

NEW CONCEPTS FOR SENSITIVE MULTICOLOR CONFOCAL MICROSCOPY

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KEY WORDS: multi-color, confocal, spectral, CCD, sensitivity, prism

The limitations of confocal fluorescence microscopy are resolution and low signal. Signal is limited by dye concentration and photobleaching. Therefore, increasing the detection efficiency is a prominent goal of instrument design [1].

Simultaneous imaging of multiple structures requires the ability to separately detect a large number of fluorochromes. This requires a sufficient number of excitation sources and flexible detection channels.

Here we present three approaches to improve the detection efficiency of multicolor confocal microscopes.

- A novel beam-splitter uses 3 dispersing prisms and a structured reflection mask to separate an arbitrary number of laser lines from the emission light. This setup achieves an edge steepness of less than 2 nm and has a transmission above 90% (see Figure 1).
- A Scan-head with a stationary beam on both scan-mirrors achieves a constant illumination of the objective back aperture. A new digital controller with unprecedented speed and accuracy moves the scan mirrors at the physical limit of the galvo.
- A prism spectrograph with a sensitive CCD camera is used as detector. The camera has a peak quantum efficiency of >60% at 520 nm. The spectrum is spread over 40 to 60 pixels. Arbitrary detection channels can be defined electronically by on-chip binning. The detection channels can be redefined without delay between two scan lines. Figure 2 shows the fluorescence signal of a single voxel of a *Tetraspeck* sample with 40 detection channels.

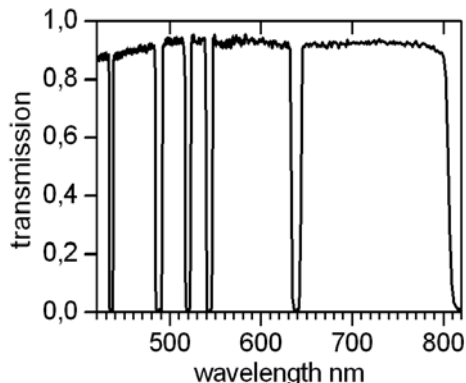


Fig. 1: Transmission spectrum of the beam-splitter.

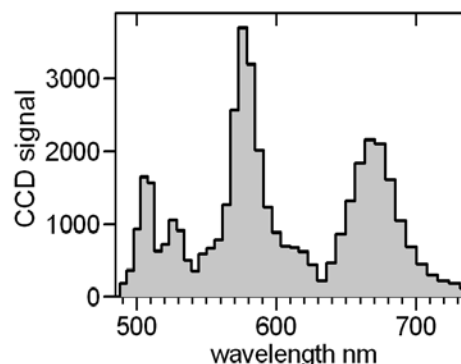


Fig. 2: Spectral composition of a voxel containing three dyes.