Whole body fluorescence imaging of Medaka fish by wide-field two-photon DSLM

Sota Takanezawa[1], Takashi Saitou[1,2], Takeshi Imamura[1]
[1]Department of Molecular Medicine for Pathogenesis, Ehime University Graduate School of Medicine, Toon 791-0295, Ehime, Japan.
[2] Translational Research Center, Ehime University Hospital, Toon 791-0295, Ehime, Japan
E-mail: s_takane@m.ehime-u.ac.jp

KEY WORDS: wide-field 2p-DSLM, whole body fluorescence imaging, 4D Ca^{2+} imaging, Medaka fish

Recently, there has been a growing interest in observing whole body of animal models to study different disease process and complex biology *in vivo*. Medaka fish, one of the vertebrate animal models, has been used not only for biological research but also for medical field, especially for drug screening and cancer research. In order to achieve fluorescence imaging of whole body of Medaka with high-resolution and high-speed, we developed wide-field two-photon digital scanned light sheet microscopy (DSLM)

In order to satisfy the specs for the in vivo wide-field microscopy with high-resolution and high-speed, it is required with 1) low-photo toxic excitation for longer observation, 2) large field of view (few mm size), 3) high spatial resolution to distinguish the single cells (2-3 μm), and 4) high-speed imaging for 4D imaging (10-100 ms / image). Among various fluorescence imaging techniques, two-photon (2p) DSLM is one of the candidates to solve these requirements [1,2]. Generally, field of view and spatial resolution were trade-off relation in conventional 2p-DSLM, those properties depend on the numerical aperture of the irradiation laser. Thus, we adopted the Bessel beam in the irradiation optical pathway to keep the field of view and spatial resolution.

As the result, over 5.5 mm Bessel beam with 5~6 um axial resolution, in which 200 nm fluorescence beads was measured to define the resolution, was obtained. This beam indicated that 2p-DSLM with the large field of view is possible to make with a high-power laser and large camera. Then, to improve the spatial resolution and efficiency of 2p-excitation, we adjusted the ring diameter of the beam at the irradiation lens such that Bessel beam equals to 1.47 mm. Here, the 1.47 mm corresponded to field of view defined by the objective lens and detector size in our system.

For whole-body fluorescence imaging of Medaka, image of the lymphatic vessels and blood vessels with better spatial resolution (axial and longitudinal resolution, ~2um) was achieved by 2p-DSLM although tiling measurements were required. In addition, high-speed 4D Ca^{2+} imaging was also obtained in the Medaka embryo. This system is useful for not only the study of Medaka whole body imaging but also large biological specimens. Furthermore, the development of wider 2p-DSLM is possible with the high-power laser.

[1] T. Imamura; T. Saitou, and R. Kawakami, "In vivo optical imaging of cancer cell function and tumor microenvironment.," *Cancer Sci.,* **109,** 912-918 (2018).
[2] A. Maruyama; Y. Oshima; H. Kajiura-Kobayashi; S. Nonaka; T. Imamura, and K. Naruse. "Wide field intravital imaging by two-photon-excitation digital-scanned light-sheet microscopy (2p-DSLM) with a high-pulse energy laser.," *Biomed. Opt. Express,* **5,** 3311-3325 (2014).