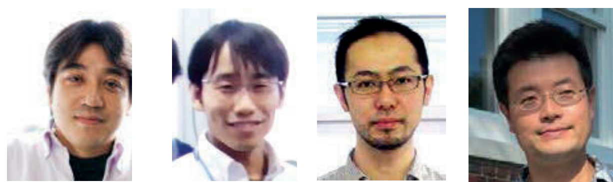
High speed and multi-color imaging with a spinning disc confocal microscopy utilizing LED as a light source



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Laser-scanning confocal microscopy is one of the most standard techniques for bioimaging of living specimen. To acquire the confocal image in conventional single-point scanning microscope utilizing Galvano meter mirrors, a coherent laser is generally used as the light source to be focused onto a small spot with diffraction-limited size. Besides the single-point scanning confocal microscope, multipoint scanning confocal microscope can be employed by using a spinning disc with multiple pinholes. The most popular multi-point scanning confocal microscope is the Yokogawa CSU. In this system, an expanded laser beam is illuminated on the Nipkow disc with tandem pairs of microlens and pinhole array. When the disk is spun, each microlens/pinhole sweeps the excitation laser beam across the object through the objective lens, producing a raster scan of multiple laser beams. This scanning system provides two advantages for confocal imaging; 1) faster frame acquisition rate (up to 360 Hz), 2) reduction of photo-induced damages as the excitation light power of each scanning point can be reduced to as low as 1/1200 of that of the single-point scanning system. However, the requirement of lasers in the Yokogawa CSU strictly limits the choice of fluorescent dyes because of the limitation of the number of laser lines introduced into a single confocal system. To overcome this problem, we developed an illumination system utilizing LEDs that were introduced into a multi-mode fiber to obtain a homogeneous light from the fiber output[1,2]. This illumination provides an incoherent light of continuous wavelength enabling selection of a wide range of fluorophores to be observed. Moreover, we constructed an optics in which off-axis light can pass through pinholes surrounding the pinhole located on the optical axis of the collimator lens (Fig.1). This optics enhanced the utility of not only the on-axis but also the off-axis light such that the available incident light was considerably increased. With this innovation, we achieved a substantial increase in the intensity of the excitation light without any loss in spatial resolution, and succeeded high-speed confocal imaging (Fig. 2) at a time resolution of up to 300 Hz and five-color time-lapse imaging (Fig. 3), both with a satisfactory S/N ratio unachievable with the previous system.

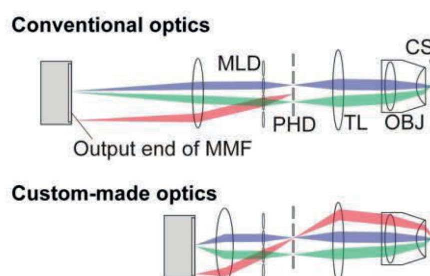

Fig. 1. Schematic of light rays emitted from the end of multi-mode fiber (MMF) passing through micro-lens disc (MLD) to cove slip and sample (CS). PHD: pinhole array disc, TL: tube lens, OBJ: objective lens.

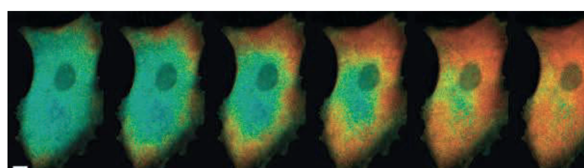

Fig. 2. High-speed Ca^{2+} imaging of a live cell expressing yellow cameleon 3.60. Series of pseudo-colored, IMD mode images are shown. Scale bars represent 10 μm..

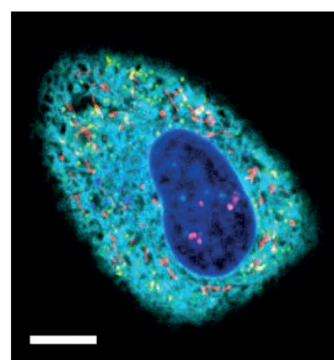

Fig.3. Five color imaging of cellular compartments in a living cell. Scale bars represent 10 μm.

Reference

1. Saito K, et al. Conjugation of both on-axis and off-axis light in Nipkow disk confocal microscope to increase availability of incoherent light source. **Cell Struct Func**, 36, 237-246, 2011
2. Saito K, et al. A mercury arc lamp-based multi-color confocal real time imaging system for cellular structure and function. **Cell Struct Funct.** 33: 133-141, 2008

Laser less Multicolor confocal system, “MESSIA®”, with infinite possibility

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On May 2012, Professor Takeharu Nagai, the Japan’s top-ranking expert in the field of bioimaging study, gave a talk at luncheon seminar at Joint meeting of the 45th annual meeting of Japanese Society of Developmental Biology & the 64th annual meeting of the Japan Society for Cell Biology held in Kobe, Japan.
He talked about the development of new system with reasonable mercury lamp, finding of high-power LED, “Light Engine”, applying it to optogenetics and challenging to spying minority in biological phenomena. Following is a summary about original report.

● ”Swim against the tide” is his motto.

He says, “Swim against the tide is my motto in research and development. Actually, I’ve been making many challenging developments. It was one of them to use mercury arc lamps instead of lasers for CSU, the Confocal Scanner Unit. It was a challenge which no one has succeeded but he indeed did to get a confocal image with mercury arc lamp. It was in 2008.

● Introduce of “Light Engine” and new optical system

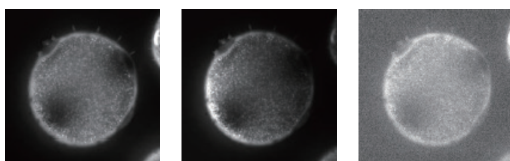
However, a problem has been remained that the power of excitation light from mercury arc lamp was absolutely weak. Dr. Nagai has considered the improvement and came up with some schemes. One is to use high-power LEDs. He says “Fortunately I got to know that Opto-Line had started to deal in “Light Engine” that is applied high-power LED. I have used it and turned out that “Light Engine” is much brighter than mercury arc lamp.” After inventing new optical system to effectively couple the incoherent light from Light Engine to the CSU system, laser less Multicolor confocal system, “MESSIA®” has come into the world in this way. Opto-Line started to deal in this system in 2011. Dr. Nagai says “Many researchers should have not used CSU efficiently until now. This system can be said “MESSIA®” as its name.”

● Application for biomanipulation and optogenetics

Dr. Nagai is now working on development of other applications using this system except for bio imaging. For example, he is addressing a technical development of physiological operation with optogenetic tools such as channelrhodopsin-2 (hereafter ChR2) . This protein puts cation into a cell and activates certain signaling system when it absorbs blue light. “Light Engine” is suitable for switching this activation. Lighting blue light on ChR2 expression in a neuron, it is possible to generate action potentials in light-dependent manner.

Furthermore, Dr. Nagai is promoting the development of analytical method called “CALI (Choromophore-Assited Light Inactivation)” by which protein of interest fused with photosensitizing dye could be inactivated by light illumination. He has succeeded in inactivating Aurora-2 function.
This technology is getting used widely in many researches. While the technology combined optical science and genetics, the latter technology, is called “Optogenetics”. It is attracted many researchers’ attention as a new method.

Back to the subject, “MESSIA®”. It can be used for existing CSU, and “MESSIA®-α”, which can be used for existing laser(s) and CSU, was launched out. They, “MESSIA®” and “MESSIA®-α”, allowed researchers to enjoy confocal imaging with CSU not only practically but also cost-effectively.
“MESSIA®” can be a messiah literally also in these new fields.



[1] Light Engine (Lumencor) [2] 488nm laser [3]100W mercury arc lamp

Sample : Pumpkin pollen
Microscope : Nikon Ti-E
Objective : 40 x Oil NA 1.30 (Nikon)
CCD Camera : ImagEM Enhanced (Hamamatsu)
- Normal mode (non EM mode)
- Exposure time : 500 ms
Confocal unit : CSU-X1 (Yokogawa)
-DM : Di01-T405/488/568/647-13x15x0.5 (Semrock
-BA : FF01-520/35-25 (Semrock)

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