8^{th} Regional Biophysics Conference

Book of Abstracts

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16^{th} to 20^{th} May 2018

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SLOVENIAN BIOPHYSICAL SOCIETY

Laboratory of Biophysics
Condensed Matter Physics Department
Jožef Stefan Institute

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Successful Ti:Sapphire laser cell surgery of Phycomyces blakesleeanus cell wall

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Application of patch-clamp method for investigation of membrane ion channels of filamentous fungi is nontrivial task due to presence of chitinous cell wall. Complete removal of the wall patch is needed to make the membrane accessible to glass pipette. We use the model filamentous fungus organism, Phycomyces blakesleeanus which is undertaken to the cell surgery procedure by means of tightly focused femtosecond laser beam. The hyphae were grown on glass coverslips coated with collagen plasmolysed and imaged by homemade nonlinear laser scanning microscope by detecting two photon excitation fluorescence signal. Although intrinsic autofluorescence of chitin enables imaging of the cell wall the hyphae were stained by Calcofluor White dye prior to the imaging in order to improve signal to noise ratio. Ti:Sa laser, used for both imaging and surgery, was operating at 730nm, with 76MHz repetition rate and 160fs pulse duration. Carl Zeiss, EC Plan-NEOFLUAR, 401.3 oil immersion objective was used for tight focusing of the laser beam and for the collection of the fluorescence signal. A visible interference filter (415nm - 685nm) was placed in front of detector in order 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). The hyphae were kept in azide throughout the experiment in order to block the regeneration of the cell wall. After the surgery, hyphae were slowly deplasmolysed to induce exit of a portion of the protoplast through the laser made incision in the cell wall.

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Keywords: Cell surgery; Ti:Sapphire

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