1-1-6. Single-Molecule Spectroscopy

Overview

Single-molecule spectroscopy is a technique that enables the optical detection and analysis of individual molecules, thereby directly revealing molecular heterogeneity and fluctuations that are averaged out in conventional ensemble measurements. Since its first report in 1989, single-molecule spectroscopy has expanded from elucidating the photophysical behavior of single fluorophores to investigating reaction mechanisms of biomolecules and photophysics of semiconductor nanomaterials. With the subsequent development of super-resolution microscopy, single-molecule spectroscopy has become established as a fundamental tool in modern science, with future applications anticipated in molecular medicine and quantum sensing technologies.

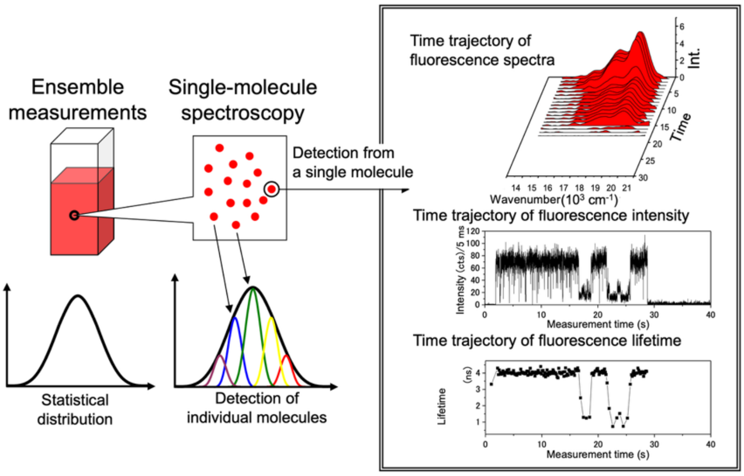

Figure 1. Schematics of single-molecule spectroscopy.

Current Status and Frontiers

Single-molecule spectroscopy originated in 1989 when Moerner and Kador detected the absorption of a single molecule under cryogenic conditions. In the following year, Orrit and Bernard succeeded in observing the fluorescence of a single molecule under the same conditions, opening a new avenue for single-molecule spectroscopy. By the mid-1990s, room-temperature detection became feasible, leading to rapid advancement. Early studies focused on phenomena such as fluorescence blinking and spectral diffusion of single fluorophores—behaviors inaccessible to ensemble measurements. Later, the approach was extended to more complex systems, including dendrimer-based multichromophoric molecules for probing FRET and electron transfer, single conjugated polymer chains, quantum dots, and organic nanocrystals. In catalysis and photochemical reactions, single-molecule methods revealed variations in reaction pathways and transient intermediates hidden in ensemble average. Notably, single-molecule spectroscopy enabled the first observation of photon antibunching, a hallmark of single-photon emission, which is crucial for emerging quantum information technologies.

In life science, single-molecule spectroscopy has provided powerful insights into enzyme reaction pathways, stepping motions of molecular motors, and fluctuations in transcription and translation at the single-molecule level. Advances in single-molecule FRET, total internal reflection fluorescence microscopy (TIRFM), fluorescence correlation spectroscopy (FCS), Raman spectroscopy, along with the development of single-photon detectors, high-sensitivity CCD cameras, and microfluidic devices, have enabled the extraction of multidimensional information.

A landmark achievement derived from single-molecule detection is super-resolution microscopy, reported by Betzig and colleagues in 2006. By exploring single-molecule blinking and reconstructing fluorophore positions with nanometer precision, methods such as PALM (Photoactivated Localization Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy) surpassed the diffraction limit of conventional optical microscopy. Together with the STED (Stimulated Emission Depletion Microscopy) technique developed by Hell, these advances were recognized with the 2014 Nobel Prize in chemistry awarded to Moerner, Betzig, and Hell, highlighting the significance of single-molecule spectroscopy and its derivative technologies. Today, single molecule spectroscopy has become a key spectroscopic technique, widely applied in chemistry, material science, biology, and medicine.

References

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Future Perspective and Directions

Challenges to be addressed and goals for the next decade: Improvement of temporal and spatial resolution, observation under physiological conditions, and stabilization of fluorescent probes. Progress in these areas is anticipated to accelerate applications not only in fundamental science but also in medicine, material science, and quantum technologies.

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