Super-Resolution Imaging and Analysis of Subcellular Structures In Cardiac Myocytes

Shirley Zhang

Super-resolution imaging allows for clear visualizations of the complex protein distributions and structures at the subcellular level. Knowing these structures and how they can be affected under certain abnormal conditions is a crucial first step in understanding the biological basis for conditions such as heart failure.

I. Introduction

During this study, mouse cardiomyocytes from wild type control and transgenic knockdown groups were labeled and imaged for structures such as t-tubules, ryanodine receptors, and junctophilin-II using a method called dSTORM (direct stochastic optical reconstruction microscopy). Specific locations and detailed structures of these subcellular structures could not be resolved using traditional light microscopy, which encounters an optical resolution limit due to light diffraction at a scale of several hundred nanometers. dSTORM allows the use of conventional fluorophores and labeling of multiple structures at once to image with a single laser to achieve much finer resolution. Laser excitation causes the fluorophores to undergo stochastic "blinking" as they alternate between "on" and "off" states controlled by oxidation and reduction reactions. By limiting these blinking events to a manageable number per frame, computer analysis can compile the data and give an accurate depiction of the structures under the microscope. The readily available materials and relatively simple procedures make this method a good alternative to expensive and complicated electron microscopy to see these structures within cells.

II. Methods

Isolated mouse cardiomyocytes were first treated with 1% Triton and a blocking solution containing normal goat serum. They were then labeled with a mouse and a rabbit primary antibody which singled out the specific proteins of interest, and were then labeled with a goat anti-mouse or anti-rabbit secondary antibody attached to Alexa fluorophores in near-infrared wavelengths like Alexa 680 and Alexa 750. Labeled cells were mounted on slides using a switching buffer containing 10 mM MEA, glucose oxidase, catalase, and 10% glucose in glycerol. Cells are illuminated with a 671 nm laser and the data is gathered and analyzed using a custom python program.

III. Results/Discussion

Figure 1 below shows images of the labeled cellular structures in mouse cardiomyocytes, with color added by computer for visualization of two different labels.

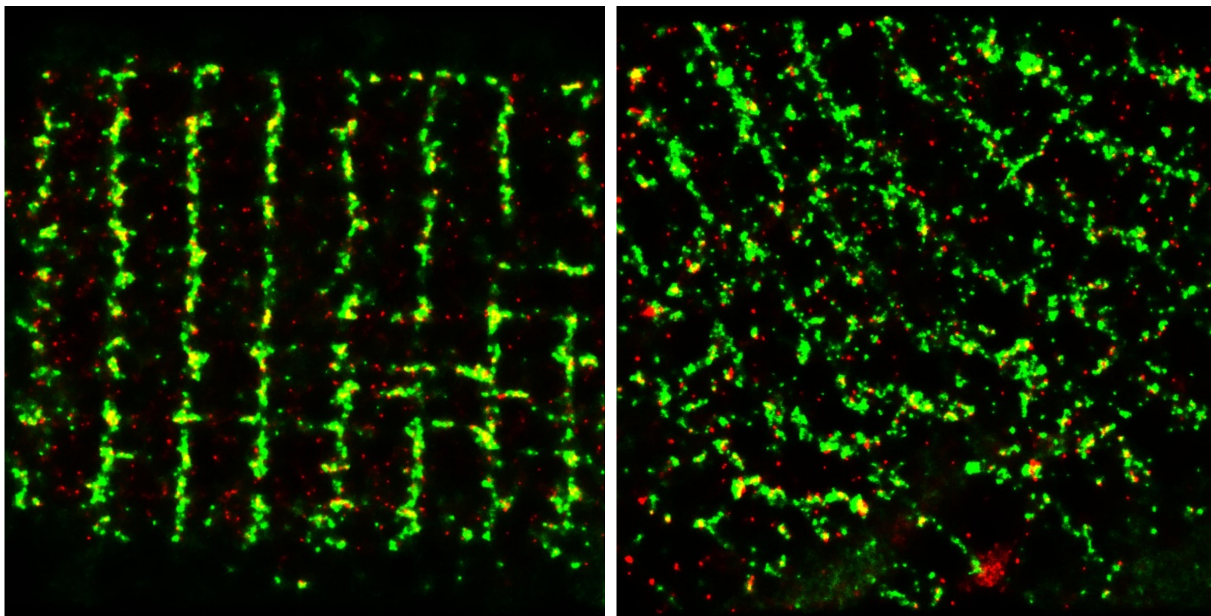

Figure 1 Mouse cardiomyocytes labeled for ryanodine receptors (green) and junctophilin-II (red) in wild type **(left)** and junctophilin knockdown mice **(right)**.

This data was far from conclusive but it did show that these antibodies worked well together under the conditions for these cells without interference from an already present membrane protein tdTomato. There were also indications that the t-systems of cells with reduced expression of junctophilin had less organized patterns, and it is known that distributions of ryanodine receptors in a cell are somewhat associated with the distribution of junctophilin. This could help further the understanding of the role that junctophilin protein plays within cells and how it affects the form and function of other associated structures.

Other cells were also used to label for caveolin-3 and a t-tubule cocktail consisting of the cav 3 antibody as well as one for Na/Ca exchange. Imaging concluded that the cocktail worked better to label the t-system than either antibody alone.

Some potential problems include the inherent variability between cells from the same sample. One cell on a slide may look very different to another, so a larger sample size would be necessary to establish what would be considered a typical cell representative of that sample. The mounting media, which contain active enzymes that lose activity over time, also could vary by batch. A poor mounting medium results in minimal "blinking" activity leading to low output of data and poor image quality. These could lead to issues in duplicating results in the future. Experiments were also done to investigate the impact of fluorophore wavelengths on the quality of imaged structures but there seemed to be no consistent differences depending on which structure was labeled using which fluorophore. Another problem which could lead to error in the final image results came from shift, which occurs if the cells move during imaging. This could occur if the slide was not properly placed on the microscope, or if there was too much mounting

media under the coverslip which could cause cells underneath to move around. If shift was minimal it could be calculated and compensated for by computer program.

IV. Conclusion

Due to time constraints, what was mentioned above was just the start for more work to be done in the future by those at the University of Auckland with more collaborations from the University of California, San Diego. Super-resolution imaging allows researchers to finally see in detail the subcellular structures which contain a wealth of information that can lead to further advances in science and medicine.

References

Baddeley D, Crossman D, Rossberger S, Cheyne JE, Montgomery JM, et al. (2011) 4D Super-Resolution Microscopy with Conventional Fluorophores and Single Wavelength Excitation in Optically Thick Cells and Tissues. PLoS ONE 6(5): e20645. doi:10.1371/journal.pone.0020645

Schermelleh L, et al. (2010) A guide to super-resolution fluorescence microscopy. Journal of Cell Biology 190: 165-175. Doi: 10.1083/jcb.201002018