1 Low-Temperature Studies in Solids

1.1 Physical Principles and Methods of Single-Molecule Spectroscopy in Solids

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1.1.1 Introduction – why do single-molecule studies in solids?

Over the past few years, the power of optical spectroscopy with high-resolution laser sources has been extended into the fascinating domain of individual impurity molecules in solids. In this regime, the single molecule acts as an exquisitely sensitive probe of the details of the immediate local environment (which may be termed the “nanoenvironment”) [1–8]. Using techniques described in this section and illustrated in the rest of Chapter 1 of this book, exactly one molecule hidden deep within a solid sample can now be probed at a time by tunable laser radiation, which represents detection and spectroscopy at the ultimate sensitivity level of $1.66 \times 10^{-24}$ moles of material, or 1.66 yoctomole.$^1$

Optical experiments in this new regime are generating much interest for a variety of reasons. Most importantly, single-molecule measurements completely remove the normal ensemble averaging that occurs when a large number of molecules are probed at the same time. Thus, the usual assumption that all molecules contributing to the ensemble average are identical can now be directly examined on a molecule-by-molecule basis. On the theoretical side, since no ensemble averaging need be done before computing an observable quantity, stronger tests of truly microscopic dynamical theories can be completed. Finally, since this is a previously unexplored regime, new physical and chemical behavior is likely to be observed, and many examples of this are presented in the chapters to come.

Single-molecule spectroscopy (SMS) in solids is related to, but distinct from, the fascinating and well-established field of spectroscopy of single electrons or ions confined in electromagnetic traps [10–12]. The vacuum environment and confining fields of an electromagnetic trap are quite different from the environment of a single molecule in a solid. The trap experiments must deal with micromotion in the confining trap potential and, to date, no single molecule has been cooled sufficiently to be bound by an electromagnetic trap. In SMS, however, the interactions with the lattice act to constrain the molecule, hindering or preventing molecular rotation. At the same time, the single molecule is continuously bathed in the phonon vibrations of the

$^1$Since a single molecule is the smallest unit of a molecular substance, a more appropriate unit in this case would be the guacamole, which is the quantity of moles exactly equal to the inverse of avocado's number [9]. (With apologies to Amadeo Avogadro)
solid available at a given temperature, and can interact with the electric, magnetic
and strain fields of the nanoenvironment.

Useful comparison may also be made to another important field, the direct probing of atoms or molecules on surfaces with scanning tunneling microscopy (STM) [13] or atomic force microscopy (AFM) [14, 15]. In STM and AFM of single molecules, a fairly strong bond must exist between the molecule and the underlying surface in order for the molecule to tolerate the perturbing forces from the tunnelling electrons or the tip. Of course, the spatial resolution of these methods is much higher as a result of the relatively short electron wavelength of a few tenths of a nanometer, and the tunneling or force tip must be placed correspondingly close to the molecule, and the molecule must be on the surface. On the other hand, SMS usually operates noninvasively in the optical far-field with a corresponding loss in spatial resolution to a value on the order of the optical wavelength (1 μm), but with no loss in spectral resolution. Moreover, single molecules can be studied below the surface in the body of the sample, and different single molecules can be selected by simply changing the optical wavelength used. In 1993, single-molecule imaging was achieved with near-field optical techniques with 100 nm resolution at room temperature [16, 17]. These near-field optical studies at room temperature and other near-field studies at liquid helium temperatures [18] will be described in chapter 2 of this book.

This section presents an overview of the physical principles and methods of high-resolution spectroscopy of single impurity centers in solids. The emphasis is on single, isolated molecular impurities, although many of the concepts described are also applicable to the possible future study of single ions as well [19]. The presentation will concentrate on the far-field regime, in which the spatial resolution is limited by diffraction effects to beam diameters on the order of 1 μm. Chapter 2 of this book treats the optical near-field, sub-wavelength realm, and Chapter 3 describes single-molecule detection in liquids. Section 1.1.2 describes the fundamental requirements for high-resolution SMS [20]. Section 1.1.3 describes the various experimental methods used to achieve SMS in solids, with selected examples. In the remaining sections of Chapter 1, specific results on external perturbations [21, 22], microscopic imaging [23], spectral shifting (also called spectral diffusion [3, 24], optical modifications of single molecules (which may eventually lead to a single-molecule optical storage) [25], correlation properties of the emitted photons [26, 27], quantum optical effects [28, 29], vibrational modes [30, 31], and single-spin magnetic resonance [32, 33] will be described in more detail. The reader may consult one of several recent reviews for more information [5–8].

The significant features of these SMS studies are: (i) new, unexpected physical effects have been observed in the single-molecule regime as a result of the nanoenvironmental sensitivity of the single-molecule lineshape, (ii) it is now possible to probe the members of the usual ensemble average one at a time, and therefore to directly measure the distribution rather than only measure its moments, (iii) as a result of the ability to follow spectral changes of a single molecule in real time, it is now possible in a single nanoenvironment to directly probe the connection between specific microscopic theories [34, 35] of local structure, dynamics, and host–guest interactions and the statistical mechanical averages that are measured in conventional experiments, (iv) the door to measurements on a single molecular spin has
been opened for the first time, which should lead to unprecedented information on local magnetic interactions, and (v) quantum optical studies may now be performed in solids where the normal transit time or micromotion effects present in trap or beam experiments are absent.

1.1.2 Physical principles and optimal conditions

1.1.2.1 General considerations

One may ask: how is it possible to use optical radiation to isolate a single impurity molecule hidden deep inside a host matrix? To answer this question concisely, single-molecule optical spectroscopy is accomplished by selecting experimental conditions such that only one molecule is in resonance in the volume probed at a time. More precisely, a combination of small probing volume, low concentration of the impurity molecule of interest, and spectral selection is necessary to insure that only one molecule is pumped by the laser beam. In addition, it is important to select a fairly stable host-guest combination and detection technique such that the detected optical signal from one molecule can be observed with sufficient signal-to-noise in a reasonable averaging period.

To proceed from one mole of material (6.02 x 10^23 molecules) to only one molecule, many orders of magnitude must be spanned. The spatial coherence available from modern laser sources facilitates the probing of a small volume by providing focal spot sizes on the order of one to a few μm in diameter. It is best to use small sample thicknesses no larger than the Rayleigh range of the focused laser spot (3–10 μm). Thus, in most experiments a small volume of sample on the order of 10–100 μm^3 is probed. This action alone represents an effective reduction of the number of molecules potentially in resonance by some 11 to 12 orders of magnitude, depending on the actual molar volume of the material.

Single-molecule experiments generally utilize samples in which the molecule of interest is present as a dopant or guest impurity in a transparent host matrix. Obviously, then, if the concentration of the guest is sufficiently small, only one molecule of interest will be in the probing volume. For experiments at room temperature where no spectral selection method is available, it is indeed necessary to reduce the concentration of the impurity dramatically to 10^{-12} moles/mole or lower, and to be very sure that no other unwanted impurity in the probed volume is capable of producing a signal that would overwhelm that from the single guest molecule of interest. The experiments in Chapter 2 of this book with near-field optics at room temperature and those in liquids described in Chapter 3 must work precisely in this regime—by extreme reduction of concentration one and only one guest molecule is allowed at a time in the volume pumped by the laser.

However, for high-resolution SMS at low temperatures which is the focus of the first Chapter of this book, such extreme reductions in concentration are not generally required, and samples are usually doped with the guest at concentrations in the range 10^{-7} to 10^{-9} mol/mol. This additional 7 to 9 orders of magnitude reduction in the number of potentially resonant molecules is insufficient to guarantee that only one impurity molecule in the probed volume is in resonance with the laser at a time. The
Figure 1. Schematic representation of the electronic energy levels of a molecule showing the ground singlet state $S_0$, the first excited singlet state $S_1$, and the lowest triplet state $T_1$. For each electronic state, several levels in the vibrational progression are shown. Laser excitation pumps the $(0-0)$ transition with energy $h\nu$. The intersystem crossing rate from the singlet manifold to the triplets is $k_{isc}$, and the triplet decay rate is $k_T = (\tau_T)^{-1}$. Fluorescence emission shown as dotted lines originates from $S_1$ and terminates on various vibrationally excited levels of $S_0$.

requisite additional selectivity on the order of a factor of $10^4$ or so is provided by spectral selection, which involves carefully selecting the guest and host and using the well-known properties of inhomogeneously broadened absorption lines in solids, to be described next.

1.1.2.2 Spectral selection using zero-phonon lines and inhomogeneous broadening

There is an important physical effect which facilitates the detection and spectroscopy of single molecules in solids at low temperatures, known as inhomogeneous broadening. This effect occurs most clearly when the optical transition pumped by the laser is a purely electronic, zero-phonon line (ZPL), from the lowest vibrational level of the electronic ground state to the lowest vibrational level of the electronically excited state. (We assume that the placement of the guest molecule in the solid effectively hinders rotation of the molecule.) Such a so-called $(0-0)$ transition at energy $h\nu$ shown in Fig. 1 often has a very long lifetime, because the de-excitation to the ground state manifold requires a large number of phonons to be emitted, and such a high-order emission process is improbable. At zero temperature, such a transition could have a lifetime-limited transition width of tens to a few hundred MHz (for an electric-dipole-allowed transition in the visible, even smaller for a partially forbidden transition).

At finite but low temperatures, the width of the ZPL can still be close to the lifetime-limited value. One may wonder why the time-varying perturbations due to the phonons of the solid do not broaden such transitions dramatically. First considering linear coupling to the phonons, it is in fact the extremely high frequency of the phonons compared to the radiative width of the ZPL which places the fluctuations of the optical transition frequency in the motionally-narrowed regime [36], so no broadening of the transition occurs. From the point of view of recoil, the ZPL is often regarded as the optical analog of the Mössbauer line, so that the entire solid sample recoils during optical absorption [37]. As a result of these considerations, a ZPL transition can only dephase and broaden by second-order coupling to the phonons, that is, by phonon scattering (two-phonon processes) [38]. At liquid helium temper-
atures, few phonons are present to produce phonon scattering, so the homogeneous width $\gamma_H$ of zero-phonon optical transitions in crystals approaches the lifetime-limited value of some tens of MHz mentioned above. Since the optical transition frequency is near 500 THz, the $Q$ or quality factor of such a narrow transition is very large, near $10^8$. In amorphous materials, other low-frequency excitations arising from two-level systems are present, and the homogeneous width is somewhat larger \[43\], but still far narrower than at high temperatures. It is the extremely high $Q$ of single-molecule lines in solids that leads to exquisite sensitivity to nanoscopic changes - very weak perturbations produced by electric, magnetic, or strain fields in the nanoenvironment can easily produce a detectable shift in the single-molecule absorption.

Now consider what happens for the collection of guest molecules which are located in the probed volume. If the sample were a perfect crystal and all local environments were identical, the optical absorption would be a single narrow Lorentzian line of width $\gamma_H$. However, these conditions are seldom met in real solid samples. The optical absorption spectrum that is actually measured for such an assembly is far broader than $\gamma_H$. The extreme narrowness of the ZPL for each of the molecules is obscured by a distribution of center frequencies for the various guest molecules, and the resulting overall profile is termed an inhomogeneously broadened line \[39, 40\] (see Fig. 2). The distribution of resonance frequencies is caused by 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 crystals, as long as $\gamma_H$ is small enough to reveal the inhomogeneous distribution. In the simplest case of inhomogeneous broadening, the overall line profile of width $\Gamma_1$ is caused by an approximately Gaussian (normal) distribution of center frequencies for the individual absorbers that is broader than the homogeneous lineshape. Inhomogeneous broadening is a universal feature of high-resolution laser spectroscopy of guest molecules in solids \[41, 42\] and of other cases where ZPLs are probed such as Mössbauer and magnetic resonance spectroscopy. It is for this reason that methods such as spectral hole-burning \[44\] and coherent transients \[45\], most often photon echoes, have been utilized to learn about the homogeneous line-width hidden under such inhomogeneous spectral profiles.

Fortunately, the normally troublesome phenomenon of inhomogeneous broadening facilitates the spectral selection of individual molecules for SMS. In effect; the spread of center frequencies means that different guest molecules have different resonance frequencies, so if the total concentration is low enough, one simply uses the tunability of a narrowband laser to select different single molecules. This spectral selection must be done in a region of the frequency spectrum where the number of molecules per $\gamma_H$ is on the order of or less than one. In general, this may be accomplished in several different ways: (a) by using a sample with a low doping level, (b) by using a sample with a very large $\Gamma_1$, or (c) by tuning out into the wings of the inhomogeneous line as shown in the right side of Fig. 2. A useful analogy is provided by

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2 The normalized Lorentzian absorption profile centered at $\omega_0$ with full-width at half-maximum $\gamma_H$ is

$$\frac{(\gamma_H/2\pi)/[(\omega - \omega_0)^2 + (\gamma_H/2)^2]}$$

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Figure 2. Schematic showing an inhomogeneous line at low temperatures and the general principle of single-molecule detection in solids. The entire line is formed as a superposition of (generally Lorentzian) homogeneous profiles of the individual absorbers, with a distribution of center resonance frequencies caused by random strains and imperfections which can be normally distributed (Gaussian). In the upper right, several dopant molecules are sketched as rectangles with different local environments produced by strains, local electric fields, and other imperfections in the host matrix. The lower part of the figure shows how the number of impurity molecules in resonance in the probed volume can be varied by changing the laser wavelength. The laser line-width is negligible on the scale shown. Although this figure shows that wavelengths in the wings of the line are required to achieve the single-molecule limit, this is not essential if either the concentration of guest molecules is lowered or if the inhomogeneous linewidth is increased.

The problem of tuning in a radio station when one is out in the country where only a few stations can be received. As the receiver is tuned, mostly static is received (no signal) until the exact frequency of a distant station is reached. Similarly, when inhomogeneous broadening causes the different single molecules in the probed volume to have different resonance frequencies, and the molecules are spaced apart by more than $\gamma_H$ on average, single molecules can be pumped selectively, one at a time, simply by tuning the laser.
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To provide a slightly more realistic picture of the inhomogeneous broadening phenomenon, Fig. 3 shows a simulated inhomogeneous lineshape produced by the superposition (summation) of a large number of individual homogeneous Lorentzian absorption profiles. In this simulation, $\Gamma_1/\gamma_H$ was chosen to be 10 for simplicity. (In many physical systems, the ratio $\Gamma_1/\gamma_H$ is much larger, often in the range $10^3$ to $10^6$.) In trace (a), only ten centers were superposed to produce the entire "inhomogeneous" line, and the identification of structures corresponding to single molecules is clearly evident. The other traces show the result of successively increasing the number of centers $N$ in the probed volume. The "spectral roughness" called statistical fine structure [46] on the peak of the profile is clearly evident, although it is decreasing in relative magnitude as $1/(\sqrt{N})$. In real experiments, statistical fine structure is often observed before proceeding to the SMS regime, in order to optimize the detection system. Indeed, the early observation of statistical fine structure for pentacene in $p$-terphenyl crystals [46, 47] provided useful encouragement that the single-molecule regime could eventually be reached. With difficulty, statistical fine structure has also been observed for ions in inorganic materials [48, 49].

1.1.2.3 Peak absorption cross section

A central concept which is crucial to single-molecule spectroscopy in solids is the role of the peak absorption cross section of the guest molecules $\sigma_p$. Study of this parameter helps to answer the question: Why is the signal from a single molecule large enough to be detected above background in a reasonable period of time? As will be shown below, the signal-to-noise ratio for SMS depends crucially on maximizing the value of $\sigma_p$, no matter which technique is used for detection. For focal spots of area greater than or equal to the diffraction limit (approximately $\lambda^2$, with $\lambda$ the optical...
wavelength), the rate at which the resonant optical transition is pumped is given by the product of the incident photon flux (in photons s\(^{-1}\) cm\(^2\)) and \(\sigma_p\) (in cm\(^2\)). Stated differently, the probability that a single molecule will absorb an incident photon from the pumping laser beam is just \(\sigma_p/A\), where \(A\) is the cross-sectional area of the focused laser beam. High \(\sigma_p\) means that the photons of the incident light beam are efficiently absorbed and background signals from unabsorbed photons are minimized.

To understand what controls the value of \(\sigma_p\), we recall that for molecules with weak electron-phonon coupling with appreciable oscillator strength in the lowest purely electronic transition, the optical homogeneous linewidth \(\gamma_H\) becomes very small at low temperatures. The key point to remember is that due to well-known sum rules on optical transitions, the peak cross section depends inversely on \(\gamma_H\), so that the narrow linewidth of a ZPL translates into a very large peak absorption cross section. For allowed transitions of rigid molecules, the value of \(\sigma_p\) becomes extremely large, approaching the ultimate limit of \(a^2\).

Although the importance of the peak absorption cross section has been recognized since the first SMS experiment [1], it is useful to review how \(\sigma_p\) can be estimated using sum-rule techniques. The standard integrated absorption sum rule [50–54] may be written

\[
S = \int \alpha(\tilde{\nu}) d\tilde{\nu} = \left(\frac{\pi e^2}{mc^2}\right) \frac{F}{n} f N_{tot} = \frac{8\pi^2 \tilde{v}_0}{ch(F/n)} \left(\frac{|\mu|^2}{3}\right) N_{tot}
\]

where \(e\) is the electronic charge, the local field factor is \(F = [(n^2 + 2)/3]^2\), \(n\) is the index of refraction, \(c\) is the speed of light, \(N_{tot}\) is the number density of absorbers producing the integrated absorption \(S\) (units cm\(^{-2}\)), \(f\) is the oscillator strength, \(\tilde{v}_0\) is the frequency at the center of the band in wavenumbers, and \(|\mu|\) is the transition dipole moment magnitude. Because the oscillator strength (or dipole moment) is generally independent of temperature, the ratio \(S/N_{tot}\) is a constant which may either be evaluated for the homogeneous band at room temperature or for a single Lorentzian profile at low temperature. Applying Eq. 1 to the single-molecule Lorentzian absorption profile of width \(\Delta \tilde{\nu} = (\pi e T_2)^{-1} = \gamma_H/(2\pi c)\) (where \(T_2\) is the dephasing time) and identifying the peak absorption divided by the number density of absorbers producing that absorption as the peak cross section (per center) yields the standard formula [20]

\[
\sigma_p = \left(\frac{2\pi e^2 T_2}{mc}\right) \frac{L}{n} f_{ZPL} = \left(\frac{16\pi^2 T_2 \tilde{v}_0}{h}\right) \frac{(L/n)}{f_{ZPL}} \left(\frac{|\mu|^2}{3}\right)
\]

\[
= 2c T_2 \left(\frac{S}{N_{tot}}\right)_{ZPL}
\]

If the integral to determine \(S\) in Eq. 1 is performed for a room-temperature liquid
solution, the resulting value of oscillator strength $f$ applies to the entire electronic transition, including all the phonon and vibrational sidebands of the ZPL. To obtain the oscillator strength of the ZPL only, $f_{ZPL}$, for use in Eq. 2, the value of $f$ must be multiplied by $F_{FC}F_{DW}$, where $F_{FC}$ is the Franck-Condon factor and $F_{DW}$ is the Debye-Waller factor. Similar considerations apply to the dipole moment and integrated absorption strength $S$. Thus, with measurement of the low temperature line-width (or $T_2$) and either the oscillator strength, dipole moment, or the value of $S/N_{tot}$ for the transition, $\sigma_p$ can be determined. For an equivalent alternative approach based on radiative lifetimes, see Ref. 55.

For molecules like pentacene, perylene, or similar rigid aromatics with a strongly allowed lowest electronic transition (see Section 1.1.3.3 for structures), the net result is that at low temperatures where $T_2$ is large (some tens of ns), the peak absorption cross section increases to levels as high as $10^{-11}\text{ cm}^2$, approximately 4000 times the (van der Waals) area of a single molecule! Thus, even though any dimension of a single molecule is much smaller than the optical wavelength, the effective area of the molecule for optical absorption is not nearly so small, and a zero-phonon optical transition of a molecule in a solid can be made to absorb light quite efficiently if the sample is cooled to low temperatures. To give a specific example for the case of pentacene, the low-temperature homogeneous width is 7.8 MHz [56], the measured ZPL dipole moment without orientational averaging is 0.7 Debye [57] where one Debye is defined as $3.33 \times 10^{-30}\text{ Cm}$, and thus the value of $\sigma_p$ is estimated to be near $9 \times 10^{-12}\text{ cm}^2$.

### 1.1.2.4 Other important requirements for single-molecule spectroscopy

This section briefly describes several additional requirements necessary to insure that the signal from a single molecule dominates over all background signals (for full details see Refs. 5 and 20).

In the case of fluorescence detection, the quantum yield for photon emission per absorption event $\phi_p$ should clearly be high, as close to unity as possible. Extreme care should be taken to minimize scattering backgrounds which may arise either from Rayleigh scattering, or from Raman scattering from the sample and the substrate.

A further requirement on the absorption properties of the guest molecule stems from the general fact that higher and higher laser power generally produces more and more signal, as long as the optical transition is not saturated. When saturation occurs, further increases in laser power generate more background rather than signal, and this is true for both fluorescence and absorption detection methods. The saturation intensity is maximized when absorbing centers are chosen that do not have strong bottlenecks in the optical pumping cycle (see Fig. 1). In organic molecules, intersystem crossing (ISC) from the singlet states into the triplet states represents a common bottleneck, because both absorption of photons and photon emission cease for a relatively long time equal to the triplet lifetime when ISC occurs. This effect results in premature saturation of the emission rate from the molecule and reduction of the absorption cross section $\sigma_p$ compared to the case with no bottleneck [58]. For
later reference, the saturation intensity \( I_S \) for a molecule with a triplet bottleneck may be written [59, 60]

\[
I_S = \frac{h\nu}{2\sigma_p T_1} \left[ \frac{1 + (k_{isc}/k_{21})}{1 + (k_{isc}/2k_T)} \right]
\]

where \( T_1 = 1/k_{21} \) is the inverse of the rate of direct decay from \( S_1 \) to \( S_0 \), \( k_{isc} \) is the rate of intersystem crossing as shown in Fig. 1, and \( k_T \) is the total decay rate from the triplet back to \( S_0 \). The factor outside the brackets is the two-level saturation intensity if there were no triplet bottleneck which represents an upper limit for the saturation intensity. Thus, minimizing the triplet bottleneck means small values of \( k_{isc} \) and large values of \( k_T \), requirements which may be easily satisfied for rigid, planar aromatic dye molecules.

A final requirement for SMS is the selection of a guest–host couple that allows for photostability of the impurity molecule and weak spectral hole-burning, where by spectral hole-burning we include any fast light-induced change in the resonance frequency of the molecule caused either by frank photochemistry of the molecule or by a photophysical change in the nearby environment [44]. For example, most fluorescence detection schemes with overall photon collection efficiency of 0.1% to 1% require that the quantum efficiency for hole-burning be less than \( 10^{-6} \) to \( 10^{-7} \). This is necessary to provide sufficient time averaging of the single-molecule signal before it changes appreciably or moves to another spectral position.

It should be noted that the additional requirements for SMS stated in this section represent a “best-case” in order to produce the highest possible signal-to-noise in a high-resolution experiment. If some loss in signal-to-noise or spectral resolution can be tolerated, these requirements can be weakened accordingly. Specific forms of the SNR for various detection methods will be presented in the next section.

1.1.3 Methods

1.1.3.1 Geometrical configurations for focusing and fluorescence collection

A variety of different configurations have been used for achieving the required focusing of the radiation in a small sample at liquid helium temperatures. If a direct absorption method is used, one need only collect the transmitted light and direct it to the detector. In the case of fluorescence detection, it is necessary to carefully collect the emitted fluorescence photons from the single molecule over as large a solid angle as possible, without collecting unwanted transmitted pumping radiation or scattered light. Because of the large number of experiments utilizing fluorescence detection, all configurations described here refer to the fluorescence method.

One useful experimental setup is shown in Fig. 4(a), the “lens–parabola” configuration. All the components shown can be immersed in superfluid helium. The focal spot from the pumping laser is produced by a small lens of several mm focal length placed directly in the liquid helium. Generally, some provision must be made for adjusting the focus at low temperatures. In one solution to this [58], the lens is mounted on a thin stainless steel plate, which can be flexed by a permanent magnet
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Figure 4. Various experimental arrangements for SMS in fluorescence excitation: (a) lens-paraboloid (after Ref. 58), L - lens, P - paraboloid, S - sample, B - beam block, M - magnet, C - coil electromagnet, (b) fiber-paraboloid (first used in Ref. 3), (c) pinhole (after Ref. 61), (d) paraboloid focus and collection (after Ref. 65).

The sample is mounted on a transparent substrate, ideally an alkali halide, whose center of symmetry prevents first-order Raman scattering by the substrate. After passage through the sample, the transmitted pumping radiation is blocked by a small beam block. The emitted fluorescence is collected by a paraboloid with numerical aperture near 1.0, and directed out of the cryostat. Using a standard two-element achromatic lens, a spot size of 3–5 μm diameter can be produced. The size of the focal spot is limited by the distortion and aberrations produced by cooling a lens designed for operation at room temperature. With optimized optics able to tolerate liquid helium temperatures, smaller spot sizes should be achievable with this configuration. One feature of this
configuration is the ability to scan the position of the focal spot at low temperatures across the sample by tilting of the incident pumping laser beam [58].

A second useful configuration (termed "fiber-parabola") used in the first fluorescence excitation SMS [3] takes advantage of the small spot size produced automatically by the core of a single mode optical fiber as shown in Fig. 4(b). Here the thin crystalline or polymeric sample is attached to the end of the optical fiber using epoxy- or index-matching gel and held by capillary action. The fiber end with the sample is again placed at the focus of a high numerical aperture paraboloid for collection of the emission. The spot size in this case is controlled completely by the fiber core diameter, and mode diameters of 4 μm are common. This configuration avoids the need to adjust the position of the focus, but does not allow any change of the volume probed by the laser after cooldown. In addition, some strain can be introduced into the sample as a result of the gluing process.

A third experimental configuration involves the use of a small pinhole aperture [61] in a thin metal plate, as shown in Fig. 4(c). The sample is mounted directly against a thin stainless steel plate with a 5 μm pinhole in it. The pinhole is illuminated with the laser beam from the opposite side. The fluorescence is collected with small lenses or a 0.85 NA microscope objective placed in the liquid He (not shown). While this method is relatively easy to implement, the sample can be easily strained during mounting resulting in very broad inhomogeneous lines. A more fundamental problem results from the strong oscillations in the local intensity of the laser beam produced by the nearby conducting aperture [62] – in general it is more difficult to determine the laser intensity at the position of the single molecule with this approach.

In order to study the vibronic structure of the single molecule, researchers have spectrally dispersed the emission by collecting the emitted light from the fiber-parabola setup and focusing it on the slit of a spectrometer [63]. A liquid-nitrogen-cooled CCD with very high quantum efficiency is used to detect the spectrally-dispersed photons. The result is actually a resonance Raman experiment on a single molecule [64] which contains useful information about possible molecular distortions produced by the local environment. In a recent enhancement of this technique shown in Fig. 4(d) [65], a high-quality diamond-turned Al paraboloid was used both to focus the emitting radiation, as well as to collect the emission in an epi-fluorescence configuration. The sample was placed at the focus of the paraboloid by flexing a thin metal plate. The focal spot diameter was estimated to be less than 2 μm, and the total sample volume probed as small as 10 μm².

For experiments in which microwave and magnetic fields are required to pump transitions between spin sublevels, a sophisticated enhancement of the lens-parabola design has been described [66] which allows low temperature positioning of the sample along two axes without use of electro- or permanent magnets.

1.1.3.2 Detection techniques

Direct absorption (frequency-modulation spectroscopy with secondary modulation)

The first single-molecule spectra were recorded in the pentacene in p-terphenyl system in 1989 using a sophisticated zero-scattering-background absorption technique,
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Figure 5. Schematic of frequency-modulation spectroscopy with Stark secondary modulation (FM-Stark). The top of the figure shows the light spectrum at the output of the dye laser (DL), after the electro-optic phase modulator (EO), and after the cryostat (C, from left to right), the arrows indicating the relative amplitudes and phases of the electric light fields. The rf source drives the EO modulator and produces the local oscillator signal (LO) for the mixer M. The output of the avalanche photodiode (APD) drives the R port of the mixer. The simple FM signal is present at the I port of the mixer. For secondary Stark modulation, a high-voltage source (HV) at reference frequency \( f \) produces a time-varying electric field across the sample. A lock-in amplifier detects the output of the I port of the mixer, and the resulting signal is averaged on a digital oscilloscope (DS). After Ref. 48.

Frequency-modulation (FM) spectroscopy [67, 68], combined with either Stark or ultrasonic modulation of the absorption line [1, 2]. Rather than describe the complete details of the FM technique here, it is more useful to describe the basic characteristics of the method, and the reader may consult Refs. 2 and 5 for more information. Referring to Fig. 5, using an electro-optic modulator (EO), the dye laser (DL) at frequency \( \omega_c \) is phase-modulated at the local oscillator (LO) radio frequency \( \omega_m \) which produces two sidebands on the laser carrier at \( \omega_c + \omega_m \) and at \( \omega_c - \omega_m \) with opposite phase. Any amplitude modulation of the laser beam exiting the sample is detected using a fast photodiode (for example, an APD) and a phase-sensitive radio frequency lock-in (mixer M) driven at \( \omega_m \). If no narrow spectral features are present, there is only a dc signal at the detector output, since a perfect phase-modulated laser beam has no amplitude modulation. The background noise level at the I port of the mixer is produced by detector noise and by the laser noise at \( \omega_m \). For LO frequencies above about 1 MHz, the limiting noise source can be quantum (shot) noise if no excess noise is introduced by the detector. Generally, the laser power at the detector must be above a certain minimum value for shot noise to dominate over other noise sources.
If a narrow spectral feature is present with linewidth on the order of or less than \( \omega_m \), the unbalancing of the two sidebands will convert the phase-modulated laser beam into an amplitude-modulated beam which produces a strong oscillating photocurrent at \( \omega_m \) at the detector output. More precisely, the detected signal at the 1 port of the mixer (in the absorption phase) is proportional to \( a(\omega_c + \omega_m) - a(\omega_c - \omega_m) \) where \( a \) is the absorption coefficient and \( L \) is the sample thickness. Thus the FM signal measures the difference in \( aL \) at the two sideband frequencies. For a spectral feature narrower than \( \omega_m \), two copies of the absorption line appear, one positive and one negative, as each of the two sidebands is swept over the absorption.

In actual practice, it is generally difficult to produce absolutely pure phase modulation of the laser beam, and a frequency-dependent interfering signal called residual amplitude modulation (RAM) is often present. While many methods have been proposed to eliminate RAM [69–70], internal secondary modulation of the spectral features by Stark-shifting or ultrasound have proved most useful in SMS experiments [2]. For the Stark-FM approach illustrated in Fig. 5, an oscillating high voltage (HV) source driven at an audio frequency \( f \) impresses a time-varying electric field across the sample. This will produce a periodic shifting of the spectral feature. Since the RAM does not oscillate at frequency \( f \), the RAM may be removed by detection of the mixer I-port output with a lock-in amplifier (LIA) driven at \( f \) (for a linear Stark shift) or at \( 2f \) (for a quadratic Stark shift). The resulting lineshape will appear as the appropriate spectral derivative of the FM signal (Ref. 1).

The signal-to-noise ratio for a single molecule detected by FM spectroscopy has been described in detail in Ref. 5. Since this is an absorption technique, clearly single molecules with higher absorption cross section lead to larger FM signals, and detectors with internal gain such as APD's are helpful in reducing detector noise. In contrast to fluorescence methods, Rayleigh scattering and Raman scattering are unimportant. Only the shot noise of the laser beam contributes to the background, assuming RAM and detector noise are properly controlled.

Fig. 6 shows examples of the optical absorption spectrum from a single molecule of pentacene in p-terphenyl using the FM Stark method. Although this early observation and similar data from the FM ultrasound method served to stimulate much further work, there is one important limitation to the general use of FM methods for SMS. As was shown in the early papers on FM spectroscopy [67, 68], extremely low absorption changes as small as \( 10^{-7} \) can be detected in a 1 s averaging time, but only if large laser powers on the order of several mW can be delivered to the detector to reduce the relative size of the shot noise. This presents a problem for SMS in the following way. Since the laser beam must be focused to a small spot, the power in the laser beam must be maintained below the value which would cause power broadening of the single-molecule lineshape. As a result, it is quite difficult to utilize laser powers in the mW range for SMS of allowed transitions at low temperatures – in fact powers below 100 nW are generally required. This is one reason why the SNR of the original data on single molecules of pentacene in p-terphenyl in Fig. 6 was only on the order of 5. (The other reason was the use of relatively thick cleaved samples, which produced a larger number of out-of-focus molecules in the probed volume. This problem has been overcome with much thinner samples in modern experiments.) If either materials with higher saturation intensity or squeezed light beams
1.1 Physical Principles and Methods of Single-Molecule Spectroscopy in Solids

Figure 6. The first single-molecule optical spectra, showing use of the FM/Stark technique for pentacene in p-terphenyl. (a) Simulation of absorption line with (power-broadened) linewidth of 65 MHz. (b) Simulation of FM spectrum for (a), $\omega_m = 75$ MHz. (c) Simulation of FM/Stark line-shape. (d) single-molecule spectra at 592.423 nm, 512 averages, 8 traces overlaid, bar shows value of $2\omega_m = 150$ MHz. (e) Average of traces in (d) with fit to the in-focus molecule (smooth curve). (f) Signal far off line at 597.514 nm. (g) Traces of SFS at the $O_2$ line center, 592.186 nm. After Ref. 1.

with reduced shot noise become easily available in the future, the utility of the FM method will improve.

Fluorescence excitation spectroscopy

In 1990, Orrit et al. also began experiments on the pentacene in p-terphenyl system and demonstrated that fluorescence excitation spectroscopy produces superior signals if the emission is collected efficiently and the scattering sources are minimized [3]. Most subsequent experiments have used this technique, in which a tunable narrowband single-frequency laser is scanned over the absorption profile of the single molecule, and the presence of absorption is detected by measuring the fluorescence emitted. A long-pass filter is used to block the pumping laser light, and the fluorescence shifted to long wavelengths is detected with a photon-counting system, usually a photomultiplier and discriminator. The detected photons generally cover a broad range of wavelengths, because the emission from the ground vibrational level of the electronically excited state terminates on various vibrationally excited (even) levels of the electronic ground state as shown in Fig. 1.

In fluorescence excitation, the detection is background-limited and the shot noise of the probing laser is only important for the signal-to-noise of the spectral feature, not the signal to background. For this reason, it is critical to efficiently collect photons (as with a paraboloid or other high numerical aperture collection system), and to reject the pumping laser radiation. To illustrate suppose a single molecule of pentacene in p-terphenyl is probed with 1 mW cm$^{-2}$, near the onset of saturation of the absorption due to triplet level population. The resulting incident photon flux of $3 \times 10^{15}$ photons s$^{-1}$ cm$^{-2}$ will produce about $3 \times 10^4$ excitations per second. With a fluorescence quantum yield of 0.8 for pentacene, about $2.4 \times 10^4$ emitted photons...
can be expected. At the same time, $3 \times 10^8$ photons/s illuminate the focal spot 3 μm in diameter. Considering that the resonant 0–0 fluorescence from the molecule must be thrown away along with the pumping light, rejection of the pumping radiation by a factor greater than $10^5$ to $10^6$ is generally required, with minimal attenuation of the fluorescence. This is often accomplished by low-fluorescence glass filters or by holographic notch attenuation filters.

The attainable signal-to-noise ratio (SNR) for single molecule detection in a solid using fluorescence excitation can be approximated by the following expression [71]:

$$\frac{S_1}{(\text{noise})_{\text{rms}}} = \frac{(D\phi_F\sigma_p P_0 \tau)/(A h\nu)}{\sqrt{(D\phi_F\sigma_p P_0 \tau)/(A h\nu) + C_b P_0 \tau + N_d \tau}}$$

where the numerator, $S_1$, is the peak detected fluorescence counts from one molecule in an integration time $\tau$, $\phi_F$ is the fluorescence quantum yield, $\sigma_p$ is the peak absorption cross section on resonance as defined above, $P_0$ is the laser power, $A$ is the focal spot area, $h\nu$ is the photon energy, $N_d$ is the dark count rate, and $C_b$ is the background count rate per Watt of excitation power. The factor $D = \eta_Q F_p F_f F_1$ describes the overall efficiency for the detection of emitted photons, where $\eta_Q$ is the photomultiplier tube quantum efficiency, $F_p$ is the fraction of the total emission solid angle collected by the collection optics, $F_f$ is the fraction of emitted fluorescence which passes through the long pass filter, and $F_1$ is the total transmission of the windows and additional optics along the way to the photomultiplier. The three noise terms in the denominator of Eq. 4 represent shot noise contributions from the emitted fluorescence, background, and dark signals, respectively. For a detailed discussion of the collection efficiency for a single molecule taking into account the dipole radiation pattern, total internal reflection, and the molecular orientation, see Ref. 60.

Assuming the collection efficiency $D$ is maximized, Eq. 4 shows that there are several physical parameters which must be chosen carefully in order to maximize the SNR. First, as stated above, the values of $\phi_F$ and $\sigma_p$ should be as large as possible, and the laser spot should be as small as possible. The power $P_0$ cannot be increased arbitrarily because saturation causes the peak absorption cross section to drop from its low-power value $\sigma_o$ according to [72]

$$\sigma_p \rightarrow \sigma_p(I) = \sigma_o/(1 + I/I_s)$$

where $I$ is the laser intensity and $I_s$ is the characteristic saturation intensity. The effect of saturation in general can be seen in both the peak on-resonance emission rate from the molecule $R(I)$ and in the single-molecule linewidth $\Delta \nu(I)$ according to [58]:

$$R(I) = R_\infty \left[ \frac{I/I_s}{1 + (I/I_s)} \right]$$

$$\Delta \nu(I) = \Delta \nu(0)[1 + (I/I_s)]^{1/2}$$
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Figure 7. Signal-to-noise ratio for fluorescence excitation of pentacene in $p$-terphenyl versus probing laser power and laser beam cross-sectional area. After Ref. 5.

The maximum emission rate is given by

$$R_\infty = \frac{(k_{21} + k_{isc})\phi_F}{2 + (k_{isc}/k_T)}$$

Eqs. 5 and 7 show that the integrated area under the single-molecule peak falls in the strong saturation regime. However, at higher and higher laser power, more and more scattering signal is produced in proportion to the laser power, so the difficulty of detecting a single molecule increases. The dependences of the maximum emission rate and linewidth on laser intensity in Eqs. 6 and 7 have been verified experimentally for individual single molecules [58].

To illustrate graphically the tradeoffs inherent in Eqs. 4 and 5, parameters for the model system pentacene in $p$-terphenyl will be used, for which $\phi_F = 0.78$ [57] and $D \approx 0.01$ in the lens-parabola geometry. Measured values for the background scattering level, dark count rate, and other parameters are given in Ref. 5. Eqs. (4) and (5) then yield a relationship between the SNR, $P_o$, and $A$. Assuming 1 s integration time, Fig. 7 shows the SNR versus laser power and beam area. It is clear that for a fixed laser spot size, an optimal power exists which maximizes the tradeoff between the saturating fluorescence signal and the linearly (with $P_o$) increasing background signal. For fixed spot size, the SNR at first improves, because the signal increases linearly with laser power and the shot noise from the power-dependent terms in the denominator only grow as the square root of the laser power. As saturation sets in, however, the SNR falls because the signal no longer increases. Another relationship illustrated in Fig. 7 is that the best-case SNR at smaller and smaller beam areas levels off (the flattening of the ridge at small beam area). This is due to the effect of saturation and shot noise – at smaller and smaller areas the power must be reduced eventually to the point where the SNR is controlled by the shot noise of the detected signal (first term in the denominator of Eq. 4). In any case, SNR values on the order of 20 with 1 s averaging time are quite useful for spectroscopic studies. However, more information about dynamical effects can be obtained if the SNR is increased further, which is one continuing challenge to the experimenters in this field. From another point of view, improvements in the SNR would allow probing at lower laser
another point of view, improvements in the SNR would allow probing at lower laser power so that materials with non-optimal photophysics (such as higher hole-burning quantum efficiency) may be studied.

To provide an example of specific experimental spectra using the fluorescence excitation method, we again turn to the model system of pentacene in p-terphenyl for simplicity. Fig. 8 shows fluorescence excitation spectra at 1.5 K for a 10 μm thick sublimed crystal of p-terphenyl doped with pentacene using the lens-paraboloid setup [7]. The 18 GHz spectrum in Fig. 8(a) (obtained by scanning a 3 MHz line-width dye laser over the entire inhomogeneous line) contains 20,000 points; to show all the fine structure usually requires several meters of linear space. The structures appearing to be spikes are not noise; all features shown are static and repeatable. Near the center of the inhomogeneous line, the statistical fine structure (SFS) characteristic of $N > 1$ is observed. It is immediately obvious that the inhomogeneous line is far from Gaussian in shape and that there are tails extending out many standard deviations from the center both to the red and to the blue. Fig. 8(b) shows an expanded region in the wing of the line. Each of the narrow peaks is the absorption profile of a single molecule. The peak heights vary due to the fact that the laser transverse intensity profile is bell-shaped and the molecules are not always located at the center of the laser focal spot. Even though these spectra seem narrow, they are in fact slightly power-broadened by the probing laser.

Upon close examination of an individual single-molecule peak at lower intensity (Fig. 8(c)), the lifetime-limited homogeneous linewidth of $7.8 \pm 0.2$ MHz can be observed [73]. This linewidth is also termed "quantum-limited", since the optical linewidth has reached the minimum value allowed by the lifetime of the optical excited state. This value is in excellent agreement with previous photon echo mea-

**Figure 8.** Fluorescence excitation spectra for pentacene in p-terphenyl at 1.5 K measured with a tunable dye laser of linewidth 3 MHz. The laser detuning frequency is referenced to the line center at 592.321 nm. (a) Broad scan of the inhomogeneously broadened line; all the sharp features are repeatable structure. (b) Expansion of 2 GHz spectral range showing several single molecules. (c) Low-power scan of a single molecule at 592.407 nm showing the lifetime-limited width of 7.8 MHz and a Lorentzian fit. After Ref. 7.
measurements using large ensembles of pentacene molecules [56, 57]. Such well-isolated, narrow single-molecule spectra in Fig. 8 are wonderful for the spectroscopist: many detailed spectroscopic studies of the local environment can be performed, because such narrow lines are much more sensitive to local perturbations than broad spectral features.

It is instructive at this point to compare the signal-to-noise ratio for SFS ($N \gg 1$) to that for one single molecule (Eq. 4). Defining $N_H$ as the number of molecules with resonance frequency within one homogeneous width of the laser frequency, and recalling that the SFS signal excursions scale as the square root of the number of molecules in resonance (see Fig. 3),

$$S_{SFS}^{\text{rms}} = \frac{\sqrt{N_H(S_1)}}{\sqrt{(N_H S_1) + C_b P_0 \tau + N_d \tau}} \approx \sqrt{D \phi_F \frac{(\sigma^2)}{A} \left( \frac{P_0 \tau}{h \nu} \right)}$$

where the last approximation assumes that background and dark counts are negligible. In this limit, the SNR is independent of the number of molecules in resonance. Therefore, when detection of SFS has been accomplished, the SNR at that point is a good estimate for the SNR for one single molecule, which degrades only when large background and dark counts are present. It is also interesting that the SNR for SFS scales as the inverse square root of the beam area. Of course, since fluorescence excitation is not a zero-background method, the SFS signal is still a small signal with a relative size $S_{SFS}/S_{TOTAL} = 1/(\sqrt{N_H})$ which must be detected on a large background, thus laser amplitude drifts and low frequency noise must be minimized in order to see the fine structure. (It is precisely this last point which stimulated the original SFS experimenters [46] to utilize FM spectroscopy.)

**Single-molecule imaging in frequency and space**

With the ability to record high-quality single-molecule absorption lineshapes such as those in Fig. 8, it becomes interesting to acquire spectra as a function of the position of the laser focal spot in the sample. Clearly, a single molecule should also be localized in space as well as absorption frequency. Using the lens–parabola geometry, the laser focal spot can be scanned over a small range in the transverse spatial dimension, and spectra can be obtained at each position. Fig. 9 shows such a three-dimensional “pseudo-image” of single molecules of pentacene in $p$-terphenyl [73]. The $\varepsilon$-axis of the image is the usual fluorescence excitation signal, the horizontal axis is the laser frequency detuning (300 MHz range), and the axis going into the page is the transverse spatial dimension produced by scanning the laser focal spot (40 $\mu$m range). There are three, large, clear single molecule peaks localized in both frequency and position at the center, upper left, and upper right. The resolution of this image in the spatial dimension is clearly limited by the 5 $\mu$m diameter laser spot; in fact, the single molecule is actually serving as a highly localized nanoprobe of the laser beam diameter itself. However, in the frequency dimension the features are fully resolved. It is clear that single-molecule peaks are localized in both frequency and space. Extensions of this concept to true diffraction-limited “fluorescence microscopy” using two
spatial dimensions will be described in Chapter 1.2, and extensions providing spatial resolution beyond the optical diffraction limit will be described in Chapter 2.

*Measurement of spectral trajectories of single molecules*

When a new regime is first opened for study, often new physical effects can be observed. In the course of the early SMS studies of pentacene in $p$-terphenyl, an unexpected phenomenon appeared: resonance frequency shifts of individual pentacene molecules in a crystal at 1.5 K [24], called “spectral diffusion” by analogy to similar shifting behavior long postulated for amorphous systems [74]. Here, spectral diffusion means changes in the center (resonance) frequency of a defect due to configurational changes in the nearby host which affect the frequency of the electronic transition via guest–host coupling. In the pentacene in $p$-terphenyl system, two distinct classes of single molecules were identified: class I, which have center frequencies that are stable in time like the three large molecules in Fig. 9, and class II, which showed spontaneous, discontinuous jumps in resonance frequency of 20–60 MHz on a 1–420 s time scale, an example of which is responsible for the distorted single-molecule peak in the center right region of Fig. 9.

Spectral shifts of single-molecule lineshapes are common in many systems, including crystals, polymers, and even polycrystalline Shpol’skii matrices [75, 76]. Spectral diffusion effects will be described in detail experimentally in Section 1.4, with theoretical analysis in Section 1.5. In this section and in the next, the principal experimental methods for studying this behavior will be described. Again taking pentacene in $p$-terphenyl as an example, Fig. 10(a) shows a sequence of fluorescence excitation spectra of a single molecule taken as fast as allowed by the available SNR. The laser was scanned once every 2.5 s with 0.25 s between scans, and the hopping of this molecule from one resonance frequency to another from time to time is clearly evident.
Figure 10. Examples of single-molecule spectral diffusion for pentacene in p-terphenyl at 1.5 K. (a) A series of fluorescence excitation spectra each 2.5 s long spaced by 2.75 s showing discontinuous shifts in resonance frequency, with zero detuning = 592.546 nm. (b) Trend or trajectory of the resonance frequency over a long time scale for the molecule in (a). (c) Resonance frequency trend for a different molecule at 592.582 nm at 1.5 K and at (d) 4.0 K. After Ref. 7.

One useful method for studying such behavior is the measurement of the spectral trajectory \( \omega_0(t) \) [24]. By sequentially acquiring hundreds to thousands of fluorescence excitation spectra and utilizing the power of digital processing to retain a record of the resonance frequency position of each such spectrum, a trajectory or trend of the resonance frequency versus time \( \omega_0(t) \) can be obtained as shown in Fig. 10(b) (for the same molecule as Fig. 10(a)). For this molecule, the optical transition energy appears to have a preferred set of values and performs spectral jumps between these values that are discontinuous on the 2.5 s time scale of the measurement. The behavior of another molecule is shown in Fig. 10(c) at 1.5 K and in Fig. 10(d) at 4.0 K. This molecule wanders in frequency space with many smaller jumps, and both the rate and range of spectral diffusion increase with temperature suggesting a phonon-driven process.

The first question which should be asked when such behavior is observed is this: is the effect spontaneous, occurring even in the absence of the probing laser radiation, or is it light-driven, i.e., produced by the probing laser itself. To answer this question, it is usually necessary to observe the spectral shifting behavior as a function of the

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**Figure 10.** Examples of single-molecule spectral diffusion for pentacene in p-terphenyl at 1.5 K. (a) A series of fluorescence excitation spectra each 2.5 s long spaced by 2.75 s showing discontinuous shifts in resonance frequency, with zero detuning = 592.546 nm. (b) Trend or trajectory of the resonance frequency over a long time scale for the molecule in (a). (c) Resonance frequency trend for a different molecule at 592.582 nm at 1.5 K and at (d) 4.0 K. After Ref. 7.

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probing laser power. In the case of the type II pentacene molecules in p-terphenyl, the spectral diffusion appeared to be a spontaneous process rather than a light-induced spectral hole-burning effect [73], but other materials have shown light-induced shifting behavior [25, 75, 80], which may be regarded as the single-molecule analog of the nonphotochemical spectral hole-burning process [77].

Since the optical absorption for pentacene in p-terphenyl is highly polarized [78] and the peak signal from the molecule does not decrease when the spectral jumps occur, it is unlikely that the molecule is changing orientation in the lattice. Since the resonance frequency of a single molecule in a solid is extremely sensitive to the local strain field, the conclusion from these observations is that the spectral jumps are due to internal dynamics of some configurational degrees of freedom in the surrounding lattice. The situation is analogous to that for amorphous systems, which are postulated to contain a multiplicity of local configurations that can be modeled by a collection of double-well potentials (the two-level system or TLS model [43]). The dynamics results from phonon-assisted tunneling or thermally activated barrier crossing in these potential wells. One possible source for the tunneling states [58] could be discrete torsional librations of the central phenyl ring of the nearby p-terphenyl molecules about the molecular axis. The p-terphenyl molecules in a domain wall between two twins or near lattice defects may have lowered barriers to such central-ring tunneling motions.

Spectral trajectories contain much information about the stochastic behavior of the single molecule. If a simple measure of the average time scale of spectral shifts is required, it is useful to calculate the autocorrelation of the spectral trajectory, $C_{\omega}(\tau)$, given by

$$C_{\omega}(\tau) = \int \omega(t)\omega(t+\tau)dt$$

which is the Fourier transform of the power spectral density of the frequency fluctuations. More complex measures can be computed, such as the survival probability for the resonance frequency to stay at its initial value, and examples of this type of analysis are presented in Section 1.5.

Although the measurement of the spectral trajectory in principle contains all the dynamical information about the system, there are practical limitations to the information that can be obtained. The principal shortcoming of the spectral trajectory measurement results from the time required to scan the absorption line with sufficient SNR to determine the resonance frequency. In many systems, this minimum scanning time is limited by the photon emission rate to times on the order of 10–100 ms, which means that dynamical behavior on faster time scales is not adequately represented. A partial solution to this problem is provided by direct time correlation measurements on the emission signal itself to be discussed in the next section. Nevertheless, the direct observations of the dynamics of a nanoenvironment of a single molecule by the spectral trajectory method have sparked fascinating new theoretical studies of the underlying microscopic mechanism [34, 35, 79], described in detail in Section 1.5. It is worth noting that such spectral trajectories cannot be obtained when a large ensemble of molecules is in resonance. The individual jumps are gen-
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Figure 11. Schematic of the temporal behavior of photon emission from a single molecule showing bunching on the scale of the triplet lifetime (upper half) and antibunching on the scale of the inverse of the Rabi frequency (lower half).

Erally uncorrelated, thus the behavior of an ensemble-averaged quantity such as a spectral hole would only be a broadening and smearing of the line.

Time correlation of single-molecule emission signal

A useful route to single-molecule information on shorter time scales lies in the actual photon emission process itself. Let us assume that the pumping laser frequency is held fixed in resonance with the single molecule. The stream of photons emitted by the molecule contains information about the system encoded in the arrival times of the individual photons. Fig. 11 schematically shows the time-domain behavior of the photon stream for a single molecule with a dark triplet state, here taken to be pentacene for definiteness (see Fig. 1). While cycling through the singlet states $S_0 \rightarrow S_1 \rightarrow S_0$, photons are emitted until intersystem crossing occurs. Since the triplet yield is $0.5\%$ [57], 200 photons are emitted on average before a dark period which has an average length equal to the triplet lifetime, $\tau_T$. This causes "bunching" of the emitted photons as shown in the upper half of the figure. Even though the actual length of time in the triplet state is a random variable with an exponential distribution, the bunching can be easily detected by measuring the autocorrelation of the photon emission signal $S(t)$, which is defined by

$$C_S(\tau) = \int S(t)S(t+\tau)dt$$

(11)

Autocorrelation analysis has long been recognized as a useful method for statistical study of stochastic dynamical processes which may be obscured by noise [81, 82]. By definition, the autocorrelation measures the similarity (overlap) between the function and a copy of the function delayed in time by a lag time $\tau$. Because the noise component of the signal is uncorrelated, its contribution to $C_S(\tau)$ decays quickly, leaving information about the (average) time-domain correlations of $S(t)$. In practice, when photon counting is used, a commercial digital correlator is employed to measure $C_S(\tau)$ by keeping track of the arrival times of many photon pairs. Modern correlators can do this over a huge logarithmic time scale covering many decades in time, a feature that is very useful for studying the dispersive dynamics characteristic of amorphous systems.

The decay in the autocorrelation of the emitted photons for pentacene in $p$-terphenyl due to the triplet bottleneck was first reported by Orrit et al. [3], and this
method has been used to measure the changes in the triplet yield and triplet lifetime from molecule to molecule [26] which occur as a result of distortions of the molecule by the local nanoenvironment. For single molecules in polymers with complex dynamics driven by TLS’s in the host matrix, correlation measurements are also quite useful [27, 83]. In this case, the amplitude fluctuations in the single-molecule fluorescence signal resulting from shifts of the resonance frequency can produce a characteristic fall-off in the autocorrelation which yields information about the TLS flipping rate distribution. Detailed examples of the use of this method to study spectral diffusion will be presented in Section 1.4.

Correlation measurements can extract information about the single molecule on much shorter time scales (down to the μs range) than the frequency scans described in the previous section. At the same time, however, the dynamical process must be stationary, that is, the dynamics must not change during the relatively long time (many s) needed to record enough photon arrivals to generate a valid autocorrelation. In addition, since the laser frequency is held fixed, when the molecule hops out of resonance with the laser, all information about the new resonance frequency of the single molecule is lost.

Turning now to the nanosecond time regime (lower half of Fig. 11), the emitted photons from a single molecule can provide still more useful information. On the time scale of the excited state lifetime, the statistics of photon emission from a single quantum system are expected [84] to show photon antibunching, which means that the photons “space themselves out in time”, that is, the probability for two photons to arrive at the detector at the same time is small. This is a uniquely quantum-mechanical effect, which was first observed for Na atoms in a low-density beam [85].

Antibunching is fundamentally measured by computing the second-order correlation of the electric field $g^{(2)}(\tau)$ (which is simply the normalized form of the intensity-intensity correlation function $C_{\gamma}(\tau)$), which shows a drop below the uncorrelated value of unity when antibunching is present [86]. For a single molecule, antibunching is easy to understand as follows. After photon emission, the molecule is definitely in the ground state and cannot emit a second photon immediately. A time on the order of the inverse of the Rabi frequency $\chi^{-1}$ must elapse before the probability of emission of a second photon is appreciable. At sufficiently high laser intensity, Rabi oscillations can be observed as the laser coherently drives the single molecule into and out of the excited state before emission occurs.

In actual practice, in order to overcome time limitations caused by the dead time of photomultipliers, two identical detectors are used to measure the distribution of time delays between the arrival times of consecutive pairs of emitted photons as in the classical Hanbury–Brown–Twiss experiment [86], which is described in more detail in Section 1.2. The expected antibunching in single-molecule emission was first observed in the author’s laboratory for pentacene in p-terphenyl [28], demonstrating that quantum optics experiments can be performed in solids and for molecules for the first time. Of course, if more than one molecule is emitting, the antibunching effect as well as the bunching effect both quickly disappear since the various resonant molecules emit independently. The observation of high-contrast antibunching is strong proof that the spectral features are indeed those of single molecules. Careful
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1.1.3.3 Materials systems and structures

This section briefly lists the materials systems in which high-resolution, low-temperature SMS studies have been performed, to be described in more detail in the remainder of Part I of this book. To date, the guest impurity molecules have been selected exclusively from the class of rigid conjugated hydrocarbons with specific cases shown in Fig. 12: (a) pentacene, (b) perylene, (c) terrylene, (d) tetra-(t-butyl)terrylene (TBT), (e) diphenyloctatetraene (DPOT), (f) 7,8,15,16-dibenzoterrylene (DBT), and (g) 2,3,8,9-dibenzanthanthrene (DBATT). These molecules have strong singlet–singlet absorption, excellent emission properties, and weak triplet bottlenecks. They also feature the weak Franck–Condon distortion necessary to guarantee a strong (0–0) electronic transition. In most cases, workers have concentrated on the fairly large aromatic hydrocarbons (AHCs) (a)–(d), (f), (g) in order to place the lowest electronic transition in the mid-visible. This allows standard tunable single-frequency dye lasers to be utilized for pumping the transition. The one exception, DPOT (e), represents a special case which was pumped either with two-photon excitation in the near IR [88], or with doubled light at 444 nm from the output of a cw Ti-sapphire laser.

The choice of the host material is generally dictated by the need to maintain a weak electron–phonon coupling and to prevent high-efficiency spectral hole-burning. The latter requirement has so far prevented hydrogen-bonded matrices from being suc-...
I Low-Temperature Studies in Solids

Table 1. Host–guest combinations studied by single-molecule spectroscopy^

<table>
<thead>
<tr>
<th>Class</th>
<th>Guest</th>
<th>Host</th>
<th>ZPL Wavelength (nm)</th>
<th>Linewidth (MHz)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHC: single crystals</td>
<td>pentacene</td>
<td>p-terphenyl</td>
<td>592.32 (O₁), 592.18 (O₂)</td>
<td>7.8 (O₁)</td>
<td>[1, 3, 24, 58]</td>
</tr>
<tr>
<td></td>
<td>naphtalene</td>
<td>602.8</td>
<td>29</td>
<td></td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>terrylene</td>
<td>p-terphenyl</td>
<td>580.4 (X₁), 578.5 (X₂), 578.3 (X₃), 577.9 (X₄)</td>
<td>48.1 (X₂)</td>
<td>[90]</td>
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<tr>
<td>AHC: polymers</td>
<td>DBT</td>
<td>PE</td>
<td>757.7</td>
<td>25-35</td>
<td>[92]</td>
</tr>
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<td></td>
<td>perylene</td>
<td>PE</td>
<td>~442</td>
<td>52-142</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>terrylene</td>
<td>PE</td>
<td>569</td>
<td>60-150</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>PVB</td>
<td>562</td>
<td>200-2000</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PMMA</td>
<td>557</td>
<td>200-2200</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PS</td>
<td>566</td>
<td>600-4000</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>AHC: Shpol'skii matrices</td>
<td>TBT</td>
<td>PE</td>
<td>~ -</td>
<td>50-320</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>DBT</td>
<td>PIB</td>
<td>567.6</td>
<td>40-370</td>
<td>[93]</td>
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<td>hexadecane</td>
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<td>40</td>
<td>[75, 76]</td>
</tr>
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<td>nonane</td>
<td>~440</td>
<td>-</td>
<td>[94]</td>
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<td></td>
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<td>tetradecane</td>
<td>444 (1-photon)</td>
<td>30</td>
<td>[88]</td>
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<td></td>
<td>DBATT</td>
<td>hexadecane</td>
<td>589.1</td>
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<td>[95]</td>
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^
For abbreviations, see text.

cessfully utilized for high-resolution, low-temperature SMS. The actual host–guest combinations fall into three categories: AHCs in crystals, AHCs in polymers, and AHCs in Shpol’skii matrices. Table 1 lists a variety of host–guest combinations that have been studied, along with the position of the ZPL, the single-molecule linewidth (FWHM, generally at ~2K), and leading references. The favorite single crystal hosts have been p-terphenyl, naphtalene, and anthracene, all of which can be sublimed to form clear micron-thick samples. Generally, single molecules in these hosts are relatively stable, with occasional spontaneous spectral diffusion driven by defects in the host crystal (see Sections 1.4 and 1.5). The polymer hosts have been selected from poly(ethylene) (PE), poly(i-butylene) (PIB), poly(vinylbutyral) (PVB), poly(styrene) (PS), and poly(methyl methacrylate) (PMMA). These materials are characterized by highly-dispersive spectral shifting phenomena, both spontaneous and light-driven. In Table 1, a range of linewidths indicates a distribution, and the original references should be consulted for more detail on the shape of this distribution. Finally, various Shpol’skii matrices such as frozen hexadecane, nonane, and tetradecane have also been examined. These convenient polycrystalline hosts yield relatively stable single-molecule spectra, with a slow, light-driven spectral shifting observed in several cases.

To date, the total number of systems which have been studied by high resolution
SMS techniques is approximately 16. Work is in progress on new materials, such as other AHCs and additional Shpol'skii matrices. It is to be expected that some laser dye molecules in appropriate hosts will also have the strong fluorescence, weak triplet bottlenecks, and near-absence of spectral hole-burning required for SMS studies.

1.1.4 Summary and outlook

Application of the concepts described in this chapter have formed the basis for many of the fascinating experiments which have been performed with single molecules in condensed matter, the description of which occupies the remainder of Chapter 1.

The attainment of SMS in solids opens up a new frontier of single-absorber experiments in which the measured properties of the absorbing center are not averaged over many "equivalent" absorbers. The significance of such experiments is fourfold. First, the properties of a single absorber are measured without ensemble averaging, which means that tests of specific theoretical models are much stronger. Second, the sensitivity to specific properties of the nanoenvironment such as the local phonon modes and the true local fields is extremely high. This means for example that the identity of the mysterious two-level systems in amorphous materials may finally be determined. Third, it provides a window into the spectral hole-burning process on a molecule by molecule basis. Thus, the exact local coupling through which optical pumping of a single molecule gives rise to changes in the nanoenvironment which shift the resonance frequency may be studied. Fourth, this regime is essentially unexplored, which means that surprises and unexpected physical effects can occur (such as the observation of spectral diffusion in a crystal).

While as a general technique high-resolution SMS is not applicable to all molecular impurities, it can be applied to the large number of absorbing molecules (and perhaps ions) in solids that have zero-phonon transitions, reasonable absorption strength, and efficient fluorescence. The detectability of the resulting single-center signal, which ultimately depends upon the specific sample and weak or absent spectral hole-burning, must be evaluated in each case. SMS signals should be observable at higher and higher temperatures if the concentration and background are both reduced sufficiently. One successful method for doing this at room temperature has been to use near-field excitation to reduce the scattering volume and increase the single-center signal, to be described in Chapter 2.

One experimental technique that has only recently been utilized for SMS is two-photon fluorescence excitation. In this approach, a long-wavelength source is used to pump the 0–0 transition via two-photon absorption, and the emission is collected as usual. Since two-photon cross sections are much smaller than one-photon cross sections for an electric dipole-allowed transition, high pumping power in the 100 mW to 1 W range is required. This difficulty is offset by the ease with which the pump radiation is separated from the emission. First results for Rhodamine B in water at room temperature have recently been reported [96] using a high-peak-power pulsed laser. At low temperature, the narrowing of the optical absorption yields a huge increase in the two-photon cross section, and pumping with a cw laser has recently produced two-photon optical spectra of single molecules of diphenyloctatetraene in n-tetradecane [88].
Other fascinating future experiments may be contemplated based on the principles and methods presented here. Detailed study of the spectral diffusion process in crystals and polymers will help to eventually identify the actual microscopic nature of the two-level systems. The door is open to true photochemical experiments on single absorbers, quantum optics, and even the possibility of optical storage using single molecules [7]. Future efforts to increase the number of probe-host couples which allow SMS will lead to an even larger array of novel observations.

One experiment that is now possible would be to use the emission from a single molecule as a light source of sub-nm dimensions for near-field optical microscopy [97]. Of course, this would involve the technical difficulty of placement of the single emitting molecule at the end of a pulled fiber tip or pipette. In all cases, improvements in SNR would be expected to open up a new level of nanoscopic detail and possibly new applications. Because this field is relatively new, the possibilities are only limited at present by the imagination and the persistence of the experimenter and the continuing scientific interest in the properties of single quantum systems in solids.

References

References


1 Low-Temperature Studies in Solids


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[95] A.-M. Boiron, B. Lounis, and M. Orrit, in press.
