ULTRAFAST ELECTRO-OPTICAL SCANNING FOR STED NANOSCOPY

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KEY WORDS: electro-optical deflector, ultrafast beam scanning

The scanning system is one of the most important components of a laser scanning fluorescence microscope. Its speed determines the pixel dwell time and the frame rate during image acquisition. In most cases, high frame rate with short pixel dwell times are desired to enable observation of fast dynamic processes and to lessen the triplet state occupation of fluorophore molecules.

Today, galvoscaners or acousto-optical deflectors (AOD) are typically employed to deflect the laser beam. However, galvoscaners are relatively slow and the AODs show wavelength dependency [1].

An electro-optical deflector (EOD) capable of deflecting light based on the Pockels effect overcomes these limits, in principle, since it operates independent of the wavelengths and is very fast. Even so, their implementation in scanning microscopes has been scarce since the deflection angle achieved with these devices is low. In diffraction limited microscopy only a few tens of resolvable features can be imaged. However in diffraction unlimited scanning techniques such as stimulated emission depletion microscopy (STED), EOD are very useful scanning tools. Since STED nanoscopy is able to resolve about ten times better than the diffraction limit [2], many hundred resolvable features can be addressed with EOD.

Nevertheless, very high voltages in the kV range have to be applied to the crystal to achieve reasonable deflection angles. Hence, high voltage generation at high frequencies becomes a challenge.

In the framework of this study, a compact electro-optical deflection system along with its appropriate driver are developed and integrated into a STED microscope. The driver generates triangle waveforms with amplitudes up to 2kVpp at 250kHz. In our optical configuration, this enables a linear scan over 6μm length within 2μs, which is ~1000 times faster than most galvoscaners. The pixel dwell time is shortened to 6.25ns and the frame rate is increased up to >1500 frames per second at 320x320 pixels.