Light Sheet Ultramicroscopy

High-resolution 3D Microscopic Views Beneath the Surface

We developed a new ultramicroscopy design equipped with modified optics to achieve 3D-vizualizations of specimens with μm-resolution. The optical unit consists of elements with complex surface structures to create an ultra-thin light sheet. Diffraction and other unwanted optical effects are minimized, while the laser energy is used more efficiently. This enables us to obtain marked enhancements in resolving fine details of specimens (e.g. fruit-flies, entire mouse brains, and mouse hippocampi).

Ultramicroscopy: Efficient Imaging Technique

Ultramicroscopy (UM) is a powerful tool for visualizing large biological samples providing micrometer resolution [1-3]. It uses nondestructive optical sectioning by laser light sheets. Beside its application in UM, the generation of such laser light sheets is a subject of interest for scientists in various research fields, such as microbial oceanography, and fluid mechanics [1]. In 2007, Dodt et al. presented a standard UM system in which the specimens are illuminated by two identical light sheets from two sides. Each light sheet is produced by an optical unit, consisting of a rectangular slit aperture, and one cylindrical lens [1]. By truncating a magnified Gaussian beam, the slit aperture generates an output beam with semi-uniform intensity distribution. Then the cylindrical lens of focal length \( f \) focuses this beam in one direction, approximately providing no changes in the perpendicular direction. As a result, the laser beam is focused into a thin light sheet [1]. The uniformity of laser intensity along the focusing line, and its length are important issues in UM [4].

It has been shown that the optical characteristics of such a light sheet (i.e. Rayleigh Range, power density, and beam width) depend on the size of the slit aperture [4]. For example the combination of a large rectangular aperture of 8 mm width and a cylindrical lens of 80 mm focal length produces a light sheet with a beam diameter of ~ 4 microns at the focus, which diverges rapidly. This results in a short Rayleigh range, and poor uniformity along the axes of the light sheet.
On the contrary, usage of a small aperture (e.g. 2 mm width) prolongs the Rayleigh range, but increases the thickness of the light sheet to about 12 microns at the focus (fig. 1A). Additionally, this results in a major loss in illumination power. To overcome the drawbacks of the standard system, more sophisticated optical designs have been developed. In 2010, we introduced a combination of one plano-concave spherical lens, and three cylindrical lenses being placed in parallel/cross positions (fig. 1B) [4]. Since this system works without any hard-edge aperture, loss of laser light energy is markedly reduced (fig. 1C). Here, we describe further improved light sheet generator, which utilizes elements with complex surface structures such as aspheric lenses [5]. These components reshape an annular symmetric Gaussian beam emitted from a 500 mW Sapphire laser (488nm, ~1mm beam width, and M² ≅ 1.1) into a thin sheet of light with optimized optical parameters. The laser beam is divided by a 50% beam splitter, and guided toward two identical optical units containing several optical elements with an aspheric surface. It reshapes the beam into two co-axial light sheets illuminating a thin section within the specimen [5].

Each optical unit includes two aspheric lenses with particular optical characteristics to reshape the incident beam towards an elliptical beam with semi-flat-top profile. Then a condensed aspheric lens and an aspheric cylindrical lens stabilize and focus the reshaped beam. The focus of the last lens can be adjusted by a computer controlled movable stage, which can be shifted along the beam propagation axis. This allows adjusting the light sheet providing the best possible uniformity for each specimen. For optical sectioning, a second computer controlled stage allows to move the specimen chamber vertically through the light sheet, while a microscope with modified infinitive objectives projects an image on the CCD-chip of a high resolution camera. Finally, 3D-reconstruction of the specimen is done using the software package Amira (Visualization Science Group, USA).

**Imaging Fluorescence Signals in Biological Specimen by** **UM**
For UM imaging, it is a prerequisite that the specimens are translucent. Since biological tissues are usually opaque, specimens have to be chemically cleared prior to microscopy. Chemical clearing media approximately match the refractive index of proteins, thereby reducing light scattering inside of biological tissues [6]. In contrast to other techniques like electron microscopy, UM enables us to visualize and analyze the outer surface, as well as inner structure of small and large specimens, simultaneously. Fluorescence labeling, as well as unspecific autofluorescence can be utilized for image generation [5]. UM has already been successfully applied for human tissues, as well as for model animals as mouse and fruit-fly. Figure 2 shows a subset of neurons expressing green fluorescent protein (EGFP) inside a whole mouse brain recorded with different magnifications. Figure 3 presents images of a fruit-fly (*Drosophila melanogaster*) using a 10x objective. A supplementary movie demonstrates fine details of the fruit-fly [7].

**Conclusion**

We presented an optical design, which allows improved optical sectioning of biological samples, via UM. Compared to the standard system, it allows resolving finer details of biological samples such as mouse brains and fruit-flies.

**Reference**

[7] [www.tuwien.ac.at/aktuelles/news_detail/article/7892/](http://www.tuwien.ac.at/aktuelles/news_detail/article/7892/)

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