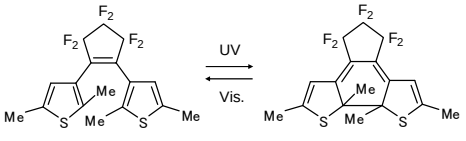
業績紹介：ラマン分光によるフォトクロミック分子 1,2-bis(2,5-dimethyl-3-thienyl)perfluorocyclopentene 開環体結晶中の閉環体の安定性の研究

関谷　博　　（九大院理・計画研究代表者）
南部伸孝　　（九大情報基セ・計画研究分担者）

論文題目：”Raman spectroscopic study on isomers of photochromic 1,2-bis(2,5-dimethyl-3-thienyl)perfluoro-cyclopentene in crystal and stability of the closed-ring forms in the open-ring forms”
著者：Kenichiro Saita, Seiya Kobatake, Tuyoshi Fukaminato, Shinkoh Nanbu, Masahiro Irie, Hiroshi Sekiya
雑誌巻号：*Chem. Phys. Lett.* **488**, (1-3), 159-163 (2008)

筆者らのグループは気相におけるクラスター分子の構造とダイナミクスの研究を行っているが、本論文は、結晶中の分子の電子・幾何構造と分子間相互作用に関する研究成果である。結晶の電子・振動分光による研究からは孤立気相状態のように分子の内部状態について詳細な情報を得ることは困難である。しかしながら、光異性化によって生じた分子が結晶中で安定に存在する場合には、結晶中の分子間相互作用を近似的に光異性化した分子を周囲の分子が取り囲んでいるクラスター分子内の相互作用とみなすことができる。分光測定と量子化学計算を組み合わせると、結晶中における特異的な分子間相互作用についての調査が可能である。

ジアリールエテン類は、紫外光または可視光を照射することによって開環―閉環反応が生じ、開環体(**1a**)と閉環体(**1b**)の両方が熱的に安定である。したがって、結晶中の分子構造、分子間相互作用および反応ダイナミクスの詳細な調査に適している。本研究は、結晶中のフォトクロミック反応における分子間相互作用およびエネルギー散逸過程を調査するための電子・振動分光法を確立するとともに、分子の構造変化が結晶の形態変化にどのように影響を及ぼすかについて解明することを目指した。



1a　　**1b**

1,2-bis-(2,5-dimethhyl-3-thienyl)perfluorocyclopentene (DMTF) 閉環体結晶中において閉環体結晶の S…S 距離は、閉環体結晶中の歪んでいない閉環体に比べて 0.24 Å 長く、分子構造が歪んでいることが知られている。歪んだ分子が安定に存在する原因を解明するためにラマン分光法を適用した。

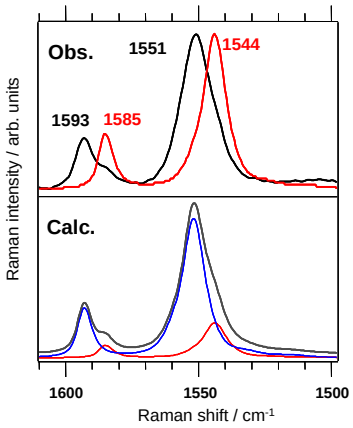


Fig. 1．上図は DMTF 閉環体微結晶のラマンバンド(赤線)と開環体微結晶に UV 光を照射したときの差スペクト(黒線)．下図において、閉環体結晶のバンド(赤線）と歪んだ閉環体のバンド（青線）との重ね合わせで、観測されたスペクトル(黒線)が再現された。

DMTF 開環体の微結晶に UV 光を照射してラマンスペクトルを観測した。開環体と閉環体のラマンバンドを明瞭に区別できただけでなく、Fig.1 に示すように歪んだ閉環体と歪んでいない閉環体ラマンバンドを分離して観測することに成功した。歪んだ閉環体と歪んでいない閉環体は同じ結晶内に存在し、これらの生成比を、定常状態において約４：１と決定した。歪んだ閉環体がなぜ開環体結晶中で安定であるかについて、閉環体が周囲の開環体に取り囲まれたクラスターモデルを考え、クラスター形成による安定化エネルギーを量子化学計算（MP2/6-31G**）によって求めた。その結果、歪んだ閉環体を含むクラスターの方が歪んでいない閉環体を含むクラスターよりも明らかに安定であることが示された。Mulliken charge の計算から、歪んだ閉環体では、チオフェン環の C=C、C−S 結合に分極が生じ、開環体チオフェン環の間の静電相互作用が増加することが示唆された。

本研究は、分子性結晶中の分子間相互作用をクラスター化学の視点から調査することによって、新規な情報が得られることを示している。

Biophotonics International 誌が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

熊崎茂一　（京大院理・計画研究代表者）

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

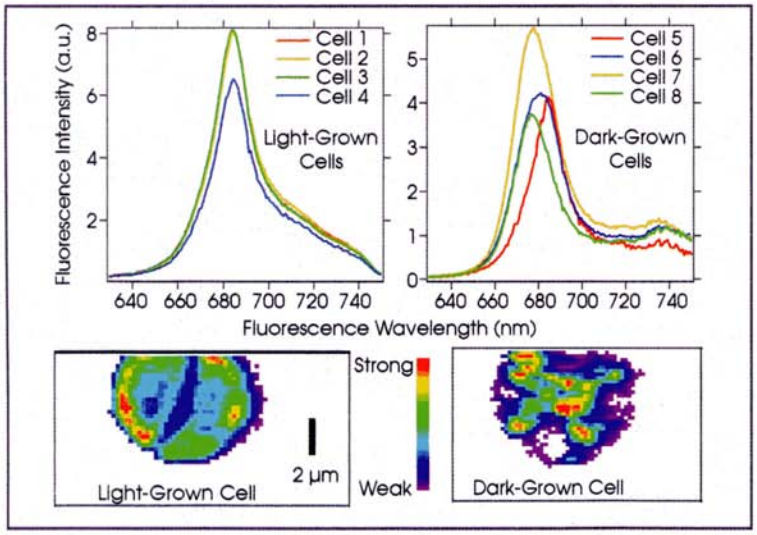
Biological research increasingly calls for simultaneous imaging at multiple wavelengths — to study complex interactions and conformational changes occurring at the same time, for example. To achieve this, fluorescence microscopes should be able to record broadband spectra with a relatively high resolution. 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.

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



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.

58 · BIOPHOTONICS INTERNATIONAL · JANUARY 2008

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