

Single Molecule Detection

Introduction

The ultimate limit to analytical sensitivity is the reliable detection of single molecules. Recent technical advances in optical detection and manipulation have made the detection of isolated, light emitting probe molecules a reality. Thus we are witnessing a burgeoning interest in the imaging and spectroscopy of single molecules, particularly within the fields of cell biology and drug discovery. Of particular importance to biology is the possibility of direct, real-time visualization of single biological macromolecules and their assemblies under native physiological conditions, offering great promise for enhancing our understanding of the behavior, interactions, mechanisms and trafficking of individual biological macromolecules within the living cell. Such studies have increased medical and pharmaceutical significance within the developing post-genomic era of proteomics, providing the means to track the behavior, kinetics and mechanistic involvement of biochemically relevant single proteins, such as enzymes.

It is reasonable to ask why it is interesting to observe the emission from a single fluorophore, since numerous fluorophore labels can be detected within a diffraction-limited spot. There was a time when the scientific community was content with detecting the combined signal of a vast number of fluorescent (or Raman active) molecules from a point of interest within the sample. There is a rapidly growing realization however, particularly within the life science and materials science fields, that the information content afforded by imaging the single fluorophore markedly exceeds that offered by the "bulk" ensemble measurement, yielding invaluable insight into individual molecular properties and their micro-environment. Experiments on single molecules have attracted vivid interest in many branches of fundamental research because they allow for the study of molecular properties normally disguised in inhomogeneous distributions of an ensemble. In contrast to observing the position of several labeled molecules at a given position, single molecule detection provides additional information, such as:

- Polarization or image spot shapes indicate orientation and reorientation.
- Time traces of intensity, emission spectrum, or fluorescence lifetime provide information on local dynamics and diffusion.
- Fluorescence Resonance Energy Transfer (FRET) provides information on the proximity of specific labeled sites less than 10nm apart, allowing for detailed probing of reaction mechanism.
- Position sensitivity allows an investigator to locate a molecule and follow simultaneously the translational motion, re-orientational motion, and the internal dynamics of the individual molecules.
- Single molecule detection has opened the way to a new effective approach for achieving sub-diffraction limit 'super-resolution' microscopy ('nanoscopy'), based on the ability to employ centroiding of the single fluorophore signals to enable highly accurate pinpointing of their position within the labeled cell.

Single molecule studies are uniquely poised to yield information about molecular motion, behavior, and fluctuations over time and space. There are many biological molecules that can avail from examination at this level, typical subjects being key members of a system that are receptive to specific cellular signals, environmental perturbations or drug intervention. Cellular mechanisms that have been examined include ion channel activity, protein folding, enzyme activity, membrane structure, molecular motors/motility and vesicle transport. Single molecule detection is a way to study detailed physical and chemical properties that allows for scrutiny of fundamental principles and mechanisms, and may lead to technological and methodological developments. Single molecule techniques also have key potential in material development. The single molecule is an excellent probe of local (nanoscale) properties since it is a quantum light source with spectrum and lifetime that is sensitive to its chemical and physical environment. The rotational and translational motion of single molecules can be measured and used to understand the local mechanical properties of the material in which the molecules are embedded. Thus groups are seeking to understand and catalog the great variety of behaviors of single molecules in technologically relevant environments to better use these probes to study the small-scale structure.