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On the possibility of observation of single quadrupoles by fluorescence resonance energy transfer scanning near-field optical microscopy

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Abstract

The possibility to observe single quadrupoles by the recently proposed fluorescence resonance energy transfer scanning near-field optical microscopy (FRET SNOM) is analyzed. When an excited dipole donor center of the SNOM tip is scanned close to a quadrupole molecule of the sample (at a distance of 1–2 nm), the probability of donor-quadrupole FRET becomes close to unity. Thus a single quadrupole can be imaged as a donor fluorescence quenching or by observing dielectric tip-enhanced fluorescence of the quadrupole. To the best of our knowledge, this is the only existing possibility to visualize single quadrupoles. © 2001 Published by Elsevier Science B.V.

Keywords: Fluorescence resonance energy transfer; Near-field optics; Quadrupole transitions; Single molecules

1. Introduction

Recently the idea to use the fluorescence resonance energy transfer (FRET) to improve the spatial resolution and sensitivity of scanning near-field optical microscopy (SNOM) has been proposed [1,2]. It is based on the fact that when the distance between donor and acceptor molecules becomes smaller than the characteristic radius of resonant energy transfer, R_0 (which for typical donor–acceptor pairs ranges 2–10 nm [3]), the probability of dipole–dipole energy transfer between these molecules is close to unity (see, for instance, Refs. [3,4] for a review). One should prepare a tip containing a single fluorescent center in the apex and scan it in close proximity to the sample surface (see Fig. 1 for an illustration). If the donor fluorescent centers of the imaging tip are excited and the fluorescence of the acceptor centers of the sample is monitored (or vice versa), the spatial resolution will be governed not by the aperture size of the microscope but by the value of R_0. Thus the spatial resolution can be improved by up to two orders of magnitude: practically it is not possible to work with apertures smaller than 30–50 nm because the intensity of light "seeping" through the nanoaperture decreases very rapidly when the aperture size decreases (see, e.g., Ref. [5] for a recent review of SNOM).

Very recently the first experiments implementing the FRET SNOM were performed [6–11]

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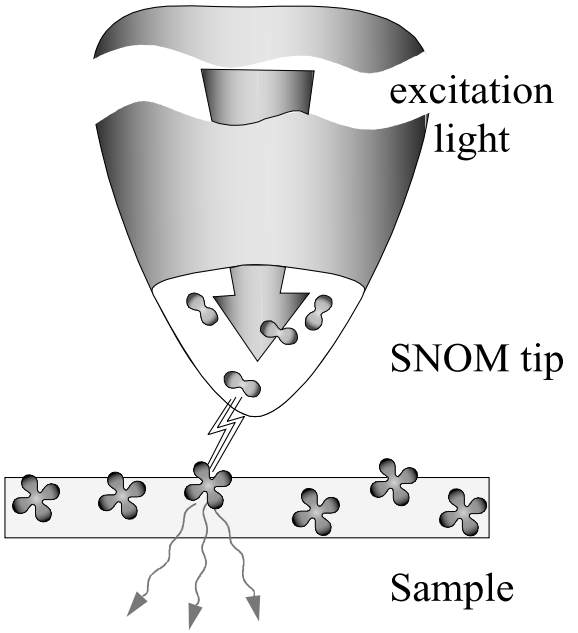

Fig. 1. Illustrating the idea of the fluorescence resonance energy transfer scanning near-field optical microscope. Excitation energy can be efficiently transferred from the tip's dipoles to the sample's quadrupoles in the same manner as to the sample's dipoles (see text), and thus single quadrupoles can be imaged as a donor fluorescence quenching or by observing dielectric tip enhanced fluorescence of the quadrupole.

which makes it timely to consider further possibilities of the method. In this paper we would like to elaborate on the fact that not only single acceptor molecules which have optical transitions of the electric dipole type in the suitable spectral range, but also single molecules which have *electric quadrupole* transitions in this spectral range, can also be imaged by the FRET SNOM method.

2. Probability of a single quadrupole excitation by the resonance energy transfer process

It can be shown, that although the optical absorption and emission cross-sections for the quadrupole transitions are six or more orders of magnitude smaller than those for the electric dipole transitions (which practically excludes the possibility of the direct optical observation of a single molecule quadrupole transition), the probability of the FRET from a donor molecule of the electric dipole type to an acceptor molecule of the quadrupole type can be equal to unity when the distance between them is of the same order as R_0 characteristic for the dipole–dipole FRET. Such a circumstance has been recognized already by Dexter in his famous paper [12] published in 1953, and this can be understood from the following consideration.

To describe the usual dipole–dipole FRET one needs to start from the expression for the electric field of the donor's dipole d^{D} at a distance R (see, e.g. Ref. [13]; for the simplicity we consider the medium with the refraction index equal to unity throughout the paper):

$$E^{\mathrm{D}} = -\frac{k^2}{R^3}\left[R\times(R\times d^{\mathrm{D}})\right] - \frac{\mathrm{i}k}{R^4}\left[3R(Rd^{\mathrm{D}}) - d^{\mathrm{D}}\right] + \frac{1}{R^5}\left[3R(Rd^{\mathrm{D}}) - d^{\mathrm{D}}\right] \quad (1)$$

and then to use the Hamiltonian $H_0 = -d^{\mathrm{A}}E^{\mathrm{D}}$ to calculate the rate of the FRET from donor to acceptor molecules, γ_{FRET}, in accordance with the Fermi golden rule:

$$\gamma_{\mathrm{FRET}} = \frac{2\pi}{\hbar}\langle f|H_0|i\rangle^2\rho(\omega) \quad (2)$$

Here, d^{A} is the dipole moment of the acceptor molecule, k is a wavenumber, $\rho(\omega)$ is the density of final states for the electromagnetic field and $\langle f|$ and $|i\rangle$ are the final and initial states of the acceptor molecule. The Förster radius, R_0, is defined as such the donor–acceptor distance, for which the FRET rate is equal to the radiation transition rate of the donor: $\gamma_{\mathrm{rad}} = (4\omega^3/3\hbar c^3)\times(d^{\mathrm{D}})^2$.

Such a consideration gives the known formula to evaluate the value of R_0 [4]:

$$R_0 = \left(\frac{3q}{4\pi}\int\frac{c^4}{\omega^4}F_{\mathrm{D}}(\omega)\sigma_{\mathrm{A}}(\omega)\,\mathrm{d}\omega\right)^{1/6} \quad (3)$$

where $F_{\mathrm{D}}(\omega)$ is the unity-normalized fluorescence lineshape of the donor ($\int F_{\mathrm{D}}(\omega)\mathrm{d}\omega = 1$), $\sigma_{\mathrm{A}}(\omega)$ is the optical absorption cross-section of the acceptor, and q is the donor's fluorescence quantum yield. As it was already mentioned, for all suitable FRET pairs R_0 ranges from 1 to 10 nm.

If, however, the dipole moment of the acceptor molecule is equal to zero, then the Hamiltonian H_0 should be replaced by $H_1 = -\sum_{i,j}(\partial E_i^{\mathrm{D}}/\partial x_j)Q_{ij}^{\mathrm{A}}$, where Q^{A} is the quadrupole moment tensor of the acceptor molecule [14]. Thus, in accordance with Eq. (2), the γ_{FRET} is now proportional to the square of the field derivative rather than to the square of the field itself.

Owing to the wave nature of light an electromagnetic field cannot be focused better than to an area of the order of the size of the wavelength. Hence, when the molecule is excited by propagating light in the far-field region the field derivative is determined by the phase factor $\exp(\mathrm{i}kx)$: $\partial E/\partial x \cong \mathrm{i}kE$. Because typically the quadrupole moment of a molecule is of the order of its dipole moment times the size of the molecule $a : Q \approx da$, the probability of the direct optical excitation of the quadrupole transition is $\sim(\mathrm{d}E)^2/(Q(\partial E/\partial x))^2 = (ka)^{-2}$ times smaller than that of the direct excitation of the dipole transition [14]. For the typical values $a = 0.1$ nm, $\lambda = 500$ nm it is easy to see that this ratio is equal to $\sim 10^6$.

The situation, however, is completely different for the dipole–quadrupole FRET. In this case the ratio of the field derivative to the field itself is determined not by the phase factor but by the near-field term $E^{\mathrm{D}} \cong d^{\mathrm{D}}/R^3$ of Eq. (1) and is equal to $\sim 3/R$. Thus the rate of the dipole–quadrupole FRET is of the order of $(3a/R)^2$ in comparison with its dipole–dipole analogue, which for $a = 0.1$ nm, $R = 3$ nm gives much larger value of the order of 0.01. Further, the same near-field term defines the dependence of the dipole–quadrupole FRET rate on the donor–acceptor distance R. Due to the aforementioned dependence of the γ_{FRET} on the square of the field derivative rather than the field itself, the rate of the dipole–quadrupole FRET increases as R^{-8} when the distance R decreases. For the example given above, it means that if the dipole–dipole FRET Förster radius was 3 nm, then for the same dipole–quadrupole spectral overlapping and donor–acceptor distance, the rate of the dipole–quadrupole FRET is $\sim 1\%$ of the donor radiation rate. The value of the characteristic radius, i.e. such a donor–acceptor distance when the FRET rate is equal to the donor radiation rate, is $\sim 100^{1/8} = 1.8$ times smaller i.e. 1.7 nm. These simple arguments show that the value of the Förster radius for dipole–quadrupole FRET is of the same order that this for dipole–dipole FRET.

A quantitative consideration by Dexter [12] gives the following formula to calculate the characteristic radius of the dipole–quadrupole FRET:

$$R_0^{\mathrm{D\text{–}Q}} = \left(\frac{135\pi\alpha q c^8}{4\tau_{\mathrm{A}}}\int\frac{f_{\mathrm{A}}(\omega)F_{\mathrm{D}}(\omega)}{\omega^8}\mathrm{d}\omega\right)^{1/8} \qquad (4)$$

Here $\alpha = 1.266$ is a numerical coefficient appearing as a result of the averaging over all possible dipole–quadrupole orientations, q is the donor's fluorescence quantum yield, and both spectra, the donor's emission $F_{\mathrm{D}}(\omega)$ and quadrupole's absorption $f_{\mathrm{A}}(\omega)$, are unity normalized. An acceptor resonance fluorescence time τ_{A} is introduced as a measure of the quadrupole transition strength. If desirable, this time can be replaced by the quadrupole moment using the known relation between them (see, e.g. Ref. [15]): $\gamma_{\mathrm{rad}}^{\mathrm{Q}} = Q^2k^5/60\hbar$, where Q is the largest component of the quadrupole tensor in a coordinate system where it is diagonal.

Although quadrupole transitions were observed for free atoms starting from the beginning of the century (not to mention that they are quite common in nuclear physics), only very limited data is available till now about the *molecule* quadrupole transition cross-sections and fluorescence times. Quadrupole transitions were observed for a few free gas-phase molecules by the electron energy loss spectroscopy (see, e.g. Ref. [16] and references therein), and were observed for a few metal ions in a condensed phase [17,18]. The theory of the molecule quadrupole transitions can be found in Ref. [19]. The general conclusion which can be made when analyzing these data in combination with the relation (4) is, of course, the same as has been already stated: the characteristic radius of the dipole–quadrupole FRET for well spectrally overlapping pairs is roughly the same (a factor of two smaller) as that of the dipole–dipole FRET pairs. Thus, single molecule dipole–quadrupole FRET SNOM can be realized exactly in the same manner as the earlier described single molecule dipole–dipole FRET SNOM [1,2].

3. Prospects of an experimental observation

When a SNOM tip containing single excited dipole centers in the apex is passing in the vicinity of a single quadrupole molecule of the sample (see Fig. 1), the radiation will not be emitted from the tip; instead, excitation will be transferred to the sample. (The necessary "contact" scanning methods and efficient light detection schemes are well elaborated in the SNOM [5].) The possibility of an experimental observation depends on the rate of the nonradiative and radiative energy dissipation from the FRET-excited quadrupoles. Modern detection techniques enable to attain a total efficiency of the photon detection of the order of $\sim 1\%$ with the noise level of $\sim 100\ s^{-1}$ (see, e.g. Ref. [20] for a recent review). This means that an excitation rate of donor fluorescence centers as small as $\sim 10^4$ times per second will be sufficient for the observation of a single quadrupole on the sample surface, provided that the energy dissipation from the quadrupole is fast enough (i.e. that the excited state time of life is smaller than 0.1 ms).

The radiative emission time for the free quadrupole molecule should be of the order of 1–100 ms which seems not enough for its observation, but for the experimental conditions considered here (room temperature, molecules into the matrix) the time of 0.1 ms is large enough for the nonradiative energy transfer from the quadrupole to the host medium to take place in most cases. The presence of a single quadrupole under the SNOM tip in such a case can be observed as the donor fluorescence quenching. The possibility to use FRET SNOM in such a quenching regime has been envisaged from the very beginning [1,2]. In the case of usual (not FRET) fluorescence SNOM such a quenching approach has been already experimentally realized [21].

Even more exciting is the possibility to observe these single quadrupole molecules in an emission mode. Recently it has been predicted, that the probability of the spontaneous radiation emission of a quadrupole can be increased $\sim (kb)^{-2}$ times in comparison with the free space case when the quadrupole is located in the vicinity of a small dielectric microsphere with the diameter b [22,23]. (The reason of such an increase is indeed the same as that of the more efficient dipole–quadrupole FRET in comparison with the direct optical excitation of a quadrupole transition: such a transition is sensitive to the field gradient rather than the field itself, and this gradient can be essentially increased when a dielectric microsphere is present.) Because the SNOM tip is rather similar to a small dielectric (glass) sphere, we can see that for the sharpest tips with the radius of curvature less than 50 nm the radiation time for the quadrupoles can become as small as 0.01 ms and even shorter. This will enable the observation of single quadrupoles by the detection of their fluorescence in complete analogy to the dipole–dipole FRET SNOM.

4. Conclusions

Thus, it is shown that single quadrupoles could be observed with a nanometer spatial resolution when using FRET SNOM in the same manner as single dipoles can be observed by this technique. To the best of our knowledge, this is the only existing possibility to visualize single quadrupoles. Recent achievements in the field of the FRET SNOM, which include not only the observation of nanolocal FRET [6–11], but also the demonstration of the "one fluorescent center containing" FRET active tips [1,2,24], enable to envisage a fast experimental realization of such a possibility.

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