Reviewer’s report

Title: Ultrasonic spectrum analysis for in vivo characterization of tumor microstructure changes in the evaluation of tumor response to chemotherapy using diagnostic ultrasound

Version: 2 Date: 10 April 2013

Reviewer: Ronald Kumon

Reviewer’s report:

I. Specific Comments (see Revision lists)

II. Conclusion

In general, the changes made by the authors have improved the manuscript. The authors have made important clarifications, and the limitations of the study are better articulated. Based on these revisions, I have suggested some additional revisions prior to publication of this study to better place in context and better differentiate it from previous work.

Major Compulsory Revisions

1. Background/Discussion: The authors have added the references suggested in the previous review, but the placement of the references has downplayed the previous work in a way that is potentially misleading. The authors state that “to the best of our knowledge, there is no article presenting the value of ultrasonic spectral analysis in the evaluation of tumor chemotherapy in vivo using diagnostic ultrasound.” This is simply not correct, given the study by Sadeghi-Naini et al. which employs an ultrasound system by the same manufacturer at nearly the same diagnostic frequency as the current study and is deployed with in vivo chemotherapeutic treatment of human patients. The authors need to recognize this study in the Introduction, and then clearly explain what is different and novel about their study. Perhaps the sentence that was added in the 4th paragraph of the Discussion can be moved to the Introduction, and the authors can provide motivation for their own study thereafter.

2. Results: The addition of Figure 5 is helpful to understand the results. It suggests that while slope does a better job than midband fit of distinguishing the
control from the treatment cases, neither parameter provides perfect ability to separate the two cases. The authors should try plotting slope vs. midband fit with the points color coded by the control and treatment cases. It would then be possible to perform a straightforward linear discriminant analysis with SPSS and compute the sensitivity, specificity, and accuracy of the resulting analysis for classification purposes (e.g., using a “leave one out” approach). This analysis would potentially add to the significance of the paper by providing some quantitatively-assessed predictive ultrasound criteria and would also help to further distinguish it from previous studies.

Minor Essential Revisions

1. Figure 2 is a helpful addition. Can you add a scale bar or square of reasonable size (e.g., 10 x 10 pixels or whatever looks appropriate) on Figure B to give the reader a visual sense of the area threshold used for the counting of the nuclei?

Discretionary Revisions

1. With the additional image analysis provided in the 2nd paragraph of the Results, it may be worth pointing out in the Discussion that the current study suggests that, in a “carefully performed study with consistent imaging conditions”, grayscale alone might provide an indicator for chemotherapeutic response. While the spectrum analysis method is better controlled through the use of calibration, it also requires access to the RF data, which is not available on most clinical systems. In contrast, a grayscale threshold would be straightforward to implement on many existing systems through post-processing of the image data.

Minor Issues Not for Publication

1. Background, Paragraph 1, line 1: Change to “Tumor malignancy is”.
2. Methods, Paragraph 1, line 3: Change to “care of laboratory animals”.
3. Methods, Paragraph 1, line 3: Change to “24 5- to 6-week old BALB/c nude female mice.”
4. Methods, Paragraph 7: Change “40x powers” to “40x power” (similar for “400x” elsewhere in the paragraph).
5. Discussion, Final Paragraph, line 4: Change to “the largest cross-sectional planes”.
6. Discussion, Final Paragraph, line 8: Change to “below the skin surface, attenuation compensation will not likely lead to significant changes in the results for a 6 MHz transducer.”

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.