Reviewer's report

Title: Infrared Micro-Spectral Imaging: Automatic Distinction of Cell Types in Axillary Lymph Node Histology

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Reviewer: Richard Mendelsohn

Reviewer's report:

The MS by Bird et al addresses an important issue in a form that should be accessible to a wide variety of target audiences. At the outset, I congratulate the authors for a well presented, important piece of work. There are some minor questions which the authors might like to consider prior to publication.

The MS deals with the feasibility of a technology, termed “spectral pathology” using infrared microscopic imaging to acquire many thousands of IR spectra at microscopic resolution, followed by multivariate statistical methods (cluster and neural net analysis) to provide a diagnosis. This is clearly an issue of importance in biomedical diagnostics, and the groups presenting this MS are leaders in the application of vibrational spectroscopy in this area.

The methods described above are appropriate. Some form of multivariate statistics are certainly required to analyze the thousands of IR spectra from each image.

The authors chose not to show any primary data, but to do so would be to duplicate what they and other laboratories already have published, and the availability of high quality primary IR spectral data is not an issue here. The quoted S/N ratios (>200) are more than adequate for the processing methods (second derivatives) that are utilized.

I have three questions of minor significance about the multivariate statistics and sample preparations.

1) The HCA method is stated (page 9 lines 8,9) to be completely unsupervised. Perhaps the algorithm is. But the question of how many clusters to select perhaps is more subjective. A comment about why 5 clusters were selected in the current instance might be helpful. Would the same number be chosen without a stained section or an a priori
histopathology classification?

2) I assume the information from the neural net analysis cannot exceed the information in the training set. So what are the consequences of a spectral pattern not in the training set? I assume a “can’t tell” result would be forthcoming (but I am not sure of this) or would a misclassification result and a better training set be required?

3) Is it known whether differing treatments of the section affect the analytical results. That is, which form (de-paraffinized section, vs frozen section) of preparation gives better measurements. Is the de-paraffinization pretty certain to remove all the paraffin. Otherwise the presence of methylene modes might confound part of the analysis?

One final trivial point. My count of the “red” pixels in Figure 2d is 9 rather the 8 stated in the text (page 17). Only 2 of these are removed from edges of unrecognized tissue. I am extremely impressed that only 2 spectra of 11,000 have the potential for misclassification. The authors might want to look at the spectra from these 2 sections to see if there is something “unusual “ about them (e.g. weak intensities, distorted background, etc.).

Overall, this MS is clearly a strong contribution to the literature and following the Journal’s code, I suggest that my comments fall under the heading of discretionary revisions, which the authors could ignore should they choose to do so.

Evaluation: Excellent piece of work, integrating and extending prior studies from the labs involved.
Recommendation: Accept for publication - discretionary revisions