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Label-Free Third Harmonic Generation Imaging and Quantification of Lipid Droplets in Live Filamentous Fungi

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Abstract. Lipids in oleaginous filamentous fungi are considered to be a valuable alternative resource for various biotechnological applications. *In vivo* label-free imaging enables monitoring of fungi lipid droplets (LD) accumulation with the minimal unwanted effects on the metabolism. LDs are the main source of contrast [1] in the Third Harmonic Generation (THG) microscopy method [2] due to their optical properties (high refractive index). The THG phenomenon is utilized in nonlinear laser scanning microscopy that employs ultra-short laser pulses for imaging. We present *in vivo* and label-free THG imaging of the individual hyphae of *Phycomyces blakesleeanus* (Figure 1), an oleaginous filamentous fungus with very rapid growth rate. The THG signal was detected in the forward direction (transmission arm) by PMT through Hoya glass UV filter with peak transmission at 340 nm. The Yb KGW laser, wavelength 1040 nm, pulse duration 200 fs, and repetition rate 83 MHz, has been the source of the infrared femtosecond pulses. The LDs from THG images were quantified by two image analysis techniques: Image Correlation Spectroscopy (ICS) and software particle counting – Particle Size Analysis (PSA). We used hyphae that undergo nitrogen starvation, which is known to cause alterations in lipid metabolism and increase of cellular lipid reserve. The two analysis methods gave similar results. The applicability of the described procedure can be easily extended to other unicellular organisms for the *in vivo* quantification of LDs since there is no need for sample labeling, fixation or any other specific preparation. In addition, we demonstrate that the ICA is suitable for THG images, although it is primarily developed and have been mostly used for fluorescence signals so far.

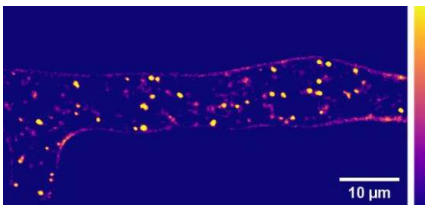

Figure 1. Label-free THG image of live *Phycomyces blakesleeanus* hyphae (4.5h nitrogen-depleted conditions). The bright structures are lipid droplets. The image was taken with Zeiss 40x 1.3 oil objective lens. The average laser power: 20 mW. Color intensity bar: deep blue – the lowest, yellow - the highest THG signal.

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