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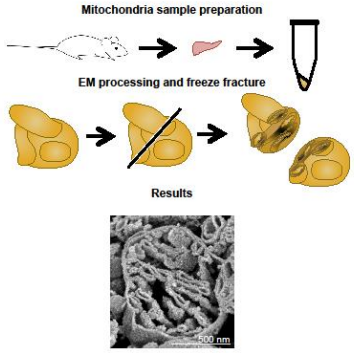
Graphical abstract



Graphical Abstract

Abstract:
Electron microscopy as a methodology for the study of mitochondria based on morphological features is a standard technique that has experienced little evolution over the course of several decades. This technology has identified heterogeneity of mitochondria populations across both whole tissues, as well between individual cells, using primarily ultrathin sections for transmission electron microscopy (TEM). However, this technique constrains the evaluation of a sample to a single two-dimensional plane. To overcome this limitation, scanning electron microscopy (SEM) has been successfully utilized to observe three-dimensional mitochondria structures within the complex microenvironment containing total cellular components. In response to these dual technical caveats of existing electron microscopy protocols, we developed a methodology to evaluate the three-dimensional ultrastructure of isolated mitochondria, utilizing a freeze-fracture step and rigorous preservation of sample morphology. This protocol allows for a more high-