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ULTRASENSITIVE MOLECULAR FLUORESCENCE SPECTROSCOPY IN LEVITATED MICRODROPLETS

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The extreme sensitivity of fluorescence spectrophotometry results from the fact that a molecule can undergo many excitation-emission cycles before destruction by photochemical degradation. For example, Rhodamine 6G (R6G) can emit in excess of 10^5 photons before photolysis takes place (1). The fraction of emitted photons collected and converted to countable pulses can be as high as 10^{-3}, although 10^{-4} is more readily attainable. Therefore, sufficient signal exists for single molecules to be detectable. Detection limits for molecules in solution have been limited by background signal from solvent Raman scattering and fluorescence. This background signal adds noise to the measurement and has effectively restricted the detectable concentration to about 10^{-13} *M*. Over the past decade, advances in detection of fewer molecules have all been made by reducing the measurement volume and/or increasing the measuring time. Given the above concentration detection limit a reduction of the measurement volume to 1 pL leads to a minimum observable quantity of ≈ 1 molecule. The ability to detect a single molecule in condensed phase could have many important applications in addition to being an interesting problem. The obvious application of this approach is to situations where small quantities of material are available for analysis. The capability to reliably detect a single fluorophore might also allow the screening and/or sorting of a collection of molecules. Such abilities would have application to many biological problems such as DNA sequencing and detection of DNA adducts.

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Measurement volumes have been reduced to date by modifying a flow cytometry apparatus (2). A sheath flow cuvette is used to hydrodynamically focus sample solution to a narrow (10-μm diam.) stream. This narrow stream of sample can be crossed with a focused laser beam to define an analysis volume of a few picoliters. This approach has made it possible to observe as few as 800 R6G molecules (3). In an attempt to minimize solvent background, Kirsch evaporated samples on 10-μm silica spheres and observed them with a fluorescence microscope (4). Their technique allowed the detection of 8000 R6G molecules. Keller more recently reported single molecule detection in condensed phase using the sheath flow cuvette, by use of a protein molecule (MW $\approx$ 250,000) which has a fluorescence yield equivalent to 25 R6G molecules (5).

In our experiments the measurement volume is reduced by observing a single microdroplet of solution (6). A 10-μm diameter spherical droplet has a volume of 0.5 pL. Such a droplet of sample solution can be generated on demand for fluorescence analysis. These droplets contain a large number of elemental charges on their surface and thus can be influenced by an electric field. The charged droplets are trapped in an electrodynamic trap that holds them stationary in space to within a fraction of a diameter. A laser is then used to excite fluorescent molecules contained within the droplet. There are a number of potential advantages of this approach over the sheath flow cuvette technique. The measurement time can be independently controlled in the case of the trapped droplet. The velocity and measurement volume determine the measurement duration for flowing streams. Diffusion of the analyte from the analysis volume is less likely for a levitated droplet. In addition, sorting at the molecular level is possible with the individual droplets.

In our initial experiments, solutions of R6G in glycerol have been studied. Sample solutions are made by diluting stock R6G/Methanol solutions in 99.8% glycerol which are then further diluted 9:1 with HPLC grade H_2O. The water is added to improve droplet formation and is assumed to completely evaporate in a few seconds reducing the droplet volume. The water is neglected in determining the solution concentrations and number of molecules in a droplet although the droplets contain several percent H_2O at equilibrium with the laboratory atmosphere. Experiments have been performed with as few as 11 $\pm$ 3 analyte molecules in 13-μm diameter droplets of glycerol. An average of 340 photons are detected per R6G molecule. Detection is limited by blank fluorescence signal. Presently, the blank corresponds to a signal of $\approx$ 0.8 p*M* R6G or $\approx$ 0.5 molecules per droplet. The detection limit (figure of merit) is defined as the noise in the blank counts divided by the counts per molecule giving $\approx$ 0.4 R6G molecules for these experiments.

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