<u>Reviewer Comments 1</u>

This paper describes a method for removing 2H signal from extracranial lipids without affecting the brain lactate 2H signal based on 1H MRI-based spatial prior knowledge of brain and skull regions. Magnetic field heterogeneity was accounted for by using surrogate B1 and B0 maps based on the 2H water shift and signal intensity respectively in each pixel across a 2D slice and by dividing the skull region into smaller compartments. Although the lipid signal from subcutaneous lipid is very low this is enhanced by proximity to the receiver elements of the coil and the benefits of removing this signal are illustrated by the images shown in figure 3 D & E. The method gave a 91% suppression of the skull lipid signal in the human brain images while retaining essentially 100% of the brain glucose and glutamate/glutamine signals albeit with greater standard deviations when compared to simulations.

The paper is well written and the presentation of the approach very clear. Not clear to me that this will be widely adopted since the correction is small and only necessary for regions close to the skull (see figure 3 C). Nevertheless, a useful method for 2H MRSI of the human brain.

<u>Author rebuttal 1</u>

The thank reviewer 1 for the positive review of the manuscript. We agree that this is only a small correction that appears to be superficial. However, processing of DMI data on >30 patients repeatedly revealed 'hot spots' in Lac/Glx maps in locations where one would not expect them. This is especially problematic when the lipid-related hotspots are immediately adjacent to a tumor-lactate-related hotspot (see also rebuttal to comments from reviewers 2 and 3). While manual adjustment and/or removal of the lipid signal is possible in some cases, it is a time-consuming and labor-intensive process that cannot readily be integrated in an automated processing pipeline needed in a clinical setting with increased patient flow. The outline algorithm allows for objective and automated lipid removal with minimal to no user input, making it an ideal candidate to be integrated into a robust DMI processing workflow.

<u>Reviewer Comments 2</u>

General comments

The authors present a clever and practical application of the SLIM method for lipid suppression in DMI scans. The problem is well-motivated as the described artifact potentially impacts all DMI scans of the brain, and the proposed solution is well-justified. This work has immediate potential impact that is easily accessible to the DMI research community given its open-source implementation. The results on the *in vivo* dataset are qualitatively impressive and highlight the promise of this technique. Simulations assessing the impact of B0 and B1 inhomogeneities are thorough and accompanied by a well-reasoned discussion. Some additional simulation results assessing SLIM's ability to disambiguate lipids from Lac in superficial lesions could give further confidence that the proposed technique is robust across a variety of DMI scenarios.

<u>Author rebuttal 2</u>

We thank reviewer 2 for their constructive review of the manuscript. The suggestions have led to additional simulations and figures and have improved the overall manuscript. Detailed answers to the raised critique are given below each individual comment in italic.

Specific comments

1. Pages 5-6: The author describes the challenge of non-homogenous ROIs, and several techniques (magnetic field heterogeneity correction; smaller regions) to address this issue. However, subsequent experiments assign the entire non-skull brain a single ROI, despite the expected heterogeneity in the true metabolic DMI signal across brain tissue. This seems to violate a major assumption made by SLIM of compartment homogeneity, and is a deviation from previously cited works using SLIM (e.g. Dong & Hwang 2006 where fat is also subdivided into micro-compartments, but muscle is sub-divided into medium-sized compartments). While simulation results seem to indicate that this choice does not hinder algorithm performance, I think the paper may benefit from further explanation as this result is not necessarily intuitive given the provided explanation of SLIM and its assumptions. In particular, even though the skull-only compartments are subtracted leaving the brain spectra untouched, would the violation of the homogenous compartment assumption in the brain have any non-local effects on the skull quantification due to its spatial response function?

1. This comment is closely related to comment 5. We have performed additional simulations to demonstrate that brain subdivision has a minimal effect on metabolite retention. In addition to the text added for comment 5, we have added the following text for this comment:

It should be noted that the brain ROI was not divided into smaller, tissue-specific ROIs (GM, WM, CSF, pathology) in any of the simulations. Preliminary simulations have shown that metabolite retention in separate tissue-specific ROIs is only marginally different if the brain is considered as one compartment (N_{brain} = 1, 98.9 ± 3.7 % across all tissue ROIs) or as four compartments (N_{brain} = 4, 99.7 ± 1.5%). ***An intuitive explanation for this observation is that even though signal leakage between brain compartments does occur, the final step in the algorithm is the reconstruction and subtraction of a skull-only DMI dataset, leaving the brain signals largely unperturbed. It should also be noted that compartmental differences found in vivo are much smaller than those used in simulations (i.e., either signal or no signal) leading to reduced leakage.*** *As the focus of the current work is on lipid removal, the decision was made to consider the brain as one compartment.*

2. Page 7, Line 6: Are scenarios considered where the pathological ROI position results in partial-voluming with skull lipids in the DMI dataset? This would seem to be the most challenging, as well as most clinically useful, application of SLIM, as it could directly disambiguate lipids from Lac and therefore impact clinical assessment of the Lac fraction of a lesion. Such an experiment would further give confidence in using SLIM in scenarios where a superficial lesion is present and partial-voluming with the skull is strongly suspected. For this reason, I think this scenario may be deserving of special consideration within the simulation analysis.

2. We completely agree with the reviewer that good performance of the algorithm in the presence of partial voluming is clinically one of the most useful features. This was insufficiently demonstrated and discussed in the first submission. We have now included new simulations (Fig. 3) to address this aspect. As a result of including a new Fig. 3, the old Fig. 3 now becomes Fig. 4.

The following text has been added:

The results in Fig. 2 were primarily focused on lipid/skull suppression and metabolite retention in voxel locations with minimal partial voluming. However, one of the most important clinical applications of the proposed algorithm is Lac retention in pathologies immediately adjacent to the skull where partial voluming can be significant. Fig. 3 summarizes Lac retention in the presence of partial voluming by using skull and brain-specific reference signals to monitor the amount of partial volume and the quality of lipid suppression. The amount of partial voluming was adjusted by spatially shifting the ROI compartments relative to the fixed MRSI grid. Figs. 3A and B show the ROI constellations to achieve ~20% (Fig. 3A) and ~55% (Fig. 3B) skull contribution to the indicated MRSI voxel. Figs. 3C and D show MR spectra at the indicated MRSI voxel location extracted from a 2D MRSI dataset obtained with standard or SLIM processing. With standard, FFT-based processing (red line) the spectra contain four signals corresponding to a skull reference signal, a brain/pathology reference signal (e.g., Glx) and a combined signal from Lac and lipids. As the reference signals have equal amplitudes per unit volume, the intensity of the skull and brain reference signals is indicative of the partial volume effect. After employing the SLIM algorithm with $N_{skull} = 1$ (blue line), the skull reference and lipid signals are largely removed. However, in agreement with Fig. 2, the removal is incomplete in the presence of B_0 and B_1 heterogeneity, leading to an overestimation of the lipid signal and thus an underestimation of the Lac signal. Fig. 3E shows the Lac-to-brain reference ratio as a function of the partial volume. With standard processing (red line), the ratio quickly increases as the large lipid contribution is included in the estimated Lac. With SLIM processing ($N_{skull} = 1$, blue line) the ratio stays close to one for small partial volumes but decreases as the lipid contribution is overestimated. When the SLIM algorithm is executed with skull subdivision ($N_{skull} = 23$, green line in Figs. 3C-F) the lipid removal becomes much less sensitive to B_0 and B_1 heterogeneity, leading to near-complete lipid removal and a correct Lac retention. The Lac-to-brain reference ratio stays close to one for all partial volumes (Fig. 3F). Note that a partial volume of 90% only contains 10% brain/pathology for which the ^2H sensitivity would typically be too low to provide useful data.

3. Page 7, Line 20: How are DMI datasets generated from the given simulated brain ROIs? In particular, what DMI metabolite values are assigned to each tissue type? And what noise level(s) are chosen?

3. The basic SLIM algorithm operates in the spatial (k-space) domain and generates ROI-dependent weights that are used as multiplication factors on the raw MRSI data to provide ROI-specific signals. The actual spectral content does not play a role, such that the spectral signals (frequencies, amplitudes) can be chosen freely. The frequencies were chosen as to avoid spectral overlap of signals, thus enabling straightforward signal quantification by spectral integration.

However, in the presence of magnetic field heterogeneity and signal leakage several aspects of the spectral domain become important. In the presence of B_0 heterogeneity, the spectral line widths become important, as narrower lines are more sensitive to frequency shifts than broader lines. For the current study, the

linewidths were chosen to approximate those observed in DMI datasets in vivo. This corresponds to T_2 relaxation times of 30 ms. As heterogeneity leads to imperfect lipid subtraction, the lipid intensity compared to the metabolite intensities needs to be chosen realistically. Unless specified otherwise, lipids and metabolites were used with equal amplitudes.

The Methods section already contained a description on the well-resolved signals and integration. We have added the following text on the linewidths and amplitudes:

The linewidths were chosen based on previously reported in vivo values ((De Feyter et al., 2018), T_2 = 30 ms). Unless specified otherwise, the amplitude per unit volume was identical for all signals (RC2.3).

4. Page 8, line 7: How are the anatomical MRIs spatially registered to the DMI datasets? Are the MR scans taken with the DMI coil in-place, or is the patient re-positioned between scans? I would anticipate that SLIM may be sensitive to registration errors, so I think this is worth mentioning.

4. For the current study there was no need to spatially register the MRI and DMI datasets as both were acquired in the same scan session with the RF coil assembly in place. Simulations (data not shown) revealed that, as expected, the reconstruction is very sensitive to a positional mismatch. A mismatch corresponding to 10-20% (2-4 mm for DMI) of the MRSI voxel dimensions is still acceptable, but the lipid suppression quickly deteriorates beyond that range. We have now added text to highlight this sensitivity and provide a recommendation:

In the current study the anatomical MRI and DMI datasets were acquired with the same RF coil assembly without subject movement. As a result, no spatial co-registration between MRI and DMI was necessary, and the MRI-derived spatial prior knowledge was accurate. However, in the presence of significant subject movement, or when using MRIs acquired from a separate scan session (e.g., clinical MRIs), a mismatch between MRI-derived spatial prior knowledge and DMI can lead to greatly diminished lipid suppression performance and even a distorted metabolite profile. Simulations (data not shown) have indicated that a mismatch of 10-20% of the nominal DMI voxel size can still retain a high level of lipid suppression, but that performance quickly degrades with larger mismatches. It is therefore recommended to acquire MRIs and DMI during the same session with an identical subject position. Spatial co-registration between MRI and DMI is possible but was not further pursued in this study. When subject movement during MRI/DMI scanning is anticipated, additional measures (e.g., motion tracking, additional immobilization) need to be taken to ensure accurate spatial prior knowledge.

5. Page 8, Line 16: The author states that "*the brain ROI was not divided into tissue-specific compartments (e.g. GM, WM, CSF) as simulations (see Results) demonstrated that this step did not affect lipid suppression and metabolite retention*"However, I do not see this experiment in the results section. While the Appendix shows *metabolite retention* for each brain tissue type, I do not see the impact of sub-dividing the brain into distinct compartments on algorithm performance. I think this result would help shed light on Point 1.

5. We thank the reviewer for catching this oversight. As indicated, the simulation outcome was planned to be reported in the Results section but was accidently omitted. We have now added the following text to the Results section (in addition to the text added for Comment 1):

It should be noted that the brain ROI was not divided into smaller, tissue-specific ROIs (GM, WM, CSF, pathology) in any of the simulations. Preliminary simulations have shown that metabolite retention in separate tissue-specific ROIs is only marginally different if the brain is considered as one compartment (N_{brain} = 1, 98.9 ± 3.7 % across all tissue ROIs) or as four compartments (N_{brain} = 4, 99.7 ± 1.5%). An intuitive

explanation for this observation is that even though signal leakage between brain compartments does occur, the final step in the algorithm is the reconstruction and subtraction of a skull-only DMI dataset, leaving the brain signals largely unperturbed. It should also be noted that compartmental differences found in vivo are much smaller than those used in simulations (i.e., either signal or no signal) leading to reduced leakage. As the focus of the current work is on lipid removal, the decision was made to consider the brain as one compartment.

6. Page 8, Line 27: The author states that *"only pure brain pixels are considered for metabolite retention".* In the case of partial-voluming of lipids with a glycolytic lesion, however, it would be interesting to see metabolite retention in this scenario as a special case (see Point 2).

6. The additional simulations described under Point 2 have demonstrated that the metabolite retention remains high even in the presence of large partial voluming. The additional figure (Fig. 3) on partial voluming addresses both points 2 and 6.

7. Page 12, Line 8: Signal retention for Glc and Glx are shown, but signal retention for Lac would also be of interest (see Point 2, 6).

7. We agree that signal retention for Lac would be of interest – perhaps even of the highest interest. However, the DMI dataset shown in Fig. 4 has only two voxels with visual Lac of which one voxel has a small amount of partial voluming with the skull. Even if the Lac signal retention is 100%, it would appear to be lower due to removal of the overlapping lipid. We have added the following text:

Lac retention cannot be quantitatively determined due to spectral overlap with lipid signals. However, the outlined simulation results on high metabolite retentions together with a close visual agreement of Lac in Fig. 4C provide confidence that the Lac signal is preserved by the lipid removal algorithm.

Technical correction

1. Page 4, Line 12: First use of the acronym ROI, spell out "region of interest".

2. Page 4, Line 20: "a N_{enc} xN_{ROI} encoding matrix" should be "an N_{enc} xN_{ROI} encoding matrix".

3. Page 8, Line 28: "…partial skull pixels are also considered even though can lead to an underestimation..." should be "…partial skull pixels are also considered even though they can lead to an underestimation..."

Corrections 1 – 3 have all been incorporated into the revised manuscript.

Reviewers Comments 3

This is a very nice manuscript describing the use of a SVD-based isolation of lipid signals in deuterium metabolic imaging. The technique is potentially important, since there could be significant signal contamination in the head, especially in the vicinity of lipids, which would lead to incorrect metabolic profiling. While I do find the topic and the manuscript interesting, I feel that the authors are not up-front with providing the reviewer with the central result, or the central issue, namely the partial volume effects. Although the strategy taken with the use of prior spatial knowledge seems to work well, as demonstrated, the authors do not provide a simple statement about the partial volume effects, which would summarize the work and its value succinctly. One would normally expect such a statement in the Conclusion and perhaps also in the abstract, and I strongly suggest that this should be done as a service to the reader.

Author rebuttal 3

The reviewer is thanked for this suggestion, which was also raised by reviewer 2. The SLIM algorithm removes signal from ROI-defined spatial (skull) positions and in our minds partial volume effects were never a separate issue – lipid removal should work in the absence or presence of partial volumes. However, we do appreciate the suggestion and do agree that an additional statement on partial volume effects will add a higher level of clarity. In addition to new simulations (Fig. 3), we have now also added additional text to the Discussion section that reads:

While lipid removal is desirable for all spatial positions to eliminate 'hot spots' in the resulting lactate map (see Fig. 4), it is especially prudent for skull areas immediately adjacent to the pathology (e.g., brain tumor) where partial volume effects would lead to a distorted lactate signal. The SLIM algorithm is based on spatial prior knowledge from high-resolution MRIs and is intrinsically suitable to separate skull from brain signals even in the presence of partial volume effects caused by the lower DMI resolution. This feature was systematically investigated with simulations (Fig. 3) and experimentally demonstrated (Fig. 4C) and confirmed constant lipid suppression performance in the absence or presence of partial volume effects.