doi:10.1017/S1431927620013501
Microsc. Microanal. 26 (Suppl 2), 2020

Determining Protein Organisation within the Z-Disc Using 3D Super-Resolution Microscopy and Pattern Recognition Analysis.

Alistair Curd[1], Joanna Leng[1], Brendan Rogers[1], Hari Shroff[2] and Michelle Peckham[1]

[1]University of Leeds, Leeds, England, United Kingdom, [2]National Institutes of Health, Bethesda, Maryland, United States

Cellular proteins are often assembled into large complexes with highly ordered structures. Examples include the nuclear pore, centrioles, and muscle Z-discs. Z-discs are narrow dense structures found at the ends of muscle sarcomeres. Sarcomeres are ~2 microns long and arranged in series in structures known as myofibrils, which run from one end of the muscle fiber to the other. Z-discs are important biological structures, which anchor actin and myosin filaments in the sarcomere, and contain many signaling proteins. However, it is challenging to uncover the organization of proteins within the Z-disk, as structures are typically only 100nm wide, below the resolution limit of confocal microscopy. Moreover, the thickness of the myofibrils makes this challenging as an electron microscopy project, and typically has had to be done using sections [1] in which proteins are difficult to identify in the resulting tomograms.

To overcome this approach, we have started to use a super-resolution microscopy approach, 3D STORM (stochastic optical reconstruction microscopy). As it is difficult for antibodies to penetrate the Z-disc, we have additionally combined this approach with novel non-antibody binding proteins called Affimers [2]. Affimers are isolated by screening a phage-display library against a target protein of interest. Once isolated, the Affimers can be expressed and purified using *E. Coli*. These small proteins are ~10kDa in size, and can be specifically dye-labelled using a unique cysteine placed at either the N- or the C-terminus. This places the dye molecule close to the target protein [3], reducing 'linkage error'. The small size of the Affimer additionally improves its ability to penetrate dense structures over antibodies [2]. Moreover, we can crystalize the Affimer in complex with its target protein to determine precisely how the Affimer binds to its target.

To image a key Z-disc protein, α-actinin-2 (ACTN2), we expressed and purified the calponin-homology domains of this protein, and isolated 7 Affimers. These were tested in standard widefield and confocal imaging (Fig. 1), and the best candidates taken forward and tested in 3D dSTORM. Alexa 647 was used for the dye label and isolated adult cardiomyocytes as the sample. We compared the Affimer data to that obtained using an mEos-α-actinin-2 expression construct, expressed in adult cardiomyocytes using an adenoviral vector, in confocal images (Fig. 1) and in PALM (photoactivated localization microscopy).

To analyse the organization of ACTN2 in super-resolution images, we developed and implemented a novel pattern recognition software approach, which we call PERPL (pattern extraction from relative positions of localisations). This approach compares experimental distances between molecules measured by analysing 3D dSTORM reconstruction images (diagrammed in Fig. 2 for the Z-disc) against models of ordered and disordered macromolecular geometry. Selection of the best model employed Akaike's Information Criterion. We benchmarked this approach against known structures (such as the nuclear pore). Analysis of the Z-disc data demonstrated the expected pattern for α-actinin-2, with a repeat at ~20nm in the axial direction. We anticipate that this approach will be able to reveal the organization of additional proteins within the Z-disc, and will be generally useful for determining underlying patterns in high resolution image data, including data clustering.

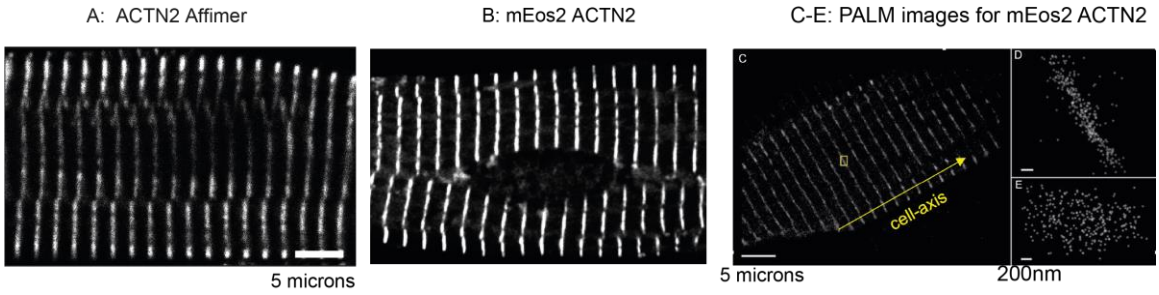

Figure 1. Figure 1: Z-Disk imaging. Confocal imaging of α-actinin-2 (ACTN2) in the Z-discs of adult cardiomyocytes using A) an Affimer to ACTN2 labelled with Alexa 647 and B) mEos2-ACTN. (Scale bar 5 μm). PALM images of mEos2-ACTN, with the cell axis shown by the yellow arrow. Yellow boxed area in C is shown expanded in D to higher magnification view. E shows a view onto the cell transverse plane (viewed along the cell axis).

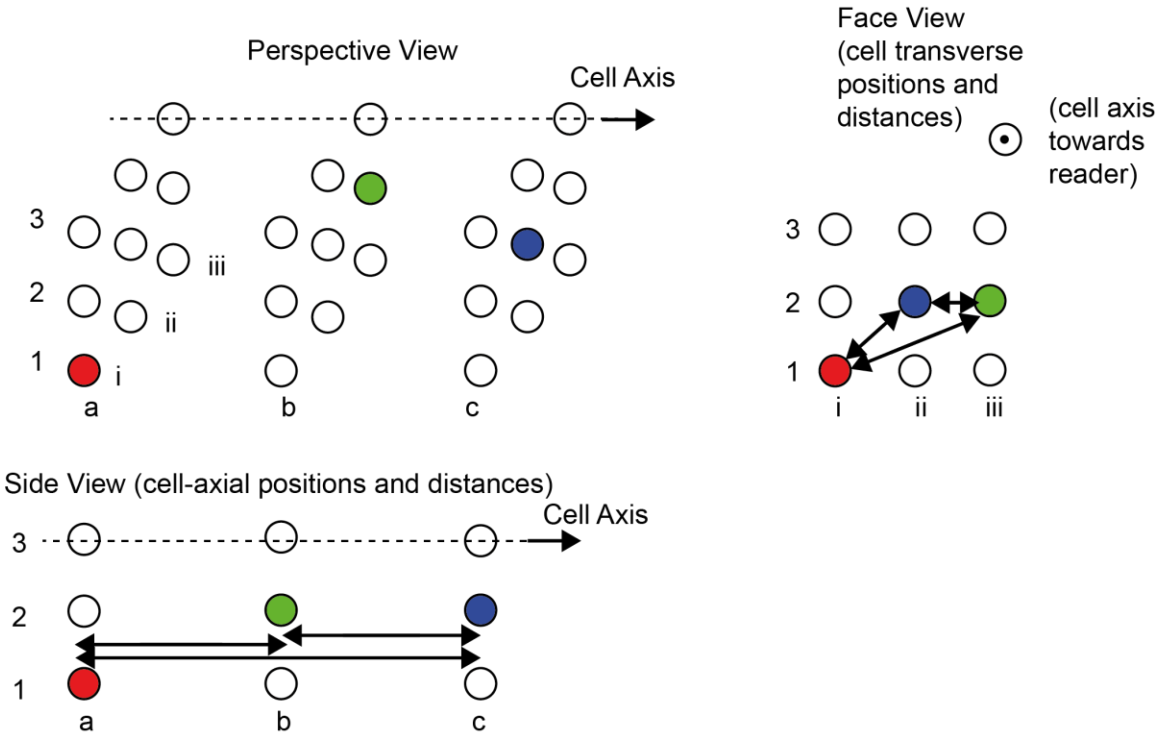

Figure 2. Figure 2: Calculating distances for relative position distributions of Z-disk localisations for PERPL analysis. Three localisations (coloured) are shown on a cuboidal lattice with a 3D coordinate system. Cell-axial distances are shown in 'Side view'. Corresponding cell-transverse distances are shown in 'Face view'. Absolute distance is used in the cell-transverse plane and directional information is discarded. This means that data will follow the same distribution when moving between Z-disk domains with different orientations and can be aggregated. Each pair of localisations has a cell-axial and a cell-transverse separation, which become 2D coordinates in the subsequent PERPL analysis.

References

[1] T Burgoyne et al., Proc Natl Acad Sci U S A **116** (2019), p. 15534.
[2] C Tiede et al., Elife **6** (2017),
[3] G Carrington, D Tomlinson and M Peckham, Mol Biol Cell **30** (2019), p. 2737.

https://doi.org/10.1017/S1431927620013501 Published online by Cambridge University Press