TWO-COLOR MULTIPHOTON SCANNED LIGHT-SHEET MICROSCOPE FOR IN VIVO INVESTIGATION OF ZEBRAFISH LARVAE

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Two-photon microscopy allows for sample excitation with low scattering and absorption due to the use of light in the infrared spectrum [1]. The sample absorbs two photons of half the energy difference between ground state and excited state. Using infrared two-photon excitation we avoid the stimulation of the visual system of living specimen thus preventing unphysiological irritation during their development.

Two-color excitation enables the simultaneous imaging of two fluorophores in the same plane of the sample. By detecting the fluorescence simultaneously with two cameras a high imaging speed is ensured. The illumination from two opposing directions allows to either enhance the contrast by reducing illumination artifacts [2] or to enlarge the illuminated region by slightly displaced foci of the illumination beams, respectively.

We extended a confocal two-photon scanned light-sheet fluorescence microscope (LSFM) equipped with two opposing illumination arms by two-color excitation and simultaneous two-color detection. The light source is an ultrashort pulsed solid-state laser with <120 femtoseconds pulse width and continuous tunable wavelengths between 680-1300 nm. The laser features a second beam output at 1041 nm for simultaneous excitation of the specimen using two different wavelengths.

We analyze the spinal cord development of double transgenic zebrafish larvae in vivo in their natural horizontal position. Given our excitation wavelengths we can use e.g. the genetically encoded fluorescent proteins eGFP and tdTomato. With this microscope we can realize long term imaging in the range of hours up to days to follow the development of fluorescent reporters in the sample. Also, we can realize ultra-fast imaging in 3D in the range of milliseconds with high resolution in two colors.

The instrument performance is characterized. Experimental data from in vivo studies in double transgenic zebrafish larvae is presented focusing on the myelinating oligodendrocytes in the spinal cord.

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