

Meeting-report

Challenges and Opportunities of Volume Electron Microscopy: SBF-SEM of *Schmidtea mediterranea*

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Volume EM has emerged as an important strategy for improving our understanding of biological processes [1]. Acquiring large EM data volumes is easier than ever, and tools for analysis like deep learning are helping to extract meaningful information more efficiently. In addition, combining strategies like on-section fluorescence labelling with array tomography for correlative light and electron microscopy (CLEM) imaging can unite molecular identification and ultrastructural detail in significant ways.

Despite its growing accessibility, the potential of volume EM can still come with challenges in obtaining a quality dataset, one of which is finding the right sample preparation strategy. Established protocols may need to be adjusted for good results in a particular sample type or for enhancement of specific features of interest. For volume EM of the planarian flatworm *Schmidtea mediterranea* (*Smed*) we explored and adjusted multiple SBF-SEM sample preparation protocols. Between two protocols that gave good conductivity in adult asexual *Smed*, we found differences in the staining intensity of two important features of interest, cell membranes and chromatin [2-5]. For each protocol, where one of these features was enhanced the other was very faint and difficult to analyse. We used a combination of staining strategies from both protocols to produce volumes in which cell membranes and chromatin were stained well and could be manually traced for morphological analysis [6].

Although the sample preparation optimization was time consuming, it led to the acquisition of multiple large SBF-SEM datasets that are being used as a continued resource for studying the general morphology of adult asexual *Smed* through both manual and deep learning analysis, and as an ultrastructural reference in combination with on section labelling CLEM experiments [7].

References

1. Christopher J. Peddie *et al*, *Nat Rev Methods Primers* 2 (2022) p. 51. <https://doi.org/10.1038/s43586-022-00131-9>
2. Yunfeng Hua *et al*, *Nature Communications* [Online] 6, 7923 (2015), <https://doi.org/10.1038/ncomms8923> (accessed February 13, 2024)
3. Juan Carlos Tapia *et al*, *Nature Protocols* 7 (2012) p. 193. <https://doi.org/10.1038/nprot.2011.439>
4. Thomas J. Deerinck, *et al*, *Protocols.io* (2022) <https://dx.doi.org/10.17504/protocols.io.36wgq7je5vk5/v2> (accessed February 13, 2024)
5. M.A. Hayat in “*Principles and techniques of Electron microscopy biological applications*”, (Cambridge University Press, Cambridge).
6. Melainia L. McClain, Stephanie H. Nowotarski in “*Methods in Cell Biology*”, ed. K. Narayan, L. Collinson, P. Verkade, (Elsevier, Cambridge) p. 213.
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