

A FAST AND SENSITIVE SPECTRAL DETECTOR FOR CONFOCAL MICROSCOPY

Joachim Walter[§], Christian Seebacher[§], Rainer Uhl^{§§}
[§]TILL I.D., Am Klopferspitz 19, 82152 Martinsried, Germany
^{§§}Bio Imaging Zentrum der LMU München,
Am Klopferspitz 19, 82152 Martinsried, Germany
E-mail: walter@biz.uni-muenchen.de

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

State of the art confocal microscopes with arbitrary beam-scanning employ photomultipliers as detectors. These devices have peak quantum efficiencies of about 30% in the UV to blue spectral region. CCD cameras can achieve quantum efficiencies of more than 90%, and the peak efficiency is in the green to red, which fits better to the emission spectra of commonly used fluorochromes. Up to now CCD cameras were not used as point detectors due to high readout noise or low readout speed [1].

Here we present a spectral detection scheme (Fig. 1) employing a CCD camera with less than 3 electrons RMS readout noise at a 10 MHz pixel clock-rate. Light from the confocal pinhole is collimated and split into its spectral components by a multi-element prism, which is designed to approach a linear dispersion. The spectrum is focused onto one column of the CCD chip. As the CCD camera only supports reading of frames, a galvanometer-driven mirror is used to distribute the signal from one scan-line in the specimen over the width of the CCD chip.

With this detection scheme, full fluorescence spectra of the sample can be recorded for each image point. Alternatively, detection channels can be flexibly defined by on-chip binning. The number and spectral region of the detection channels can be switched without delay between scan-lines. Depending on the channel setup, pixel times below 1 μ s can be achieved.

Detection efficiency can be enhanced by means of a novel beam-splitter, which uses 3 dispersing prisms and a structured reflection mask to separate the laser lines from the emission light. This setup achieves an edge steepness of less than 2 nm and has a transmission above 90% across the visible spectrum.

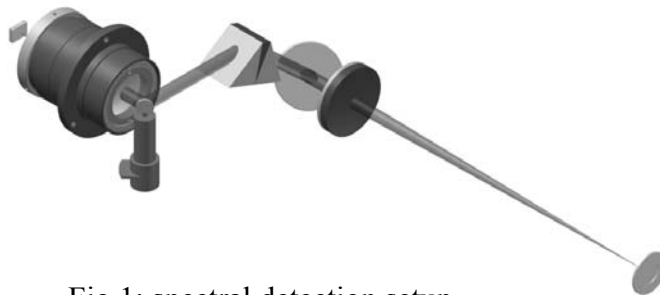


Fig.1: spectral detection setup

[1] J. B. Pawley, Handbook of Biological Confocal Microscopy, Chapter 12, 2nd edition, Plenum Publishing Corporation, New York, 1995.