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To Whom It May Concern,

I was given the task of assessing Andrew Noske's PhD thesis in early 2010, a task which I gladly accepted having met Andrew a few years prior and seen demonstrations of his contribution to cellular tomography in conferences. Written below is my written assessment of Andrew's PhD thesis. After reading this assessment Andrew suggested it was a valuable summary of his thesis for anyone considering downloading and reading it, and requested I write this brief foreword.

Although I have not worked with Andrew, I invited him as a speaker at a mitochondrial conference I organized in Melbourne Australia in 2008. He is clearly a very bright and hard-working scientist with a load of potential and also a great communicator. Feel free to contact me if you have any further questions, or e-mail Andrew if you'd like a copy of his PhD thesis "Multi-Scale, Spatio-Temporal Analysis of Mammalian Cell Tomograms".

Yours Sincerely

Michael Ryan

Examiner report of the thesis by Andrew Noske

I am incredibly impressed by the quality of this thesis. The candidate has written in a floorless style and in an engaging way even when the material is often technical and in many cases outside of the comprehension of this examiner. I have examined 9 external theses and also supervised 7 PhD students to completion. I regard this as the best thesis that I have read and feel privileged to be an examiner. I congratulate the candidate and his supervisor for producing an excellent body of work and I look forward to reading additional publications arising from this study.

Normally, I include a list of grammatical and spelling errors to be amended, however I was not able to find any. This was incredibly refreshing and for this reason I believe that the thesis should be accepted without amendments and the candidate be conferred as Doctor of Philosophy.

General comments:

The work focuses on two general aspects related to electron tomography. Firstly, the candidate outlined new approaches to more efficiently and accurately reconstruct multiple images into a 3D picture at high resolution. Secondly, the candidate used these improvements to address a biological problem – the investigation into changes in the internal architecture of pancreatic beta cells following glucose stimulation.

In Chapter 1, the candidate introduced the rather broad topic in exquisite detail. The introduction covered aspects of cellular architecture related to beta cell biology as well as concepts related to electron tomography and whole cell reconstruction. The figures were clear and used to illustrate important concepts. Aspects of this thesis could easily be adapted into two review articles.

The Materials and Methods section was very clear and interesting and had many hallmarks of being a research-based chapter in itself. The overarching strategy employed for ET was nicely outlined.

In Chapter 3, the candidate presented drawing tools to more efficiently facilitate whole cell reconstruction by allowing tomogram segmentation. Such tools including methods to calculate connectivities of cellular compartments given different shape contours that exist. In addition the candidate clearly explained ruffling problems inherent in manual segmentation and ways in which they have been overcome through the use of smooth and spherical interpolation techniques. The challenge of mapping Golgi ribbons was also overcome using linear interpolation and the efficiency of such mapping was substantially improved when compared to the previously employed manual segmentation. The improvements made resulted in a 4-fold improvement in the segmentation of a whole cell tomogram. Commendably, these techniques have now been integrated into the program IMOD for other experts to use.

In Chapter 4, the candidate presented his work on reconstructing cellular tomograms by specifically analysing mature granules. Using the knowledge that these granules are highly spherical, the candidate was able to debunk a long-held assumption related to sample decay during ET that results in section collapse. The candidate convincingly showed that the collapse was significantly less than previously thought (25% vs. 40%). The relevance of this comes when correcting for such deformation (by stretching) during reconstructions. This is most well demonstrated in Fig 4.21 (and later in chapter 6) where cell stretching (using *zScale*) is performed. The candidate also uses this chapter to report additional techniques to accurately undertake tilt-series projections to result in higher resolution tomograms. Such techniques clearly provide strategies to effectively reduce bottlenecks in electron tomography.

The technical work outlined in chapters 2-4, culminated with a whole-cell tomographic study of 4 beta cells – two which were stimulated and two which were not. This is an incredible feat and demonstrates the drastic improvements made by the candidate in refining techniques during his PhD. The candidate uses rational approaches to interpret the findings – especially given the variability between the different cells and nice hypotheses are formulated. The candidate was able to demonstrate the novel finding that beta cells do not appear to simply slowly release insulin from their granules following stimulation but rather, cells can release them more rapidly but at seemingly different rates. Besides this, there were also many interesting and seemingly unique observations that bring new questions to light (and which the candidate has touched on). For example, I was fascinated by the plasma membrane invaginations to connect with centrioles (is this already known?). The observation that the centrioles differ in size between cells might reflect beta cells being at a different stage in the cell cycle. Of course the mitochondrial localisation and the changes seen are quite unique. It will be interesting to compare these with live cells and analyse their dynamics (especially the large mitochondrion that may buffer calcium and those abutting the PM). An additional question coming from the study of cell and organelle volume is what how is this all regulated? For example, how are the organelles able to differ in size not just between beta cells (where subtle differences are observed) but also between cells of other tissues and organisms? The techniques outlined by the candidate and being employed so beautifully by the Marsh group (including the simplified 3D abstractions of spatial representations of various compartments shown in Fig. 5.33) means that accurate volume reconstructions can be undertaken which will aid in addressing this area.

The candidate concludes his thesis with an excellent discussion in Chapter 6. This was thought-provoking (again) and I was particularly excited by the potential of using principles similar to that used by Google Earth to explore the inner workings of the cell. I wait for this with bated-breath!