Analyzing the Effectiveness of Expansion Techniques for Meningeal Tissue

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Expansion Microscopy (ExM) enhances spatial resolution by physically enlarging tissues, enabling detailed visualization without requiring advanced microscopy systems. ExM has proven effective for many tissue types, but its application to highly fibrous, layered tissues remains challenging. This study investigated effectiveness and efficiency of three expansion methods, standard ExM, Magnify, and a modified version of Magnify for expanding meningeal lymphatic tissue, a structurally complex and clinically significant component of the central nervous system. Brain tissue from rats was used as a comparative control due to its known compatibility with ExM. Tissues were stained, imaged pre- and post-expansion, and quantitatively analyzed using ImageJ, with statistical comparisons performed using one-way ANOVA and follow-up tests. Results showed that standard ExM successfully and reproducibly expanded brain tissue, while meningeal lymphatic tissue exhibited minimal expansion and significant folding. Both Magnify-based methods failed to produce measurable expansion. Statistical analysis confirmed a significant (P=0.0160) overall difference among methods, though only the standard ExM method demonstrated meaningful expansion. Efficiency analysis revealed that Magnify-based methods required fewer materials and less time, despite their lack of effectiveness. These findings highlight limitations of current expansion techniques for fibrous tissues and emphasize the need for tissue-specific protocol optimization. Future work focuses on modifying digestion conditions, polymer composition, and tissue preparation to improve expansion uniformity in layered tissues like the meninges, with potential applications in disease modeling, biopsy analysis, and accessible high-resolution imaging.

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