3D Reconstruction of Fluorescence Guided Intraoperative Margin Assessment

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ABSTRACT / INTRODUCTION

Clinical Gap: Modern pathology relies on multiple imaging modalities to characterize tissue microenvironments. Brightfield H&E provides high-resolution cellular architecture, while fluorescence imaging identifies molecular markers. However, standard histopathology evaluates 3D biological structures using ultra-thin (3-5 µm) 2D sections, which discards crucial volumetric data like tumor invasion depth and vascular networks.

Challenge: Fluorescence-guided imaging captures a 2D surface signal that often lacks direct spatial correspondence with underlying 3D cellular morphology. Because tissues deform during sectioning, manual alignment of serial sections to cross-validate fluorescence against H&E ground truth is virtually impossible.

Solution: Bridging this gap by creating mathematically aligned, 3D volumetric representations of tumors directly supports the development of next-generation fluorescence-guided cancer detection and intraoperative AI tools.

RESEARCH CONTEXT

- Develop a robust tissue detection algorithm capable of differentiating tissue from background noise across varied modalities (H&E and Fluorescence).
- Develop a 2D U-Net Convolutional Neural Network (CNN) to identify tumor regions relying exclusively on fluorescence imaging.
- Perform highly accurate 2D to 3D coregistration of serial tissue sections using non-rigid transformations to account for tissue deformation.
- Reconstruct a continuous 3D volumetric model of the tissue and tumor samples to allow for cross-modality spatial querying.

Dataset: Serial frozen tissue sections from 9 distinct mouse tongue tumor specimen samples (M1 through M9); Captured as whole-slide images (WSIs) across three modalities: standard H&E (RGB), single-channel grayscale fluorescence (785Ex-820Em), and RGBTrans (Multispectral).

METHODS & PIPELINE

1. **Multimodal Tissue Detection**
   a. Gaussian blurring suppressed cell-level high-frequency noise
   b. A dual-space evaluation used HSV Otsu thresholding (Saturation channel) and RGB luminance thresholding (<230) to aggressively filter bright glass background
   c. Boolean intersection guaranteed high-confidence boundaries
   d. Multi-channel images were converted to grayscale, normalized to an 8-bit range (0-255), and segmented using automated Otsu thresholding
2. **Non-Rigid Serial Registration**
   a. Sections were processed using VALIS (Virtual Alignment of pathoLogy Image Series) to establish spatial correspondence
   b. Groupwise rigid registration corrected overall position, followed by non-rigid registration to account for local tissue deformation and distortion caused by sectioning
   c. Unalignable sections were tailed off to ensure high registration quality
3. **Volumetric & Surface Reconstruction**
   a. Binary masks were sorted by section ID, standardized using nearest-neighbor interpolation, and stacked along the z-axis to produce a binary 3D voxel array
   b. The **Marching Cubes algorithm** (isosurface level 0.5) was applied to traverse binary boundaries and generate a continuous triangular surface mesh, converting the arrays into interactive 3D HTML visualizations
4. **2D U-Net Fluorescence Tumor Segmentation**
   a. Specimens M1-M4 were used for training; M5 was saved for independent validation to ensure the model could generalize
   b. Images and masks were divided into overlapping 512 x 512 pixel patches with a stride of 256 pixels
   c. Random 90-degree rotations and horizontal/vertical axis flips were applied to the training dataset to introduce spatial and intensity variation

RESULTS

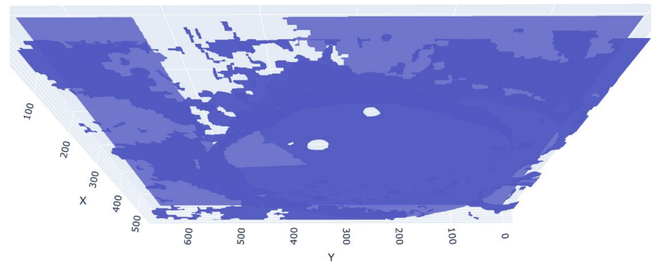

Figure 1. M9 3D Tissue Reconstruction. 3D surface mesh of the entire tissue volume for specimen M9.

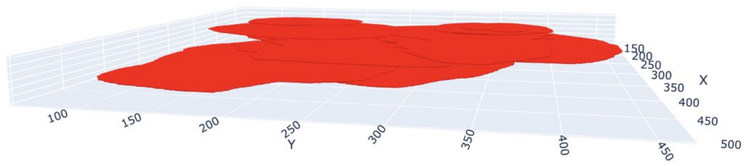

Fig 2. M9 3D Tumor Reconstruction. Volumetric representation isolating the spatial distribution of the annotated tumor region for specimen M9.

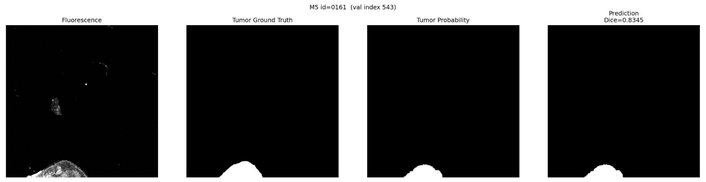

Fig 3. Validation of U-Net tumor segmentation on independent specimen M5, demonstrating isolation of tumor boundaries relying only on fluorescence.

DISCUSSION/CONCLUSION

This automated, multimodal pipeline successfully overcomes the fundamental spatial disconnect of traditional 2D pathology. By mathematically linking surface-level molecular signals to their underlying cellular ground truth, this work establishes that targeted fluorescent agents can act as highly accurate proxies for standard histopathology. Moving forward, reconstructing continuous invasion depths and structural margins provides the necessary computational foundation for next-generation intraoperative technologies, directly supporting augmented-reality surgical platforms that guide complete, real-time tumor eradication.

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