Whole body 3D Morphometrics of Medaka Fish using Wide-field 2-photon Light-sheet Microscopy

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

KEY WORDS: Light-sheet microscopy, 2-photon excitation, Bessel beam, whole body imaging, medaka fish, quantitative morphometrics

There has been a growing interest in observing whole body of animals to study biological and disease processes which occurred across the entire body. Medaka fish, a model animal of vertebrate, is a suitable animal for such an analysis because of its small size and high transparency. In order to perform fluorescent whole body imaging of Medaka, we developed a wide-field 2-photon digital scanned light-sheet microscopy (Figure 1). We used near-infrared laser for 2-photon excitation of light sheet because this has clear advantages of lowering phototoxicity and improving penetration depth. However, this suffers from its narrow excitation range. In order to address this issue, we employed near-infrared Bessel beam for illumination optics. Consequently, our optical system achieved ~1.5 mm field of view with <2 um axial and <1 um lateral resolution when using the ×10 magnification objective lens, which makes it possible to perform large scale imaging in a single cell resolution.

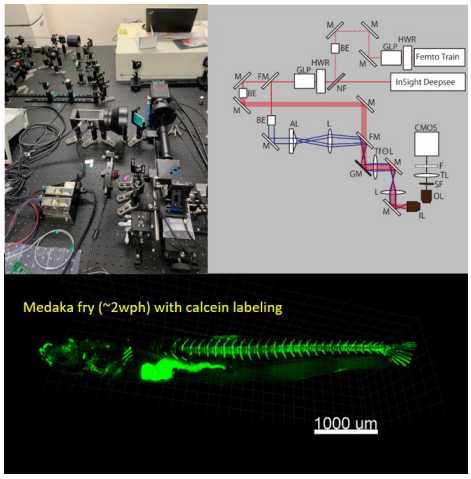

Figure 1: Wide Field 2-Photon DSLM and whole body fluorescent imaging of a medaka larva.

We applied this system to analyze cellular morphology over whole body of Medaka fish. Here, focusing on skeletal morphology, we studied (semi-)automated image processing method, which include image registration, morphological feature extraction, and analysis of variations between different medaka strains (Figure 2). The image registration transformed the 3D image into a series of (transverse) sections perpendicular to the manually-defined body axis, and this image form improved the comparability of images between individuals and different strains of medaka. 3D morphometrics can be applied to the registered images to quantify the skeletal morphology such as size and number of vertebrae and the acquired parameters differentiated the medaka strains. Therefore, this approach provides an image processing pipeline for digital whole body 3D morphometrics of medaka fish.

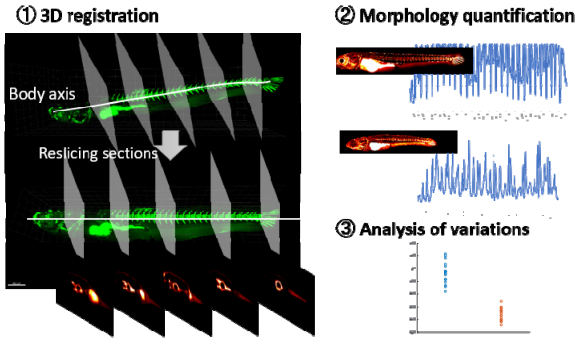

Figure 2: Tailored 3D image processing for medaka whole body morphometrics.