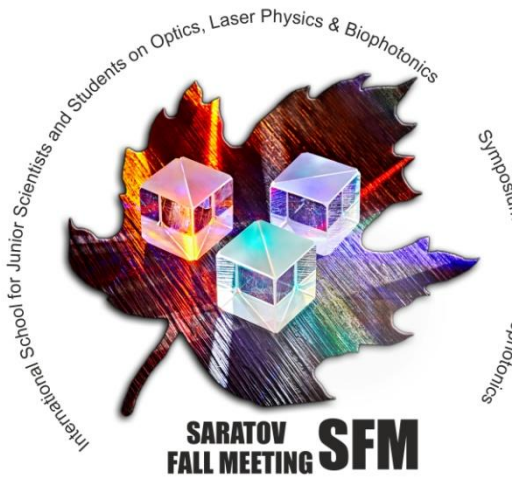


Two-Photon Microscopy based Oral lesion classification using AI models

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Introduction

Oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMDs) remain a major global health burden, with patient outcomes closely tied to how early the disease is caught. Conventional histopathology, though still the diagnostic gold standard, is held back by sampling bias, staining variability, and the subjectivity of manual interpretation. Label-free optical approaches — including Raman spectroscopy, diffuse reflectance, and autofluorescence imaging — have emerged as promising alternatives, offering biochemical specificity without the cost or invasiveness of staining. Multiphoton microscopy in particular, encompassing two-photon fluorescence (TPF) and second-harmonic generation (SHG), provides sub-micron optical sectioning and draws contrast directly from endogenous tissue components such as collagen, elastin, NADH, and FAD, making dye-free "virtual biopsy" imaging possible.

Most earlier work applying two-photon imaging to cancer diagnosis relied on qualitative, visual assessment of tissue morphology. The rise of deep learning has changed this, enabling automated detection of subtle structural and biochemical changes at a scale and consistency manual review cannot achieve. Building on this shift, the present study integrates label-free TPF imaging with a ConvNeXt-Small deep feature extractor and a multiclass SVM-RBF classifier to objectively distinguish Inflammatory/Control, OPMD, and Malignant oral tissue — with the broader aim of developing a rapid, near real-time screening tool that could help triage lesions before biopsy is needed.

Results

- Overall accuracy:** 90% — macro-average precision 0.88, recall 0.91, F1-score 0.89 (multiclass SVM-RBF on ConvNeXt-Small features).
- Class-wise F1-scores:** Inflammatory/Control = 0.99, OPMD = 0.78, Malignant = 0.91 — strongest discrimination for control and malignant tissue; OPMD hardest due to biological heterogeneity.
- Confusion matrix:** Inflammatory/Control correctly classified 97.4% of the time (2.6% misclassified as Malignant); OPMD 88.9% correct (11.1% → Malignant); Malignant 86.1% correct (13.9% → OPMD).
- ROC-AUC:** 1.00 (Inflammatory/Control), 0.94 (OPMD), 0.96 (Malignant).

Discussion

Label-free imaging matches reported DL benchmarks

The pipeline achieved 90% accuracy without exogenous dyes, in line with (or exceeding) prior deep-learning oral-lesion studies reporting sensitivities/specificities in the 85–90% range, reinforcing label-free TPF + AI as a viable objective screening alternative to biopsy-dependent histopathology.

SVM-RBF on deep features was the best classifier tested

Among 8 classifiers evaluated on identical ConvNeXt-Small features (SVM-RBF, Decision Tree, Random Forest, Extra Trees, Gradient Boosting, KNN, Naïve Bayes, MLP), SVM-RBF gave the best overall accuracy and class balance — supporting the use of classical ML on deep features for small, imbalanced medical imaging datasets where end-to-end deep training risks overfitting.

OPMD is the diagnostic bottleneck; fixation is a key limitation

OPMD's lower F1 (0.78) reflects genuine biological overlap between dysplastic and malignant phenotypes. Additionally, FFPE fixation is known to alter NADH/FAD autofluorescence signals (per Sánchez-Hernández et al. 2023), which may limit generalization to fresh/in vivo tissue — motivating future work on fresh-tissue validation, FLIM for metabolic specificity, and multicenter datasets.

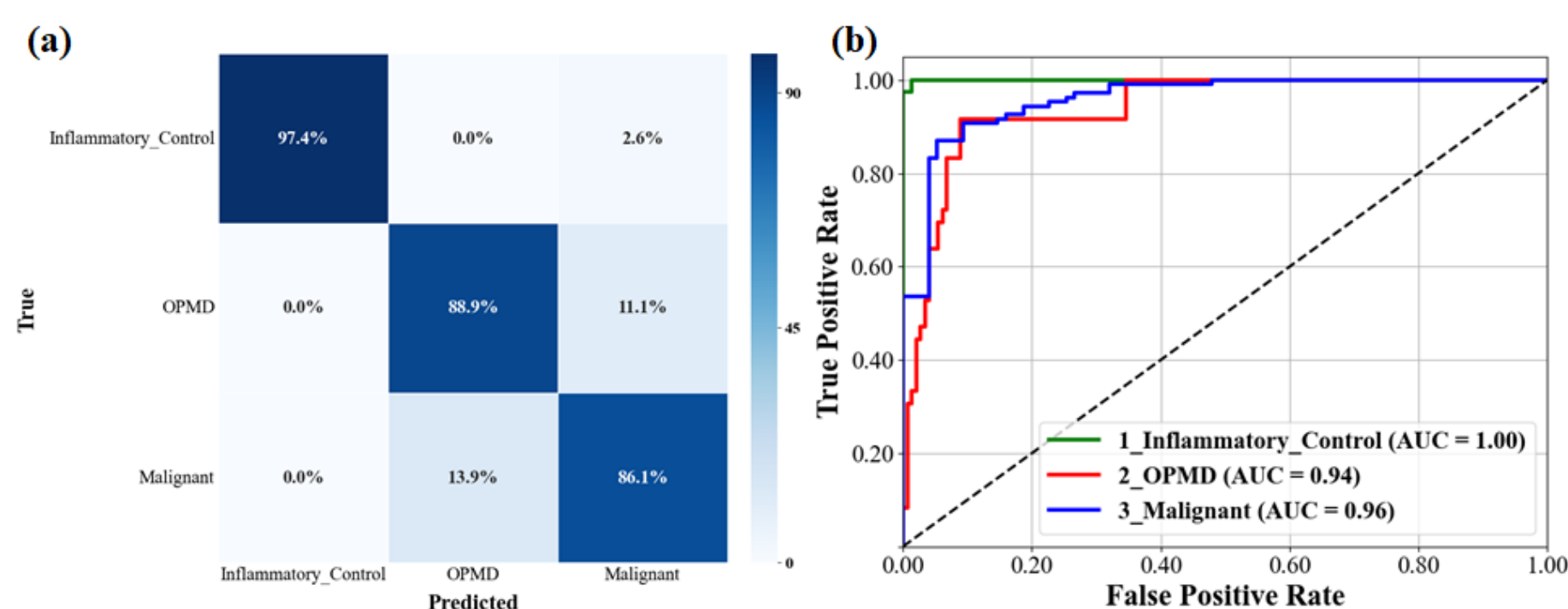


Fig. 2. Model performance evaluation. (a) Confusion matrix showing classification accuracy across three classes: Inflammatory/Control, OPMD, and Malignant. (b) Receiver operating characteristic (ROC) curves with AUC values of 1.00 for Inflammatory/Control, 0.94 for OPMD, and 0.96 for Malignant, indicating high sensitivity and specificity.

Conclusion

Key Finding 1

A label-free two-photon imaging + deep learning pipeline (ConvNeXt-Small features + SVM-RBF) achieved 90% accuracy classifying oral tissue into Inflammatory/Control, OPMD, and Malignant categories — without any exogenous stains.

Key Finding 2

The system operates near real-time (<0.5 s inference per image), supporting its feasibility as a rapid, objective triage/pre-screening tool ahead of invasive biopsy, including potential intraoperative margin assessment.

Key Finding 3

OPMD remains the key diagnostic challenge due to its biological heterogeneity; future work should pursue multimodal imaging (e.g., FLIM, SHG/CARS/THG), explainability, and prospective multicenter validation for clinical translation.

Reference

- M. Karnati et al., "Integration of Deep Learning and Machine Learning for Label-Free Two-Photon Imaging-Based Classification of Oral Lesions: A Pilot Study," in IEEE Photonics Journal, vol. 18, no. 4, pp. 0601006-0601006, Aug. 2026, Art no. 0601006, doi: 10.1109/JPHOT.2026.3701723.

Methodology

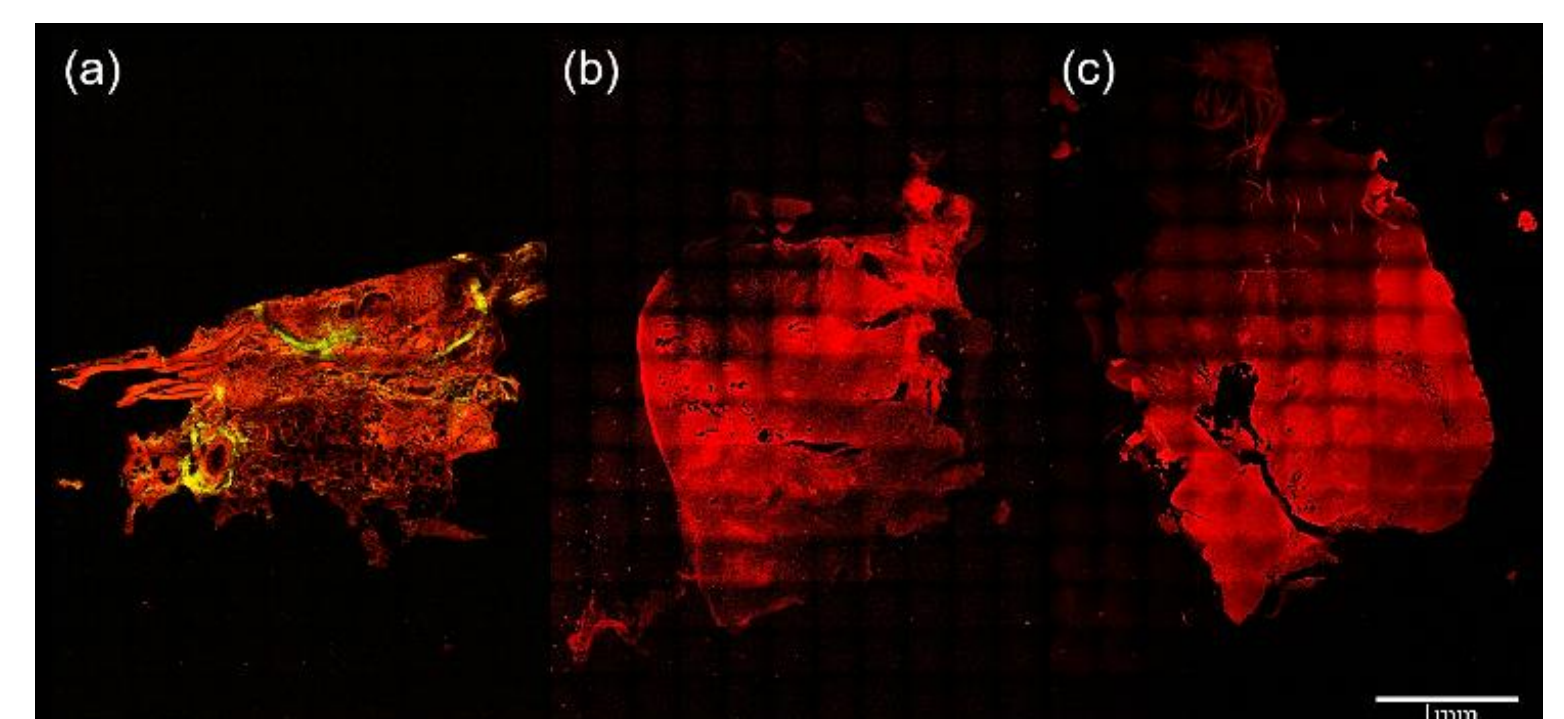
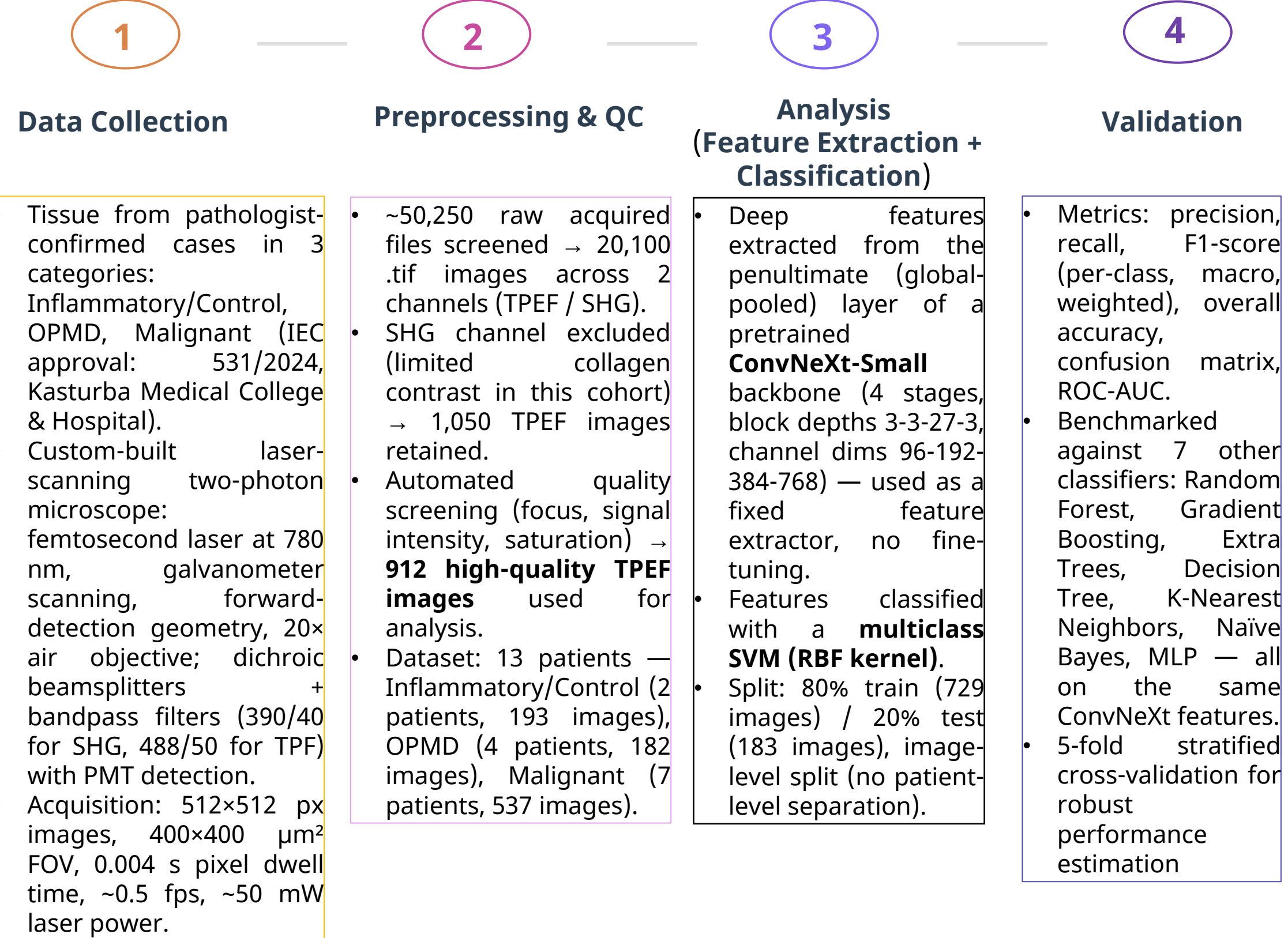


Fig. 1. Representative TPF images from oral tissue categories: (a) Inflammatory-Control; (b) OPMD; and (c) Malignant (OSCC), magnification 20x. TPF in red and SHG in green (Scale-1mm).

Table 1. Performance evaluation of all models

Model	Accuracy	Macro Precision	Macro Recall	Macro F1-score
Support Vector Machine (RBF)	0.90	0.88	0.91	0.89
Decision Tree	0.73	0.69	0.66	0.67
Random Forest	0.81	0.79	0.71	0.71
Extra Trees	0.79	0.76	0.67	0.67
Gradient Boosting	0.86	0.85	0.80	0.82
K-Nearest Neighbors	0.86	0.84	0.83	0.83
Naive Bayes	0.73	0.70	0.75	0.71
Multi-Layer Perceptron (MLP)	0.88	0.88	0.84	0.86