Using AI Based Nuclear Segmentation to Infer Toxicologic Pathology Outcomes such as Liver Hypertrophy from Cell Density Maps

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Background

⇒ Changes in cellular architecture induced during drug safety assessment can be difficult to see at whole slide level, requiring pathologists to scan over the whole tissue at high magnification, a time consuming process. As such, the reliable identification of liver hypertrophy within toxicologic pathology workflows, informed by subtle differences in individual hepatocyte size and extent, is challenging.

⇒ Application of digital pathology tools, such as AI-enabled nuclear segmentation in combination with cellular density maps, can help with the reliable identification of such lesions, enabling pathologists to identify hypertrophic regions at low magnification with speed and accuracy, in an unsupervised fashion using a pretrained model which requires no additional annotations for the studies under investigation.

Objectives

⇒ A methodology for the visualization of changes in cellular density within tissues is presented, which can provide a pathologist reviewer with a heatmap visualizable at low magnification that captures changes happening at the cellular level (Fig. 2), which would normally require higher magnification views to observe directly.

⇒ Use cases for nuclear segmentation and density view are investigated to find regions of centrilobular hypertrophy within liver sections (Fig. 3).

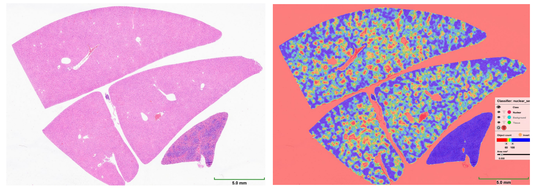

Fig.2 Interactive cell density view. Cell density map view (right) provides more cellular-level context, i.e. low density areas in red, comparing to tissue view (left) at whole slide view.

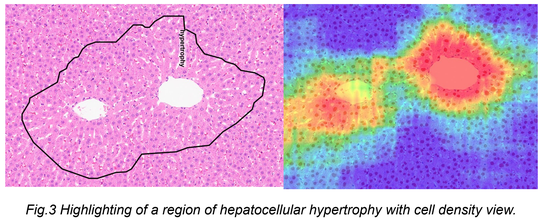

Fig.3 Highlighting of a region of hepatocellular hypertrophy with cell density view.

Methods

⇒ **Data:** Whole slide H&E images containing representative lesions of interest were scanned and made available within the Patholytix Study Browser. 5 distinct studies containing hypertrophic lesions have been investigated here, with the lesions of interest annotated by pathologists.

⇒ **Nuclear segmentation**: StarDist [1,2], a published convolutional neural network trained to segment individual nuclei was applied to the images. Patholytix Polymask technology automatically calculated features (including area, shape, color, and x and y coordinate) of each nucleus at the time of detection.

⇒ **Hepatocyte filtering:** A range of features that distinguished hepatocytes from other liver-resident cells was identified. Filtering nuclei with these features allows us to calculate density of hepatocytes specifically.

⇒ **Cell density mapping**: A "Cell Density" overlay is introduced to show changes in cellular density across the tissue which can be customized via density settings to highlight regions with differences in cellular counts within a particular region of interest. The area over which cell density was measured here was standardized to 0.05mm^2.

Fig.1 Workflow for detecting high or low cell density areas in liver using cell density map approach proposed in this work.

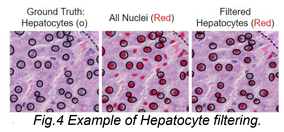

Fig.4 Example of Hepatocyte filtering.

Results

⇒ The performance of the nuclear segmentation algorithm is compared against annotations performed on selected regions of interest within the liver images, showing predicted hepatocyte nuclei match well against pathologist annotations. After quantitative features filtering, Hepatocyte nuclei are successfully isolated from other liver-resident cells. Hepatocyte filtering helps to exclude cells that have no impact on hypertrophy (Fig. 4-6).

⇒ Density map allows a good indication of the low cell density areas (Fig. 3, 10 -12). Cell density calculated with predicted hepatocytes nuclear per area indicates clear separations between normal and hypertrophy regions for two studies under investigation (Fig.6-7). A good correlation between the reduction in cell density with increasing severity of hypertrophy is observed (Fig.8), as is also observed in representative screenshots (Fig. 10).

⇒ Qualification of the predicted low density regions in 5 studies shows that low density findings scored by manual observation of the density map with a fixed window of density threshold of 80-100 cells counts per area appears to separate control from hypertrophy tissue well (Fig.9). For slides annotated by pathologists as containing minimal to moderate hypertrophy, 16 of 20 exhibited low density (Fig.9) An example high and low resolution view showing hepatocyte filtering and cell density is shown in Fig. 11, demonstrating a clear difference from control slides.

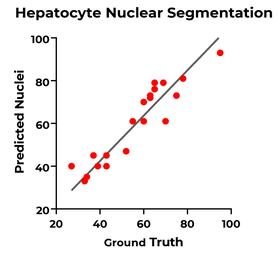

Fig.5 Predicted Hepatocyte nuclei after feature filtering against ground truth

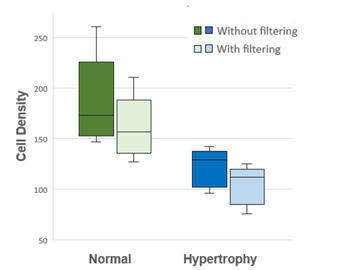

Fig.6 Predicted cell counts before and after hepatocyte filtering for normal and hypertrophy regions.

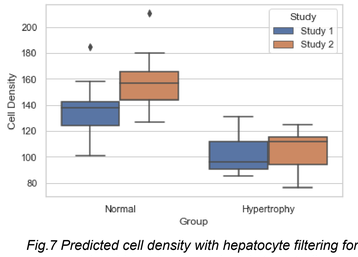

Fig.7 Predicted cell density with hepatocyte filtering for normal and hypertrophy regions in two studies.

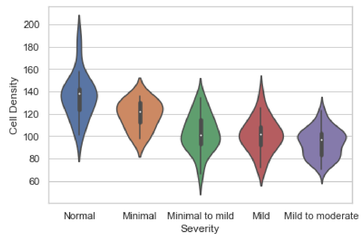

Fig.8 Cell density versus severity of hypertrophy.

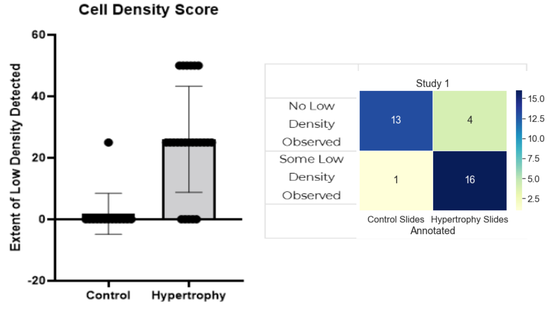

Fig.9 Scoring of Hepatocellular Hypertrophy Study on Whole Slide Images, and Confusion matrix of the cell density approach for detecting slides containing hypertrophy in study 1 with density range of 80-100.

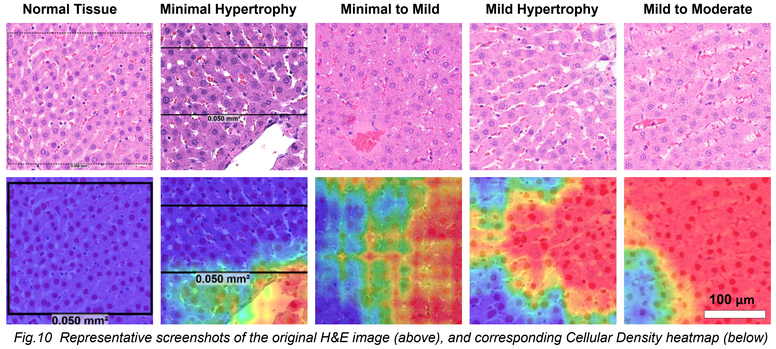

Fig.10 Representative screenshots of the original H&E image (above), and corresponding Cellular Density heatmap (below)

Conclusion

⇒ Nuclear Segmentation with a well-understood AI classifier (StarDist) provides the ability to accurately detect cells; and Quantitative Filtering can localize Hepatocytes within the liver.

⇒ Cell Density View provides an unbiased assessment of potential hypertrophic regions of interest to investigate further from a whole slide perspective.

⇒ This methodology permits the detection of differences in cell density, identifying lesions with variations in density when compared to normal tissue, without a need to manually annotate lesion data and train a supervised lesion detection algorithm.

⇒ By adjusting viewing thresholds for cell density maps, density based lesions can be visualized as a heatmap and used as a decision support tool that could increase the efficiency and consistency of hypertrophy scoring.

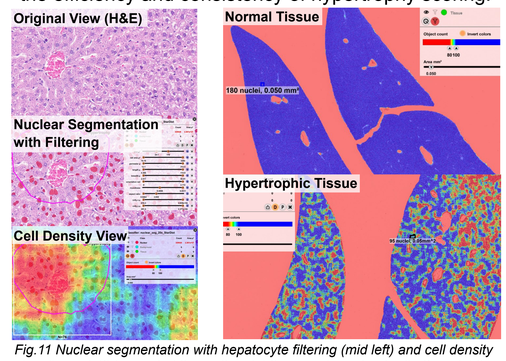

Fig.11 Nuclear segmentation with hepatocyte filtering (mid left) and cell density view (lower left) for control tissue (top right) versus hypertrophy (bottom right)

Future Directions

⇒ This approach could also be utilized to detect other cell density lesions in liver, e.g: Vacuolation, Atrophy, Hydropic degeneration, Karyomegaly and etc.

⇒ Detection of increased cell density in immune infiltrate.

⇒ Applications in other organs, such as decreased cellularity in thymus, lymph nodes and bone marrow.

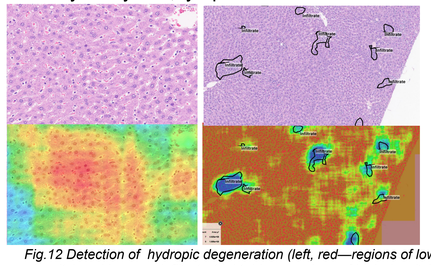

Fig.12 Detection of hydropic degeneration (left, red—regions of low density) and immune infiltrate (right, blue—regions of high density)

Contact Information

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https://deciphex.com https://twitter.com/deciphex

References

[1] M. Weigert et al.: Cell Detection with Star-convex Polygons., in Int. Conf. on Medical Image Computing & Computer Assisted Intervention, Granada Spain (2018) DOI: 10.1007/978-3-030-00934-2_30 .

[2] M. Weigert et al.: Star-convex Polyhedra for 3D Object Detection and Segmentation in Microscopy, in The IEEE Winter Conference on Applications of Computer Vision (WACV), Snowmass Village, Colorado (2020) DOI: 10.1109/WACV45572.2020.9093435

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