EFFICIENT EXCITATION OF BLUE EMITTING DYES BY TWO-PHOTON MICROSCOPY AT VISIBLE WAVELENGTHS

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An important advantage of multi-photon microscopy over more traditional single-photon imaging techniques is the elimination of high-energy laser sources that can cause significant photo-damage to delicate biological specimens. This is particularly acute when short wavelength excitation is required.

A number of papers (including [1,2]) have investigated the optimum two-photon excitation wavelengths for short-wavelength excitable molecules and have reported that for several fluorochromes the two-photon cross-section increases rapidly at shorter wavelengths. However, most papers thus far have used a Ti:Sapphire laser, which has a wavelength tuning range of 680-1080nm, and therefore few (and mainly cuvette based) data of two-photon excitation efficiency is available for excitation wavelengths shorter than 680nm.

Using a new wavelength tunable optical parametric oscillator and frequency mixing system (Coherent Chameleon OPOVis), we have studied the wavelength dependence of two-photon excitation efficiency of a number of common UV excitable dyes, such as the nuclear stain DAPI, starch staining dye Calcofluor White and Alexa 350, in the wavelength range 530nm to 770nm. We will report details of the optical system and biomedical specimens used to make our measurements, together with a summary of our results.

One key finding is that DAPI, a very commonly used nuclear marker, when excited at 590nm, results in a 7 times increase in the fluorescence signal output when compared to excitation at 680nm with the Ti:Sapphire laser. We also find that although the rate of photo-bleaching increases at shorter wavelengths, it is still possible to acquire many images with higher fluorescence intensity. This is particularly useful for applications where the aim is to image the structure, rather than monitoring changes in emission intensity over extended periods of time.


Figure 1: The two-photon excitation spectrum of the nuclear stain DAPI, extracted from images of individual 3T3 cells. Also indicated are the wavelength ranges available with a Ti:Sapphire laser.