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Electron tomography of rabbit cardiomyocyte three-dimensional ultrastructure

Eva A. Rog-Zielinska [a, *], Callum M. Johnston [a], Eileen T. O'Toole [b], Mary Morphew [b], Andreas Hoenger [b], Peter Kohl [a, c]

[a] National Heart and Lung Institute, Imperial College London, UK
[b] Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA
[c] Institute for Experimental Cardiovascular Medicine, University Heart Centre Freiburg – Bad Krozingen, Medical School of the University of Freiburg, Germany

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ABSTRACT

The field of cardiovascular research has benefitted from rapid developments in imaging technology over the last few decades. Accordingly, an ever growing number of large, multidimensional data sets have begun to appear, often challenging existing pre-conceptions about structure and function of biological systems. For tissue and cell structure imaging, the move from 2D section-based microscopy to true 3D data collection has been a major driver of new insight. In the sub-cellular domain, electron tomography is a powerful technique for exploration of cellular structures in 3D with unparalleled fidelity at nanometer resolution.

Electron tomography is particularly advantageous for studying highly compartmentalised cells such as cardiomyocytes, where elaborate sub-cellular structures play crucial roles in electrophysiology and mechanics. Although the anatomy of specific ultra-structures, such as dyadic couplons, has been extensively explored using 2D electron microscopy of thin sections, we still lack accurate, quantitative knowledge of true individual shape, volume and surface area of sub-cellular domains, as well as their 3D spatial interrelations; let alone of how these are reshaped during the cycle of contraction and relaxation. Here we discuss and illustrate the utility of ET for identification, visualisation, and analysis of 3D cardiomyocyte ultrastructure such as the T-tubular system, sarcoplasmic reticulum, mitochondria and microtubules.

Contents

Abbreviations: ER, endo-plasmic reticulum; ET, electron tomography; FIB, focused ion beam; Mito, mitochondria; MT, microtubules; SBF, serial block face; SEM, scanning electron microscopy; SL, sarcomere length; (j)SR, (junctional) sarcoplasmic reticulum; (n)SR, (network) sarcoplasmic reticulum; TEM, transmission electron microscopy; T-tub, T-tubules; 2D, two-dimensional; 3D, three-dimensional.

* Corresponding author. Cardiac Biophysics and Systems Biology Group, Harefield Heart Science Centre, Hill End Road, Harefield, UB9 6JH London, UK.
E-mail address: e.rog-zielinska@imperial.ac.uk (E.A. Rog-Zielinska).

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1. Introduction

The predominantly reductionist scientific approach, whilst offering important insight that otherwise might be obscured, is not without limitations (Asano, et al., 2016), as even the most detailed knowledge of individual components alone is often insufficient to predict the behaviour of complex biological systems. However, despite the fact no biological system exists in isolation, we often approach and study biological structures as if they were independent of their environment.

A cell is one example of a complex system whose individual components interact with one another, and with their environment, in time and space. These interactions influence function and structure of each individual component. Cellular processes are performed by molecular assemblies that form complex functional modules that convoluted biochemical pathways that themselves require a non-random spatial organization of components to proceed efficiently (Alberts, 1998; Aloy and Russell, 2004; Hartwell et al., 1999). Successful scientific exploration of cellular organization should involve not only the identification and detailed characterization of parts, but also of their spatio-temporal interactions (Kohl et al., 2010). In order to understand the behaviour of an integrated system, such as a cell, we must consider how interacting networks (e.g. proteins, membrane structures, filaments, etc.) are arranged in 3D, how their arrangement enables and restricts their dynamics, and how these spatial relations are affected by both normal function (particularly in a contractile organ such as the heart) and pathological remodelling. Determination of *in situ* structure requires the development of cutting edge methods allowing the cells to be preserved as close to their native state as possible, thus providing an accurate snapshot of the often unstable and transient molecular assemblies.

Many gaps in our current knowledge stem from the fact that structures we are studying are complex 3D objects, which are reduced to (at best - serial) 2D representations when analysed using conventional approaches. Consequently, there is increasing appreciation and desire for detailed, high resolution, and – importantly – quantitative 3D analysis of structural and ultrastructural topology of biological samples. Unfortunately, many of the structures of interest are of dimensions equivalent to, or below, the diffraction limit of standard light microscopy, while at the same time being too complex to be easily studied by ultra-high resolution methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

Conventional transmission electron microscopy (TEM) offers an optimal X-Y resolution of subcellular structures (1 nm in X-Y plane), yet it is inherently a 2D approach, with low maximum sample thickness (samples are typically 40–80 nm thick) leading to poor Z-resolution. An advancement of this technique into the 3D domain came with the introduction of serial sectioning, allowing for pioneering insight into 3D cardiomyocyte ultrastructure (De Maziere et al., 1992; Johnson and Sommer, 1967). This approach is limited, however, in its Z-resolution, suffers from challenges in 3D integration of inherently deformed 2D sections, while also being very labour-intensive.

Over the last few years, scanning electron microscopy (SEM) has been used to overcome some of the limitations of conventional TEM. SEM uses an electron beam to visualise surface topology of a sample and is able to achieve resolution of 5 nm in X-Y (Sulkin et al., 2014; Glancy et al., 2015; Pinali and Kitmitto, 2014). When combined with serial block-face imaging (SBF-SEM), for example using focused ion beam technology to remove surface matter after each scan (FIB-SEM), SEM is a method of choice for 3D nano-scale visualisation of biological structures over extended (for electron microscopy techniques) volumes, potentially linking observations from nano- to micro-scales (Denk and Horstmann, 2004; Heymann et al., 2009; Knott et al., 2008).

SBF-SEM development built on use of an ultramicrotome mounted inside the vacuum chamber of a scanning electron microscope. Following imaging, a thin section of the freshly-scanned surface (usually 25 nm–100 nm) is cut from the block and discarded, before the 'new' block-face is imaged again (Peddie and Collinson, 2014). In FIB-SEM, instead of an ultramicrotome, an ion beam is used to burn-off, rather than mechanically cut, a thin layer (typically 5 nm–50 nm) of material from the sample surface (Knott et al., 2008; Peddie and Collinson, 2014). In both cases, one obtains stacks of inherently co-registered images that then can be aligned and reconstructed to reveal the 3D structure of interest within the spatial context of a cell or tissue (Villinger et al., 2012). Recently FIB-SEM has been successfully employed to visualize the nano-topography of muscle cells (Sulkin et al., 2014; Glancy et al., 2015; Pinali and Kitmitto, 2014), revealing the complex network of cardiac T-tubules (T-tub) as well as of a mitochondrial "reticulum" both in cardiac and skeletal muscle cells. Technical challenges of the approach are associated with protecting the sample from degradation or warping by ion beam (heat damage) or blade interactions (mechanical damage). Additionally the device requires extreme levels of isolation from external sources of interference such as building vibrations, temperature fluctuations, ventilation drafts, etc. Furthermore, both SBF-SEM and FIB-SEM destroy the tissue sample as part of the observation process, and no re-imaging can be performed.

The introduction of electron tomography (ET) to biological research has allowed 3D reconstructions that are non-destructive in as far as the sample is concerned (Frank, 1992). The theory behind ET is very much like that of more well-known types of tomography (computed tomography or positron emission tomography) used routinely in clinical practice, with the differences that ET is based on electron beams (rather than X-rays or γ-rays), and that, in ET, the sample is rotated (rather than the beam source). In practice, ET involves s serial TEM, acquired at different angular orientations (usually between -70° and $+70^{\circ}$) of a thick (typically 200 nm–300 nm) section. As for technical constrains, the prolonged electron beam exposure affects the plastic used for biological sample embedding, resulting in specimen deformation. This can be corrected for post-acquisition. Also, the overall volume covered is limited, but tiling and stacking of serial high-resolution ET is possible, for example to reconstruct small cells (Hoog et al.,

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