First glance at a multitude of ion currents on filamentous fungus *Phycomyces blakesleeanus* protoplasts obtained by femtosecond laser microsurgery

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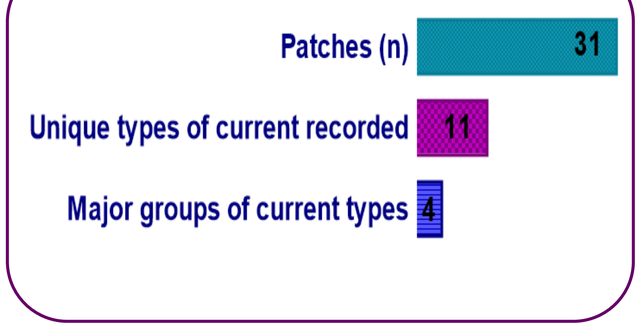
Introduction

The good quality of the membrane is of critical importance for successful patch clamp method recording of membrane ion currents. It is a direct consequence of mechanical integrity, cleanliness of the membrane and the physiological fitness of the cell. For instance, cells entering apoptosis or necrosis, or suffering oxidative stress do not have good quality membrane.

Advanced imaging techniques that enable more controllable surgery process could result in cell wall microsurgery minimally damaging to protoplast membrane. In pursuit of the reproducibly high quality membrane of "de-walled" protoplasts, we undertook the final rounds of protocol optimization described in poster presentation **Stevanovic *et al.*,** this conference (**B.7**). Optimized microsurgery protocol gave rise to protoplasts prone to form contacts of high electrical resistance (GΩ) with a patch pipette.

Here we present overall results of the first time ever recording of the rich ion channel current activity from filamentous fungus *Phycomyces blakesleeanus* protoplasts.



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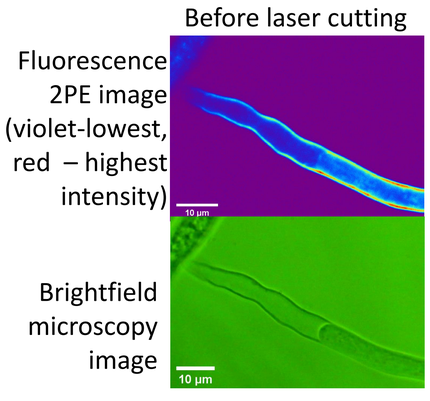
Methods

Ti:Sa LASER MICROSURGERY

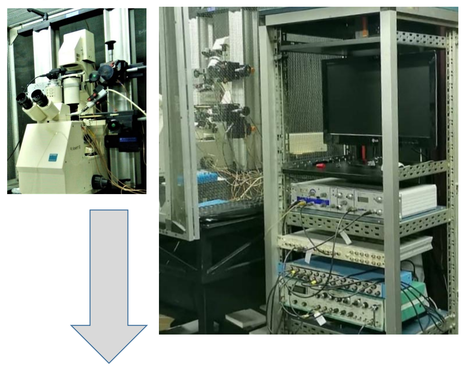
(730 nm; 76 MHz, 160 fs pulse duration)



transferring the chamber with hypha to patch-clamp rig

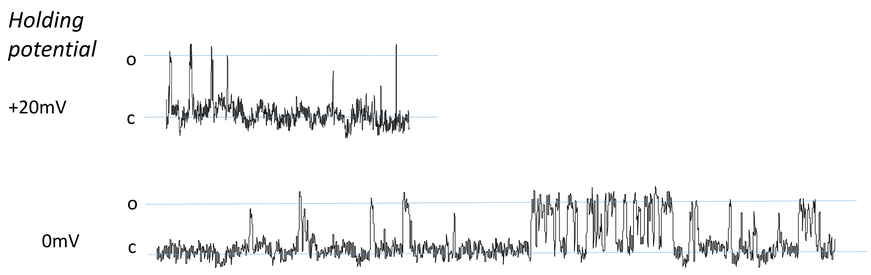
PATCH CLAMP RECORDING

(inside-out; outside-out and whole cell voltage clamp)

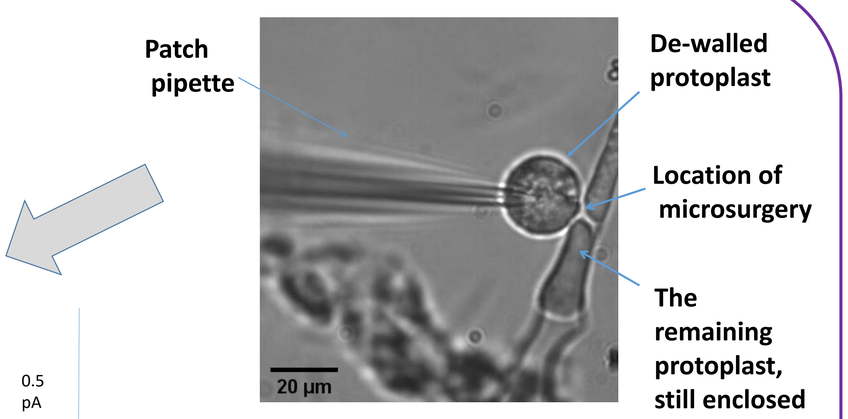


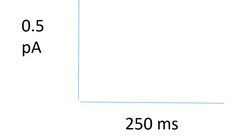
SINGLE CHANNEL CURRENTS

Currents were recorded from the patch after excision from a protoplast obtained after microsurgery of cell wall, much like the one depicted in the panel left.



C: channel closed;
O-channel opened



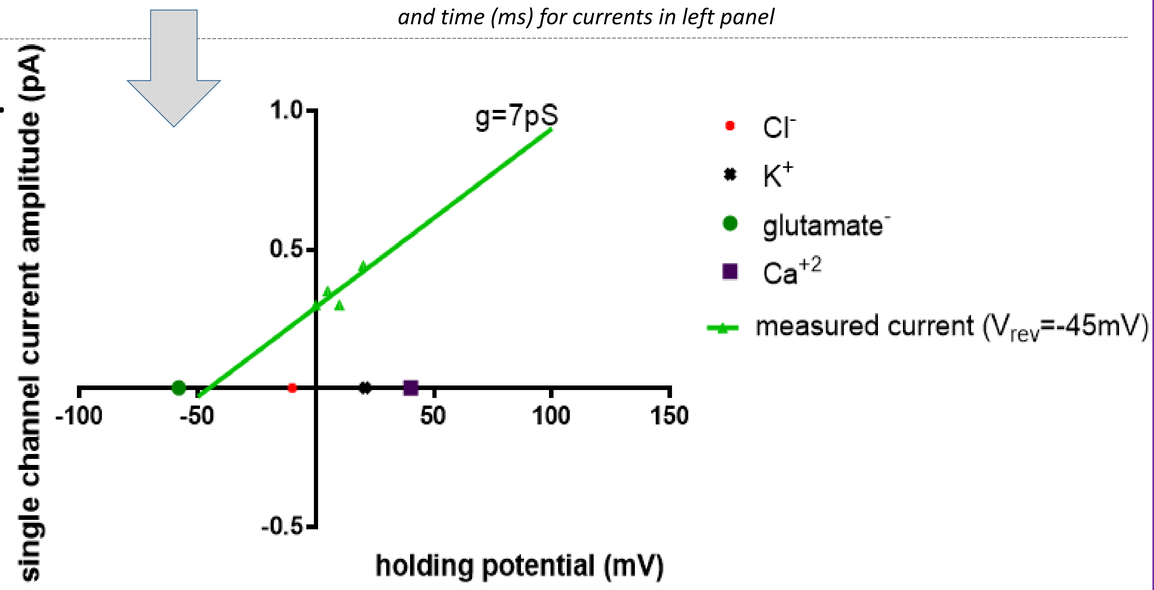


Scale bars for current amplitude (pA) and time (ms) for currents in left panel

A.

	Bath (mM)	Pipette (mM)	Nernst eqilibrium (mV)
Cl-	83	56	**-10**
K+	125	55	**21**
glutamate-	49	5	**-58**
Ca++	24	1	**40**
Mg++	2	2	**/**
NO3-	45	0	**-300**
Cl+NO3	128	56	**-21**

B.

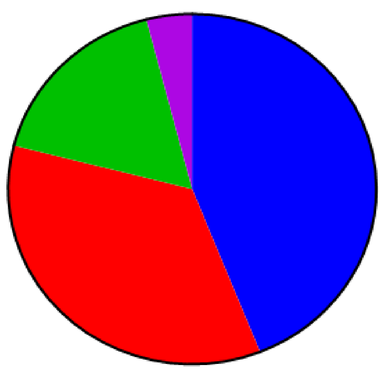


The example of the I/V graph constructed from single channel current amplitudes at several membrane potentials.
A.) Ion concentrations in external (bath) and intra (pipette) solutions used in the experiment shown in B;
B.) Linear fit of current-voltage dependence gives **conductance (g)** in pS and the **potential of current equilibrium (V_{rev})** in mV. Current ion selectivity is derived from V_{rev} position in respect to the Nernst equilibrium expected values for each ion present. Nernst equilibrium values for each ion are marked in B on x axis.

Conclusions

- Utilization of the Ti:Sa femtosecond laser with optimizations of the cell wall microsurgery protocol **resulted in protoplasts that were prone to form contacts of high electrical resistance** (GΩ) with a patch pipette.
- By far, **the most frequent types of conductance were anionic**. It is possible that high calcium concentration prevented detection of potassium currents that would normally be expected to be found aside anionic ones.
- The presence of **anion channels that favor permeation of organic acids** to chloride on the filamentous fungi protoplast membrane, has been shown for the first time.

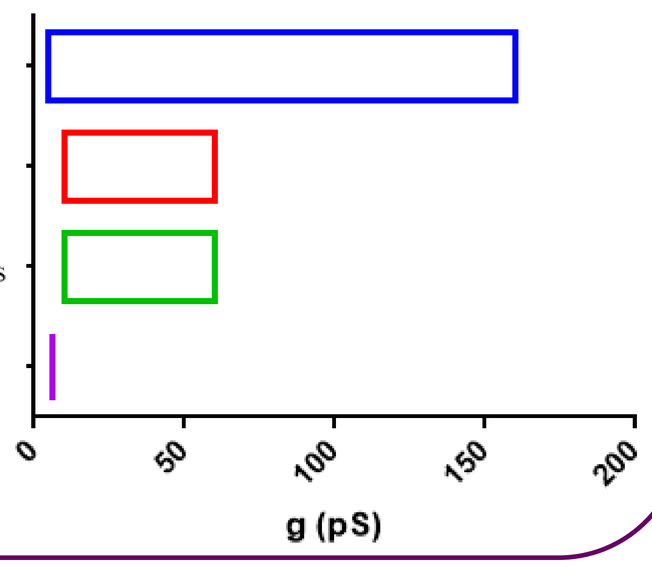
4 major groups of current types were identified:



Relative frequency of occurrence

- 44.00% Unselective anion currents
- 35.00% Chloride selective anionic currents
- 17.00% Organic acid selective anionic currents
- 4.00% Calcium selective cation currents

Range of conductances



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