Development of an AI method for rapid immunohistochemical stained image structure auto-segmentation

Najah Curtis[1], Ethan Cramer[2], Ioannis Verginadis[1], Michele M. Kim[1], Cameron Koch[1], Rodney D. Wiersma[2]
The University of Pennsylvania, Philadelphia, PA[1], University of California, Los Angeles, Los Angeles, CA[2]

2026 JULY 19–22 VANCOUVER, BC
JOINT AAPM | COMP MEETING

ABSTRACT

Purpose: Manual segmentation of immunohistochemical (IHC) stained images is a time-consuming task that typically takes 1-2 workdays to segment all images needed for analysis. Deep learning-based methods were employed to create an AI model to automatically segment IHC stained images of the different subregions (crypts, stroma, and villi) of murine small intestine within seconds.

Methods: For each mouse, approximately 5 cm of jejunum was frozen in a "Swiss Roll" format, sectioned, stained, and imaged. Tissue sections were stained for gamma-H2AX (detecting double strand DNA breaks), total DNA content (using DRAQ5), and hypoxia content (using EF5 binding). A total of 56 DRAQ5 stained images were manually segmented using ImageJ. 45 images were randomly shuffled into training (85%) and validation (15%) datasets, and 11 were evaluated by dice similarity coefficient (DSC). Multi-class masking was employed to assign a value and weight to each structure for segmentation predictions: background (weight = 0.0), crypts (weight = 4.0), villi (weight = 1.0), and stroma (weight = 4.0). The predicted segmentations can be manually modified before saving. The auto-segmented images were then used as binary mask overlays to segment their respective DRAQ5 and gamma-H2AX stained images using a separate in-house Python program.

Results: Overall, the model does well with segmenting the subregions of the intestine. The mean DSC for 11 images for each structure is reported: stroma 0.866±0.012, crypts 0.870±0.011, and villi 0.97±0.005. The segmentation (without modifications) takes seconds. However, modifications of varying degrees are generally required before the final segmentation is saved. Continued work is ongoing to further automate this process.

Conclusion: This algorithm has high potential to improve workflow for rapid IHC stained image structure auto-segmentation. Although this method was trained on intestinal IHC images, it is a general method that could be applied to other tissue types for segmentation as well.

CONTACT

Najah Curtis
University of Pennsylvania – Department of Radiation Oncology
3400 Civic Center Blvd, Philadelphia, PA, 19104
Najah.Curtis@pennmedicine.upenn.edu

BACKGROUND AND PURPOSE

Immunohistochemistry (IHC) images are a necessary component of radiobiology research; these allow for the evaluation of tissue architecture and structural changes as well as changes in expression and localization of specific proteins in response to various radiation modalities.

The intestine is an example of a highly researched tissue due to its clinical relevance and is known to exhibit the FLASH effect[1,2]. There are varying levels of hypoxia in the intestine along with many different cell types, which could affect how these areas within the intestine respond to radiation and potentially contribute to the oxygen-related component of the FLASH effect.

Therefore, there is a need to segment the intestine to study the response of radiation to the different subregions of the intestine (stroma, crypts, villi), however, manual segmentation is very time consuming, limiting the number of images that can be used. **Therefore, this deep learning model would decrease the time required to segment these images and aid in the workflow of such studies.**

METHODS AND MATERIALS

Experimental Procedure:

- Mice were:
  - Preinjected intravenously with EF5
  - Irradiated with FLASH **(FR)** or standard **(SR)** radiation using:
    - A 230 MeV beamline
    - 2cm x 2cm square field size to irradiate the whole abdomen
    - 5 Gy per mouse at 120 Gy/s for FLASH and 0.66 Gy/s for standard irradiations
  - Euthanized 30 minutes post irradiation for tissue collection of their jejunum.
  - The tissue was frozen then sectioned, stained, and imaged for use in the program.
  - These tissue sections were stained for:
    - **γ**-H2AX (identifying double strand DNA breaks),
    - Total DNA content (using the DRAQ5 stain), and
    - Hypoxia content (through EF5 binding)

Deep Learning Algorithm Development:

- **61 EF5** and **56 DRAQ5** stained images were manually segmented using ImageJ
- 51/45 images for EF5/DRAQ5, respectively, were randomly shuffled into training (87.5% / 85%) and validation (12.5% / 15%) datasets
  - 10/11 images were evaluated by dice similarity coefficient (DSC).

Deep Learning Algorithm Development:

- Multi-class masking was employed to assign a value and weight to each structure for segmentation predictions:
  - background (weight = 0.0)
  - crypts (weight = 4.0)
  - villi (weight = 1.0)
  - stroma (weight = 4.0).
- The predicted segmentations can be manually modified before saving.
- The auto-segmented images were then used as binary mask overlays to segment their respective EF5 and gamma-H2AX stained images

RESULTS

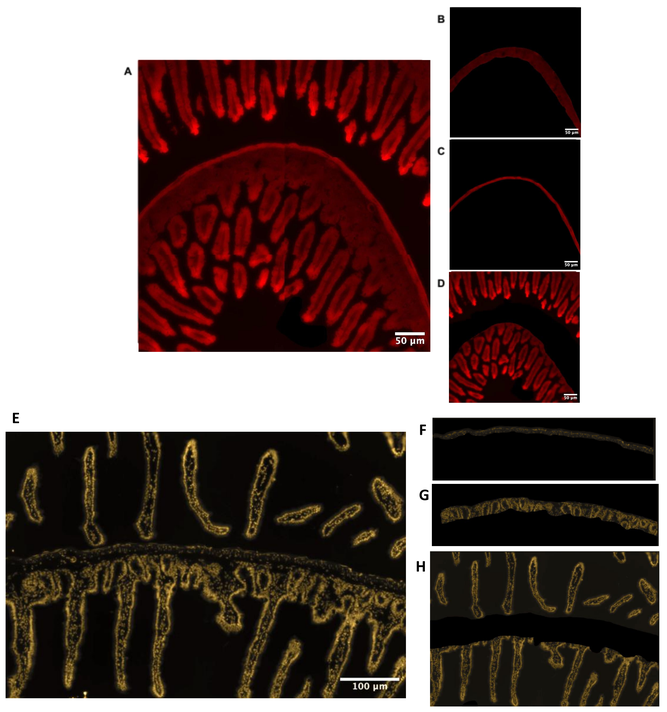

Figure 1 Manual segmentation of an EF5 stained image (panels A-D) and a DRAQ5 image (panels E-H). Note that villi tips are the brightest (most hypoxic), stroma is the second brightest, and crypts are the dimmest (least hypoxic) in the EF5 stained images.

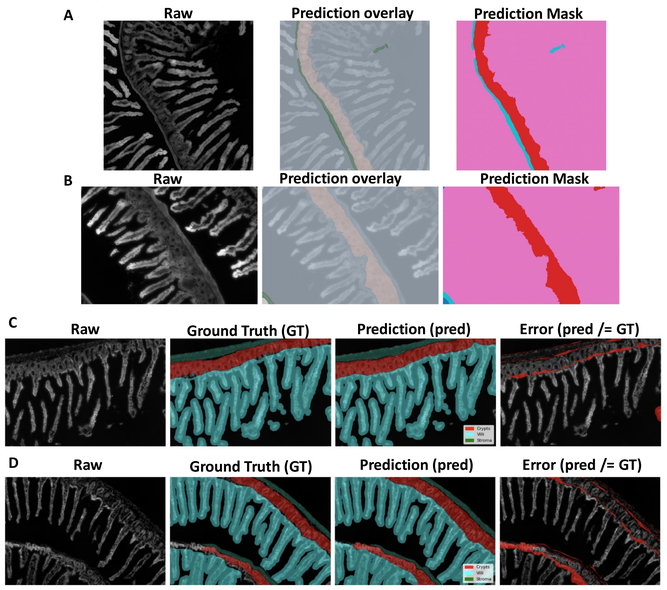

Figure 2 Predictions of AI auto-segmentation algorithm with varying levels of accuracy. Panels A and B are predictions using the EF5 trained algorithm and panels C and D are using the DRAQ5 trained algorithm.

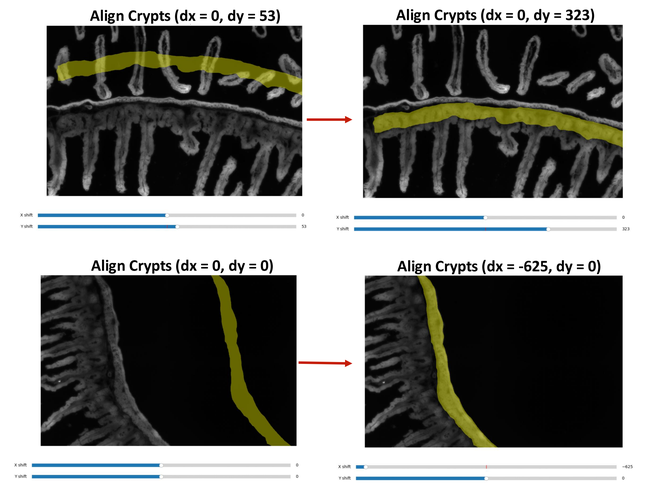

Figure 3 Mask overlays for the EF5 image based on the DRAQ5 auto-segmentation and allowing for alignment adjustments of the crypts (top) and the stroma (bottom) when the image size of the segmentation doesn't match the original image size.

The mean DSC for the 10 EF5 images for each structure is reported: stroma 0.961, crypts 0.947, and villi 0.942.

The mean DSC for the 11 DRAQ5 images for each structure is reported: stroma 0.866±0.012, crypts 0.870±0.011, and villi 0.97±0.005.

The EF5 trained algorithm is based on signal intensity, and would mistake the crypts region for the villi, or mistake the villi for the stroma since those areas can have similar signal intensities **(Fig. 1A-D, 2B).** The DRAQ5 trained algorithm is based on shape and can at times struggle with identifying the crypts and stroma, mostly due to the presence of Peyer's patches (not depicted).

Manual segmentation of the crypts, stroma, and villi for a one section of the three stains takes about 10 minutes. Auto-segmentation (without modifications) of each structure for one set of the three stains takes about 4 minutes, which would drastically decrease the time required for segmentation as the number of images increase. However, modifications of varying degrees are generally required before the final segmentation can be saved for both EF5 and DRAQ5 images.

CONCLUSIONS

This algorithm has high potential to improve workflow for rapid IHC stained image structure auto-segmentation. Although this method was trained on intestinal IHC images, it is a general method that could be applied to other tissue types for segmentation as well.

REFERENCES

1. Kim MM, Verginadis II, Goia D, et al. Comparison of FLASH Proton Entrance and the Spread-Out Bragg Peak Dose Regions in the Sparing of Mouse Intestinal Crypts and in a Pancreatic Tumor Model. *Cancers*. 2021;13(16):16. doi:10.3390/cancers13164244
2. Diffenderfer ES, Verginadis II, Kim MM, et al. Design, Implementation, and in Vivo Validation of a Novel Proton FLASH Radiation Therapy System. *International Journal of Radiation Oncology*Biology*Physics*. 2020;106(2):440-448. doi:10.1016/j.ijrobp.2019.10.049