Fluorescence Spectral Imaging of Individual Pigment-Protein Complexes

Application Note

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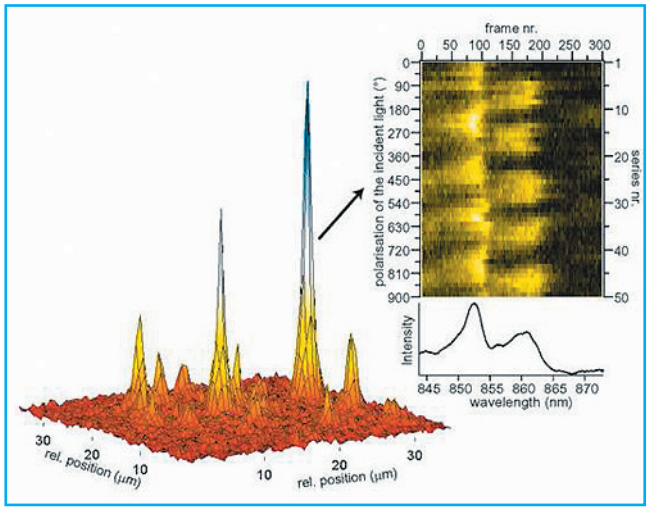
Researchers at the University of Bayreuth are investigating highly specialized pigment protein complexes, so called light-harvesting antennas, involved in the primary steps of light absorption in the photosynthesis of purple bacteria. Applying single molecule techniques enables them to obtain detailed information about the character of the electronically excited states that play a prominent role in the biological function of the aggregate.

Previously, the experiments were limited to the investigation of one complex per day due to the sequential character of the experiment when using a sensitive photodiode for detection. Switching to the Andor iXon DV887 has enabled the investigation of as many complexes as can be simultaneously imaged on the CCD. The key feature of the camera is that it combines high sensitivity and low noise without sacrificing the fast read-out that is essential for this approach.

The figure below shows a three-dimensional representation of a fluorescence microscopy image of a part of the sample. Each peak corresponds to the diffraction-limited image of a single pigment-protein complex. A fluorescence-excitation spectrum from the complexes is recorded by reading out the CCD camera while scanning the wavelength of the excitation laser, consequently the CCD-frame number corresponds to wavelength. Furthermore, by changing the polarization of the excitation light after each laser scan, they are able to obtain valuable details about the nature of the excited states of the pigment protein complexes.



An example is shown for one of the peaks on the right hand side of the figure that displays the CCD frame number (i.e. wavelength) versus polarization of the excitation light, the fluorescence intensity given by the color code. Underneath, the total spectrum corresponding to the sum of all individual scans is shown. From the polarization pattern it becomes evident that the two prominent features in the spectrum can be excited at different polarization angles of the incident light. Since this information can be extracted from each peak that has been simultaneously imaged by the iXon, it has been shown that this new multiplexing detection scheme has enhanced the efficiency of the experiment by a factor of 50!

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