Ratio Imaging - Simultaneous detection of fluorescence images excited by two wavelengths

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INTRODUCTION

An innovative detection unit using a fiber bundle for image delay is presented. The technique opens up new possibilities for the simultaneous detection of fluorescence signals of identical emission, but different excitation spectra. In that context, the technique can be used for measurements of intracellular redox states and for studies concerning apoptosis and necrosis. Since illumination is realized by a SPIM module [1] the technique is ideally suited for studies on 3-dimensional multicellular spheroids [2] used e.g. in tumor research. Both, detection unit and SPIM module, are mounted to a conventional inverse microscope.

SETUP

Two wavelength excitation is realized by two pulsed laser diodes with a repetition rate of 40 MHz and a time shift of Δt = 12.5 ns between one another in a light sheet module adapted to a conventional inverse microscope [1]. The detection unit includes a dual-view device and an image transmitting fiber bundle delaying one of the images by Δt = 12.5 ns. The gated camera is triggered by a master pulse generator, and images are integrated over several time frames in the ns-range (with the fluorescence image excited by λ_1 being on side 1 and that excited by λ_2 on side 2 of the detection area). Since the samples, in particular 3-dimensional multicellular spheroids are located in micro capillaries, measurements can be performed under flow conditions as well [1]. The setup is depicted in Figures 1 and 2.

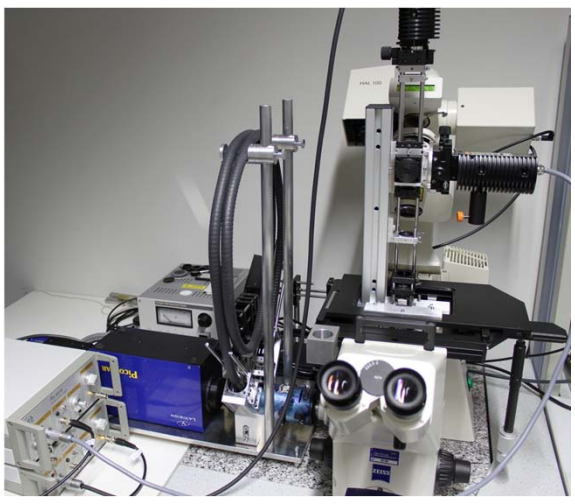

Figure 1. Modules for SPIM and ratio imaging mounted to an inverse microscope.

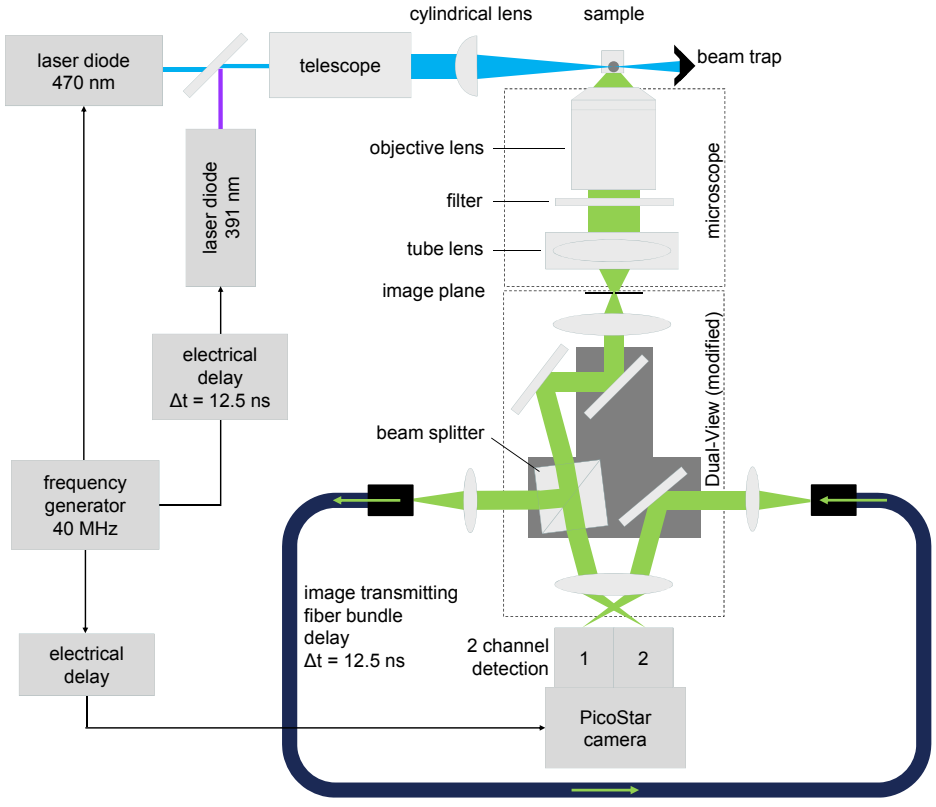

Figure 2. Scheme of the optical setup - SPIM combined with nanosecond ratio imaging.

APPLICATIONS AND RESULTS

Applications include characterization of active agents in tumor therapy, studies concerning apoptosis and necrosis and measurements of intracellular redox states giving information on the presence of reactive oxygen species, e.g. in tumors. Furthermore, the detection technique is presently modified for online fluorescence lifetime measurements.
Spheroids of U251MG-L106 glioblastoma cells permanently transfected with the glutathione sensitive green fluorescent redox sensor Grx1-roGFP2 were used for measurements of the intracellular redox state. The excitation spectrum of the redox sensitive GFP variant roGFP2 (data extracted from [3]) is shown in Figure 3. Oxidation was induced by 50 µM hydrogen peroxide (H_2O_2) in salt solution (EBSS) pumped through the micro-capillary in which the spheroid was located. Ratio images (Figure 4) were calculated by dividing the fluorescence intensities excited at 391 nm by those excited at 470 nm pixel by pixel. The time curve of the mean ratio values of this layer is depicted in Figure 5. It is shown that oxidation of glutathione is nearly completed after 90 s exposure to H_2O_2, predominantly in the outer cell layer of the spheroid.

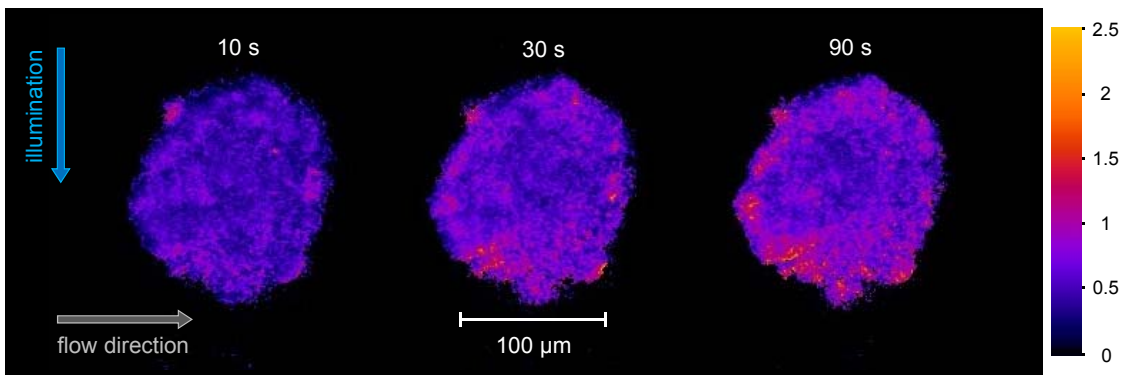

Figure 4. Ratio images of one layer (z = 70 µm) of a spheroid of U251MG-L106 glioblastoma cells permanently transfected with the glutathione sensitive green fluorescent redox sensor at various times in the flow of EBSS containing 50 µM H_2O_2.

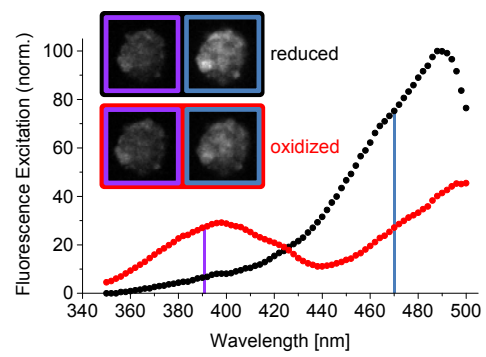

Figure 3. Excitation spectrum of roGFP2 (spectral data extracted from [3]).

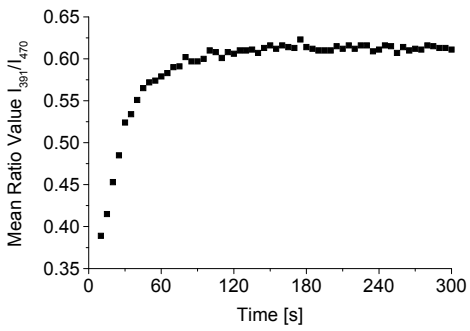

Figure 5. Time curve of the mean ratio values.

SELECTED REFERENCES

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[3] C.T. Dooley, T.M. Dore, G.T. Hanson, W.C. Jackson, S.J. Remington, and R.J. Tsien, "Imaging Dynamic Redox Changes in Mammalian Cells with Green Fluorescent Protein Indicators," *J. Biol. Chem.* 291(21), 22284-22293 (2004).

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