Reviewer's report

Title: Resolution of the three dimensional structure of components of the glomerular filtration barrier

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Reviewer: Tom Van Agtmael

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This manuscript by Arkill et al describes the comprehensive EM analysis of the glomerular filtration barrier in 3 dimensions. This manuscript describes an impressive technical advantage that has the potential to significantly impact the electron microscopy analysis of the filtration barrier in kidney disease. This is urgently required as to fully understand the mechanisms of kidney disease it is necessary to understand the renal structure in three dimensions. While classical two dimensional analysis of the filtration barrier is valuable it does not provide the overall level of information regarding potential defects in the filtration barrier. For example from a low number of sections it is impossible to evaluate morphological defects and how significant they may be without an estimate of their size. The analysis described in this manuscript paves the way for this type of analysis.

The manuscript itself is well written and I have only a couple of minor essential comments.

The clarity of the results section related to Figure 3 can be improved. For example there is reference to Figure 3B but this is not annotated on the figure itself.

While the discussion focuses on the technical aspects of the analysis, it would be appropriate to comment on some of morphological features of the analysed kidney samples. For example the knobby appearance of the glycocalyx is striking...what would the impact of this be on its function, given its active role in providing barrier function? Also is this entirely due to the variability in thickness or does it reflect the un-evenness on the underlying cell surface of the endothelial cell and is the thickness of the glycocalyx more consistent? I realise this is based on a very limited sample number but a comment would be appropriate.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.