Improved contrast in confocal microscopy by using a blue frequency doubled diode-pumped solid-state laser

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Abstract: The standard Ar ion laser in a confocal microscope was replaced with an intra-cavity frequency doubled Nd:YAG laser operating at 473 nm. The fluorescence image quality suggests that excitation at 473 nm could be preferable.

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Fluorescent labelling enables detection and imaging with exquisite sensitivity. In confocal microscopy, fluorescently labelled tissue samples and live cells can be studied in detail. One of the most common fluorofores is fluorescein, which absorbs around 494 nm and emits around 519 nm [1,2]. Normally, fluorescein excitation in confocal microscopes and biomolecule separation systems is achieved with an Ar ion laser emitting at 488 nm. The same excitation system is used for e.g. the genetically engineered intra-cellular fluorofore green fluorescent protein (GFP).

Replacing the Ar laser with a compact frequency doubled diode-pumped solid-state laser (DPSSL) operating at 473 nm has several advantages. Apart from the obvious advantages of compactness, lower noise, longer lifetime, and potentially lower cost, the replacement can also give potential higher signal-to-noise ratio due to better filter function as excitation and emission wavelengths have a larger wavelength separation. Another advantage with excitation with 473 instead of 488 nm is the lower absorption dependence on pH, giving a system more insensitive to variations in buffers or sample preparations [3], Fig. 1. A drawback with excitation at 473 nm is the lower absorption compared to at 488 nm. However, we believe that the lower noise of the DPSSL combined with improved filter function will compensate for the lower absorption, resulting in a preference for excitation with the DPSSL.

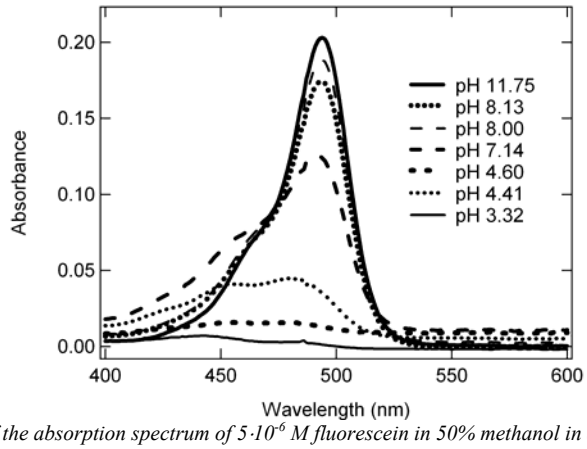

Fig. 1. pH dependence of the absorption spectrum of $5 \cdot 10^{-6}$ M fluorescein in 50% methanol in deionized water [3].

In a demonstration experiment, a commercial 50 mW intra-cavity frequency doubled Nd:YAG laser operating at 473 nm [4] was used for excitation in a Zeiss LSM5 Pascal confocal microscope. The same optical fiber as used for the standard Ar laser was used for coupling the 473 nm line into the microscope. An 80/20 beamsplitter was used in the

position of the primary dichroic. Fluorescence from the sample was separated in two wavelength bands with a dichroic mirror at 545 nm and further selected by emission filters at 505-530 and >560 nm. Figure 2 shows fluorescence from cell walls in the *convallaria* root, a stardard test sample. The apparent image quality was assessed to be equal or better as with the original Ar ion laser. Quantitative measurements are underway to compare the performance of Ar laser and DPSSL in a confocal microscope set-up.

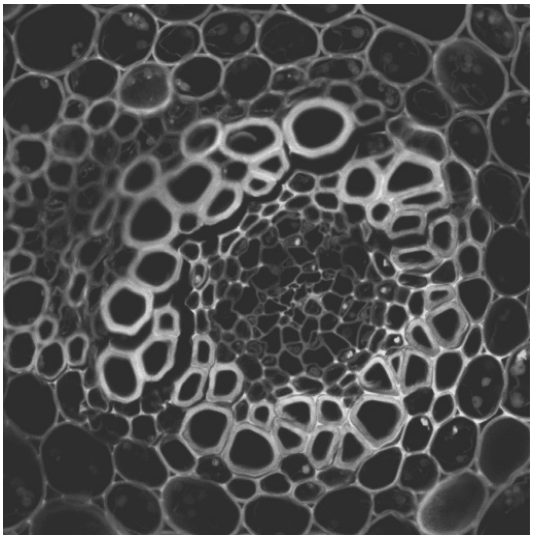

Fig. 2. Confocal microscopy image of *convallaria* root. The image side is 250 µm.

[1] LightCycler-Fluorescein CPG product information sheet (3 113 906), Roche Diagnostics GmbH, Mannheim, Germany.
[2] R Sjöback, J. Nygren, M. Kubista, "Absorption and fluorescence properties of fluorescein", Spectrochimica Acta A, **51**, L7-L21 (1995).
[3] Personal communication with S. Franzen, Department of Chemistry,North Carolina State University, Raleigh, NC 27695, USA.
[4] Cobolt Blues, data sheet obtained from http://www.cobolt.se on 031117.