# Application of light-sheet microscopy to cell and development biology

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**Abstract** Light-sheet microcopy is a powerful method to visualize thick living specimens such as embryos and tissues, with low photodamages and fast image acquisition. The very basic one, however, suffers from sample vibration and light-sheet degradation. We have developed two microscopes, ezDSLM and 2P-DSLM, to improve each of the problems.

**Keywords:** light-sheet microscopy, live imaging, mouse development, amoeba, two-photon microscopy

## 1. Introduction

From the 1990s to now, confocal microscopy has been widely used to acquire three-dementional information of thick biological specimens including cells and embryos. One of the most serious problems of this method is photodamage such as bleaching and phototoxicity. It illuminates excitation light throughout the whole depth of the specimen, and obtains an optical section by disposing the fluorescent light emitted from the outside of the focal plane. Thus the photodamage will accumulate proportional to the number of the sections.

Light-sheet microscopy has emerged as an alternative method these days. As shown in Fig.1, it is characterized by perpendicularly placed objective lenses, each of them for solely illumination and detection whereas the other microscopes use single objective for both purposes, and the illumination objective gives sheet-shaped excitation light to the focal plane of the detection objective. Since the excitation occurs only at the focal plane, the causal photodamage is greatly reduced to the minimum. Besides, the light-sheet microcopy has an advantage of high temporal resolution because the fluorescent light is obtained by 2D image sensor(s) i.e. CCD/CMOS camera(s), while the confocal microcopy typically uses point scanning. Keller et al. demonstrated the powerfulness of this method by tracking cleavage and migration of more than 10,000 cells in a zebrafish embryo [1].

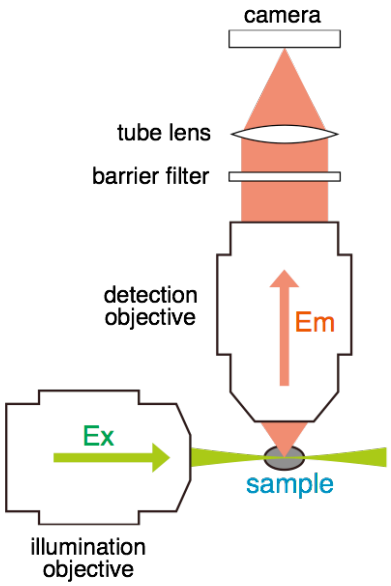


Fig. 1. Principle of light-sheet microscopy.

The light-sheet microscopy however suffers with several peculiar shortcomings such as specimen mounting and sheet degradation.

The specimen mounting problem comes from the arrangement of objectives. Standard procedure of the specimen preparation needs embedding it into a gel rod in order that the specimen's position can follow the movement of motorized stages for z-scan. This procedure is relatively complicated to many biologists who are used to work with a slide glass and a coverslip, and for some living specimens embedding itself is harmful. The softness of the gel causes swinging problem for ultrafast imaging that the specimen cannot immediately follow the movement of the stage.

The sheet degradation problem comes from scattering and irregular refraction of the excitation light sheet within the specimen. The scattered light illuminate outside of the focal plane to increase background noise, and the irregular refraction produces stripe-patterned uneven illumination.

## 2. Technical improvements to overcome the problems in light-sheet microscopy

We have worked to solve or improve some part of the above problems. For swinging problem, we have built a new set of light-sheet microscope, namely ezDSLM, where the position of light-sheet and the detection objective move for z-scan, instead of the specimen, as shown in Fig.2. Since ezDSLM excludes the swinging component, it can be used for ultrafast 4D imaging to visualize fast moving protozoa *Amoeba proteus* up to 2 volumes/sec, as shown in Fig.3 [2][3].

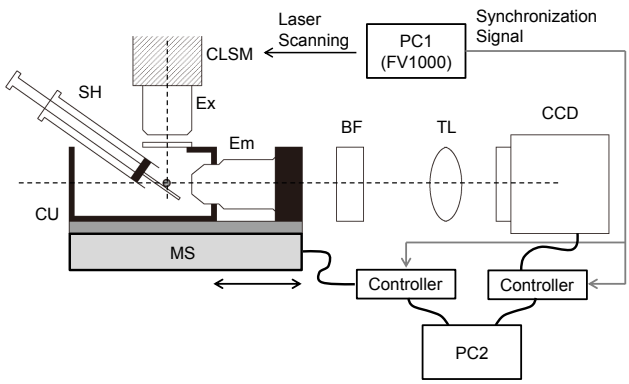


Fig. 2. Schematic diagram of ezDSLM.

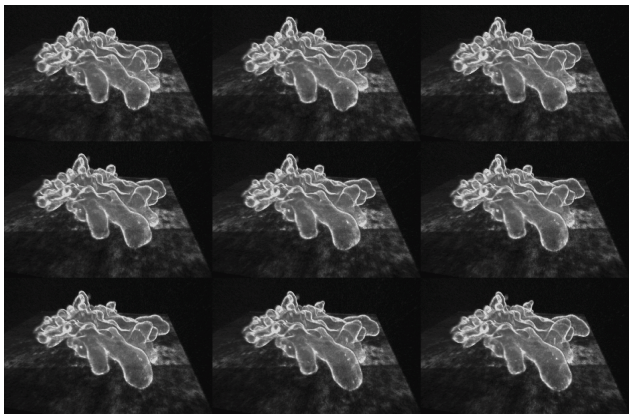

Fig. 3. Movement of *Amoeba proteus*.

We also worked to improve the sheet degradation problem. A previous work showed that combination with two-photon excitation (TPE) is useful: the scattered excitation light cannot contribute to TPE since occurrence of TPE needs high photon density [4]. It had suffered narrow field-of-view because high numerical aperture (NA) objective was necessary to obtain thin beam waist, in order to obtain enough high photon density. We overcame this dilemma by using a fiber laser with high peak power (pulse width 350 fsec, repetition 100 kHz) compared with conventional titanium:sapphire laser (~100 fsec, 80 MHz), as illustrated in Fig. 4. We built a set-up to test this idea, and successfully obtained wider field-of-view (~0.7 mm) and improved contrast with imaging of living medaka [5].

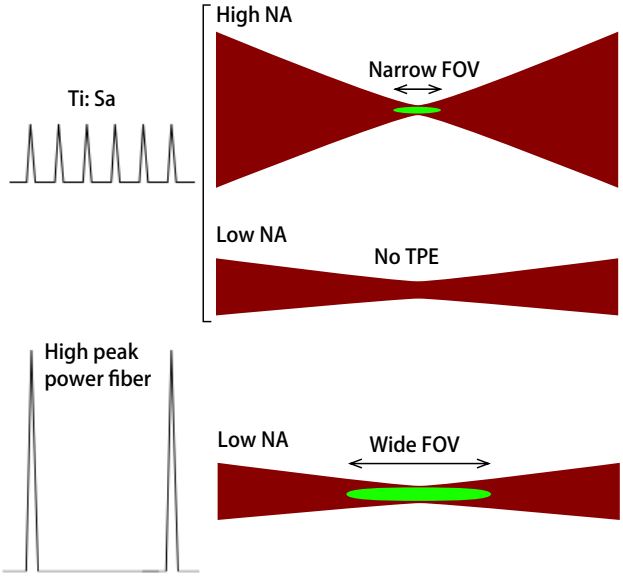


Fig. 4. Idea for wide field-of-view TPE light-sheet.

## 4. Conclusion

Light-sheet microscopy is very powerful method for bioimaging in principle, but several things need to be done for wider biological applications. Here we show some of our efforts.

## References

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