Author's response to reviews

**Title:** Label-free blood serum detection by using surface-enhanced Raman spectroscopy and support vector machine for the preoperative diagnosis of parotid gland tumors

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

On behalf of the authors of this manuscript, I would like to appreciate your evaluable suggestions very much. During the process of revision, we try our best to answer and revise the questions and problems by point-to-point, and we hope that our manuscript is suitable for the publication. It is our honor to introduce our study and research through BMC Cancer.

The followings are the responses to the reviewers and the revisions are all shown in the blue color.

1. Experimental methods

Our expression may be vague and we have deleted the word ‘Significantly’. The patients participated in this study never achieved any treatment before in order to reduce the effects of the drugs and treatments.

And we use the 4th polynomial function and Savitzky-Golay smoothing in the LABSPEC 2.0 to subtract the autofluorescence background and smooth the spectra, which we have added in the department ‘Data analysis’.
2. **Data analysis**

The figure 2 showed the raw spectra which were not normalized, because we wanted to show the subtracted and smoothed spectra first in our study. So the y-axis is not the normalized intensity. And then the fig. 3 showed the normalized spectra.

Dear reviewer, we all appreciated your advice on using a leave-one-patient-out cross validation method, and we had a discussion about it before we reply to you. At last, all the authors come to an agreement that we used both of the leave-one-sample-out and leave-one-patient-out methods in this study to get a comparison. We give these following explanation and we hope you can agree:

Our study is just a preliminary study to research the detection of the serum samples by Raman spectroscopy and SVM, our study just prove the potential to diagnoses the serum samples, so the sample was selected as serum. But the diagnostic method developed by the SERS and SVM would be applied in clinic in the future, so we also should use the leave-one-patient-out method.

In this study, when we used the leave-one-patient-out method, all the spectra from the same patient would be left out to test the prediction performance of the classification model. Though the accuracy, sensitivity and specificity decreased, the results were
similar to the ones carried out by leave-one-sample-out method that the malignant tumor MEC could be easier to diagnose than other tumors. But in this study, we have not enough confidence to give an exact conclusion when we used the leave-one-patient-out method due to the clinical significance not the statistical method. The origin purpose of this study was to detect the serum from the patients and this experiment was just a preliminary study. We needed further studies in order to build up the relation between the patients and serum samples. We thought the only 4-6 serum samples could not represent the patients completely, and we needed more studies to explore the method. Now, we have proved the potential of Raman spectroscopy to detect the serum samples in this study. So we have added the limitation of our study in the 'Discussion' to give readers a discussion.

3. **Comparison of the spectra**

The serum SERS spectra from our study, Li's report and Lin's report have been compared again. We also found some differences existing among these spectra. The reasons are not very clear and we think the differences may be related to the various nano-particles, the different preparation of serum and
equipment parameters. So the comparison of the spectra with those reported in the literatures has been added in the discussion department.

A. Lin et. (Optics Express 2011, 14, 13565).  B. Li et (J Biomed
4. Limits of this work

We appreciate for your suggestions and find out several limits of our study. Due to the limits of sample size and epidemiological characters, the differences of age and gender in some groups could not be reduced easily. It is needed a large sample size experiment to give an explanation. So the above limits have been rewritten in the revised manuscript. And we hope our study gives a start, and more hospitals and laboratories could participate in this research.