EUROPEAN MOLECULAR IMAGING MEETING

18th Annual Meeting of the European Society for Molecular Imaging

EMIM 2023 | 14th-17th March

Salzburg Congress, Austria

Programme Overview Session: PW36

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Isabelle Heing-Becker[1], Ria Thielhorn[1], Nadja Hümpfer[1], Jakob Rentsch[1], Rainer Haag[1], Kai Licha[1], Helge Ewers[1]

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Mucus Permeation of Lipid Coated Nanoparticles through 3D Human Lung Epithelial Model

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Third harmonic generation imaging of live fungal cells – quantifying lipid droplets dynamics during nitrogen starvation

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Introduction

Studies of lipid droplet (LD) physiology in fungi are still in their infancy but their quantitation has relevance to issues in biomedicine, agriculture and industrial waste. Third Harmonic Generation (THG) microscopy is non-invasive, produces inherently confocal images and doesn’t require fixation or external labeling, which make it suitable for *in vivo* LD imaging [1, 2]. We present *in vivo* and label-free imaging of LD in individual fungal cells by THG microscopy to assess the effects of nitrogen starvation. The LD quantification was performed by two image analysis techniques.

Methods

THG microscopy was applied for the first time to a filamentous fungus and our choice was the oleaginous fungus *Phycomyces blakesleeanus.* To observe the changes in LD number, the 22h old hyphae culture was divided into control and nitrogen starved groups (N-starved). A home built nonlienar microscope with Yb:KGW laser at 1040 nm (200 fs pulses, 83 MHz repetition rate) was used for THG imaging of live unstained hyphae [3]. THG signal was detected by PMT in the transmission arm after passing through a Hoya glass UV filter with the peak at 340 nm. 2D THG images of LDs (Fig. 1a) were analyzed by Image Correlation Spectroscopy (ICS) measuring spatially-correlated fluctuations [4] and software particle counting – Particle Size Analysis (PSA).

Results/Discussion

The small volume of hyphae suspension was placed between two coverslips of 170 μm thickness in order to meet the criteria for the best numerical aperture of the objective lens and for better transmission of THG signal. The high resolution of the microscopic system, the hyphae thickness (ca 10 μm) and medium transparency made it possible for the whole hyphae to be optically sectioned and a 3D model to be reconstructed (Fig. 1b and video). Since ICS was primarily developed for fluorescent images and was not used to analyze THG images, we have tested it by comparing the results to the PSA. Nitrogen starvation as expected [5] increased LD number compared to control which was confirmed by both

methods and obtained results are in good agreement. The overall increase of LDs during growth without available nitrogen is found to be between 3 and 4.5 h time point, followed with the loss of population of larger-than-average LDs during prolonged starvation.

Conclusions

THG microscopy is suitable for imaging and quantification of changes in lipid droplet number, brought upon by complete removal of nitrogen, from such low density/diameter baseline. 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.

Acknowledgement

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Disclosure

I or one of my co-authors have **no financial interest** or **relationship** to disclose regarding the subject matter of this presentation.

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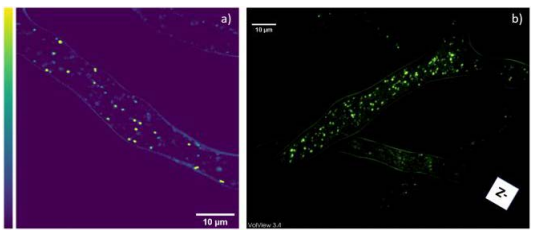

Figure 1. Label-free imaging of Phycomyces blakesleeanus hyphae.

(a) one THG slice and **(b)** 3D model of 23 THG slices 0.9 µm apart. The average laser power at sample plane was 23–26 mW.

Keywords: label-free, third harmonic generation microscopy, lipid droplets, image correlation spectroscopy, filamentous fungi

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Laser nano-surgery of fungal cell wall to enable patch clamping

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Nonlinear Optical Imaging and Quantification of the Pathological Changes in Gastrointestinal Tumor Nonlinear Optical Imaging and Quantification of the Pathological Changes in Gastrointestinal Tumor

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