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FINAL REPORT

MODELING SINGLE MOLECULE DETECTION PROBABILITIES IN MICRODROPLETS

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Modeling fluorescence collection from single molecules in liquid microspheres

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Optimization of molecular detection efficiencies is important for analytical applications of single molecule detection methods. In microdroplets some experimental limitations can be reduced, primarily because the molecule cannot diffuse away from the excitation and collection volume[1]. "Digital molecular detection" using a stream of microdroplets has been proposed as a method of reducing concentration detection limits by several orders of magnitude relative to conventional measurements. However, the bending and reflection of light at the microdroplet's liquid-air interface cause the illumination intensity and fluorescence intensity collected to be strongly dependent on the position of the molecule within the droplet. Our goal is to model the detection of single molecules in microdroplets so that we can better understand and optimize detection efficiencies.

In the first year of this modeling effort we studied the collection of fluorescence from unit-amplitude dipoles inside of spheres[2]. The frequency-integrated normalized fluorescent power collected by an objective was integrated over a normalized Lorentzian lineshape function. We found that the position dependence of the fluorescence collected was markedly decreased as the N.A. of the collection optics was increased. We also found that the very low intensities collected at most frequencies from some regions near the outer edge of the sphere were dramatically increased when the linewidth of the dipole spanned several morphology dependent resonances (MDRs) of the sphere and when the NA of the lens was increased. In the first year we also initiated our efforts to study the effects of excitation inhomogeneities, and the effects of illuminating with counterpropagating plane waves.

In this second year we modified our analysis to accurately model the effects of excitation inhomogeneities, including effects of molecular saturation, motion of the droplet, and phase variations between the two counter-propagating waves that illuminate the droplet. We showed that counter-propagating plane wave illumination can decrease the variations in the intensity which excites the molecules[3].

Also in this second year we simulated (using a Monte Carlo method) the detection of fluorescence from many droplets, each of which may contain zero, or one (or at higher concentrations, a few) fluorescent molecules. The model includes the effects of molecular diffusion and photobleaching, illumination and collection geometry, detector noise, and interference from Raman emission. We also discussed detection limits in microdroplets. We made detailed calculations of photocount statistics for single-molecules in microdroplets and examined the effects of fluorophore diffusion and illumination geometry on the distribution of single-molecule photocounts in viscous (glycerol) and non-viscous (water) liquid microdroplets. These two examples represent limiting cases in which the RMS diffusion length (on the time scale of the measurement) is either small or large compared to the droplet diameter and result in significant differences in calculated single-molecule photocount distributions and molecular detection efficiencies. The calculated distributions illustrate the effect of spatial inhomogeneities in the fluorescence collection from single molecules within the droplet, and allow quantitative estimates of molecular detection efficiencies as a function of average signal-to-noise ratio.

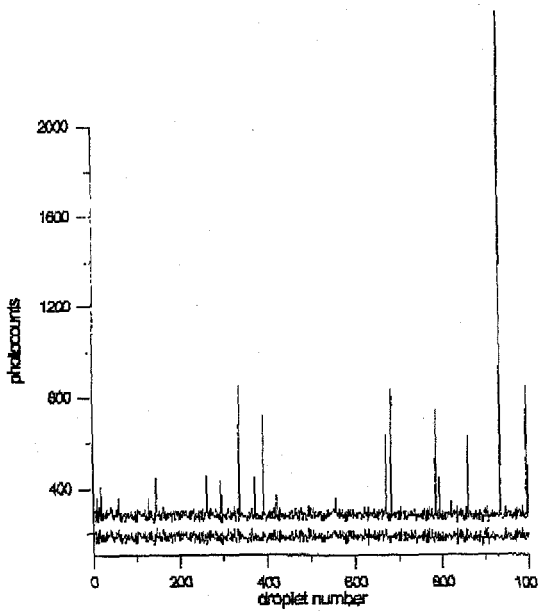

Figure 1: Photocounts as a function of droplet number for a series of 1000 droplets. The lower curve is for blank droplets (each with no fluorescent molecule). The upper curver has been offset for comparison.

The results calculated were selected to: 1) model previously reported[1, 4, 5] and ongoing experiments, 2) illustrate variations in some of the parameters in ways that would be extremely time-consuming to do in the laboratory, 3) help in understanding the effects of optical inter-

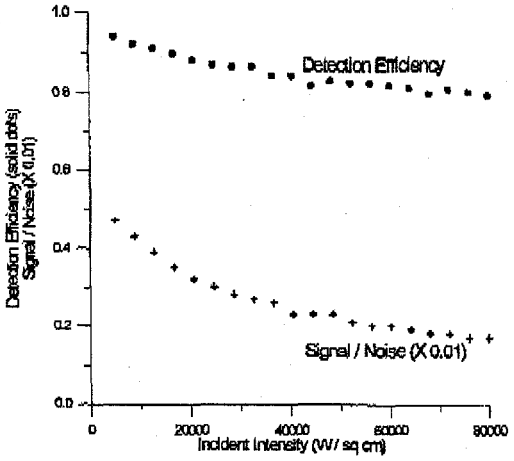

Figure 2: Detection efficiency and signal to noise ratio as a function of incident intensity.

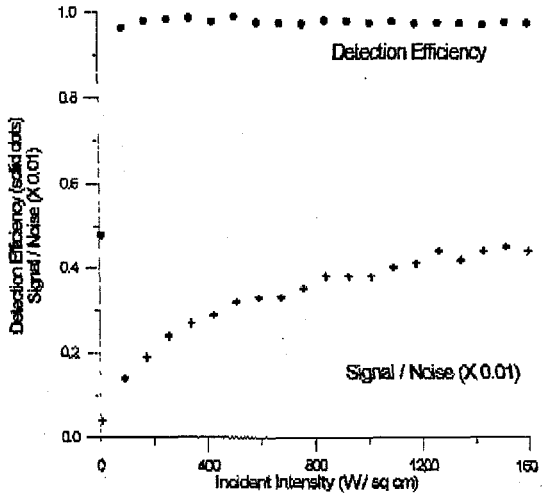

Figure 3: Detection efficiency and signal to noise ratio as a function of incident intensity.

actions with the droplet on the detection probability, and 4) suggest experimental variations in which the detection efficiencies may be increased.

For the results shown, the incident waves propagate in the $\pm z$ directions. The collection lens is centered on the x axis and has a NA of 0.42. Results are shown for two classes of droplets: water droplets with ~ 0.75 μm diameters, and glycerol/water (85 % glycerol and 15 % water) droplets with 6.5 μm diameters.

Figure 1 illustrates the number of photons collected per droplet from a sequence of 1000 glycerol/water (85 %) droplets. Each has a diameter of 6.5 μm. The average number of R6G molecules per droplet is 0.02. Droplets having 0 or 1 molecules are observed. The lower curve shows counts from the blank (no R6G) droplets. The upper curve has been offset by 200 counts to allow comparison. The illumination intensity is 40,000 W/cm^2, and the illumination/collection time is 100 ms. The Raman scattering from the droplet is more important than the scattering from gasses in the cell.

A primary figure of merit for a single molecule detection scheme, is the molecular detection efficiency, the probability that the photons detected from a single molecule exceed some threshold. A typical value for the threshold, the value of the threshold used here, is the sum of the background ("blank") signal and three times the standard deviation of background.

Figures 2 and 3 show the molecular detection efficiency and the signal-to-noise ratio as a function of the incident intensity. In Figs. 2 and 3 the droplets are glycerol/water (85%), are in the beam for 100 ms, and have 6.5 μm diameters. The incident intensities in Fig. 3 were chosen to illustrate the detection efficiencies within the range of intensities typically used in experiments. The intensities in Fig. 3 were chosen to illustrate the range where the detection efficiency is largest and where the detection efficiency and S/N show the most variation.

Figures 2 and 3 suggest that the models can help in opimizing experimental arrangements for single molecule detection in microdroplets.

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