization by means of microscope images is shown.
3.1. EXPERIMENTAL SETUP

3.1.1. Optical setup

The setup used for one- and two-photon excitation single-molecule experiments is shown in Fig. 3.1. The sample is located in a liquid helium cryostat at a temperature of about 1.7 K. The $S_0 \rightarrow S_1$ zero-phonon line of the probe molecules can be selectively excited either by one or two photons using a single-mode Ti:Sapphire laser (CR899-29) emitting around 888 nm and an external resonator (LAS, Wavetrain) for second harmonic generation. Laser frequency scans are produced by a ramp generator (analog: Stanford Research Systems, DS345, or digital using an IO Tech Dig488 input/output interface). The IR and the blue beam are both vertically polarized and the excitation polarization may be varied using the polarization rotator (Newport, PR-550). The two laser beams are focused to the same spot of about 2 μm diameter with a microscope objective (Newport, 60x NA=0.85) which is integrated into the sample holder (Fig. 3.2). The objective can be approached to and withdrawn from the sample by a stepper motor which operates in superfluid helium. The design of the sample holder has been described in detail in ref. [56]. The fluorescence emitted by the sample is collected by the same objective. This confocal arrangement provides a nearly diffraction limited excitation volume, a high collection efficiency and a low background. An aperture of 75 μm diameter is placed in the conjugate focal plane of the objective outside the cryostat. The magnification in this plane is about 40x. Only light emitted from the same sample volume $V$ of about 10 μm$^3$ independent of the excitation process is collected. The 2 μm diameter of $V$ is given by the aperture’s projection inside the sample, the depth of $V$ is defined by the size of the laser waist. Moreover, this aperture is indispensable for background reduction in one-photon excitation experiments. Laser light scattered by the sample is rejected using a holographic notch filter (Kaiser, HNPF-457 for blue light) and cut-off filters (Schott, KV-470 for blue light and KC-17, KC-19$^{(i)}$ for IR light). A Peltier cooled photomultiplier (PM, Hamamatsu R4220P) is used for single photon counting of the fluorescence and the signal is recorded as a function of the laser frequency using either a multichannel analyzer (Stanford Research Systems, SR430) or a home-built binary counter. Including the solid angle of collection, optical losses and the quantum efficiency of the detector, the overall quantum yield of photon detection $\Lambda_{tot}$ is approximately 1% [51]. Automation of the experiment includes the synchronization of the frequency ramp generator, the counter and the data storage.

(i) Purchased from Azov’s Optical Mechanical Factory, Ukraine
Experimental Setup

Figure 3.1: Experimental setup for one- and two-photon SMS. Dark and bright thick lines correspond to laser beams at 888 nm (two-photon excitation) and 444 nm (one-photon excitation), respectively. Dashed lines are electronic connections. LPC is a liquid-crystal based laser power controller. BS is a beam splitter, L a lens and PM a Peltier cooled photo-multiplier. The sample holder with microscope objective is sketched in more detail in Fig. 3.2.

Figure 3.2: Detail view of the sample holder. The sample is sandwiched between two microscope cover glasses. It is mounted in the focus of a microscope objective which can be moved with respect to the sample by a stepper motor (not shown here). The excitation light (the case of two-photon excitation, $\lambda = 888$ nm, is shown) is focused through the objective which also collects the emission. This confocal design simultaneously yields a small excitation volume and a high collection efficiency.
More details about the electronics setup are reported in refs. [55] and [56]. In the following paragraphs, the light sources, the reference cavity, the cryostats and the detector are described in more detail.

### 3.1.2. Light sources

An argon ion laser (Coherent, Innova-200), operated in multi-line mode with a maximal output power of 20 W, is used to pump the Ti:Sapphire ring laser (Coherent, CR 899-29). The Ti:Sapphire laser is run in the mid wavelength range tunable from 795 to 905 nm. Single-mode operation is achieved by means of two intra-cavity elements: a birefringent filter (BRF), and an intra-cavity assembly (ICA) consisting of a thin and a thick etalon. Active frequency stabilization of the laser resonator is obtained using fringe locking to an external low finesse invar cavity which is temperature stabilized. The feedback signal is applied to a piezo transducer (PZT) onto which one of the folding mirrors is mounted. The locking yields a bandwidth of less than 500 kHz rms \(^{(ii)}\). A scanning brewster plate and the PZT serve to perform continuous frequency scanning over a range of up to 30 GHz. The locking is stable up to scan rates of 1 GHz/20 ms. A wave meter, which is based on the combination of an optical activity monochromator and a Vernier etalon, measures the laser frequency with an absolute precision of ±200 MHz and a reproducibility of 50 MHz. The BRF, ICA, PZT and Brewster plate are computer controlled, yielding automatic frequency scanning over the entire tuning range (Autoscan operation). Single-mode operation is verified by monitoring the transmission peaks of a Fabry-Perot Etalon (Coherent, model 240) with a free spectral range of 1.5 GHz. Fluctuations in the laser output power are stabilized using a laser power controller (Cambridge Research & Instrumentation, LPC) based on a liquid crystal device. The power available for the experiment was typically 1 W.

Second harmonic generation (SHG) of the laser light is accomplished in an LBO \(^{(iii)}\) crystal which is located in the waist of a commercially available external ring cavity (LAS, Wavetrain). The frequency of this cavity is stabilized to maximum transmission using a PZT mirror. The Wavetrain is passively scanned as a slave of the Ti:Sapphire laser. Its tuning range without mode-hopping is about 3 GHz. The conversion efficiency of SHG is about 5%. The Ti:Sapphire laser system and the Wavetrain cavity for SHG have been described in many details in chapter 3 of ref. [56].

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\(^{(ii)}\) Specified by the manufacturer

\(^{(iii)}\) Lithium triborate, LiB\(_3\)O\(_5\)
3.1.3. Reference cavity

Long term drifts of the laser frequency on the order of 100 MHz per hour are not eliminated by the commercially provided locking techniques described before. Therefore, the laser itself cannot be used as a frequency standard for the measurement of single-molecule spectral dynamics over many hours. Such investigations require a relative accuracy of \( \frac{dv}{v} < 10^{-7} \), corresponding to less than 30 MHz (homogenous linewidth) at an absolute frequency of \( 3.4 \times 10^{8} \) MHz (888 nm). Considering an independent reference cavity of length \( l = 463 \) mm (as used in this work), the variation in length must be less than \( lv/l = 40 \) nm over the duration of the experiment. This shows that the requirements on the thermal expansion and acoustic stability are quite severe.

It has turned out that these requirements are well met by the cavity design sketched in Fig. 3.3. A zerdur tube is chosen for its low thermal expansion. A temperature stabilized copper shield is used as a thermal buffer (\( T = 306 \) K). To reduce mechanical strain forces between the zerdur and copper, the two surfaces are polished and a dry lubricant (Molykote) is applied. A lot of care has to be taken over the gluing of the mirrors. A very thin layer of glue is of crucial importance, in order not to destroy the outstanding thermal expansion properties of zerdur. Therefore, the mirrors are glued under mechanical pressure and all residual glue at the mirror edges is removed. The epoxy glue is chosen so that its glass transition is way above the working temperature. All material properties important for the cavity design are listed in Table 3.1.

**Figure 3.3:** Design of the confocal reference cavity using a zerdur tube.
Table 3.1: Properties of the materials used for the reference cavity.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz mirrors</td>
<td>Neva Technology (St. Petersburg)</td>
<td>Reflectivity ( R = 80% , (750-900 \text{ nm}) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radius ( r = 1 ) (confocal)</td>
</tr>
<tr>
<td>Zerodur tube</td>
<td>Schott (Mainz)</td>
<td>Length ( l = 463 \text{ mm} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermal Expansion ( \alpha = 5 \times 10^{-8} \text{ K}^{-1} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specific heat ( c_p = 0.8 \text{ J/(gK)} )</td>
</tr>
<tr>
<td>Copper shield</td>
<td></td>
<td>Thickness 5 mm, Thermal Expansion ( \alpha = 2 \times 10^{-5} \text{ K}^{-1} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specific heat ( c_p \approx 0.4 \text{ J/gK} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermal conductivity ( \Lambda_{\text{therm}} = 390 \text{ W/mK} )</td>
</tr>
<tr>
<td>Epoxy glue,</td>
<td>CIBA (Basel)</td>
<td>Thermal Expansion ( \alpha = 6 \times 10^{-5} \text{ K}^{-1} )</td>
</tr>
<tr>
<td>Araldit 2019</td>
<td></td>
<td>Glass transition temperature ( T_G = 56.8^\circ \text{ C} )</td>
</tr>
<tr>
<td>Heater foil</td>
<td>Hotsil</td>
<td>80 W, 42 V dc, 22 ( \Omega )</td>
</tr>
</tbody>
</table>

In an everyday experiment, the direction of incidence is typically out of the cavity axis. There are two beams exiting from the cavity, each of them being four times reflected between the mirrors. The beam paths through the cavity are schematically shown in Fig. 3.4. When only one of the two beams is detected, the cavity is characterized by the parameters calculated in eqs. (3.1) – (3.3), where \( I_0 \) and \( \lambda \) denote the intensity and wavelength of the incident beam, respectively, \( l \) is the cavity length and \( R \) the reflectivity of the mirrors.

**Figure 3.4:** Trajectories of rays in a confocal cavity with off-axis and inclined incidence. Two diverging beams exit from the cavity.
Transmitted intensity $I$:

$$I(\lambda) = \frac{I_0 (1 - R)^2}{(1 - R^2)^2 + 4R^2 (\sin(4\pi l/\lambda))^2}. \tag{3.1}$$

Free spectral range FSR and finesse $F$:

$$\text{FSR} = \frac{c}{41} = 162 \text{ MHz} \quad , \quad F = \frac{\text{FSR}}{\Delta\nu} = \frac{\pi R}{1 - R^2} = 7.0 \tag{3.2}$$

Bandwidth (FWHM) $\Delta\nu$:

$$\Delta\nu = \frac{1 - R^2}{4\pi l R} = 7.7 \times 10^{-4} \text{ cm}^{-1} = 23.2 \text{ MHz}. \tag{3.3}$$

The measured cavity transmission fringes at a wavelength of 888 nm are shown in Fig. 3.5. The FSR is 163 MHz and the bandwidth 18 MHz, corresponding to a finesse of 9.06.

![Figure 3.5: Transmission fringes of the reference cavity at 888 nm. FWHM = 18 MHz, FSR = 163 MHz, Finesse = 9.06.](image)

To check for the stability of the cavity, the $6s(6^2S_{1/2}, F=4) \rightarrow 6p(6^2P_{3/2}, F'=4,5)$ hyperfine transition lines of Cs atoms were used as an absolute frequency reference. The optical linewidth of an atomic gas in a vapor cell at room temperature is dominated by Doppler broadening. Saturated absorption spectroscopy (see ch. 10 in ref. [80]) removes the Doppler broadening by enhancing the signal of atoms which have a zero velocity component along the laser beam axis. The experimental setup is shown in Fig. 3.6. The resulting Doppler-free narrow peak is called “Lamb dip”. To understand the Lamb dip we consider a first laser beam at frequency $\Omega$ that travels through the vapor cell along the (+x)-direction (pump beam). The pump beam excites all atoms with velocity component $v_x = c(\Omega - \omega_0)/\omega_0$, where $\omega_0$ is the atomic transition frequency. A second beam at the same frequency (probe beam) – usually the retroreflection of the pump beam (see Fig. 3.6) – is sent
Figure 3.6: Experimental setup for saturated absorption spectroscopy. BS is a beam splitter, PD a photodiode, VFC a voltage to frequency converter.

Figure 3.7: Transmission of the Cs vapor cell. Laser wavelength at 852.347 nm, power 70 mW. The Lamb dips of the $6^2S_{1/2}, F=4 \rightarrow 6^2P^0_{3/2}, F'=4$ and the $6^2S_{1/2}, F=4 \rightarrow 6^2P^0_{3/2}, F'=5$ hyperfine transitions are marked by arrows. In the frequency interval 530 - 640 MHz the probe beam was fully absorbed, except for the Lamb dip due to saturation (see inset). For relative frequencies below 350 MHz and above 790 MHz the transmission was so strong that the photodiode was saturated.
through the gas coincident with the first beam in (-x)-direction. The probe beam excites the atoms
with $v_{(-x)} = c(\omega_0 - \Omega)/\omega_0$. Atoms having $v_x = v_{(-x)} = 0$ are excited by both beams. The laser
power is chosen such that for these atoms the probe beam saturates the excited state population. The
absorption of the probe beam is thus reduced (transmission enhanced) if compared with atoms
having non-zero velocity along the beam axis. This decrease in absorption gives rise to a Doppler-
free peak which is the Lamb dip. Fig. 3.7 shows the Lamb dips of the $6^2S_{1/2}, F'=4 \rightarrow 6^2P^{0}_{3/2}, F'=4$ and the $6^2S_{1/2}, F'=4 \rightarrow 6^2P^{0}_{3/2}, F'=5$ hyperfine transitions of Cs (at 852.347 nm), their linewidth is
about 10 MHz.

The deviation of the cavity transmission maximum with respect to the Cs line was measured
for nine hours (Fig. 3.8a). The long term drift of the cavity was 0.02 ($\pm$ 0.02) MHz/h and is thus

![Graph](image.png)

**Figure 3.8:** (a) Temporal stability of the reference cavity. The temperature is stabilized to 306 K.
The cavity transmission maximum is measured relative to the Cs absorption line at 852.347 nm
($3.52 \times 10^8$ MHz). Long term drift (thick grey line): 0.02 ($\pm$ 0.02) MHz/h. Standard deviation of
frequency fluctuations: 1.4 MHz. (b) Histogram of frequency fluctuations relative to the average
frequency (grey line in (a)). The thick grey line is a Gaussian fit. The 95% confidence interval of
frequency fluctuations is 2.7 MHz.
negligible. Frequency fluctuations on a shorter time scale occurred within ±2.7 MHz (95% confidence), a histogram of these fluctuations is shown in Fig. 3.8b. In conclusion, this cavity meets all requirements for a frequency standard in single-molecule experiments, where linewidths are typically above 10 MHz.

3.1.4. Cryostats

All experiments at constant temperature were carried out in a home-built liquid helium bath cryostat, where the sample holder is immersed into the cooling liquid. The temperature of about 1.7 K is reached by pumping the sample chamber to a vapor pressure of 12 mbar. At this pressure the helium is in the superfluid phase below the λ-point (2.17 K). Thus, bubble formation which considerably disturbs single-molecule detection is avoided.

In experiments requiring temperature variation, a helium-flow cryostat (Janis, SVT-200) was used. The liquid helium is stored at ambient pressure in a reservoir which is connected to the sample chamber through a capillary. The sample chamber is pumped continuously, the pressure is regulated by the gas inlet valve. The cooling power and sample temperature depend on the pressure. A temperature sensor located on the sample mount and a heater inside the helium capillary allow for regulation of the sample temperature using a PID controller (LakeShore, 300 Autotuning).

3.1.5. Detector

A photo-multiplier tube (PM, Hamamatsu R4220P), Peltier-cooled to -24°C, was used to detect the photons emitted by the sample. At an anode voltage of 1200 V, the measured dark count rate was approximately 5 counts/s at a discriminator voltage of -30 mV. The quantum efficiency specified by the manufacturer is about 20% at 440 nm. The temporal response of the apparatus is determined by the temporal response of the PM, which depends on the position of photon incidence onto the photo-cathode. The prompt response was measured using a time-correlated photon counting technique [81] by moving a pulsed laser beam (pulse duration ~4 ps, λ = 820 nm) across the photo-cathode. The response of the PM is shown in Fig. 3.9 as a function of time and as a function of one spatial coordinate. Remarkably, the sensitivity shows a deep minimum when looking along the spatial direction. This “hole” is presumably due to a local damage of the photo-cathode. At the optimized spatial position, the response time of the PM is 0.5 ns (Fig. 3.9, inset).


3.2. Collection Efficiency and Simulation of Statistical Fine Structure

3.2.1. Problem and implementation

When molecular parameters such as the linewidth, saturation intensity or saturated emission rate are determined from single-molecule data, a comparison with corresponding ensemble measurements is of great interest. The representativeness of an individual molecule for the entire ensemble can be rated or, if a large number of molecules is measured, the distribution of a parameter can be related to the ensemble average value.

In the apparatus shown in Fig. 3.1, the aperture placed in the confocal plane of the laser focus strongly reduces the collection efficiency for molecules located outside the volume \( V \), which is defined laterally by the aperture's projection inside the sample and longitudinally by the size of the laser waist. It is assumed that single-molecule peaks are obtained from emitters located in the center of \( V \), because they give rise to the strongest signals. For these molecules, the fraction of fluorescence gathered by the microscope objective is entirely imaged into the aperture. Thus, single-molecule peaks are all detected at about the same efficiency (assuming equal orientations of the transition...
When an ensemble of molecules is probed, as in the case of the SFS, the photon emission from each molecule is collected with a different efficiency according to its position relative to the center of V.

For these reasons, the SFS signal recorded by our apparatus is simulated numerically. In the simulations, molecules are randomly and homogeneously distributed in a cylindrical sample of 5 μm radius and 18 μm thickness. A Gaussian excitation beam with an amplitude equal to the measured saturation intensity is focused into the center of this cylinder. Because the focus was not exactly diffraction limited in real experiments, the exciting wave is defined as the convolution of a propagating TEM$_{00}$ wave (divergence given by the 0.85 numerical aperture of the focusing objective) and a lateral Gaussian broadening function, yielding a total waist diameter of 4 μm (in accordance with one-photon excitation experiments). Each molecule is treated as an isotropically emitting point source. This approximation is justified, because the total emission rate is normalized to the measured single-molecule count rate. The following molecular parameters, which were obtained from one-photon excitation single-molecule spectra (see 6.2.1.), are used for the simulations: $R_\text{ss} = 1900 \text{ s}^{-1}$, $I_\text{sat} = 3 \text{ W/cm}^2$, $\Gamma_0 = 36 \text{ MHz}$ (see 2.2.2. for definitions). The convolution of the emitted wave with the detection optics transfer function is described by a Gaussian wave which is centered at the position of the molecule. In this case, the waist diameter corresponds to the detection optics resolution. This emitted wave is now integrated over the pinhole area for each molecule $^{(iv)}$. The value of the integral corresponds to the amplitude of the molecular Lorentzian line profile. Its width is calculated from the experimental value $\Gamma_0$ taking into account saturation. All these Lorentzians are randomly dispersed over an 8 GHz spectral interval and are finally summed up, yielding the simulated SFS. To keep the computation time in a reasonable range, the number of molecules in the sample volume was limited to 100,000 (corresponding to a concentration of about $1.5 \times 10^{-6} \text{ M}$). The chromophore concentration in real samples was about 200 times higher ($2-4 \times 10^{-5} \text{ M}$). However, the ratio between the average single-molecule emission rate and the number of molecules per homogeneous linewidth coincided satisfactorily between experiment and simulation. Thus, no significant error was made by reducing the number of molecules for the calculations. More details about the SFS simulations are given in Appendix A.

$^{(iv)}$ For simplicity, the emitted wave and the integration over the pinhole area are formally treated in the object space (inside the sample) and not in the image space which is defined by the detection optics. In this way, the magnification of the optics need not be considered.
3.2.2. Simulation of Statistical Fine Structure

In the simulations, the radius of the excitation beam was varied between 1.3 \( \mu \text{m} \) and 1.95 \( \mu \text{m} \), that of the emitted beam between 0.75 \( \mu \text{m} \) and 1.3 \( \mu \text{m} \), without a significant effect on the result. The average single-molecule count rate obtained from the simulation was \( R_{\text{av}} = 135 - 170 \text{ cps} \), while the number of molecules per homogenous linewidth, \( N_{\text{H}} \), was around 60. In the specific example shown in Fig. 3.10, \( N_{\text{H}} = 62 \) and \( R_{\text{av}} = 158 \text{ cps} \). In a real SFS experiment, \( N_{\text{H}} = 65 \) and \( R_{\text{av}} = 160 \text{ cps} \) were determined (see 6.2.3.). Hence, the different collection efficiencies of individual molecules and the consequences for SFS measurements are well understood and are described by the above simulation procedure.

![Simulated SFS](image)

**Figure 3.10:** Simulation of SFS over an 8 GHz spectral interval. 100'000 molecules are randomly distributed in a sample volume of 90 \( \mu \text{m}^3 \). The photophysical molecular parameters are given in the text. The pinhole diameter was 1 \( \mu \text{m} \) (projection to focal plane of objective), the radii of the excitation and emitted beams were 1.65 \( \mu \text{m} \) and 1.3 \( \mu \text{m} \), respectively.
3.3. **THE SAMPLE**

3.3.1. **Shpol’skii systems**

Different types of host materials are used in low-temperature spectroscopy of dopant chromophores: organic and inorganic glasses, polymers, gas deposited solid matrices, isomorphic crystalline matrices and poly-crystalline Shpol’skii matrices. In crystalline systems, isolated guest molecules occupy unique vacancies in the crystal lattice of the host. These chromophores in their solvent cages are often slightly distorted and may occupy different sites in the host matrix. This causes a multiplet structure of the vibronic spectra. In this work Shpol’skii matrices are used and in the following they are briefly characterized. A comprehensive introduction to the properties and spectroscopy of Shpol’skii systems is given in ref. [82].

Shpol’skii and co-workers discovered the occurrence of highly resolved vibronic spectra of polyaromatic hydrocarbons, when these compounds were dissolved in suitable n-alkanes and rapidly crystallized by immersion into a cryogenic liquid [83]. Such systems showed quasi-line spectra with linewidths around 10 GHz. These lines were assigned to optical zero-phonon transitions. This so-called “Shpol’skii effect” arises only when the crystallization takes place under non-equilibrium conditions. A Shpol’skii system thus corresponds to a metastable thermodynamic non-equilibrium system [82]. The formation of such a metastable phase preferably occurs in the case of merely partial guest-host steric conformity (low degree of molecular isomorphism), high crystallization rates and, in many cases, at high concentration. The Shpol’skii effect allows an enormous degree of supersaturation of the solute compound and is by no means an effect of low concentrations. In general, several chromophores substitute several solvent molecules in Shpol’skii systems. On the contrary, systems with isomorphic components form an equilibrium phase of molecularly dispersed mixed crystals where one chromophore substitutes one solvent molecule. In such a case, low concentrations and slow cooling rates are required. The Shpol’skii effect and the type of guest – host system built at a given concentration strongly depend on the cooling rate. Correspondingly, a well defined cooling procedure is a basic requirement for reproducible spectroscopic properties of these systems.
Diphenyloctatetraene in n-tetradecane

The chromophore studied throughout this work is all-trans-1,8-diphenyl-1,3,5,7-octatetraene (DPOT), the molecular structure is shown in Fig. 3.11. The over-all length of DPOT is about 18.1 Å and is very similar to that of n-tetradecane (~20.1 Å). N-tetradecane (TD) crystals are triclinic with one molecule per unit cell. The unit cell dimensions are $a = 4.29$ Å, $b = 4.82$ Å, $c = 18.58$ Å, $\alpha = 93.1^{\circ}$, $\beta = 78.8^{\circ}$, $\gamma = 107.0^{\circ}$, and the unit cell volume is $361$ Å$^3$ [84]. The crystal structure of DPOT is orthorombic with $a = 10.196$ Å, $b = 7.504$ Å, $c = 19.579$ Å, and a unit cell volume of $1498$ Å$^3$. TD is chosen as a suitable host material to form a Shpol’skii system with DPOT guest molecules. From a comparison of the TD unit cell dimensions with the geometrical structure of DPOT, it must be expected that DPOT is slightly distorted in the TD crystal.

![Molecular structure of the centrosymmetric DPOT (ground state), measured at 173 K [85]. Bond lengths are in Å, angles in degrees. The plane of the polyene chain makes an angle of about 5.4° with the planes of the benzene rings.](image)

3.3.2. Sample preparation and characterization

The samples were prepared by dissolving a small amount of solid DPOT (Aldrich) in TD (Fluka, puriss.) at room temperature. Chromophore concentrations were generally around $2 \times 10^{-5}$ M. A droplet of solution was placed between two microscope cover glass plates. After mounting this sandwich to the sample holder (Fig. 3.2), it was immediately cooled to form a Shpol’skii matrix by immersing it directly into liquid helium. This procedure was kept reproducible. However, samples of varying quality were obtained with regard to the suitability for SMS experiments, and only about 20% of the samples could be used.

Standard microscope images using white light illumination (Fig. 3.12) and fluorescence images (Fig. 3.13) provided a preliminary characterization of the samples. A typical sample consists of several TD micro-crystals. It has turned out that in samples suitable for two-photon SMS (denoted as “good samples”), these micro-crystals are of rectangular shape and are aligned uniaxially.
The distribution of DPOT showed considerable inhomogeneities. Large clusters giving rise to brightly fluorescent spots as well as "empty regions" with no DPOT were found. Even in "good samples" most of the fluorescence originated from the borders of the micro-crystals (Fig. 3.13). Two reasons may account for this observation. First, the emitted light is preferably scattered at crystal defects such as borders or cracks. Second, the chromophore concentration may be higher at the borders due to a zone refining effect that could occur despite of the rapid crystal growth. Variations of the local DPOT concentration by a factor of about 10 were indeed observed within individual micro-crystals. In several cases, the concentration was higher at the borders than in the center of the crystal.

While fluorescence images could be obtained from almost all samples, two-photon excitation of DPOT was only observed with good samples. In bad samples, a drastic decrease of the two-photon absorption rate may be caused presumably by distortions of the excitation beam due to a high density of crystal defects and a rough surface. Moreover, unfavorable orientations of the chromophores are more likely in bad than in nicely grown good samples. Once a suitable micro-crystal was selected,

(v) In the laboratory slang we called this structure which was characteristic for good samples "chess board structure".
the IR beam was focused onto it by moving the microscope objective, yielding a focal diameter down to 1.5 μm at λ = 888 nm (Fig. 3.12, right).

Figure 3.13: Fluorescence image of the sample shown in Fig. 3.12, excitation wavelength at 444 nm. Remarkably, most of the fluorescence intensity originates from the borders of the micro-crystals.
Seite Leer / Blank leaf
4. **SPECTROSCOPIC CHARACTERIZATION OF DIPHENYLOCTATETRAENE**

To provide a more solid basis for the understanding of single-molecule spectra, the electronic properties of DPOT are summarized, and the spectral characteristics are studied by means of one-photon and two-photon fluorescence excitation spectroscopy. A remarkable sensitivity of the line intensities on the matrix, as well as molecular sites of different symmetries are observed. It is shown that the spectral features of DPOT in n-alkane matrices can be interpreted in the framework of a vibronic coupling between the first and second excited singlet states. DPOT molecules of a symmetry-broken site in tetradecane have proven to be very suitable for two-photon excitation studies on the single-molecule level. Finally, measurements of the excited-state lifetime are reported for the DPOT/tetradecane system.
4.1. INTRODUCTION

A linear polyene is a chain of conjugated carbon-carbon double bonds with a quasi-one-dimensional unbranched π-electron system. The resonance structure of the ground state is represented by alternating double and single bonds. Historically, much of the interest in linear and diphenyl-polyenes has developed from a desire to understand the spectral and photochemical properties of the visual pigments. There is a high similarity among all polyenes (i) regarding the electronic structure and the photochemical properties of the excited state. Therefore, diphenyl-polyenes are considered as simple model systems of carotenoids or retinoids, which represent natural polyenes [86,87]. The chemical structures of a few examples are shown in Fig. 4.1.

![Chemical structures](image)

Figure 4.1: Chemical structure of (a) β-carotine, (b) all-trans-retinal and (c) all-trans-diphenyloctatetraene (DPOT).

Carotenoids [88,89], whereof β-carotine is the most known representative, are the largest group of natural polyenes. They have two functions in the photosynthetic apparatus of bacteria, algae and green plants. One function is an accessory light-harvesting pigment which transfers its excitation energy to chlorophyll or bacteriochlorophyll [90]. The second role is to protect the photosynthetic cells from harmful photo-oxidation reactions [91]. Retinoids, another group of natural polyenes, play a crucial role in vision [92]. The visual pigment (e.g. rhodopsin in the rod cells of the retina) is a complex consisting of a retinal chromophore and an opsin protein. The primary step in the visual process after the absorption of a photon is the excited-state isomerization of 11-cis-retinal to all-trans-retinal. This photochemical reaction is supposed to go via the triplet state [93]. The cis-to-trans isomerization leads to a spatial separation of retinal and the opsin. Subsequently a train of events is released that finally produces an amplified electric pulse in the optic nerve.

(i) except for the shortest ones (stilbene, butadiene, diphenylbutadiene) which show properties deviating from the general behavior of polyenes.
The molecular environment in bio-polymers such as rhodopsin is relatively rigid, particularly on the time scale of electronic excitation. Therefore, the biological environment is much more like the environment in an organic solid than in an organic liquid. Linear and diphenyl-polyenes in n-alkane solid solutions are thus regarded as good models for studying the photophysical and photochemical behavior of the visual pigments. However, the interest of this chapter is not the biological role of polyenes, but it is focused on their electronic structure that makes diphenyl-polyenes very favorable candidates for two-photon excitation SMS.

4.2. ELECTRONIC PROPERTIES OF DIPHENYLOCTATETRAENE (DPOT)

4.2.1. Overview of the electronic structure

Chromophores of the octatetraene type have been widely investigated. Numerous reports have been published about the electronic structure, spectroscopy and photochemistry of linear and diphenyl-polyenes. Three comprehensive reviews [86,94,95] and three articles [96-98] which are of fundamental importance for this work are cited here. While refs. [86] and [94] are focused on the electronic structure, ref. [95] also emphasizes the photochemical properties of the excited state. Ref. [96] is the first elaborate work on the excited-state characteristics of DPOT, [97] corresponds to the first report about high-resolution two-photon excitation spectra (for octatetraene), and in [98] the most important photophysical parameters of DPOT are determined.

The most significant feature of the electronic structure of all polyenes is a weakly absorbing excited singlet state (assigned to $S_1$) below the strongly absorbing state which is assigned to $S_2$. This was first discovered by Hudson and Kohler in 1972 [99] for the case of $\alpha,\omega$-diphenyloctatetraene. Schulten and Karplus [100] showed that this ordering of excited states reflected the importance of electron correlation in these molecules. In particular, the lowest excited singlet state ($S_1$) was proven to be a state of correlated electrons that includes a double electron excitation.

The lowest excited singlet states of linear polyenes are appropriately built from $\pi$ molecular orbitals ($\pi$-electron approximation). Due to the $C_{2h}$ symmetry of linear all-trans-polyenes, their states are characterized in terms of the A and B representations. The $S_0$ and $S_1$ states are of $A_g$ symmetry and $S_2$ is of $B_u$ symmetry. Accordingly, electric-dipole transitions from $S_0$ to $S_1$ are forbidden by the $g \leftrightarrow u$ selection rule, while transitions from $S_0$ to $S_2$ are strongly allowed. This pattern of excited singlet states is a general property of polyenes and is also accurate in the presence
of small distortions of the molecules \([96]\). Therefore the labels \(1^{1}A_g\) for \(S_0\), \(2^{1}A_g\) for \(S_1\) and \(1^{1}B_u\) for \(S_2\) are also adequate even when the \(C_{2h}\) symmetry is not strictly realized. In particular, the interaction with the environment may break the molecular symmetry. A symmetry breaking leads to a mixing of \(S_1\) and \(S_2\). A more detailed discussion of this subject follows in paragraph 4.2.2. An overview energy level diagram of DPOT is schematically shown in Fig. 4.2.

In the \(1^{1}A_g\) ground state all bonding molecular orbitals are doubly occupied. The \(1^{1}B_u\) state is generated from \(S_0\) by promoting a single electron from the HOMO (highest energy occupied molecular orbital) to the LUMO (lowest energy unoccupied molecular orbital). \(2^{1}A_g\) is a superposition of two doubly excited \(\pi\)-to-\(\pi^*\) configurations. One of them corresponds to a double excitation from HOMO to LUMO (two electrons promoted), the other one to a double jump from HOMO to LUMO+1 of a single electron. \(A_g\) states are assigned to “covalent” states in which the electrons are correlated (they tend to avoid each other). \(B_u\) states have more “ionic” character because of a lower electron correlation; the electrons are less likely to avoid one another, resulting in an increased local electron density. The most important difference between the equilibrium geometries of the \(S_0\) and \(S_1\) state is a change in carbon-carbon bond lengths. In \(S_1\) the order of
double and single bonds is supposed to be reversed with respect to the order in $S_0$ [94,101]. Experimental evidence for a significant change of geometry upon $S_0 \rightarrow S_1$ excitation is given in ref. [97], where the relative vibronic intensities are measured in a two-photon excitation spectrum of octatetraene in n-octane. The vibrational frequencies in $S_1$ are different from those in $S_0$.

The generality of the electronic structure of polyenes has already been mentioned above. The properties of the excited state and the photophysical parameters depend on the polyene chain length. For example, the $S_0 - S_2$ energy splitting increases strongly with increasing chain length and the $S_0 \rightarrow S_1$ transition becomes progressively weaker. So far, linear and diphenyl-polyenes have been discussed on equal footing. Indeed, results obtained for linear polyenes are correct at least qualitatively for the corresponding $\alpha,\omega$-diphenylpolyenes as well. Unsubstituted polyenes are rather poor emitters [102,103], whereas $\alpha,\omega$-diphenylpolyenes are highly fluorescent and are therefore preferably investigated by means of fluorescence excitation techniques.

4.2.2. $S_1 - S_2$ vibronic coupling and intensity borrowing

It is well established that the low lying weak transition ($1A_g \rightarrow 2A_g$) gains its intensity from a mixing of the $2A_g$ and $1B_u$ state. The interaction occurs even in free molecules due to vibronic coupling, and the $1A_g \rightarrow 2A_g$ 0-0 transition could be observed for octatetraene in a free jet expansion [104]. In solid solutions, the $2A_g - 1B_u$ interaction is usually enhanced on account of environmental distortions of the molecule by the surrounding crystal. The $S_1$ symmetry is broken and a transition dipole moment $\langle S_1|\hat{h}|S_0 \rangle$ is induced which causes a partially radiative decay of $S_1$. Details of the $2A_g - 1B_u$ coupling have been widely discussed [105-113]. In the model of vibronic coupling presented here, environmental distortions of the molecular geometry are taken into account by an additional static potential which is implicitly included in an effective electronic Hamiltonian.

We consider the vibronic Hamiltonian $\hat{H}$ of a molecule in the force field of the solid environment

$$\hat{H} = \hat{h}^{\text{eff}} + \hat{T} ,$$

(4.1)

where $\hat{h}^{\text{eff}}$ is an effective semi-empirical Hamiltonian for the electronic states of the molecule, including the electronic kinetic energy, the total intramolecular potential and a static external perturbation by the surrounding medium. $\hat{T}$ is the operator of the nuclear kinetic energy. The electronic basis functions
\[ \phi^e(\{r_i\};\{Q_j\}) \quad , \quad e \in \{G,A,B\} \quad (4.2) \]

depend on the electronic coordinates \( \{r_i\} \) and parametrically on the nuclear coordinates \( \{Q_j\} \). \( e = G, A, B \) indicate the electronic states \( 1A_g \) (G), \( 2A_g \) (A) and \( 1B_u \) (B). Though \( \hat{h}^{\text{eff}} \) is in general not of \( C_{2h} \) symmetry, the eigenfunctions are formulated in a representation of the \( C_{2h} \) point group. Thus, according to the properties of unperturbed DPOT, the states G, A and B are primarily of \( g, g \) and \( u \) symmetry, respectively. A detailed discussion of the symmetry aspects of \( \hat{h}^{\text{eff}} \) is found in ref. [110]. In the basis \( (4.2) \) the expectation values are

\[ \langle \phi^e | \hat{h}^{\text{eff}} | \phi^{e'} \rangle = \delta_{e,e'} (E^e + V^e(\{Q_i\})) , \quad (4.3) \]

where \( E^e \) are the electronic energies at the equilibrium position of the ground state and \( V^e \) are diabatic nuclear potential surfaces. Within the harmonic approximation we write

\[ V^e(\{Q_i\}) = \frac{1}{2} \sum_i (\omega_i^e Q_i^2) + 2 (\omega_i^e)^2 y_i^e Q_i , \quad (4.4) \]

where the normal coordinates \( Q_i \) of all electronic states are assumed to be coaxial, so that only displacements along the axes have to be considered. In eq. (4.4) \( \omega_i \) is the frequency of the \( i \)-th normal mode and \( y_i \) is the spatial displacement of the harmonic potential with respect to the ground state equilibrium (see Fig. 4.3). A crucial feature of the effective Hamiltonian \( \hat{h}^{\text{eff}} \) is the coupling between the A and B states which is introduced by

\[ \langle \phi^A | \hat{h}^{\text{eff}} | \phi^B \rangle = V^{BA} , \quad (4.5) \]

while all other off-diagonal elements of \( \hat{h}^{\text{eff}} \) are set to zero. Since an effective Hamiltonian has been considered, the source of the coupling \( V^{BA} \) need not be further specified. \( V^{BA} \) may be due to an asymmetric environmental perturbation (crystal field) or may be of purely vibronic nature. The electronic transition dipole moments are

\[ \langle \phi^B | \mu | \phi^G \rangle = \mu^{BG} \]
\[ \langle \phi^A | \mu | \phi^G \rangle = 0 \quad (4.6) \]

The \( G \to A \) transition moment is set to zero according to the selection rule of the isolated molecule.

Within the Born-Oppenheimer approximation, the following vibronic basis functions are considered:
The index $i$ denotes the vibrational normal mode, $v_i$ corresponds to its quantum number. $|v_i^{G}(Q_i)\rangle$ are the vibrational eigenfunctions of the Hamiltonian

$$\hat{T} + \hat{V}_{i}^G = \frac{\Delta E_i^G}{2\pi} \left( v_i^G + \frac{1}{2} \right) |v_i^G\rangle,$$

where $\Delta E_i^G$ is the energy shift caused by the spatial displacement of the harmonic potential with respect to the ground state equilibrium. Taking the electronic transition moment $\mu_{BG}^G$ independent of the nuclear displacements (Franck-Condon approximation), the vibronic transition dipole moments are given as

$$\langle \Psi^B | \hat{\mu} | \Psi^G \rangle = \mu_{BG}^G \prod_i \langle v_i^B | v_i^G \rangle.$$

For the absorption only the $\{v_i^G\} = \{0\}$ state is of importance, and the corresponding vibronic transition moments are

$$d_{\{v_i^G\}} = \mu_{BG}^G \prod_i \langle v_i^B | v_i^G = 0 \rangle.$$
Accounting for the interaction $V^{BA}$ between the A and B states, we can now set up the vibronic Hamiltonian matrix in the basis (4.7) (Fig. 4.4). The diagonal elements correspond to the energy levels of the molecule in the solid environment without A–B coupling. They are given by

$$H_{\{v^{\alpha}\}, \{v^{\beta}\}} = E_0^e + \sum_i \frac{\hbar \omega_i^e}{2\pi} v_i^e, \quad e \in \{A, B\},$$

where $E_0^e$ is the energy difference between the zero-vibrational level of state $e$ and the electronic ground zero-vibrational state (see Fig. 4.3). The non-zero off-diagonal elements are

$$H_{\{v^{\alpha}\}, \{v^{\beta}\}} = V^{BA} \prod_i \langle v_i^A | v_i^B \rangle.$$

The matrix $H$ is now diagonalized, resulting in eigenvalues $\{E_j\}$ and eigenvectors $\{U_j\}$ which include the A–B coupling. The eigenfunctions $\{U_j\}$ are mixed states of $A_g$ and $B_u$ type and are no longer a representation of $C_{2h}$. It is important that merely the $B_u$ components of $U_j$ have a non-zero transition moment to $1A_g$. The resulting transition moments are given by the transformation

$$\tilde{d}_1 = \sum_j U_{1j} d_j,$$

where the sum is taken over the vibronic states ordered according to the states used to set up the matrix $H$. The intensities of the observed one-photon vibronic transitions are

$$I_{1} \sim (\tilde{d}_1)^2.$$

To determine the two-photon vibronic transition intensities $I_{1}^{TP}$, the electric dipole transition moment $\mu^{BG}$ in eq. (4.10) is replaced by the two-photon transition polarizabilities $S_{GA}$ and $S_{GB}$.
which are given by eq. (5.7) and will be discussed in section 5.2. The two-photon vibronic transition moments are written as

\[
d_{\{\nu_i^n\},\nu_i^m} = S_{GA} \prod_i \langle \nu_i^A | \nu_i^G = 0 \rangle
d_{\{\nu_i^n\},\nu_i^m} = S_{GB} \prod_i \langle \nu_i^B | \nu_i^G = 0 \rangle
\]  

(4.15)

Carrying out the transformation (4.13), we get

\[
I_{TP} \sim \left( \sum_j U_{ij} d_{ij} \right)^2 .
\]  

(4.16)

In contrast to one-photon excitation, \( G \to A \) is the allowed two-photon transition and \( S_{GB} \) vanishes in general. \( S_{GB} \) is only non-zero if the \( G \to B \) transition is accompanied by a change in static dipole moment (see eq. (5.14)). Thus, the role of the borrowing and lending states is in principle inverted in the case of two-photon excitation. However, under the conditions which lead to \( A \to B \) vibronic coupling, the molecular symmetry is broken and \( S_{GA}, S_{GB} \neq 0 \) occurs intrinsically. Therefore, the borrowing is definitely less pronounced for two-photon transitions. But since the transition tensors of the \( G \to A \) and \( G \to B \) transitions (see eq. (5.7)) are combined by the transformation (4.16), constructive and destructive interferences may occur. As a consequence, the sign convention between the tensor elements of \( S_{GA}, S_{GB} \) and \( V^{BA} \) is important. Choosing positive signs for \( S_{GA} \) and \( S_{GB} \), a negative sign had to be fixed for \( V^{BA} \) in the calculations described below, in order to achieve a reasonable agreement with the experimental two-photon excitation spectra.

In sections 4.3 and 4.4 experimental spectra will be fitted according to the model described above. In all calculations shown, the \( S_0 \) (G), \( S_1 \) (A), and \( S_2 \) (B) vibrational manifolds were each represented by three harmonic modes \( |v_i^G, v_i^A||v_i^B, v_i^A| (e \in \{G, A, B\}) \). The vibronic basis set was restricted to \( v_i^e \leq 7 \) and to the 250 lowest vibronic energy levels. The following adjustable parameters were optimized with respect to the spectral data [114]: vibrational frequencies \( \omega_i^e \) in the electronic states \( G, A, B \); displacements \( y_i^e \) of the vibrational potentials for \( e = G, A, B \); 0-0 excitation energy \( E_0^A \); A-B energy splitting \( E^{BA} = E_0^B - E_0^A \); vibronic coupling constant \( V^{BA} \).

The following normal modes are considered for the analysis of the one- and two-photon spectra: a symmetric torsional vibration at \( \sim 260 \) cm\(^{-1}\) (T), the C-C single bond symmetric stretch vibration at \( \sim 1200 \) cm\(^{-1}\) (S), and the C=C double bond symmetric stretch vibration at \( \sim 1650 \) cm\(^{-1}\) (D). Additionally, a non-totally-symmetric mode of about 60 cm\(^{-1}\) (U) appeared to be important in inducing the forbidden \( A \to G \) luminescence in centrosymmetric DPOT.
4.3. DPOT IN N-OCTANE

One-photon excitation and emission spectra of DPOT in n-octane were measured at a temperature of about 5 K. For these measurements, a standard fluorescence spectrometer was used. The excitation light was generated by a xenon lamp and filtered by a double slit monochromator. Another double slit monochromator was used to filter the fluorescence. The sample was located in a helium flow cryostat at a temperature of about 5 K. It was prepared by immediate cooling of a 3 mm diameter tube containing the liquid solution. All spectra were measured at a resolution of about 10 cm\(^{-1}\), corresponding to 0.25 nm at a wavelength of 440 nm. In excitation spectra, the fluorescence was collected in a 60 cm\(^{-1}\) spectral window centered at the peak emission frequency.

The vibronic transitions observed in a one-photon excitation (OPE) spectrum (Fig. 4.5a) can be assigned by means of a fit according to the vibronic coupling model (see 4.2.2.) [114]. Starting

![Figure 4.5: One-photon excitation spectrum of DPOT in octane-H\(_{18}\). (a) Experimental spectrum. The \(S_0 \rightarrow S_1 0-0\) line is marked by a circle. (b) Fit according to the vibronic coupling model with a coupling constant of \(V^{BA} = -500\) cm\(^{-1}\). The \(S_0 \rightarrow S_1\) and \(S_0 \rightarrow S_2\) origins are marked. (c) Simulated spectrum assuming no symmetry breaking, vibronic coupling is set to \(V^{BA} = 0\). The assignment of the lines is summarized in Table 4.1.](image)
from model parameters reported in the literature [110], a set of parameters producing an optimal fit of the experimental spectrum was determined. The result is given in Table 4.2. The convolution of the calculated line spectrum with the line shape function shown in Fig. 4.6 yields the fitted spectrum which is shown in Fig. 4.5b. The effect of the \( S_1 - S_2 \) coupling becomes clear by the comparison with a calculation at \( V^{BA} = 0 \) (Fig. 4.5c). \( S_0 \rightarrow S_1 \) vibronic transitions are forbidden if \( V^{BA} = 0 \). They gain intensity in presence of the perturbation, whereas the \( S_0 \rightarrow S_2 \) vibronic lines become slightly weaker. This is known as “intensity borrowing”. Moreover, the \( S_0 \rightarrow S_2 \) 0-0 line is blue-shifted by about 200 cm\(^{-1}\) due to the \( S_1 - S_2 \) interaction. The assignments of all lines observed in Fig. 4.5 are summarized in Table 4.1.

The emission spectrum of DPOT in octane (Fig. 4.7) shows the 0-0 ZPL and the ground state S- and D-vibrations at 1160 cm\(^{-1}\) and 1580 cm\(^{-1}\), respectively. The torsional mode (T, 268 cm\(^{-1}\)) is weak but also seen in \( S_0 \). The phonon sideband structure of the ZPL is dominated by local modes at 27 cm\(^{-1}\) and 56 cm\(^{-1}\). Whereas the 27 cm\(^{-1}\) mode is also observed for the transition to the D vibrational level, the phonon wing associated with the transition to the S vibrational state is much broader and differently structured. Apparently, the S-vibration couples more strongly to the phonons than the D-vibration.

**Figure 4.6:** Line shape function used in the simulated spectra of Fig. 4.5. This function corresponds to an asymmetric Gaussian. The line shape mimics the ZPL and the phonon side-band, where the latter is taken into account by the asymmetry.

**Figure 4.7:** Emission spectrum of DPOT in octane-H18. Excitation at 408 nm (24510 cm\(^{-1}\)).
Observed frequency [cm\(^{-1}\)] | Relative frequency [cm\(^{-1}\)] | Calculated frequency [cm\(^{-1}\)] | Calculated relative intensity | Assignment | Electr. state | Vibr. mode
---|---|---|---|---|---|---
22358 | 0 | 22382 | 0.76e-2 | \(S_1\) | 0-0 |
22614 | 256 | 22644 | 0.25e-2 | \(S_1\) | T |
23597 | 1239 | 23596 | 0.32e-1 | \(S_1\) | S |
23828 | 1470 | 23858 | 0.13e-1 | \(S_1\) | S + T |
24033 | 1675 | 24031 | 0.58e-1 | \(S_1\) | D |
24284 | 1926 | 24290 | 0.31e-1 | \(S_1\) | D + T |
24489 | 2131 | 24489 | 0.27 | \(S_2\) | 0-0 |
24740 | 2382 | 24753 | 0.19e-1 | \(S_2\) | T |
25285 | 2927 | 25279 | 0.24e-1 | \(S_1\) | D + S |
25548 | 3190 | 25539 | 0.18e-1 | \(S_1\) | D + S + T |
25717 | 3359 | 25712 | 0.13 | \(S_2\) | S |
26021 | 3663 | 25974 | 0.24e-1 | \(S_2\) | S + T |
26267 | 3809 | 26175 | 0.11 | \(S_2\) | D |
26898 | 4540 | 26894 | 0.97e-2 | \(S_2\) | \(S^2\) |
26966 | 4608 | 26971 | 0.17e-1 | \(S_1\) | \(D^2 + S\) |
27364 \(a\) | 5006 | 27329 | 0.76e-2 | \(S_1\) | \(D^3\) |
27364 \(a\) | 5006 | 27364 | 0.96e-2 | \(S_1\) | \(S^4\) |
27364 \(a\) | 5006 | 27407 | 0.39e-1 | \(S_2\) | D + S |
27830 | 5472 | 27870 | 0.16e-1 | \(S_2\) | \(D^2\) |

\(a\). The line at 27364 cm\(^{-1}\) is the superposition of three lines which are not resolved.

**Table 4.1:** Vibronic frequencies in one-photon excitation spectra of DPOT in octane. Vibrational modes are abbreviated as T (torsion), S (C-C single bond stretch), D (C=C double bond stretch), for frequencies see Table 4.2.

Two-photon excitation of DPOT in octane was not efficient enough for the measurement of a spectrum. The main problem was probably the laser light scattering at the sample surface which caused a bad focusing of the beam. The preparation of a sample with a smooth surface was a crucial problem in the case of octane. Moreover, the two-photon excitation signal generally appeared to depend very sensitively on the matrix.
4.4. DPOT IN N-TETRADECANE

4.4.1. Two-photon excitation spectroscopy

For two-photon spectroscopy on bulk samples, the apparatus described in section 3.1 was used. The spectral resolution was determined by the interval of the laser scan, which was set to 5 GHz (0.16 cm⁻¹) for bulk experiments.

One-photon excitation (OPE) and two-photon excitation (TPE) spectra of the region around the 0-0 line of DPOT in TD are shown in Fig. 4.8. The TPE spectrum reveals the existence of two molecular sites with the 0-0 ZPLs at 444.0 nm (22523 cm⁻¹) for site I and at 442.2 nm (22614 cm⁻¹) for site II (ii). Site I is observed under both one- and two-photon excitation. The shape and position of the OPE line overlaps with its TPE counterpart within experimental accuracy. The relatively broad bands peaked at 443.5 nm are the phonon wings which are also similar. Contrary to site I, site II is

(ii) In the case of TPE, the excitation wavelength corresponds to half of the laser wavelength.
only seen under TPE and is missing in the OPE spectrum. These observations lead to the hypothesis that site II is built from centrosymmetric and thus unperturbed molecules, whereas site I corresponds to molecules with broken symmetry. In the following, the different characteristics of the two sites will be studied and the hypothesis about their symmetries will finally be confirmed. The relative intensity of site II altered from sample to sample, and even when the exciting laser beam was moved within one micro-crystal of TD. The ratio of the site II to site I peak intensities varied between 0.1 and 2, and was mostly around 0.3.

The multiple-site structure is confirmed by a TPE spectrum over a wide frequency range (Fig. 4.9a). All lines with relative frequencies below 1500 cm\(^{-1}\) appear as a doublet with almost identical splitting of \(96 \pm 3\) cm\(^{-1}\). The existence of several sites is typical for Shpol’skii systems and results from the superposition of identical spectra with an offset corresponding to the frequency shift of their 0-0 lines. Thus, the site splitting is in principle reproduced for all vibronic transitions. In the DPOT/TD system however, the doublet structure disappears above 24000 cm\(^{-1}\). In this frequency range the \(S_1 - S_2\) coupling becomes very important for site I. As a consequence, the energy levels of the \(S_1\) and \(S_2\) states are shifted and the \(S_0 \rightarrow S_2\) transitions borrow intensity from the \(S_1\) vibronic lines in the case of TPE.
Figure 4.9: Experimental and simulated TPE spectra of DPOT in TD-H30. (a) Experimental spectrum, the doublets (marked by double arrows) have a splitting of about 95 cm$^{-1}$. (b) Sum of both sites. (c) Fit of site II only (centrosymmetric), vibronic coupling constant $V^{BA} = 0$. (d) Fit of site I only (distorted molecules), vibronic coupling constant $V^{BA} = -350$ cm$^{-1}$. (e) Simulated spectrum of site I assuming no symmetry breaking, $V^{BA} = 0$. The assignments of the lines are given in Table 4.3.

Most of the lines observed in Fig. 4.9a can be assigned by fitting the vibronic coupling model to each site independently [114]. The model parameters were optimized by simultaneously fitting the TPE (Fig. 4.9a), OPE (Fig. 4.14a) and emission (Fig. 4.11a) spectra. The resulting parameters are given in Tables 4.5 and 4.6 for sites I and II, respectively (at the end of this section). The convolution of the calculated line spectra with the line shape function in Fig. 4.10 yields the simulated TPE spectra of Fig. 4.9b-e. The spectrum of the centrosymmetric site II (Fig. 4.9c) is dominated by the
S$_0$ $\rightarrow$ S$_1$ vibronic lines of the totally symmetric torsional (T), C-C single bond stretch (S) and C=C double bond stretch (D) vibrations [98] (see Table 4.3). For site I (Fig. 4.9d), which arises due to symmetry-broken DPOT molecules, a static dipole moment change upon electronic excitation must be considered. Thus, the electric-dipole transition S$_0$ $\rightarrow$ S$_2$ gains two-photon absorptivity according to eq. (5.14). In Fig. 4.9d, the S$_0$ $\rightarrow$ S$_2$ 0-0 line (at 24306 cm$^{-1}$) is remarkably strong. It not only borrows intensity from the nearby S$_0$ $\rightarrow$ S$_1$ vibronic transitions (e.g. line at 24366 cm$^{-1}$), but also possesses an intrinsic two-photon transition moment. The latter was estimated to correspond to about 30% of the S$_0$ $\rightarrow$ S$_1$ two-photon transition moment, in order to obtain a satisfactory fit to the data. The strong intensity of the line at 24366 cm$^{-1}$ results from a constructive interference between the elements of the S$_0$ $\rightarrow$ S$_1$ and S$_0$ $\rightarrow$ S$_2$ transition polarizability tensors. The S$_1$ - S$_2$ coupling constant of $|V^{BA}| = 350$ cm$^{-1}$ obtained for site I is slightly weaker than the one determined for the octane host (500 cm$^{-1}$). Further, it is in reasonable agreement with the value of 380 cm$^{-1}$ that was calculated from an analysis of electro-optic absorption measurements [110]. The effect of the symmetry breaking becomes evident when the S$_1$ - S$_2$ coupling is switched off in the calculation of the site I spectrum (Fig. 4.9e). In this case, the site I and site II spectra are almost identical, except for the 95 cm$^{-1}$ site splitting. The assignments of the lines observed in Fig. 4.9 are listed in Table 4.3. These assignments are the over-all result of the analyses of all TPE, OPE, emission and one-photon absorption spectra.
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4. SPECTROSCOPIC CHARACTERIZATION OF DIPHENYLOPTATETRAENE

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<th>Site</th>
<th>El. state</th>
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<td></td>
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<td>D</td>
<td>OPE</td>
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<tr>
<td>26220</td>
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<td>26232</td>
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<td>I</td>
<td>S(_1)</td>
<td>D(^2) + T(^2)</td>
<td>OPE</td>
</tr>
<tr>
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<td>S(^2)</td>
<td>OPE</td>
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<td>OPE</td>
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<td>26725</td>
<td>0.39e-2</td>
<td></td>
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<td>S(_1)</td>
<td>D + S(^2)</td>
<td>OPE</td>
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<td>S(_2)</td>
<td>D(^2)</td>
<td>OPE</td>
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</table>

a. These two lines are not clearly resolved in the spectrum.
b. These three lines are not clearly resolved in the spectrum.

Table 4.3: Vibronic frequencies in the excitation spectra (TPE: Fig. 4.9, OPE: Fig. 4.141). Vibrational modes are abbreviated as T (torsion), S (C-C single bond stretch), D (C=C double bond stretch), for frequencies see Table 4.5 (site I) and Table 4.6 (site II). Transitions of site II are marked in grey.

Next, we focus on the emission spectra which are shown in Fig. 4.11. Spectrum I (Fig. 4.11a) is observed when the exciting laser wavelength is tuned to 888.0 nm (TPE of site I); spectrum II (Fig. 4.11c) when the excitation is at 884.4 nm (TPE of site II). It is emphasized that, though the excitation is a two-photon process, the luminescence spectra always result from one-photon transitions. The emission spectrum I in Fig. 4.11a consists of several sharp lines. These lines correspond to the T-, S- and D-modes and their combinations up to the second overtone in the electronic ground state. Spectrum I is simulated according to the vibronic coupling model, using the parameters in Table 4.5 and the line shape function in Fig. 4.12. The calculated spectrum is shown in Fig. 4.11b. The resulting assignments of the observed lines are summarized in Table 4.4. Some of the assignments are uncertain and are marked by a question mark (?). The emission upon OPE (only site I can be excited) will be discussed in Fig. 4.17. It is found to be identical to its TPE counterpart in Fig. 4.11a.

The emission spectrum II shown in Fig. 4.11c is the superposition of two parts. The first part is similar to the spectrum I in Fig. 4.11a. This part appears due to excitation of site I through its phonon wing. The second contribution comes from site II itself. In the site-II-luminescence, the 0-0
ZPL is absent (Fig. 4.13). This is consistent with the absence of the 0-0 line in the OPE spectrum (see Fig. 4.8). Therefore, the luminescence II starts from a false origin at 22552 cm\(^{-1}\) (asterisk in Fig. 4.13), which probably corresponds to a non-totally symmetric mode of about 62 cm\(^{-1}\), designated as the "U-mode" in the following (U for "ungerade"). Such a vibration was also observed as a hot excitation in supersonic jet spectroscopy of 1,6-diphenylhexatriene [111]. The emission spectrum of site II is obtained by subtraction of luminescence I from luminescence II and is shown in Fig. 4.11d. It is completely different from the site I emission in Fig. 4.11a and is presumably built up by combination lines of non-totally symmetric vibrations and the modes observed in luminescence I. An assignment of the observed peaks was only possible by introducing an additional mode X of 103 cm\(^{-1}\), which however could not be identified as a fundamental vibration.

**Figure 4.11:** Luminescence spectra I (a) and II (c) observed under TPE when the laser is tuned to 888.0 nm (11261 cm\(^{-1}\), site I) and 884.4 nm (11307 cm\(^{-1}\), site II), respectively. Spectra (a) and (c) are scaled so that the peak at 22520 cm\(^{-1}\) has the same intensity. Luminescence II is a superposition of the emission of sites I and II because of a partial excitation of site I through the phonon wing. (d) Emission of site II exclusively, obtained by subtraction of spectrum (a) from (c). (b) Fit of the site I emission using the parameters in Table 4.5, \(V_{BA} = -350\) cm\(^{-1}\).
Figure 4.12: Line shape function used for the simulated emission spectrum in Fig. 4.11b. The line shape mimics the ZPL and the phonon side-band, where the latter is taken into account by the asymmetry. The 0-0 line and the T and D vibronic lines are described by an asymmetric Gaussian with a standard deviation ratio of 1:3. For the S-vibronic transitions the lines are broader. This is presumably due to a stronger coupling of mode S to the phonons and is taken into account by making the asymmetry linearly dependent on the vibrational quantum number of mode S.

Figure 4.13: Detailed view of the emission in the 0-0 region. The thin black line and thick grey line correspond to luminescence I and II, respectively. The two spectra are normalized to the strongest line which is the 0-0 ZPL of site I. The arrow marks the excitation frequency for spectrum II which corresponds to the $S_0 \rightarrow S_1$ 0-0 ZPL of site II. The U-mode, which induces the site II emission is marked by the asterisk ($\ast$).

<table>
<thead>
<tr>
<th>Observed frequency [cm$^{-1}$]</th>
<th>Relative frequency [cm$^{-1}$]</th>
<th>Calculated frequency [cm$^{-1}$]</th>
<th>Calc. relative intensity</th>
<th>Assignment</th>
<th>Excitation process</th>
</tr>
</thead>
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<td>22614$^a$</td>
<td>-93 (0)</td>
<td>-</td>
<td>0</td>
<td>II</td>
<td>0-0</td>
</tr>
<tr>
<td>22552$^b$</td>
<td>-31 (62)</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>U</td>
</tr>
<tr>
<td>22521</td>
<td>0</td>
<td>22524</td>
<td>0.15e-2</td>
<td>I</td>
<td>0-0</td>
</tr>
<tr>
<td>22484</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td>P</td>
</tr>
<tr>
<td>22449</td>
<td>72 (165)</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>U + X</td>
</tr>
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</table>
Table 4.4: Vibronic frequencies in the emission spectra (two-photon excited emission: Fig. 4.11, 
one-photon excited emission: Fig. 4.17). Vibrational modes are abbreviated as T (torsion, 268 cm$^{-1}$ in 
$S_0$), S (C-C single bond stretch, 1159 cm$^{-1}$ in $S_0$), D (C=C double bond stretch, 1583 cm$^{-1}$ in $S_0$), P 
(effective local phonon, 35 cm$^{-1}$), U (inducing mode of ungerade symmetry, 62 cm$^{-1}$), X (unassigned 
mode, 103 cm$^{-1}$). Transitions of site II are marked in grey.

<table>
<thead>
<tr>
<th>Observed frequency [cm$^{-1}$]</th>
<th>Relative frequency [cm$^{-1}$]</th>
<th>Calculated frequency [cm$^{-1}$]</th>
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<th>Excitation process</th>
</tr>
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<tr>
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<td>U + T$^2$ (?)</td>
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<tr>
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<td>21365</td>
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<td>S</td>
</tr>
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<td>21090</td>
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<td>21097</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>U + X + D (?)</td>
</tr>
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<td>-</td>
<td>II</td>
<td>U + D + T (?)</td>
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<td>?</td>
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<td>S$^2$</td>
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<td>D + S</td>
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<td>3163</td>
<td>19358</td>
<td>0.21e-2</td>
<td>I</td>
<td>D$^2$</td>
</tr>
</tbody>
</table>

- Site II origin, absent in the emission spectrum
- False origin of site II emission

4.4.2. One-photon excitation spectroscopy

One-photon excitation and emission spectra of DPOT in TD were measured using the 
apparatus described in section 4.3. The transitions observed in the OPE spectrum of Fig. 4.14a are 
assigned by the same procedure that has been applied for the analysis of TPE spectra, i.e. by fitting 
the vibronic coupling model for each site independently [114]. The optimized model parameters for 
sites I and II are again given in Tables 4.5 and 4.6, respectively. For the calculation of the site I 
spectrum, the line shape function shown in Fig. 4.15a was used, where different inhomogeneous 
broadenings were considered for the $S_1$ and $S_2$ states. The line shape shown in Fig. 4.15b provides a 
satisfactory parametric description of the site II spectrum; it was inspired by the shape of an
absorption spectrum (Fig. 4.16), which will be discussed below. The resulting simulated OPE spectra are shown in Fig. 4.14b-d. The assignments of the lines are listed in Table 4.3. As expected, the $S_0 \rightarrow S_1$ 0-0 transition is absent in the site II spectrum (Fig. 4.14d). It only appears in the presence of vibronic coupling, so in site I (circle in Fig. 4.14a,c). If compared with octane, the $S_0 \rightarrow S_1$ 0-0 line is blue-shifted by 173 cm$^{-1}$ and $S_0 \rightarrow S_2$ is red-shifted by 117 cm$^{-1}$ in TD. Correspondingly, the $S_1 - S_2$ splitting (including the perturbation splitting) decreases by 290 cm$^{-1}$. Further, the 0-0 ZPL is about a factor of two weaker compared to octane. These observations are consistent with the decrease of the $S_1 - S_2$ coupling constant from 500 cm$^{-1}$ to 350 cm$^{-1}$ when changing from an octane to a TD host.

Now we focus on the absorption spectrum shown in Fig. 4.16. Remarkably, the peaks observed in the excitation scan of Fig. 4.14a are not all reproduced in the absorption spectrum. Further, the intensity ratio of the $S_0 \rightarrow S_1$ 0-0 line of site I (2252 cm$^{-1}$) and the $S_0 \rightarrow S_2$ 0-0 line of...
site II (24223 cm$^{-1}$) is approximately 10 times smaller in the absorption compared to the excitation spectrum. According to Fig. 4.14, site I shows strong lines for the transitions $S_0 \rightarrow S_2$ 0-0 (at 24356 cm$^{-1}$) and $S_0 \rightarrow S_1$ D+T vibronic (at 24387 cm$^{-1}$). These transitions are not at all observed in Fig. 4.16. The absorption signal is proportional to the molecular concentration times the absorption cross section of the corresponding state. The excitation spectrum is proportional to the fluorescence quantum yield of the corresponding emitting state. The emission spectra in Fig. 4.11 show that the

![Figure 4.15:](image)

**Figure 4.15:** (a) Line shape function used in the simulated spectrum of site I. The ZPL is defined as a delta-function, the phonon wing as a multi-phonon structure of one mode given by a parabola centered at 35 cm$^{-1}$ from the ZPL origin (grey line). Inhomogeneous broadening is introduced by convolution of this function with a Gaussian with width of 6 cm$^{-1}$ and 60 cm$^{-1}$ in the $S_1$ and $S_2$ states, respectively. The black line shows the $S_1$ line shape. (b) Line shape of site II, which mimics the ZPL, a multi-phonon wing structure and a non-Gaussian inhomogeneous broadening. A sum of two exponentials defined by $\exp\{-x/a\}$ is chosen, with a ratio of 1:1.7 between the decay constants. The function was inspired by the absorption lines in Fig. 4.16.

![Figure 4.16:](image)

**Figure 4.16:** Top (black): Absorption spectrum of DPOT in TD. Data are taken from ref. [82]. Bottom (grey): Simulated fluorescence excitation spectrum of site II (identical with Fig. 4.14d). The same frequency range as in Fig. 4.14 is displayed.
fluorescence yield is considerably smaller in site II than in site I. Thus, lines of site II should be much stronger in absorption than in excitation. For these reasons, all strong lines in the absorption spectrum are assigned to $S_0 \rightarrow S_2$ vibronic transitions of site II. In Fig. 4.16, the grey curve is the simulation of the site II spectrum based on the model parameters in Table 4.6 and using the line shape function in Fig. 4.15b.

The emission spectrum upon OPE at 410.0 nm (24390 cm$^{-1}$, $S_0 \rightarrow S_1$ D+T vibronic, site I) is shown in Fig. 4.17. The spectra upon excitation at 383.0 nm (26110 cm$^{-1}$, $S_0 \rightarrow S_2$ D vibronic, site I), 390.5 nm (25608 cm$^{-1}$, $S_0 \rightarrow S_1$ D+S+T vibronic, site I), 410.0 nm (24390 cm$^{-1}$, $S_0 \rightarrow S_1$ D+T vibronic, site I), 415.5 nm (24067 cm$^{-1}$, $S_0 \rightarrow S_1$ D vibronic, site I) and 420.5 nm (23781 cm$^{-1}$, $S_0 \rightarrow S_1$ S vibronic, site I) are identical; they roughly scale with the peak intensities at the corresponding frequencies in the excitation spectrum (see Fig. 4.14a). This is expected because all noted excitation lines belong to site I. Surprisingly, the scaling also applies for excitation at the $S_0 \rightarrow S_2$ 0-0 transition of site II at 413.0 nm (24213 cm$^{-1}$). Apparently, the relative line intensities in the emission spectra are independent of the excited site, provided the excitation occurs at energies above the 0-0 transition of either site. There is another striking observation. The emission following OPE at the $S_0 \rightarrow S_1$ 0-0 line of site I at 444.0 nm (22523 cm$^{-1}$) differs from the spectra at all other excitation frequencies by the absence of the progression built on the “U-mode” (recalling that the U-mode is a non-totally symmetric vibration that induces the emission of site II; it thus appears as a false origin in the two-photon excited luminescence of Fig. 4.11c,d). OPE at 444.0 nm leads to an emission spectrum which is identical with the two-photon excited luminescence of site I (spectrum I in Fig. 4.11).

These results are now summarized. In the emission spectra, the progression built on the U-mode, which is characteristic for the site II emission, is also present upon the excitation of vibronic lines of site I. The U-mode is only absent upon excitation at the origin of site I (at 444.0 nm). In accordance with the broad absorption line shapes in Fig. 4.16, OPE of site II becomes more and more efficient at increasing excitation frequency (starting from 22523 cm$^{-1}$). Thus, there seems to be a mixing of the two sites in the excited state. This claim is consistent with the observation of identical emission lines for the excitation of site II (at 413.0 nm) and site I (at 420.5 nm, 415.5 nm, 410.0 nm, 390.5 nm, 383.0 nm). The model suggested in Fig. 4.18 accounts for all these results.
**Figure 4.17:** Emission spectrum. OPE at 410.0 nm (24390 cm\(^{-1}\)). For assignments see Table 4.4.

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<th>Electronic state (site I)</th>
<th>Vibrational mode (^a)</th>
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<td></td>
<td><strong>T</strong></td>
<td><strong>S</strong></td>
</tr>
<tr>
<td>Vibrational frequency, (\omega_i^c/2\pi c) ((c \in \text{G, A, B} ; i \in \text{T, S, D}))</td>
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</tr>
<tr>
<td></td>
<td>A ((S_1))</td>
<td>265 cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td>B ((S_2))</td>
<td>265 cm(^{-1})</td>
</tr>
<tr>
<td>Displacement of vibrational potential, (y_i^b)</td>
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</tr>
<tr>
<td></td>
<td>A ((S_1))</td>
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<tr>
<td></td>
<td>B ((S_2))</td>
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<td>Ratio of two-photon transition tensors (^b)</td>
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<tr>
<td>Coupling constant (V^{BA})</td>
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\(^a\) T: torsion; S: C-C single bond stretch; D: C=C double bond stretch.

\(^b\) In dimensionless units (as in ref. [110]).

Table 4.5: Parameters of the vibronic coupling model adjusted for site I. The TPE (Fig. 4.9a), OPE (Fig. 4.14a) and emission spectra (Fig. 4.11a) were fit simultaneously.
Parameter & Electronic state (site II) & Vibrational mode

<table>
<thead>
<tr>
<th>Parameter</th>
<th></th>
<th></th>
<th>T</th>
<th>S</th>
<th>D</th>
</tr>
</thead>
<tbody>
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<td>G (( S_0 )) &amp; - &amp; - &amp; -</td>
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<td></td>
<td>A (( S_1 )) &amp; 265 cm(^{-1}) &amp; 1240 cm(^{-1}) &amp; 1630 cm(^{-1})</td>
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<tr>
<td></td>
<td>B (( S_2 )) &amp; 265 cm(^{-1}) &amp; 1260 cm(^{-1}) &amp; 1650 cm(^{-1})</td>
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<tr>
<td>Displacement of vibrational potential, ( \gamma^b )</td>
<td>G (( S_0 )) &amp; 0 &amp; 0 &amp; 0</td>
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<tr>
<td></td>
<td>A (( S_1 )) &amp; 0.75 &amp; 2.1 &amp; 2.1</td>
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<tr>
<td></td>
<td>B (( S_2 )) &amp; 0.4 &amp; 1.0 &amp; 1.2</td>
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<tr>
<td>Ratio of two-photon transition tensors (^b)</td>
<td>(</td>
<td>S_{GB}/S_{GA}</td>
<td>= 0 )</td>
<td></td>
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</tr>
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<td>0-0 excitation energy of ( S_1 ) (( E^A_{0} ))</td>
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</table>

Table 4.6: Parameters of the vibronic coupling model adjusted for site II. The TPE (Fig. 4.9a) and OPE spectra (Fig. 4.14a) were fit simultaneously. No ground state data were available for site II (emission spectrum). Footnotes have the same meanings as in Table 4.5.

### 4.4.3. Discussion of results and open questions

**Hypothesis: Shpol'skii sites in dynamic equilibrium**

The analyses of the one-photon excitation (Fig. 4.14) and absorption (Fig. 4.16), the two-photon excitation (Fig. 4.9) and the emission spectra (Fig. 4.11, Fig. 4.17) lead to the following all-inclusive hypothesis about the properties of the two molecular sites of DPOT in TD. The suggested two-site model is sketched in Fig. 4.18. Sites I and II correspond to different configurations of DPOT molecules inside the TD host. The two sites can convert into one another and are in a dynamic equilibrium. The symmetry of site I is distorted due to interactions with the environment. Correspondingly, the states \( S_1 \) (A) and \( S_2 \) (B) are coupled (\( |V^{BA}| \neq 0 \)) and their energy difference is larger than in the unperturbed configuration. For site I, the \( S_0 \rightarrow S_1 \) transitions gain intensity due to the \( S_1 \rightarrow S_2 \) coupling and \( S_1 \) is the fluorescing state. Centrosymmetric DPOT molecules which are of unperturbed symmetry give rise to site II. Here, \( S_0 \rightarrow S_1 \) is a one-photon-forbidden transition and only two-photon excitation is strongly allowed. For site-II-molecules the direct \( S_1 \rightarrow S_0 \) emission is weak and is only possible through inducing vibrations of ungerade symmetry. As an alternative
relaxation path, site II can convert to site I over a small barrier on the $S_1$-potential surface and subsequently decay radiatively to the site I ground state. It is assumed that most of the site II emission originates from this path. Since no depopulation of site II was observed upon irradiation, the I $\leftrightarrow$ II inter-conversion in the $S_0$ state must occur at the same probability as in the $S_1$ state.

Figure 4.18: Two-site model of DPOT in tetradecane.

This model is in agreement with the following observations:

- Site I is observed in OPE and TPE spectra, whereas site II only shows up under TPE.
- The $S_1$ state of site I is strongly fluorescent. For site II, the $S_1 \rightarrow S_0$ fluorescence is weak and the emission spectrum is built on a false origin (observed upon TPE of the $S_0 \rightarrow S_1$ 0-0 ZPL).
- Provided that the excitation occurs to states higher than the zero vibrational level of $S_1$, the emission spectrum of site I shows weak lines corresponding to the progression built on the U-mode of site II. This is consistent with the I $\leftrightarrow$ II inter-conversion over a barrier on the $S_1$-potential surface. Analogously for site II, where excitation above the $S_0 \rightarrow S_1$ 0-0 transition energy results in an emission spectrum similar to the one of site I.
- No intensity redistribution was observed between the two sites, consistent with equal probabilities for the I $\leftrightarrow$ II inter-conversion in the $S_0$ and $S_1$ states.
If compared with site I, the population of site II is presumably about 10 times larger and its fluorescence quantum yield is by a factor of about 10 smaller. This leads to similar intensities of the $S_0 \rightarrow S_1$ 0-0 lines in TPE spectra, but results in a 10 times higher contribution of site II to the intensities in the absorption spectrum.

Open questions

The approach outlined above still leaves some questions unanswered. In particular, a couple of assumptions were made for fitting the experimental spectra according to the vibronic coupling model. These assumptions have not been justified so far and are discussed in the following. (i) Based on the vibrational frequencies reported in the literature [98], we have assumed that three fundamental vibrations (T, S, D) are of importance. However, a splitting of the D-mode into two fundamental modes was proposed for dithienyl-polypenes based on ab-initio calculations [115]. Such a splitting could in principle also occur for certain diphenylpolycnes [116]. However, calculations of the vibrational frequencies of DPOT that could give evidence for a splitting of the D-mode are not at disposal at present. (ii) For the simulation of the emission spectrum in Fig. 4.11b, a broader line shape was chosen for the S-mode than for the D-mode in order to account for the different linewidths observed. The appearance of the C-C vibration as a "complex multiplet" was also reported by other authors [94]. We argued that a stronger coupling of the C-C vibration to the phonons could cause a corresponding broadening of the vibrational line. However, the broad feature observed in the emission spectrum at around 21360 cm$^{-1}$ in Fig. 4.11 could also result from two unresolved vibronic lines, one corresponding to the S- and the other to a fourth fundamental mode. Such an explanation would be in accordance with the above mentioned splitting of the D-mode. Ab-initio calculations of the vibrational frequencies of DPOT in the ground and excited state are necessary to clarify these uncertainties.

Another point that requires some discussion is a possible correlation between the peak intensity of the 0-0 line of site II (at 22618 cm$^{-1}$) and the peak intensity of the strongest line in the TPE spectrum (at 24366 cm$^{-1}$, marked by the asterisk in Fig. 4.9a) that has not been mentioned so far. However, this intensity–intensity correlation is based on the measurement of a few spectra only and cannot be analyzed quantitatively. Moreover, its significance depends considerably on the way how the background is taken into account in the spectra, and integrated intensities should be correlated instead of peak intensities. By the spectral calculations, the line at 24366 cm$^{-1}$ has been assigned to the $S_0 \rightarrow S_1$ D+T vibronic transition of site I, but not to a transition of site II. Thus, the predictions of our vibronic coupling model would contradict with such an intensity–intensity
correlation. At the moment, there is no satisfactory explanation for this potential discrepancy. Further investigations are required to find out its relevance and to solve it if necessary.

**Concluding remarks**

In conclusion, one- and two-photon excitation spectroscopy of DPOT in n-alkane Shpol'skii matrices are well understood in the framework of a vibronic coupling between the $S_1$ and $S_2$ states. This interaction leads to non-zero transition moments of the $S_0 \rightarrow S_1$ vibronic transitions for OPE and the $S_0 \rightarrow S_2$ vibronic transitions for TPE. For the systems DPOT/TD and DPOT/octane a parameter set has been optimized to reproduce the observed spectra by a model calculation. The assignments of the lines observed in excitation (Table 4.3) and emission (Table 4.4) spectra of DPOT in TD substantially agree with the literature [98]. Our spectral calculations for the two sites of the DPOT/TD system lead to the model presented in Fig. 4.18, which considers two molecular conformations in a dynamic equilibrium. This description is only on good terms when the $T$-, $S$- and $D$-modes are the only important fundamental vibrations and no fourth mode has to be taken into account (see above). Further, the intensity correlation between the 0-0 line of site II and a strong line assigned to site I, that was qualitatively observed in TPE spectra, may be an indication of shortcomings of our spectral fits. The questions that should be clarified have been noted above.

### 4.5. Effect of matrix deuteration

Unexpectedly, the OPE and TPE spectra of DPOT change upon deuteration of the host material. The effect appears more significantly in TD than in octane, as seen in Fig. 4.19. The most striking feature is a drastic intensity decrease of the 0-0 line with respect to the vibronic lines (Fig. 4.19a). Moreover, the “U-modes” which induce the direct emission of site II are much stronger in TD-D30 than in TD-H30. This indicates a smaller population of site I in the deuterated system. In octane, the effect of matrix deuteration is almost vanishing (Fig. 4.19b). In contrast to TD, the $S_0 \rightarrow S_1$ 0-0 line is stronger in the deuterated than in the protonated octane (concluded from an excitation spectrum which is not shown here). The weaker sensitivity to matrix deuteration in octane may be related to the fact that there is only one Shpol’skii site in this host, whereas two sites show up in TD. TPE spectra at higher resolution is shown in Fig. 4.20. In TD-D30, the $S_0 \rightarrow S_1$ 0-0 transition is blue-shifted by about 15 cm$^{-1}$ with respect to TD-H30. Additionally, the intensity of the 0-0 line under TPE is about 10 times weaker in TD-D30 than in TD-H30. In the case of OPE, this intensity
loss amounts to at least a factor of 2 (Fig. 4.19a). The observation of single molecules was not achieved in TD-D30. This is of no surprise, considering the poor emission rates in this system.

In the following, an attempt of a simple explanation for these observations is given. The unit cell dimensions of a crystal mostly depend on the molecular charge distributions and are thus not expected to vary with the isotopic composition. Since C-D bonds are slightly shorter than C-H

![Figure 4.19: Emission spectra of DPOT in protonated and deuterated matrices: (a) TD-H30 and TD-D30 (excitation at 410 nm (=24390 cm⁻¹)). (b) octane-H18 and octane-D18 (excitation at 408 nm (=24510 cm⁻¹)). The spectra are normalized to equal intensities of the 0-0 lines (marked by black dots).]
bonds, DPOT molecules substituted into TD-D30 probably have a somewhat larger pocket as if substituted into TD-H30. As a consequence, the interaction between chromophore and matrix becomes weaker for the deuterated host. The DPOT molecules are less distorted in TD-D30 and their properties are closer to the vacuum properties than in TD-H30. This argument may account for the blue shift of the 0-0 line in TD-D30. Further, if the chromophore – host interaction is weaker, the extent of mixing of the $S_1$ and $S_2$ states is expected to be smaller and accordingly the $S_0 \rightarrow S_1$ transition becomes weaker. Thus, the properties of site-I-molecules are closer to the ones of site-II-molecules in TD-D30 than in TD-H30.

![Figure 4.20: Two-photon excitation spectra of the 0-0 lines of DPOT/ TD-H30 (thick line) and DPOT/ TD-D30 (thin line, multiplied by a factor of 10). The 0-0 line in TD-D30 is blue-shifted by 0.6 nm, corresponding to about 15 cm$^{-1}$ on the molecular frequency scale. Site II is very weak in this sample. The DPOT concentrations are about equal in both samples. The wiggles on the spectra are artifacts which occur due to interference from the cryostat windows.](image)
4.6. EXCITED-STATE LIFETIME

The lifetime of the $S_1$ state of DPOT in TD was measured at 1.8 K for both one- and two-photon excitation. DPOT was excited to the $S_2$ vibrational manifold by a short laser pulse. $S_2$ decays to $S_1$ by internal conversion with a time constant of about 0.6 ps (measured in a cyclohexane solution at room temperature [117]), and the subsequent slow fluorescence decay to the $S_0$ ground state was recorded. The lifetime experiments did not allow for a discrimination of the two sites.

For pulsed two-photon excitation of the $S_2$ manifold, a Styrl 9M (iii) dye laser was used which was pumped by a frequency-doubled mode-locked Nd:YAG laser with a pulse duration of about 70 ps [81] (Coherent Antares 76, 1.7 W at 532 nm, 76 MHz repetition rate). The pulse duration of the dye laser was approximately 4 ps and lasing up to 860 nm was achieved (upper wavelength threshold). Unfortunately, there was no lasing at 888 nm for two-photon excitation of the 0-0 transition of $S_0 \rightarrow S_1$. For one-photon excitation, the second harmonic of the pulsed IR beam was generated in a LiIO$_3$ crystal. The $S_1$ emission signal was recorded by a time-correlated single photon counting technique. Details of the apparatus are described in ref. [81]. The decay curves were deconvolved with the prompt response of the apparatus (shown in Fig. 3.9). When the whole emission was collected, the deconvolved signals were a superposition of the single exponential fluorescence decay and a remaining instantaneous response originating from an unknown source (iv) (Fig. 4.21). The long decay time obtained from a double exponential fit to these data was assigned to the $S_1$ lifetime. When exclusively the emission at 444 nm was detected, the average $S_1$ lifetime upon OPE at 410 nm was $\tau_{\text{OPE}} = 12 \pm 2$ ns (Fig. 4.22). This decrease if compared with the above value of $\tau_{\text{OPE}} = 12 \pm 2$ ns is probably due to the absence of the prompt response signal and a correspondingly lower background, which may be important for the fitting procedure. Within the accuracies of the measurements, the lifetimes are equal under OPE and TPE, with $\tau_{\text{TPE}} - \tau_{\text{OPE}} = 0 \pm 2$ ns.

(iii) Styrl 9M (Lambda Physik) was dissolved at a concentration of 2g/l in a mixture of 70% vol. Ethylene Glycol and 30% vol. Propylene carbonate.

(iv) This prompt response may be due to scattering in the filters which were positioned in front of the detector to reject the excitation light.
Figure 4.21: Data file of lifetime measurement. TPE at 820 nm, laser power 160 mW, accumulation time 683 s.

Figure 4.22: Fluorescence decay of $S_1$, OPE at 410.0 nm. Emission detected at 444 nm. Single exponential fit yields fluorescence decay time of $\tau_{\text{OPE}} = 11.35 \pm 0.15$ ns.
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A general theory of two-photon absorption is reviewed. The focus is put on the ac-Stark shift of the ground and excited state energy levels that is intrinsic for two-photon optical transitions. Two-photon excitation SMS is demonstrated experimentally. Of importance here is the right choice of chromophore–matrix system, as well as the use of a confocal optical design that provides a low background and high photon collection efficiency. A proof for the presence of a double photon transition is given by the study of the polarization dependence of the excitation rate and by the optical saturation behavior at increasing laser intensity. A red-shift of the single-molecule lines with increasing laser power is observed, which at least partially results from the ac-Stark effect. A detailed discussion follows in chapter 6.
5.1. INTRODUCTION

In most single-molecule experiments up to date [11-13] the molecule had to absorb only one photon to make a transition from its ground to the excited state, i.e. the interaction between the single quantum system under study and the electromagnetic wave was linear. In the experiments presented in this chapter, a nonlinear interaction between an SM and light occurs by simultaneous absorption of two photons, each carrying half of the optical transition energy. Such double quantum jumps are fundamental processes of nonlinear optics and here their first direct observation on a single quantum system is reported. Other non-linear optical experiments with single molecules have been published, the off-resonant ac-Stark effect [54] and hyper-Raman resonances [118].

Two-photon SMS also opened a way to two-photon confocal microscopy [29-31] on the SM level. Compared to conventional one-photon confocal microscopy, the two-photon technique reduces the background and improves the resolution. Two-photon fluorescence excitation microscopy has been applied to room-temperature SM imaging [119,120] and will supplement other SM imaging techniques [14,35].

A major problem of two-photon spectroscopy on the SM level is the extremely small cross section $\sigma^{(2)}$ of two-photon absorption. The largest two-photon cross sections are found in polar molecules with a widely delocalized electronic system, and design strategies for compounds with high $\sigma^{(2)}$ have been worked out [121]. Most of these chromophores are based on stilbene, the shortest diphenylpolyene. In our experiments, the $S_0 \rightarrow S_1$ transition of diphenyloctatetraene was studied. Two-photon SMS was feasible because of a high photon count rate, a low background and favorable triplet state properties (see 2.3.2.). Besides the right choice of the chromophore–host system, a nearly diffraction limited focusing of the high power laser radiation together with a strong cooling power of the cryostat were other particular experimental requirements for a successful two-photon single-molecule experiment.
5.2. Theory of two-photon absorption

The initial theory of double-photon transitions was developed by Göppert-Mayer [122] using a standard perturbation approach. A simple selection rule for two-photon transitions can only be given in the case of centrosymmetric molecules where the ground and excited state have to be of equal \( g \leftrightarrow u \) symmetry. Additionally, the relative polarizations of the two photons must be appropriate if they stem from two different laser beams. The latter condition becomes void in the case of single-beam two-photon absorption. In this section, the two-photon excitation rate is derived for a chromophore in a solid matrix. In a solid, the crystal field generally breaks the parity symmetry and the dopant may have a static dipole moment. In the following, we assume that the molecule possesses a two-photon allowed transition between the ground state \( |g\rangle \) and an excited state \( |e\rangle \), without considering details about the nature of the molecular eigenfunctions. This assumption is fulfilled for the \( S_0 \rightarrow S_1 \) transition of DPOT in TD. The electronic states of DPOT have been discussed in detail in paragraph 4.2.2.

5.2.1. Hamiltonian and two-photon absorption cross section

The Hamiltonian responsible for the interaction of a two-level molecule with a quantized electromagnetic field, which is defined by the operator of the electromagnetic vector potential \( \hat{A} \), is (written in Coulomb gauge, see ch. 14 in [48])

\[
\hat{H}_1 = -\frac{e}{m} (\hat{p} \cdot \hat{A}) + \frac{e^2}{2m} \hat{A}^2 = \hat{H}_1' + \hat{H}_1'' ,
\]

(5.1)

where \( \hat{p} \) is the momentum operator of the molecular electron. The vector potential \( \hat{A} \) is developed in a series of plane field modes with wavevectors \( \hat{k} \) and polarizations \( s \) (see e.g. [48])

\[
\hat{A}(\hat{r}, t) = \sum_{k, s} \left( \frac{\hbar}{4\pi \omega \varepsilon_0 V} \right)^{1/2} \hat{\epsilon}^{\dagger}_{k, s} \left( \hat{\alpha}^\dagger_{k, s} e^{i(\hat{k}_|\hat{r} - \text{zent}| \omega t)} + \hat{\alpha}_{k, s} e^{-i(\hat{k}_|\hat{r} - \text{zent}| \omega t)} \right),
\]

(5.2)

where \( \hat{\alpha}_{k, s} \) and \( \hat{\alpha}^\dagger_{k, s} \) denote the annihilation and creation operators for the field mode \( \hat{k}, s \), \( \hat{\epsilon}^{\dagger}_{k, s} \) corresponds to its polarization vector and \( V \) is the volume of the cavity confining the field. \( \hat{A} \) fulfills the Maxwell equations

\[
\nabla^2 \hat{A}(\hat{r}, t) - \frac{1}{c^2} \frac{\partial^2}{\partial t^2} \hat{A}(\hat{r}, t) = 0 \quad \text{and} \quad \nabla \hat{A}(\hat{r}, t) = 0 .
\]

(5.3)
The operators of the electric field $\mathbf{E}(\mathbf{r}, t)$ and magnetic induction $\mathbf{B}(\mathbf{r}, t)$ are defined by

$$\mathbf{E}(\mathbf{r}, t) = \frac{\partial A(\mathbf{r}, t)}{\partial t} \quad \text{and} \quad \mathbf{B}(\mathbf{r}, t) = \nabla \times A(\mathbf{r}, t).$$  \hfill (5.4)

Explicitly, the quantized electric field reads

$$\hat{\mathbf{E}}(\mathbf{r}, t) = \sum_{k, s} \left( \frac{\hbar \omega}{4\pi\varepsilon_0 \sqrt{V}} \right)^{1/2} \hat{e}_{k, s} \left( i\hat{a}_{k, s} e^{i(k \cdot \mathbf{r} - \omega t)} - i\hat{a}^\dagger_{k, s} e^{-i(k \cdot \mathbf{r} - \omega t)} \right).$$  \hfill (5.5)

In the electric dipole approximation, the $\hat{H}_{I''} \propto \hat{A}^2$ term of the interaction Hamiltonian (5.1) only contributes to Rayleigh scattering in first order perturbation theory [123]. The number of photons in the field is thus not changed and there is no absorption. Double photon transitions may take place by means of the $\hat{H}_I \propto \hat{p} \cdot \hat{A}$ part of the Hamiltonian in second order perturbation theory.

In the electric dipole approximation $\hat{H}_I'$ can be written as the product of the molecular dipole moment $\hat{\mu}$ and the electric field $\hat{E}$

$$\hat{H}_I' = -\frac{\hat{e}}{m}(\hat{p} \cdot \hat{A}) = -\hat{\mu} \cdot \hat{A} = -\hat{\mu} \cdot \hat{E}.$$  \hfill (5.6)

The application of second order perturbation theory, using the perturbation operator $\hat{H}_I'$, yields the cross section $\sigma^{(2)}_{ge}$ for a transition from the ground state $|g\rangle$ to an excited state $|e\rangle$ under simultaneous absorption of two photons of frequency $\omega_L$. The energy conservation law requires

$$2\omega_L = 2\pi(E_e - E_g)/\hbar = \omega_{ge}.$$  

We use the abbreviations

$$S_{ge} \equiv \left( \frac{\hbar^2}{4\pi^2} \right)^2 \sum_i X_{i, ge} \quad \text{and} \quad X_{i, ge} \equiv \frac{\left( \tilde{e} \cdot \tilde{e} \right)_{il}}{E_{gi} - \hbar \omega_L/2\pi},$$  \hfill (5.7)

where $\mu_{gi} = \langle g|\hat{l}|i\rangle$ and $\tilde{e}$ is the unit vector along the laser electric field polarization. The two-photon absorption cross section $\sigma^{(2)}_{ge}$ is given by [123-126]

$$\sigma^{(2)}_{ge} = \frac{(2\pi)^4 g(2\omega_L - \omega_{ge})}{\tilde{e}^2 \varepsilon_0^2 \omega_L^2} \cdot |S_{ge}|^2,$$  \hfill (5.8)

where $g(2\omega - \omega_{ge})$ is the line shape function of the optical transition, e.g. a normalized Lorentzian. The unit of $\sigma^{(2)}_{ge}$ is $m^4 s$. According to eq. (5.7), the calculation of $\sigma^{(2)}_{ge}$ involves an infinite sum over so-called intermediate states $|i\rangle$, where the states $|g\rangle$ and $|e\rangle$ must be included in the sum. Assuming
\( \mu_{gi} \parallel \mu_{ie}^{(i)} \) and a direction cosine \( \cos \phi \) between \( \vec{\varepsilon} \) and the transition dipole moments, \( \tilde{X}_{i,ge} \) reads

\[
\tilde{X}_{i,ge} = \tilde{X}_{i,ge}(\cos \phi)^2 \quad \text{with} \quad \tilde{X}_{i,ge} = \frac{\mu_{gi} \mu_{ie}}{E_{gi} - \hbar \omega_L/2\pi}.
\] (5.9)

Hence, we obtain the following expression for \( \sigma^{(2)}_{ge} \):

\[
\sigma^{(2)}_{ge} (\omega_L) = \frac{\omega_L^2 g (2\omega_L - \omega_{ge})}{\epsilon_0^2 \omega_L^2} \cdot (\cos \phi)^4 \left| \sum_i \tilde{X}_{i,ge} \right|^2.
\] (5.10)

Correspondingly, the rate of two-photon excitation \( W^{(2)}_{ge} \) is given by

\[
W^{(2)}_{ge} (\omega_L) = \sigma^{(2)}_{ge} (\omega_L) \cdot \left( \frac{2\pi I}{\hbar \omega_L} \right)^2 = \frac{4\pi^2 I^2 g (2\omega_L - \omega_{ge})}{\hbar^2 \epsilon_0^2} \cdot (\cos \phi)^4 \left| \sum_i \tilde{X}_{i,ge} \right|^2,
\] (5.11)

where \( I \) is the laser intensity in W/m², and \( 2\pi I/\hbar \omega_L \) corresponds to the photon flux. The two-photon excitation rate depends on the laser intensity as \( I^2 \) and on the polarization angle of the laser light as \( (\cos \phi)^4 \).

The contributions from the \( |i\rangle = |g\rangle \) and \( |i\rangle = |e\rangle \) state to the sum in eqs. (5.8),(5.10) may be separated. Discarding for the moment the \( (\cos \phi)^2 \) factor in eq. (5.9) and following the discussion in ref. [126], we can write

\[
S_{ge} = \Delta S_{ge} + \frac{\hbar^2 \omega_L^2}{4\pi^2} \sum_{i,g,e} \tilde{X}_{i,ge}, \quad \text{with}
\]

\[
\Delta S_{ge} = \frac{\hbar \omega_L}{2\pi} \mu_{ge} (\mu_{ee} - \mu_{gg}) = \frac{\hbar \omega_L}{2\pi} \mu_{ge} \Delta \mu_{\text{stat}}.
\] (5.12),(5.13)

\( \Delta \mu_{\text{stat}} \) is the static dipole moment difference between the excited and the ground state. Thus, the contribution \( \Delta S_{ge} \) becomes particularly important if \( |g\rangle \to |e\rangle \) is a one-photon allowed transition \( (\mu_{ge} \neq 0) \) and if the system under consideration has no center of symmetry \( (\Delta \mu_{\text{stat}} \neq 0) \). The contribution \( \Delta \sigma^{(2)} \) to the peak absorption cross section that originates from \( \Delta S_{ge} \) becomes

\[
\Delta \sigma^{(2)} = \frac{e^2}{\pi mc^2} \frac{T_1 (\Delta \mu_{\text{stat}} e)^2}{\Delta E},
\] (5.14)

(i) This is a reasonable assumption for DPOT, where the strong transition dipole moments are parallel to the long molecular axis [127,128].
where $f$ is the oscillator strength of the transition $|g\rangle \rightarrow |e\rangle$, $\Delta E = E_{\text{ge}}$ the excitation energy and $T$ the excited state lifetime. A Lorentzian of width $T^{-1}$ has been used for the line shape function $g$. Using the units cm$^{-1}$ for $\Delta E$, Debye for $\Delta \mu_{\text{stat}}$ and measuring $\Delta \sigma^{(2)}$ in $10^{-20}$ cm$^4$s, we obtain

$$\Delta \sigma^{(2)} = 0.63 \cdot \frac{fT_{\Gamma}(\Delta \mu_{\text{stat}})^2}{\Delta E}. \quad (5.15)$$

### 5.2.2. ac-Stark effect

When two states of an atom or molecule are coupled to a radiation field at a double photon resonance, there is a shift of the molecular eigenstates. This effect is called “light shift” or “ac-Stark effect” and arises due to the nonlinear optical interaction between molecule and laser field. To the lowest order of approximation, the ac-shifts of the ground ($|g\rangle$) and excited ($|e\rangle$) state energy levels are given by [125,129]

$$\Delta E_e(v_l) = -\frac{1}{2} \sum_i \left| \langle g|i\rangle \right|^2 \frac{2E_i}{E_i^2 - (\hbar v_l)^2} \cdot \vec{F} = \frac{1}{4} \alpha_e(v_l) \cdot \vec{F},$$

$$\Delta E_c(v_l) = -\frac{1}{2} \sum_i \left| \langle e|i\rangle \right|^2 \frac{2(E_i - E_e)}{(E_i - E_e)^2 - (\hbar v_l)^2} \cdot \vec{F} = \frac{1}{4} \alpha_c(v_l) \cdot \vec{F}. \quad (5.16)$$

The sums run over all states $i$ with energy $E_i$ and wave function $|i\rangle$. For resonant two-photon transitions $\hbar v_l = E_e/2$. $\vec{F}$ is the laser electric field amplitude at the location of the molecule. $\alpha_g(v_l)$ and $\alpha_e(v_l)$ correspond to the dynamic polarizability tensors of the ground and excited states at the laser frequency $v_l$. In a SM experiment, the resonance frequency shift $\Delta v_{\text{Stark}}$ due to the ac-Stark effect (here written as an observable in Hz) is proportional to the polarizability difference $\Delta \alpha(v_l) = \alpha_e(v_l) - \alpha_g(v_l)$. Because in the case of DPOT the strong transition dipole moments are parallel to the long molecular axis, only the tensor component $\Delta \alpha$ along this axis is important. Correspondingly, $\vec{F}$ can be replaced by its projection $F$ onto the long axis and we write $\Delta v_{\text{Stark}}$ in the form

$$\Delta v_{\text{Stark}} = \frac{1}{4\hbar} \Delta \alpha(v_l) F^2 = -\frac{1}{2\hbar c \varepsilon_0} R^2 L^2 (\cos \beta)^2 \Delta \alpha(v_l) I, \quad (5.18)$$

where $\cos \beta$ is the direction cosine of the field polarization and the molecular axis, and $I$ the laser intensity measured outside the sample in units of Wm$^{-2}$. In eq. (5.18) dielectric field corrections
have also been considered: $R = 2/(n + 1)$ accounts for the reflection of the incident field at the sample surface and $L = (n^2 + 2)/3$ is the Lorentz field correction, where $n$ is the refractive index.

The ac-Stark effect also occurs when a two-level atom (or molecule) is strongly coupled to an intense laser field at a one-photon resonance. In this case, the light shift is adequately described in the dressed atom picture \[125\]. The Stark shift can be measured by pump — probe experiments, where a strong pump beam at fixed frequency "dresses" the molecular states with laser photons and a weak probe beam is scanned to measure the changes in the optical transition frequency of the dressed system. The light shift depends inversely and with opposite sign on the pump detuning from the bare molecular resonance. Thus, the closer the pump beam is tuned to the resonance, the more the molecular transition is shifted away. This kind of experiment has been reported for single molecules of terylene in p-terphenyl \[54\] and for dibenzanthanthrene in naphthalene \[118\]. When the pump beam was tuned into resonance with the molecule, a complicated W-shaped absorption profile was observed \[54\] that was assigned to an effect similar to the Autler-Townes splitting \[125\].

The case of two-photon excitation obviously differs from the above “pump — probe approach”, because pump and probe beams cannot be distinguished and the laser frequency is very far from resonance with the molecular transition. Moreover, the sign of the ac-Stark shift depends on the sign of the polarizability difference $\Delta \alpha$, rather than the sign of the laser detuning (which is intrinsically to the red in our case).

### 5.2.3. Optical saturation

Here we consider the saturation of the two-photon excitation rate and the line broadening that are associated with the presence of a triplet bottle-neck state in the optical pumping cycle of the SM. The rate equations characterizing two-photon excitation of a 3-level molecule (ground state, excited fluorescent state and dark bottle-neck state) are identical to the equations describing one-photon excitation \[50\], if $2 \sigma(2) L/(h \nu_0)$ is replaced by $\sigma$. $\sigma(2)$ and $\sigma$ are the cross sections for two-photon and one-photon absorption given by eqs. (5.10) and (2.6), respectively. The count rate $R$ in the center of a SM line and the molecular linewidth $\Gamma$, which corresponds to twice the measured spectral width, are given by

$$R = \frac{A_{\text{tot}} \delta f}{2} \cdot \frac{K}{1 + \frac{(h \nu_0)^2 K}{2 \sigma(2) \Gamma^2}}, \quad \text{and} \quad (5.19)$$
\[ \Gamma = \Gamma_0 \sqrt{1 + \frac{2\sigma^{(2)} I^2}{K(h\nu_0)^2}} \tag{5.20} \]

where the same notation as in 2.2.2. is used. \( K = \left[ T_1 (1 + k_{\text{ISC}} / 2 k_r) \right]^{-1} \), \( k_{\text{ISC}} \) is the intersystem crossing rate, \( T_1 = k_{21} + k_{\text{ISC}} \) the total decay rate of the excited singlet state, \( k_r \) is the total decay rate from the triplet to the ground state. \( \phi_f \) is the quantum yield of fluorescence, \( A_{\text{tot}} \) the total photon collection efficiency, and \( \Gamma_0 \) is the low-power limit of the linewidth. We introduce the high-power count rate \( R_{\text{sat}} = A_{\text{tot}} \phi_f K / 2 \) and the saturation intensity \( I_{\text{sat}} = h\nu_0 \sqrt{K / 2 \sigma^{(2)}} \); in the case of two-photon excitation \( I_{\text{sat}} \) is defined in a different form if compared with one-photon excitation (eq. (2.13)). Eqs. (5.19) and (5.20) are accordingly written as

\[ R = \frac{R_{\text{sat}}}{1 + \frac{I^2}{I_{\text{sat}}^2}} \tag{5.21} \]

\[ \Gamma = \Gamma_0 \sqrt{1 + \frac{I^2}{I_{\text{sat}}^2}} \tag{5.22} \]

At low laser intensity (\( I \ll I_{\text{sat}} \)) we get

\[ R = A_{\text{tot}} \phi_f \sigma^{(2)} \left( \frac{1}{h\nu_0} \right)^2 = \frac{R_{\text{sat}}}{I_{\text{sat}}^2} I^2. \tag{5.23} \]

5.3. RESULTS AND DISCUSSION

5.3.1. Two-photon excitation single-molecule spectra

In this paragraph, the first observation of two-photon single-molecule spectra will be presented. As a preliminary study, the two-photon excitation spectrum of the inhomogeneously broadened ZPL of DPOT in n-tetradecane is investigated (Fig. 5.1). The line maximum is at about 888.0 nm and the linewidth is approximately 120 GHz. The average intensity of the band was proportional to the square of the laser intensity and was roughly proportional to the concentration of DPOT at room temperature. Since the local concentration in the solid matrix may deviate from the concentration of the liquid solution (see 3.3.2), the intensity of the inhomogeneous band varied by a factor of about five when the laser spot was moved from one place on the sample to another. With the laser tuned to the maximum of the inhomogeneous line at 888.0 nm, the dependence of the excitation
rate on the polarization angle $\phi$ of the laser light was investigated, the result is shown by the thick line in Fig. 5.2. The $(\sin \phi)^4$ dependence that is expected according to eq. (5.11) is observed in Fig. 5.2. The high contrast between the maxima and minima of the polarization curve and the

![Graph](image1.png)

**Figure 5.1:** Excitation scan over the inhomogeneously broadened two-photon absorption band. The concentration of DPOT is $2.5 \times 10^{-3}$ M, the laser intensity is about 4 MW/cm$^2$. The phonon wing is not shown.

![Graph](image2.png)

**Figure 5.2:** Thick lines: Dependence of the fluorescence signal on the polarization angle $\phi$ of the excitation light. Thin lines: Dependence of the fluorescence signal on the orientation of a polarizer in front of the detector. The solid lines are experimental data, the dashed lines indicate the fits.
excellent fit between the experimental data (solid thick line in Fig. 5.2) and the $(\sin \varphi)^4$ prediction (dotted thick line in Fig. 5.2) indicates that most of the molecules are aligned parallel. In contrast to the excitation, the fluorescence emission is caused by a one-photon transition. Correspondingly, the fluorescence intensity varied according to $(\sin \varphi)^2$ when rotating a polarizer in front of the detector. This is shown by the thin lines in Fig. 5.2. The phase shift between the polarizations of the excitation rate and the emitted light is only ±2°. Thus, the important components of the two-photon transition tensor (eq. (5.7)) are oriented parallel to the emission dipole moment $\mu_{ge}$ within the experimental accuracy. This observation is confirmed by a study of the orientation of dipole moments for DPOT in polymer films [127,128].

The band shape of the inhomogeneous line in Fig. 5.1 is dominated by the statistical fine structure (SFS, see 2.2.3). The SFS is caused by fluctuations of the number of molecules per frequency interval in the excited volume and is reproducible from scan to scan (Fig. 5.3a). From the SFS, the concentration of DPOT in the sample can be estimated [52]. The effective volume excited by two photons is $10^{-11} \text{cm}^3$. The intensity fluctuations in the center of the inhomogeneous band are about 30% of the average intensity. The corresponding number of molecules in the excited volume per frequency interval, which is equal to the measured linewidth ($\Delta v_0 = 30 \text{ MHz}$, see below), is 10. This corresponds to a DPOT concentration of $0.7 \times 10^{-5} \text{ M}$, which is in reasonable agreement with the concentration of $2.5 \times 10^{-5} \text{ M}$ measured at room temperature before the sample preparation.

A high resolution scan measured in the red wing of the inhomogeneous band (Fig. 5.3b) shows three SM lines with a signal to background ratio of about 10. The background was proportional to the square of the laser power and was probably caused by non-resonant second harmonic generation in the substrate or sample itself. The light intensity of the second harmonic propagating in the direction of the excitation beam was measured to be less than $4 \times 10^4$ photons/s. About 5% of these photons could be reflected from the sample surface and collected by the microscope optics integrated in the sample holder.

Some of the SM lines showed spectral jumps and the signal disappeared suddenly after a few scans. This behavior has been observed in many SM experiments (see also 2.3.3), particularly in Shpol'skii systems [55]. Others were very photostable and could thus be measured at different excitation powers. Fig. 5.4 shows the power dependence of the line intensity and linewidth of molecule B in Fig. 5.3. It is assumed that the Lorentzian line-shape function is given by
Figure 5.3: First observation of two-photon excitation of single molecules. a) High resolution scan at an excitation wavelength of 888.0 nm showing statistical fine structure. b) High resolution spectrum of three single-molecule lines (A,B,C), wavelength at 888.3 nm.

\[
g(v_L) = \frac{1}{(v_0 - 2v_L)^2 + \Gamma^2 / 4},
\]

where \( v_L \) is the laser frequency and \( v_0 \) is the transition frequency of the SM line. Notice that the molecular linewidth \( \Gamma \) corresponds to twice the experimentally measured linewidth \( \Delta v \).

At low power, the count rate \( R \) is proportional to the square of the laser intensity \( I \), but at higher power \( R \) increases more slowly than \( I^2 \), indicating a saturation. The measured linewidth \( \Delta v \) varies only little upon increasing the laser intensity from 0.6 MW/cm\(^2\) up to 2 MW/cm\(^2\). At the
highest intensity (~ 5 MW/cm²), R is about 4 times smaller than the value extrapolated from low-power data (line R ~ I²), whereas the line broadening is only 50% and does not compensate the reduction in the line intensity. To explain the saturation, the triplet bottleneck state in the optical pumping cycle of the SM must be taken into account. A least squares fit of eqs. (5.21) and (5.22) to the experimental data in Fig. 5.4 yields the parameters \( R_\infty = 1900 \) counts/s, \( \Gamma_0 = 60 \) MHz and \( I_{\text{sat}} = 4.1 \) MW/cm². Based on the measurement of a few molecules only, variations of about 20% were observed to \( R_\infty, \Gamma_0 \) and \( I_{\text{sat}} \). For DPOT, \( T_1 = 11 \) ns (see 4.6.) and \( \phi_f = 0.15 \) [130], yielding \( T_{\text{rad}} = T_1/\phi_f \approx 70 \) ns. The molecular linewidth is more than a factor of three larger than the lifetime limited value of \( (2\kappa T_1)^{-1} = 14 \) MHz. This difference requires further investigations and is discussed with regard to IR induced spectral diffusion in section 7.3. Spectral diffusion processes could also explain the fluctuations of the experimental values of \( \Gamma \) in Fig. 5.4b. From eq. (5.23) and the experimental data it follows that \( \sigma^{(2)} \approx 4 \times 10^{-45} \) cm⁴ s at 2 K. This value is about four orders of magnitude larger than that measured at room temperature [131] because of a drastic line narrowing at

Figure 5.4: Dependence of the count rate (a) and the linewidth (b) of the molecule B on the laser intensity. A least squares fit of eqs. (5.21) and (5.22) to the experimental data is shown by solid lines. The dashed lines \( R \sim I \) and \( R \sim I^2 \) are shown for comparison.
low temperature. The parameters \( K \) and \( k_T \) can be estimated as well, since \( K = 2R_\omega/\phi_f A_{\text{tot}} \) and \( k_{\text{ISC}} = 8 \times 10^5 \text{ s}^{-1} \) [132]. With a value of \( A_{\text{tot}} \approx 0.01 \) for the collection efficiency (see 3.1.1.) we get \( K = 2.5 \times 10^6 \) and \( k_T = 1.1 \times 10^4 \text{ s}^{-1} \). The value for \( k_T \) is in reasonable agreement with the triplet decay rate measured for shorter diphenylpolyenes (2.5 \times 10^3 \text{ s}^{-1} for diphenylhexatriene and 4.1 \times 10^2 \text{ s}^{-1} for diphenylbutadiene [133]).

In conclusion, these observations are a clear manifestation that the recorded signals correspond to the emission of DPOT molecules excited by two photons. It was not clear at the beginning of this project whether two-photon excitation of a single molecule is feasible at all. The extremely low cross section of two-photon absorption, thermal heating under excitation by strong laser light and low count rates due to saturation were anticipated obstructions for such experiments. The very low background under two-photon excitation and the use of a confocal optical setup made this experiment possible.

### 5.3.2. Light induced frequency shift

SMS becomes particularly exciting when the line shape of the single quantum system under study can be manipulated in a reproducible way. In two-photon experiments, light induced SM resonance frequency shifts in the order of 200 MHz were observed which were controlled by the laser intensity. The spectral peaks moved to the red with increasing laser power, accompanied by a broadening which was about an order of magnitude smaller than the shift (ii).

Fig. 5.5 shows a significant light induced frequency shift of about -170 MHz for three single molecules when the laser power was increased from 125 mW to 400 mW. Remarkably the corresponding broadening of these lines was only 50 MHz. Frequency shifts were investigated for 11 molecules. To avoid a systematic error due to the laser frequency drift, the laser power was alternated between high and low levels several times (Fig. 5.6a). For all molecules, the frequency shift could be satisfactorily fit with a linear function of power. The average shift coefficient for the investigated molecules was -600 MHz/W. In contrast to the line frequencies, the linewidths were only weakly affected by the laser power. An enhanced broadening was observed at powers above 250 mW that could be explained by optical saturation due to the triplet bottleneck (see Fig. 5.4b). To exclude

---

(ii) Remembering the discussion of dynamic SM line shapes in section 2.4, it must be emphasized that the line shape depends on the time resolution of the measurement and that the definition of a "linewidth" and a "peak frequency" is not always trivial. These terms are used with the model of an approximate Lorentzian line shape in mind.
Figure 5.5: TPE spectrum of the molecules depicted in Fig. 5.3 (molecules 6, 7, 8 in Table 5.1), measured at 400 mW and 125 mW of laser power.

Figure 5.6: Power dependencies of SM frequency shifts. The numbers near the data points show the order they were measured. The lines are linear fits. (a) Molecule 1 in Table 5.1, inset shows line shapes at 320 mW (left, thick line) and 90 mW (right, thin line) with Lorentzian fits. (b) Molecule 5 in Table 5.1, inset shows line shapes at 360 mW (left, thick line) and 150 mW (right, thin line) with Lorentzian fits.
saturation broadening, only linewidths measured below 250 mW have been taken into account in the analysis. In this range, the broadening was at least 5 times smaller than the line shift (see Table 5.1).

In addition to this systematic and continuous line shift, discrete spectral jumps were observed (data points 5 and 6 in Fig. 5.6b). The laser power dependence of the jump rate was not studied. For some molecules, this rate was so high that the contribution to the regular power-shift could not be evaluated. Such molecules are not included in Table 5.1 where the results are summarized.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Line shift [MHz/W] a</th>
<th>$\Gamma_0$ [MHz] (linear fit)</th>
<th>Line broadening [MHz/W] b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$-800 \pm 25$</td>
<td>$120 \pm 40$</td>
<td>$150 \pm 160$</td>
</tr>
<tr>
<td>2</td>
<td>$-600 \pm 120$</td>
<td>$60 \pm 30$ c</td>
<td>- c</td>
</tr>
<tr>
<td>3</td>
<td>$-900 \pm 300$</td>
<td>$80 \pm 30$ c</td>
<td>- c</td>
</tr>
<tr>
<td>4</td>
<td>$-520 \pm 80$</td>
<td>$50 \pm 20$</td>
<td>$30 \pm 50$</td>
</tr>
<tr>
<td>5</td>
<td>$-600 \pm 45$</td>
<td>$55 \pm 15$</td>
<td>$60 \pm 60$</td>
</tr>
<tr>
<td>6</td>
<td>$-630 \pm 90$</td>
<td>$63 \pm 6$</td>
<td>$30 \pm 40$</td>
</tr>
<tr>
<td>7</td>
<td>$-500 \pm 80$</td>
<td>$60 \pm 18$</td>
<td>$60 \pm 60$</td>
</tr>
<tr>
<td>8</td>
<td>$-600 \pm 60$</td>
<td>$36 \pm 18$</td>
<td>$160 \pm 100$</td>
</tr>
<tr>
<td>9</td>
<td>$-580 \pm 80$</td>
<td>$55 \pm 25$</td>
<td>$70 \pm 120$</td>
</tr>
<tr>
<td>10</td>
<td>$-600 \pm 50$</td>
<td>$100 \pm 50$ c</td>
<td>- c</td>
</tr>
<tr>
<td>11</td>
<td>$-680 \pm 80$</td>
<td>$140 \pm 40$ c</td>
<td>- c</td>
</tr>
</tbody>
</table>

a. Experimental data are extrapolated to 1 W.
b. Optical saturation broadening is excluded. Only measurements below 250 mW are taken into account and the results are extrapolated to 1 W.
c. The linewidth fluctuations from scan to scan have not allowed to estimate the line broadening and $\Gamma_0$. The mean value of $\Gamma$ and its standard deviation (measured at low IR power) is given instead of $\Gamma_0$.

Table 5.1: Power dependence of line frequency and linewidth for 11 single-molecules. The excitation power was varied between 60 and 400 mW, shift and broadening were extrapolated to 1 W. $\Gamma_0$ is the low-power limit of the linewidth.

In order to achieve two-photon excitation very high fields are required. As a consequence, nonlinear optical interactions between light and molecule become important on the one hand, and considerable amounts of energy may be dissipated into the lattice on the other hand. The power dependent SM frequency shift observed in Fig. 5.6 may be of various origins. The line shift resulting from the ac-Stark effect is intrinsic for two-photon excitation and is considered as a potentially important contribution. However, the ac-Stark effect does not give rise to a line broadening. Other
contributions to the shift may be related to a laser induced heating and to non-equilibrium conditions inside the spot of excitation. Such contributions are partly associated with a line broadening and would thus account for the linewidth increase as a function of power below optical saturation. SM frequency shifts and line broadenings under two-photon excitation will be discussed in detail in section 6.4.
6. SPECTRAL DYNAMICS IN ONE- AND TWO-PHOTON EXCITATION OF SINGLE MOLECULES

By a comparison of one- and two-photon spectra of the same electronic transition, spectral dynamics specific for two-photon SMS are studied. From measurements of the temperature dependence of the one-photon and the power dependence of the two-photon excitation spectra complementary information is obtained. The analysis shows the importance of four mechanisms which contribute to line shifts and broadenings in two-photon SMS and which are discussed in detail: the ac-Stark effect, a laser induced heating due to matrix absorption in the excited volume, the interaction with non-equilibrium phonons, and the acceleration of TLS dynamics by the IR irradiation and the non-equilibrium conditions.
6.1. Overview of spectral dynamics

6.1.1. One-photon excitation

For one-photon excitation (OPE) only a weak laser field is required. The SM line shape is governed by the interaction with bulk and local phonons at thermal equilibrium. Bulk phonons are known to cause line shifts and broadenings following a $T^4$ and $T^7$ law, respectively [78]. Below 10 K however, shifts and broadenings by bulk phonons are usually negligible if compared with the contributions from local phonons. The temperature dependent line broadening and frequency shift of a SM optical transition coupled to local phonons are given by eqs. (2.36) and (2.37), respectively.

The role of local phonons has been elucidated in various studies. Hesselink and Wiersma [134] investigated the dephasing of photon echoes for mixed molecular crystals of pentacene in naphthalene and p-terphenyl in the range of 4.5 K to 20 K. The comparison of the echo signals of protonated and perdeuterated pentacene in naphthalene revealed that the dephasing was dominated by a librational mode. Its nature was assigned to a rotational oscillation of the guest molecule in the cage formed by the surrounding host. A librational mode was also identified for tetracene in p-terphenyl by Kryschi et al. [135]. Voelker et al. [136] studied the temperature dependent shape of photochemically burned spectral holes for free-base porphyrin in n-octane crystals between 1.7 K and 4 K. Frequency shift and width of the spectral holes increased according to an activation law with the same activation energy, indicating the coupling of the chromophore to a single local phonon in the limit of fast exchange. An example of slow exchange was reported by Dicker et al. [137].

Surprisingly, only very few studies have so far taken advantage of the SM resolution to investigate the temperature dependence of both frequency shift and line broadening [138,139]. Most of the investigations have concentrated merely on the linewidths [50,58,140-143], so that an unambiguous identification of local phonons was not possible.
6.1.2. Two-photon excitation

Very high laser intensities are required in order to achieve two-photon excitation (TPE) of the molecules. As a consequence different mechanisms come into play if compared with one-photon excitation. In section 6.4, four types of mechanisms will be discussed which are important for the analysis of SM spectra: the ac-Stark effect, laser induced heating, the interaction with non-equilibrium phonons, and IR-induced TLS dynamics.

1. The ac-Stark effect is an intrinsic property of the molecule under TPE \([125,129]\) and has already been described in paragraph 5.2.2. The SM frequency shifts due to the nonlinear interaction between molecule and laser field. To the lowest order of approximation the ac-shift is given by eq. (5.18). The ac-Stark effect causes a light shift but does not give rise to a line broadening.

2. The high-intensity IR irradiation may be weakly absorbed by overtone vibrations of the matrix molecules. In TD for example, the C-H vibrations have an absorption coefficient of \(1.7 \times 10^{-2} \text{ cm}^{-1}\) at 888 nm (see Fig. 6.10). By vibrational relaxation and thermalization of the absorbed energy the sample is heated up inside the volume of laser irradiation. This leads to an increased activation of the local phonons. The temperature within the focal spot is governed by the heat transport to the rest of the crystal and to the crystal boundaries which are in contact with liquid helium. This transport is mediated by acoustic phonons and represents a complicated mechanism. The scattering of the acoustic phonons depends on the temperature and the heat transport may follow a ballistic or a diffusional behavior.

3. Non-equilibrium conditions and long living non-equilibrium phonons (NQP) \([144]\) have to be considered in the laser focal volume. NQP can propagate through the lattice and interact with higher-frequency local modes, which have a significant amplitude only at the sites of the probe molecules. Such modes in turn can lead to an increased optical shift and broadening if compared with the local modes activated at thermalized conditions.

4. The dynamics of slow TLSs can be accelerated by the high laser power. Such an acceleration results in a faster spectral diffusion and thus in an increased line broadening. The spectral diffusion depends on the distribution of the TLS relaxation times and the broadening saturates when the measuring time exceeds the slowest TLS relaxation.
Summarizing this introductory overview, the interpretation of two-photon excitation spectra is more involved than the corresponding one-photon spectra. This is due to the laser induced heating and the non-equilibrium conditions in the spot of strong IR irradiation, and also due to the ac-Stark effect. In the discussion of two-photon single-molecule spectra in section 6.4 we will consider all four mechanisms which have been outlined above.

6.2. ONE- AND TWO-PHOTON SMS OF DPOT

SMS has revealed remarkable differences between one- and two-photon spectra of DPOT in TD, which did not show up using high-resolution ensemble techniques such as SFS or spectral hole burning. In this section, the OPE and TPE single-molecule spectra are characterized. Their dependences on the laser intensity are studied for site I (broken symmetry, $\lambda_{S_0-S_1} = 444.0$ nm), where SM data are compared with corresponding ensemble values. Ensemble averaged data are obtained from investigations of the statistical fine structure (SFS). SFS studies of site II (centrosymmetric, $\lambda_{S_0-S_1} = 442.2$ nm) are also included for comparison. Further, the response of OPE spectra to intense IR irradiation by means of a second laser beam is investigated. The findings are compared with TPE spectra where the IR beam itself produces the excitation of the molecules. Finally, the linewidths under one- and two-photon excitation are compared.

6.2.1. One-photon SMS

OPE single-molecule spectra were investigated in different samples – an example is shown in Fig. 6.1. Usually, the SM lines were not very stable and spectral dynamics such as discrete frequency jumps were generally present. This phenomenon has also been observed in other Shpol'skii systems [11,55,145]. A few molecules were photo-stable enough to be investigated over a longer period of time. For one of them (molecule I in Fig. 6.1), the dependences of the line intensity and width on the excitation intensity are shown in Fig. 6.2. The solid lines in Fig. 6.2 are simultaneous least-squares fits with eqs. (2.12) and (2.11) for (a) and (b), respectively. The fit yields the saturation intensity $I_{sat} = 3.0$ W/cm$^2$, the high-power count rate $R_\infty = 1900$ cps and the low-power linewidth $\Gamma_0^{OPE} = 16$ MHz. The relative errors of ±43% for $I_{sat}$, ±18% for $R_\infty$, and ±68% for $\Gamma_0^{OPE}$ were estimated as the ranges in which the error function was doubled from its minimum value under variation of the corresponding fit parameter. The first two points of the linewidth data (filled circles
Figure 6.1: One-photon excitation spectrum of four DPOT single molecules in the tetradecane matrix. The excitation wavelength was at 444.175 nm (Ti:Sapph. laser at 888.35 nm), the intensity was 3.2 W/cm². The mirror symmetry, which is produced by the symmetric scan of the laser frequency, shows the reproducibility of the spectrum.

Figure 6.2: Dependences of the line intensity (a) and the linewidth (b) on the excitation intensity for molecule 1 in Fig. 6.1. The solid lines are simultaneous least squares fits with eqs. (2.12) for (a) and (2.11) for (b).
in Fig. 6.2b) were excluded from the fit, because they indicate broadening mechanisms other than optical saturation. The value of $R_m = 1900$ cps is about twice the corresponding value for two-photon excited molecules (see 5.3.1.), taking into account the 50% reduction of the total collection efficiency by the notch filter which was used to suppress the excitation light in OPE experiments (for details about the experimental setup see section 3.1).

The one-photon peak absorption cross section which is given by eq. (2.6) is estimated to $\sigma^{(1)} \approx 2 \times 10^{-12}$ cm$^2$, using the following photophysical parameters: fluorescence quantum yield $\phi_f \approx 0.1$, Debye-Waller factor $C_{DW} = 0.43$ (determined from an excitation spectrum, Fig. 4.8), intensity ratio of the 0-0 line to the full luminescence $C_{FC} = 0.1$ (determined from an emission spectrum, Fig. 4.11a), yielding a total Franck-Condon factor of $C_{DW}C_{FC} \approx 0.04$ for the purely electronic transition. The angle between the laser field polarization and the molecular emission dipole, which is parallel to the absorption dipole moment and to the long axis of the molecule [127,128], was estimated to be $\beta \approx 60^\circ$ [146]. Including all losses, the solid angle of collection, and the orientation of the molecular dipole, the photon emission rate at high power is estimated to $R_m = 3 \times 10^5$ s$^{-1}$ and the saturation intensity at the location of the molecule is estimated to $I_s = 0.7$ W/cm$^2$ [146]. Because of linewidth fluctuations within $\pm 30\%$ from scan to scan with very little correlation to the laser power, the data in Fig. 6.2b allowed only for an estimate $\Gamma_0^{OPE} = 15-25$ MHz of the homogeneous linewidth at low power. Taking into account 12 investigated molecules in different samples, $\Gamma_0^{OPE}$ was distributed between 20 MHz and 40 MHz with a distribution maximum around 30 MHz. The narrowest observed linewidth was 16 MHz, which is in reasonable agreement with the lifetime limited value of 14 MHz. The observed widths $\Gamma_0^{OPE}$ were confirmed by spectral hole burning data. Spectral holes could be burned under OPE, but not under TPE. The low-power hole width, which is twice the molecular linewidth, was usually around 60 MHz, the narrowest hole had a width of 32 MHz. The saturated hole depth was about 50%.

The fluctuations in the measured linewidths reflect the presence of spectral dynamics on a scale shorter than the accumulation time of one scan which is about 200 s. Similar distributions of linewidths were also observed in other Shpol'skii matrices [147,148]. Remarkably, the scatter in the line intensity (Fig. 6.2a) is much smaller than in the linewidth (Fig. 6.2b). This may indicate the presence of light induced spectral jumps. In this case, a molecule would change its transition frequency when the exciting laser is close to resonance. Thus, when accumulating about 100 scans to record a SM line, the measured line intensity is close to its real maximum, whereas the linewidth strongly depends on the jump distance.
### 6.2.2. Two-photon SMS

In the same sample, the SM linewidth under TPE was $\Gamma_{\text{TPE}} = 75 \text{MHz}$, in agreement with the results in paragraph 5.3.1. Already at the lowest excitation powers, the linewidths probed by TPE were generally two to three times broader than in OPE spectra. Moreover, $\Gamma_{\text{TPE}}$ fluctuated within $\pm 25\%$ from scan to scan. Most likely, spectral diffusion processes are responsible for the linewidth increase in TPE spectra with respect to OPE. Contrary to the spectral dynamics seen under OPE, these dynamics are probably enhanced by the IR light. However, such an effect must be saturated in the investigated power range. The difference between SM linewidths in OPE and TPE spectra is discussed in more detail in 6.2.5.

### 6.2.3. Statistical fine structure investigations of site I (distorted DPOT)

Using single-molecule spectroscopy, preferentially the strongest absorbers are detected, and therefore a specific group of molecules is investigated. Their properties might differ from the ensemble properties. For this reason, the ensemble features of the one- and two-photon excited molecules were compared by studying the statistical fine structure (SFS, see 2.2.3.). The SFS shows up in high-resolution scans in the center of the inhomogeneous 0-0 line and is caused by fluctuations of the number of chromophores per frequency interval. SFS investigations at different excitation powers allow for the determination of the spectral number density, the average homogeneous linewidth and the average SM emission rate for an ensemble of molecules.

Assuming the same saturation intensity $I_{\text{sat}}^{\text{ens}}$, linewidth $\Gamma_{\text{ens}}$ and high-power count rate $R_{\text{ens}}$ for all individual molecules, the correlation function of the excitation spectrum is a Lorentzian with FWHM $2\Gamma_{\text{ens}}(I)$ at any excitation intensity $I$. The power dependence of this linewidth is identical to the power dependence of the SM linewidth. For OPE, the expression for $\Gamma_{\text{ens}}(I)$, which is similar to eq. (2.11), reads

$$\Gamma_{\text{ens}}(I) = \Gamma_{0,\text{ens}}^{\text{OPE}} \sqrt{1 + I/I_{\text{sat}}^{\text{ens}}} .$$

The average fluorescence intensity $R_{\text{ens}}$ increases linearly (quadratically in the case of TPE) with $I$ for $I < I_{\text{sat}}^{\text{ens}}$. Because molecules out of resonance gain line intensity above $I_{\text{sat}}^{\text{ens}}$, $R_{\text{ens}}$ increases proportional to $\sqrt{I}$ (~$I$ for TPE) at high power. $R_{\text{ens}}(I)$ of an ensemble excited at the laser frequency $\Omega$ is obtained by integration of the homogeneous lineshape function $R_{\text{ens}}^{\text{ens}}(I, \Omega)$ over all
molecular resonance frequencies \( \omega \) at fixed \( \Omega \). Under OPE, assuming equal saturation intensities \( I_{\text{ens}}^{\text{sat}} \) for all absorbers, \( R_{\text{ens}}^{\text{sat}}(I, \Omega) \) and \( R_{\text{ens}}^{\text{sat}}(I) \) are [149]

\[
R_{\text{ens}}^{\text{sat}}(I, \Omega) = R_{\text{ens}}^{\text{sat}} \frac{I}{I_{\text{ens}}} \frac{\left(\Gamma_{\text{ens}}^{\text{OPE}}/2\right)^2}{(\Omega - \omega)^2 + (1 + I/I_{\text{ens}}^{\text{sat}})(\Gamma_{\text{ens}}^{\text{OPE}}/2)^2},
\]

(6.2)

\[
R_{\text{ens}}(I) = \frac{\pi R_{\infty} N_H}{2 I_{\text{ens}}^{\text{sat}}} \frac{I}{\sqrt{1 + I/I_{\text{ens}}^{\text{sat}}}} = F \frac{I}{\sqrt{1 + I/I_{\text{ens}}^{\text{sat}}}}.
\]

(6.3)

In the case of OPE, the investigated ensemble had a number density of 1.1 molecules/ MHz. The SFS and its correlation function are shown in Fig. 6.3. The dependences of \( R_{\text{ens}}^{\text{sat}} \) and \( \Gamma_{\text{ens}}^{\text{OPE}} \) on the excitation intensity are plotted in Fig. 6.4a and Fig. 6.4b, respectively. Both data sets were fit simultaneously, yielding the saturation intensity \( I_{\text{ens}}^{\text{sat}} = 2.7 \text{ W/cm}^2 \), the average high-power count rate per single molecule \( R_{\infty} = 160 \text{ cps} \), and the low-power limit of the average homogeneous

Figure 6.3: OPE spectrum showing SFS at a wavelength of 444.0 nm, excitation power: 3.5 W/cm\(^2\). The number of molecules per homogeneous linewidth was \( N_H = 65 \), yielding a number density of 1.1 molecules per MHz. Again, the mirror symmetry shows the reproducibility of the fine structure signal. Inset: Cross-correlation of the two symmetrical halves of the double scan, which was calculated instead of the auto-correlation of a single scan, with the advantage that the \( \delta \)-function peak originating from the noise was absent. The thick line is a Lorentzian fit with a FWHM of 116 MHz, corresponding to an average homogeneous linewidth of \( \Gamma_{\text{ens}}^{\text{OPE}} = 58 \text{ MHz} \).
Figure 6.4: Analysis of the SFS at different laser intensities. (a) Power dependence of the average fluorescence intensity $R_{\text{ens}}$. The solid line is a least squares fit to eq. (6.3) where a linear term $B \cdot I$ was added to account for additional background signal. (b) Power dependence of the average homogeneous linewidth, deduced from the cross correlation of the two symmetrical halves of a scan. The solid line is a least squares fit to eq. (6.1). Both data sets were fit simultaneously, resulting in the values $\Gamma_{\text{sat}}^{\text{ens}} = 2.7 \text{ W/cm}^2$, $F = 6000 \text{ cm}^3 \text{ W}^{-1} \text{ s}^{-1}$, $B = 100 \text{ cm}^5 \text{ W}^{-1} \text{ s}^{-1}$ and $\Gamma_0^{\text{OPE}} = 33 \text{ MHz}$. The relative errors of $\pm 59\%$ for $\Gamma_{\text{sat}}^{\text{ens}}$, $\pm 18\%$ for $F$ and $\pm 21\%$ for $\Gamma_0^{\text{OPE}}$ have the same meaning as in Fig. 6.2.

The ensemble linewidth is strongly power dependent (Fig. 6.4b) and shows much less fluctuations than a single-molecule linewidth (see Fig. 6.2b), because the spectral dynamics experienced by single absorbers are hidden in the ensemble. The representativeness of the SM data in the previous paragraph is shown by the good agreement between $\Gamma_{\text{sat}}^{\text{ens}}$ and $\Gamma_{\text{sat}}$, and between the above $\Gamma_0^{\text{OPE}}$ and the estimated $\Gamma_0^{\text{OPE}}$. The average $R_{\infty}$ calculated from the ensemble measurements is 12 times smaller (160 cps) than the corresponding value obtained from SM data (1900 cps). This difference arises due to a different collection efficiency for each molecule that contributes to the SFS. This problem has been discussed in section 3.2 where the collection efficiency of the apparatus has been simulated. The average SM count rate calculated from that simulation of the SFS was $135 - 170$ cps. This agrees with the measured value of 160 cps. For the simulation, the molecular parameters $R_{\infty} = 1900 \text{ cps}$, $I_{\text{sat}} = 3 \text{ W/cm}^2$, $\Gamma_0^{\text{OPE}} = 36 \text{ MHz}$ were...
In the case of TPE, the number density of the ensemble was 0.5 molecules/MHz. This number is about a factor of two smaller if compared with OPE. A similar ratio was found for the number of observed lines in TPE and OPE single-molecule spectra. This reduction of SM lines in TPE spectra will be discussed in more detail in 6.2.5. The average homogeneous linewidth was about 95 MHz with fluctuations of ±10% from scan to scan, and there was no power dependence of the linewidth. The value of $\Gamma_{\text{TPE}} \approx 75\text{MHz}$ from SM data is in reasonable agreement with the ensemble value. Saturation of the fluorescence intensity could not be achieved, since the laser power available was not high enough. Thus, the highest laser power at hand serves as a lower estimate for the saturation intensity, $I_{\text{ens}}^\text{sat} > 20\text{MW/cm}^2$. For comparison, the SM saturation intensity $I_{\text{sat}}$ was in the range $7 - 13\text{ MW/cm}^2$ in this sample. A few reasons are pointed out for this discrepancy which did not appear in OPE experiments. Because the TPE rate is proportional to the square of the probing laser intensity, it depends more strongly on the position of the molecule with respect to the beam center than the OPE rate. Moreover, the TPE rate is more sensitive to the angle between the wavevector of the laser field and the molecular transition dipole. The detected single molecules may have a slightly larger tilt than the ensemble average. Additionally, the blue light intensity available was much higher than $I_{\text{ens}}^\text{sat}$ and therefore even molecules in unfavorable conditions could be excited at a high rate in one-photon SFS studies. This was not the case for TPE where, as a consequence, SMs with lowest saturation intensities were selected, and $I_{\text{sat}} < I_{\text{ens}}^\text{sat}$ is not surprising.

6.2.4. Statistical fine structure investigations of site II (centrosymmetric DPOT)

In the site of undistorted centrosymmetric DPOT molecules (site II), SM detection was not possible because the photon emission rate from the $S_1$ state was too low. The $S_1 \rightarrow S_0$ transition is forbidden for site II and thus the luminescence is very weak (see Fig. 4.1Id). A further characterization of this site can be obtained from investigations of the SFS. High-resolution TPE spectra over a range of 14 GHz, measured in the center of the inhomogeneous line at 442.2 nm, are displayed in Fig. 6.5a. As expected, they show strong and reproducible fluctuations in the fluorescence intensity corresponding to the SFS. The relative amplitude of the SFS is about 18% in Fig. 6.5a (taking into account that site II and the phonon wing of site I contributed approximately equally to the total count rate of about 1000 counts/260 ms). From this amplitude we can calculate
the number of molecules per homogenous linewidth, which is $N_H \approx 30$ in Fig. 6.5a. With this number the average count rate per molecule is about 70 cps in site II. This value is about an order of magnitude lower than the average count rate per single molecule in site I. The cross correlation function of the two scans in Fig. 6.5a is shown in Fig. 6.5b. The peak near the origin can be fitted by a Lorentzian with an FWHM of $220 \pm 30$ MHz, corresponding to an average SM linewidth of $110 \pm 15$ MHz for DPOT in site II. The width of site-I-molecules in the same TD micro-crystal was about 40% smaller. Single-molecule experiments for site I yielded linewidths of 60 – 70 MHz.

At first sight, broader lines are rather expected for distorted molecules which have a stronger coupling to the environment, thus for site I. Within site I however, two to three times broader linewidths were measured in TPE than in OPE spectra. Therefore, broadening mechanisms must be considered which are specific for TPE but are inactive under OPE. Arguing on the basis of results which will be presented later (see paragraph 6.2.5 and chapter 7), an acceleration of TLS dynamics by the powerful IR irradiation is presumably responsible for an enhanced spectral diffusion in TPE.

![Figure 6.5: (a) Two high-resolution TPE spectra recorded in the center of site II (442.2 nm). The upper trace is shifted along the vertical axis for clarity. The highly reproducible structure is the SFS. The laser intensity was approx. 4 MW/cm². The horizontal axis corresponds to twice the laser frequency detuning. (b) Cross-correlation function of the two traces shown in (a). The fit is a Lorentzian with a width of 220 MHz, corresponding to a homogeneous linewidth of 110 MHz.](image)
spectra. Because of the centrosymmetric configuration of the chromophores in site II, these molecules are supposed to have more empty space inside the pocket formed by the surrounding host molecules. From the opposite point of view, neighboring matrix molecules have more space to undergo re-arrangements in site II if compared with site I (in site I the DPOT-TD configuration is expected to be more rigid). Thus, barrier heights for TLS flips are assumed to be smaller in site II and spectral diffusion processes are more easily activated in this case. We thus find the reasonable argument that the linewidth under TPE is broader in site II than in site I because of a more efficient acceleration of spectral diffusion by the IR light.

6.2.5. **Comparison of one- and two-photon excitation spectra**

The inhomogeneous line shapes of the OPE and TPE spectra of DPOT in TD are identical (see Fig. 4.8). However, this similarity does not necessarily imply that the properties of the single molecules building up those lines are also similar with regard to the two excitation processes. Therefore, the observation of one and the same molecule under both excitation processes is of great interest. In an ideal experiment, a few SM lines should be measured under OPE using a blue laser beam (second harmonic of IR beam), while the power of the fundamental IR beam, which is used for TPE, is continuously increased in small steps. Eventually, the TPE rate of the molecules exceeds the OPE rate and the blue beam can be turned off without any change of the SM spectra - the OPE spectrum has gradually gone over in a TPE spectrum. This kind of experiment turned out to be very difficult, since it required a very long observation time. However, the photo-stability was rather poor for DPOT in TD, particularly under IR irradiation (for details see Fig. 6.8), and no molecule could ever be observed over a time long enough to see a continuous OPE-to-TPE transition. Nevertheless, important information has been obtained from the attempts of such an experiment.

In advance, spectral hole burning was applied to study whether the same molecules could be observed in OPE and TPE spectra at all. Using OPE, spectral holes were burned in the center of the inhomogeneous band. These holes could then be observed in OPE spectra (Fig. 6.6, upper trace). Deep holes of about 50% relative depth could be observed under both one- and two-photon excitation (Fig. 6.6, lower trace). The two-photon hole detection was complicated by a reduction of the hole depth during TPE readout ("hole filling"). This hole filling disabled the burning of persistent holes using TPE. The small red-shift of the hole position in TPE spectra is related to the light induced SM frequency shift reported in 5.3.2. Except for this shift, the holes are similar for OPE and TPE. This experiment shows that at least some of the molecules are sensitive to both excitation
processes. The similarity of the OPE and TPE spectra mirrored in the inhomogeneous line shapes is confirmed by the shapes of the spectral holes.

Figure 6.6: Hole burning experiment at $\lambda = 444.0$ nm. Some of the molecules can be detected in both OPE (upper trace) and TPE (lower trace) spectra. The thick grey lines are Lorentzian fits to the spectral holes.

Figure 6.7: Two scans at SM resolution manifest a much more complicated picture ($\lambda = 444.0$ nm). More molecules are seen in the OPE spectrum and no obvious correlation between the spectral positions of SM lines in the two spectra is observed. The laser frequency was scanned by a triangular voltage ramp producing a mirror symmetry in the spectra (indicated by the dashed line).
SMS however reveals a more involved picture. The one- and two-photon excitation SM spectra, shown in the upper and lower traces of Fig. 6.7 respectively, are remarkably different. The most striking difference is that the number of lines observed under OPE is approximately twice the number observed under TPE. This ratio is confirmed by SFS studies where the number density of an ensemble was typically about 0.5 molecules/ MHz in the case of TPE and about 1 molecule/MHz for OPE (see 6.2.3.). Moreover, SM lines are generally broader in TPE than in OPE spectra. This was already stated in paragraph 6.2.2. where the power dependence of the linewidth was studied. In Fig. 6.8 three different types of SM spectra over the same frequency range are compared: (a,d) TPF using the fundamental (IR) laser wavelength of 888.4 nm, (b) OPE using the second harmonic (blue) laser wavelength at 444.2 nm with simultaneous illumination with a second beam at the IR wavelength, (c) OPE only. The contribution from TPE itself to the spectrum 6.8b is only 25% and Fig. 6.8b is not a superposition of Figs. 6.8a and 6.8c. The number of observed SM lines is dramatically reduced upon IR irradiation. This effect is independent of the excitation process itself and is observed in spectra (a), (b), and (d). The gradual transformation from spectrum (c) to (b) while steadily increasing the power of the IR beam has been observed only qualitatively, but no SM line could be followed during this time consuming process – the rate of spectral jumps was too high in the TD Shpol’skii matrix. The number of SM lines showing up in the OPE spectrum (c) is again approximately twice the number in the spectra (a), (b), and (d). Apparently, the high-power IR light induces spectral diffusion processes which result in lines so broad that about 50-70% of the molecules are no longer detected.

IR-induced spectral diffusion was found to be a resonant process in the spectral hole-burning experiments reported in refs. [150,151]. Additional H2O molecules embedded into a polymer hole-burning matrix could perform flips or reorientations when resonantly illuminated with IR light. As a consequence, solvent shifts of adjacent dye molecules changed and an overall broadening of the hole profile was observed. Less than 10 excitations of a fundamental vibration per H2O molecule were enough to fully saturate that broadening. In our experiment, the IR light is weakly absorbed by overtone C-H vibrations of the TD matrix (for details see Fig. 6.10). Each TD molecule is excited vibrationally at least 10 times during the recording time of one scan when the sample is irradiated with 10 MW/cm² of IR at 888 nm. TLS dynamics are assumed to be triggered by the release of

(i) This ratio was found to vary from sample to sample. It also depended on the laser spot position on the micro-crystal and on the alignment of the aperture in the confocal plane. The highest ratio measured was 6:1.

(ii) In the laboratory slang we have called this effect of the IR irradiation a "cleaning effect" because the "unstable" molecules seem to be "cleaned" away from the spectrum.
One- and two-photon SMS of DPOT

this vibrational excitation energy into the lattice. The resulting spectral diffusion, which must be saturated on the time scale of a TPE single-molecule measurement (~ 10 s), can explain the line broadening in the presence of high-power IR light.

Figure 6.8: Single-molecule spectra measured at different conditions. (a) TPE with 350 mW at the fundamental laser wavelength (λ = 888.4 nm). (b) OPE with 500 nW at the second harmonic laser wavelength (λ = 444.2 nm) when the sample was simultaneously illuminated with 350 mW of IR using the fundamental beam at 888.4 nm. (c) Pure OPE spectrum (λ = 444.2 nm, 500 nW). (d) TPE (λ = 888.4 nm, 350 mW) to check for the reproducibility of scan (a). Spectra (a), (b), and (c) are shifted up for clarity. The spectra were measured in the order (a) to (d). The comparison of (a) and (d) shows that there was no laser frequency drift (see molecules number 3 and 4). In the TPE spectra (a) and (d) the vertical scale is expanded by a factor 4. The contribution from TPE itself to the spectrum (b) was less than 25%. The SM lines number 3 and 4 are observed under both OPE (b) and TPE (a,d). Taking into account the light induced shift, molecule 3 most likely originates from one of the two molecules labelled by asterisks in the pure OPE spectrum (c). Molecule 1, the strongest peak under OPE (b), did not manifest itself in the pure TPE spectra. The shoulder marked by 2 and the molecule 5 in the spectra (a) and (d) were not observed in the spectrum (b). The vertical lines are guides for the eye. Places where corresponding lines are missing, are labelled by the arrows.
From Fig. 6.8, three different classes of DPOT molecules can be distinguished on a phenomenological basis. "Class 1" molecules can be detected by OPE, whereas TPE is impossible or very inefficient (e.g. molecule 1 in Fig. 6.8). "Class 2" molecules can be detected upon simultaneous absorption of two photons, while OPE is very inefficient (SMs 2 and 5 in Fig. 6.8). "Class 3" molecules can be detected in both one- and two-photon excitation spectra (SMs 3 and 4 in Fig. 6.8). The number of one-photon excited molecules, which may belong to class 1 or class 3, is generally two to three times the number of two-photon excited molecules, being of class 2 or class 3. The presence of spectral dynamics made a reliable identification of class 3 molecules difficult, and has disabled their detailed study. In the specific example of Fig. 6.8, one tenth of the one-photon excited molecules and correspondingly one third of the ones detected under TPE were of class 3. This classification is of phenomenological nature and is not based on a microscopic model. It is assumed that all these observations are related to the different sensitivity of SMs to the spectral diffusion processes which are accelerated by the IR illumination.

All linewidths measured in one- and two-photon single-molecule and SFS experiments are summarized in Fig. 6.9. The linewidth is two to three times broader when high power IR is irradiated.

![Figure 6.9: Summary of linewidths in one- and two-photon spectra. TPE: Large empty squares with error bars are data points from SFS measurement, small filled squares originate from SM data (a saturation curve according to eq. (5.22) is fitted). OPE: Large empty circles are from SFS, small filled circles from SM data (a saturation curve is fitted to eq. (2.11)). The linewidth distribution observed under OPE with additional IR irradiation (~ 300 mW) is marked by the double arrow. The triangle denotes the lifetime limited value of 14.5 MHz.](image-url)
onto the sample, as in the case of TPE (squares) or when additional IR is switched on in an OPE experiment (double arrow). IR-induced spectral diffusion processes which are probably responsible for this broadening are further investigated in section 7.3, where the linewidths under TPE are studied in dependence of the measurement’s time resolution. But even in pure OPE spectra (circles), the linewidth at low power is broader than the lifetime limit (triangle). Hence, additional spectral dynamics on a timescale faster than the measuring time (~ 10 s) must be active at the sample temperature of 1.8 K.

6.2.6. Summary

Single molecules of DPOT in TD have been investigated with regard to their behavior under one- and two-photon excitation. The number of observed SM lines was two to three times larger in OPE than in corresponding TPE spectra. The homogeneous linewidths probed by TPE were two to three times broader than under OPE. Particularly, this was also the case for molecules which were detectable under one- and two-photon excitation simultaneously (“class 3”). The broadening likely occurs owing to spectral diffusion induced by vibrational excitations of the matrix upon the high-power IR irradiation. The effect must be saturated in the investigated power range, because no significant dependence of $\Gamma_{\text{TPE}}$ on the laser intensity was observed.

6.3. Temperature dependence of single-molecule spectra

6.3.1. Laser induced heating in two-photon excitation experiments

An absorption spectrum of pure TD measured at room temperature is shown in Fig. 6.10. The third overtone of the C-H vibration (band center at 932 nm (= 10730 cm$^{-1}$)) has an absorption coefficient of $1.7 \times 10^{-2}$ cm$^{-1}$ at 888 nm. This corresponds to an excitation rate per TD molecule of about 3000 s$^{-1}$ at a laser intensity of 10 MW cm$^{-2}$ (approximately upper limit of intensity in our experiments). For two-photon excitation of single molecules, a laser intensity of about 1 MW cm$^{-2}$ was required to obtain a sufficient signal-to-noise ratio. Thus, under the conditions of a two-photon experiment the third-overtone C-H vibrations of the matrix are excited by the laser. This vibrational energy is released to the lattice and the matrix is heated up within the hot-spot of the focused laser irradiation. The effective temperature within the focal spot is governed by the heat transport
properties of the crystal. The TD microcrystals have many cracks and a rough surface which is in contact with the superfluid helium.

A peculiarity of the heat transfer in superfluid He is the He film boiling at the interface with the solid that occurs above a critical heat flux [152]. In our experiments, such a regime was observed at laser powers above 400 – 500 mW focused to the excitation spot of 2 μm diameter. The film boiling was visible by the formation of tiny bubbles. These bubbles occurred due to evaporation of superfluid He that had creeped into the cracks and roughness of the sample surface. So the bubble formation was restricted to the thin zone between sample surface and cover glass. The average temperature increase in the sample chamber was negligible (< 0.05 K), the film boiling was not associated with a raise of pressure in the cryostat. To find a relation between the heat flux into the crystal and the steady-state temperature increase in the laser spot, a temperature dependent thermal conductivity and the nonlinear Kapitza resistance at the interface of the solid with the superfluid He must be taken into account. The effective temperature $T_{\text{eff}}$ inside the crystal as a function of the laser power $P$ can be estimated from steady-state heat transport conditions using the Kapitza relation [152,153]

$$T_{\text{eff}} = (T_b^4 + \Delta P)^{1/4}, \quad (6.4)$$
where $T_b$ is the He-bath temperature of 1.8 K, $P$ the laser power in watts, and $\Lambda$ is a parameter with units $K^4 W^{-1}$. $\Lambda$ depends on the absorption coefficient of the C-H vibrations, the thermal conductivity, and on the size, molar mass and Debye temperature of the crystal.

In TPE experiments, the temperature raise within the spot of irradiation leads to an enhanced activation of the local phonons and thus to line broadenings and line shifts. A power dependent line shift has been reported for TPE spectra of DPOT in TD in section 5.3. However, those results alone do not allow for a definite discrimination of the local-phonon induced shift from other contributions, such as the optical Stark shift or line shifts resulting from other relaxation pathways of the matrix vibrations. One way to shed light on these questions is to use single-photon excitation. In this case, nonlinear optical effects are suppressed and less energy is dissipated into the lattice. Temperature dependent studies of line shapes aim at a characterization of thermal effects in the absence of laser induced heating.

In the following, the temperature dependence of SM line shapes is investigated for one-photon excitation spectra of DPOT in TD at temperatures below 10 K. We will learn that for one-photon excitation the observed red shifts and line broadenings at higher temperatures are well understood in the framework of a thermally activated local phonon mode. In section 6.4, these temperature dependences will then be compared with the power dependent line shifts observed in TPE single-molecule spectra.

### 6.3.2. Local phonons in the DPOT/tetradecane system

A two-photon excitation spectrum of DPOT in TD recorded at 1.8 K is shown in Fig. 6.11. The $S_0 \rightarrow S_1$ 0-0 ZPL at 22521 cm$^{-1}$ is assigned to the symmetry-broken site I. All results reported in the following have been obtained for this site. The ZPL is accompanied by a phonon wing with two peaks at 25 cm$^{-1}$ and 33 cm$^{-1}$ with widths of approximately 10 cm$^{-1}$. These peaks are tentatively assigned to two librational modes. They could not be resolved in the one-photon excitation spectra. A local mode of 32 cm$^{-1}$ was observed for the similar system of octatetraene in n-hexane [154]. The line at around 22625 cm$^{-1}$ (442.1 nm) is due to DPOT located at the centrosymmetric site II.
6.3.3. Temperature dependence of single-molecule line shapes

Using one-photon excitation, frequency shifts and linewidths of several individual molecules were measured in the temperature range of 1.8 K to 6.6 K. For the temperatures 1.8 K and 5.5 K the spectra of five molecules (A, E, F, G, H) are displayed in Fig. 6.12. An increase of the linewidth with increasing temperature is clearly visible. Accordingly, the molecules F and G close to each other in frequency cannot be resolved at the higher temperature. For all temperatures Lorentzians were fitted to the observed SM lines. The corresponding $\Delta \Gamma(T)$ and $\Delta \nu(T)$ dependences were then fit to eqs. (2.36) and (2.37), respectively. We recall

$$\Delta \Gamma(T) = \Gamma(T) - \Gamma_0 = \beta_\Gamma \cdot \exp\left(\frac{-E}{kT}\right), \quad (2.36)$$

$$\Delta \nu(T) = \nu(T) - \nu_0 = \beta_\nu \cdot \exp\left(\frac{-E}{kT}\right), \quad (2.37)$$

where

$$\beta_\Gamma = 2\tau \delta \left[ \frac{\delta/2\pi}{1 + \tau^2 \delta^2} \right] \quad \text{and} \quad \beta_\nu = \left[ \frac{\delta/2\pi}{1 + \tau^2 \delta^2} \right], \quad \text{with} \quad |\beta_\Gamma/\beta_\nu| = 2\tau|\delta|. \quad (6.5)$$
For molecules A and D the fitted linewidths and shifts are shown in Fig. 6.13. Below 3K there was no significant variation of the line frequency and linewidth for all molecules. Therefore, $v_0$ and $\Gamma_0$ were set to the average of the values between 1.8 K and 2.5 K. The fitted parameters of 9 single molecules are collected in Table 6.1. In general, good agreement was obtained. Tentatively the linewidths deviate from the fits towards larger values at higher temperatures. The local phonon frequencies ($\omega/\hbar c$) of the 9 molecules are between 14 cm$^{-1}$ and 19 cm$^{-1}$ and the local phonon

![Figure 6.12: Spectra of molecules A (a) and E, F, G, H (b) at different temperatures. The laser wavelength is at 444.2 nm. Molecules A, E and H exhibit a significant frequency shift of about -300 MHz upon a temperature increase to 5.45 K (a) and 5.65 K (b), respectively. Molecules F and G are no longer resolved at 5.65 K. The upper spectra are shifted along the vertical axis for clarity.](image.png)
lifetimes within 1–3 ps. Similar results were observed for octatetraene in n-hexane [154]. The ratios $|\Delta \Gamma/\Delta \nu| < 1$ indicate an intermediate to fast exchange behavior.

Three remarks are in order here. (i) The local modes of Table 6.1 are not confirmed by the structure of the phonon sideband in Fig. 6.11, where the maxima appear at $2 \times (E/hc) = 30 \text{ cm}^{-1}$. One can argue that local vibrations crucial for the shift and broadening need not necessarily be strongly active modes in allowed electronic transitions. In a hole-burning study of phthalocyanine doped TD, Rebane et al. [155] also observed that the peak of the phonon sideband was at about twice
the frequency of a thermally activated local vibration. These authors claimed that this was due to its non-totally symmetric nature. Applying the same arguments to our system, the local modes in Table 6.1 are presumably of low symmetry. (ii) In SM spectroscopy, for obvious reasons, the strongest and most photo-stable absorbers are preferentially detected. This may lead to a non-representative set of molecules. SFS investigations provide high-resolution information on an ensemble of molecules. Comparing the parameters of the 9 molecules with those from SFS data, also presented in Table 6.1, a good agreement is observed. Therefore molecules A - J likely form a representative set. (iii) The frequency shift due to the thermal expansion of the matrix should also be considered. From the Grüneisen formula [156], a thermal expansion coefficient of $2 \times 10^{-8} \text{K}^{-1}$ for TD and a corresponding blue shift of less than 1 MHz/K is calculated at 2 K. Hence, although the shift is proportional to $T^3$ at low $T$ [156], a negligible contribution from the temperature induced matrix expansion is expected.

| Molecule | $\Gamma_0$ [MHz] | $E$ [cm$^{-1}$] | $\beta_T$ [GHz] | $\beta_v$ [GHz] | $|\Delta\Gamma/\Delta v|$ | $\delta/2\pi$ [GHz] | $\tau$ [ps] |
|----------|-----------------|----------------|----------------|----------------|---------------------|----------------|---------|
| A        | 27.5            | 17.2 (4)       | 11.8 (18)      | -31.4 (31)     | 0.38                | -32.5          | 0.9     |
| B        | 18.0            | 17.9 (4)       | 24.1 (67)      | -25.3 (46)     | 0.96                | -31.0          | 2.5     |
| C        | 35.0            | 17.3 (23)      | 15.7 (34)      | -24.4 (36)     | 0.64                | -26.9          | 1.9     |
| D        | 58.5            | 14.6 (6)       | 13.5 (27)      | -18.0 (24)     | 0.76                | -20.5          | 2.9     |
| E        | 25.7            | 18.9 (2)       | 18.6 (30)      | -34.8 (22)     | 0.54                | -37.3          | 1.1     |
| F        | 22.2            | 17.0 (13)      | 9.4 (44)       | -20.2 (118)    | 0.48                | -21.3          | 1.8     |
| G        | 26.6            | 15.9 (4)       | 7.0 (7)        | -15.8 (11)     | 0.44                | -16.6          | 2.1     |
| H        | 29.2            | 16.7 (6)       | 11.0 (31)      | -21.1 (33)     | 0.56                | -22.5          | 1.8     |
| J        | 32.0            | 18.9 (3)       | 13.6 (25)      | -34.2 (64)     | 0.40                | -35.5          | 0.9     |
| average A - J | 30.5 | 17.2 (14) | 14 (5) | -25 (7) | 0.57 (19) | -27 (7) | 1.7 (7) |
| SFS (same sample) | 25.0 | 20.7 | 16.8 | -38 | 0.44 | - 40 | 0.9 |
| SFS (other sample) | 26 | 19.7 | 15 | -42 | 0.36 | -43 | 0.7 |

**Table 6.1:** Results of the temperature dependent measurements of the 9 single molecules A - J. Ensemble averaged values obtained from SFS investigations of two different samples are also included.
The temperature dependences of the linewidths in Fig. 6.13 show that broadening mechanisms other than the coupling with local phonons are present. First, the linewidths $\Gamma_0$ (at $T = 0$ K) of Table 6.1 are compared with the lifetime limited width of 14.5 (±0.7) MHz (see section 4.6). This value, although measured for the bulk, is considered as a lower bound for the linewidth of the individual chromophores. Remarkably, all $\Gamma_0$ values in Table 6.1 exceed this limit, typically by about 15 MHz. TLS dynamics which are active at temperatures below 3 K, are presumably responsible for the additional broadening beyond the lifetime limited value. As an alternative explanation, this broadening may be caused by light-induced saturated spectral diffusion. Such a process was observed on a time scale of about one second in two-photon spectroscopy, where high laser intensities are present (see chapter 7). Second, at temperatures above 6 K the linewidths deviate significantly from the fit for molecule A (Fig. 6.13a, open circles). The effect is also present but less distinct for molecule D (Fig. 6.13b). Because the deviations are more pronounced for the broadening than for the shift, we argue that above 6 K, bulk phonon scattering becomes important for which the broadening grows faster than the shift with increasing temperature.

6.4. DISCUSSION OF TWO-PHOTON SINGLE-MOLECULE SPECTRA (PART I.)

The TPE investigations of the DPOT/TD system presented in section 5.3 are now discussed thoroughly. In SM spectra, power dependent red shifts of 500 – 900 MHz/W were observed while the corresponding line broadenings of 30 – 140 MHz/W were about one order of magnitude smaller. These results are now compared with the temperature dependence observed under one-photon excitation. In fact, with the temperature dependence of the one-photon and the power dependence of the two-photon excitation spectra complementary information is obtained. As a characteristic quantity for the discrimination between the different processes contributing to the TPE line-shape (see 6.1.2.), the ratio $\frac{\Delta \Gamma}{\Delta \nu}$ is considered. According to eqs. (2.36) and (2.37), $\frac{\Delta \Gamma}{\Delta \nu}$ does not depend on the temperature.

In Fig. 6.14, the temperature dependence of one-photon spectra (OP data) is compared with the power dependence of two-photon excitation spectra (TP data) under conditions of approximately equal line shifts. We focus on the ratio $|\Delta \Gamma/\Delta \nu|$. In the OP data of Fig. 6.14b the linewidth increases by 110 MHz and the frequency shifts by -175 MHz for molecule E upon a temperature change from 1.8 K to 5.15 K. This corresponds to $|\Delta \Gamma/\Delta \nu|_{\text{OP}} \approx 0.6$ which is compatible with the values in
Table 6.1. In the TP data of Fig. 6.14a, a shift of -160 MHz and line broadening of 50 MHz is observed upon a laser power increase from 125 to 400 mW. Accounting for an optical saturation broadening of about 35 MHz obtained from independent measurements (see Fig. 5.4), we find that $|\Delta \Gamma / \Delta \nu|_{\text{TP}} \approx 0.1$. As a consequence, $|\Delta \Gamma / \Delta \nu|_{\text{OP}}$ is about 6 times larger than $|\Delta \Gamma / \Delta \nu|_{\text{TP}}$ for the

![Diagram](image)

**Figure 6.14:** Comparison of OP and TP data. (a) TP data at a laser wavelength of 888.3 nm: Two-photon excitation spectrum of molecules 6, 7, 8 (from left to right) in Table 5.1 at 1.8 K. The excitation power is 125 mW (lower trace) and 400 mW (upper trace, shifted for clarity), respectively. The line shift associated with this power increase is about -160 MHz, the corresponding line broadening is about 50 MHz (average of molecules 6 and 7). (b) OP data at a laser wavelength of 444.2 nm: One-photon excitation spectrum of molecules E, F, G, H at 1.8 K (lower trace) and 5.15 K (upper trace, shifted for clarity). The line shift associated with this temperature increase is between -175 MHz (molecule E) and -215 MHz (molecule H), the corresponding line broadenings are about 110 MHz.
molecules shown in Fig. 6.14. Taking into account the uncertainties of $\Delta \Gamma$ in the TP data (error intervals in Table 5.1), we estimate that $|\Delta \Gamma/\Delta \nu|_{\text{TP}} \leq 0.3$ for all molecules observed. From Table 6.1 we find that the average $|\Delta \Gamma/\Delta \nu|_{\text{OP}}$ is $0.5 - 0.6$ and thus at least by a factor of 2 larger than $|\Delta \Gamma/\Delta \nu|_{\text{TP}}$. This indicates very strongly that there is a significant difference between the results obtained from the two excitation methods.

The question arises whether different molecular ensembles are probed under one- and two-photon excitation and whether this would account for different $|\Delta \Gamma/\Delta \nu|$ values. It is emphasized that the molecular transition is the same for the two excitation techniques, so that in principle the same set of molecules is expected to be observed in one- and two-photon spectra. However, 2-3 times less SM lines were detected in two-photon than in one-photon spectra (see Fig. 6.8). This result has been explained by an accelerated spectral diffusion under IR illumination resulting in lines so broad that about 50% to 70% of the molecules are no longer detectable. One could argue that the ratio $|\Delta \Gamma/\Delta \nu|_{\text{OP}} = 0.6$ is related to this particular group of molecules that vanish in a two-photon experiment and are exclusively seen under one-photon excitation. The validity of this argument becomes questionable when statistical reasoning is applied. We compare data sets of 9 molecules under one-photon and 7 molecules under two-photon excitation, and thus the probability for two disjoint sets is very small. Therefore, the possibility is ruled out that the difference in $|\Delta \Gamma/\Delta \nu|$ originates from disjoint ensembles, and hence the difference is significant.

While only one mechanism is predominantly responsible for the temperature dependence of OP data, namely the activation of local phonons, several mechanisms contribute to the laser power dependence of TP data (see Table 6.2). We therefore write for the line broadening

$$\Delta \Gamma(P) = \Delta \Gamma_{\text{th}}(P) + \Delta \Gamma_{\text{NQP}}(P) + \Delta \Gamma_{\text{TLS}} + \Delta \Gamma_{\text{sat}}(P),$$  \hspace{1cm} (6.6)

where $\Delta \Gamma_{\text{th}}$ results from the local phonon activated by the laser induced heating with the power dependence given by eq. (6.4), $\Delta \Gamma_{\text{NQP}}$ denotes the broadening due to the non-equilibrium phonons, and $\Delta \Gamma_{\text{TLS}}$ is caused by IR-induced TLS dynamics. $\Delta \Gamma_{\text{sat}}$ is the optical saturation broadening which has been studied previously in 5.3.1. Analogously to eq. (6.6), the frequency shift is

$$\Delta \nu(P) = \Delta \nu_{\text{Stark}}(P) + \Delta \nu_{\text{th}}(P) + \Delta \nu_{\text{NQP}}(P).$$  \hspace{1cm} (6.7)

The ac-Stark shift $\Delta \nu_{\text{Stark}}$ and the NQP contribution $\Delta \nu_{\text{NQP}}$ depend linearly on $P$, whereas $\Delta \nu_{\text{th}}$ follows a non-linear $P$-dependence according to eq. (6.4). The TLSs do not contribute to the shift. The TP data alone do not allow for the discrimination between the different effects. However, by identifying the ratio $|\Delta \Gamma_{\text{th}}(P)/\Delta \nu_{\text{th}}(P)|$ of the TP data with $|\Delta \Gamma(T)/\Delta \nu(T)|$ of the OP data, one
can estimate the effective temperature in the system which then allows for the independent determination of $\Delta\nu_{\text{th}}$ and $\Delta\Gamma_{\text{th}}$. In the following the broadenings and shifts will be discussed in terms of eqs. (6.6) and (6.7).

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>SM frequency shift ($\Delta\nu$)</th>
<th>SM line broadening ($\Delta\Gamma$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no broadening</td>
<td>$\Delta\nu = 0$</td>
<td>$\Delta\Gamma = 0$</td>
</tr>
<tr>
<td>ac-Stark effect</td>
<td>see eq. (5.18)</td>
<td>see eq. (2.36)</td>
</tr>
<tr>
<td>Interaction with thermally activated local photon</td>
<td>$\Delta\nu = 150 \text{ MHz/W}^a$</td>
<td>$\Delta\Gamma = 30 - 40 \text{ MHz}$</td>
</tr>
<tr>
<td>NQP</td>
<td>small ($&lt; 10%$ of $\Delta\nu_{\text{NQP}}$)</td>
<td>$100 \text{ MHz/W} &lt; \Delta\Gamma_{\text{NQP}} &lt; 7 \text{ MHz/W}^a$</td>
</tr>
<tr>
<td>Interaction with spectral diffusion due to accelerated TLS dynamics</td>
<td>$\Delta\nu_{\text{TLS}} = 7 - 30 \text{ MHz}$</td>
<td>$\Delta\Gamma_{\text{TLS}} = 30 - 40 \text{ MHz}$</td>
</tr>
<tr>
<td>Saturation of optical transition</td>
<td>$\Delta\nu_{\text{Sat}} = 0$</td>
<td>$\Delta\Gamma_{\text{Sat}} = 25 \text{ MHz}$</td>
</tr>
</tbody>
</table>

$a.$ for molecule 1 in Table 5.1.

Table 6.2: Contributions of various mechanisms to the frequency shift and line broadening in two-photon excitation spectra of single molecules.
6.4.1. ac-Stark effect

The resonance frequency shift $\Delta v_{\text{Stark}}$ due to the quadratic optical Stark effect depends according to eq. (5.18) on the difference $\Delta \alpha(v_L) = \alpha_e(v_L) - \alpha_g(v_L)$ between the excited ($|e\rangle = |S_2\rangle$) and ground ($|g\rangle = |S_0\rangle$) state polarizabilities, evaluated at the laser frequency $v_L$. The polarizabilities $\alpha_g(v_L)$ and $\alpha_e(v_L)$ are given by eqs. (5.16) and (5.17), respectively, they explicitly read

$$\alpha_g(v_L) = \sum_i \frac{2E_i}{E_i - (hv_L)^2}, \quad (6.8)$$

$$\alpha_e(v_L) = \sum_i \frac{2(E_i - E_e)}{(E_i - E_e)^2 - (hv_L)^2}. \quad (6.9)$$

For the sake of clarity eq. (5.18) is repeated here:

$$\Delta v_{\text{Stark}} = -\frac{1}{2\hbar c \varepsilon_0} R^2 L^2 (\cos \beta)^2 \Delta \alpha(v_L), \quad (5.18)$$

where the dielectric field corrections $R$ and $L$ have been defined in paragraph 5.2.2.

Now we consider two ways to estimate $\Delta v_{\text{Stark}}$. The first method is based on the expression

$$|\Delta v_{\text{Stark}}| \approx 40 \text{ MHz/W} \quad (iii).$$

With $m = 5$ as a reasonable number of intermediate states $|i\rangle$ taken into account in eqs. (6.8) and (6.9). Considering $|i\rangle = |S_2\rangle$ as a single intermediate state, the approximation of eq. (6.10) yields

$$|\Delta v_{\text{Stark}}| \approx 100 \text{ MHz/W}. \quad (6.11)$$

The second method is based on approximating the ac polarizabilities by the corresponding dc values, which are $\alpha_{dc}^g = 100 \times 10^{-40} \text{ CV}^{-1} \text{ m}^2$ [158] and $\alpha_{dc}^e = 165 \times 10^{-40} \text{ CV}^{-1} \text{ m}^2$ [110] along the long molecular axis of DPOT, yielding $\Delta \alpha_{dc} \approx 6.5 \times 10^{-39} \text{ CV}^{-1} \text{ m}^2$. Taking into account the laser spot size of 2 $\mu$m diameter, the angle $\beta$
of approximately 60° between the electric field and the molecular axis (iv) and a refractive index of 
\( n = 1.45 \), we get \( \Delta v_{\text{Stark}} = -180 \text{ MHz/W} \). A frequency shift of \( \Delta v_{\text{Stark}} = -420 \text{ MHz/W} \) is 
obtained when using the more recent experimental result \( \alpha_{\text{dc}} = 25.3 \times 10^{-39} \text{ CV}^{-1} \text{ m}^2 \) of ref. [160]. 
However, a large contribution to \( \Delta \alpha_{\text{dc}} \) is from the second electronically excited singlet state 
\( |j\rangle = |S_2\rangle \) at 24390 cm\(^{-1}\). Considering that this contribution is roughly five times smaller and of 
opposite sign at the laser frequency of \( v_L/c = 11261 \text{ cm}^{-1} \), the value of \( -180 \text{ MHz/W} \) over-
estimates the power dependent shift. Therefore, \( \Delta v_{\text{Stark}} \approx -100 \text{ MHz/W} \) obtained from the first 
method is taken as a reasonable guess for the ac-Stark shift.

One of the transitions between \( |S_1\rangle \) and a higher excited singlet state \( |nB_u\rangle \) might be resonant 
with the laser frequency \( v_L \) and could thus give rise to a dominant term in the sum of eq. (6.9). 
Resonances are not taken into account in the two estimates above. In the following an estimate for 
the contribution to \( \Delta \alpha(v_L) \) from such a resonance is given. Assuming a Lorentzian line-shape of 
width \( \gamma_{nB_u} \), the contribution of a resonant state \( |nB_u\rangle \) is largest when the laser frequency is detuned 
from the \( nB_u \leftrightarrow S_1 \) transition such that \( |E_{nB_u} - E_{S_1} - hv_L| = h\gamma_{nB_u} / 2 \) (\( \gamma_{nB_u} \) in units of cm\(^{-1}\)). In 
this case, the corresponding term in the sum of eq. (6.9) is \( \left| \langle S_1 | \hat{\mu} | nB_u \rangle \right|^2 / h\gamma_{nB_u} \). Assuming a width 
of \( \gamma_{nB_u} = 1000 \text{ cm}^{-1} \), the resonance occurs at \( (E_{nB_u} - E_{S_1})/hc = 11760 \text{ cm}^{-1} \). Assuming further a 
dipole moment of \( \langle S_1 | \hat{\mu} | nB_u \rangle \approx 5 \text{ Debye} \) the contribution to \( \Delta \alpha(v_L) \) is about \( 10^{-38} \text{ CV}^{-1} \text{ m}^2 \), 
corresponding to a light shift of \( \Delta v_{\text{Stark}} \approx -400 \text{ MHz/W} \). By adding this value to the “non-
resonant” term of \(-100 \text{ MHz/W} \) estimated above, we obtain \( \Delta v_{\text{Stark}} \approx -500 \text{ MHz/W} \) which can be 
regarded as an upper limit for the optical Stark effect in DPOT. Excited-state absorption spectra 
recorded in the range of 12800 cm\(^{-1}\) to 23800 cm\(^{-1}\) show strong bands for the \( 5B_u \leftrightarrow S_1 \) transition at 
\( (E_{5B_u} - E_{S_1})/hc = 15520 \text{ cm}^{-1} \) and the \( 8B_u \leftrightarrow S_1 \) transition at \( (E_{8B_u} - E_{S_1})/hc \approx 21360 \text{ cm}^{-1} \) 
[161]. However, these transitions are not resonant with the laser frequency \( v_L \) and thus do not 
support the assumption of a resonance that would significantly enhance the shift. From these 
considerations one can conclude that the ac-Stark shift is in the range of \(-500 \text{ MHz/W} < \Delta v_{\text{Stark}} < -100 \text{ MHz/W} \).

(iv) This angle is observed to be rather reproducible in different Shpol’skii samples of DPOT in TD, if the 
samples are prepared in the same way. The angle was estimated in ref. [159].
6.4.2. Laser induced heating

In paragraph 6.3.1 we have considered the laser induced heating in the spot of excitation that is caused by a weak absorption of the third overtone C-H vibrations of the matrix molecules. The temperature increase is assumed to result from the Kapitza resistance [152,153] and is described by eq. (6.4). It is claimed that the local mode of $E = 17 \text{ cm}^{-1}$, which was considered for the OP data, is activated by the laser induced heating and thus causes the broadening in the power dependent two-photon spectra. This mode is associated with the ratio $|\beta_r/\beta_v| \approx 0.5$. For molecule 1 of Table 5.1 the observed linewidth increase is $\Delta \Gamma_{th}(P) = 150 \text{ MHz}/\text{W}$, while the frequency shift is $\Delta \nu(P) = -800 \text{ MHz}/\text{W}$. Using the above ratio we find $|\Delta \nu_{th}| = \Delta \Gamma_{th}/0.5 \approx 300 \text{ MHz}/\text{W}$. In Fig. 6.15 the corresponding power dependent frequency shift is shown. The experimental shifts are reduced by 60% in order to comply with the above estimate of $\Delta \nu_{th}$. These 60% of the total shift result presumably from the contributions $\Delta \nu_{\text{Stark}}$ and $\Delta \nu_{\text{NQP}}$, which are not or only negligibly associated with a broadening (see eqs. (6.6),(6.7) and Table 6.2). The data displayed in Fig. 6.15 are expected to correspond to $\Delta \nu_{th}(P)$. Inserting $T_{\text{eff}}$ from eq. (6.4) into eq. (2.37) yields

\[ \frac{P}{G_a} \gg \frac{G_a}{\alpha \Gamma_{\text{opt}}} \]

Figure 6.15: Two-photon excitation: Power dependence of the thermally induced line shift $\Delta \nu_{th}$ of molecule 1 in Table 5.1. The measured shifts are reduced by 60% to subtract the ac-Stark effect and the NQP-induced shifts. The solid line is a least squares fit to eq. (6.11) with $\beta_v = -30 \text{ GHz}$, $E/\hbar c = 17 \text{ cm}^{-1}$ and $\Lambda = 900 \text{ K}^2 \text{ W}^{-1}$. These parameters agree with the OP data.
\[ \Delta v_{\text{th}}(P) = \beta_v \cdot \exp \left\{ -\frac{E}{k(T_h + \Delta P)^{1/4}} \right\}. \tag{6.11} \]

Eq. (6.11) was fitted to the data in Fig. 6.15 by varying \( \Delta \) and the frequency offset \( v_0 \) while \( E \) and \( \beta_v \) were fixed. With \( E = 17 \text{cm}^{-1} \) and \( \beta_v = -30 \text{GHz} \) taken from the OP data in Table 6.1 the fit yields \( \Delta = 900 \text{ K W}^{-1} \). The result is given as the solid line in Fig. 6.15. At the highest laser power of \( P = 320 \text{mW} \) an effective temperature of \( T_{\text{eff}} \approx 4.1 \text{K} \) is calculated from eq. (6.4). At \( P = 320 \text{mW} \) the linewidth of the molecule in Fig. 6.15 is about 180 MHz, corresponding to 60 MHz broadening with respect to zero power. 20 MHz thereof are assigned to optical saturation, resulting in \( \Delta \Gamma_{\text{th}} \approx 40 \text{ MHz} \). This thermal broadening is consistent with the average line broadening of 35 MHz at a sample temperature of \( T_{\text{eff}} = 4.1 \text{ K} \) for the molecules measured under one-photon excitation.

6.4.3. Non-equilibrium phonons

A more elaborate relaxation scenario of the overtone C-H vibrations in the matrix includes the generation of non-equilibrium phonons (NQP, for an introduction see Eisenmenger and Kaplyanskii [144]). The NQP are assumed to directly excite higher energy localized vibrations which are not thermally activated below 10 K. The cascading relaxation processes are schematically shown in Fig. 6.16. The third overtone of the C-H vibration (band center at 10730 cm\(^{-1}\), see Fig. 6.10) is excited by the laser. The vibrational energy is released by an unspecified relaxation path including molecular internal and lattice vibrations. Because multi-phonon transitions play presumably a dominant role, the population of the phonons takes place primarily at the upper bound of the phonon density of states, i.e. at the Debye frequency. Phonons, including higher frequency NQP, propagate through the crystal according to their mean free path and interact with the chromophores by activating localized vibrations. The line shift \( \Delta v_{\text{NQP}} \) caused by coupling to the higher frequency local mode is calculated from eq. (2.35),

\[ \Delta v_{\text{NQP}} = W_c \tau \delta / 2\pi = n_{\text{LP}} \delta / 2\pi, \tag{6.12} \]

where the denominator in eq. (2.35) is discarded since \( (\tau \delta)^2 \) decreases rapidly with the increasing local phonon frequency \( \omega_{\text{LP}} \), \( (\tau \delta)^2 \approx \omega_{\text{LP}}^{-6} \) (see Appendix B). \( W_c \) in eq. (6.12) is the excitation rate by the NQP and again \( \tau \) is the local mode lifetime. \( n_{\text{LP}} \) is the corresponding local mode population, \( n_{\text{LP}} = W_c \tau \cdot \). As shown in Appendix B, the excitation rate is
We = \frac{4\pi s^3}{3\omega_{NQP}^2} \cdot Q \tau_{NQP}, \quad (6.13)

where \( \omega_{NQP} \) is the NQP frequency resonant with the local phonon under consideration (\( \omega_{NQP} = \omega_{LP} \)). \( s \) is the sound velocity that is estimated to be 2200 m/s from eq. (C.3), and \( Q \) is the NQP generation rate which is proportional to the laser intensity \( I \) (eq. (B.8)). \( \tau_{NQP} \) is the NQP lifetime, the estimate in Appendix C yields a value between 6 ns and 90 ns. Local phonons at \( \omega_{LP} = 30 \text{ cm}^{-1} \) are observed in the two-photon excitation spectrum of DPOT in TD (Fig. 6.11). For this phonon mode and for a laser intensity of \( I = 25 \text{ MW/cm}^2 \) (corresponding to 1 W focused to a spot of 2 \( \mu \text{m} \) diameter) we calculate \( W_e = 9 \times 10^{-9} - 1.3 \times 10^{-11} \text{ s}^{-1} \). Here the lower and upper limits correspond to \( \tau_{NQP} = 6 \text{ ns} \) and \( \tau_{NQP} = 90 \text{ ns} \), respectively. Based on scaling laws, the ratio \( \frac{|\Delta \Gamma_{NQP}/\Delta \nu_{NQP}|}{2\tau|\delta|} = 0.1 \) is calculated for a local mode of \( \omega_{LP} \approx 30 \text{ cm}^{-1} \) (see Appendix B). Thus, we get \(-1000 \text{ MHz/W} < \Delta \nu_{NQP} < -70 \text{ MHz/W}\).

![Figure 6.16: Schematic representation of the NQP generation, relaxation and interaction with the chromophore’s local mode. The coupling between the local mode and the dopant molecule is described by an effective 4-level-system (see also Fig. 2.7).](image-url)
6.4.4. TLS dynamics

In two-photon excitation of DPOT in TD a SM line broadening of 30 - 40 MHz was observed if compared with one-photon excitation, independent of the laser power in the range of 90 mW to 400 mW (see Fig. 6.9). This linewidth increase was tentatively assigned to saturated spectral diffusion. TLS dynamics are assumed to be accelerated by the non-equilibrium conditions inside the laser spot, which are generated by the IR absorption of the matrix and the subsequent relaxation processes shown in Fig. 6.16.

Until now, only qualitative arguments have been given for the acceleration of TLS dynamics and the IR-induced line broadening. In chapter 7, TPE single-molecule spectra at different time resolutions will be investigated using the novel Intensity-Time-Frequency-Correlation technique for SMS. The dependence of the SM linewidths on the measuring time will reveal the time scale of the IR-induced TLS dynamics. Further, it will be shown that all TPE experiments presented so far have been carried out on a time scale where the spectral diffusion is in a saturated regime.

6.4.5. Summary and conclusions

Four mechanisms which affect the SM line shape in two-photon excitation spectra have been discussed and the main results are collected in Table 6.2. The ac-Stark effect leads to the frequency shift $\Delta \nu_{\text{Stark}}$ but causes no broadening. Predictions of this shift are difficult because they rely on the dynamic polarizabilities of the ground and excited state and accurate data are not at disposal. The strong laser powers applied in two-photon experiments lead to a heating of the matrix and to non-equilibrium conditions inside the laser spot. As a consequence, the lowest frequency local phonons are activated according to the effective temperature in the laser spot, which results in the line broadening $\Delta \Gamma_{\text{th}}$ and shift $\Delta \nu_{\text{th}}$. This effect is equivalent to the temperature dependence of the line shape under one-photon excitation. NQP excite higher frequency local phonons which cause a shift $\Delta \nu_{\text{NQP}}$ whereas the associated broadening $\Delta \Gamma_{\text{NQP}}$ is small. Independent of the laser power an additional linewidth increase $\Delta \Gamma_{\text{TLS}}$ has been observed if compared with one-photon excitation. This enhancement of the spectral diffusion is assigned to TLS dynamics accelerated by the non-equilibrium conditions.

The present analysis demonstrates that the shifts due to the ac-Stark effect and the NQP depend linearly on the laser power while the laser induced heating leads to a nonlinear dependence through the effective-temperature activation of the local phonons. The observed line shifts show a
linear behavior within the experimental accuracy. This indicates that line shifts are presumably
dominated by the ac-Stark effect and the higher frequency local phonons triggered by NQP.

For the particular case of molecule 1 in Table 5.1 the total frequency shift is
\[ \Delta \nu(P) = -800 \text{ MHz}/\text{W}. \]
A contribution of \( \Delta \nu_{\text{th}} = -300 \text{ MHz}/\text{W} \) is estimated for the coupling to
the thermally activated local phonons. For the other two mechanisms only bounds can be given:
\[-500 \text{ MHz}/\text{W} < \Delta \nu_{\text{Stark}} < -100 \text{ MHz}/\text{W} \]
and \[-1000 \text{ MHz}/\text{W} < \Delta \nu_{\text{NQP}} < -70 \text{ MHz}/\text{W}, \]
where \( \Delta \nu_{\text{Stark}} \approx -100 \text{ MHz}/\text{W} \) is regarded as the most reliable value for the optical Stark shift.

Further studies of single-molecule spectral dynamics under two-photon excitation should
include an independent examination of each of the mechanisms involved. For instance, the ac-Stark
shift does not significantly depend on the matrix and primarily depends on the molecular properties.
Therefore this nonlinear optical effect should be investigated independent of the shifts due to the
local vibration dynamics. This could be accomplished by studying single molecules of the same
species in two different matrices, where the influence of local phonons is strong in the one and
negligible in the other host. Another possibility would be to measure the ac-Stark effect in the gas
phase. Moreover, precise knowledge of the effective temperature \( T_{\text{eff}} \) inside the laser spot is
desirable. \( T_{\text{eff}} \) could be measured using a single molecule as a temperature sensor provided its
temperature characteristics are known.

The spectral dynamics due to NQP have demonstrated the possibility of producing highly
sensitive NQP sensors on the basis of SM line shifts. In such a measurement, the line of a probe
molecule would be continuously measured using a first laser. By means of a second laser, which is
preferably resonant with a matrix absorption, the NQP would be generated. The NQP population in
function of the spatial position and the wavelength of the second laser beam could then be measured
at the location of the SM probe by analyzing the line-shape variations. Ideally, no other mechanisms
give rise to additional spectral dynamics.
A novel approach to SMS is presented, which is based on the autocorrelation of fast frequency scans. The technique, which is called Intensity-Time-Frequency-Correlation, allows for the measurement of single-molecule spectral shapes at so far unattainable time resolutions down to micro-seconds. It is applied to determine the linewidth as a function of the measuring time in two-photon single-molecule spectra of DPOT. The results show a line narrowing at short times, where the short-time linewidth is in agreement with the width observed in one-photon spectra. The analysis shows that the intense IR irradiation accelerates spectral diffusion processes which saturate on a time scale of about 1 s and accordingly cause a broadening on this time scale. These results complete the discussion of single-molecule spectral dynamics under two-photon excitation given in chapter 6.
7.1. INTRODUCTION

A common characteristic feature of single molecules is that each successive spectral measurement of the same molecule can reveal a new spectrum, even when the macroscopic conditions do not change, i.e. even under equilibrium conditions. In solids, temporal resonance frequency fluctuations occur mainly due to interactions of the chromophore with its environment. These frequency changes lead to the phenomenon of spectral diffusion and to line shapes that are in general non-Lorentzian. Dynamic SM line shapes have already been discussed in section 2.4. According to the tunnelling two-level system (TLS) model [65-67], the environment is represented by a set of TLSs with flip rates distributed from microhertz to gigahertz. Thus, experimental studies of fast spectral dynamics and SM – matrix interactions require a technique that combines high time resolutions with the spectral sensitivity provided by SM probes. However, the implementation of SMS at high time resolution has been considered impossible so far, because the number of detected photons emitted by an individual chromophore is small and fluctuates according to a Poisson distribution. Even when the exciting laser power saturates the transition and the emission rate is at maximum, the peak signal rarely reaches $10^5$ counts/s (only in the case of a strong emitter, e.g. terylene [162]). To measure the linewidth, the laser power should be well below saturation, so that only $10^4$ counts/s are detected. Thus in the best case, a recording time on the order of 10 ms is required to determine the entire line shape. For molecules with 100 times lower emission rates (pentacene, DPOT, and many others), this time can be as long as a few seconds. The loss of spectral information at decreasing scan time is shown in Fig. 7.1.

![Count rate vs. scan time](image)

**Figure 7.1:** Two-photon excitation spectrum of DPOT in TD. The same spectral interval is measured at different scan rates of the laser frequency. In the slowest scan (left, 55 s/scan), the SM lines 1 and 2 appear at a high signal-to-noise ratio and the line shape may be determined. These lines are no longer observed in a 20 times faster scan (center, 2.7 s/scan). Here, the SM line 3 gives rise to the strongest signal, but its line shape cannot be determined unambiguously. SM 3 is not clearly resolved in the scan to the left. In the fastest scan (right, 0.36 s/scan), only single counts are detected, and no spectral lines are identified. Thus, 55 s/scan is approximately the time resolution limit in this example.
Using a conventional photon correlation technique [163,164], insight is gained into fast SM dynamics but no spectral information is provided. In this chapter, Intensity-Time-Frequency Correlation (ITFC) spectroscopy is introduced. ITFC is a new approach to SMS which allows for the measurement of line shapes at time resolutions down to milli- or micro-seconds.

Strictly speaking, time and frequency are not independent of each other. They are conjugate variables linked by the Fourier transformation, so that “time dependent spectra” and “spectral dynamics” are in principle self-contradictory terms. In this respect, the SM transition frequency $\omega(t)$, which has been defined in eq. (2.27), does not designate a spectrum and does not result from a Fourier transformation. $\omega(t)$ must be regarded as a time-dependent parameter that describes the change of the molecule’s optical response due to its stochastic interaction with the environment.

7.2. **Intensity-Time-Frequency-Correlation (ITFC) Spectroscopy**

7.2.1. Principles of ITFC

In ITFC spectroscopy, instead of recording one spectrum with an accumulation time long enough to have the required signal/noise ratio, $N$ very fast scans over the same spectral region are acquired. Each of them is a SM spectrum with a high time resolution but very small signal/noise ratio. When this ratio is improved by summing up many scans, the time resolution is lost (conventional SMS). Summation yields a SM line which is broadened by spectral dynamics that occur on time scales longer than the single-scan time and shorter than the total recording time of all traces. To improve the signal/noise ratio preserving the single-scan time resolution, autocorrelation functions (ACFs) are calculated for each scan and then these functions are averaged. We define the ITFC signal by

$$S_{\text{ITFC}}(\Omega') \equiv \langle \Psi_k(\Omega') \rangle \equiv \frac{1}{N} \sum_{k=1}^{N} \Psi_k(\Omega'), \quad (7.1)$$

where $\Psi_k(\Omega')$ corresponds to the ACF

$$\Psi_k(\Omega') \equiv I_k(\Omega') \star I_k(\Omega') \equiv \int_{-\Omega_0/2}^{\Omega_0/2} I_k(\Omega)I_k(\Omega + \Omega') d\Omega. \quad (7.2)$$
In this equation $I_k(\Omega)$ is k-th single-scan spectrum and $\Omega$ is the angular laser frequency. The scan interval $\Omega_0$ is chosen such that during the experiment the line intensity is negligible outside the scan range. In this case, the integration limits can be set to $+\infty$ and $-\infty$. The ACFs $\Psi_k$, though quite noisy in the case of fast scans, have the important peculiarity that their maximum is always at the origin. In the ACFs the frequency scale is shifted so that the peak frequency position of the SM line in the spectrum $I_k(\Omega)$ becomes the origin in $\Psi_k(\Omega')$. Therefore, even if the molecular frequency “jumps” to a new position from scan to scan, the ACFs will “ignore” these jumps and only faster dynamics will define the shapes of the functions $\Psi_k$. Hence, the averaging of ACFs preserves the time resolution of a single scan.

This method has been called “ITFC”, because the fluorescence Intensity is measured as a function of frequency at a certain Time resolution, and this spectrum is then Correlated with itself in Frequency space. $S_{\text{ITFC}}(\Omega')$ is a Frequency-Correlation spectrum. The gain of the ITFC spectrum $S_{\text{ITFC}}(\Omega')$ compared with the conventional spectrum $\langle I_k(\Omega) \rangle$ lies in the time resolution of the line-shape measurement. However, the information about the absolute center frequency of the molecule is lost by the calculation of $\Psi_k(\Omega')$, i.e. the frequency “trajectory” is lost. Because time and frequency are conjugate variables, this information cannot be extracted at arbitrary accuracy and at any desired time resolution. The ITFC method is illustrated in comparison to conventional SMS in Fig. 7.2.

The time resolution of $S_{\text{ITFC}}(\Omega')$ depends on the scan rate $r$ of the laser frequency and does not have a trivial definition ($r$ is in units of Hz/s or s$^{-2}$). If $S_{\text{ITFC}}(\Omega')$ differs significantly from zero only for $0 < \Omega' < \Omega'_{\text{max}} < \Omega_0$, part of the scan interval $\Omega_0$ consists of no signal and $\Omega_0$ can be narrowed without losing the “jumping” molecule. Thus, $\Omega'_{\text{max}}/r$ can be defined as the time resolution, no matter how large $\Omega_0$ is (for an example see Fig. 7.4, more details about the figure are given in 7.2.2). The average cross correlation of the k-th and (k+p)-th scan is defined as

$$S_{\text{ITFC}}(\Omega', p) = \frac{1}{N-p} \sum_{k=1}^{N-p} I_k(\Omega') \star I_{k+p}(\Omega'), \quad (7.3)$$

and yields an ITFC spectrum as well. The time resolution of $S_{\text{ITFC}}(\Omega', p)$ is given by the lag of recording time between the k-th and (k+p)-th scan, which corresponds to $\Delta = p\Omega_0/r$ (assuming zero dead time in between subsequent scans). Thus, ITFC spectra at different time resolutions can be obtained from a single set of scans.

(i) This averaging presupposes no significant time-irreversible changes in the sample.
When considering the time resolution, one should be aware that SM line shapes are generally very complicated functions which cannot be described by a single well-defined parameter such as a linewidth or $\Gamma_{\text{max}}$. In practical data analysis however, the observed time-dependent non-Lorentzian line shape is usually fitted to a Lorentzian function. Using this approximate procedure, an effective linewidth is determined which depends on the scan rate or time resolution, respectively.

In the SM excitation spectra $I_k(\Omega)$, the signal is proportional to the population of the excited state. This population is a solution of the optical Bloch equations when the molecular resonance frequency $\omega$ and the laser frequency $\Omega$ are functions of time [48,77]. The time dependence of $\Omega$ is given by $\dot{\Omega}(t) = -\Omega_0/2 + \Gamma_0 t$, where $t$ is measured from the start of the corresponding scan and the
frequency scan interval \( \Omega_0 \) is chosen such that the line intensity is negligible outside the scan range during the whole experiment. \( \omega(t) = \omega_0 + \omega'(t) \) is a stochastic function according to eq. (2.27). We consider an approximation which is valid if the time between frequency jumps as well as \( (rT_1)^{-1} \), the time to scan the frequency interval \( (T_1)^{-1} \), are both longer than \( T_1 \). In this case, \( \omega(t) \) and \( \Omega(t) \) are slowly varying parameters and the steady-state population of the exited state can be used, which is a Lorentzian function of \( (\Omega(t) - \omega(t)) \). Thus, \( S_{\text{ITFC}}(\Omega') \) reads

\[
S_{\text{ITFC}}(\Omega') \sim \left\{ \int_{-\infty}^{\infty} \left[ \frac{1}{(\Omega - \omega_0)^2 + \left( \frac{\Gamma_0}{2} \right)^2} \cdot \frac{1}{(\Omega + \Omega' - \omega_0)^2 + \left( \frac{\Gamma_0}{2} \right)^2} \right] \right\} d\Omega', \tag{7.4}
\]

where the time \( t \) is substituted by \( \Omega/r \) and \( N\Omega_0/r \) is the total recording time. The linewidth \( \Gamma_0 \geq (T_1)^{-1} \) approximately includes dephasing on time scales shorter than \( T_1 \) (if present). We recall eq. (2.27) to describe temporal frequency changes,

\[
\omega'(t) = \sum_m \zeta_m(t) v_m, \tag{2.27}
\]

where \( \zeta_m(t) \) are stochastic functions. The functions \( \zeta_m(t) \) can be divided into two groups with correlation times shorter and longer than \( \Omega_0/r \) (time to scan one trace). When evaluating the integral in eq. (7.4), only \( \zeta_m(t) \) with correlation times shorter than \( \Omega_0/r \) are significant. Functions with long correlation times can be replaced by a constant. This constant will not affect the integral when its limits are \( \pm \infty \). If all \( \zeta_m(t) \) have long correlation times, the integral is a Lorentzian with a width of \( 2\Gamma_0 \), as if there were no spectral dynamics. This task is illustrated by the numerical simulations in the next paragraph.

### 7.2.2. Numerical simulation of spectral dynamics

The evolution of the ITFC signal as a function of the frequency scan rate is simulated numerically for the case of one single molecule interacting with a set of 12 TLSs. In this case, the stochastic functions \( \zeta_m(t) \) take values -1 or +1 when the m-th TLS is in its ground or excited state, respectively. \( \zeta_m(t) \) has a correlation time of \( \tau_{c,m} \). \( |2v_m| \) is the frequency shift of the SM line, when the m-th TLS flips between the two states. Here, for simplicity, the “up” and “down” transition rates are equal for each TLS (high temperature limit). The TLS parameters are chosen as follows: \( v_m = \{3, 4, 5, 6, 8, 10, 11, 12, 16, 20, 22, 23\} \) and \( \tau_{c,m} = \{0.05, 0.15, 0.1, 0.25, 25, 75, 45, 35, 125, 38, 40, \)
The only important molecular parameter is $\Gamma_0$ which is set to 5. The scan range is $\Omega_0 = 256$. All of these numbers are given in arbitrary units (a.u. (ii)).

When the SM spectrum is measured in the conventional way at a high time resolution, i.e. at a high frequency scan rate of $r = 500$, the line shape is Lorentzian and "jumps" to a new center frequency from scan to scan (Fig. 7.3, right). Notice that in a real experiment this signal would be below the noise level. At a 500 times lower scan rate ($r = 1$), the SM line shape is strongly distorted from a Lorentzian and varies from scan to scan (Fig. 7.3, left).

Fig. 7.4 displays the ITFC signal calculated for different scan rates $r$ and for different sub-sets of TLSs being active. The ITFC at $r = 0.005/1$ (frequency units/time units) is the long-time limit for the spectral diffusion (curve 1). Curve 2 represents the case $r = 1/1$. Curve 3 (dotted) is obtained for the same scan rate, but with all TLSs switched off which have correlation times $\tau_{c,m} > 1$. Curve 4 simulates a very fast scan with $r = 500/1$. For curves 2 and 4, the time resolution is $\Omega_{\text{max}}^{(2)}/r (= 22)$ and $\Omega_{\text{max}}^{(4)}/r (= 0.02)$, respectively, but not $\Omega_0/r$ (256 and 0.5, respectively). The comparison of curves 2 and 3 with curve 1 shows that scanning at the rate $r = 1/1$ removes contributions from slow TLSs. Curve 4, where $\Omega_{\text{max}}^{(4)} = 2\Gamma_0 = 10$, indicates that the $2\Gamma_0$.

![Figure 7.3: Numerical simulation of three subsequent measurements of a SM line. Two frequency scan rates are compared (left: $r=1$, right: $r=500$). The time resolutions of the spectra are given by $\tau = \Omega_{\text{max}}/r$. The corresponding ITFC spectra are shown in Fig. 7.4, curves 2 and 4, respectively.](image)

(ii) In the literature, the abbreviation "a.u." is often used for "atomic units". In contrast, "a.u." stands for "arbitrary units" here.
limit of the observed linewidth is achieved for the fastest scan \((r = 500/1)\). At this scan rate the ITFC has a Lorentzian shape. This is not generally true for relatively slow scans, where the shape of the ACF depends on the properties of the functions \(\zeta_m(t)\).

**Figure 7.4:** Numerical simulation of the ITFC signal for a single molecule which is coupled to 12 TLSs. Curve 1 corresponds to a slow measurement with scan rate \(r = 0.005\), for curves 2 and 3 the scan rate is \(r = 1\), and curve 4 is obtained for a very fast scan with \(r = 500\).

### 7.2.3. Analogy between ITFC and 3-pulse photon echoes

A SM optical line shape depends on the start time \(t_0\) (absolute time) as well as on the duration \(t_m\) of the experiment. The \(t_0\)-dependence reflects that the SM spectrum is different for each successive measurement. In contrast, the line shape of an ensemble of molecules is reproducible on all time scales. The time resolution \(t_m\) of the ITFC signal is determined by the scan rate \(r\) of the laser frequency. A rigorous solution of the Bloch equations results in a remarkable analogy between \(S_{\text{ITFC}}\) and the signal in a three-pulse photon echo (3PPE) experiment (a comprehensive introduction to photon echoes is given in ref. [165]). This statement requires further explanation, because ITFC is a frequency-domain SM technique and photon echoes are time-domain signals obtained only from bulk samples. Each individual chromophore is characterized by a specific microscopic environment and a corresponding frequency trajectory \(\omega'(t_0 + t)\). Here we consider an ensemble of dopant molecules, where each of them interacts with a statistically identical set of TLSs. In a "gedanken" 3PPE experiment on such a sample, the echo amplitude can be related to the Fourier transform of \(S_{\text{ITFC}}\) generated by one molecule out of this hypothetical ensemble. The derivation of this relation
will be briefly outlined below, for a more detailed mathematical treatment of ITFC spectroscopy see ref. [166].

Because the probability for photon emission by the molecule is proportional to the time-dependent population $\rho_{22}(t)$ of the excited state, the frequency-domain integral $I_k(\Omega') \ast I_{k+p}(\Omega')$ in eq. (7.3) is proportional to a time-domain integral over $[\rho_{22}(\Omega/r)\rho_{22}^\Delta(\Omega/r + \Omega'/r)]$, where $t = \Omega/r$ and $\Delta = p\Omega_0/r$ ($\Delta$ is the delay time between the start of the k-th and (k+p)-th frequency scan). Accordingly, $S_{ITFC}(\Omega', p)$ can be expressed as a function of $\rho_{22}(\Omega/r)$ and $\rho_{22}^\Delta(\Omega/r + \Omega'/r)$, which are obtained from solutions of the Bloch equations. Now we consider a “gedanken” 3PPE experiment on a sample of statistically identical molecules, where $\tau$ is the delay time between the first and second pulse and $T_w$ the waiting time between the second and third pulse. Calculating the 3PPE signal $S_{3PPE}(\tau, T_w)$ in terms of $\rho_{22}(t)$ [165] and comparing the expressions for $S_{3PPE}(\tau, T_w)$ and $S_{ITFC}(\Omega', p)$ yields the relation [166]

$$S_{ITFC}(\Omega', p) \sim \text{Re} \left\{ \int_0^\infty e^{-\Omega' \tau} \cdot S_{3PPE}(\tau, T_w) d\tau \right\}, \quad (7.5)$$

where $T_w = \Omega'/r + \Delta = (\Omega + p\Omega_0)/r$. Eq. (7.5) relates the signal $S_{3PPE}$, which is produced by an ensemble of statistically identical molecules, to the signal $S_{ITFC}$, which is obtained from a single molecule out of that hypothetical sample.

Next we discuss the limit $T_1^2 \tau \ll 1$ (slow scan on the time scale of the excited state lifetime). If $\Delta \gg \Omega'/r$ (iii), $S_{3PPE}(\tau, T_w)$ is independent of $\Omega'$. Taking into account that $S_{3PPE}(\tau, T_w)$ decays with $e^{-\tau/T_1}$, $S_{ITFC}$ is approximated by

$$S_{ITFC}(\Omega', p) \sim \text{Re} \left\{ \int_0^\infty e^{-\Omega' \tau} \cdot S_{3PPE}(\tau, (p\Omega_0/r)) d\tau \right\}. \quad (7.6)$$

Thus, $S_{ITFC}(\Omega', p)$ is just the Fourier transform of $S_{3PPE}(\tau, T_w)$ with $T_w = p\Omega_0/r$. Moreover, the right hand side of eq. (7.6) also corresponds to the shape of a spectral hole [76,166] in such a particular sample. For $\Delta = 0$ ($p = 0$, corresponding to an autocorrelation), $S_{ITFC}$ can no longer be interpreted as the Fourier transform of a single-shot 3PPE amplitude $S_{3PPE}(\tau, T_w)$. In this case, $S_{ITFC}(\Omega')$ is represented by Fourier transforms of a series of $S_{3PPE}(\tau, T_w)$ with different waiting times $T_w$. Each $T_w$ corresponds to a different point $\Omega'$ on the frequency axis according to $T_w = \Omega'/r$.

(iii) Unless $\Delta = 0$ this is always fulfilled, since $\Delta \gg \Omega'/r \gg \Omega'/r$. 

In conclusion, there is a remarkable analogy between the ITFC spectrum of a single molecule and the 3PPE amplitude of an ensemble of statistically identical molecules. Thus, ITFC provides spectral information on the whole temporal range that is accessible in photon echo experiments. The analogy between ITFC and photon echoes can also be understood from an empirical point of view. For both methods, the gain in time resolution is paid up by losing the information about the absolute resonance frequency of the molecule. Only relative frequencies—linewidths or dephasing times, respectively—are obtained, but at high time resolutions. Further, the analogy between ITFC and photon echoes is related to the identity between time- and ensemble-averages (ergodic theorem). The ITFC signal is generated by one molecule whose resonance frequency is time dependent. In a long-time limit, the probability distribution of frequencies is given by the inhomogeneous spectral broadening. Contrary for the 3PPE signal, which results from a population of many molecules. Their frequencies are assumed stable and the whole population builds up the inhomogeneous spectral line.

Since the ITFC method is intrinsically time-dependent, there is no need to artificially account for finite measuring times when probing fluctuating environments, and no additional assumptions about TLS flip rates are required. Ref. [76] shows that by using ITFC, distributions of the TLS parameters can be recovered from SM linewidth distributions without any additional approximations.

7.2.4. Limitations of ITFC

The question arises what the time resolution limit is for the ITFC method. How fast can we scan over the molecular resonance? First we note that the scan should be slow on the time scale of the excited state lifetime, in order to avoid a “fast passage broadening” of the resonance, so \( r \ll T_1^{-2} \). Next we consider the minimum number of detected photons required in a scan so that \( S_{\text{ITFC}}(\Omega') \) is still well defined. To calculate the ACF, the frequency scan \( I_k(\Omega) \) must at least consist of two photo-counts. Scans with zero or one count must be discarded.

When the average number of detected photons in a scan is \( R_{\text{tot}} \), the probability to have at least two counts in this scan is

\[
Z = \sum_{i=2}^{\infty} \phi(i, R_{\text{tot}}) = 1 - \phi(1, R_{\text{tot}}) - \phi(0, R_{\text{tot}}).
\]

In this equation \( \phi(i, R_{\text{tot}}) \) is the Poisson distribution, giving the probability of counting \( i \) photons when the average number of photons is \( R_{\text{tot}} \). With regard to the ITFC signal, \( Z \) gives the fraction of
the number of scans which contribute to the ITFC signal. Z as a function of $R_{\text{tot}}$ is shown in Fig. 7.5. Assuming a Lorentzian SM line of width $\Gamma$ which is excited at optical saturation and scanned at a rate $r$ ($\Gamma/r$ corresponds to the scan time per linewidth), the average number of counts per scan is

$$R_{\text{tot}} = \int_{-\infty}^{\infty} \frac{(\Gamma_\omega^2 / 2)^2 (R_\infty / r)}{(\Omega - \omega)^2 + (\Gamma_\omega / 2)^2} d\Omega = \frac{\pi R_\infty \Gamma_\omega}{2r}, \tag{7.8}$$

where $R_\infty$ is the high-power count rate at the peak of the Lorentzian. Neglecting intersystem crossing, we have $R_\infty = A_{\text{tot}} \phi_f / 2T_1$, where $A_{\text{tot}}$ is the total collection efficiency of the apparatus and $\phi_f$ the fluorescence quantum yield. Setting $\Gamma_\omega = T_1^{-1}$ and taking the condition $r \ll T_1^{-1}$ into account, $R_{\text{tot}}$ reads

$$R_{\text{tot}} = \frac{\pi A_{\text{tot}} \phi_f}{4T_1^2 r} \geq \frac{\pi A_{\text{tot}} \phi_f}{4}. \tag{7.9}$$

For two-photon excitation of DPOT we have $R_\infty = 1900 \text{ s}^{-1}$ and $\Gamma_\omega \approx 3.7 \times 10^8 \text{ rad s}^{-1}$ ($\Gamma \approx 60 \text{ MHz}$). $R_{\text{tot}} = \{0.01, 0.1, 1, 5, 10\}$ correspond to $r = \{110, 11, 1, 0.2, 0.1 \text{ GHz/ms}\}$, respectively. To determine the signal-to-noise of the ITFC signal, the number of photons $R_{\text{ITFC}}$ which contribute to $S_{\text{ITFC}}$ must be considered. The relative number of photons $R_{\text{ITFC}} / R_{\text{tot}}$, where

**Figure 7.5:** Fraction $R_{\text{ITFC}} / R_{\text{tot}}$ of "useful photons" and fraction $Z$ of number of scans contributing to the ITFC, when the average number of detected photons per scan is $R_{\text{tot}}$. 

![Graph](image_url)
\( R_{\text{ITFC}}/R_{\text{tot}} \) is again the average number of counts in a single scan, is given by

\[
R_{\text{ITFC}}/R_{\text{tot}} = \frac{1}{R_{\text{tot}}} \sum_{i=2}^{\infty} \frac{i}{R_{\text{tot}}} \cdot \varphi(i, R_{\text{tot}}) = 1 - \frac{1}{R_{\text{tot}}} \cdot \varphi(1, R_{\text{tot}}) - \frac{0}{R_{\text{tot}}} \cdot \varphi(0, R_{\text{tot}}). (7.10)
\]

\( R_{\text{ITFC}}/R_{\text{tot}} \) is also displayed in Fig. 7.5. For instance when \( R_{\text{tot}} = 5 \), 99.3% of the detected photons contribute to the ITFC. When the scan rate is 20 times higher and accordingly \( R_{\text{tot}} = 0.1 \), only 10% of the counts contribute to the ITFC and 90% of the detected photons must thus be discarded. As long as the scan rate is so slow that \( R_{\text{ITFC}}/R_{\text{tot}} = 1 \), the number of scans required to keep the signal-to-noise constant increases proportional to \( r^1 \). At fast scan rates, when \( R_{\text{ITFC}}/R_{\text{tot}} < 1 \), the number of required scans increases according to \( (rR_{\text{ITFC}}/R_{\text{tot}})^{-1} \).

In conclusion, as long as \( r \ll T_{1}^{-2} \), the time-resolution limit of the ITFC technique is related to the photo-stability of the chromophore, because the total time of the experiment increases dramatically for \( R_{\text{ITFC}}/R_{\text{tot}} < 1 \), i.e. for \( R_{\text{tot}} < 2 \).

### 7.3. Two-photon excitation ITFC spectroscopy of single DPOT molecules

The ITFC technique is now applied to study spectral dynamics in the two-photon excitation spectra of DPOT in TD. Curve (a) in Fig. 7.6 shows a TPE spectrum of DPOT measured at a time resolution of about 500 s. The laser light (wavelength at 888.0 nm) was focused to a spot of approximately 3 μm diameter, the excitation power was about 140 mW. The intensity of the one-photon emission was recorded as a function of the laser frequency. The spectral feature at 3030 MHz represents a SM line with a width of about 60 MHz, a typical linewidth in TPE spectra (see section 5.3). This linewidth is more than twice the average linewidth of 26 MHz measured in OPE spectra (see section 6.2). In the ITFC spectrum of this molecule (Fig. 7.6, right), recorded with a time resolution of about 40 ms, a significant line narrowing down to 28 MHz is observed. This time resolution can only be achieved using the ITFC technique. The SM line cannot be seen in the single scan (b).

In Fig. 7.7, the linewidth \( \Gamma \) averaged over about 10 molecules, whose resonance frequencies are within a total scan range of 3.7 GHz, is shown as a function of the time resolution \( t_m \). To get this average, the ACF can be calculated for the whole 3.7 GHz trace at once. On a logarithmic scale, the observed time dependence of \( \Gamma \) appears as a step function. This indicates that in contrast to glasses,
Two-photon excitation ITFC spectroscopy of single DPOT molecules

Figure 7.6: Left: (a) TPE spectrum obtained by averaging 200 single scans recorded with frequency steps of 3.6 MHz and 5 ms accumulation time at each frequency position. The spectrum has a time resolution of about 500 s. The SM line at 3030 MHz has a width of 62 MHz (the grey line is a Lorentzian fit). (b) Example of a single scan. In this fast scan, the molecule at 3030 MHz produces a signal of about 1.3 counts in the maximum. Right: Average of 200 ACFs of single scans, calculated for the spectral region between arrows A and B. The ITFC has a time resolution of 40 ms, and the decay corresponds to a linewidth of 28 MHz (a Lorentzian fit is shown).

Figure 7.7: Time dependence of the linewidth (half width at half maximum of the ITFC) in TPE spectra. Each data point represents the average linewidth of 10 single molecules. The solid line corresponds to \( \Gamma = 31 \text{ MHz} + 33 \text{ MHz} \cdot \left( 1 - \frac{1}{1 + 0.5 \text{ Hz} \cdot t_m^2} \right) \), obtained from a least squares fit to eq. (7.11).
the distribution of TLSs in the DPOT/TD system is remarkably different from the one assumed in the standard TLS model. Fig. 7.7 shows that there is no spectral diffusion on the time scales $10^{-3} - 10^{-2}$ s and $10 - 200$ s, where the linewidth remains almost constant (31 MHz and 65 MHz, respectively). These values are still broadened by 3–5 MHz due to a small remaining saturation of the optical transition. Thus, the linewidth is 26 – 28 MHz on a short time scale, in agreement with OPE data. OPE spectra do not show any spectral dynamics in the time range $10^{-3} - 10^{-2}$ s. This confirms that the difference between the SM linewidths in OPE and TPE spectra is caused by spectral diffusion processes, which are accelerated by the strong IR irradiation at 888 nm – a hypothesis discussed already in section 6.4.

The observed spectral diffusion significantly varies from molecule to molecule. Because of additional frequency jumps of 0.1 – 10 GHz, which occurred on the time scale of $10^3 - 10^4$ s, the time range presented in Fig. 7.7 could not be entirely covered by observing the same molecule. Such jumps were observed under both OPE and TPE and were already mentioned in section 6.2. Due to these dynamics and conventional photo bleaching, the molecular ensemble under study usually changed after one or two hours, even if the spectral range and the laser beam position were preserved. For this reason, only the average linewidth of 10 molecules is shown in Fig. 7.7. It must be emphasized that this averaging is not related to the ITFC method itself, which allows for the study of single molecules, provided that they are photo-stable.

IR induced spectral diffusion was also observed in spectral hole burning experiments on dye doped polymethylmethacrylate and was explained by interaction of probe molecules with water molecules embedded in the polymer and flipping between two states under IR illumination. Those flip-flop dynamics were modelled by a set of TLSs with a narrow distribution of the flip rates. An approximate relation for the time dependent line broadening of the spectral holes was deduced [151]:

$$
\Gamma = \Gamma_0 + \gamma \left(1 - \frac{1}{(1 + \kappa t_m)^2}\right),
$$

where $\gamma$ describes the coupling between a probe molecule and the TLSs, $\kappa$ corresponds to an average TLS flip rate, and $t_m$ is the measuring time. According to eq. (7.6), the shape of a spectral hole in a sample of statistically identical chromophores is similar to the shape of the ITFC signal of an individual molecule out of that ensemble. Therefore, assuming a narrow distribution of the TLS flip rates, eq. (7.11) is a good approximation for the time dependence of the ITFC linewidth of a single molecule which interacts with one effective TLS. The experimental data in Fig. 7.7 are fitted to eq. (7.11), yielding $\Gamma_0 = 31$ MHz for the linewidth at short measuring times and $\kappa = 0.5$ Hz.
for the TLS flip rate. This $\kappa$ should be considered as a rough estimate. At higher laser powers a shift of the time scale of line broadening towards shorter times was observed, in agreement with an acceleration of the IR-induced spectral diffusion.

7.4. **Discussion of two-photon single molecule spectra (Part II.)**

In two-photon excitation of DPOT in TD a SM line broadening of 30–40 MHz has been observed if compared with one-photon excitation. The broadening is independent of the laser power in the range of 90 to 400 mW (see Fig. 6.9). Using the ITFC technique, this broadening can be assigned to spectral diffusion processes that are accelerated by the IR light at 888 nm. In one-photon experiments with additional IR illumination using a second laser beam, the SM line broadening has also been observed. This spectral diffusion saturates on a time scale of 1 s (at a laser power of 140 mW). The underlying TLS dynamics must be triggered by the non-equilibrium conditions in the sample, which result from the strong IR irradiation. The TLS flips take place on a time scale of milliseconds to seconds, and thus the broadening increases on this time scale and saturates at longer times (see Fig. 7.7). The distribution of the TLS flip rates is so narrow that the data in Fig. 7.7 are satisfactorily fit with eq. (7.11) using a single flip rate of 0.5 Hz.

Remarkably, the SM linewidth of 30 MHz measured in OPE spectra and in TPE ITFC experiments at short times $t_m$ is still broader than the lifetime limited width of 14.5 MHz (see Fig. 6.9). Other TLSs with shorter relaxation times are presumably responsible for this additional linewidth increase. These TLSs are also triggered by the blue excitation light used for OPE, in contrast to the TLSs which cause the broadening on the time scale of a second in the case of TPE.
“Now I began a study of the comparative virtues of wisdom and folly, and anyone else would come to the same conclusion I did — that wisdom is of more value than foolishness, just as light is better than darkness; for the wise man sees, while the fool is blind. And yet I noticed that there was one thing that happened to wise and foolish alike — just as the fool will die, so will I. So of what value is all my wisdom? Then I realized that even wisdom is futile. For the wise and the foolish both die, and in the days to come both will be forgotten. So now I hate life because it is all so irrational; all is foolishness, chasing the wind. .... In my search for wisdom I observed all that was going on everywhere across the earth — ceaseless activity, day and night. Of course, only God can see everything, and even the wisest man who says he knows everything, doesn’t!” — King Solomon (965-926 B.C., Book of Ecclesiastes 2:12-17 & 8:16-17)
The work presented here focussed on three subjects within the field of single-molecule spectroscopy in solids. First, two-photon fluorescence excitation was established as an additional technique for SMS investigations. The response of single-molecule optical lines to the specific conditions created in the samples during a two-photon experiment was studied. Second, a new approach to SMS was developed that allowed for the investigation of spectral dynamics at previously unattainable time resolutions. Third, polyenes—a physiologically important class of molecules—were introduced as a new system for single-molecule studies. Polyenes in n-alkanes appeared to be appropriate systems to achieve and examine two-photon excitation spectroscopy of single molecules.

By means of standard fluorescence excitation and emission spectroscopy, two molecular sites were identified for DPOT in n-tetradecane at 1.8 K. Site I corresponds to distorted symmetry-broken DPOT molecules, which presumably do not have enough space in the tetradecane lattice. For this site, the transition strength between the ground (S₀) and the lowest excited singlet (S₁) state is considerably high for both one- and two-photon transitions. In contrast, site II originates from undistorted centrosymmetric chromophores, where S₀ → S₁ is only observed under two-photon excitation. The spectral properties of the two sites are well understood in the framework of a vibronic coupling between S₁ and the second excited singlet state. The appearance of an undistorted centrosymmetric and a symmetry-broken site in one and the same organic crystal makes DPOT/tetradecane a promising system to further investigate the characteristics of mixed parity states in general and the excited singlet states of polyenes in particular. Site-I molecules of DPOT in tetradecane have proven to be suitable for two-photon excitation studies on the single-molecule level.

Using a confocal optical design, which provides a low background and a high photon collection efficiency, two-photon spectroscopy of single DPOT molecules was accomplished at cryogenic temperatures. The choice of the chromophore–matrix pair was of crucial importance for the success of the experiment. The demonstration of two-photon SMS on DPOT has opened the way to single-molecule studies on the physiologically important polyenes. Further, two-photon confocal microscopy has become applicable as an alternative method to conventional single-molecule imaging techniques.

Insight into spectral dynamics specific for two-photon SMS was provided by a comparison of one- and two-photon spectra of the same electronic transition. With the temperature dependence of the one-photon and the power dependence of the two-photon excitation spectra complementary information was obtained. Four mechanisms were found to be of particular importance for the analysis of two-photon single-molecule spectra: (i) the ac-Stark shift at strong laser fields, (ii) the activation of local phonons due to a temperature increase within the spot of the highly focussed IR
irradiation, (iii) the generation of non-equilibrium bulk phonons (NQP) which in turn activate higher frequency local phonons, and (iv) IR-induced dynamics of lattice two-level systems (TLSs) leading to saturated spectral diffusion. The experimental results and analyses did not allow for an unambiguous discrimination between the NQP-induced line shift and the ac-Stark shift. Further experiments have been proposed whose aim is an independent examination of each of the mechanisms.

The laser-induced heating in the excitation spot may be viewed as a drawback of two-photon spectroscopy. It could be circumvented by a careful choice of the host material, e.g. by using a perdeuterated matrix to avoid light absorption by the C-H vibrations (i). If this is not possible, the effective temperature inside the laser spot should be determined. A single molecule, which can be of a different species than the dopant under study, could act as a very sensitive temperature sensor. The possibility of producing NQP sensors on the basis of single-molecule line shifts has also been demonstrated and an example of a possible experiment has been outlined.

A novel spectroscopic technique called “Intensity-Time-Frequency-Correlation” (ITFC) has been developed. It is based on the autocorrelation of fast frequency scans and allows the determination of single-molecule line-shapes at time resolutions down to microseconds. ITFC studies of two-photon excitation spectra of DPOT revealed a line narrowing with decreasing measuring times, whereas the linewidth in corresponding one-photon spectra was independent of the time resolution. The effect was explained by spectral diffusion processes accelerated by the intense IR irradiation in two-photon experiments. The underlying TLS dynamics seem to occur on a narrow time scale. ITFC spectroscopy combines the high spectral sensitivity inherent for SMS with the time resolution accessible in three-pulse photon echo studies. The ITFC technique has proven to be a powerful tool for the investigation of all kinds of single-molecule time trajectories, and will potentially find a broad range of applications.

The field of optical probing of individual molecules is rapidly developing and diversifying and has attracted the interest of researchers from various disciplines. Two-photon confocal microscopy is an important tool for the investigation of single bio-molecules and their function under physiological conditions. From this perspective, the field of biophysics could presumably benefit

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(i) In the case of DPOT in TD, single molecules could only be detected in protonated tetradecane-h30, but not in deuterated tetradecane-d30. The $S_1 \leftrightarrow S_0$ transition strength of DPOT was found to be strongly sensitive to the matrix. Even per-deuteration resulted in a loss of emission rate that made single-molecule detection impossible.
from the techniques and results presented in this work. Further, the application of the novel method of ITFC spectroscopy will probably lead to new insights into the low-temperature dynamics of amorphous solids.
A. Simulations of statistical fine structure

In the apparatus shown in Fig. 3.1, the aperture placed in the confocal plane of the laser focus strongly reduces the collection efficiency for molecules located outside the volume $V$, which is defined laterally by the aperture’s projection inside the sample and longitudinally by the size of the laser waist. Thus, the photon emission of different molecules is collected with different efficiencies. This has important consequences for statistical fine structure (SFS) experiments. In this appendix, detailed information is given about the simulation of SFS signals.

Generation of the sample

The sample is defined as a two-dimensional array of the molecular $r$- and $z$-positions, corresponding to randomly dispersed molecules inside a cylindrical volume. The sample is shown schematically in Fig. A.1. A cylindrical symmetry is assumed without loss of generality. $(r_j, z_j)$-pairs are randomly distributed within the given volume. Because the molecular density is constant in the $z$-dimension, the distribution $\{z_j\}$ is equal to a uniform distribution function $\mathcal{R}(j)$ multiplied by the sample thickness. In the radial dimension the molecular density is proportional to $r$. Thus, the distribution $\{r_j\}$ is equal to $\sqrt{\mathcal{R}(j)}$ times the sample radius. In order to maintain a reasonable computation time, the number of molecules in the sample was limited to $100'000$ (sample thickness $18 \mu m$, sample radius $5 \mu m$).

Figure A.1: Schematic representation of the cylindrical sample. The pinhole (PH) located in the conjugate image plane outside the cryostat is projected into the sample. The emission of molecule 1 (black) is only weakly blocked by the PH because it is located close to the $z$-axis and close to the PH. The emission of molecule 2 (grey) is strongly blocked by the PH.
The excitation wave

The center position of the excitation beam is in the center of the sample, i.e. in the center of the pinhole, which corresponds to the origin of the coordinate system \((r,z)\). The amplitude of the excitation wave is normalized to the measured excitation intensity in \(W/cm^2\). Due to imperfections of the optics, the size of the focused laser spot is not diffraction limited. The excitation wave is defined as the convolution of a diffraction limited TEM_{00} wave with a radial Gaussian broadening function. The beam radius of the TEM_{00} beam is \(w\) and its divergence is determined by the numerical aperture of the focusing objective. The broadening function has a beam radius \(b_{ex}\). Thus, the excitation wave has an effective beam radius of \(R_{ex} = \sqrt{b_{ex}^2 + w^2}\) and is given by

\[
I_{ex}(r, z) = I_0 \cdot \frac{1}{1 + y} \cdot \exp\{-\rho\},
\]

with

\[
\rho = \frac{2r^2}{R_{ex}^2 + w^2 y}, \quad y = \left(\frac{z \cdot \lambda/n}{\pi w^2}\right)^2, \quad w = 1.22 \cdot \frac{\lambda}{2 \cdot NA},
\]

where \(NA\) is the numerical aperture of microscope objective \((= 0.85)\), \(n\) the refractive index of the cover glass \((= 1.5 \approx \text{refractive index of tetradecane})\), \(R_{ex}\) the effective radius of the excitation beam, \(I_0\) is the laser intensity and \(\lambda\) the wavelength of 444nm. \(w\) is given by the first minimum of the Airy function. The intensity distribution of the exciting beam in the vicinity of the origin is shown in Fig. A.2.

![Intensity distribution of the excitation beam. \(R_{ex} = 1.95 \mu m\). (a) Three-dim. plot, (b) contour plot.](image)
The wave emitted by the molecules

The center position of the emitted beam is in each case at the position \((r_j, z_j)\) of the molecule. The origin of the coordinate system \((r, \phi, z)\) is in the center of pinhole. For simplicity, the emitted wave is defined as the convolution of a diffraction limited TEM\(_{00}\) wave with a radial Gaussian broadening function (similar to the exciting wave). The emitted wave is normalized so that the cross sectional area of the waist corresponds to the pinhole area. Again, the TEM\(_{00}\) wave is given by the waist radius \(w\) and the divergence determined by the numerical aperture of the collecting objective. The beam radius of the broadening function is \(b_{em}\). The resulting emitted wave has an effective beam radius of \(R_{em} = \sqrt{b_{em}^2 + w^2}\), which corresponds to the resolution of the detection optics. Since the sample is about 100 times thinner than the focal length of the objective, the collection angle is approximately equal for all molecules. The wave emitted by molecule \(j\) is defined by

\[
l_em^j(r, \varphi, z) = l_0^j \cdot \frac{1}{1 + y} \cdot \exp(-\rho).
\]  

The fraction of intensity which is emitted by molecule \(j\) and passes through the pinhole (located at \(z = 0\)) is

\[
l^j_{em, total} = \frac{1}{N} \cdot \int_0^{2\pi} \int_0^{2\pi} l^j_{em}(r, \varphi, z=0) r dr d\varphi,
\]  

where

\[
\rho = \frac{2(r_j^2 + r^2 - 2rr_j \cos(\varphi))}{b_{em}^2 + w^2(1 + y)} = \frac{2(r_j^2 + r^2 - 2rr_j \cos(\varphi))}{R_{em}^2 + w^2 y} ,
\]

\[
y = \left(\frac{(z - z_j) \cdot \lambda / n}{\pi w^2}\right)^2
\]

\[
N = \frac{\pi R_{em}^2}{2} \cdot \left(1 - \exp\left(- \frac{2 R_{ph}^2}{R_{em}^2}\right)\right) ,
\]

\[
I_0^j = \frac{R_\infty}{1 + I_{sat}/(I_{ex}(r_j, z_j))}.
\]  

In these equations, \(R_{em}\) is the radius of the emitted beam, \(R_{ph}\) is the pinhole radius (= 1 \(\mu\)m) and \(I_0^j\) the total emission rate of molecule \(j\). \(N\) is a normalization factor so that the full emission (= \(I_0^j\)) is collected for a molecule located at \((r_j, z_j) = (0, 0)\). The intensity distribution of the emitted beam in the vicinity of the molecular position is shown in Fig. A.3.
Figure A.3: Intensity distribution of the beam emitted by each molecule. $R_{em} = 1.3 \mu m$. (a) Threedim. plot, (b) contour plot.

**Generation of the SFS**

For each molecule, a Lorentzian line shape is calculated. Its peak intensity is given by the corresponding intensity $I_{em, total}^j$. For the linewidth (FWHM) saturation broadening is taken into account, $\Gamma^j = \Gamma_0 \cdot \sqrt{1 + I_{ex}(r_j, z_j)/I_{sat}}$. The line centers of the Lorentzians are randomly distributed over a frequency interval of 8 GHz, using the uniform random distribution function $\mathcal{R}(j)$. Summation of all these Lorentzians yields the SFS.
B. Non-equilibrium phonons (NQP)

In this Appendix the stationary NQP population is studied for a system where energy is fed into the phonon bath within the laser spot by IR absorption. The phonon bath energy is governed by the energy supply at the Debye frequency \( \omega_D \) and by the heat diffusion out of the spot. The population of the individual phonon modes within the laser spot is controlled by the cascading decay of high vibrational phonons into lower frequency modes and by the propagation of the individual modes out of the laser spot. The cascading decay results from the intrinsic anharmonicity scattering of one mode into two phonon modes of typically similar frequency. The propagation is primarily controlled by elastic scattering at lattice imperfections. The description of such a system is complicated because anharmonicity scattering and the mean-free path between scattering events depend on the phonon frequency. Scaling laws are usually applied while the estimate of absolute values remains approximate \([167, 168]\).

We concentrate on the activation of higher local phonon modes by the NQP. Thus, the central question is whether higher local modes are more efficient in broadening and shifting of the molecular transitions and whether the ratio \( |\Delta \Gamma / \Delta \nu | \) is altered upon the activation by NQP, if compared with activation at thermal equilibrium. The latter question can be discussed in terms of the following scaling approach. From eqs. (2.36) and (2.37) one has

\[
|\Delta \Gamma / \Delta \nu | = 2|\delta|\tau. \tag{B.1}
\]

We assume that the shift \( \delta \) of the local mode upon electronic excitation scales linearly with the phonon frequency, \( |\delta| \sim \omega_{LP} \), and that \( \delta \) is of negative sign. The lifetime of the local mode is assumed to behave according to an elastic phonon scattering process, so that the following scaling applies \([168]\):

\[
\tau \sim \omega_{LP}^{-4}. \tag{B.2}
\]

Thus, eq. (B.1) results in

\[
|\Delta \Gamma / \Delta \nu | \sim \omega_{LP}^{-3}. \tag{B.3}
\]

This law indicates that an increase of the local mode frequency reduces dramatically the ratio \( |\Delta \Gamma / \Delta \nu | \). For instance, the activation of a mode of 30 cm\(^{-1}\), motivated by the lines observed in the sideband of Fig. 6.11, instead of the 17 cm\(^{-1}\) mode from Table 6.1, would reduce the ratio of \( |\Delta \Gamma / \Delta \nu | = 0.6 \) obtained from one-photon data down to 0.08. This result is tentatively in accord
with the observation that the ratio $|\Delta \Gamma / \Delta \nu|$ is smaller for two-photon excitation than for one-photon excitation.

Next, we study the efficiency of the NQP in producing a local mode excitation rate $W_e$. The activation of a local mode by NQP is highest at resonance. We therefore consider solely the particular phonon modes at resonance with the local mode and do not discuss the cascading system. The resonant NQP modes are clearly below the Debye frequency and above the thermalization frequency and are thus intermediate states of the cascading system. In the following, the subscript NQP denotes the phonon at resonance with the local mode ($\omega_{\text{NQP}} = \omega_{\text{LP}}$). We assume a given spot volume and write for the time evolution of the local phonon population $n_{\text{LP}}$ and the NQP population $n_{\text{NQP}}$ respectively:

$$\frac{dn_{\text{LP}}}{dt} = -\frac{n_{\text{LP}}}{\tau} + W_e,$$  \hspace{1cm} (B.4)

$$\frac{dn_{\text{NQP}}}{dt} = -\frac{n_{\text{NQP}}}{\tau_{\text{NQP}}} + Q + c_{\text{LP}}\left( -W_e + \frac{n_{\text{LP}}}{\tau} \right),$$  \hspace{1cm} (B.5)

where $\tau$ and $\tau_{\text{NQP}}$ are the local phonon and NQP lifetime, respectively, $Q$ is the NQP generation rate and $c_{\text{LP}}$ the local phonon density which is assumed equal to the chromophore concentration. $W_e$ results from the cross section $\sigma_{\text{NQP}}$ of NQP absorption which is given by [167]

$$\sigma_{\text{NQP}} = \frac{4\pi}{3} c_{\text{NQP}}^2 \omega_{\text{NQP}}^2,$$  \hspace{1cm} (B.6)

and hence

$$W_e = \sigma_{\text{NQP}} s n_{\text{NQP}},$$  \hspace{1cm} (B.7)

where $s$ denotes the sound velocity (see Appendix C). For an estimate of the NQP generation rate $Q$ we assume that all the energy absorbed at the incident IR wavelength passes through the phonon modes under consideration. We thus write

$$Q = \frac{2\pi I c}{\hbar \omega_{\text{NQP}}},$$  \hspace{1cm} (B.8)

where $I$ is the laser intensity and $c$ is the IR absorption coefficient of $1.7 \times 10^{-2}$ cm$^{-1}$ at 888 nm. In the stationary case eqs. (B.4) and (B.5) yield

$$n_{\text{LP}}^{\text{stat}} = \tau W_e \quad \text{and} \quad n_{\text{NQP}}^{\text{stat}} = \tau_{\text{NQP}} Q.$$  \hspace{1cm} (B.9)
Inserting eqs. (B.6), (B.8) and (B.9) into eq. (B.7) we find

\[
W_e = \frac{8\pi^2 3}{3\hbar\omega_{\text{NQP}}} \cdot \tau_{\text{NQP}} = \frac{4\pi s^3}{3\omega_{\text{NQP}}} \cdot Q \tau_{\text{NQP}}.
\] (B.10)

The crucial quantity in this expression is the NQP lifetime $\tau_{\text{NQP}}$ which is discussed in more detail in Appendix C.
C. NQP LIFETIME

As mentioned in the introductory paragraph of Appendix B, the NQP lifetime is limited by the two processes of inelastic anharmonicity scattering and propagation out of the illuminated volume. An expression for the inelastic scattering time $\tau_p$ of a phonon with frequency $\omega_{NQP}$ is given by [169]

$$\tau_p \equiv \frac{128 \rho s_L^5}{\hbar \varphi^2} \left(1 + 2 \left(\frac{s_L}{s_T}\right)^3\right) \omega_{NQP}^{-5} \quad (C.1)$$

where $s_L$ and $s_T$ are the longitudinal and transverse sound velocities which may significantly differ from each other, $\rho$ is the mass density and $\varphi$ an anharmonicity parameter [169]. The propagation characteristics are mainly determined by Rayleigh-type elastic scattering at impurities. The corresponding scattering time $\tau_i$ is [170]

$$\tau_i = \frac{1}{C_i a_0^3 \xi \omega_D} \left(\frac{\omega_D}{\omega_{NQP}}\right)^4 \quad (C.2)$$

where $C_i$ is the crystal defect density, $a_0^3$ the unit cell volume and $\xi$ is a parameter approximately equal to unity for lattice imperfections.

Now we consider the phonon propagation length $L$ and the corresponding propagation time $t_L \equiv L/s$. For $t_L \ll \tau_p$, $\tau_i$ the phonons are not scattered at all and they propagate “ballistically” during the time interval $t_L$. In the case $t_L = \tau_p$, $L$ corresponds to the intrinsic mean free path, $L = s \tau_p$. If $t_i \ll \tau_p \ll \sqrt{\tau_p \tau_i}$ the phonons are scattered by impurities many times before they decay and the propagation follows a diffusion process with a diffusion constant $D = \tau_i s^2/3$. In this case, the propagation length during the intrinsic lifetime $\tau_p$ is $L = \sqrt{D \tau_p} = s \sqrt{\tau_p \tau_i}/3$. In our experiment, the volume of NQP generation is given by the volume $V_{laser}$ of the laser waist. If $V_{laser} < L^3$, the NQP escape out of the generation volume before they decay. Their propagation time out of $V_{laser}$ corresponds to $t_L = V_{laser}^{1/3}/s$ for ballstic and $t_L = V_{laser}^{2/3}/D$ for diffusive motion, respectively.

Finally, we estimate the relevant NQP lifetime $\tau_{NQP}$ for an effective phonon with the frequency $\omega_{NQP}/2\pi c = 30\text{cm}^{-1}$ in our system. A guess for the average sound velocity $s$ is obtained from the relation

$$s \approx \left(\frac{\omega_D^3}{6\pi^2 N}\right)^{1/3} \quad (C.3)$$
where \( N \) is the number of molecules per unit volume. \( N \) is equal to \( a_0^{-3} \) in a primitive unit cell. For TD we get \( s = 2200 \text{ m/s} \), assuming a Debye frequency of \( \omega_D/2\pi c = 60 \text{ cm}^{-1} \) [171] and taking \( N = 2.3 \times 10^{21} \text{ cm}^{-3} \) (calculated from the mass density of \( \rho = 0.762 \text{ g/cm}^3 \) and the molar mass of 198.4 g/mol). The ratio \((s_L/s_T)\) between the longitudinal and transverse sound velocity is about 2 and we take \( s_L \approx s \) for the estimate of \( \tau_p \). The anharmonicity parameter \( \varphi \) is not accurately known, \( \varphi \) is guessed to be between 0.5 and 2 [169]. Accordingly, the range of \( \tau_p = 6 - 90 \text{ ns} \) is calculated for the intrinsic NQP lifetime from eq. (C.1). Considering that \( \tau_p \) scales as \( s_L^5 \) and \( \varphi^{-2} \), and that \( s_L \) (or \( s \)) and \( \varphi \) are not known experimentally, the broad range of \( \tau_p \) is justified. To estimate the phonon-impurity scattering time \( \tau_i \) we take \( C_i = 4 \times 10^{18} \text{ cm}^{-3} \) as a guess for the density of crystal defects. This concentration corresponds to 1 defect per \( 10^4 \) unit cells which is a reasonable value for Shpol'skii matrices. Accordingly, we get \( \tau_i \approx 150 \text{ ps} \). Thus, the NQP propagation is of diffusive nature on the scale of the generation volume \( V_{\text{laser}} \approx 100 \mu\text{m}^3 \). As a consequence, the NQP lifetime is governed by the intrinsic decay and not by the escape out of the probing volume. In conclusion, we estimate that \( \tau_{\text{NQP}} \) is in the range of 6 – 90 ns.
REFERENCES
References to chapter 1


References to chapter 2


**References to chapter 3**


References to chapter 4


REFERENCES

[114] All calculations have been performed by Dr. Gert Zumofen, Physical Chemistry Laboratory, ETH Zürich.
For the interpretation of the spectra, we have chosen a “visual” optimization procedure. The experimental and calculated data were plotted one upon the other and the match was empirically rated.

References to chapter 5

References to chapter 6

[146] According to the method described in ref. [51].

These authors assume that each TLS gives the same contribution to the broadening. This assumption is equivalent to a model where each dopant chromophore interacts with a statistically identical ensemble of TLSs. Further, spectral diffusion during “burning” and “reading” times is neglected. In this case, the ensemble average of the transition frequency (measured in hole-burning) is equal to its time average (measured in SM spectra).

[157] Eq. (6.10) corresponds to eq. (4.38) of ref. [129] which was deduced for atoms. The Franck-Condon factor $C_{FC}$ is introduced to account for the vibronic relative to the purely electronic transition strength. The approximation $\langle g|\hat{\mu}^2|g\rangle \approx \langle g|\hat{\mu}^2|e\rangle \approx \langle e|\hat{\mu}^2|e\rangle$ is also used.

References to chapter 7

References to the Appendix
Curriculum Vitae

Personal Data

Name: Daniel Walser
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April '84 - Sept. '88 "Mathematisch-Naturwissenschaftliches Gymnasium", Kantonsschule Sargans (SG). Matura Typus C.
July '95 - Sept. '99 Graduate studies in the group of Prof. Urs P. Wild at the Physical Chemistry Laboratory, ETH Zürich. Research area: High-resolution laser spectroscopy of single molecules in solids. Teaching: Assistant in laboratory and exercise courses for undergraduate students in physical chemistry.