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Indirect Correlative Light and Electron Microscopy – A High Throughput Approach with Robust Quantitative Capabilities

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Background: Correlative light and electron microscopy (CLEM) is emerging as a powerful tool for nanoscale structural studies in both the biological and physical sciences. However, the challenges associated with obtaining precisely registered images of nanoscale neighborhoods within a sample on multiple microscopes severely limit the adoptability and throughput of CLEM. Additionally, the low throughput and lack of robust image analysis lead to CLEM being largely used as a qualitative approach with limited ability to assess structural heterogeneity or identify subtle changes associated with early stages of disease. Thus, high-throughput CLEM approaches with robust quantitative capabilities are needed.

Approach: We have developed *indirect* CLEM [*iCLEM*] as a low-cost, high throughput option with extensive quantitative capabilities. By taking advantage of structural fiducials / landmarks identifiable via both light and electron microscopy, *iCLEM* sidesteps the rate-limiting steps of conventional CLEM – preparation of samples suitable for both light and electron microscopy and precise registration of nanoscale images. Instead, *iCLEM* entails correlation of distributions of morphometric measurements made in relation to structural fiducials, collected from independent sets of light and electron microscopy samples. This approach readily lends itself to integration with computational modeling, providing a clearly defined pipeline to uncover structure-function relationships. Additionally, the approach is agnostic to specific imaging modalities and allows us to multiple techniques targeting different spatial scales to be seamlessly combined.

Example Application: We have used *iCLEM* to undertake systematic investigation of the structural underpinnings of electrical signal propagation in the heart, a critical process that coordinates the contraction of billions of muscle cells during each heartbeat. Motivation for this work derives from that fact that disruption of this process leads to life-threatening disruptions in the heart's rhythm (arrhythmias). Therefore, we used *iCLEM* to study the intercalated disk (ID), a specialized structure that affords electrical and mechanical coupling between muscle cells in the heart. Although, the ID is widely recognized as a complex, heterogeneous structure, the lack of quantitative structural data has forced computational models to omit it or simplify it to a homogenous 2D circle. Using *iCLEM*, we have obtained the first-ever quantitative picture of the ID, enabling us to construct realistic 3D computational models. Transmission electron microscopy (TEM) enabled us to quantify ID ultrastructure from micro- through nano-scales and thereby, construct populations of finite element models of IDs, capturing both intra- and inter- individual variability. We then populated these meshes with electrogenic proteins whose spatial distributions were derived from super-resolution microscopy: 1) sub-diffraction confocal microscopy (sDCI) coupled with **m**orphological **o**bject **l**ocalization (MOL; a distance transformation-based spatial analysis approach) for microscale information and STORM-based Relative Localization Analysis (STORM-RLA) of single molecule localization data for nanoscale information. By incorporating these data into computational models of electrical signal propagation, we are uncovering previously unappreciated structure-function relationships that determine the regularity of the heart's

rhythm. These predictions, along with functional imaging studies of electrical signal spread in the heart, are providing the basis for the development of novel classes of anti-arrhythmic drugs.

Conclusions: Rich and robust quantitative data from high-throughput *iCLEM* approaches offer major advantages: 1) Assessment of intra- and inter- individual variability to improve reproducibility in the life sciences, 2) Integration into computational models to uncover structure-function relationships, and 3) Development of diagnostic and therapeutic strategies for the earliest stages of disease. Additionally, the ability to utilize previously available tools with few, if any, constraints on the specific imaging modalities used provides unprecedented flexibility at low cost and significantly lowers the barrier to entry.

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