Image Scanning Microscopy and Metal Induced Energy Transfer: Enhancing Microscopy Resolution in All Directions

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11h00
SÃ©minaire de JÃ¶rg Enderlein (Georg August UniversitÃ¤t, GÃ¶ttingen, Allemagne), organisÃ© par Antoine Delon (LIPHY).

Classical fluorescence microscopy is limited in resolution by the wavelength of light (diffraction limit) restricting lateral resolution to ca. 200 nm, and axial resolution to ca. 500 nm (at typical excitation and emission wavelengths around 500 nm). However, recent years have seen a tremendous development in high- and super-resolution techniques of fluorescence microscopy, pushing spatial resolution to its diffraction-dictated limits and much beyond. One of these techniques is Image Scanning Microscopy (ISM). In ISM, the focus of a conventional laser-scanning confocal microscope (LCSM) is scanned over the sample, but instead of recording only the total fluorescence intensity for each scan position, as done in conventional operation of an LCSM, one records a small image of the illuminated region. The result is a four-dimensional stack of data: two dimensions refer to the lateral scan position, and two dimensions to the pixel position on the chip of the image-recording camera. This set of data can then be used to obtain a super-resolved image with doubled resolution, completely analogously to what is achieved with Structured Illumination Microscopy. However, ISM is conceptually and technically much simpler, suffers less from sample imperfections like refractive index variations, and can easily be implemented into any existing LSCM. I will also present recent results of combining ISM with two-photon excitation, which is important for deep-tissue imaging of e.g. neuronal tissue, and for performing non-linear coherent microscopy such as second-harmonic generation.
A second method which I will present is concerned with achieving nanometer resolution*along*the optical axis. It is called Metal Induced Energy Transfer or MIET and is based on the fact that, when placing a fluorescent molecule close to a metal, its fluorescence properties change dramatically. In particular, one observes a strongly modified lifetime of its excited state (Purcell effect). This coupling between an excited emitter and a metal film is strongly dependent on the emitter’s distance from the metal. We have used this effect for mapping the basal membrane of live cells with an axial accuracy of ~3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other super-resolution microscopy techniques which enhance the lateral resolution of imaging. Moreover, it is even applicable to localizing individual molecules, thus offering the prospect of three-dimensional single-molecule localization microscopy with nanometer isotropic resolution for structural biology.

Infos pratiques

Lieu

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