AN OPTOGENETICS TOOLBOX FOR TWO PHOTON MICROSCOPY

Markus Wewer, Bernd Müller-Zülow, Uwe Schröer, Thomas Pingel, Heinrich Spiecker
LaVision BioTec GmbH
Astastrasse 14, 33617 Bielefeld, Germany
Email: wewer@lavisionbiotec.com

Key Words: two photon microscope, optogenetics, phototreatment, FRAP, resonant scanner, trigger sequencer

The optogenetic method uses techniques from optics and genetics. This technique becomes very popular in neuroscience to control and observe the activities of single neurons and networks in living tissues or animals. It is even possible to measure these effects of manipulation in real time [1]. In optogenetics light sensitive proteins like rhodopsins play a major role. These proteins can be stimulated by visible light. On the other hand a millisecond temporal precision is needed to get the full biological information from the process. We will discuss how an advanced concept of controlling light sources, scanner hardware and optics provides flexibility to adapt the microscope to this rapidly developing field.

LaVision Biotec as a leading manufacturer for enhanced two photon microscopes now introduces a new concept to cover the needs for optogenetic methods in two photon microscopy. Three independent scanners can be used for imaging and stimulation. The stimulation with visible light can be done with three laser wavelength in the visible range. These lasers can be modulated in a μs time scale while imaging continue with either a normal galvanometric scanner or a resonant scanner. The imaging laser wavelength range is from 690nm – 1500nm. Very fast switching (in the millisecond regime) between imaging and stimulation modes becomes feasible. In combination with a newly developed trigger sequencer complex sequences of stimulation of regions of interest and even single cells are now possible. We also present a new spectral detector with the possibility of detecting up to six colors simultaneously. The PMTs are protected with a fast shutter to prevent those detectors from damage when stimulating with visible light.