Reviewer's report

Title: Quantitative metric profiles capture three-dimensional temporospatial architecture to discriminate cellular functional states.

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Reviewer: Stephen Lockett

Reviewer's report:

Previously the authors developed a graph theoretical-based method for capturing structural characteristics of histopathological images in order to distinguish healthy, cancerous and damaged tissues. The manuscript describes an extension of this method to analyze structural characteristics of cancer cell lines grown in 3D collagen-based hydrogels. Results show that discriminatory metrics for 3D hydrogels were generally the same as those for 2D histology data, but living 3D hydrogels have the advantage that changes in the metrics could be followed over time.

Major Compulsory Revisions

1) It is not possible to understand the abstract, because it does not explain the rationale for performing structural analysis in 3D hydrogels and it uses terminology (for example, “our cell graph method”) that would only mean something to people who have read previous papers by the same authors.

2) The main body of the manuscript needs to provide a strong rationale for analyzing 3D hydrogels. Clearly, 3D hydrogel analysis will not provide information that pathologist can access. On the other hand such analysis could benefit understanding of cancer models provided the metrics can be interpreted in terms of underlying molecular mechanisms of cancer progression.

3) A key experiment is lacking from the manuscript; that is the 3D analysis of thick histological tissue samples fluorescence labeled for nuclei and imaged using confocal microscopy. It appears from the literature cited that the authors may already have these data.

4) The validation in the results sections is incomplete. The validation that is provided shows agreement between 2D analysis of histological images and 3D analysis of hydrogels, but the metrics should be validated by showing their discriminatory power over a panel of cancerous and non-cancerous tissues when performing 2D analysis.

5) While it is plausible that their data is capturing features of EMT, an experiment specifically targeting this hypothesis really needs to be included.

6) While the temporal experiments reported in figure 5 are substantial, the experiment for each cell line needs to be repeated 3 to 5 times for validation.

Minor Essential Revisions
7) The first paragraph of the results section is methods.

8) Figure 4 very nicely illustrates the visual differences between images for the discriminating metrics. However, this contradicts the claim in the text that the computational approach is detecting differences that are indistinguishable by eye. A figure should be included, which illustrates data sets that are indistinguishable by eye but are discriminated by their computational approach.

9) The section of text on page 12 starting with “We elected to use graph theory-based methods …” should be in the background section.

10) Table 3: Further explanation of the metrics is needed.

Discretionary Revisions

11) No need to go into details about Otsu’s method.

12) Table 2: DU145 is missing. Could merge tables 1 and 2 to save space.

13) Figure 3: Please state what “A”, “B” and “C” mean.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests