

# OPTICALLY SECTIONED IMAGING BY OBLIQUE PLANE MICROSCOPY

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Selective plane illumination microscopy (SPIM) [1] is an elegant method for performing optically sectioned and 3D fluorescence imaging. However, SPIM requires that the sample is illuminated by a lens placed at  $90^\circ$  to the collection objective and therefore conventional sample preparation techniques, e.g. microscope slides, cannot normally be used. Recent work has addressed this issue and *in vivo* imaging of neuron activity has been demonstrated [2].

This paper demonstrates and discusses an alternative method that uses a single high numerical aperture objective lens to both illuminate and image the specimen and to achieve optically sectioned imaging without the use of any beam scanning or image processing [3]. The experimental setup is shown in fig. 1. The image (P1) of the obliquely illuminated specimen is demagnified by a second ‘microscope’ (L3-4) to form a second image of the specimen where the axial and lateral magnifications are equal (P2). Finally, a third microscope (L5-6) placed at an angle to the second is used to obtain the final optically sectioned image (P3).

Results obtained using the oblique plane microscopy (OPM) system will be presented, together with an analysis of the potential performance of such systems, both in terms of resolution and collection efficiency. OPM can readily be implemented as an add-on to a conventional fluorescence microscope and therefore can be employed in combination with other established microscopy techniques, e.g. bright field and phase contrast imaging. This makes OPM an attractive technique for biologists wanting to use multiple microscopy techniques on the same specimen and field of view. OPM may be attractive for time-lapse monitoring of biological samples, e.g. the early development of embryos. Its potential for high speed imaging and its ability to image through coverslips and substrates could also be exploited to provide high-speed optically sectioned images of processes occurring within microfluidic devices or high throughput imaging of multiwell plate or biochip sample arrays.

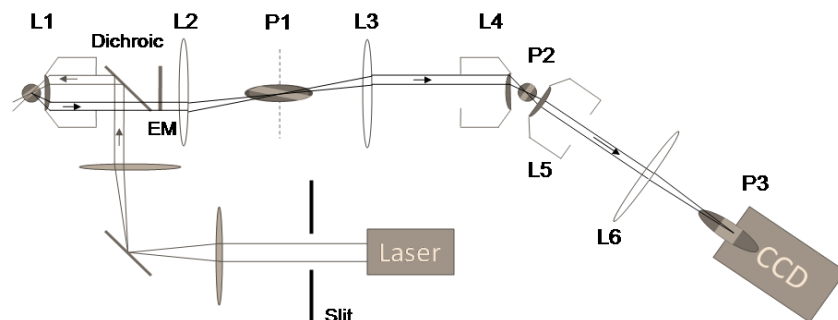


Figure 1: Experimental setup for oblique plane microscopy.

L1-6, lenses; EM, emission filter; P1-3, image planes.

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[2] T. F. Holekamp, D. Turaga, and T. E. Holy, "Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy," *Neuron* **57**, 661-672 (2008).

[3] C. Dunsby, "Optically sectioned imaging by oblique plane microscopy," *Optics Express*, **16** (25), 20306-20316 (2008).