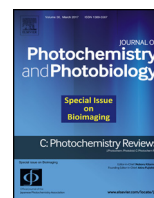




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Invited Review

Single-molecule studies beyond optical imaging: Multi-parameter single-molecule spectroscopy

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ABSTRACT

Single molecule spectroscopy has undergone a remarkable development in the past few decades, and its ability to unmask the unique features of individual molecules has found increasing use in research of soft matter and polymers, in chemistry, as well as in biophysics and biology. In this concise review we bring an overview of the synergy effects that result from combinations of single-molecule and single-particle fluorescence spectroscopy with other techniques, such as electron microscopy and scanning probe microscopy, or with external-field effects including hydrostatic pressure and electric and magnetic fields.

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1. Introduction

Single molecule spectroscopy (SMS) has undergone a remarkable development from its origins as a technique in low-temperature high-resolution spectroscopy [1,2] to applications in super-resolution fluorescent microscopy [3]. Its ability to unmask the unique features of individual molecules has found use in low-temperature physics and optics [4,5], in research of soft matter [6], polymers [7,8], semiconducting nanocrystals [9] and materials [10], in the general field of chemistry [11,12] including catalysis [13] and physical chemistry [14], and increasingly in biophysics and biology [15–17], with a particular emphasis on super-resolution imaging [18–20]. Detection of single molecules allows the measurement of various photophysical parameters related to fluorescence such as spectra and spectral diffusion, fluorescence intensity fluctuations (intermittency or blinking), fluorescence lifetime, excitation and emission polarization, and photon anti-bunching. In the field of, e.g., polymer physics measurement of these phenomena on the level of single chains lead to an unprecedented insight into photophysics of conjugated polymers [21–24]. Apart from the photophysical properties, microscopy of individual molecules enables precise determination of the molecular orientation and location, and using again the example of polymer physics measurement of these parameters reveals nanoscale mechanism of polymer diffusion and relaxation [25–27]. Molecular localization with sub-diffraction precision [28] has ultimately lead to the development of the super-resolution fluorescence microscopy [29–31].

Even though the localization-based super-resolution fluorescence microscopy has brought a qualitative improvement in spatial resolution, there is still a considerable gap between the size of many nano-objects and the practically attainable resolution in fluorescence. It is, therefore, challenging to use the same technique and instrumentation for both imaging the size and structure of

a nano-object and for studying its optical properties. A possible solution to this dilemma is a use of synergetic combination of high-resolution microscopy and single molecule spectroscopy. The synergy of the sub-nanometer to atomic resolution in structural imaging and single-molecule sensitivity in photophysical characterization will enable addressing some of the outstanding issues in chemistry, physics or materials science. In the field of chemistry, for example, it can bring the ability to accurately predict the structure of complex synthetic macromolecules or supramolecular constructs and to correlate such structures to physical-chemical properties and location-sensitive reactivity. In solid-state catalysis it may enable correlation of catalytic activity with the catalyst crystal structure and composition, or with its porous dimensionality and structure. In physics, it will bring together the atomic-level structure of semiconducting nanocrystals, plasmonic nanoparticles, 2D materials and other low-dimensional systems with their optoelectronic properties. In addition, scanning probe techniques will add the possibility of examining or modifying the optoelectronic functionality of such systems and their prototypical devices. As will be shown below, the first steps of many of these envisioned applications have been realized, including works on different types of semiconducting nanocrystals of mainly the II–VI group as well as on the emerging halide perovskites, or on plasmonic nanostructures.

One of the aims of this short review is to provide an overview of recent efforts in this direction of research. The Section 2 summarizes technical developments and results obtained by combined transmission electron (TEM) and scanning electron (SEM) microscopies and fluorescence microscopy on individual nano-objects. In Section 3 the combination of single-molecule spectroscopy with scanning probe microscopies is reviewed. Unlike electron microscopy, fluorescence and scanning probe microscopies can be combined within one instrument. This brings an advantage of the possibility of simultaneous imaging with the two techniques, and also of possible manipulation of the nano-object by the probe and its immediate effect on the fluorescence properties. Such manipulations may include mechanical distortions or applications of electric field and current. These types of external effects have been applied on single molecules and nano-objects also by means other than the probes of scanning microscopes, and such works are reviewed in Section 4, with an emphasis on the effects of hydrostatic pressure, electric field and magnetic field on the photophysical properties and processes in single molecules.

2. Single-molecule spectroscopy and electron microscopy

2.1. SMS and TEM

Combined imaging by fluorescence microscopy and spectroscopy and by transmission electron microscopy can be done by subsequent measurements of well-marked samples in the two instruments. The biggest challenge is posed by the differences in resolution and view-fields in both methods. Use of very thin silicon nitride plates that are non-fluorescent and transparent for electron beams as substrates for measurements of single semiconductor quantum dots [32] enabled a systematic investigation of the correlation between crystal structure and photophysical properties of individual colloidal quantum dots of CdSe capped with ZnS shell. The results indicated that presence of stacking faults or even polycrystallinity in individual nanoparticles do not cause fluorescence quenching or decrease of quantum yield. Fluorescence polarization studies showed that a 2-dimensional transition dipole resulting in elliptically polarized emission is a generally property of CdSe nanocrystals. Even single-crystal nanoparticles with almost perfect spherical shapes show fluorescence with a significant degree of elliptical polarization (Fig. 1), and the degree appears to increase

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