A109 A Semi-Automated Multiparametric Pipeline for Mitochondrial Segmentation and Quantification to Evaluate Metabolic Dysregulation

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Introduction: Metabolic dysfunction is emerging as a critical priming process of numerous age-associated diseases such as Age-related Macular Degeneration (AMD). As mitochondrial functional and structural parameters are intertwined, analysis of mitochondrial network morphology and dynamics have been proposed as key biological markers for quantitative description of a cell metabolic state. The aim of this study was to better characterize the efficacy and accuracy of a novel automated mitochondrial segmentation and quantification pipeline for rapid and reproducible morphometric analysis of the mitochondrial network. This methodology was validated using conditions known to induce mitochondrial dysfunction in RPE, the primary cell target in AMD. **Methods:** Human RPE, ARPE-19, were exposed to TGFβ2 (10 ng/mL) and TNFα (10 ng/mL) for 72 hours. The mitochondria network was stained using MitoTracker Orange and imaged using confocal imaging (SP8). Images were pre-processed through a series of ratiometric filtering to extract fluorescent biostructure signal from background noise, thresholded to isolate mitochondrial network into computer memory, and post-processed removing out-of-field artifacts. Three-dimensional particle analysis provided insight to mitochondrial volume, surface area, and sphericity, while final skeletonization processing provided quantification of branch characteristics. **Results:** Both TGFβ2 and TNFα, induced a pathological reorganization of the mitochondrial network, marked by significant increase in sphericity (weighted) (3.2x $p = 0.0163$, 3.3x $p = 0.0003$) and a reduction in branches per mitochondria (0.23x $p = 0.0155$, 0.20x $p = 0.0096$) respectively normalized to the untreated control. **Conclusions:** Super-resolution fluorescence microscopy image analysis permitted efficient extraction of 3D mitochondrial network parameters established as important biological markers of metabolic dysfunction pathogenesis (per cell or per mitochondria depending on analysis configuration). The non-specific nature of this automated pipeline approach serves as an attractive methodology for rapid, large-batch exploration and characterization of pathologies driven by metabolic dysregulation.