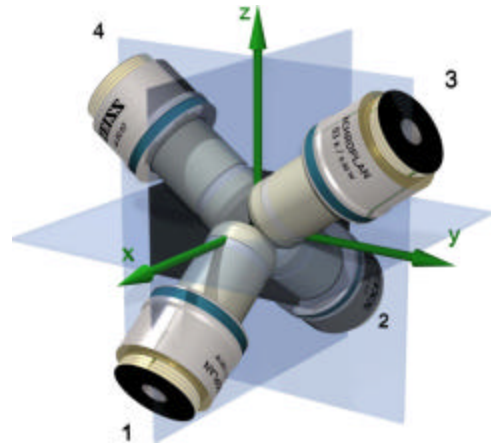


# Isotropic resolution and optical manipulation in multiview microscopy

Jan Huisken, Jim Swoger, and Ernst H.K. Stelzer  
Cell Biology and Cell Biophysics Programme  
European Molecular Biology Laboratory  
Meyerhofstrasse 1, 69117 Heidelberg, Germany  
E-mail: *lastname@embl-heidelberg.de*

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High-resolution fluorescence microscopy is an essential tool in modern molecular biology studies. The basic element of the Multiple Imaging Axis Microscope (MIAM)<sup>1</sup> described here is a set of four objective lenses arranged in a tetrahedral geometry around the sample, permitting observation from all sides. The objectives are water-dipping NA = 0.9 lenses with a working distance of ~1.5 mm, which allow the observation of millimeter-sized samples. Despite the relatively low NA of the individual objective lenses, the computational combination of images simultaneously acquired along the multiple axes of the MIAM allows the generation of a combined image with a nearly isotropic resolution of ~220 nm. The hardware and the technical implementation of the MIAM as well as the post-processing steps required to generate a single high-quality image from the four individual views, are discussed. Examples of specimens imaged with the MIAM are presented. These illustrate the power of the system as a tool for the optical investigation of thick biological samples.



Trapping and active movement of the sample in the microscope is gently performed by applying optical forces. In our Differential Active Optical Manipulator (DAOM)<sup>2</sup> four beams are arranged along the axes of a tetrahedron and are approximately collimated within the vicinity of their point of intersection. The DAOM makes use of a distinctive advantage of collimated light; in contrast to optical tweezers, this instrument allows movement of the confined particle over long distances (i.e. 100s of  $\mu\text{m}$ ) without mechanical scanning. The beams' scattering forces superimpose in their common volume, and the total force (magnitude and direction) on the particle can be set by adjusting the beams' intensities. This can be done quickly and independently for each beam, allowing us to move a particle along arbitrary paths within a volume of about 1nl. Active feedback ensures that the sample can be held in place or transported to a specified position as desired. It provides focusing and lateral translation of the microscopic sample.

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<sup>1</sup> J. Swoger, J. Huisken, E.H.K. Stelzer: Multiple imaging axis microscopy improves resolution for thick-sample applications, *Opt. Lett.* **28**, 1654 (2003)

<sup>2</sup> J. Huisken, J. Swoger, E.H.K. Stelzer: Three-dimensional optical manipulation using four collimated intersecting laser beams, submitted