The power of single molecule microscopy: from nanoparticle investigations to microbiome analysis.

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Optical microscopy has been a tool of choice ever since van Leeuwenhoek used Hooke's microscope to observe biological specimens. Chief among its advantages is the fact that imaging is noninvasive. In combination with the straightforward sample preparation and general convenience, optical microscopes remain essential to many aspects of modern-day research.
The advent of fluorescence microscopy further increased the importance and applicability of optical microscopy. Observing fluorescence emission instead of measuring light transmission dramatically increases the sensitivity and selectivity of the technique, and fluorescence microscopy has become a widely used and fundamental tool in the study of complex biological and biochemical problems. The sensitivity argument has accumulated in the development of single molecule microscopy. As a result, a large variety of techniques for labeling carefully targeted molecules with fluorescent dyes has been developed. Unfortunately, the price that is paid for the convenience and capability of fluorescence microscopy is that of a relatively poor spatial resolution when compared to electron or X-ray microscopy. The smallest distance between two objects that can be resolved is known as the *resolution* of the microscope, and in optical microscopy this fundamental limitation is roughly equal to half the wavelength of the used light, which typically corresponds to about 250 nm for blue-green excitation light (500 nm). In modern high-end research microscopes this *resolution limit* is not due to technical or design issues, but solely determined by this fundamental law of nature.
Unhindered by this limitation, living cells and organisms are characterized by a complex and elaborate ordering and structuring at length scales below the diffraction limit. An example of this are the microtubules in living cells, which are highly structured and essential components of the cytoskeleton but have a diameter of only about 24 nm. The same is true of modern materials, that often display strong heterogeneity at nanometer length scales (e.g. polymer matrices), that directly impact the material properties. While this nano-organization in complex system is obviously a fundamentally important concept, it is seemingly inaccessible by direct optical means. By contrast, it can be revealed through other microscopy techniques, such as scanning probe, electron or X-ray microscopy, which do enable the required spatial resolution. However, these techniques are limited by an elaborate and destructive sample preparation or measurement, or an inability to visualize details beyond the surface of the sample (e.g. scanning tunneling microcopy, STM). The noninvasiveness and extreme contrast and sensitivity of fluorescence microscopy are unmatched by other microscopy techniques.
Because of the unique nature of fluorescence microscopy, particularly its high sensitivity and selectivity, compared to similar technologies, several schemes have been proposed in recent years to circumvent the diffraction limit in fluorescence microscopy. Single molecule localization microscopy such as PALM, STORM, NASCA and other schemes are well established now. Other approaches such as STED (stimulated emission depletion), SIM (structured illumination) and SOFI (stochastic optical fluctuation of intensity), just to name a few, have resulted in strongly improved resolution as compared to diffraction limited fluorescence microscopy.

In this contribution, I will discuss two different applications of single molecule/super-resolution microscopy, the study of perovskite nano-particles and super-resolution optical mapping of DNA.

After seminal reports of their interesting physical properties (published in JACS 2009 and Science 2012), an explosion of scientific interest into metal halide perovskites (MHPs) in the past decade has seen this family of materials emerge as the most exciting avenue for next-generation solar cells. The strong promise for MHP materials arise from their fundamental physics; from high absorption coefficients at visible wavelengths, long carrier diffusion lengths and small exciton binding energies, to its simple solution-based processing. Justifiably, an early surge of research activity was inspired by an empirical race to produce photovoltaic devices with ever-higher photo-conversion efficiencies.
Consequently, early research saw perovskite engineering significantly outpace the understanding of their physical properties. In response, the focus of researchers is steadily shifting toward the intrinsic properties of perovskites, as these will define their performance in photonic applications. In this light, we aim at connecting the microstructure of perovskite crystals with their physical properties, by addressing following goals: (1) Development of protocols for controlled and reproducible synthesis of a variety of highly crystalline, monodisperse and defect-poor perovskite crystals; 2) development of new microscopy modalities to unravel perovskite fundamental physics. Recent progress on our work will be reported. (1-7).
Next, I will show how we developed super resolution optical mapping of DNA, coined by us as FlUOROCODE. DNA sequencing methodologies rely on massively parallel DNA sequencing approaches, which sequence short regions of the genome, from 30 up to 1500 bases in length, followed by a computationally-intensive assembly of these fragments into a genomic DNA sequence. This method requires large amounts of DNA and is labour intensive, relative to optical mapping technologies. For example, sample preparation for so-called next-generation sequencing experiments requires a full day to complete. For some experiments, this is a price that is well worth paying. However, single-base resolution of the DNA sequence is often unnecessary, as genomic differences between species (e.g., microorganisms) or structural variations between individuals within a given species (e.g., humans) can be distinguished using lower-resolution mapping approaches. While optical (restriction) mapping is easier than sequencing, it suffers from two important limitations, namely the scale on which information can be obtained and the speed. The scale is limited by the use of enzymes that break the DNA, into fragments of around 10kb in length. Since sequence reads typically run to around a few hundred bases, there is a void in the scale of information that can be derived from these techniques. Genes are typically of the order of 1 kilobase in length but can run up to several tens of kilobases, placing genetic elements exactly within this gap. An alternative to restriction mapping was developed in our lab (8), the so-called DNA FLUOROCODE. In this technique a DNA methyltransferase is used to direct the labelling of the DNA at sequences reading 5'-GCGC-3' with a fluorescent probe. The DNA is then analyzed using a wide-field fluorescence microscope with sub-diffraction limit localization of the emitters. This technique allows for a much higher labelling density compared to restriction enzymes, and an unparalleled resolution. In this contribution, I will describe the progress that was made with this concept in terms of labelling, surface deposition of DNA, superresolution imaging …I also will discuss alternatives that we develop for stretching the DNA on a surface and applications envisioned. (8-10)

References:

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