about the forces between organelles, the elastic properties of biological samples. This installation allows you to solve the following tasks:
- Laser dissection of tissues and cells.
- Optical transfection (introduction of external genetic material into cells through channels created in the cell membrane).
- Artificial laser fusion of two or more cells.
- Laser inactivation of cell chromosomes.
- Therapeutic laser cloning.
- Study of the elastic properties of biological objects at different stages of development; interaction forces between individual parts of a biological system (organelles, organelles and membranes, etc.).

A fundamentally new technology has been developed and the necessary material and technical equipment has been developed for minimally invasive nanosurgery of mammalian embryos using lasers with radiation in the transparency window of biological tissue. A promising technology has been developed for obtaining genetically modified preimplantation mammalian embryos.

S6.388. High intensity of FAD autofluorescence as an indicator for detecting cellular pathology

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Optical imaging using endogenous fluorescence (FAD), which is involved in such processes as fatty acids oxidation, Krebs cycle and other redox reactions, is one of the promising ways to study the metabolic status of cells. The possibility of simple and non-invasiveness FAD determination is based on its autofluorescence, with an excitation spectrum in the wavelength range of 350-500 nm with two peaks - at 370 and 450 nm, and the emission spectrum falls in the region of 500-600 nm with a maximum at 525 nm.

According to literature sources, cells in different physiological states have different levels of FAD intensity in green-blue spectrum. To determine the physiological state of cells by the difference in the FAD intensity, in this work, a culture of skin fibroblasts was studied after after 20-day cultivation in DMEM-based growth medium (Gibco, UK) containing 4.5 g/l glucose, 10% fetal bovine serum (Biological Industries LDT, Israel), penicillin (100 units/ml) (Gibco, USA), streptomycin (100 μg/ml) (Gibco, USA), in a CO2 incubator (Thermo Scientific) at 37°C, 100% relative humidity and 5% CO2 content (Eppendorf AG). Studies were performed using a ZEISS LSM 900 laser scanning confocal microscope with Airyscan 2 system (Carl Zeiss AG, Germany) at a wavelength of 488 nm.

The first study stage, fibroblasts were planted on 0.5 mm thick coverslips with pre-applied mesh to locate the individual cells. As the performed analysis shows, cell culture contained cells with high and low fluorescence intensity. This could be explained by the difference in metabolic status of cells. This made it possible to divide the cells into two subgroups based on the intensity of the autofluorescence signal: with a high autofluorescence signal (presumably senescent or pathological) and with a low signal.

After 24 hours, the proportion of necrotic cells in the culture was analyzed by double staining with Hoechst 33342 (5 μM) and propidium iodide (20 μM) for 30 min at 37 °C. To count the total number of cells, Hoechst 33342 was used, which stains the nuclei of cells in any physiological state. Propidium iodide, unable to penetrate entire membranes and stain viable cells, was used for staining and counting necrotic cells. The proportion of necrotic cells among cells with a high FAD autofluorescence intensity was 47.4%, whereas among cells with a low autofluorescence signal – 27.3%. Intense FAD signal can be associated with highly oxidized state of a coenzyme included in the structure of redox enzymes.

At the next stage of the study, to determine the structure of the FAD signal, different solutions were alternately introduced: adrenaline (10 μM) to activate MAO-A, selegiline (20 μM) to inhibit this enzyme, FCCP (2 μM), which is a protonophore and leads to the separation of mitochondrial respiration, and KCN (1 μM), which is an inhibitor of complex IV electron transport chain. This made it possible to determine the level of the FAD signal associated with MAO, as well as the total pool of FAD complex II of mitochondria. It was found that in cells with a high autofluorescence intensity, the pool MAO was 12.3% ± 2.1 of the total autofluorescence signal, whereas in cells with a low intensity this value was 6.4% ± 0.9. The combination in cells of a higher level of the MAO pool and a reduced level of the mitochondrial FAD pool may be a consequence of the formation of the corresponding aldehydes during oxidative deamination of monoamines, which can inhibit succinate dehydrogenase. As a result, the expression of this enzyme decreases, thus, the functioning of the complex II electron transport chain is impaired.

This study shows the possibility of early diagnosis of various diseases by detection of the FAD autofluorescence signal and by finding the cells with high fluorescence intensity, which are mostly necrotic. Low level viability of cells with high fluorescence intensity in a green-blue spectrum can be a marker for early diagnosis of various diseases, determining the exact localization and prevalence of pathology in the tissue. At the same time, the increased FAD signal is due to high MAO activity, which makes it possible to further find tools for influencing this enzyme, preventing the development of a pathological state of cells. The proposed approach has important advantages, including non-invasiveness, high sensitivity, safety and can be used for early diagnosis of various diseases and monitoring of patients' response to therapeutic interventions, including in real time.

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S6.389. Influence of fermentation on prolonged hydrogen photoproduction by Chlamydomonas reinhardtii cells under mineral starvation

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Lack of macroelements such as nitrogen, phosphorus, sulfur, iron and magnesium is one of the key factors that play an essential role in the processes of phytoplankton life activity. The shortage of these minerals has a particular impact on the process of long-term photogenesis of hydrogen in some green microalgae that are part of phytoplankton communities, which is their specific response to stress conditions. The basis of this response of algae is the interaction between aerobic and anaerobic processes inside the cell which provide microorganisms with energy under conditions of mineral starvation. At the same time, the participation and role of primary processes of photosynthesis as well as starch storage (and consumption) during hydrogen production in light by green microalgae cells have been studied in sufficient detail so far. At the same time, the influence of fermentation reactions on hydrogen photogenesis in starved cultures of eukaryotic microorganisms has been studied to a lesser extent.

The aim of this work was to study the effect of fermentation processes in the cells of green microalgae Chlamydomonas reinhardtii of wild type and mutant strain on continuous photogenesis of molecular hydrogen under sulfur deficit conditions. For this purpose, the following measurements and comparative analysis were performed in photoreactors under constant light and absence of sulfur in the medium: hydrogen content in the reactor gas phase, starch content in cells, measurement of photosynthetic activity and respiration rate in cultures of mutant