Ultrasensitive Laser Spectroscopy in Solids: Single-Molecule Detection

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ULTRASENSITIVE LASER SPECTROSCOPY IN SOLIDS: SINGLE-MOLECULE DETECTION

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Abstract In spite of detection intensity constraints necessary to avoid power broadening, the optical absorption spectrum of *single* molecules of pentacene in *p*-terphenyl crystals can be measured by (1) using laser FM spectroscopy combined with Stark and/or ultrasonic double modulation (to remove residual amplitude modulation) and (2) recording spectra far out in the wings of the inhomogeneous line to reduce the number of molecules in resonance to one.

INTRODUCTION

Recent progress in the optical detection and spectroscopy of single ions in vacuum confined in electromagnetic traps has led to novel measurements that test our understanding of quantum physics. For example, various workers have achieved direct measurement of quantum jumps, Doppler sidebands, and other fundamental phenomena such as ion crystallization [1,2]. By using laser-induced fluorescence and a novel hydrodynamically-focused flow to confine the molecules and reduce the scattering volume, single molecules of the protein B-phycoerythrin with the equivalent of 25 rhodamine 6G chromophores have also been detected[3].

The detection and spectroscopy of a single absorber in a solid (called in the case of a molecular absorber single-molecule detection, or SMD) would provide a useful tool for the study of local host-absorber interactions where the absorbing center is essentially at rest, confined by the host lattice, and where the normal averaging over many "equivalent" centers is removed. Generally, absorbing guest centers in solids with zero-phonon transitions form inhomogeneously broadened lines, [4] where the overall line profile is caused by a (usually Gaussian) distribution of center frequencies for the individual absorbers that is broader than the (usually Lorentzian) homogeneous lineshape of the individual absorbers (See Figure 1, left side). The distribution of center frequencies is caused by dislocations, point defects, or random internal electric and strain fields and field gradients in the host material. Strong inhomogeneous broadening generally requires
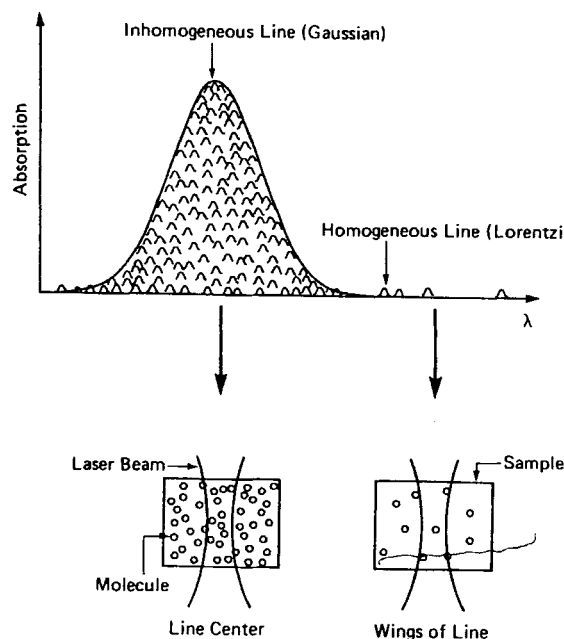
FIGURE 1   Schematic showing an inhomogeneous line at low temperatures
and the principle of single-molecule detection in solids. The lower part of
the figure shows how the number of impurity molecules in resonance in the
probed volume can be varied by changing the laser wavelength. The laser
linewidth ($\simeq$ 3 MHz) is negligible.

low temperatures, because the homogeneous zero-phonon lines become much
narrower than the inhomogeneous distribution only when the host phonons are
quenched. In such lines, the centers within a homogeneous width of a given
frequency are located at that particular spectral position for a variety of possible
reasons in general. This intrinsic multi-dimensional inhomogeneity cannot be
removed with spectral hole-burning or coherent transient techniques. However,
with SMD the absorption spectrum of an individual absorber can become directly
accessible, as long as no other centers are present at the same frequency. This
is a primary reason to pursue the spectroscopy of individual centers in condensed
matter. For convenience in our case, one absorber will be selected for spec-
troscopy by proceeding out into the wings of the inhomogeneous line as shown
on the right side of Fig. 1.[5]   Since the center frequencies for these absorbers
are displaced very far from the most common center frequencies near the center

of the inhomogeneous line, such absorbers have highly unique and improbable strained sites.

Compared to the other single-absorber experiments, SMD in solids provides a different set of experimental challenges. The problem can be likened to finding a needle in a haystack, because unlike the ion-trap experiments, for example, the molecule of interest is hidden within a solid containing a large number ($\simeq$ $10^{12} - 10^{18}$) of "non-absorbing", potentially interfering host molecules within the laser focal volume. If laser-induced fluorescence excitation were used for SMD and the host molecules had appreciable Raman (or Rayleigh) scattering cross sections, the signal from the one absorbing molecule could be swamped by the scattering signal from the host. Unlike the hydrodynamic focusing experiments, it is not always possible to reduce the host scattering volume. Furthermore, it would be intriguing to measure the absorption spectrum of a single absorber instead of simply detecting its presence in a digital fashion at a fixed laser wavelength.

Rather than utilizing fluorescence excitation methods, where the chief experimental problem is the reduction of interfering scattering signals, we have chosen an alternative route to SMD, namely, laser FM spectroscopy [6] with double modulation. This paper describes the past efforts toward achieving SMD, and summarizes the recent single-molecule spectra we have obtained in solids using FM-Stark and FM-ultrasound double-modulation techniques [7,8] for the model system composed of pentacene substitutional impurities in p-terphenyl crystals at 1.5K. The pentacene molecules can substitute for any one of the four p-terphenyl molecules in the low-temperature unit cell[9], giving rise to four $S_1 \leftarrow S_0$ 0-0 optical absorption origins near 593 nm, named O_1, O_2, O_3, and O_4. We focus on the origins O_1 and O_2, because the homogeneous linewidths are smaller here than for the other origins.

PROGRESS TOWARD SMD: STATISTICAL FINE STRUCTURE

Due to the randomness associated with the imperfections in solid hosts, inhomogeneous absorption lines (at least near their centers) are often approximated by smooth, Gaussian profiles[4]. However, since the inhomogeneous line on a microscopic scale is simply a superposition of discrete homogeneous lines with widths as much as 1000 times narrower than the overall inhomogeneous profile, the true shape of the inhomogeneous line cannot be a smooth function in reality. In fact, unavoidable number fluctuations in the density of absorbers per unit wavelength interval should give rise to a "spectral noise" on the overall Gaussian

background that scales as the square root of the mean number of centers in resonance. To be precise, defining the average number of centers in the probed volume within one homogeneous width of the laser wavelength as $\bar{N}_H$, there should be a statistical fine structure (SFS) present on the absorption profile scaling in absolute magnitude as $\sqrt{\bar{N}_H}$ (in the limit of $\bar{N}_H >> 1$). Since SFS arises from the absorption of many overlapping impurity absorptions, the absolute magnitude of the SFS is clearly larger than a single-molecule absorption signal (where $\bar{N}_H \simeq 1$). Therefore, observations of SFS would be expected to precede true single-molecule detection.

Recent observations of SFS in the pentacene in p-terphenyl system provided a crucial first step toward single-molecule detection and spectroscopy[10]. This was achieved using the high-resolution and high-sensitivity technique of laser frequency-modulation spectroscopy (FMS) [6] to probe the optical absorption in a zero-background manner sensitive only to narrow spectral features. This detection method will be described in more detail in the next section.
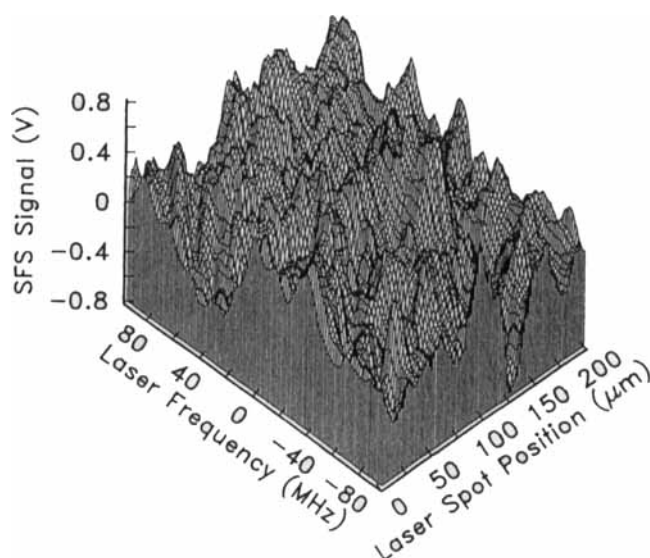

FIGURE 2   SFS structure versus laser spot position and laser frequency near the inhomogeneous line center for pentacene in p-terphenyl. A sequence of 100 spectra were obtained, moving the 20μm laser spot by 2μm after each spectrum, and the results plotted as a contour plot of the SFS signal to show the SFS "landscape".

Since its source is a random process, the SFS spectral structure changes for different probe volumes, and an "SFS landscape" of the inhomogeneous line can be generated by acquiring SFS spectra as a function of laser spot position, as in Figure 2. The bumps and valleys in this figure may be regarded as the "fuzz" on the top of the "haystack" represented by the pentacene inhomogeneous line that results from the statistics of independent, additive random variables. We note in passing that a crucial physical parameter may be derived directly from SFS measurements: by computing the autocorrelation of the measured spectra[10], the homogeneous width of the underlying Lorentzian profiles may be determined.

Subsequent to the first observations of SFS, other researchers moved closer to single-absorber detection in solids by observing SFS at increasingly lower impurity concentrations. Lange et al. [11] relied upon fluorescence excitation of Sm^{2+} ions in CaF_2 at 77K with a fixed frequency laser in tightly focused spots. These researchers saw Poisson fluctuations in the detected fluorescence as a function of the position of the focal spot and concluded that they had reached the level $\bar{N}_H = 5$. Another novel approach, developed by the Yen group at the University of Georgia[12], used laser fluorescence excitation in a glass fiber doped with Nd^{3+} ions. Here the fiber geometry effectively maintains a small focus and a small probing volume in order to reduce background signals from the host. The measured SFS led these researchers to conclude that they had reached $\bar{N}_H$ values on the order of a few tens of ions. In both cases, special detection geometries were necessary to avoid host background fluorescence.

EXPERIMENTAL APPROACH TO SMD

Our approach to SMD for pentacene in p-terphenyl avoids the problem of fluorescence background by using a powerful absorption technique, laser FM spectroscopy (FMS)[6]. The basic operation of FMS is illustrated in the upper part of Figure 3. A tunable single-frequency cw Rhodamine 6G laser beam at ω_c is passed through an electro-optic phase modulator EO to produce light that is frequency-modulated at a rf frequency ω_m in the MHz range. This FM light beam has the spectrum shown in the upper center of the figure: a carrier at the original frequency, plus two sidebands displaced by $\pm\omega_m$ from the carrier. If this FM light beam were sent directly to a high-speed photodiode detector (which measures the envelope of the power absorbed), no photocurrent at ω_m would be detected. In other words, an FM light beam has no amplitude modulation (AM). In the frequency domain, this lack of AM can be understood by noting that one of the

two sidebands is in phase and the other out of phase with respect to the carrier. Therefore, the beat signals caused by each sideband interfering with the carrier cancel.
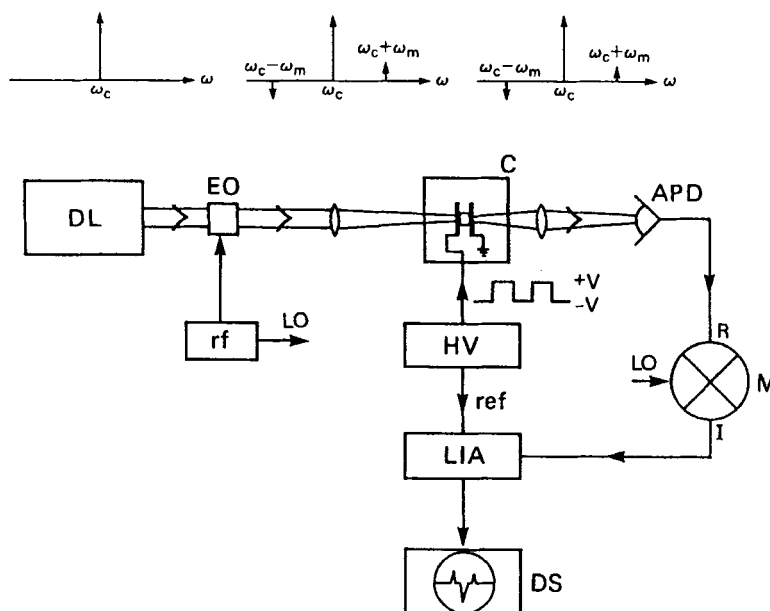

FIGURE 3   Schematic showing the principle of FM spectroscopy with Stark secondary modulation. The upper part of the figure shows the dye laser (DL) spectrum before the electro-optic phase modulator (EO), after the EO, and after the sample in the cryostat C. Legend: rf - rf oscillator at ω_m, APD - avalanche photodiode detector, M - double-balanced mixer, HV - high voltage source, LIA - lock-in amplifier, DS - digital storage and averaging oscilloscope.

Now when a spectral feature is present that disturbs the balance between the two sidebands, a rf photocurrent appears that is proportional to the difference in optical absorption at the upper and lower sidebands. This photocurrent at ω_m may be detected (phase-sensitively) by a rf lock-in composed of a mixer M driven with a local oscillator derived from the original rf source. Thus, the low frequency or baseband signal at the I port of the mixer is the standard FMS signal, with the following properties[13]: (1) it appears on a background that is derived from the laser noise at ω_m, which may be at the quantum limit if no excess noise is in-

troduced by the detector, (2) the size of the signal is largest when the spectral feature is narrower in width than ω_m, and (3) for wide spectral features the FMS signal is proportional to the derivative of the feature with an amplitude approaching zero for increasing width of the feature. Point (1) is responsible for the quantum-limited performance of FM spectroscopy that has been achieved with simple (non-avalanche) photodiodes[14]. Points (2) and (3) are particularly important for SMD, because any undesired optical absorption from other impurities or from the p-terphenyl host that is broad compared to ω_m is not detected with appreciable amplitude. With our values of $\omega_m/2\pi = \nu_m = 50 - 90\,\mathrm{MHz}$, only rigid molecules like pentacene with homogeneous widths less than $\simeq 100$ MHz will be detected.

There is one background signal from FMS that must be avoided. Since the method directly senses the conversion of FM into AM, any residual AM (also called RAM) from imperfections in the modulator can give rise to a spurious background signal. To overcome this, we utilized a secondary modulation of the spectral feature itself. Figure 3 shows the case where the secondary modulation is produced by an electric field oscillating at a low frequency in the kilohertz range. The electric field shifts the absorption profile twice each cycle via the quadratic Stark effect. Then the output of the mixer, processed by a final lock-in amplifier LIA at 2f, yields a detected signal that is free from RAM background limitations. In addition to this FM/Stark technique, we also separately utilized[7] a different secondary modulation to achieve SMD, ultrasonic (stress) modulation, which operates in a similar fashion by shifting the homogeneous line in a periodic fashion. It is clear that other local perturbations of the impurity molecule might also be used for double-modulation detection.

The size of the expected absorption signal from a single molecule is straightforward to estimate[7]. The change in absorbance, $(\Delta\alpha)L$, is given by the probability of absorption of a photon in the incident beam by the molecule, σ/A, where σ is the peak absorption cross section and A is the area of the laser beam. Clearly, then, one would prefer tightly focused laser spots and molecules with strong absorptions. In our experiment, the focal spot was $\simeq 3\ \mu$m in diameter, and the peak (low-temperature) absorption cross section for pentacene is $9.3 \times 10^{-12}\,\mathrm{cm}^2$, yielding an absorbance change of $\simeq 10^{-4}$. This is not an extremely small signal except that detection must be performed with a light intensity that does not produce extreme power broadening. To meet this constraint in a tightly focused spot, we performed measurements with only 0.1 μW of light at the detector. Even at this level we chose to accept some amount of power broadening.

Such a low light level requires an avalanche photodiode to avoid detector John-son noise. However, avalanche photodiodes have an excess multiplicative noise which requires operation somewhat above the quantum limit.

RESULTS: FM/STARK TECHNIQUE

In spite of these limitations, the FM/Stark and FM/US methods can be used to detect the optical absorption of a single molecule of pentacene in a solid crystal of p-terphenyl. Figure 4 shows examples of the spectra for the FM/Stark case.
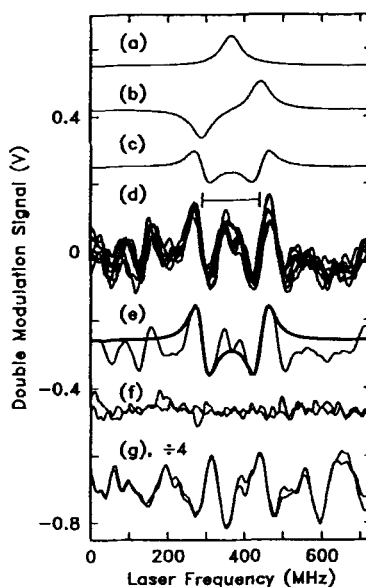

FIGURE 4   Single-molecule spectra using FM/Stark technique (quadratic Stark effect) (a) Simulation of absorption line. (b) Simulation of FM spectrum for (a), v_m = 75 MHz. (c) Simulation of FM/Stark double-modulation lineshape. (d) SMD spectra at 592.423 nm, 512 averages, 8 traces overlaid, bar shows value of $2v_m$ = 150 MHz. (O_1 line center is at 592.326 nm .) (e) Average of traces in (d) with fit to the in-focus molecule (smooth curve). (f) Signal very far off line at 597.514 nm, same conditions. (g) Traces of SFS at the O_2 line center, 592.186 nm, 128 averages each.

The first three traces show simulations to explain the expected single-molecule lineshape for either Stark or ultrasonic double modulation. Trace 4(a) shows a Lorentzian absorption profile of width y, and trace 4(b) shows the expected simple FM signal[13]: two copies of the absorption line with opposite sign, spaced by

$2\nu_m = 150$ MHz (in the limit $\gamma << \nu_m$). With secondary modulation that causes frequency shifts less than the linewidth, the resulting double-modulation lineshape is the derivative of the simple FM lineshape (trace 4(c)). Thus the signature of a single molecule is a "W"-shaped feature with a large negative slope and a large positive slope separated by $2\nu_m$.

In a typical experiment, the laser frequency was set near the center of the inhomogeneous line, and the resulting strong SFS signal was used to optimize the optical and electronic configuration. Then as the laser wavelength was moved out into the wings of the line, the SFS amplitude dropped uniformly. Eventually spectra that appear to be superpositions of 2-5 single-molecule spectra like trace 4(c) were observed. Finally, sufficiently far out into the wings of the line, true single-molecule spectra could be recorded. Trace 4(d) shows a set of eight FM/Stark double modulation spectra of a strong in-focus molecule far out in the long-wavelength edge of O_1, along with several unavoidable weak repeatable features from out-of-focus molecules at the left and right edges of the laser scan range. These out-of-focus features were caused by molecules not located at the laser waist position (see Fig. 1) and may be suppressed in future experiments with thinner samples. The fiducial bar marks a spectral range equal to $2\nu_m$. Trace 4(e) shows the average of the eight scans in 4(d), along with a fit to the central feature generated by a simple model for the double-modulation process[8]. The fit to the essential features of the SMD lineshape is reasonable, and the homogeneous width required by the fitting process is somewhat larger than the low-power homogeneous width, as expected.

Trace 4(f) shows the detected signal from a laser wavelength so far away from the pentacene site origins that no molecules are expected to lie in the laser scan range; this is the background shot and avalanche noise. In samples of un-doped pure p-terphenyl, only a baseline noise level similar to the off-line data in Fig. 4(f) was observed, even near the center of the inhomogeneous line. Trace 4(g) shows spectra of the strong SFS observed near the center of the inhomogeneous line using a smaller number of averages. This spectrum is composed of a superposition of many "W" profiles like Figure 4(d) with many different center frequencies, illustrating the qualitative difference between spectra of large numbers of molecules (Fig. 4(g)) and spectra of one molecule (Fig. 4(d)).

Using the related FM/US technique, [7, 8] when the modulating frequency ν_m is varied, the single-molecule lineshapes can be observed to expand and contract as required to maintain the proper spacing between the inner edges of the "W". Such clear variations in the recorded spectrum cannot be observed when many

molecules are present in the laser scan. This, in conjunction with the shape of the observed features, the position relative to the pentacene in p-terphenyl origins, the lack of such signals in undoped samples, and the appearance of single-molecule spectra with both the FM/Stark and FM/US techniques, leads us to the conclusion that the recorded spectra are due to single molecules of pentacene.

DISCUSSION

One experimental shortcoming of zero-background techniques is the difficulty in absolute calibration of the observed signals. We attempted to carefully do this by using a FM signal of known amplitude, but the amount by which the absorption lines were shifted by the secondary modulation could only be estimated. In spite of these limitations, the size of the single-molecule signals appears to be somewhat larger than expected given our level of power broadening. This intriguing observation (which needs to be confirmed in future experiments) suggests that pentacene molecules in the highly strained, improbable sites far out in the wings of the inhomogeneous line may have reduced intersystem crossing rates.

The attainment of single-molecule detection and spectroscopy 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. Here the absorbing entity is exquisitely sensitive to the symmetry and perturbations introduced by the local environment such as the local vibrational modes and the true local fields. While as a general technique the method presented here is not applicable to all molecular impurities, it can be applied to the large number of absorbing ions and molecules in solids that have zero-phonon transitions. The detectability of the resulting single-center signal, which ultimately depends upon the absorption strength and upon quantum noise limits, must be evaluated in each case. For situations in which the molecular linewidth is large, recent important advances in two-tone FM spectroscopy [15, 16] make FMS practical with very large sideband spacings.

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5. The reader will recognize that it would also be possible to drastically lower the impurity concentration and work near the center of the inhomogeneous line. We chose the present approach, because by tuning to the center of the inhomogeneous line where N is large, a convenient alignment signal exists that can be used to optimize the detection system. Then, by tuning out into the wings, single molecule absorptions can be observed.
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