



Intraoperative Label-Free Fluorescence Lifetime Imaging for Real-Time Delineation of Occult Primary Oropharyngeal Carcinoma

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Importance

- Carcinoma of unknown primary origin evades primary site identification after exhaustive clinical, radiographic, and surgical evaluation.¹
- Successful patient surgical outcomes depend on the identification and complete removal of cancer; however low detection rates have motivated the search for novel approaches to facilitate the detection and excision of these elusive tumors.²

Objective

- To determine whether a label-free Fluorescence Lifetime Imaging (FLIm) technique, integrated into the da Vinci SP transoral robotic surgical platform (TORS), can be used intraoperatively to delineate unknown primary carcinoma of the oropharynx.

Design

- Optical parameters derived from FLIm measurements of oropharyngeal tissues suspected of occult primary were validated against gold-standard hematoxylin and eosin histopathology and used to evaluate the discriminatory performance of these parameters derived through a random forest machine learning classification approach.
- The classifier was trained on a larger 55-patient cohort of known primary oropharyngeal tumors and used to estimate the probability of healthy vs. cancer for each spectral point measurement.

Participants

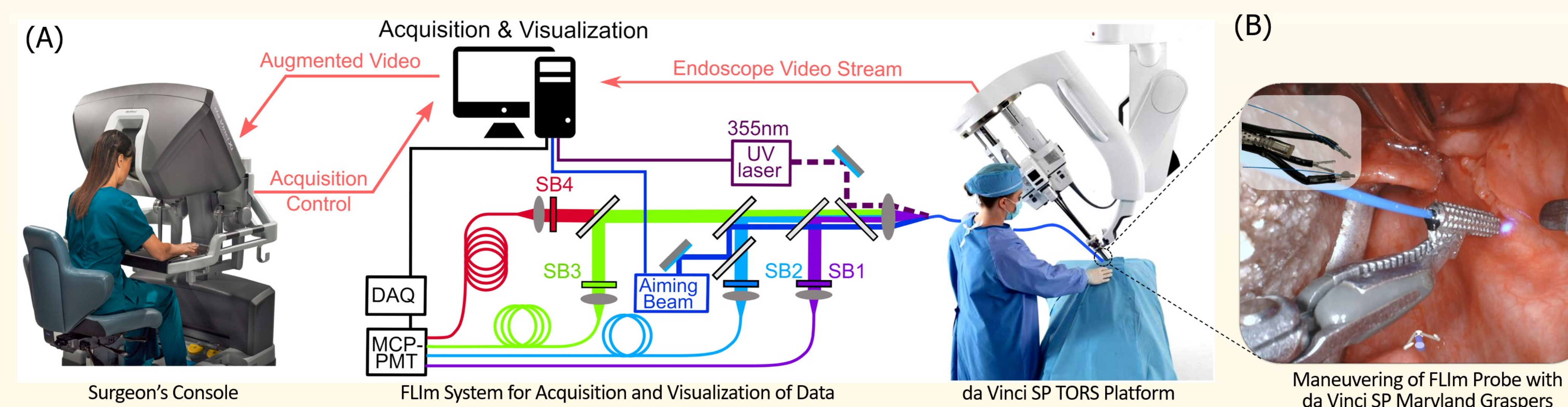
- N=8 patients presenting with a preoperative diagnosis of p16+ Squamous Cell Carcinoma (SCC) metastatic to head and neck with unknown primary site.

Hypothesis

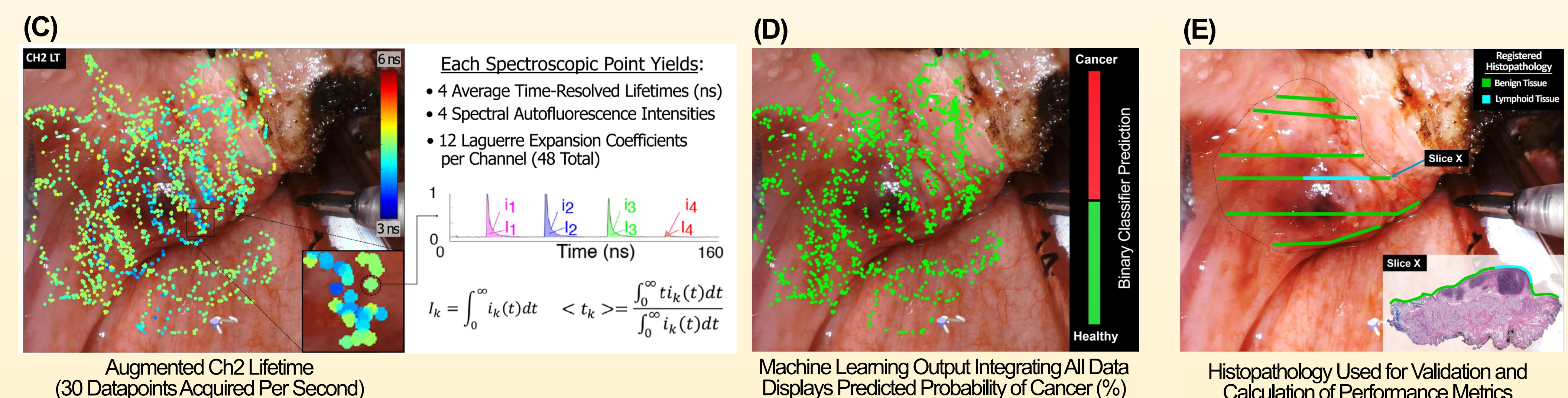
- **Prospective Hypothesis:** Intraoperatively acquired FLIm measurements output from a machine learning classifier can successfully detect p16+ occult primaries presenting in the mucosa (<250 μ m from tissue surface) and differentiate such malignancies from healthy tissue.
- **Primary Outcome Measurements:** Patient-level receiver operating characteristic area-under-the-curve (ROC-AUC) to quantify coregistered histologic true positive rate vs. false positive rate of the data, along with sensitivity and specificity.

FLIm Instrumentation

Figure 1: Instrumentation Schematic



(A) Schematic of the custom-built FLIm instrument and corresponding integration with TORS instrumentation. The instrument features a 355 nm excitation laser delivering light through a multi-mode fiber optic cable to a surgical tissue region of interest. Resulting autofluorescence is transmitted back to a wavelength selection module, comprising 4 channels which capitalize on the peak autofluorescence of collagen, NADH, FAD, and porphyrins. (B) Illustration of the integration of the fiber optic probe into the da Vinci SP surgical robot, held by Maryland Bipolar Forceps. The operator at the surgical console manipulates the fiber optic probe with the robotic instruments to conduct a scan of approximately 1 minute over a surgical region of interest. An aiming beam is used to create blue visible laser light on the tissue to localize where point-spectroscopic measurements are acquired.

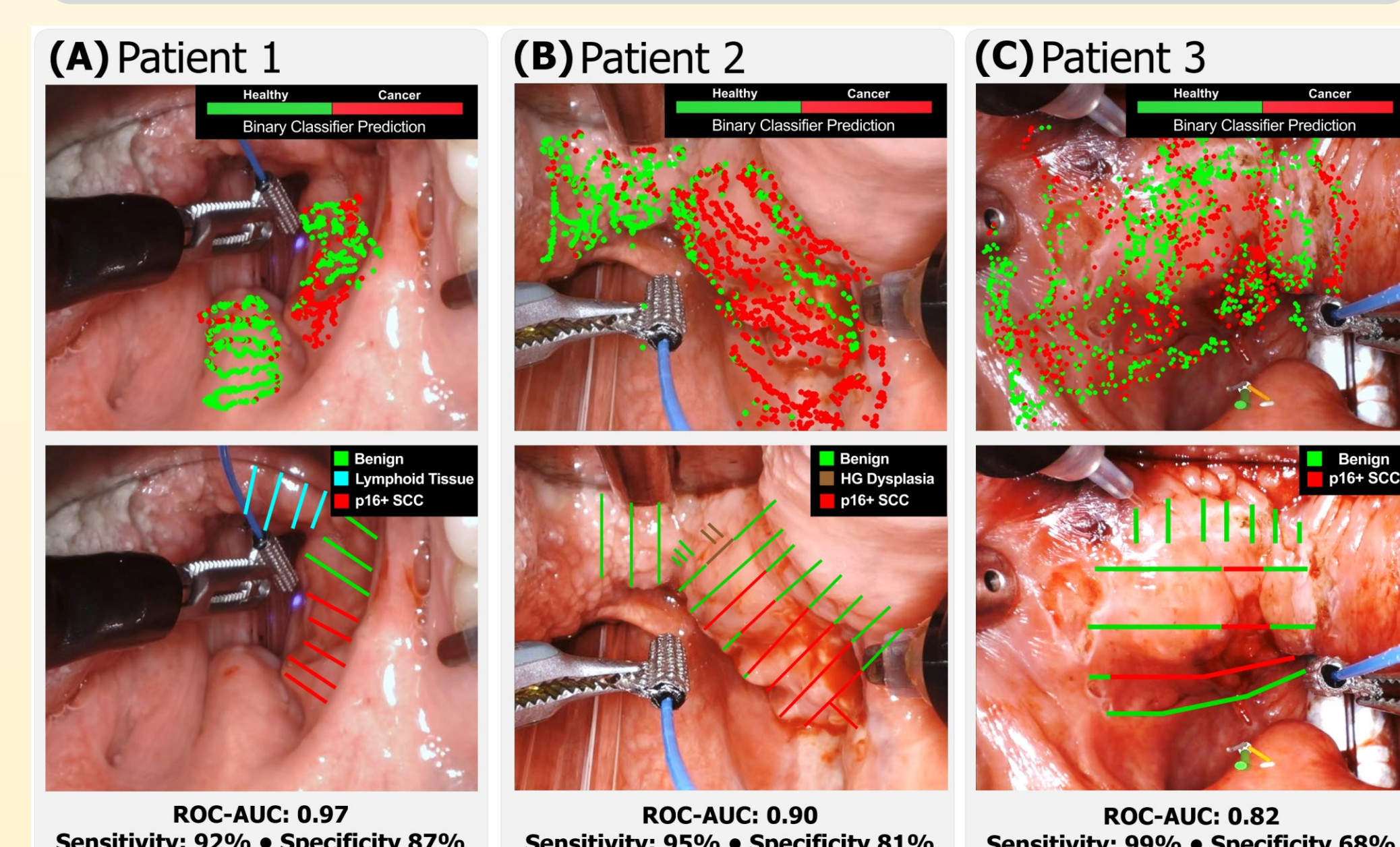


(C) Illustration of 1 of 56 possible metrics output from the FLIm instrument, namely channel 2 (NADH) time-resolved autofluorescence lifetime, with corresponding example waveforms and calculations for spectral intensity I_k and lifetime $\langle t_k \rangle$ demonstrated on the right. (D) Machine learning output from integrating all data at each spectroscopic point-measurement to perform an expected probability of cancer. (E) Histopathology from the excised specimen registered *in vivo* to validate and quantify performance metrics (ROC-AUC, sensitivity, and specificity) of the FLIm device.

Results

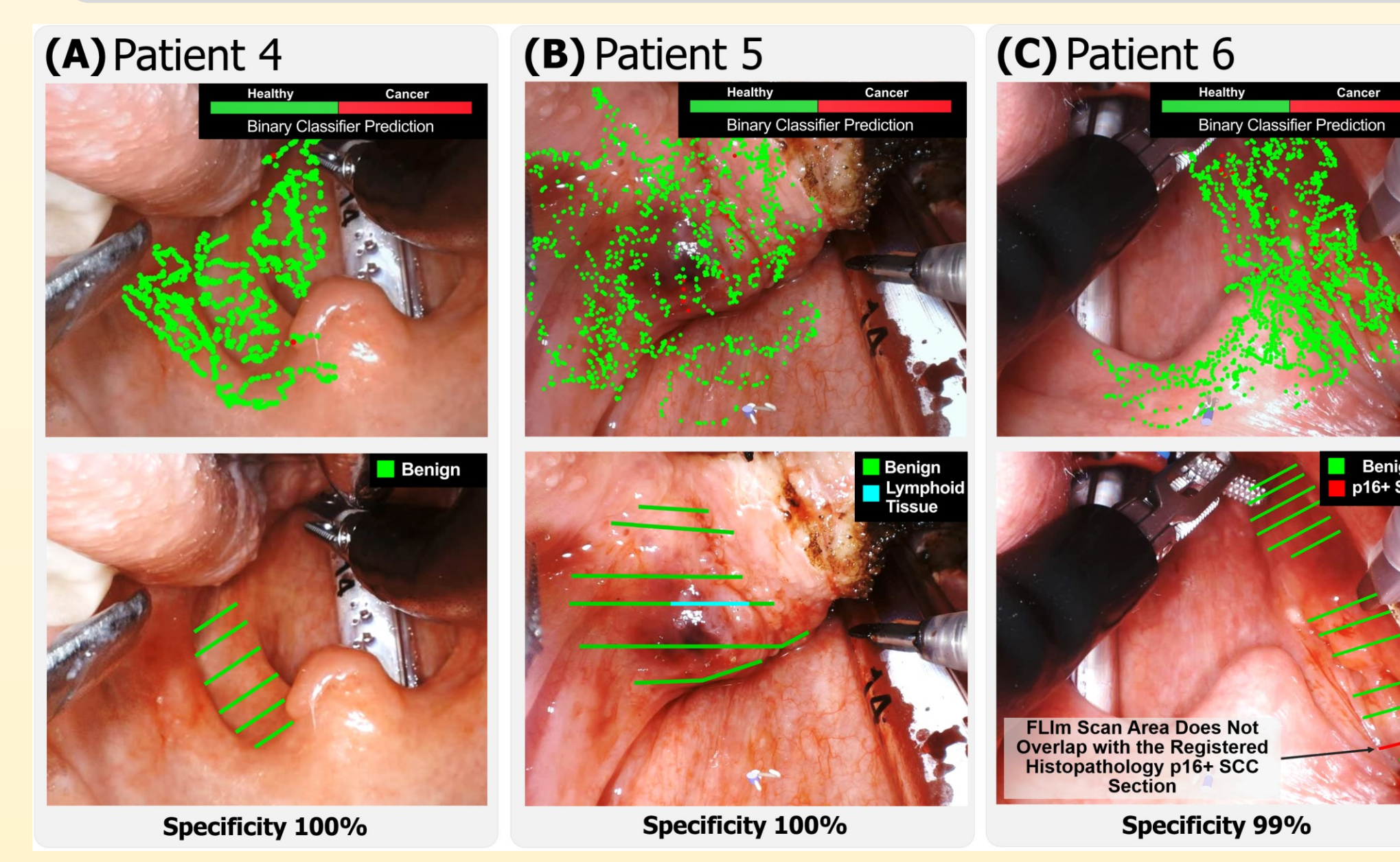
- *In vivo* FLIm data was acquired bilaterally on palatine tonsil and base of tongue tissues for all 8 patients. SCC of unknown primary origin (all p16+) presented within the regions of three patient's FLIm scans, representing one palatine tonsil and two base of tongue tissues.
- Four patients had no oropharyngeal primary identified in final pathology. One patient was omitted from further analysis due to study exclusion criteria.
- Among the analyzed 7 patients, p16+ H&N SCC of unknown primary was correctly detected using FLIm in all 3 patients where the occult primary presented in the scan area, yielding strong discrimination with an ROC-AUC of 0.89 ± 0.07 . Collectively, the mean sensitivity of the method was found to be $95 \pm 3.5\%$, and specificity $90 \pm 11.4\%$.

Figure 2. Case Studies of Patients with p16+ SCC within the Imaged FLIm Area.



Case studies of the 3 patients presenting with p16+ SCC within the imaged FLIm area. Each patient case study displays both the binary probability of cancer for each acquired spectral point-measurement and the patient's corresponding registered histopathology *in vivo*. (A) Patient 1 with p16+ SCC of the right palatine tonsil. (B) Patient 2 with p16+ SCC of the left base of tongue. (C) Patient 3 with cancer of right base of tongue.

Figure 3. Case Studies of Patients with Benign Tissue Only within the Imaged FLIm Area



Selected case studies for 3 of the 4 patients where FLIm scan areas comprised only benign tissue. (A) FLIm scan for Patient 4 of a benign left palatine tonsil. (B) FLIm scan for Patient 4 of benign right base of tongue tissue. (C) Patient 6 case study illustrative of a challenging scenario where the FLIm scan area does not overlap with the area of cancer. This highlights the importance of scanning the full surgical area.

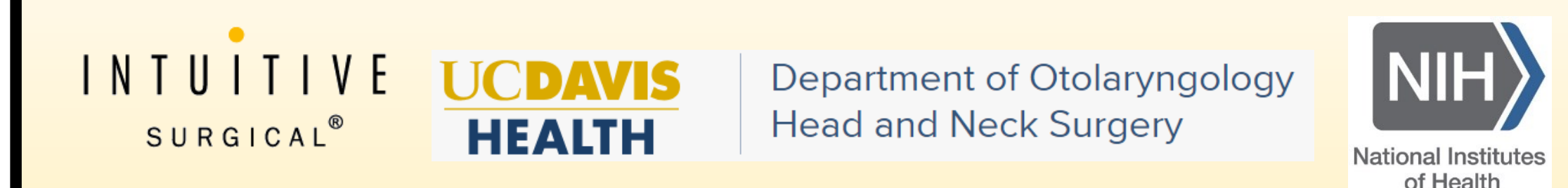
Conclusions

- Our results demonstrate strong value proposition in the clinical management of occult HNSCC by clearly demarcating tissues uninvolved with carcinoma as benign, thus demonstrating potential to improve patient care and surgical outcomes.
- For all patients presenting with p16+ unknown primary HNSCC in the FLIm scan region, the method correctly identified the presence and location of the occult primary with overall strong performance.
- Our current results demonstrate that for mucosa-presenting (<250 μ m from tissue surface) p16+ SCC of unknown primary, FLIm holds great promise in aiding a surgeon to screen, intraoperatively delineate, and excise these elusive tumor cohorts, while preserving uninvolved healthy functional tissue.

Next Steps

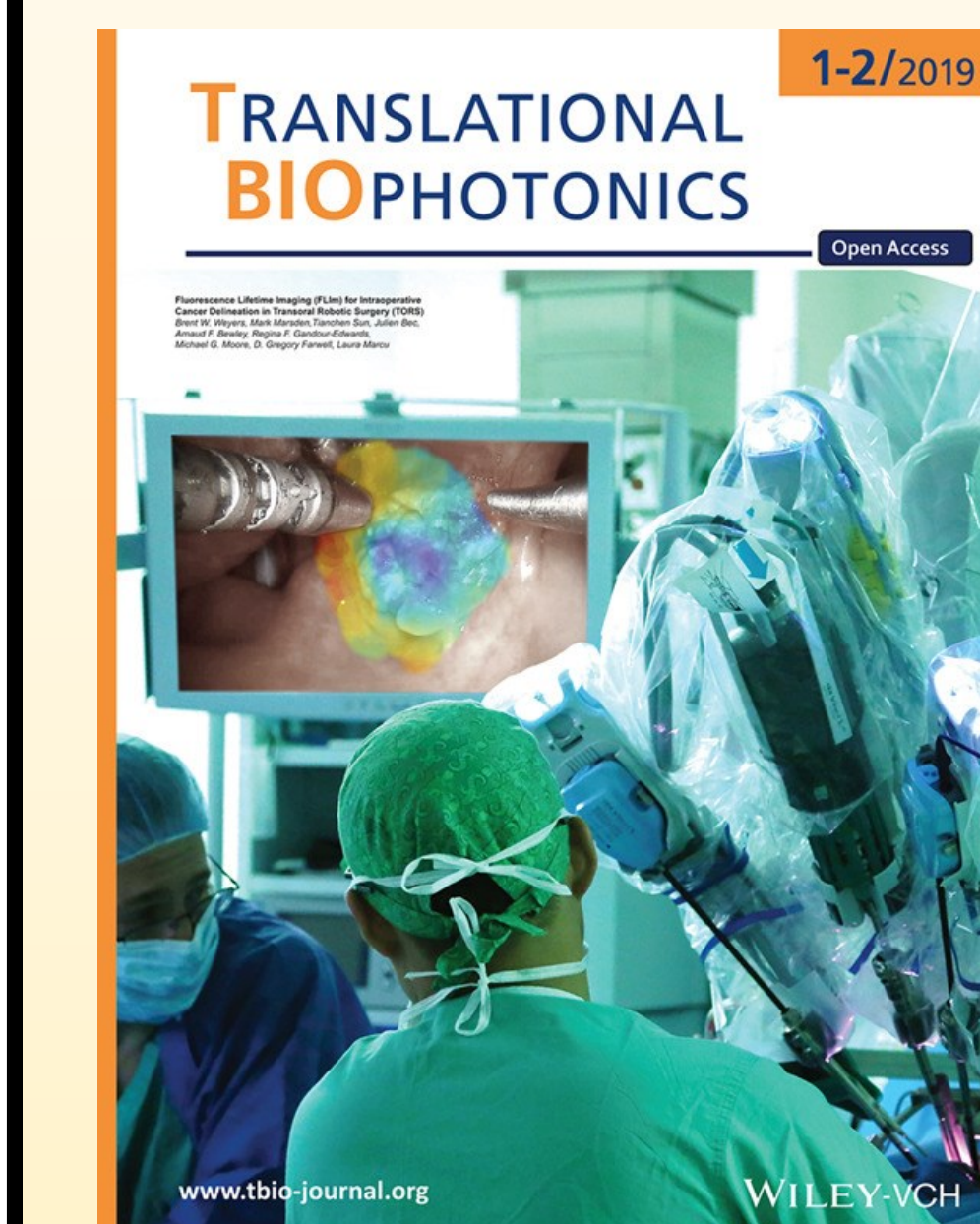
- Continue to expand the patient database.
- Add more nuanced tissue characteristics into the classifier (dysplasia, lymphoid tissue, etc.).
- Implement real-time methods for intra-operative motion correction and machine learning classification.

Acknowledgements



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2. Fu, T. S., Foreman, A., Goldstein, D. P. & De Almeida, J. R. The role of transoral robotic surgery, transoral laser microsurgery, and lingual tonsillectomy in the identification of head and neck squamous cell carcinoma of unknown primary origin: A systematic review. *Journal of Otolaryngology - Head and Neck Surgery* 45, (2016).