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# Tissue Omics & Optical Technologies

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Session chair: **Dale Waterhouse** (London, UK); **Alan Race** (Unterschleißheim, Germany)

Shortcut: **PW36**

1095 # Laser nano-surgery of fungal cell wall to enable patch clamping

**Tanja Pajić**[1], Katarina Stevanović[1], Nataša V. Todorović[2], Steva Lević[3], Svetlana Savić Šević[4], Dejan Pantelić[4], Miroslav Živić[1], Mihailo D. Rabasović[4], Aleksandar J. Krmpot[4, 5]

[1] University of Belgrade, Faculty of Biology, Institute of Physiology and Biochemistry, Belgrade, Serbia
[2] University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, Belgrade, Serbia
[3] University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
[4] University of Belgrade, Institute of Physics Belgrade, National Institute of the Republic of Serbia, Belgrade, Serbia
[5] Texas A & M University at Qatar, Doha, Qatar

**Introduction**

Electrophysiology studies of ion channels, in live filamentous fungi by patch clamp method are not possible due to presence of rigid chitinous cell wall that prevents patch clamp pipette to access the plasma membrane. We present laser nano-surgery of the fungal cell wall that enables patch clamp electrophysiology studies. Similar approaches as one-time reports utilizing nanosecond laser pulses long time ago were not pursued further [1,2]. Here, we demonstrate reproducible method using femtosecond lasers accompanied by two-photon excitation fluorescence (TPEF) imaging of hyphae.

**Methods**

A wild-type strain of filamentous fungus *Phycomyces blakesleeanus* (Burgeff) [NRRL 1555(-)] were grown on glass coverslips with hand-etched grid, coated with a thin layer of 50% collagen type I as an immobilizer. Home built nonlinear laser scanning microscope [3,4] utilizing Ti:Sa tunable fs laser was used for TPEF imaging of hyphae and the cell surgery. The latter is enabled with the custom made add-on in software. Coverslip with hyphae is transferred to another microscope setup for patch clamp, consisting of micromanipulators and precise electronics for pA current measurements. The surgical incisions and released protoplasts were additionally imaged by scanning electron microscopy for which treated hyphae had to undergo critical point drying procedure.

**Results/Discussion**

Hyphae were stained by Calcofluor White and treated with an exocytosis inhibitor (brefeldin A) and a respiration inhibitor (sodium azide) to prevent cell wall regeneration. Since the cell wall and the plasma membrane are in the close contact [4] hyphae were kept in hyperosmotic solution to retract the cytoplasm from the cell wall. Surgical spot-wise pattern was precisely positioned at TPEF image of selected hypha at the place where the plasma membrane was retracted. The dwell time (1s) and the laser power (4-15mW) were set with fixed repetition rate (76MHz), pulse duration (160fs) and laser wavelength (730nm). Upon the surgery, hyphae were gently deplasmolysed. A protoplast with plasma membrane accessible for the patch clamp pipette was released through the surgical incision (Fig 1). The >GΩ seal resistance was achieved. Numerous ion channels are recorded in different configurations (on cell, inside-out, whole cell and out-out) (Fig 2).

**Conclusions**

The whole process (cell surgery + patch clamping) is rather complex and specific steps have to be strictly followed for high success rate and reproducibility. Also, chemicals concentrations, solutions osmolarity, timing and cutting parameters have to be kept in the specified narrow range. Obtained current recordings provide valuable information on fungal cell membrane ionic channels.

**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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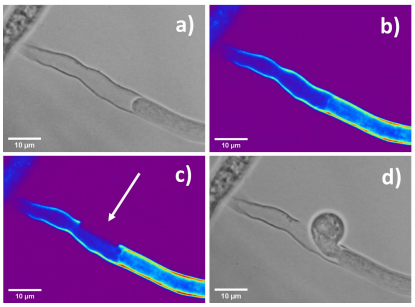


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Bright field **(a)** and TPEF **(b)** image of plasmolised hyphae *Phycomyces blakesleeanus* prior to the surgery. **c)** TPEF image of the same hyphae after the surgery. Surgical incision is pointed by the arrow. **d)** Bright filed image of the same hyphae with the protoplast released through the surgical incision.

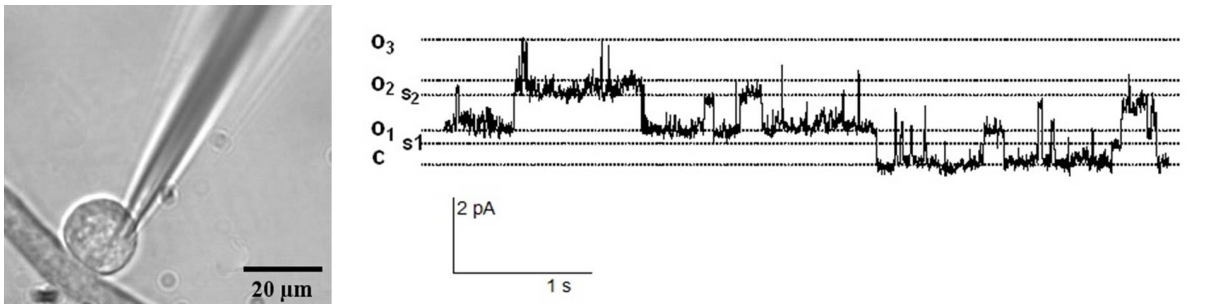


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**Fig 2. Patch clamping upon the cell nano-surgery.**

**Left:** Bright filed image of the patch clamp pipette in contact with membrane of the protoplast released through the surgical incision. **Right:** representative single channel current recording in "outside/out" configuration at voltage -10mV. o: open channel current level; c: closed channel current level; sub-conductivity level. Calibration bar is at the bottom

Keywords: laser nanosurgery, patch clamp, electrophysiology, two-photon imaging, cell surgery