

3D Laser-Nanosurgery with Selective Plane Illumination Microscopy
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ABSTRACT:

Modern research in the fields of cell biology and biophysics increasingly requires imaging techniques with improved temporal and spatial resolutions to observe the sample in vivo. Furthermore it is often necessary to specifically interact with the sample to gain information about dynamic processes (e.g. microtubule-(de)polymerization).

The length scales of such processes can range from the order of hundreds of nanometers in subcellular compartments to the order of some millimeters in embryos or organs, ideally observed over time scales ranging from less than a second to several hours or even days.

The development of the Selective Plane Illumination Microscope (SPIM) [1] allows the generation of optically sectioned images with an almost isotropic spatial resolution, at fast recording times and at a substantially reduced light exposure to the fluorophores leading to less photobleaching. However this instruments lacks the possibility to optically interact with the specimen.

Current laser-based nanosurgery [2] can be performed with high efficiency, sub-micrometer precision and at high speed, but could still benefit from an imaging system with optical sectioning capabilities.

An ideal instrument therefore would incorporate both the properties of the laser-nanoscalpel (to interact with the sample) and the properties of the SPIM (to observe it).

The combination of an UV-laser-based nanodissection system with a Selective Plane Illumination Microscope enables us to perform diffraction limited nanosurgery in live biological material while observing the development of the sample during the intervention. It will be a very worthwhile tool for research in current fields of biology, e.g. developmental biology, 3D cell cultures, cytoskeleton-dynamics or neuroscience.

This presentation shows the development of the instrument and a proof of feasibility of ablating and imaging biological material in three dimensions.

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[1]

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