雑誌掲載：Biophotonics International が２光子励起ラインスキャン蛍光スペクトル顕微鏡を紹介

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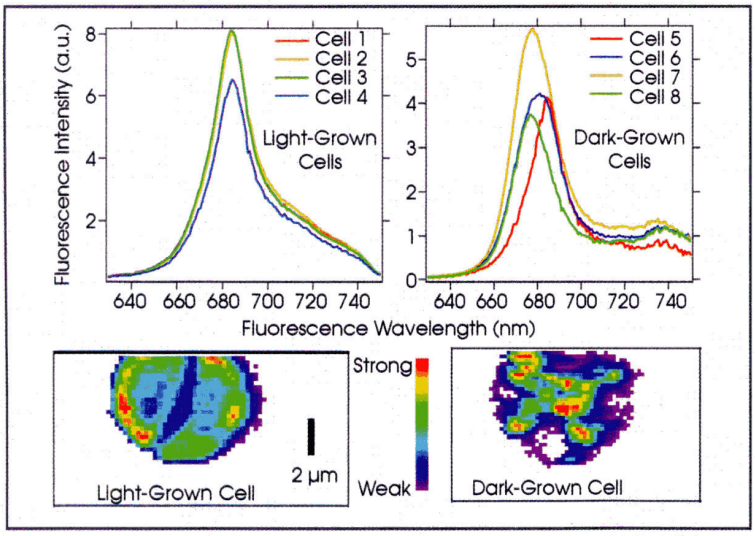
先にニュースレター１１月号に紹介した研究業績（J. Microsc. 228, 240 - 254 (2007)）が Biophotonics International (Laurin Publishing)の記者の目に留まり、２００８年１月号の Biophotonics Research 欄（58-59 ページ）で紹介されました。

New approaches to multicolor fluorescence imaging

Technique advances acquisition of broadband fluorescence spectra

Biological research increasingly calls for simultaneous imaging at multiple wavelengths — to study complex interactions and conformational changes occurring at the same time, for example. To achieve this, fluorescence microscopes should be able to record broadband spectra with a relatively high resolution. Until recently, though, most commercial microscopes offered only single-channel detection using photomultiplier tubes (PMTs) or avalanche diodes, allowing the acquisition of multicolor information only by changing filters or tuning a prism.

A handful of companies now offer spectroimaging systems in which the output fluorescence and/or the Raman scattering of a confocal microscope are transmitted into a polychromator outfitted with a multichannel detector. These typically rely on point-by-point acquisition of fluorescence, however, and, when users want to increase the scan rate, they can do so only by stepping up the laser power — possibly leading to faster photobleaching or phototoxicity.



Researchers have reported a technique for acquiring broadband fluorescence spectra, enabling simultaneous fluorescence imaging at multiple wavelengths. Using a line scan of the illumination beam enabled them to increase the scan rate relative to that typically achieved with point-by-point acquisition, and thus to perform imaging of complex interactions and conformational changes, without increasing the potential for photobleaching or phototoxicity. Shown here, for example, are fluorescence spectra and chlorophyll fluorescence images of a type of algae. The right and left panels correspond to algal cells grown under different light and nutrient conditions. In the left panel, the spectra are constant among the different cells, while in the right panel, the spectra vary considerably.

Several groups have reported enhancing the scan rate using a line scan of the illumination beam, thus obviating the need to increase the laser power. In the November issue of *Journal of Microscopy*, researchers with Kyoto University and Osaka University, both in Japan, reported such an approach. The paper describes two ways to achieve line-scan illumination: via either a cylindrical lens or a rapid-scan mirror. In the current study, the researchers reasoned that they could achieve better depth sectioning using a rapid-scan mirror.

In the experimental setup, the source of the multiphoton excitation was a femtosecond pulse train generated by a titanium sapphire laser oscillator made by Clark-MXR Inc. of Dexter, Mich., pumped by an argon-ion laser made by Spectra-Physics of Mountain View, Calif. The pulse train was transmitted into an inverted microscope made by Olympus Corp. of Tokyo. A resonant scan mirror made by Electro-Optical Products Corp. of Glendale, N.Y., oscillating at 7.9 kHz modulated the incident angle of the beam as it traveled to a 1.4-NA, 100× objective lens, also made by Olympus. An electron-multiplying CCD camera made by Andor Technology of Belfast, UK, detected the resulting fluorescence spectra by way of an imaging polychromator made by Bunkou-Keiki of Hachiouji, Japan.

Shigeichi Kumazaki, the first author of the study, noted two main challenges in

developing the system: finding the best imaging polychromator and correcting the unavoidable image distortion. "I compared three imaging polychromators and selected the best one, from Bunkou-Keiki," Kumazaki said. "I made a homemade program to correct the remaining image distortion and recover a true spectral image with an estimated error of only 80 nm in the whole view."

The researchers tested the system by imaging thykaloid membranes in a cyanobacterium, an oxygen-evolving photosynthetic organism with a variety of pigment-protein complexes. Obtaining broadband fluorescence spectra from cyanobacteria is potentially very useful because they can reveal stoichiometric ratios and the efficiencies of electronic excitation transfers with the complexes, as well as the quenching mechanisms behind the photosynthetic reactions. Also, the cyanobacterium provided a good testing ground for the technique because its multiple fluorescence bands are highly overlapping and not easily untangled by more conventional detection methods that use dichroic mirrors and bandpass filters.

The experiments proved successful, yielding detailed three-dimensional spectroimages of the cyanobacterium and even uncovering an unexpected intracellular feature. The system demonstrated wavelength resolution of 1 nm, spectral coverage of 250 nm in the current setup (this can be extended easily to 500 nm, Kumazaki said) and nearly diffraction-limited resolution, as well as an improvement in the scan speed proportional to the length of the line scan. The researchers described the full width at half maximum of the point spread function measure to be 0.33 (vertical to the line scan), 0.39 (along the line scan) and 0.59 (axial) at 685 nm.

There is still room for improvement, though. One alternative setup is a multianode photomultiplier tube — from Nikon, for example — combined with a confocal scanning microscope. "Compared to this," Kumazaki said, "our setup is inferior with respect to the anisotropic resolution. Our setup also needs a relatively long data transfer time for a single line because of the large number of pixel signals to be transferred to the PC (about 50 ms)."

He noted, however, that the first of these issues can be addressed by introducing a lens array and a pinhole array. The second can be addressed by incorporating a CCD camera with a shorter dead time in data transfer and by optimizing the imaging area and binning. The data transfer time also can be accelerated using specialized hardware. "Then, it's a matter of which is more sensitive: PMT or CCD. At the moment, CCD can achieve a higher quantum yield and a larger number of pixels, which results in a larger number of wavelength channels."

The researchers are considering implementing these changes. Kumazaki added that it also might be interesting to realize a multispectral microscope, as line scanning can be extended to allow absorption mapping, Raman mapping and second-harmonic generation. □

Gary Boas