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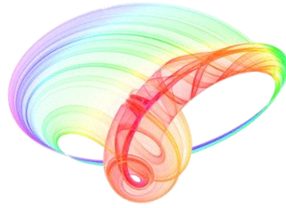

PHOTONICA2017

The Sixth International School and Conference on Photonics

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28 August – 1 September 2017

Belgrade, Serbia

Editors

Marina Lekić and Aleksandar Krmpot

Institute of Physics Belgrade, Serbia

Belgrade, 2017

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

of

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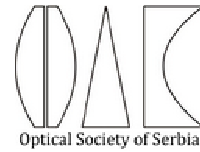

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Phycomyces blakesleeanus hypha cell wall surgery by Ti:Sapphire laser

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The ion channels on the membrane of filamentous fungi remain uninvestigated to this day due to their inaccessibility to patch-clamp pipette, brought about by sturdy cell wall. Small number of described channels is from very specific types of cells (wall less mutants or sporangiophore aerial cell membrane). The enzymatic approaches of cell wall removal, albeit successful on plant cells, failed when applied to fungi. In order to obtain clean "patchable" membrane from any type of filamentous fungal cell we undertook a task of finding the conditions for cut of the small part of the hyphal wall by laser surgery on the model filamentous fungus organism, *Phycomyces blakesleeanus*. The successful wall surgery should result in exposure of large enough portion of cell membrane with a minimal damage to the protoplast. Therefore, we performed series of experiments with cell plasmolisys in hypoosmotic media and subsequent deplasmolisys, to determine the conditions for reliable retraction of cytoplasm that could be reversed. Next, hyphae, grown on glass coverslips coated with collagen, were plasmolysed and mounted on the stage of the homemade nonlinear laser scanning microscope for imaging and cell surgery [1]. The Ti:Sapphire laser (Coherent, Mira 900-F) has been used as a light source in the microscope. It has operated at 730nm. This wavelength enables two photon excitation of auto-fluorescence in cytoplasm, as well as dye (Calcofluor white), visualizing fungi wall. We have used Carl Zeiss, EC Plan-NEOFLUAR, 40×1.3 oil immersion objective for focusing of the laser beam and collection of fluorescence. A visible interference filter (415nm - 685 nm) in front of detector has been used to remove scattered laser light. The successful cutting of cell wall could be achieved within the range of laser intensities and cutting speeds (dwell times). Throughout the experiment, fungi were kept in azide or Brefeldin A in order to block the process of depositing the new wall material. Afterwards, hyphae were slowly deplasmolysed to induce exit of a portion of the protoplast through the laser-cut hole in the cell wall. However, in some instances, the part of the protoplast bulged through the hole immediately after cell surgery, while the cell was still in hypertonic solution. In other instances, the cytoplasm remained away from the cut hyphal apex even through series of slow incrementing hypotonic solutions. Finally, when laser cutting was applied on the side of the cell only, as to cut a small hole, the successful exit of a portion of protoplast through the hole during deplasmolisys could be reliably achieved.

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[1] Mihailo D. Rabasović, et al, J *Biomed Opt* 20 016010 (2015).