Optical Trapping of Single Fluorescent Molecules at the Detection Spots of Nanoprobes

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We propose a scheme of optical trapping of fluorescent molecules, based on the strongly enhanced optical field due to surface plasmon resonances at laser illuminated metal tips or particles. A semiclassical approach is compared to a quantum-mechanical one. Attractive as well as repulsive forces are possible depending on the wavelength of the optical field. The trapping potential is shown to be strong enough to overcome the Brownian motion in water solution for common optical tweezer light intensities. Single molecule resonance Raman spectroscopy probes are particularly well suited for the trapping scheme. Finally we propose intracellular probing of the function of biomolecules as an application.

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Optical manipulation of particles has been achieved in water solution at room temperature, by the use of optical tweezers and optical scissors [1]. This technology has developed into an important research tool in cell biology. The objects manipulated are cells, organelles, and larger molecules. The spot of manipulation, or attraction, is conventionally created by confocal concentration of the optical energy supplied by a laser. Another way of concentrating optical energy is by the surface plasmon resonance at nanoprobes, which has been suggested for nanometric optical tweezers [2], capable of ''tweezing'' smaller particles than the conventional optical tweezers, particles typically down to a few nanometers. Here we investigate the possibility that the nanoprobes should also be able to tweeze even smaller particles, if they are fluorescent molecules [3].

Optical detection and spectroscopy of single molecules have been achieved in water solution at room temperature. Particularly interesting is single molecule Raman spectroscopy (SERS) or single molecule resonance Raman spectroscopy (SERRS) [4–8] made possible by the surface plasmon resonance at colloidal particles acting as antennas or transformers amplifying the signal. The Raman signal is rather rich in structure compared to other optical spectroscopies and is well suited for precise molecule discrimination. Here we investigate the possibility of attracting molecules to the detection spots by optical means. We use the theory developed in Ref. [9] and compare it to some formulas derived here from a simple classical model [10] of a fluorophore.

We first assume that the molecule has the linear polarizability $\underline{\underline{\alpha}}$, which may be a second rank tensor (single underline = vector, double underline = second rank tensor). This means that the molecule acquires an oscillating dipole moment $\underline{p} = \underline{\underline{\alpha}} \cdot \underline{E}$ in the oscillating electric field $\underline{E}$. The molecule absorbs energy and reradiates it. The force exerted on the molecule is $\underline{F} = \Re[(\underline{\underline{\alpha}} \cdot \underline{E})^{\dagger} \cdot (\underline{E}\hat{\nabla})]$ or $F_k = \Re(E_n^* \alpha_{mn}^* \partial_k E_m)$, where $\Re$ means taking the real part. This can be divided into a scattering force and a gradient force [1]. The scattering force is pointing in the direction of the incident light. The gradient force is pointing in the direction of the intensity gradient and is described by $\underline{F}_{\text{grad}} = \frac{1}{2}\Re(\alpha)\nabla|\underline{E}|^2$ and is the force we consider here. It may also be described by a potential $U = -\frac{1}{2}\Re(\alpha)|\underline{E}|^2$. The reason for considering only the gradient force is the following. For an inhomogeneous plane wave the gradient force on the molecule is directed along the imaginary part of the wave vector, i.e., along the amplitude gradient. The scattering force is directed along the real part of the wave vector. This means that for a standing inhomogeneous plane wave there is only a gradient force, since a standing wave is composed of two waves in opposite directions, concerning the real parts of the wave vectors. From previous work done by the authors [11], it seems as if the huge field enhancement due to surface plasmon resonance may be mainly considered as a standing inhomogeneous wave around the particle.

Now we introduce the simple mechanical model of the fluorophore. It consists of an electron in a three-dimensional harmonic potential, which by definition has unity oscillator strength, a characteristic of strong fluorescence transitions. The radiation damping is introduced via a damping force on the electron. The equation of motion is $m_e(\ddot{\underline{r}} + \Gamma\dot{\underline{r}} + \omega_0^2\underline{r}) = e\underline{E}$. The polarizability of such a model fluorophore is then given by $\alpha(\omega) = \frac{e^2/-m_e}{\omega_0^2-\omega^2-i\Gamma\omega}$. The radiation damping is given by Fermi's golden rule combined with the conditions for unity oscillator strength to $\Gamma = \frac{\omega_0^2 e^2}{2\pi\varepsilon c^3 m_e}$. A realistic two-level molecule has an upper limit of the polarization (and the energy) which our model does not have. Therefore we limit the energy of the oscillations to $\hbar\omega_0$ which will give a saturated polarization of $\sqrt{(2e^2\hbar)/(m_e\omega_0)}$. Compare this to the (quantum-mechanical) dipole transition moment for unit oscillator strength $p_{\text{tr}} = \sqrt{(3e^2\hbar)/(2m_e\omega_0)}$. The dependence of the potential on the field strength is quadratic for an unsaturated and linear for a saturated dipole moment, which is less efficient.

The quantum-mechanical treatment is based on the optical Bloch equations and the dressed atom approach

and is taken from Ref. [9]. Only gradient forces are considered. We consider a molecule with two electronic states in a photon field with an amplitude gradient. By Ref. [9] the gradient force is described by a potential given by

$$U = \frac{\hbar(\omega - \omega_0)}{2} \ln\left(1 + \frac{\frac{\Omega^2}{2}}{(\omega - \omega_0)^2 + \frac{\Gamma^2}{4}}\right),$$

where $\Omega = \frac{\underline{p}_{\mathrm{tr}} \cdot \underline{E}}{\hbar}$ is the Rabi frequency and $\underline{p}_{\mathrm{tr}}$ the transition dipole moment.

We also see a saturation in this case; the potential has a logarithmic dependence on the electric field when "saturated."

Now to the field enhancement due to surface plasmon resonance. The optical field enhancement may be rather large. From Refs. [4,12] the SERS signal enhancement may be 10 to 15 orders of magnitude. Since the SERS signal enhancement goes as the fourth power of the amplitude enhancement (SERS signal is power enhancement two ways), the amplitude enhancement is 300 to 5000. The enhanced field is strongly evanescent and short range. Since a broad range of photon modes is coupled to the strong field enhancement, the rate of spontaneous emission from a molecule in the enhancement region is also affected. We may change $E \rightarrow AE$ for the electric field and $\Gamma \rightarrow \gamma A^2 \Gamma$ for the rate of spontaneous emission, where A denotes the amplitude enhancement and γA^2 a weighted average of the enhancement of the different possible modes. The square dependence for the spontaneous emission rate is due to the square dependence of the transition matrix element in the Fermi golden rule. The density of photon states is not affected by the resonator since the states look the same far from the resonator as they would without it. γ is about one-third, $\langle \cos^2\theta \rangle = \frac{1}{3}$, for a field enhancement particle small compared to the wavelength and less than one-third otherwise.

Now we will compare this with the Purcell enhancement of spontaneous emission in a resonant cavity [13]. Purcell discusses a change of the density of states rather than a change of the transition matrix element which amounts to the same emission rate. Instead of a cavity we here talk about a resonator in general, capable of field enhancement. We also assume that the only losses are from radiation to the outer plane wave photon modes (this may be different from the original Purcell assumption). The Purcell enhancement factor is $f = \frac{3Q\lambda^3}{4\pi^2 V}$, which is the ratio of the spontaneous emission rate in the resonator to the spontaneous emission rate in free space. Let the energy inside the resonator be W and the power out from it be P. In steady state the same amount of power should go into the resonator. From the definition of the quality factor Q we get $P = \frac{\omega W}{Q}$. The energy is assumed generated by an emitter (atom or molecule) which is emitting at a rate proportional to Q due to the Purcell effect, i.e., $P \propto Q$. From this we get that $W \propto Q^2$, and it follows that the amplitude inside the resonator is proportional to Q. But the amplitude enhancement is the ratio of the amplitude inside to the amplitude outside the resonator. The square of the amplitude outside is proportional to P which means that the square of the amplitude enhancement is proportional to Q, i.e., $A^2 \propto \frac{W}{P} \propto Q$. By the Purcell enhancement formula we then get that the spontaneous emission enhancement factor is proportional to the square of the field enhancement, i.e., $f \propto A^2$, which agrees with the result above. See also the discussion on page 3 of Ref. [14].

The increased spontaneous emission rate affects the trapping potential capabilities by making it lower. There is also a good side of the increased spontaneous emission. In Ref. [9] a dipole diffusion coefficient for the molecule momentum due to the optical field is $D_{\mathrm{dip}} \sim \frac{\hbar^2(\nabla\Omega)^2}{\Gamma}$, and it is warned that the diffusion constant increases more rapidly with the laser intensity than the trapping potential. Fortunately, the spontaneous emission rate also increases rapidly with field enhancement and balances it out in the diffusion constant. The diffusion constant above, D_{dip}, is for the particle momentum. The diffusion constant for the particle position is given by $D_{\mathrm{pos}} = \frac{D_{\mathrm{dip}}}{K^2}$, where K is the friction coefficient of a particle moving in a viscous fluid. For a spherical particle of radius r it is given by $K = 6\pi r\eta$ where η is the viscosity. The normal diffusion constant in a viscous liquid is given by $D = \frac{kT}{K}$. For a calculation of a typical measure of the diffusion constants we use a molecule of diameter 1 nm, and the values in Fig. 2. We get $D_{\mathrm{pos}} = 3.8 \cdot 10^{-11} \frac{m^2}{s}$ which should be compared to $D = 4.1 \cdot 10^{-10} \frac{m^2}{s}$ which is about an order of magnitude larger. If the increase of the spontaneous emission rate is not considered, the diffusion constant due to the optical field may be orders of magnitudes larger than the ordinary diffusion constant which would be disastrous for the trapping.

As seen in Fig. 1 the three descriptions give rather different results. They all coincide far from the resonance. Near the resonance the saturation comes into play. It is unfortunate that the simple unsaturated classical model does not work; it should have given an easy way to manipulate the fluorophore at a very specific frequency. That effect should have been even more pronounced than in Fig. 1 if the decay rate did not increase (the decay rate also has a positive effect; see above). The modified simple model, with saturation, also shows better trapping compared to the most realistic model, from Ref. [9]. The most realistic model still shows trapping but it is not very precise in frequency.

Figure 2 shows the trapping potential at a spheroid. The calculation of the field enhancement uses spheroidal vector wave functions and is rather exact [11]. Other shapes with sharper ends would show stronger field enhancement. The enhancement is strongest at the surface of the ends, attracting molecules to the spot most sensitive for Raman spectroscopy (SERS). One may think that the

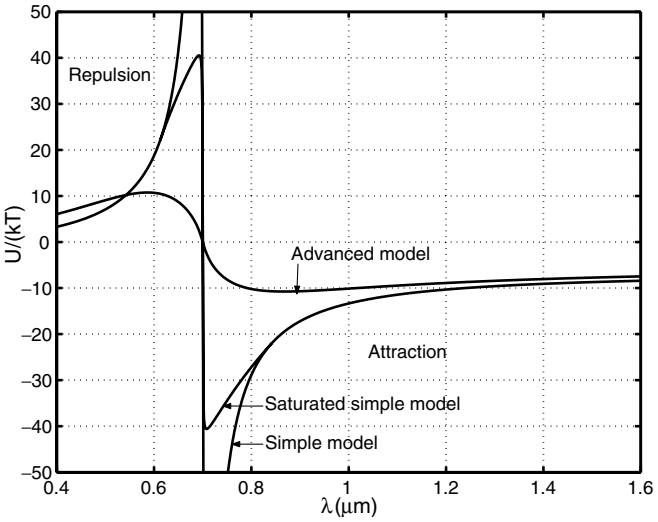

FIG. 1. The potential acting on a unit oscillator strength fluorophore. The laser intensity at the probe is 50 $\mathrm{mW}/\mu\mathrm{m}^2$, the field enhancement at the probe is 1000, the surrounding medium is water, and the resonance wavelength of the fluorophore is 0.7 μm. Unfortunately the most realistic model has the poorest trapping potential, but still it has a trapping potential of 10 kT at $\lambda = 0.8$–1 μm, which is enough to overcome the Brownian motion. A real probe has its own resonance frequency, taking out only a small portion of the diagram. The wavelength region $\lambda = 0.8$–1 μm is particularly convenient to minimize destruction of cell components.

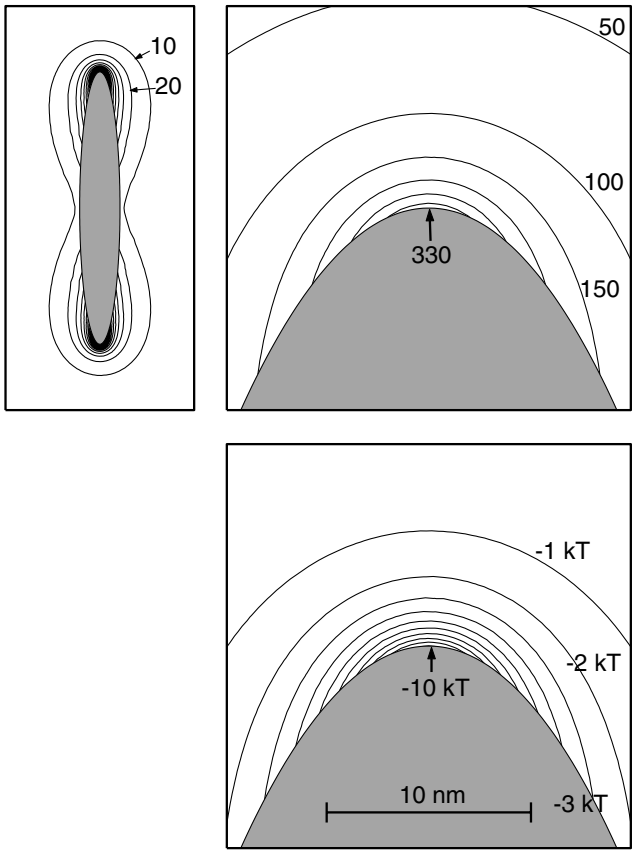

FIG. 2. This figure shows the field enhancement and the corresponding trapping potential at a silver spheroid in liquid water. The incoming beam is from the left at 90° to the main axis. The polarization is parallel to the main axis. Spheroidal length is 0.2 μm, axial ratio 6.746, wavelength 1.1 μm, field intensity before enhancement 500 $\mathrm{mW}/\mu\mathrm{m}^2$. Upper left: Overall view of the enhancement. Upper right: Magnification of the top region. Lower: The corresponding trapping potential.

resonance properties of the molecule may change near the surface for some reason, for example, the Stark effect if the probe is charged and the tip is exhibiting a strong dc electric field. In that case the force may change before the surface is reached and the molecule is trapped a small distance from the surface. In the case of a dc electric field at the tip, dc dipole forces may also come into play.

In order for this trapping technique to work well, one may have to put some effort on probe optimization. Theoretical calculations on simple shapes have been done, for example, on ellipsoids [11] where high field enhancement is achieved at the ends of the more elongated shapes. It has also been achieved between almost touching spheres [12]. High field enhancement seems to have been achieved experimentally on irregular shapes where the hot spots are believed to be at sharp irregularities or between particles [12]. Design criteria for a good fluorophore trap would be high enhancement at as large a volume as possible, together with coupling to a broad range of photon modes to increase the spontaneous emission rate.

Finally we propose intracellular probing of the function of biomolecules. A silver or gold particle, preferentially elongated, designed to have a spot for SERS (hot spot) is inserted into a cell and controlled by optical tweezers; see Fig. 3. A trapping optical field is created at the detection spot, via plasmon resonance. The plasmon resonance frequency may be altered by different polarization or angle of incidence of the incoming beam; see Ref. [11]. A fluorescent molecule is selectively attracted to the detection spot and trapped. Possible fluorescent molecules nearby may be found by regular ''single molecule fluorescence detection.'' Single molecule SERS spectra of the trapped molecule are recorded. The molecule is then released by making the optical potential repulsive. Thereafter another molecule may be selectively attracted and trapped to the detection spot.

Raman spectra are rather detailed in structure and contain lots of information. Therefore we also propose the optical probe for intracellular single DNA sequencing, using the following scheme, somewhat similar to the one suggested in Ref. [15]. The probe traps a DNA-binding protein that is doing some action on the DNA bases in sequence, for example, helicase. SERS spectra are recorded while the protein is doing its action. The bases of the DNA at the binding spot of the protein may change

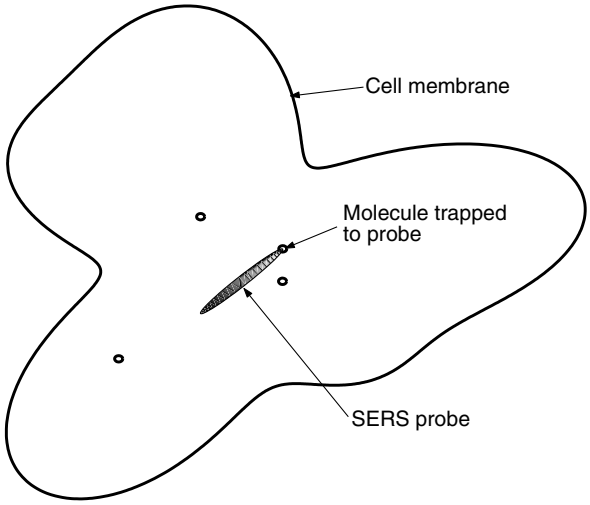

FIG. 3. A schematic picture of a probe inside a cell.

the spectra of the protein-DNA complex such that the different bases may be resolved as they pass.

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