Review of: "Saturated reconstruction of living brain tissue"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Saturated reconstruction of living brain tissue describes a pipeline based on super resolution (STED)microscopy and, more importantly2, deep learning algorithms that enables 3D *in vivo* super resolution imaging (circa 130 nm isotropic resolution) especially in brain tissues. The method is reliable and efficient, therefore I recommend the publication of this work after a proper revision. Please note: the required revision is not necessary for correcting or adding up relevant missing part but to improve the readability of the manuscript, enhancing its valence and let it accessible to a wider number of interested readers

Main Points: The authors should, in my view, make clearer in the manuscript that their methodology is valid and can be applied in combination with any 3D super resolution approach (and in general, any super resolution approach, also the 2D ones). Although I am much into using STED, for me, the choice of STED in this manuscript might be mostly due to the fact that this is the only 3D super resolution method that the author have now available in house. Indeed, 130 nm isotropic resolution should be achievable by mean of more power friendly, label flexible super resolution approaches.
More over in the described approach I believe photo-toxicity is not an issue but acquisition time is an Issue. I assume that accumulating 70 times over an image acquired with a pixel dwell time of 1µs provide the same good SNR of an Image took with 70µs dwell time but with dramatically less photo damage. Therefore, the author either can demonstrate with further evidence that their deep learning based pipeline is the only way for avoiding photo-toxicity or simply justified the usage of LIONESS as a method that enable the opportunity investigating living samples allowing to drastically reduce the acquisition time. Finally, talking about toxicity no information is given about excitation and STED laser power. For example no prove has been given that the STED power used is the minimum necessary to achieve the desired resolution

Small points:
In general, I believe that in all the Figure and Movie caption and when the data displayed in the figures is recalled in the manuscript more precise information should be provided. In deteails

1. Figure 3,5. Supplementary Figure 5,6,13-18 and wherever it occurs please justify at least once while is choose to display to projection spanning 150 nm (and please specify 3 frames). Please inform us about the reasoning of this choice and, is this not reducing the axial resolution of the image?.....
2. Starting from Supplementary Figure 4 and wherever it occurs it would be nice that besides reporting voxel size and integration time is also reported (in minutes or seconds) how long does it take to acquire the entire volumetric image.

In Page 6 manuscript where is written: *“for this organoid dataset(1,737 µm3)”* and in the figure 1 reported the size 19.3x15x6 cubic µm. I believe it is better to add up more information e.g.: *for this organoid dataset- (1,737 µm3 that correspond to a stack of 120 380x300 pixel images acquired at the rate of 0.5 images per second)*. Please note the value I wrote are my estimate and I introduced only for giving an example. This kind of additional information together with the acquisition time should be provided in all figures and videos were volumetric information are retrieved and