

Selective Plane Illumination Microscopy provides isotropic resolution in three-dimensional images of embryos as well as cells

Ernst H.K. Stelzer

European Molecular Biology Laboratory (EMBL)
Meyerhofstrasse 1, D-69117 Heidelberg, Germany, stelzer@embl.de

KEY WORDS: fluorescence, in vivo, live cell imaging, reconstruction

Confocal theta microscopy was invented about ten years [1] ago to provide a tool for the investigation of large specimens with a high and isotropic three-dimensional resolution. The fundamental principle is the detection of fluorescence light at an angle of 90 degrees to the illumination axis. A new implementation of this principle [2] takes advantage of parallel recording. This new high-resolution light microscope has been developed for the modern life sciences. It is designed to image large biological samples (e.g. embryos and three-dimensional cell cultures) down to the sub-cellular level. The fundamental principle of Selective Plane Illumination Microscopy (SPIM) remains the detection of fluorescence light perpendicular to the illumination axis. However, the illumination system selectively excites fluorophores within an entire plane, which is also the focal plane of a detection system consisting of a long working distance objective lens and a CCD camera. Selective plane illumination provides optical sectioning directly. Therefore, bleaching outside the thin volume of interest is avoided. Because of its capability to perform well with long working distance lenses and the good penetration depth of the illumination plane millimetre-sized specimens can be observed in their entirety. To further increase the resolution and the information content of the data stack, rotation of the sample changes excitation and detection axes with respect to the sample. Parts of the sample that would otherwise be hidden or obscured become accessible. Data stacks recorded at different angles can be combined in post-processing steps to yield a high-resolution image of the complete sample [3]. The 3D resolution is then dominated by the lateral resolution and resolution becomes identical along all directions, i.e. isotropic. Artefacts that are due to the elongation of the point spread function are avoided. For the past few years various different species (Medaka, mouse, drosophila, yeast, ...) were observed with the instrument. Results of a number of experiments that demonstrate the properties and excellent performance of SPIM in particular with live samples will be shown. This technology is of interest to all scientists working with large samples and trying to investigate features that require high three-dimensional resolution over a large volume. Moreover, SPIM technology opens up new dimensions in many disciplines. For the emerging field of 3D cell cultures [4] SPIM is an ideal instrument. It enables researchers to image a whole population of cells in their 3D context, to visualize their morphology in a matrix and to track cells through the context of surrounding tissue. The goal of this presentation is to introduce SPIM as a straightforward alternative to confocal and multi-photon microscopy that provides improved depth discrimination, high recording speed, and an excellent signal to noise ratio. It provides the opportunity to seriously reconsider many currently favoured approaches in the modern life sciences.

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