

High & isotropic resolution with large samples in the selective plane illumination microscope (SPIM)

Ernst H.K. Stelzer, Jim Swoger, and Jan Huisken

Cell Biology and Cell Biophysics Programme
European Molecular Biology Laboratory (EMBL)
Meyerhofstrasse 1, D-69117 Heidelberg, Germany
E-mail: *lastname@embl-heidelberg.de*

KEY WORDS: Isotropic resolution, high resolution, light microscopy, fluorescence microscopy, multiple imaging axes, embryology, three-dimensional.

Confocal theta microscopy was invented about ten years ago to provide biologists with a new tool for the investigation of large specimens with high, isotropic three-dimensional resolution. The fundamental principle used in the microscope is the detection of fluorescence light at an angle of about 90° to the illumination axis.

Our new implementation of the theta principle provides a much longer working distance such that millimeter-sized specimens can be observed in their entirety. The instrument features optical sectioning by the excitation of fluorescence in a selected volume. Detection of the fluorescence is performed perpendicular to the excitation. Rotation of the sample makes it possible to change excitation and detection axes with respect to the sample. It enables us to illuminate and image parts of the sample that would otherwise be hidden or obscured. A three-dimensional image is created by scanning the specimen through the stationary volume of illumination. Data stacks recorded at different angles can be combined in a post-processing step to yield a high-resolution image of the complete sample. Selective Plane Illumination Microscopy (SPIM) is especially well suited for the investigation of millimeter-sized samples. We have observed various different species with our instrument and show the results of a number of experiments that demonstrate the properties and performance of the microscope. This technology is of interest to all scientists working with such large specimens and who are trying to investigate features (such as gene expression patterns) that require high three-dimensional resolution over a large volume.

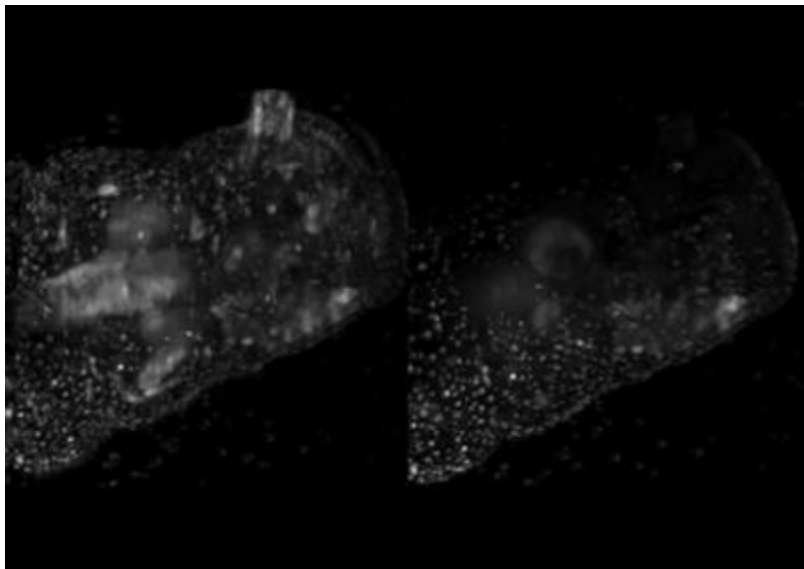


Figure 1: GFP-H2B labeled Drosophila embryos. The image on the left is a projection through a stack combined from four independently recorded stacks. It thus shows features that are 100s of μm apart along the optical axis. The image on the right is the projection through a single stack of SPIMages. The front is well resolved, the elements in the back remain invisible.