Title: **MULTIMODAL OPTICAL IMAGING AND SPECTROSCOPIC TECHNIQUES FOR CANCER SCREENING AND DIAGNOSIS**

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ABSTRACT

Conventional cancer screening methods are typically confined to large urban hospitals, presenting challenges related to expense, time consumption, and limited accessibility. Implementing fast and reliable screening tools like multi-modal optical imaging and spectroscopic devices holds promise for mitigating healthcare costs and increasing sensitivity and accuracy in the diagnosis of diseases. These non-contact or minimally invasive methods operate within the near UV, visible, and near-infrared electromagnetic spectra, collectively termed "**optical biopsy**", offering real-time and precise cancer diagnosis. This thesis aims to develop novel portable, multi-spectral, and multi-modal devices using optical techniques such as auto-fluorescence (AF) imaging, fluorescence (FL) imaging and spectroscopy, and micro-endoscopy-based point-of-care tools. AF and FL techniques exhibit high specificity and sensitivity; being directly linked with the molecular levels of human tissue, they can be used as a quantitative tool for cancer detection or diagnosis. These advancements stand to revolutionize the screening, diagnosis, treatment, and prevention of oral and breast cancer, presenting opportunities for quicker and more efficient healthcare interventions.

 First, we propose the development of portable, cost-effective, and user-friendly FL-based smartphone imaging and spectroscopic devices for detecting invasive ductal carcinoma (IDC) within tumor margins during tumor removal. These multi-modal tools leverage molecular-level FL sensitivity, exhibiting distinct behaviors in normal, cancerous, and marginal tissues. Employing simultaneous FL imaging and spectroscopy during intra-operative breast cancer procedures holds considerable advantages for identifying tumor margins and distinguishing between tumor and healthy tissues. Our observations highlight notable spectral changes, such as red-shift, variations in full-width half maximum (FWHM), and increased intensity, progressing from normal tissue toward the tumor centre. Principle component analysis (PCA) is used for the classification of invasive ductal carcinoma with an accuracy of 93 %, sensitivity of 75%, and specificity of 92.8%. We obtained an average 6.17 ± 1.66 nm red-shift for IDC with respect to normal tissue. Following the development of FL-based smartphone imaging and spectroscopic devices, these tools have also demonstrated the capability to diagnose early-stage breast cancer fibroadenomas. The experiments were performed, and we analyzed and

classified fibroadenomas, IDC, and normal tissue. The sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy for IDC and fibroadenoma compared to normal tissue (total efficiency) are 78.6 %, 90%, 91.6%, 75%, and 95%, respectively. Additionally, we also observed a red shift for fibroadenoma from normal tissue, which is 4.96±2.61 nm. The study includes a total of 11 IDC and 10 fibroadenoma patients.

Further, we report the development of multi-modal autofluorescence and fluorescence imaging and spectroscopic (MAF-IS) smartphone-based systems for fast and real-time oral cancer screening. MAF-IS system is indigenously developed and offers the advantages of being a low-cost, handy, non-contact, non-invasive, and easily operable device that can be employed in hospitals, including low-resource settings. In this study, we report the results of 43 individuals with 28 OSCC and 15 oral potentially malignant disorders (OPMDs), i.e., epithelial dysplasia and oral submucous fibrosis, using the developed devices. We observed a red shift in fluorescence emission spectra in-vivo. We found red shift of 7.72±6nm, 3 ±4.36nm, and 1.33±0.47nm in the case of OSCC, epithelial dysplasia, and oral submucous fibrosis, respectively, compared to normal. The results were compared with histopathology and found to be consistent. Further, the MAF-IS system provides results in real-time with higher accuracy and sensitivity compared to devices using a single modality. Our system can achieve an accuracy of 97% with sensitivity and specificity of 100% and 94.7 %, respectively, even with a smaller number of patients (28 patients of OSCC). The proposed MAF-IS device has great potential for early screening and diagnosis of oral cancer in the future.

Following the development of smartphone-based imaging and spectroscopic tools for breast and oral cancer, a necessity arises to observe real-time cellular changes in cancer progression at the microscopic level within living tissues and in in-vivo mode. While existing micro-endoscopes serve this purpose, their complexity and bulkiness limit their functionality to imaging alone. Addressing these, we developed a compact and user-friendly micro-spectro-endoscope (MSE) operating in both imaging and spectroscopic modalities. The MSE employs a graded index (GRIN) rod-lens for micro-endoscopy and utilizes oblique illumination to eliminate specular reflections. Notably, the MSE achieves a spatial resolution of 4.38 μm and a spectral resolution of 0.5nm, encompassing a field of view measuring 1.44 × 2.55 mm². Employing AF and FL techniques, our MSE screens OSCC, oral dysplasia, and normal tissues. Analysis reveals distinguishable FL contrast among all the cases alongside significant spectrum parameter shifts, particularly observed in OSCC and oral dysplasia scenarios.

Following the application of the MSE in FL mode, we proceeded to integrate AF into the developed MSE. This integration enabled us to investigate lupus nephritis (LN), an autoimmune disease characterized significantly by proteinuria. We employed AF spectroscopy utilizing kidney tissues from the Murphy Roth large (MRL) strain across three stages: young, antibody-positive, and proteinuria-afflicted. Our findings revealed distinct changes among these three cases, aligning notably with histopathological observations. The MSE, with its dual-modality, imaging, and spectroscopy in AF and FL modes, has effectively probed oral cancer and proteinuria-afflicted kidney samples, showcasing its versatile diagnostic potential.

Finally, we conducted a comprehensive examination involving the tagging of fluorescein sodium salt (FSS) with oral cancer tissues, previously utilized for the FL technique in our earlier research. This study encompassed both ex-vivo and in-vivo investigations of oral cancer with FSS, expanding our understanding of its application and behaviour within the context of oral cancer detection and analysis.