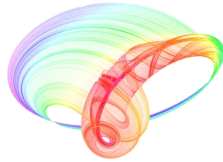


Book of abstracts



PHOTONICA2019

The Seventh International School and Conference on
Photonics, 26 August – 30 August 2019, Belgrade, Serbia

& Machine Learning with Photonics Symposium
(ML-Photonica 2019)



& ESUO Regional Workshop



& COST action CA16221



Editors: Milica Matijević, Marko Krstić and Petra Beličev

Belgrade, 2019

ABSTRACTS OF TUTORIAL, KEYNOTE, INVITED LECTURES,
PROGRESS REPORTS AND CONTRIBUTED PAPERS

of

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Technical Assistance

Danka Stojanović and Goran Gligorić

Publisher

Vinča Institute of Nuclear Sciences

Mike Petrovića Alasa 12-14, P.O. Box 522

11000 Belgrade, Serbia

Printed by

Serbian Academy of Sciences and Arts

Number of copies

300

ISBN 978-86-7306-153-5

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In vivo Third Harmonic Generation Imaging of *Phycomyces blakesleeanus*

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Third Harmonic Generation is a nonlinear optical effect in which the incident laser beam interacts with a medium producing the light of exactly three times shorter wavelength than the incidental one. THG is generated in medium that have third order nonlinearity, but it is particularly pronounced at the interfaces where the steep change of refractive index takes a place [1]. THG phenomenon is employed in laser scanning microscopy that utilizes ultrashort laser pulses for imaging. The THG microscopy is a label free techniques that provides important information on the sample such as membrane imaging and lipid droplets distribution [2]. It is mostly used for *in vivo* imaging of small model organisms like zebrafish [3] and *C. Elegance* [4].

We present THG imaging of filamentous fungus organism *Phycomyces blakesleeanus*, combined with Two Photon Excitation fluorescence (TPEF). The hyphae were grown in various conditions on glass coverslips coated with collagen and concanavalin A. For THG imaging of label-free, 16-24 hour old hyphae, we used 1040 nm, 200 fs pulses from Yb KGW laser, while for TPEF, Ti:Sa pulses at 730 nm, 160 fs duration, were used. Both laser beams were focused with the same objective lens, Zeiss Plan Neofluar 40x1.3. Detection of THG was performed by PMT through Hoya glass UV filter with peak transmission at 340nm, while for TPEF 400-700 nm band pass filter was used. THG images revealed the chitinous cell wall and the membrane that are clearly separated. The appearance of the cell wall was confirmed by colocalization with TPEF images. Most prominent observation on the THG images is presence of numerous, seemingly randomly dispersed, round shiny features throughout the cytoplasm, for which we suspect that could be lipid droplets as indicated in Débarre et al., 2006.

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