Washington University in St. Louis
Office of Technology Management

LIGHT SHEET MICROSCOPY WITH OBJECTIVE-COUPLED SELECTIVE PLANE ILLUMINATION

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Technology Description

Researchers in Prof. Timothy Holy's laboratory at Washington University in St. Louis are among the independent inventors of light sheet fluorescence microscopy and the first to use it for large-scale calcium imaging. This patented technology, OC-SPIM (objective-coupled selective plane illumination microscopy), covers the use of light sheet microscopy when collecting volumetric images by manipulating the optics rather than the sample. This widely-adopted practice has enabled three-dimensional imaging in large volumes of intact tissue without specialized sample mounting procedures.

Patents and Exemplary Claim

Objective-coupled selective plane illumination microscopy (U.S. Patent No. 8,254,020 and 9,383,568)

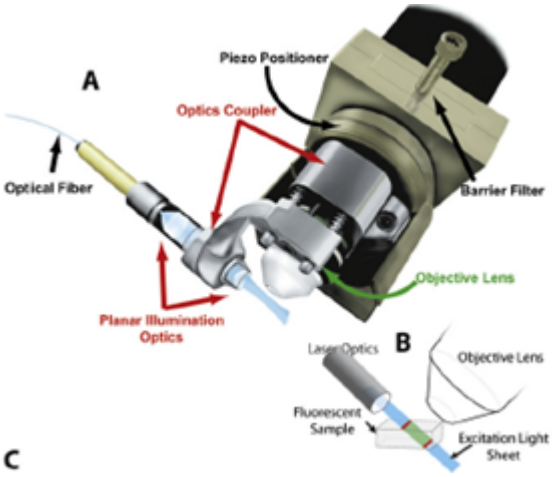
1. A system for imaging comprising:

an illumination source configured to generate and communicate a light beam to an optic assembly configured to focus the light beam into a thin light blade and to an illumination plane;

an objective having a lens and an optic axis and configured to focus at a focal plane and to move along the optic axis;

a processor in operative association with said illumination source and the objective through first and second actuators, respectively, by control signals generated by the processor, the processor being operative to independently move both the illumination source and the objective to ensure the movement is synchronized to maintain a fixed relationship between the illumination plane of the illumination source and the focal plane of the objective as the objective moves along the optic axis such that the illumination plane illuminates only the focal plane and excites only the in-focus portion of a sample; and

a detector configured to detect at least one projected image from the illuminated focal plane through the objective.



(A) Schematic of the original OCPI microscope: A thin sheet of light from a laser sections the sample. The light sheet is aligned with the objective focal plane to prevent fluorescence excitation in out-of-focus regions. A piezoelectric positioner couples the Illumination optics to the objective lens, maintaining alignment with the objective focal plane during z-axis scanning. The orientation of the microscope can be tilted to adapt to sample mounting. (B) Schematic showing the orientation of tissue, objective, and illumination optics. (Holekamp, T. F., Turaga, D., & Holy, T. E. (2008). Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy. *Neuron*, 57(5), 661-672.)

A more recent design of the same principle: Greer, Cody J., and Timothy E. Holy. "Fast objective coupled planar illumination microscopy." *Nature communications* 10.1 (2019): 1-14.

Applications

- **High speed light sheet microscopy** with end user applications in neurology, cell biology, immunology and developmental biology

Key Advantages

- **Very high speed three dimensional imaging** with minimal photodamage and low noise
- **Visualize large samples**

Related Web Links

- Timothy Holy Profile
- Holy Lab