NEAR-FIELD SINGLE MOLECULE SPECTROSCOPY

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Near-Field Single Molecule Spectroscopy

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ABSTRACT

The high spatial resolution and sensitivity of near-field fluorescence microscopy allows one to study spectroscopic and dynamical properties of individual molecules at room temperature. Time-resolved experiments which probe the dynamical behavior of single molecules are discussed. Ground rules for applying near-field spectroscopy and the effect of the aluminum coated near-field probe on spectroscopic measurements are presented.

2. INTRODUCTION

Recent advances in near-field fluorescence microscopy [1-3] make it possible to conduct single molecule spectroscopy at the nanometric scale in an ambient environment[4-7]. While there have been several far-field techniques allowing single molecule detection (not yet single molecule spectroscopy) at room temperature, e.g. in liquids with flow cytometry[8], in aerosol particles[9], in electrophoresis gels [10], as well as with confocal microscopy[11], the near-field technique offers the special advantages of high spatial resolution and high sensitivity. The high spatial resolution (20-100nm) breaks the diffraction limit inherent in far-field measurements. The high sensitivity for fluorescence detection results from the high photon flux delivered by the near-field probe, the strong evanescent wave components at the near-field and the ease of background rejection.

The unique features of near-field microscopy allow one to extend the pioneering single molecule spectroscopy work done at cryogenic temperatures[12-14] to the room temperature regime, and thus open up many new possibilities in analytical chemistry, material researches and biological sciences. From a chemical physics point of view, conducting spectroscopy on a single molecule basis removes spectral heterogeneity and enables one to probe a molecule in a specific local environment. Time resolved single molecule spectroscopy[9,10] allows one to observe chemical reactions (such as electron transfer) taking place on individual molecules, which can offer a great deal of detailed information complementary to experiments done on big ensembles of molecules.

3. NEAR-FIELD FLUORESCENCE MICROSCOPE

Our near-field fluorescence microscope [15] is similar in design to that of Betzig and Trautman [1]. The shear-force/near-field microscope is interfaced to a Nanoscope III controller from Digital Instruments. The schematic of the scanning head is shown in Figure 1, only special features of the apparatus are summarized as follows.

The shear-force feedback mechanism[16, 17] is implemented by laterally dithering the near-field probe at its resonant frequency with a bimorph. The dither amplitude is detected optically by a combination of a single mode fiber pigtailed to a 1.3 micron laser diode and a dual

InGaAs photodiode. The near-field probe splits the infrared beam and acts as an oscillating shutter. The signal from the dual diode is processed with phase sensitive detection and the amplitude output serves as the feedback signal. Such a design results in very small vertical noise ($< 3\text{\AA}$) in the shear force images. Measurements can be done either in air or under water[15].

Figure 1. Schematic of the near-field probe and the shear force feedback mechanism

The sample is raster scanned with a closed loop xy scanner (Queensgate) containing capacitance-based sensors (positioning accuracy of 1nm), which avoids complications associated with piezo hysteresis. This feature allows us to quickly and accurately position the tip above a molecule.

The scanning head and the xy scanner are mounted on an inverted fluorescence microscope (Nikon Diaphot). The emission is collected through the transparent substrate with a N.A. 1.4 oil emersion objective and the excitation light is blocked by holographic filters. The emission is confocally imaged onto the small active area of an avalanche photodiode (EG&G Canada). The analog output of the detector is fed into a 100X fast preamplifier, followed by a leading edge discriminator in order to achieve the fastest time response for time correlated photon counting.

4. FLUORESCENCE IMAGING OF SINGLE MOLECULES

Figure 2 shows a fluorescence image of single sulforhodamine 101 molecules dispersed on a glass surface. 594nm excitation light was used and the image was acquired in 8 min. The intensity profile of the features have a FWHM of 100nm, limited by the tip aperture. Linear polarized excitation light was used (with precompensation by a $1/4$ wave plate, an extinction ratio of 100:1 is observed when viewed in the far-field). The different intensities of the peaks are due to the different molecular orientations and the different excitation spectra. Following Betzig[4], we can determine the orientation of each individual molecule in all three directions. From the image patterns, we conclude that the molecular transition dipoles are parallel to the glass surface. This is intuitively consistent with the planar structure of sulforhodamine 101.

Rotation of the input polarization by 90° reverse the intensities of the peaks, allowing the determination of their orientation in the xy plane.

Figure 2. Near-field fluorescence image of single sulforhodamine 101 molecules dispersed on a glass surface. [Xie and Dunn, *Science*, **265**, (1994) 361]

5. GROUND RULES FOR NEAR-FIELD SPECTROSCOPY

Before we can extract useful information from the near-field measurements, ground rules for spectroscopy should be revisited. The essential difference between near-field and far-field spectroscopy is the evanescent wave involved in the former.

Bethe and Bouwkamp have solved the Maxwell equations for a small aperture in a conducting screen such as that shown in Figure 3.[18,19] Mapping the near-field E-field distribution of a near-field tip with single molecules has shown an excellent agreement with Bethe and Bouwkamp's treatment[4]. The nonplanar nature of the electromagnetic wave provides the longitudinal polarization components at the edges of the aperture as shown in Figure 3, which allow one to determine molecular orientations in all three dimensions.

Figure 3. The electric field line and the field vectors near a small aperture in a conducting screen.

According to Bethe and Boukamp, when the diameter of the aperture, a , is smaller than the wavelength of the light, the photon flux reaching the far-field is proportional to a^6 (instead of a^2). At the far-field, the aperture looks like a point source. At the near-field, there is a strong evanescent wave which is non-propagating and rapidly decaying with distance. This evanescent component can excite molecules,[2] and is primarily responsible for the high sensitivity of the near-field technique. The signal calculated based on the photon flux to the far-field and the absorption cross section (Beer's law) only gives the lowest estimates of signal strength. We and others[4] have experimental evidence for emission count rates higher than the signal estimated by the photon flux to the far field.

To quantitatively predict the signal strength, one needs to evaluate the transition probability which is proportional to the electric dipole matrix element in time dependent perturbation theory:

$$|\langle \psi_i(x, y, z) | \mathbf{E}(x, y, z, t) \cdot \mathbf{e} r | \psi_f(x, y, z) \rangle|^2 \quad (1)$$

In conventional spectroscopy with plane waves, the amplitude of $\mathbf{E}$ does not vary with x, y, z , and can therefore be extracted out from the spatial integration. This allows the establishment of selection rules based on molecular properties alone. In the case of a small aperture, if a molecular wave function, $\psi(x, y, z)$ spans the size of the aperture, $\mathbf{E}(x, y, z, t)$ can no longer be extracted out of the integration in Eq. 1. Grober et al pointed out that the selection rules might be different from conventional spectroscopy in these systems.[20] However, for a single dye molecule, the wave function is about 1 nm in size, much smaller than the aperture. Therefore, Eq. (1) becomes,

$$|\mathbf{E}(x, y, z, t) \cdot \langle \psi_i(x, y, z) | \mathbf{e} r | \psi_f(x, y, z) \rangle|^2 \quad (2)$$

i.e., the transition dipole can be viewed as a point dipole and the selection rules are the same as those for far-field spectroscopy. Eq. (2) also provides the basis for determining the molecular orientation of single molecules discussed in the last section.

To summarize, the ground rules for near-field single molecule spectroscopy are

- 1) The calculation of signal strength should not be based on the photon flux to the far-field (Beer's law), but instead based on the transition probability in Eq. (1).
- 2) The selection rules might be different from those of far-field experiments for molecular aggregates or semiconductor systems where the wave function spans the size of the aperture. [20] However, conventional selection rules hold for single molecule experiments.
- 3) The non-planar nature of the electromagnetic wave near the tip end provides longitudinal electric field components capable of probing molecular transitions or orientations not accessible with far-field electromagnetic waves.[4]

6. PROBING SINGLE MOLECULE DYNAMICS

There are two classes of experiments on two vastly different time scales that one can probe room temperature dynamics of single molecules.

Figure 4 show the fluorescence decay of a single oxazine 725 molecule dispersed on a glass surface. Oxazine 725 molecule is known to undergo an excited state charge transfer reaction.[22] The observed fluorescence decay is the reflection of the population decay from the local excited state, due to the twisted intramolecular charge transfer reaction. [22]

Before we can extract useful information, however, the perturbation of the tip to the spectroscopic measurements needs to be examined.

7. PERTURBATION TO SPECTROSCOPIC MEASUREMENTS

We have examined the single molecule emission lifetimes as a function of the lateral displacement of the tip relative to the molecule. With 7nm tip-molecule gap, we observed the shortening of fluorescence lifetimes of sulforhodamine 101 molecules near the tip edges, due to excited state energy transfer to the aluminum coating of the tip.[6] Trautman and coworkers have recently observed the lengthening of the fluorescence lifetime near the tip edges for a different system.[23] With a bigger tip-molecule gap (20nm), we have also observed lengthening of fluorescence lifetimes near the tip edges for sulforhodamine 101 molecules dispersed on a glass surface.

The fluorescence lifetime of a molecule in front of a metal surface has been extensively studied [24]. According to a classical model originated by Kuhn[25], the molecular dipole, μ , follows the equation of motion:

$$\ddot{\vec{\mu}} + \omega_0^2 \vec{\mu} = \frac{e^2}{m} \vec{E}_R - \gamma \dot{\vec{\mu}} \quad (3)$$

In this equation, μ is oscillating at the optical frequency ω , driven by its own reflected wave from the metal surface, E_R , and damped with the reciprocal of the fluorescence lifetime, γ .

$$\vec{\mu} = \vec{\mu}_0 \cos \omega t e^{-\gamma t/2} \quad \text{and} \quad \vec{E}_R = \vec{E}_0 \cos (\omega t - \phi) e^{-\gamma t/2} \quad (4)$$

The amplitude E_0 and the phase ϕ of the reflected wave E_R are determined by the dielectric constants of the metal and the specific geometry between the dipole and the metal surface. Knowing E_0 and ϕ , the modified fluorescence lifetime and frequency shift can be calculated as follows:

$$\gamma = \gamma_0 + \frac{e^2}{m\mu_0\omega_0} E_0 \sin \phi \quad \text{and} \quad \Delta\omega = -\frac{\gamma^2}{8\omega_0} - \frac{e^2}{2m\mu_0\omega_0} E_0 \cos \phi \quad (5)$$

Chance, Prock and Silbey have developed a theory for molecular fluorescence in front of an infinite and flat metal surface, based on Sommerfeld's treatment [26] of the reflected field E_0 and ϕ . [27] This theory allows explicit evaluation of Eq. 5 and the separation of radiative and nonradiative contributions to a fluorescence decay. For dye molecules in front of an aluminum surface, the nonradiative energy transfer dominates within 10 nm, while the radiative rate dominates at a larger separation. While CPS theory is helpful to gain an intuitive understanding of the behavior observed in near-field experiments, a quantitative understanding relies on the numeric calculation of E_0 and ϕ at the specific geometry of the near-field probe. Work is in progress toward this objective.

8. CONCLUSIONS

There have been efforts in developing techniques for conducting optical spectroscopy at the subnanometer or even atomic dimension. From a spectroscopy point of view, the atomically close tip-sample separation necessary for this high resolution would strongly perturb the spectroscopic measurements. The near-field technique, though low in spatial resolution compared to STM and AFM, is essentially a nonperturbative technique. The effects of the tip on spectroscopic measurements are summarized as follows:

1) Effects on excitation and emission spectra

The frequency shift due to interactions with the aluminum coating of the near-field tip can be estimated with CPS theory and are a fraction of a cm^{-1} . Fortunately this is not important at room temperature given the broad spectral width of electronic transitions. At cryogenic temperatures, however, such frequency shifts may become visible.

2) Effects on fluorescence lifetimes

The radiative and nonradiative energy transfer rates are significantly affected by the tip and are very sensitive to the boundary conditions. Fluorescence quenching can be minimized by centering the tip above a molecule. Fortunately, for dynamical processes that are much faster than the radiative and non-radiative energy transfer rates ($< 1.5\text{ns}$), fluorescence lifetimes can be measured in a nonperturbative way.

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