

Line numbers refer to the revised manuscript. For improved intelligibility, line numbers have also been added to the editorial comments from the manuscript, which are included at the end of this rebuttal document. When referencing specific sections of the manuscript, an underscore highlights edits.

## **Editorial comments:**

*“1. The editor has formatted the manuscript to match the journal's style. Please retain the same.”*

To also retain coherency, order of paragraphs or sentences has been adjusted where needed (refer to the first two comments from the manuscript shown below). The overall format established by the editor has been retained.

*“2. Please address all the specific comments marked in the manuscript.”*

All comments in the manuscript have been addressed. The comments have been answered in the manuscript and, for improved intelligibility, are also included in this rebuttal document.

*“3. Once done please ensure that the highlight is no more than 2.75 pages including headings and spacings.”*

The highlight for scripting and filming has been reviewed and is about 2.75 pages long.

## **Editorial comments from the manuscript:**

“We cannot have paragraphs of text in the protocol section (referring to lines 104ff.). Hence moved here.

Protocol should only be made of discrete steps showing actions.”

Instead of moving the section about precautions when handling rabies virus to the end of the introduction, where it does not really fit into, we have now combined it with the already existing precautionary paragraph about the chemical hazards of the protocol. This also eliminates the additional paragraph but retains more coherency.

*“Combined the background and note as we cannot have paragraph of text in the protocol section (lines 127ff., 164ff., 206ff., and 220ff.).”*

We changed the order of the combined paragraph (note first, background second) in all cases to improve coherency and intelligibility.

*“Please refer to the supplementary file (lines 257ff.) as well and include it in the figure legend.”*

We have now added a reference to the 3D printing file (.STL file) that can be found in the supplement of this publication. A reference was already included in the figure legend of the corresponding Figure 2.

lines 257ff. (6.1.): Using a 3D printer, print imaging chamber and lid (material: Copolyester [CPE], nozzle: 0.25 mm, layer height: 0.06 mm, wall thickness: 0.88 mm, wall count: 4, infill: 100%, no support structure; the corresponding .STL file can be found in the supplementary materials of this protocol).

*“Please expand (BABB-D15) during first time use.”*

The abbreviation has been defined on first use (line 239f.).

*“For this section, please provide all the graphical user interface, button clicks, knob turns etc. to show how is a step performed.”*

We have now added an exemplary workflow of how to process large image stacks to generate 3D projections using ImageJ (7.3.x.).

However, we did not add any further detail explanation for the image acquisition part (7.1. and 7.2.). Potential users will most likely be using a variety of microscopy set-ups from different manufacturers and varying types of acquisition software. We have specified the type of microscope and objective we used in the Table of Materials that is attached to this publication. Together with the values and parameters we already provided here (7.1.1. and 7.2.1.), we are confident that each microscope user can input these settings into their individual microscopy systems. Further explanation and detailed descriptions would only benefit users with the very same microscope and, thus, in our opinion only clutter the protocol.

*“Please provide reprint permission for this figure.”*

The figure legend for Figure 1A has been poorly phrased. The graphical representation of the workflow has not been taken from either publication but has in fact been made by us and is based on written text from the two publications already mentioned in the figure legend. We, thus, have rephrased the figure legend to avoid ambiguity.

lines 364f.: (A) Graphical representation of the workflow based on the protocols from Renier et al. (2014)<sup>14</sup> and Pan et al. (2016)<sup>13</sup>.

*“Please provide a scale bar for all the microscopic images for example RABVP and TO\_PRO3 etc.”*

It is common practice in depiction of immunofluorescence images to provide scale bars for the merge images only. This aims to declutter the overall image composition and improve comprehensibility. In all non-3D images included in this publication, a scale bar is included in the merge image. As the relative scale in the single channel images, albeit usually being depicted smaller in size, is the very same as in the respective merge image, distances and lengths can still easily be estimated. Therefore, for the reasons mentioned above, we would prefer not to include any additional scale bars in those images, if not necessary.

*“The panel C is not marked in the figure.”*

The two lower panels in Figure 6 have now been marked as Figure 6C (bottom left) and Figure 6D (bottom right) for improved referencing and intelligibility of the figure.