Silver filler pre-embedding to enhance resolution and contrast in multidimensional SEM - A nanoscale imaging study on liver tissue

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The recent emergence of serial block-face scanning electron microscopy (SBF-SEM), has allowed for the automated generation of large-volume 3-D structural information at the ultrastructural scale, providing insights previously unrealised by conventional imaging techniques. Despite the plethora of advantages achieved through SBF-SEM, a major challenge inherent to the technique is that of electron charging, which ultimately reduces attainable resolution and detracts from overall image quality. Current methods to deal with charging include (1) sample preparation protocols that involve the repeated application of multiple heavy metal stains and mordanting agents, as well as (2) imaging under variable pressure chamber conditions. Whilst both methods have been proven to mitigate charging, they are not without their limitations and compromise. In the present study, we outline a novel "silver filler pre-embedding approach" that involves infiltration of liver tissue with highly conductive silver nanoparticles suspended in gelatin. We demonstrate the elimination of charging within extracellular spaces (e.g. blood vessels) and an increase in overall specimen conductivity, which allows imaging under high-vacuum chamber conditions - enabling the attainment of superlative image contrast and resolution. Such improvements ultimately improve dataset quality, reduce imaging time and expedite manual and automated segmentation of structures of interest.