

Introduction

- Hepatocellular carcinoma (HCC) is the **fifth most common cause of cancer worldwide as of 2023**.¹
- HCC is a malignant tumor of the hepatocytes.¹
- Common causes of HCC include hepatitis B or C infection, heavy alcohol use, cigarette smoking, and hereditary hemochromatosis.^{1,2}
- Common treatment methods include tumor ablation, liver transplantation, surgical resection, systemic chemotherapy, and transarterial therapies.¹
- Diagnosis and treatment of HCC can be done via imaging studies, such as computed tomography (CT), and via biopsy, after which histopathological analysis can be performed.³
- The histopathological examination requires analysis by a pathologist, making it difficult to communicate findings to more junior individuals and medical and research personnel who aren't pathologists.³
- While metrics such as the Hounsfield unit (HU) used in CT describe the physical density of the tissue, such metrics are not currently used in histopathology.⁴
- Standardized metric must be created to analyze the tumor, surrounding tissue, and the cellular anomalies found in Hematoxylin and Eosin (H&E) histopathology slides.

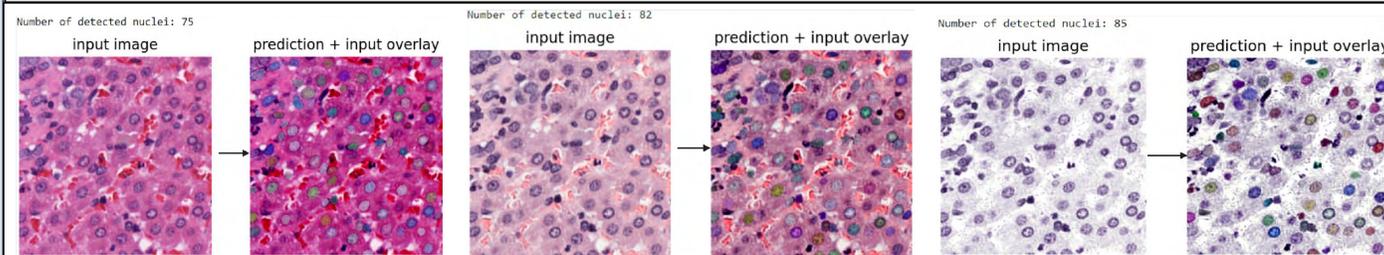
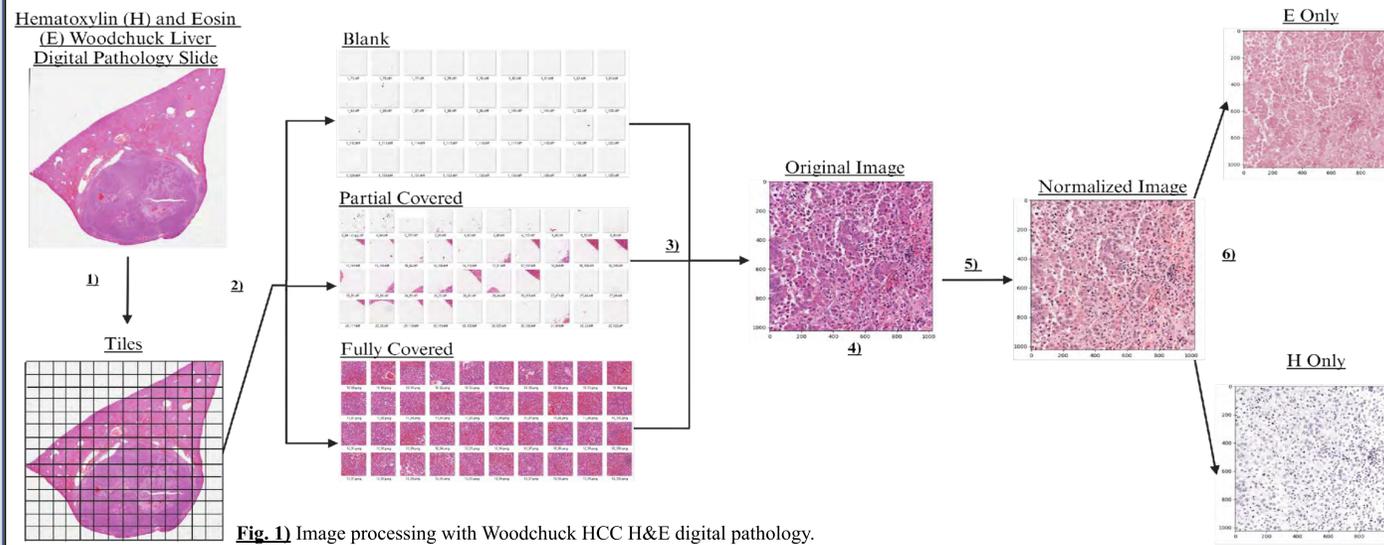
Objectives

- Create and implement image processing and image segmentation for cell count on woodchuck liver H&E digital pathology slides.
- Measure accuracy of the image segmentation model.

Materials and Methods

- Image processing Python v3.12.4 libraries (Fig.1):** Cv2, Skimage, Matplotlib, Numpy, Scipy, Openslide, Pillow
 - Tile, normalize, and digitally separating Hematoxylin and Eosin in H&E
 - Use Openslide, Pillow, numpy, and matplotlib lib to separate slide into tiles
 - Manually sort tiles as blank, partially covered, and fully covered.
 - Take average pixel value (APV) and standard deviation of pixel value (SDPV) of blank, partial, and fully covered tiles, respectively
 - Use APV and SDPV of fully covered tile to estimate the range of APV the tiles will be selected from
 - Use Openslide to normalize H and E pixel values respectively
 - Separate H from E
- Selected 3 representative tiles and 3 readers who counted the cells on each tile. Averaged readers' results to establish baseline.
- Image segmentation Python v3.12.4 libraries (Fig.2):** numpy, matplotlib, CSBDeep, Stardist, Seaborn, Pandas, Skimage
 - Input each of the original tiles, normalized tiles, and h-only tiles of the representative substantially covered tiles (SCT), covered tile with blood vessel (FCT-BV), and fully covered tile (FCT) into Stardist.
 - Use CSBDeep, matplotlib, Skimage, and Seaborn to display segmented image
 - Representative of this process is shown in Fig. 2
 - Compare results to the manual count average

Materials and Methods



Results

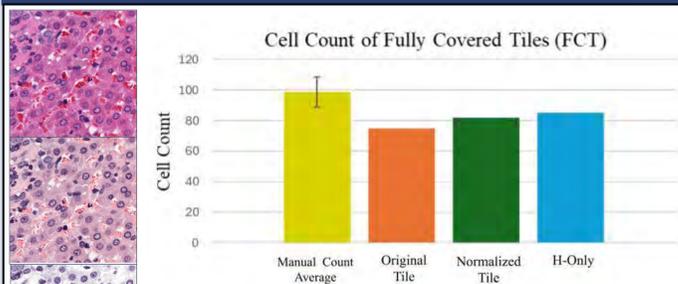


Fig. 3 Cell count of fully covered tiles. Manual count average (98.67 ± 9.87 cells), original tile (75 cells), normalized tile (82 cells), H-only (85 cells). To the left, is the representative FCT. From top to bottom is the original, normalized, and H-only of FCT.

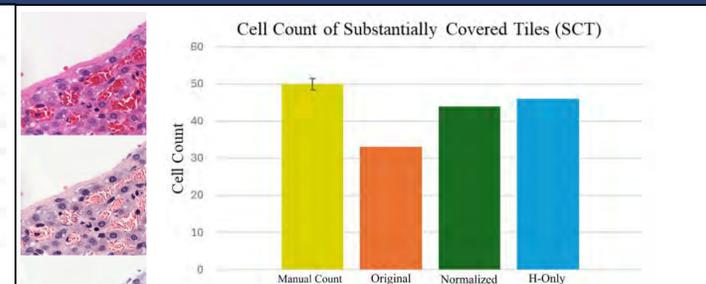


Fig. 4 Cell count of substantially covered tiles. Manual count average (50 ± 1.52 cells), original tile (33 cells), normalized tile (44 cells), H-only (46 cells). To the left, is the representative SCT. From top to bottom is the original, normalized, and H-only of SCT.

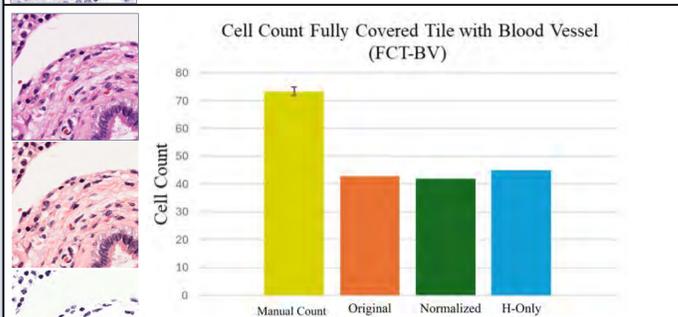


Fig. 5 Cell count of fully covered tile with blood vessel. Manual count average (73.33 ± 1.53 cells), original tile (43 cells), normalized tile (42 cells), H-only (45 cells). To the left, is the representative FCT-BV. From top to bottom is the original, normalized, and H-only of FCT-BV.

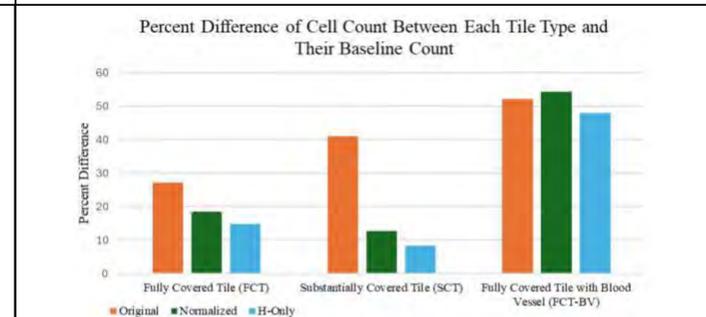


Fig. 6 Comparing percent difference of cell count between each tile type and the manual count average. Original, fully covered tile 27.26%; normalized FCT 18.45%; H-only FCT 14.89%. Original, substantially covered tiles 40.96%; normalized SCT 12.77%; H-only SCT 8.33%. Original, fully covered tile with blood vessel 52.14%; normalized FCT-BV 54.33%; H-only FCT-BV 47.77%.

Results

- Comparing SCT, FCT, percent diff., the H-Only tiles were the most accurate in each respective category.
- SCT H-only tile was the most accurate. Normalized SCT was second.
- The least accurate original, normalized, and H-only tile was FCT-BV

Discussion/Limitations/Future Direction

Discussion:

- The image segmentation model had an easier time identifying the shapes of the cells in H-only tiles because it has easily identifiable nuclei from the partially white background.
- SCT had the most accurate normalized and H-only tiles because there were fewer overlapping cells in representative slide
- StarDist image segmentation may not work best on endothelial cells present on FCT-BV because of the cell's abnormal shapes and the overlapping cells

Limitations:

- One limitation is using Openslide to normalize tiles instead of using Stardist because Stardist has its own normalization model. So, the cell count may not be the most precise
- Parameters (scale, probability threshold, and the Non-maximum suppression) were at default settings and can be optimized

Future Directions:

- Optimize the scale value, probability threshold, and non-maximum suppression to optimize it for our tiles, since default values were used for all of them.
- Identify structures on digital histopathology slides by putting tiles on a grid. Identify structures on CT scan using radiomics. Correlate Hounsfield unit of structure from CT scan to digital histopathology. See Fig. 7.

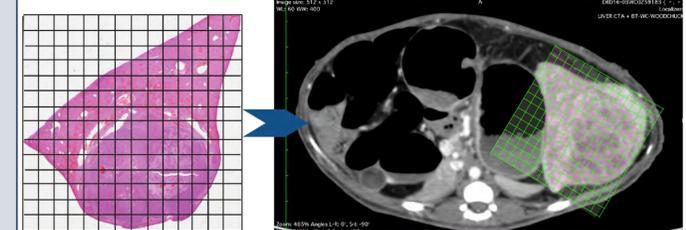


Fig. 7 Correlating histopathology slide to axial CT slice of woodchuck liver with HCC tumor.

- Add in features to analyze nuclei structures and what they mean
 - Ex. The shape of endothelial cells are distinct from any other type of cells. **Use this distinction to identify cell type.**
 - Ex. After segmenting the cells, you can get the area of the nuclei and cells. **Use the pixel measurements to differentiate the cell types**

References

- Kwabena, et. al; 2023, StatPearls
- Chuang, et.al; Cancer Letters
- Mauda-Havakuk, et. al; 2021, J Hepatocell Carcinoma
- DenOtter, et. al; 2024, StatPearls

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