

ARTICLES

High-Resolution Optical Spectroscopy of Single Molecules in Solids

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1. Introduction. Why Study Single Molecules in Solids?

In recent years, powerful new experimental methods have been developed to allow *exactly one* molecule hidden deep within a solid sample to be probed at a time by tunable laser radiation. This achievement represents detection and spectroscopy at the ultimate sensitivity level of 1.66×10^{-24} mol of material, or 1.66 ymol.¹ 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").^{2–13}

Optical experiments at the single-molecule level are currently 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. This is because single-molecule measurements directly measure the distribution of behavior, rather than only its moments. On the theoretical side, since no ensemble averaging need be done before computing an observable quantity, stronger tests of microscopic dynamical theories can be completed.^{14–16} Third, since the single-molecule regime is previously unexplored, new physical and chemical behavior is likely to be observed, and a few examples of this will be described below. The reader may consult one of several recent reviews for more information.^{7–13}

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.^{17–19} The trap experiments are performed in vacuum, and the atoms must be laser-cooled, a method that has not been applicable so far to molecules due to rotation. In SMS, however, the solid acts as a trap, preventing rotation,

but the surrounding solid represents a potential source of background signals. In addition, in SMS the single molecule is bathed in the phonon spectrum available at the measurement temperature. Useful comparison may also be made to another important field, the direct probing of single atoms or molecules on surfaces with scanning tunneling microscopy (STM)²⁰ or atomic force microscopy (AFM).^{21,22} The spatial resolution of SMS is generally poorer, on the order of the optical wavelength (1 μm) unless near-field optical methods are used.^{23–28} In the tunneling spectroscopies, however, the molecule or atom must be on the surface and

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(1) The "yocto" (y) prefix was selected by IUPAC to signify 10^{-24} . 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 avocadro's number. (with apologies to Amadeo Avogadro).

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W. E. Moerner was born on June 24, 1953, in Pleasanton, CA. After receiving three Bachelor's degrees in 1975 from Washington University, St. Louis, he obtained M.S. (1978) and Ph.D. (1981) degrees in physics from Cornell University on the optical properties of molecular impurities in solids. From 1981 until 1995, he was Research Staff Member and Manager at the IBM Almaden Research Center, San Jose, CA, where he worked on spectral hole-burning, photorefractive polymers, and single-molecule spectroscopy. In 1995, he became Distinguished Professor of Physical Chemistry at the University of California, San Diego. Current major interests include optical materials and the development of single-molecule probes of local environments.

must be able to tolerate the tip forces and/or tunneling currents. By contrast, SMS often utilizes the optical wavelength to achieve additional selectivity, and single molecules can be studied below the surface in the body of the sample.

Although single molecules have been optically detected and studied both at liquid helium temperatures and at room temperature, this Account concentrates primarily on the former, which is the regime in which high-resolution spectroscopy can be performed.

2. Basic Principles of Single-Molecule Spectroscopy

To put it simply, to achieve SMS at any temperature, one must (a) guarantee that only one molecule is in resonance in the volume probed by the laser and (b) provide a signal-to-noise ratio greater than one for a reasonable averaging time.

To proceed from 1 mol of material (6.02×10^{23} molecules) to only one molecule in resonance, many orders of magnitude must be spanned. By focusing to small spots near the diffraction limit of a few micrometers in diameter, and by using small sample thicknesses (3–10 μm), 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 11–12 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} mol/mol or lower, and to be very sure that no other unwanted impurity in the probed volume (or the host itself) is capable of producing an interfering signal. However, for high-resolution SMS at low temperatures, 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 7–9 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 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.^{29–32}

2.1. Spectral Selection Using Zero-Phonon Lines and Inhomogeneous Broadening. Inhomogeneous broadening is an important physical effect which facilitates the detection and spectroscopy of single molecules in solids at low temperatures. This

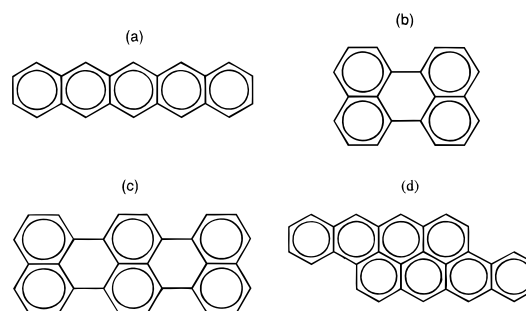


Figure 1. Structures of some of the molecules which have been studied by high-resolution SMS: (a) pentacene, (b) perylene, (c) terrylene, (d) 2,3:8,9-dibenzanthanthrene.³³

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 (0–0) transition often has a very long lifetime, because the de-excitation to the ground state manifold requires a large number of phonons to be emitted, an improbable event. At temperatures of a few kelvin, such a transition can have a lifetime-limited absorption width of tens to a few hundred megahertz (for an electric-dipole-allowed transition in the visible, even smaller for a partially forbidden transition). In addition, it is important to ensure that a large fraction of the oscillator strength of the transition is in the (0–0) component. For this reason, the best molecules for high-resolution SMS are large, flat, rigid molecules with weak electron–phonon coupling and small Franck–Condon distortion. Examples of some aromatic hydrocarbons which have been studied with high-resolution SMS are shown in Figure 1.

As is well-known, a ZPL transition can only dephase and broaden by second-order coupling to the phonons, that is, by phonon scattering (two-phonon processes).³⁴ At liquid helium temperatures, few phonons are present, so the homogeneous width γ_H of zero-phonon optical transitions in crystals approaches the lifetime-limited value. Since the optical transition frequency is near 500 THz, the Q or quality factor of such a narrow transition can be as large as 10^8 . In amorphous materials, other low-frequency excitations arising from two-level systems³⁵ are present, and the homogeneous width is somewhat larger,³⁶ 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 to the optical absorption for a 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

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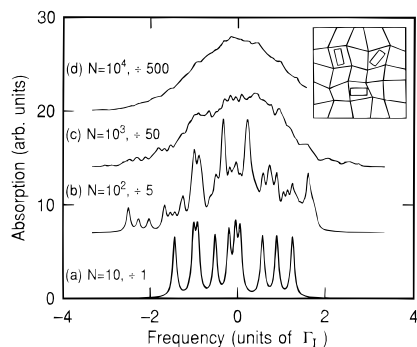


Figure 2. Simulated absorption spectra with different total numbers of absorbers N , using a Gaussian random variable to select center frequencies for the inhomogeneous distribution. Traces a–d correspond to N values of 10, 100, 1000, and 10 000, respectively, and the traces have been divided by the factors shown. For clarity, $\gamma_H = \Gamma_I/10$. Inset: several guest molecules are sketched as rectangles with different local environments produced by strains, local electric fields, and other imperfections in the host matrix.

Lorentzian³⁷ line of full width at half-maximum γ_H . However, these conditions are seldom met in actual samples. The optical absorption spectrum that is actually measured is a broad profile of width Γ_I , termed an inhomogeneously broadened line,^{29,30} and Γ_I can be 10^3 to 10^5 times larger than γ_H . Figure 2 shows a sequence of simulated inhomogeneous line-shapes for different numbers of molecules N for the simple case of $\Gamma_I/\gamma_H = 10$. The broad profile results from a distribution of resonance frequencies for the individual guests 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. Inhomogeneous broadening is a universal feature of high-resolution laser spectroscopy of guest molecules or ions in solids,^{31,32} and of other cases where ZPLs are probed such as Mössbauer and magnetic resonance spectroscopy. Historically, to overcome inhomogeneous broadening, methods such as spectral hole-burning³⁸ and coherent transients³⁹ have been utilized to learn about the homogeneous line width hidden under such broad profiles.

For our purposes, inhomogeneous broadening actually 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 narrow-band 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 γ_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 Γ_I , or (c) by tuning out into the wings of the inhomogeneous line. A useful analogy⁴⁰ is provided by 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, one hears mostly static (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 γ_H on average, single molecules can be pumped selectively, one at a time, simply by tuning the laser, as in Figure 2a.

2.2. Detection Methods and Signal-to-Noise Ratio Considerations. The first SMS experiments in 1989 utilized either of two powerful double-modulation absorption techniques, laser frequency modulation with Stark secondary modulation (FM-Stark) or frequency modulation with ultrasonic strain secondary modulation (FM-US).^{2,3} In contrast to fluorescence methods, Rayleigh scattering and Raman scattering were unimportant. While successful, these methods were limited by the quantum shot noise of the laser beam, which is relatively large at the low laser intensity required to prevent saturation of the single-molecule absorption. Subsequent work has concentrated on fluorescence excitation, an approach first utilized for single molecules in solids in 1990 by Orrit et al.⁴ In fluorescence excitation, a tunable narrow-band 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 from the tunable pump is detected with a photon-counting system.

In fluorescence excitation, the detection is usually background-limited and the noise of the probing laser is only important for the signal-to-noise ratio of the spectral feature, not the signal-to-background ratio. 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/cm², near the onset of saturation of the absorption due to triplet level population. The resulting incident photon flux of 3×10^{15} photons/(s cm²) will produce about 3×10^4 excitations per second. With a fluorescence quantum yield of 0.8 for pentacene, about 2.4×10^4 emitted photons can be expected. At the same time, 3×10^8 photons/s illuminate the focal spot 3 μ m 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. In very recent work, two-photon pumping of the single-molecule fluorescence has been used to greatly simplify rejection of the pumping light.⁴¹

2.2.1. Experimental Configurations for Fluorescence Excitation. One useful experimental setup is shown in Figure 3a, the “lens-parabola” configuration. All the components shown can be immersed in

(37) A normalized Lorentzian absorption profile centered at ω_0 with full width at half-maximum γ_H is $(\gamma_H/2\pi)[(\omega - \omega_0)^2 + (\gamma_H/2)^2]^{-1}$.

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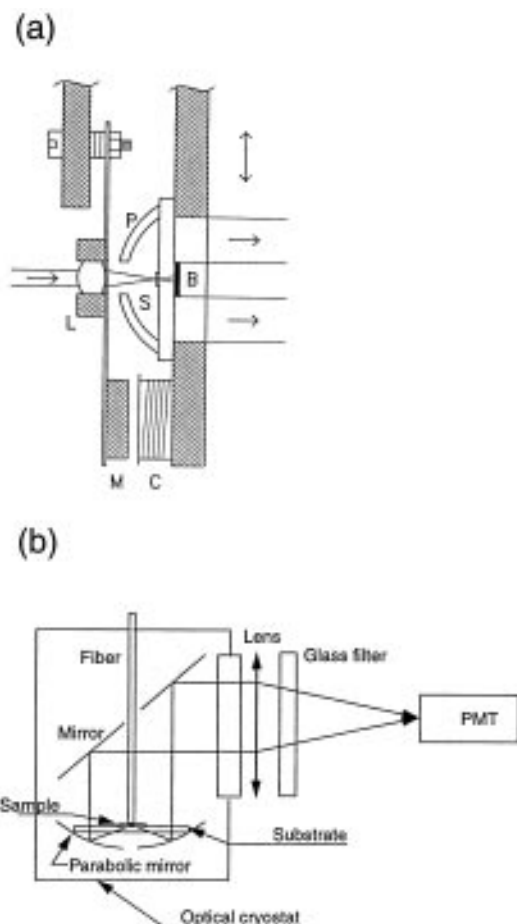


Figure 3. Examples of experimental arrangements for fluorescence excitation SMS: (a) lens-paraboloid (Reprinted with permission from ref 42. Copyright 1991 American Institute of Physics), L = lens, P = paraboloid, S = sample, B = beam block, M = magnet, C = coil electromagnet, (b) fiber-paraboloid (first used in ref 4).

superfluid helium. The focal spot from the pumping laser is produced by a small lens of several millimeters focal length. Generally, some provision must be made for adjusting the focus at low temperatures. In one solution to this,⁴² the lens is mounted on a thin stainless steel plate, which can be flexed by a permanent magnet (M)/electromagnet (C) pair to adjust the focal position. After passage through the sample mounted on a transparent substrate, 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.

A second useful configuration⁴ (termed "fiber-parabola") takes advantage of the small spot size produced automatically by the core of a single mode optical fiber as shown in Figure 3b. 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.

Another successful experimental configuration in-

volves the use of a small pinhole aperture⁴³ in a thin metal plate. If a high-precision paraboloidal mirror is available, it may be used for both focusing pump radiation and collecting emission.⁴⁴

2.2.2. Factors Contributing to the Signal-to-Noise Ratio. The detectability of the single-molecule signal is all-important. Some factors contributing to the signal-to-noise ratio will now be mentioned.⁴⁵ Since fluorescence is the primary detected quantity, the quantum yield for photon emission per absorption event ϕ_f should clearly be high, as close to unity as possible. Extreme care must 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 crucial requirement on the guest results from the photophysics of optical absorption in solids. The probability that a single molecule will absorb a photon from the incident beam is simply σ_p/A , where σ_p is the peak absorption cross section per molecule (cm^2) and A is the area of the probing laser beam (cm^2). The goal is to efficiently produce absorption events with the incident photons rather than unwanted scattering signals; thus, σ_p should be as large as possible and A as small as possible. Since σ_p depends linearly upon the oscillator strength and inversely upon the optical homogeneous line width γ_H , strong absorptions and narrow lines give the largest σ_p values. Strong absorptions are conveniently provided by electric-dipole-allowed singlet-singlet transitions in aromatic molecules where the oscillator strength of the lowest electronic transition is often near unity. Expressions for estimating σ_p are given in refs 10 and 45.

For molecules like pentacene, perylene, or similar rigid aromatics shown in Figure 1 with a strongly allowed lowest electronic transition, the net result is that at low temperatures where the dephasing time T_2 is large (tens of nanoseconds), the peak absorption cross section increases to levels as high as 10^{-11} 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 single molecule can be made to absorb light quite efficiently.

A further requirement on the absorption properties of the guest molecule stems from the general fact that although higher laser power generally produces more and more signal, saturation of the transition should be avoided. When saturation occurs, further increases in laser power generate more background rather than signal. Generally, saturation causes the peak absorption cross section to drop from its low-power value σ_0 according to⁴⁶ $\sigma_p(I) = \sigma_0/(1 + I/I_S)$, where I is the laser intensity and I_S is the characteristic saturation intensity.⁴⁵ The saturation intensity is maximized when absorbing centers are chosen that do not have strong bottlenecks in the optical pumping cycle. In organic molecules, intersystem crossing (ISC) from the singlet

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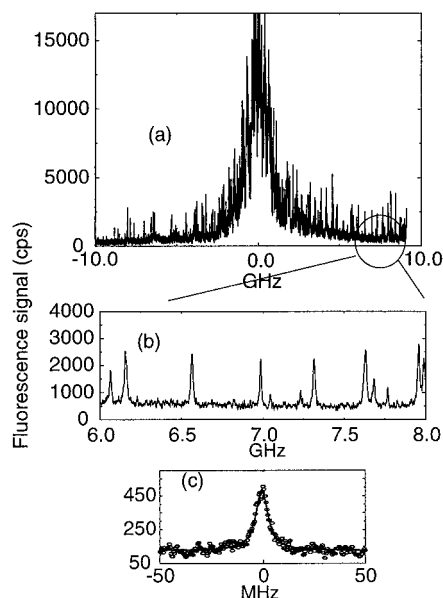


Figure 4. Fluorescence excitation spectra for pentacene in *p*-terphenyl at 1.5 K measured with a tunable dye laser of line width 3 MHz. The laser detuning frequency is referenced to the line center at 592.321 nm. (a) Broad scan of the inhomogeneously broadened line; all the sharp features are repeatable structure. (b) Expansion of the 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. Reprinted with permission from ref 9. Copyright 1994 American Association for the Advancement of Science.

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. Minimizing the triplet bottleneck means small values of the intersystem crossing rate k_{ISC} and large values of the triplet decay rate k_T , requirements which may be easily satisfied for rigid, planar aromatic hydrocarbons and laser dyes.

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.³⁸ For example, most fluorescence detection schemes with an overall photon collection efficiency of 0.1–1% 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 requirements stated in this section represent a “best case” in order to produce the highest possible signal-to-noise ratio in a high-resolution experiment. If some loss in signal-to-noise ratio or spectral resolution can be tolerated, these requirements can be weakened accordingly.

3. Experimental Examples

3.1. High-Resolution Optical Spectra of Single Molecules. To provide an example of specific experimental results, we turn to the model system of pentacene in *p*-terphenyl. Figure 4 shows fluorescence

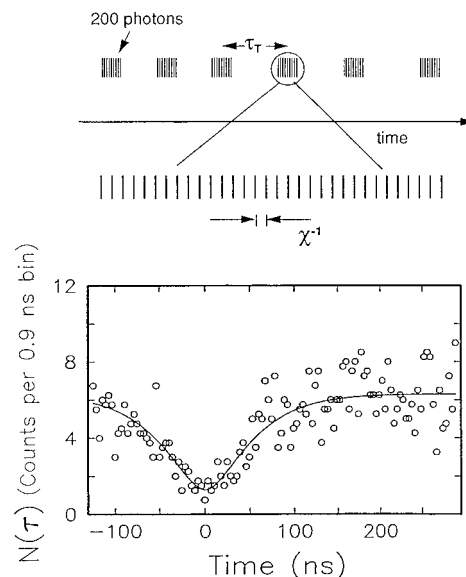


Figure 5. Schematic of the temporal behavior of photon emission from a single molecule showing bunching on the scale of the triplet lifetime (top) and antibunching on the scale of the inverse of the Rabi frequency (middle). Bottom: measured distribution of time delays between successive photons for a single molecule of pentacene in *p*-terphenyl showing antibunching at $t = 0$.⁵⁶

excitation spectra taken with the lens-paraboloid setup at 1.5 K for a 10 μm thick sublimed crystal of *p*-terphenyl doped with 10^{-9} mol/mol pentacene.⁴² The 18 GHz spectrum in Figure 4a (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 structure⁴⁷ characteristic of $N > 1$ is observed. Figure 4b 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 (Figure 4c), the lifetime-limited homogeneous line width of 7.8 ± 0.2 MHz can be observed.⁴⁸ This line width has reached the minimum value allowed by the lifetime of the S_1 excited state of 24 ns in agreement with photon echo measurements on large ensembles.^{49,50} Such well-isolated, narrow single-molecule spectra are wonderful for the spectroscopist: many detailed studies of the local environment can be performed, because such narrow lines are much more sensitive to local perturbations than broad spectral features are.

3.2. Correlation Properties of Single-Molecule Emission. Photon Antibunching. The stream of photons emitted by a single molecule contains information about the system encoded in the arrival times

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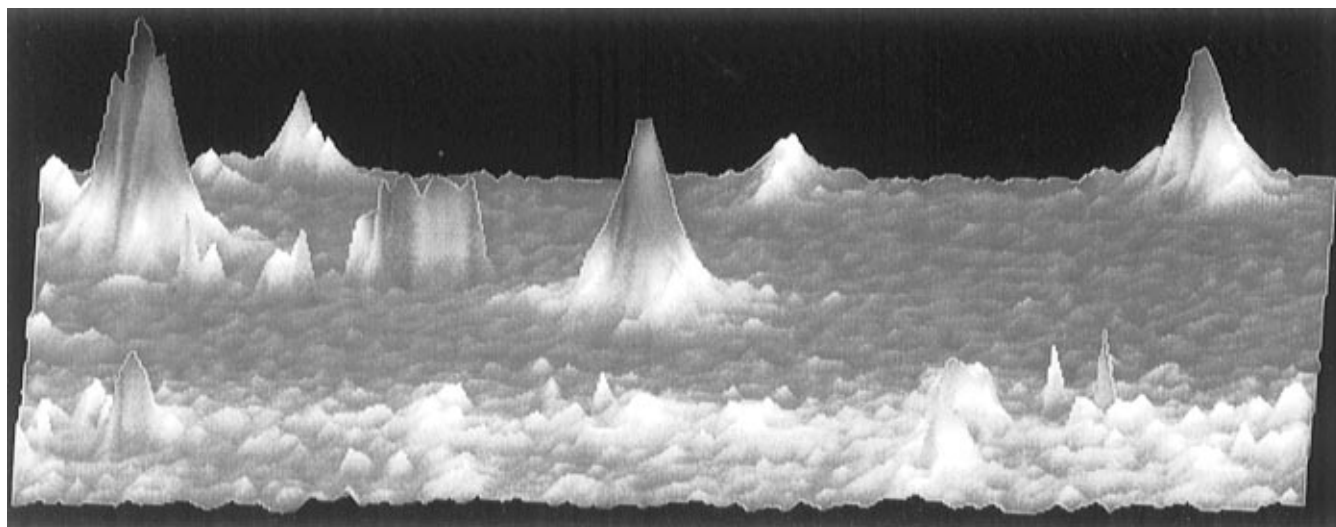


Figure 6. Three-dimensional pseudoimages of single molecules of pentacene in *p*-terphenyl. The measured fluorescence signal (*z*-axis) is shown over a range of 300 MHz in excitation frequency (horizontal axis, center 592.544 nm) and 40 μm in spatial position (axis into the page).

of the individual photons. Figure 5, top, schematically shows the time-domain behavior of the photon stream for a single molecule with a dark triplet state, here taken to be pentacene. 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%, on average 200 photons are emitted in a “bunch” before a dark period which has an average length equal to the triplet lifetime, τ_T . The corresponding decay in the autocorrelation function of the emitted photons for pentacene in *p*-terphenyl is easily observed,⁴ and this phenomenon has been used to measure the changes in the triplet yield and triplet lifetime from molecule to molecule⁵¹ which occur as a result of distortions of the molecule by the local nanoenvironment. Such correlation measurements can also extract information on wide time scales about the spectral shifting behavior which occurs in amorphous systems⁵² (see section 3.4). 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, yielding information about the two-level system (TLS) flipping rate distribution.^{52,53} For this to be valid, however, the dynamical process must be stationary; that is, the dynamics must not change during the relatively long time (many seconds) needed to record enough photon arrivals to generate a valid autocorrelation.

By contrast, in the nanosecond time regime within a single bunch (Figure 5, middle), the emitted photons from a single quantum system are expected to show 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,⁵⁴ which was first observed for single Na atoms

in a low-density beam.⁵⁵ 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 χ^{-1} must elapse before the probability of emission of a second photon is appreciable.

To observe antibunching correlations, the second-order correlation function $g^{(2)}(\tau)$ is generally measured by determining the distribution of time delay $N(\tau)$ between the arrival of successive photons in a dual-beam detector. The antibunching in single-molecule emission was first observed for the pentacene in *p*-terphenyl model system,⁵⁶ demonstrating that quantum optics experiments can be performed in solids and on molecules for the first time (Figure 5, bottom). Of course, if more than one molecule is emitting, the antibunching effect and the bunching effect both quickly disappear since the molecules emit independently. The observation of high-contrast antibunching is strong proof that the spectral features are indeed those of single molecules. Careful study of the shape of $g^{(2)}(\tau)$ in the nanosecond time regime has been used to determine both the excited state lifetime T_1 and T_2 for a single terrylene molecule in *p*-terphenyl.⁵⁷ Other quantum-optical experiments, such as measurement of the AC Stark shift,⁵⁸ have also been performed. The convenient “trap” that the solid forms for a single molecule at low temperatures will allow further studies of the interactions of single molecules with the quantum radiation field.

3.3. Single-Molecule Imaging in Frequency and Space. With the ability to record high-quality single-molecule absorption line shapes such as those in Figure 4, it becomes useful to acquire spectra as a function of the position of the laser focal spot in the sample. A single molecule should also be localized in space as well as in resonance frequency. Using the

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lens-parabola geometry, the laser focal spot can be scanned over a small range in the transverse spatial dimension, and fluorescence excitation spectra can be obtained at each position. Figure 6 shows such a three-dimensional "pseudoimage" of single molecules of pentacene in *p*-terphenyl.⁴⁸ The *z*-axis of the image is the emission signal, the horizontal axis is the laser frequency detuning (300 MHz range), and the axis going into the page is one transverse spatial dimension produced by scanning the laser focal spot (40 μm 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 μm 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. The extension of this concept to true diffraction-limited "fluorescence microscopy" using two spatial dimensions has been demonstrated,^{59,60} which allows many single molecules to be followed simultaneously.

3.4. Spectral Shifting Behavior 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 on pentacene in *p*-terphenyl, an unexpected phenomenon appeared: resonance frequency shifts of individual pentacene molecules in a crystal at 1.5 K,⁶¹ called "spectral diffusion" by analogy to similar shifting behavior long postulated for amorphous systems⁶² (although this behavior is not diffusion in the strict sense, i.e., it is not governed by a diffusion equation). Here, spectral diffusion means changes in the center (resonance) frequency of a guest 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 Figure 6, 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 left region of Figure 6; this molecule is shifting during the measurement. Figure 7a shows a sequence of fluorescence excitation spectra of a single pentacene 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 scan to scan is clearly evident.

Spectral shifting can be studied by (a) recording the observed line shapes for many single molecules,^{16,63} (b) autocorrelation,⁵² and (c) measurement of the spectral trajectory $\omega_o(t)$.⁶¹ To record $\omega_o(t)$, one analyzes many sequentially acquired fluorescence excitation spectra like that in Figure 7a and plots a trajectory

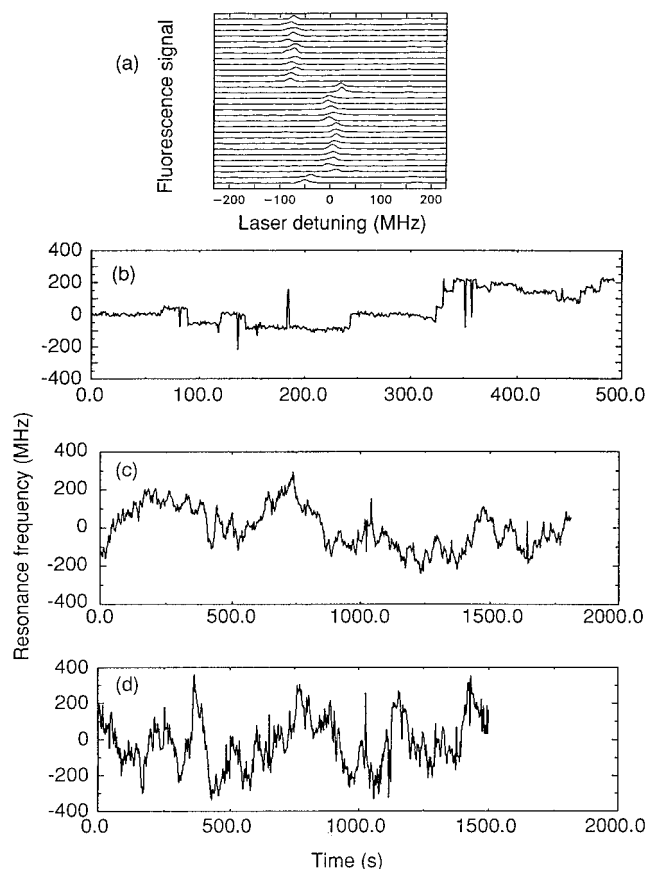


Figure 7. Examples of single-molecule spectral diffusion for pentacene in *p*-terphenyl at 1.5 K: (a) a series of fluorescence excitation spectra each 2.5 s long spaced by 0.25 s showing discontinuous shifts in resonance frequency, with a zero detuning of 592.546 nm, (b) trend or trajectory of the resonance 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 (d) that at 4.0 K. Reprinted with permission from ref 9. Copyright 1994 American Association for the Advancement of Science.

or trend of the center frequency versus time as shown in Figure 7b. The spectral trajectory shows that the optical transition energy appears to have a preferred set of values and performs spectral jumps between these values that are discontinuous on the 2.75 s time scale of the measurement. The behavior of another molecule is shown in Figure 7c at 1.5 K and in Figure 7d 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. It is worth noting that such spectral trajectories cannot be obtained when a large ensemble of molecules is in resonance. The individual jumps are generally uncorrelated; thus, the behavior of an ensemble-averaged quantity such as a spectral hole in this system would show only a broadening and smearing of the line.

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 optical excitation itself? Of course, it is not possible to say much about what the molecule is doing when it is not being observed! To distinguish spontaneous vs light-driven behavior, experimenters generally observe the average rate of spectral shifts at different probing power levels. If the rate of spectral jumps

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Table 1. Summary of What We Measure/What We Learn

measurement	information obtained
microscopy (low temperature, far-field) ^{59,60,67} polarization of excitation/emission ⁶⁸ spectral trajectory, spontaneous and light-induced spectral shifts, distribution of line widths ^{61,63,66,69-71} Stark effects—shifts of absorption spectrum by applied electric field ^{6,72} emission spectrum (resonance Raman) ^{44,73-75}	location and distribution of chromophore labels, correlations with topography orientation of molecule, identification of site dynamics of local environment, especially time-dependent configurational and bonding changes, nonradiative decay processes local site symmetry, local electric fields
autocorrelation of emitted photon stream and time-correlated photon counting ^{51,52,76} second-order correlation function $g^{(2)}(\tau)$ ^{56,58} single-spin magnetic resonance ⁷⁷⁻⁸⁰ temperature dependence of all effects	position and strength of A_g modes of electronic ground state, distortions of the probe molecule by local environment excited state lifetimes, time scale and distribution of conformational tunneling states quantum optical effects, sub-Poissonian statistics hyperfine splittings, ¹³ C interactions, local magnetic environment barrier heights to conformational change of the local environment

does not change, it is generally assumed that the phonons present in the lattice at the experimental temperature are responsible, and this form of spontaneous spectral diffusion is discussed in the next paragraph. When the rate of spectral jumps increases with laser power, the process is regarded as being light-driven. For an example of a light-driven process, we recall that fluorescence quantum yields are seldom exactly unity, so that photon emission can be accompanied by nonradiative decay or by excitation of internal vibrational modes of the ground state if a $0 \rightarrow 1$ or $0 \rightarrow 2$ etc. photon is emitted. The lifetime of such excited vibrational levels in a solid is extremely short (in the picosecond range), as emission of one or a few phonons can easily empty the level. The result can be that these locally emitted phonons change the nearby environment of the molecule slightly, resulting in a spectral shift of the optical absorption. While this model for light-driven spectral diffusion seems plausible, in fact the detailed mechanism by which the spectral shift is produced is still a mystery. It is clear, however, that light-induced spectral shifts are the single-molecule analog of the nonphotochemical spectral hole-burning process,^{36,64} generally observed for zero-phonon lines of dye molecules in many amorphous solids. Several single-molecule systems have shown light-induced shifting behavior, for example, perylene in poly(ethylene),⁶³ terylene in poly(ethylene),⁶⁵ and terylene in a Shpol'skii matrix,^{66,67} and it

is hoped that future detailed study of this effect will shed light on the microscopic mechanisms of nonphotochemical hole-burning.

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 effect.⁶¹ Since the single-molecule absorption is extremely sensitive to the local strain field, it is reasonable to expect that the spectral jumps are due to internal dynamics of some configurational degrees of freedom in the surrounding lattice, driven by the phonons present at the experimental temperature. The situation is analogous to that for amorphous systems, where the local dynamics result from the two-level systems near the guest. The dynamics result from phonon-assisted tunneling or thermally activated barrier crossing. One possible source for the tunneling states in this crystalline system⁴² 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. A theoretical study of the spectral diffusion trajectories¹⁴⁻¹⁶ has allowed determination of specific defects that can produce this behavior, attesting to the power of SMS in probing details of the local nanoenvironment.

Spectral shifts of single-molecule line shapes are common in many systems, appearing not only for certain crystalline hosts but also for essentially all polymers studied, and even for polycrystalline Shpol'skii matrices. Moreover, spectral shifting has recently been observed on a much larger frequency scale for single molecules on surfaces at room temperature for both near-field²⁵ and far-field studies.²⁸ This behavior is turning out to be a ubiquitous feature of single-molecule experiments, giving us a new window into local dynamical behavior.

4. Summary and Outlook

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. A partial listing of the physical measurements performed so far and what is being learned from such measurements is provided in Table 1. The significance of such experiments is 4-fold. First, the properties of a single

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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. The door is open to true photochemical experiments on single absorbers, quantum coupling experiments, and even the possibility of optical storage using single molecules⁹ if optical control of light-induced spectral shifts can be refined.

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