Author's response to reviews

**Title:** Towards real-time metabolic profiling of biopsy specimen during a surgical operation by 1H HRMAS-NMR: a case report

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**Author's response to reviews:** see over
Dear Editor,

Please find the revised version of our manuscript entitled:

**Towards real-time metabolic profiling of biopsy specimen during a surgical operation by $^1$H HRMAS-NMR: a case report**

We are very grateful to the two reviewers for their positive response to the manuscript.

In the following, we answer their questions and present the modifications we have made to the original manuscript. These modifications are highlighted in red in the text.

Reviewer 1:

1) The acronym HRMAS-NMR has been spelled out.

2) The operation is ‘simulated’ in the sense that the metabolic analysis was performed on an already excised colon. The manuscript has been modified.

3) A mirror sample is classically a biopsy specimen taken very close to the original biopsy. This information has been added to the text.

4) Details regarding the PLS-DA analysis have been provided.

5) Clearly, the new biopsy specimen are not part of the statistical model.

6) The Y values of the predicted samples have been added to the manuscript.

We include to this letter a SIMCA graph showing the distance to model (DmodX) results for the 9 test biopsies. This graph shows that most of the 9 biopsies fall with an acceptable range
of the model. Only biopsy number 1 appears a bit off the model. However, it appears on the extreme border region of the adenocarcinoma region in the PLS-DA plot which can be explained easily as it is the most infiltrated biopsy specimen.

7) We have added the Lancet citation to our manuscript.

Reviewer 2:

1) The reviewer is absolutely right and we are aware of all these effects. This is the main reason why our data acquisition time is limited to 14 min (to prevent sample degradation). We have replaced the term “intact biopsy” with “unprocessed biopsy”.

2) We have performed a histopathological analysis on the content of the inserts after the metabolic study and we have found the same results as those obtained on the mirror samples. This precision has been added to the text.

3) We agree that the lactate is more likely to be affected by ischaemia effects. In our study, the lactate appears in the statistical analysis as a weak discriminating metabolite. However, following the recommendations of the referee, we have removed the lactate from the list of discriminating metabolites.

4) We have provided the additional experimental conditions required by the referee.

We hope that these explanations and modifications will answer the comments made the referees.

Thanking you,
Yours sincerely,

I.J. Namer