Introduction

Biology changed its outlook with the advent of lasers in 1960s. The key revolution was in microscopy where, one could now observe features even inside cells. However, it is difficult to focus light down to a spot smaller than about half a wavelength. Stimulated emission depletion microscopy (STED) uses fluorescence to image cells beyond this diffraction limit.

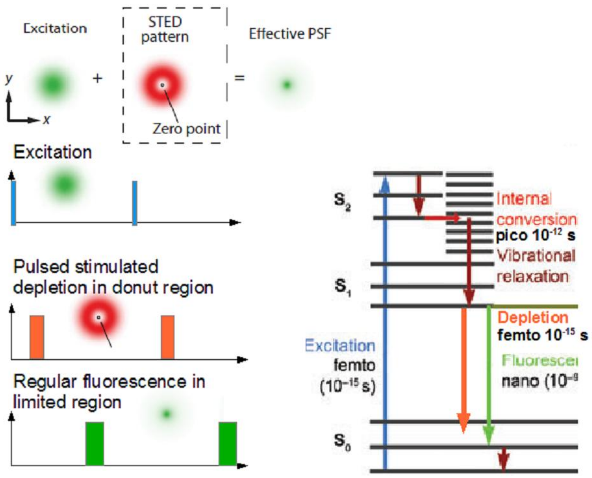

Figure 1. STED working principle with a pulsed depletion, also showing the energy levels.

In a STED microscope, we encourage molecules to make the transition from a higher energy state to a lower energy state, using light of a particular wavelength. The molecules that undergo this depletion of energy can no longer fluoresce. By selecting the region of depletion to be in the periphery of the excitation spot, the effective fluorescing spot size (denoted as PSF) is smaller than either of the excitation and depletion spots . The process is shown schematically in Figure 1.

Pulsed Depletion Laser

The power levels of a depletion laser can cause photo-bleaching of the sample. To circumvent this problem, we have developed a pulsed depletion laser, synchronous with the excitation laser.

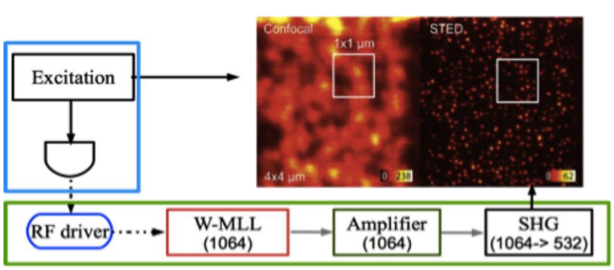

Figure 2. 532nm pulsed depletion laser design, expected to yield images with less than 100 nm resolution.

To achieve synchronization, afraction of the excitation laser-power is fed to a photo detector,. Theradiofrequency (RF) output of the detector is then fed to an appropriatedriving circuit to generate appropriate electronic pulse that can drive the next module, which is an actively mode locked laser (MLL). The MLL produces sub-nano second optical pulses of 1064 nm wavelengths in synchronization with excitation laser, which are amplified and converted to 532 nm pulses using a second harmonic generation (SHG) crystal. This is shown schematically in Figure 2.

Results

Feature	Target	Achieved
Pulsewidth (ps)	<500	less than 200 ps
Repetition rate (MHz)	80.1	80.1 with pulse input
Peak power (mW@532 nm)	250	450 at 80.07MHz

Table I
PERFORMANCE OF THE DEPLETION LASER.

Table 1 shows the specifications of the pulsed depletion laser, and Figure 3 shows the synchronized pulse train. We have constructed a fiber based pulsed depletion laser at 532 nm, and tested it for synchronization with a Ti:Sapphire laser. The effectiveness of the pulsed depletion laser will now be investigated by characterizing the flourescence life-time of suitable flourophores.

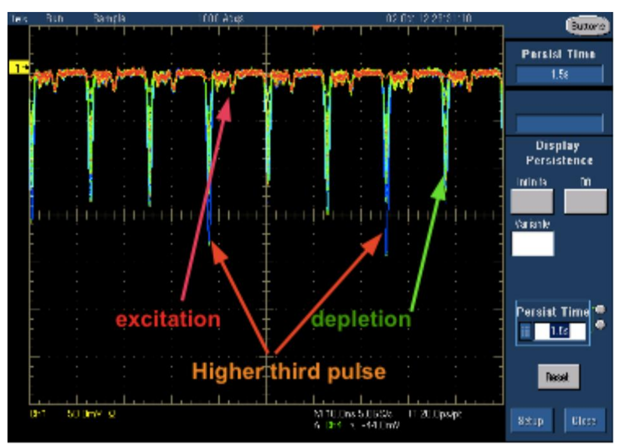

Figure 3. Synchronization between excitation and depletion pulses showing mode locking characteristics.

Publication

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