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Dear Editor,

Thanks for your response and for the reviewer's comments on our manuscript. We did, accordingly, a deep work of revision by modifying the paper in response to the extensive and insightful comments. We have rewritten many sections of the manuscript, changed some figures and we hope that this complies now with the referee's remarks. We respond to the comments point counter point.

All of the changes made with respect to the last version are marked in red.

We hope that now the manuscript could be considered acceptable for publication on PeerJ.

Yours sincerely,

Martina Campanella

(corresponding author)

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REV 1

The introduction provides an acceptable general introduction, but fails to clearly identify why an alternative is needed to existing approaches.

We better clarified this aspect in the Introduction explaining how crucial is to look for solutions different from tractography based on anatomical or functional landmarks in case of white matter architecture disruption.

The last paragraph of the introduction would benefit greatly from separating the outline of the method from the brief description of how the method is to be evaluated.

We re-arranged the Introduction by separating the outline of this study from its depiction.

The rationale of the method needs to be outlined in a suitable manner because the methods section breaks the process into steps that are difficult to follow the logic of the process.

We organized better the Method session, following the logical process that brings to the definition of the tracts of interest.

The rationale for and importance of the probabilistic evaluation should also be better stated.

We explained in a more extensive way the importance of introducing a complementary probabilistic framework to provide a further validation to the deterministic results. The advantages that probabilistic techniques have in some applications are somewhat reduced here, because we ran deterministic tractography from many starting points, which has a similar effect to the stochastic influence in probabilistic tractography. The comparative results we included in the manuscript support our choice of a simpler and quicker deterministic algorithm, more suitable for clinical routine.

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While informed consent is stated, there is no indication that the study itself had been approved by the responsible ethical committee or research review board.

We inserted the indication about the ethical approval in the proper section of the Methods.

In several points, the abstract is vague or imprecise where a bit more clarity would serve.

We changed the misleading and awkward terms in the Abstract.

The "Targets" paragraph needs some work to clarify the process.

We organized better the "Target" paragraph, following the logic process of the Target ROIs generation.

The method is reasonably described, though the sub-section on tracts of interest was not sufficiently clear for me to understand how the number of centroids generated was decided.

We clarified this aspect in the Method section, in the Target Generation paragraph.

The results section needs fairly extensive rewriting. In particular, there are numerous comments that speculate as to the reason behind individual observations (e.g. "probably as a result of the tumor mass", and several other sentences involving "probably"). All of these comments should be eliminated, and such speculation restricted to the discussion (e.g. This could indicate that the underlying axonal structures have remained intact but spatially displaced) with relevant literature cited to support the claims or suggestions. When talking about the various pathways found, it might be useful to indicate where the lesion was and where the observed tract end point centroid(s) were.

We avoided all the confusing sentences, we re-organized all the section and we moved the comments to the discussion.

The discussion also needs considerable revision, firstly to provide a more readily followed line of thought, and more to reflect on its place in context of existing literature. As part of the connection to the literature, it would be good to reflect evidence from elsewhere that disruption is particularly consistent with tumor cell infiltration and not simply edematous changes. There is a large literature on individual diffusion parameters in brain tumors, some relation with how the changes reported in those studies relate to tractography could be attempted.

We almost completely re-wrote the Discussion section. We commented our findings in contest of the literature and we discuss now more the methodological issues.

There are a large number of linguistic peculiarities and greater care is needed in providing a consistent and complete message.

We corrected all the linguistic inaccuracies through the manuscript.

REV 2

The authors use HARDI and PASMRI. Even if there is nothing wrong with that, it may sound as an overkill for patient data. The choice becomes clear later in the manuscript and in the discussion, but it could be clarified and justified here as well. Maybe the authors could briefly mention that despite other techniques being available for resolving crossings using low angular resolution data (e.g. (Peled et al, MRI 2006), (Tournier et al, NeuroImage 2007), (Sotiropoulos et al, JMRI 2008), etc) the particular choice ensures higher sensitivity to fibre crossings.

We clarified the choice of a multifibre approach in the Introduction section, and better in the Discussion as well, underlining the importance of fully capturing the complex axonal configurations.

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Probabilistic Tractography: Please provide references (e.g. Behrens et al MRM 2003, Parker et al JMRI 2003).

We inserted the right references about probabilistic tractography in the Introduction.

The choice of the imaging protocol is suboptimal. Voxels are anisotropic (suboptimal for tractography, as signal will be lower and therefore uncertainty will be higher along the smaller voxel dimensions). Also, multi-shot acquisitions are more susceptible to subject motion, which is clearly an issue for patients. The authors need to justify these choices and/or at least discuss them as limitations. Finally, the reported resolution is probably interpolated. What is the native resolution of the data? (e.g. 120mm/70 slices does not give 1.5mm that the authors report as slice thickness)

We corrected the wrong data about the DWI sequence applied (FOV) and we discussed the imaging protocol used in the Limitations of the study section.

Using five packages to do deterministic tractography is clearly unnecessary. Nothing wrong about that, but you make your life more difficult. Most of the functions you have performed using AFNI/SPM for instance can be done using FSL or SPM alone.

We discussed this issue in the Limitations of the study section.

References 9 and 35 are repeated, they are the same.

We deleted the repeated reference.

Enhanced / Unenhanced: You mean Gadolinium-enhanced T1? Please clarify.

We clarified it now in the text.

Defining tracts of interest. I feel this is an awkward way of defining tracts. Tractography can estimate paths everywhere, so what do the estimated paths in terms of anatomy and their relationship to the tumour area (particularly the ones identified between two targets)? Could you give us some insight behind this choice as opposed to focusing e.g. on specific tracts defined via strictly anatomical criteria?

We explained better in the manuscript our choice of not using seeds based on anatomical or functional landmarks for tracking (Introduction and in the Discussion sections)

Figure 5: For the Meningioma case the shown example does not clearly support the main conclusion (i.e. that tracts are displaced by the tumour). Could you please provide a probabilistic tractography result for cases M3 or M4, rather than M2? That would make your point stronger and clearer.

We provided the connection probability map for case M3, instead of M2.

REV 3

The description of the methodology is complex and it should be less redundant. The authors should try to reduce it in length.

We re-organized the Methods sections, clarifying all the logical thread to the definition of the tracts of interest. Unfortunately we were not able to shorten it significantly.

The clinical research question is broad and not very well identified.

We clarified better the main aim of our study, explaining the insight behind our data-driven approach in the Introduction, in the Discussion and in the Limitation sections.

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The authors made a big effort to use a novel approach with "user-independent" semi-automatic definition of seed and target ROIs, however their only minimal supervised method is going to leave out (or miss) several white matter tracts within and around the mass that may have an important role in brain function.

The proposed method aims at reconstructing all bundles and trajectories of tumor-involved WM tracts in relation to the contralateral healthy myeloarchitecture without any a-priori anatomical knowledge. We are aware that our approach isn't supervised by an atlas. Nevertheless, we visually inspected every reconstructed trajectory, from the seed tracking to the generation of the tracts of interest. Moreover, we decided to run a complementary probabilistic analysis to confirm the results and modeling their uncertainty. Finally, we used a multi-fibre tracking algorithm in order to overcome the underestimation of the extent of estimated tracts.

The authors stop short from identifying the name of the major bundles that are infiltrated/damaged by the lesion.

We now identify the name of the WM fiber tracts, resulting from the tracking analysis.

The choice to use the PAS functions for multi-fiber reconstruction was a good one, because this method is less sensitive than classic DTI to missing white matter tracts in areas of crossing, branching or fanning. However this method has its own limitations that the authors should acknowledge in the appropriate section of the discussion.

We now discuss advantages and limitations of the adopted reconstruction algorithm in Discussion and Limitations.

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The selection of the targets appear to be partially bias. In areas of T2WI signal abnormality diffusion imaging may miss existing fibers. There are asymmetric tracts in the human brain (i.e. arcuate fasciculus). How do the authors controlled for these two issues? They should address this issue in the M&M section.

We explained better the target generation process, clarifying how we managed the estimation of asymmetric tracts in the Methods section. In addition we commented the possibility of a loss of reconstructed WM bundles in the Discussion and Limitations sections.

A major limitation of this study was the lack of a reference index such as intraoperative electrostimulation subcortical mapping.

Unfortunately we did not have extensive subcortical data. In some cases the surgeon stimulated the tissue under the lesion but only when there was the doubt of an excessive proximity to functionally relevant tracts. We engaged this issue in the Limitations section, explaining the choice of a completely data-driven tractography approach.

LGG are known to infiltrate white matter tracts while HGG tend to dislocate them and eventually destroy them. In the discussion the authors should make a comment about what they found and what it is reported in the literature. The Discussion is weak and very brief. The authors should put their finding in contest of the literature. They should describe in greater detail potential clinical implications of their findings on planning surgery in patients harboring neoplasms infiltrating eloquent areas.

We re-wrote the Discussion, commenting the obtained alteration patterns in contest of the literature, for the three types of brain tumors evaluated. In addition we better clarified the importance of the suggested method in the clinical environment in the Conclusion section.

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In the Limitation paragraph the authors should acknowledge limitation of tracking with PAS in areas of T2-weighted signal abnormalities. They should also acknowledge possible bias of their methods in ROI target selection. Main WM tracts coursing in the proximity or within the tumor may be missed with the approach used in this study.

We discussed these issues in the Limitations sections.

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