Preparation of single-cell samples for SBF-SEM; a novel multi-technique approach using MicroCT.

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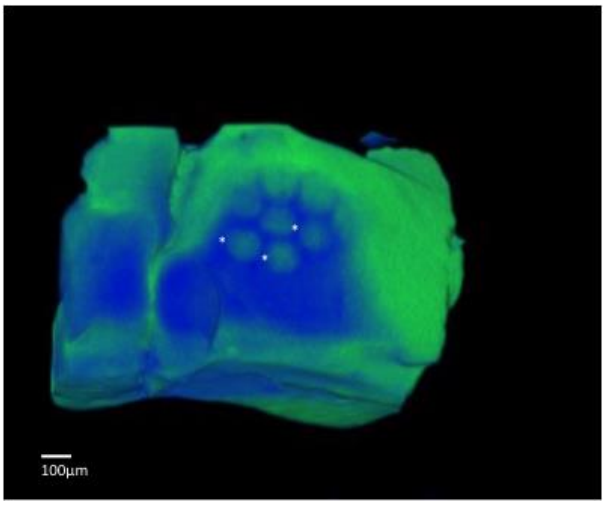
Background and Purpose: The development of serial block face scanning electron microscopy (SBF-SEM) has significantly improved our understanding of the three-dimensional (3D) structure of many different biological tissue types and cellular pathways, with particular focus on elucidation of neural networks in the brain (Helmstaedter et al., 2013). Most work conducted has involved small tissue blocks or cell monolayers, which are easily processed and sectioned to allow for subsequent 3D reconstruction. Our sample posed a greater challenge; we are studying the meiotic spindle inside individual mouse oocyte cells, and therefore needed to develop a robust method to allow us to process a single cell (of approx. 80µm in diameter) for serial sectioning. In this abstract we describe a novel approach for how microCT can be used in conjunction with SBF-SEM to accurately locate single cells in a resin sample, allowing specific dimensions to be calculated and optimal positioning and orientation of the sample for SBF-SEM.

Basic Method: Mouse oocytes (n=120) were collected and fixed with 2.5% gluteraldehyde and 2% formaldehyde. To facilitate processing of individual cells, the oocytes were encapsulated in groups of 8-10 in a drop of BSA overnight. The BSA cube containing the oocytes was then processed for SBF-SEM as described by Nixon et al (2017). Staining with heavy metals turned the sample block black and cells were no longer visible. Procure 812 resin was used for embedding and individual Sapphire coverslips placed on top of each block to ensure the surface remained flat for easier block alignment for SBF-SEM. After embedding, cells were still undetectable inside the block so the sample was removed and fitted inside the MicroCT chamber to enable precise detection of their position. MicroCT allowed for quick and clear identification of individual cells (Fig 1) such that the block dimensions and cell position could be estimated to cut the block smaller and accurately orientate for SBF-SEM.

Principal Conclusions: MicroCT offers a non-invasive, quick and accurate tool to identify single cells encapsulated in a block of unknown proportions. This novel multi-technique approach could have many potential applications when working with individual cells or hard to identify samples that require encapsulation prior to processing for many serial sectioning techniques.

Figure 1: Resin block with 7-8 individual mouse oocyte cells clearly distinguishable (*) embedded deep inside the block.



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