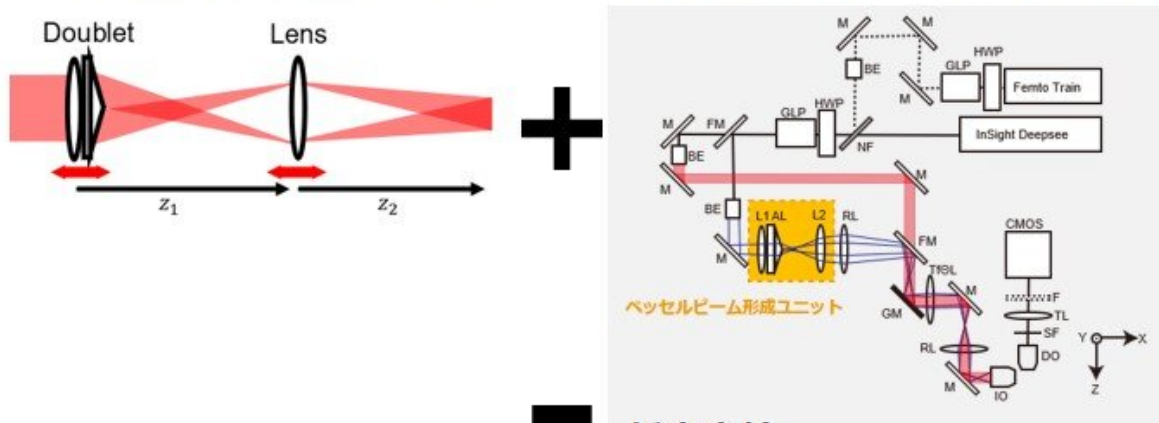


A new light-sheet microscopy unit enables an extended field of view and reduced photodamage

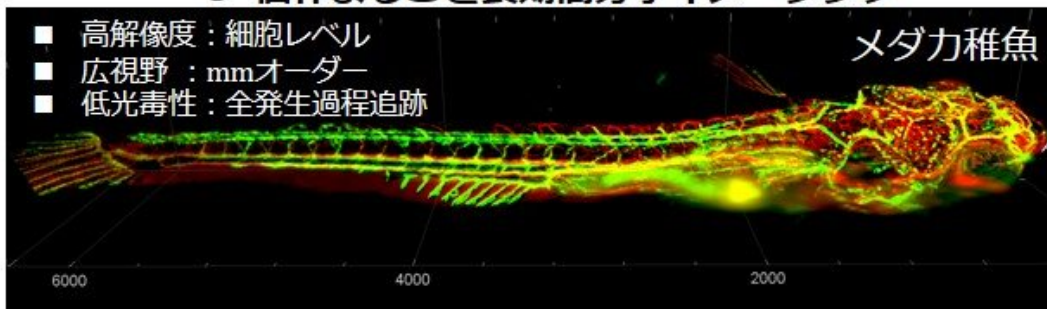
May 28 2021

A ベッセルビーム形成ユニット B 2光子励起光シート蛍光顕微鏡



↓ 低光毒性
広視野化

C 個体まるごと長期間分子イメージング



(A) The Bessel beam forming unit composed of a combination of three lenses. (B) Construction of two-photon excitation light-sheet microscopy using the Bessel beam forming unit and near-infrared lasers. (C) Whole body lymphatic

and blood vessel imaging of a medaka larva using the microscope. Credit: Takashi Saitou, Ehime University

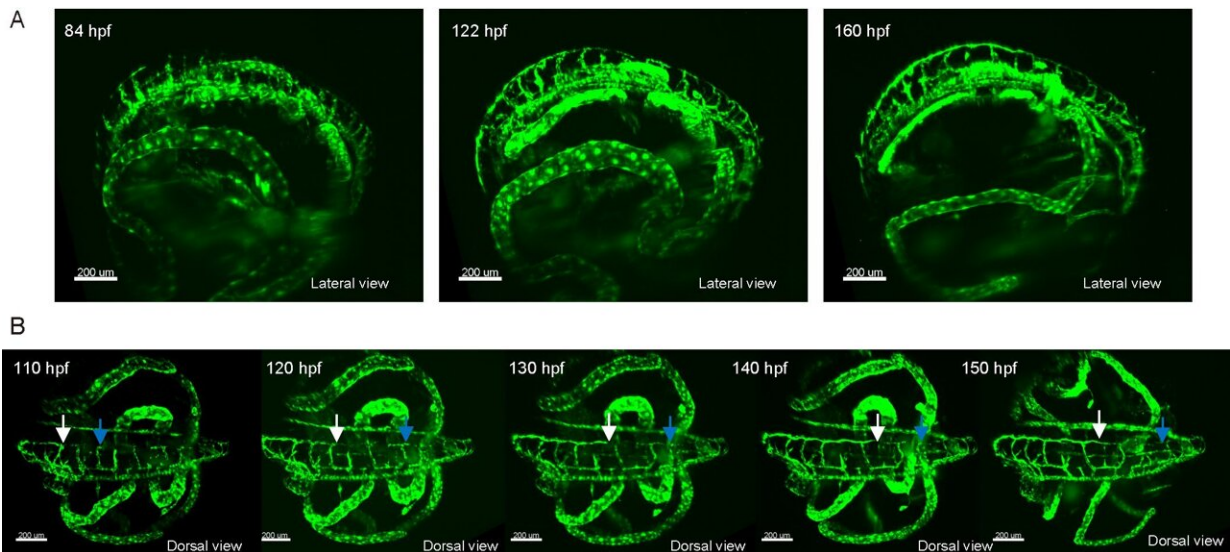
A research group led by Takashi Saito, of the Ehime University Graduate School of Medicine, developed a 2-photon excitation light-sheet fluorescence microscope which (1) lowers phototoxicity, (2) extends the field of view, and (3) heightens spatial resolution. This microscope, when used for the observation of medaka fish, made it possible to observe the whole body of the embryo (an extended field of view) at a cellular level resolution (high spatial resolution) without affecting the growth of the fish (low phototoxicity) over a three-day span of embryonic development. This result was published in the scientific journal *Nature Communications*.

The [fluorescence microscope](#) is widely used in the field of life science to observe molecules inside a cell in a non-invasive way. Light-sheet fluorescence microscopy makes it possible to record three-dimensional [images](#) with high acquisition speed and [high spatial resolution](#). However, in conventional light-sheet microscopes, it is difficult to limit photodamage to living tissues, and also difficult to simultaneously achieve wide FOV and high (cell level) [spatial resolution](#).

Development of a two-photon excitation wide-field, light-sheet microscope

The Ehime University research group of Takashi Saitou, Sota Takanezawa, and Takeshi Imamura utilized the two-photon [excitation](#) phenomenon as a key to solving this problem. The two-photon excitation microscope with infrared lasers enables gentle (low phototoxic) imaging of living organisms. However, because the light must be focused on a narrow range to induce two-photon excitation, the excitation range (in

light-sheet microscope, the field of view) is narrow. In order to solve this, the researchers developed simple illumination optics unit with a Bessel beam that expands the laser propagation range in the direction of the optic axis (Fig. 1A). This unit can stretch the beam length to 600-1000 μm while maintaining a 2-3 μm axial resolution when using a 10x magnification NA0.3 objective lens. Using this optical unit, they constructed a two-photon excitation light-sheet microscopy (Fig. 1B), which makes it possible to perform whole-body imaging of medaka larvae with cellular resolution (Fig. 1C).



Observation of a lymphatic vessel development over three days using a FLT4-EGFP embryo which expressed green fluorescent protein in the lymphatic endothelial cells. Credit: Nature Communications

The medaka is widely used as a model organism for vertebrate. It is suitable for fluorescent imaging since it is small and transparent. To evaluate the applicability of the microscope for use on living organisms, the researchers performed a phototoxicity assessment. This revealed

reduced photodamage compared with the conventional Gaussian beam light-sheet sheet microscope. It is therefore suggested to be suitable for long-term live imaging. The researchers then applied long-term time-lapse imaging of the transgenic medaka in which lymphatic endothelium is labeled with [green fluorescent protein](#), and succeeded in live imaging over three days at intervals of five minutes (Fig. 2).

In this study, the researchers developed a new high performance light-sheet fluorescence [microscope](#). Using this technology, scientists can observe almost all the embryonic growth processes of medaka fish with high cellular [resolution](#) over the whole body of the fish. This technology is expected to contribute to the molecular level understanding of embryonic development, the elucidation of pathogenesis for lifestyle-related diseases, and to further the technology of drug development.

More information: Sota Takanezawa et al, Wide field light-sheet microscopy with lens-axicon controlled two-photon Bessel beam illumination, *Nature Communications* (2021). [DOI: 10.1038/s41467-021-23249-y](#)

Provided by Ehime University

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