

# Rapid Optical Cytology with Deep Learning-Based Cell Segmentation for Diagnosis of Thyroid Lesions

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**Abstract:** We have developed and implemented a rapid, robust, and clinically viable protocol for fluorescence polarization cytopathology of thyroid nodules. The proposed approach utilizes rapid sample preparation and automated image analysis to accurately diagnose thyroid cancer. © 2023 The Author(s)

## 1. Introduction

Worldwide incidence of thyroid cancer is increasing with >586,000 new cases estimated in 2023 [1]. Fine needle aspiration (FNA) cytology is used to classify thyroid nodules into benign or malignant categories. This approach depends on subjective visual assessments and yields indeterminate findings in ~30% of thyroid FNA cases [2]. Moreover, typical turnaround times for the diagnosis range from a couple days to multiple weeks.

Recently, we showed that fluorescence polarization (Fpol) of methylene blue (MB) is significantly elevated in thyroid cancer [3]. In this work, we developed a rapid cytology sample preparation protocol with automated image analysis to facilitate clinical translation of MB Fpol technology and improve the reliability of cytopathological evaluations.

## 2. Materials and Methods

### 2.1 Sample Preparation and Imaging

Fine needle aspirates were obtained from de-identified, cancerous and benign thyroid tissues following surgery via consecutive sampling technique at BayState Medical Center, Springfield, MA, USA. The samples were transported in 1.5 mL tubes filled with 1.0 mL of Leibovitz's L-15 medium (Thermo Fisher Scientific, Waltham, MA, USA) to the Advanced Biophotonics Laboratory at the University of Massachusetts Lowell. Erythrocytes were lysed by adding 0.5 mL of RBC Lysis Buffer (Alfa Aesar, Haverhill, MA, USA) for 5 minutes, then cells were stained in 1.0 mL of 0.1 mg/mL MB solution (McKesson Corporation, San Francisco, CA, USA) for 20 minutes. Cells were extracted using a sterile syringe and smeared onto a glass slide. A coverslip was added to the slide, and the cells were imaged immediately using multimodal confocal microscopy [4]. Clinical cytology and histology were processed for each specimen.

### 2.2 Data Processing and Analysis

Fluorescence emission and polarization images were processed using the methodology described in our previous publication [3]. Cells were segmented manually. In parallel, we developed a U-Net-based deep learning system that was trained and tested on images of malignant and benign thyroid cells. Cells were segmented by the deep learning model for comparison. Fpol values were calculated for each cell using equation (1):

$$Fpol = \frac{I_{co} - G \times I_{cross}}{I_{co} + G \times I_{cross}} \quad (1)$$

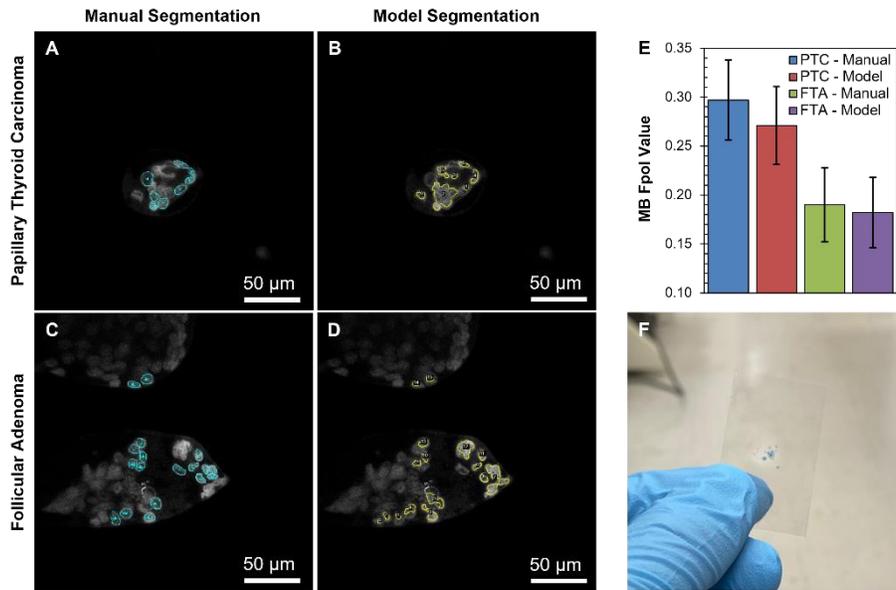
where  $I_{co}$  is the co-polarized fluorescence emission,  $I_{cross}$  is the cross-polarized fluorescence emission, and  $G$  is the system calibration factor, equal to 0.75.

Average MB Fpol and standard deviation values were determined for all the cells and specimens analyzed. Clinical diagnoses were obtained from BayState Medical Center and correlated with the optical imaging assessments.

### 3. Results and Discussion

In total, 12 fine needle aspirates (652 cells) were imaged and analyzed including 5 cancerous (272 cells) and 7 benign (380 cells). Malignant FNAs included 3 papillary thyroid carcinoma (PTC) and 2 oncocytic carcinoma (OCA), while the benign specimens included 3 follicular thyroid adenoma (FTA) and 4 multinodular goiter (MNG). The rapid sample preparation protocol reduced the time required for cell handling and imaging from about 24 hours to less than 30 minutes. Furthermore, by applying a deep learning-based model, the average time required for cell segmentation was reduced to less than 3 seconds from approximately 45 minutes required for manual segmentation. Therefore, overall time reduction for cell imaging and Fpol analysis was on the order of 95% across all samples.

Fig. 1A shows representative malignant PTC cells annotated by manual segmentation (cyan regions), whereas in Fig. 1B the same cells were outlined by the U-Net-based deep learning model (yellow regions). Figs. 1C and 1D display benign FTA cells segmented via manual and automated methods, respectively. Average MB Fpol was higher in cancerous cells as compared to noncancerous (Fig. 1E). Analysis also revealed that the differences in Fpol values obtained through manual vs. automatic cell segmentation agreed within  $\pm 15\%$ . Optical imaging results using rapid smearing protocol (Fig. 1F) correlated well with clinical histopathology.



**Fig.1.** Methylene blue fluorescence emission images of PTC cells analyzed using (A) manual and (B) automated segmentation. Methylene blue fluorescence emission images of FTA cells analyzed using (C) manual and (D) automated segmentation. (E) Histogram of MB Fpol values (error bars correspond to one standard deviation). (F) Thyroid cytology slide prepared using rapid protocol.

Results demonstrate that rapid MB Fpol cytology facilitates reliable and accurate thyroid cancer detection at the cellular level. Slide preparation by smearing technique was 24 times faster than previous methods, whereas the deep learning model significantly reduced segmentation time, with comparable Fpol values and number of segmented cells. The model proved robust against variations in image quality, although it was challenged by clusters of overlapping cells.

Future directions of rapid optical cytopathology will include improved segmentation model performance using a larger training data set, and development of a prototype Fpol imager for clinical trials.

### 4. References

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