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FINAL REPORT

MODELING SINGLE MOLECULE DETECTION PROBABILITIES IN MICRODROPLETS

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Prepared for:

Michael D. Barnes
Chemical and Analytical Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN

Prepared by:

Steven C. Hill
Physicist
Army Research Laboratory
AMSRL-BE-S
White Sands Missile Range, NM 88002-5501
(505)678-4078, 2432 (Fax)

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Modeling Fluorescence Collection from Single Molecules in Liquid Microspheres

Steven C. Hill
Army Research Laboratory White Sands Missile Range, NM 88002-5501

Final Report

Optimization of molecular detection efficiencies is of central importance in analytical applications involving single molecule detection [1]. In addition to limitations imposed on the fraction of molecules which can be detected by the average signal-to-noise ratio, experimental factors such as excitation inhomogeneity and molecular diffusion conspire to further limit "molecular detectability." Recent single molecule detection experiments in microdroplets suggest that such experimental limtations can be significantly reduced [2] primarily because the molecule cannot diffuse away from the excitation volume. However, unlike fluorescence detection from bulk streams where the fluorescence intensity is isotropic in space, the large refractive index change at the surface of microdroplets implies that the fluorescence intensity collected by a lens will be strongly dependent on the position of the molecule within the droplet. In addition, the same refractive index discontinuity at the droplet surface produces a complicated excitation intensity distribution within the droplet. Thus, issues such as whether molecules near the surface of the sphere can "hide" from the detector as a result of total internal reflection of emission near the droplet surface, or poor excitation efficiency due to the molecule being located in a "shadow" region of the droplet will have a potential effect on molecular detection efficiencies. Here we discuss numerical tools for modeling the fluorescence collected from a single molecule within a microsphere as a function of its position and orientation, the size of the droplet, the numerical aperture of the collection lens, the detection geometry, the type of illumination (planewave or counterpropagating plane wave), and the linewidth of the emitting molecule.

To model the fluorescence from single molecules (point sources) within microspheres we use a semiclassical formalism [3], in which the molecule is modeled as a dipole emitting at a single frequency. The fields radiated by the dipole are expressed in spherical coordinates. The additional fields induced inside and outside the sphere are determined by matching the boundary conditions.

Figure 1 illustrates the fluorescence collected from various points inside a 8 μm diameter droplet. The lens is positioned along the z axis and has a numerical aperture of 0.5. The dipole is assumed to rotate rapidly relative to the fluorescence lifetime, and so dipole orientation effects are averaged, and the results are independent of the azimuthal angle. Enhancement or inhibition of rates (predicted at a single frequency) has not been observed when the droplet is large enough that the fluorescent bandwidth extends over several morphology-dependent resonances (MDRs) of the droplet. To approximate the actual situation, we assume a Lorentzian lineshape function for the emission from the molecule, and integrate over the emission wavelengths. Because the MDRs of the droplet can have large effects even though their linewidths may be narrow, we approximate the fluorescence collected as a non-resonant background and a number of Lorentzian functions. The integration over the products of the Lorentzians is then done analytically.

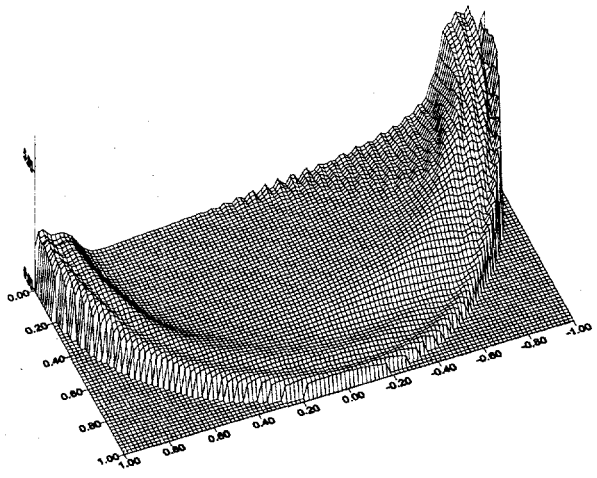

Figure 1: Fluorescence collected from randomly oriented dipoles. The emission is integrated over frequency when the linewidth is 100 cm^{-1}, and the center frequency of the transition is 16666.7 cm^{-1}. The diameter of the droplet is 8 μm, and the refractive index is 1.34. The NA of the collection lens is 0.5. The results are shown as a function of the normalized positions inside the sphere, x/a and z/a, where a is the radius of the sphere.

Figure 2 shows the internal intensity of a sphere (8 μm diameter, with a refractive index of 1.34) illuminated with a plane wave. Figure 3 shows the internal intensity of the same sphere illuminated with counterpropagating plane waves.

Figure 4 illustrates the fluorescence collected from one cross-section of a sphere illuminated with a plane wave. The fluorescence collected is the product of the internal intensity generated with a plane wave, and the normalized fluorescence collected from a randomly oriented di-

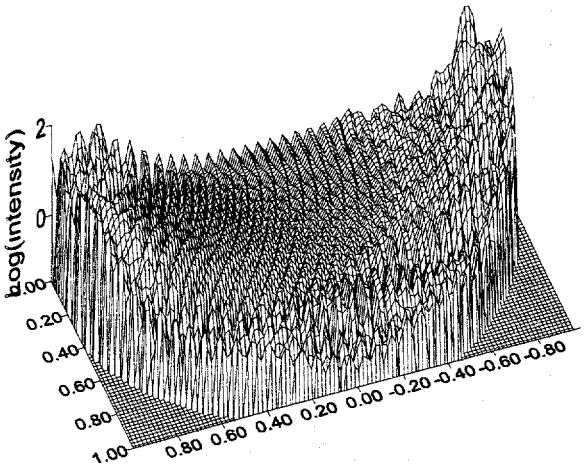

Figure 2: Internal intensity (log scale) of a 8 μm diameter sphere (with a refractive index of 1.34) illuminated with a plane wave propagating in the z direction.

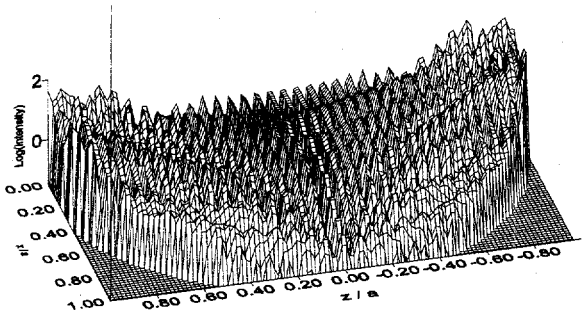

Figure 3: Internal intensity (log scale) of a 8 μm diameter sphere (with a refractive index of 1.34) illuminated with counterpropagating plane waves polarized parallel to the scattering plane.

pole (emitting with a center frequency of 16666.7 cm^{-1} and a linewidth of 100 cm^{-1}). Therefore, it has no line of symmetry as do Figs. 1-3. The shadow region is apparent, as are the high-intensity regions along the z axis. The collection of more light from dipoles on the side away from the lens is also apparent.

We find that the collected intensity depends on the position and orientation of the dipole, the numerical aperture of the collection optics, the emission wavelength(s), and the size of the sphere. When the dipole is randomly oriented, or when the NA or emission frequency bandwidth increase, the dependence on position decreases. In larger spheres there are regions from which only little fluorescence is collected. For example, a 15 μm diameter water droplet has regions from which only $1/30^{th}$ of the emission from a dipole in free space is collected, even when the NA=0.5. These results suggests that, with respect to

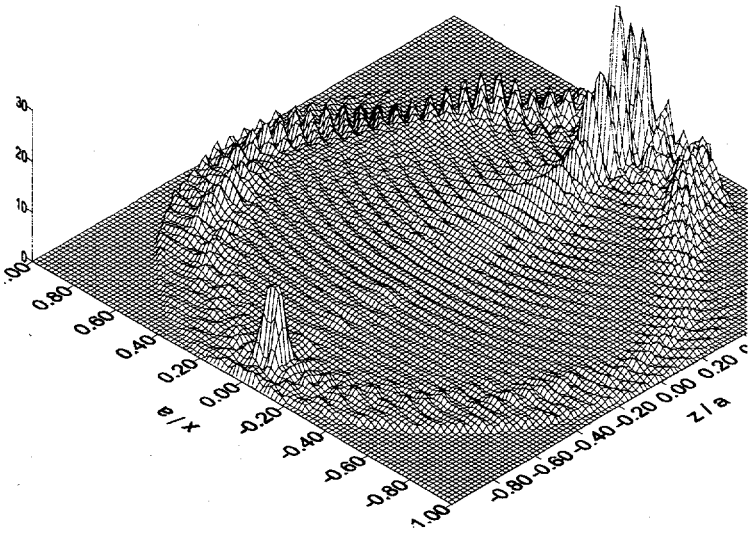

Figure 4: Fluorescence collected from randomly oriented dipoles inside a 8 μm sphere illuminated with a plane wave. The fluorescence emission is integrated over frequency. The emission linewidth is 100 cm^{-1}, and the center frequency of the transition is 16666.7 cm^{-1}. The refractive index of the droplet is 1.34. The NA of the collection lens is 0.5, and the lens is on the x axis (90 degrees from the direction of the incident wave).

collection of fluorescence, smaller droplets are more useful for single molecule detection. When the molecule is assumed to have a nonzero emission linewidth, and the emission is integrated over frequency, the strong resonance effects on the emission are much weaker as the homogeneous linewidth approaches the free spectral linewidth of the cavity modes. We are also examining the molecular diffusion, and the effects of photobleaching in order to obtain a realistic model of molecular detection efficiencies in microdroplets.

References

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[3] H. Chew, P. J. McNulty, and M. Kerker, "Model for Raman and fluorescent scattering by molecules embedded in small particles," Phys. Rev. A. **13**, 396-404 (1976).

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