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# Biological autoluminescence as a non-invasive monitoring tool for pulsed electric field effects on yeast cells

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## Abstract

The application of pulsed electric field (PEF) is nowadays becoming a very promising tool for application in medicine or food industry. However, the mechanisms of PEF interaction with living matter are still not fully elucidated. The aim of the presented work is to show the potential application of biological autoluminescence (BAL) for monitoring of pulsed electric field (PEF) biological effects on yeasts. The yeast cell culture (*Saccharomyces cerevisiae*) is exposed to the pulsed electric field (pulse duration: 0.1-5 ms, pulse number: 1-10, electric field strength: 3 $kV.cm^{-1}$) and the BAL is monitored during exposure. The results indicate detectable differences in BAL dynamics when PEF is applied. Due to its non-invasive and label-free application, the BAL could be used as a beneficial tool for monitoring of PEF biological effects.

## 1 Introduction

Although an extensive theoretical and experimental research has been carried out, the unambiguous explanation of electromagnetic field influence on living structures is still lacking. The mechanisms of pulsed electric field (PEF) interaction with biological matter are not fully established, but PEF treatments are nowadays becoming widely used in biomedicine and food industry [1]. The research challenges for PEF biological effects are still present, with potential novel applications in diagnostics, therapy as well as in industry.

Because this research field has still many open questions not possible to be answered with current tools, it is essential to find novel methods for monitoring and evaluating various facets of biological effects. Here we suggest the monitoring of biological autoluminescence (BAL), as an externally detectable manifestation of the oxidative metabolic activity of living cells [2]. The aim of this work is to show the use of BAL to detect the PEF effects on yeasts *Saccharomyces cerevisiae*.

## 2 Materials and Methods

The yeast culture *Saccharomyces cerevisiae* (genetic background BY4741, MATa) used in the experiments is stored on agar plates (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) agar, 2% (w/v) D-glucose in purified water) in a refrigerator at 4 °C. Yeasts are inoculated from agar plate into glass Erlenmeyer flask (250 mL) with 100 mL of YPD medium (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) D-glucose in purified water) and cultivated for 24 hours at 30 °C on an orbital shaker (Biocer) at 180 rpm.

The pre-cultivated cell culture is centrifuged twice at 3000 rpm, each time for 5 minutes and washed with LCB1 buffer (2.38 g/L HEPES, 85.6 g/L sucrose, 0.142 g/L $MgCl_2$, 0.066 g/L $CaCl_2$). Cell concentration is measured by a cell counter (Beckmann Coulter) and diluted in a 50 mL stock solution. The sample of yeast cell culture from stock solution (2 mL, $1\times10^9$ cells/mL) is transferred into a custom-manufactured cuvette equipped with electrodes for pulses delivering. The sample is placed into the light-tight chamber for BAL measurement.

The photomultiplier (PMT) module H7360-01, selected type, (Hamamatsu Photonics K.K.) with spectral sensitivity in the range 300-650 nm is used to detect BAL. Typical dark count (noise) of PMT module H7360-01 is about 20 counts per second (cps). The BAL intensity from the empty cuvette (background count) is about 60 cps. We set a bin of PMT counter to 50 µs (the shortest possible). In this case, the value of dark count is 0.001 counts/bin and background count 0.003 counts/bin. Measurement of the sample takes place in a light-tight chamber (standard black box, Institute of Photonics and Electronics, CZ) specially designed for the purpose of BAL measurements. The PMT module is mounted on the top of the chamber viewing the sample inside the chamber.

For PEF experiments we use the high-power pulse generator (ELECTROcell B15) to expose the samples to 1, 3, or 10 pulses with a duration (pulse width) of 0.1, 0.5, 1, and 5 ms. The electric field strength in the samples reaches 3 $kV.cm^{-1}$. The frequency of the pulsing is 1 Hz. The yeast cell culture is PEF-treated in LCB1 buffer and BAL during PEF application is measured. BAL measurement for each set of parameters (pulse width, pulse number) is performed in triplicate.

## 3 Results and Discussion

The effects of 7 different settings of PEF parameters (pulse number, pulse width, firing rate) were investigated. We aim to study direct effects during pulsing, *i.e.* we focused on the analysis of BAL kinetics directly during single pulses delivering. To be able to analyze these direct effects, we set the bin of PMT as short as possible to 50 µS. Since we applied the pulses with a duration (pulse width) in the range 0.1 – 5 mS, we have 2-100 measuring points of BAL intensity.

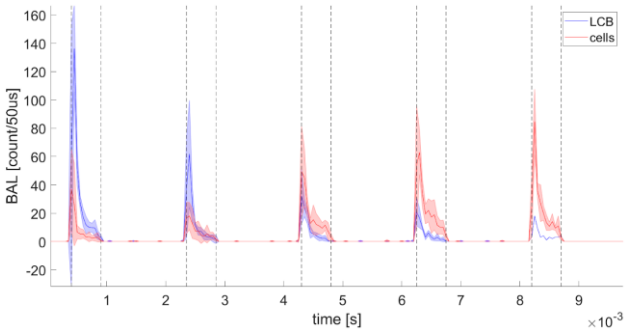


**Figure 1.** The BAL kinetics during PEF treatment (electric field strength 3 kV.cm$^{-1}$, pulse width 0.5 ms, firing rate 1 Hz, 10 pulses delivered to the solution). We display BAL only during the first 5 pulses, due to the limitation of PMT counter memory, which can record only 100000 measuring points. All pulses are zoomed horizontally, *i.e.* pauses between them are cut out. The pulse duration is marked by 2 vertical dashed black lines.

Here we show the results of BAL from the yeast cell sample treated by 10 pulses of 0.5 ms duration. It can be seen, that the BAL intensity from a buffer (LCB) only is at the beginning (first pulse) superior to the BAL intensity from cell solution (Fig. 1). But with the increasing number of pulses delivered to the solution, the BAL from cells overruns it. The interpretation of these preliminary results is difficult, but we hypothesize the rate of the cell wall and cell membrane permeabilization is raised after each pulse delivered to the solution, as it was shown in several studies [3, 4]. Then the parts of the intracellular content could be released from the cell [5], and, extracellular medium, *e.g.* ions from the solution, could be transported in the reverse direction - into the cell [4]. The answer to a question, why the BAL is increased during this transport, is not straightforward. We know that the BAL intensity can be modulated by the presence of reactive oxygen species (ROS) [6], which are formed in the cell solution under both normal and excessive oxidative processes, but also as a product of cell apoptosis [7]. Therefore, we suppose the PEF could initiate oxidative reactions in solution or even induce apoptotic processes, which are accompanied by enhanced ROS production and the following increase of BAL intensity. We can look at these processes also from the perspective of dielectric parameters variation. The permeabilization of the cell wall and cell membrane by applied PEF (electroporation), and following ions leakage from intracellular space to medium, leads to the increase of medium conductivity. The higher conductivity of medium means the higher current flow in it, which initiates reaction pathways leading to higher ROS production and BAL enhancement.

When we look at the BAL trend during a single pulse only, for both buffer and cells, we see the same descending trend of BAL kinetics. We assume this could be the response on very fast change (derivation) of the electric field in the solution and following initiation of fast oxidation of biomolecules leading to ROS production and BAL. The descending trend during pulse could represent the progressive consumption of ROS molecules due to recombination. However, we are currently not able to state any conclusion about gradually decreasing BAL intensity from buffer only.

To analyze the total BAL intensity detected by PMT during every single pulse, we calculate the sum of BAL intensity for each pulse. At first, we subtract mean noise obtained from an interval just before and after each pulse Noise background is almost negligible here: for 50 µs bin its typical mean value is 0.003. Then we make cumulation exactly only during the pulsing time. In order to compare different conditions of various time lengths, we finally make an average from it. The results are in the Fig. 2.

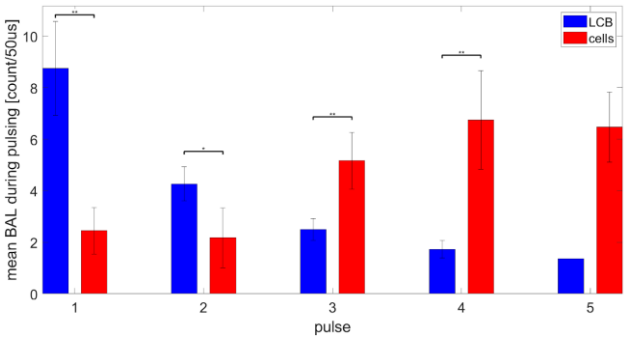


**Figure 2.** The average BAL intensity during each single pulse delivered to the solution. The bars represent mean values, calculation from 4 (LCB) or 6 (cells) measurement, $^{*}p<0.05$, $^{**}p<0.01$. The error bars represent the standard deviation.

## 4 Conclusion

We presented the application of BAL for non-invasive and label-free monitoring of PEF biological effects. The results indicated the dose-dependent response of BAL - with increasing number of pulses delivered to the cell solution, the BAL intensity was gradually enhanced. We suggested that this could be the result of progressive permeabilization of cell wall, followed by the release of intracellular content and enhanced transport of extracellular ions into the cells. These processes might be accompanied by oxidative reactions or cell apoptosis, when ROS production is enhanced, and therefore the increase of BAL is observed.

## 6 Acknowledgements

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