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Photosynthetic Structures and their Regulations: Microscopy

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In optical microscopy, the maximum resolution which may be obtained by a microscope is limited by the so-called diffraction barrier, first described by physicist Ernest Abbe in 1874. This limit is close to λ/2, λ being the wavelength used to produce the image. The beginning of this century has seen the development of optical super-resolution fluorescence microscopic methods, which yields pictures to resolutions of a few tens of nanometers. This breakthrough, which has already transformed the field of cellular biology, relies on different approaches, which are generally demanding either in terms of optics or sample preparation, and which all different experimental limitations. We have developed a simple and cheap way of performing super-resolution fluorescence microscopy, based on classical confocal microscopy, but using a detector small as compared to the image of a point fluorophore. In such conditions, confocal microscopy is pushed to its very theoretical limits, and a gain in resolution of 1.4 in every direction can be predicted. Experimentally, a set-up has been constructed, and the measured resolution using a 488 nm wavelength is 180 and 330 nm in the lateral and axial directions, respectively, without any post-processing of the recorded. Such method allows an excellent reconstruction of 3-D volumes of subcellular structures.

We have applied such approach to inner membrane of chloroplasts of different organisms, which dwells the ensemble of proteins involved in the primary steps of photosynthesis. In this case, chlorophyll fluorescence is recorded, and thus gives direct access to the organisation of the photosynthesis apparatus and its remodelling upon light changes. This methodology was also applied on cyanobacteria, to get insight on the spatial organisation of the different photosystems in the photosynthetic membrane of these organisms and suggest a complex partition of these membranes between domains ensuring different functions.