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Opracowanie roztworu optycznie oczyszczającego i rozszerzającego tkanki, służącego do precyzyjnej akwizycji i segmentacji danych pochodzących z mikroskopii fluorescencyjnej

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Streszczenie w języku angielskim

Over the past two decades, microscopy has dynamically changed from an almost exclusively qualitative technique to a highly quantitative one, providing unique avenue to understanding the spatial relationships between cells by visualizing their distribution. So far, the main limitation of the quantitative studies using confocal microscopy was the imaging depth, limited to a maximum of few cell layers because of the natural opacity of tissues - and thus a significant deterioration of data quality along with the increasing depth of imaging.

The recently presented techniques of tissue optical clearing (TOC) almost completely removed this barrier by allowing to perform the imaging of even the whole body of laboratory rodents with high, close to cellular, resolution. On the one hand, it opens up completely new set of scientific questions to be answered in the real 3D context of organs, but on the other, it exhibits the imperfections of the methods currently used during the reconstruction and segmentation of the obtained data even more vividly. Expansion microscopy (ExM) is the approach that combines the advantages of TOC with dramatically improved imaging resolution and is based on the transformation of cells/tissues into tissue-gel hybrids. Unfortunately, even if the significant level of complexity of the available protocols and the difficulty of working with the resulting delicate hybrids are omitted, it should be underlined that the minimum value of tissue volume enlargement in the case of ExM is as much as 20× (2000%), which makes the acquisition of even the thinnest sections a task practically impossible in terms of required time, workload and finally data handling. Recently, using a series of solutions consisting of imidazole and antipyrine, the first method of combined TOC and expanding in a controlled manner without the need for hydrogel synthesis, CUBIC-X, was presented. Unfortunately, when applied to mouse brain, CUBIC-X, similarly to the classic ExM methods, offers a very significant increase in organ volume of ~10×, which almost excludes it from everyday laboratory practice.

Based on the presented methodology in CUBIC-X, an attempt was made to develop the first solution that performs optical clearing and moderate, but reproducible, tissue expansion. As a result of the conducted experiments, ISEE was discovered – a first-in-class solution that guarantees high transparency, expands tissues by ~15% in each axis and significantly improves the quality of signal segmentation, both nuclear and cellular, using semi-automatic methods. ISEE was also verified compatible with immunohistochemistry using primary and secondary antibodies (including those directed against endogenous proteinaceous fluorophores). Finally,

compatibility of ISEE with imaging of brain tissue, as well as thick (~ 200 μm) sections of murine lymph nodes, was shown.