

**Диференціація клітин за поверхневим зарядом мембрани може покращити довготривале зберігання еритроцитів за умов низьких температур**

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**Discriminating Cells by Membrane Surface Charge May Improve Long-Term Refrigerated Storage of Red Blood Cells**

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During the process of refrigerated storage the red blood cells are preserved at low temperatures to maintain their viability and prevent bacterial growth. Nowadays three different methods of refrigerated RBC storage are in use. Liquid storage is the most common and convenient among them, when RBCs are stored in an additive solution at (1–6)°C for up to 42 days, but this method causes metabolic and structural changes in the RBCs over time (storage lesion), which may affect their function and safety. In cryopreservation RBCs are frozen with 40% glycerol and stored at –60°C to –80°C for up to 10 years. A still experimental and not yet widely used method is freeze-drying: RBCs are frozen and then dehydrated under vacuum to remove water and ice crystals. The freeze-dried RBCs can be stored at room temperature for several years.

RBCs are a diverse population of cells with different sizes, shapes, and deformabilities, which affect their function, survival and susceptibility to storage lesions. RBC diversity arises from the influence of i) genetic factors when different RBC phenotypes are determined by genetic variations in genes encoding for RBC membrane proteins, enzymes, and hemoglobin; ii) environmental factors such as oxygen availability, temperature, pH, and osmolarity can alter the shape and deformability of erythrocytes; iii) physiological factors: age, gender, hormonal status, and nutritional habits can affect the size and hemoglobin content of RBCs, older RBCs tend to be smaller and less deformable than younger ones. The different subsets of RBCs can be collected and analyzed for studying the effects of storage lesions, aging, or diseases on RBC properties, and the storage efficiency of subpopulation differs.

Cell surface charge is an important property of RBCs that affects their interactions with other cells and molecules. The distribution of cell surface charge in the RBC population is influenced by the expression of membrane proteins, glycoproteins, sialic acids. pH, ionic strength, temperature, and oxygen tension can alter the surface charge of RBCs by affecting the ionization of membrane groups or the binding of plasma proteins.

We have developed a procedure for automatic evaluation of individual erythrocytes electrophoretic mobility and zeta potential in the sample of healthy donors. The obtained values of the  $\zeta$ -potential coincide with the literature. Found distributions of erythrocytes by the value of the  $\zeta$ -potential correspond to the age distribution of erythrocytes in the population. If such distribution is in sync with RBC floating density and hemoglobin content distribution we could achieve a new noninvasive test for *in situ* diagnostics of a blood specimen collected for blood banking. We could quantitatively assess amount of different subgroups of RBCs in it and a forecast for its storage duration. This could enhance widespread accepted practice of refrigerated RBC storage and could be used for the improvement of new approaches for freeze dry or cryo-banking storage.

**Програмне рішення для ідентифікації та трекінгу поведінки рухомих об'єктів необхідне для вивчення механізмів адаптації риб до низьких температур**

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**Software Solution for Moving Objects Identification and Tracking Individual Behavior in Studies of Fish Adaptation for Sub-Ambient Temperatures**

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Studying the behavior of fish at low temperatures can help understand how they adapt to climate change and how this affects their life cycle, metabolism and physiology. Some fish species can survive the winter by entering a state of hibernation or dormancy. Other fish species can regulate their body temperature through heat exchange or endothermy. Still other fish species can survive in extremely cold temperatures thanks to antifreeze proteins that prevent the formation of ice in their cells. By studying these mechanisms, we can figure out how fish cope with the cold and what effects this may have on their populations and ecosystems.

Studying the individual characteristics and not the averaged characteristics of the population can be useful for understanding the diversity and variability of living organisms, as well as for the selection and conservation of species. Individual characteristics of individuals can be related to their genetics, physiology, behavior and interaction with the environment. By studying these features, one can better understand how organisms adapt to different conditions and how this affects their survival and development.

The use of individual fish monitoring reduces mortality rates, ensures the optimum use of feed, and enhances growth rates. However, individual fish identification has been a challenge, mainly because of the invasive tagging method, which is time-consuming and impractical for large quantities of fish.

A new non-invasive methodology for individual fish identification based on machine learning was tested under real-time artificial fresh water tank conditions. Two approaches for fish localization and detection were used. In the first case manual selection of the region of interest from underwater snapshots of moving fish was performed aimed at reliable identification of the unique skin patterns of fish. Another method was to train a convolutional neural network to find fish-like objects and identify them after that using a database of individuals' signatures. The second approach was selected as simple, cost-effective, reliable, robust one. Image acquisition could be done under ambient light conditions with a web-camera to collect data for identification. The detection of a fish in the proper position and within the necessary angle was done with the help of a YOLOv5 convolutional neural network. The pre-trained network was integrated into .NET code for detecting fish, and the solution was tested by ensuring the software highlights detected objects (fish) with bounding boxes. Then the software algorithm of fish identification was added. The accuracy of identification was 79.5%, and the algorithm worked efficiently even under conditions for which it was not trained. The system has the potential to enhance fish farming productivity by reducing mortality rates, optimizing feed usage, and enhancing growth rates. The simplicity and power of the approach make it an attractive option compared to other invasive methods. This solution will help precision fish farming by providing a non-invasive and efficient way to identify and track individual fish in real-time.

