

MULTIMODAL OPTICAL IMAGING AND SPECTROSCOPIC TECHNIQUES FOR CANCER SCREENING AND DIAGNOSIS

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

MAY 2024

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by

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Submitted
in fulfilment of the requirement for the degree of
Doctor of Philosophy
to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI
MAY 2024

Dedicated to,

My Grandparents

CERTIFICATE

This is to certify that the thesis entitled “**MULTI-MODAL OPTICAL IMAGING AND SPECTROSCOPIC TECHNIQUES FOR CANCER SCREENING AND DIAGNOSIS**” submitted by **Ms. PRAMILA THAPA** to the Department of Physics, Indian Institute of Technology Delhi for the award of the degree of **DOCTOR OF PHILOSOPHY**. This thesis is a bonafide record of the research carried out by her under my guidance and supervision. In my opinion, the thesis has reached the standards fulfilling the requirements for the submission relating to the degree.

The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Date:

Place: New Delhi

Prof. Dalip Singh Mehta

Department of Physics

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ACKNOWLEDGEMENTS

First and foremost, my deep gratitude goes to Prof. Dalip Singh Mehta, who expertly guided me in this five-year journey. I extend my deepest gratitude to him for his unwavering guidance, invaluable insights, and unending support throughout this journey. His mentorship has been instrumental in shaping the trajectory of this thesis. He has been supportive since I started learning PYL-760 (Biomedical optics and Bio-Photonics) at IIT Delhi. Prof. Mehta supported me not only academically but emotionally through the rough road to accomplishing the projects. His motto, “*Science is to be meant for mankind,*” has influenced me a lot and pushed me to work beyond my limits. Further, I owe a debt of gratitude to him for being an integral part of this academic journey. His unwavering commitment, scholarly wisdom, and belief in my potential have played a pivotal role in achieving the milestones of this thesis.

My heartfelt appreciation goes to Prof. Dr. Deepika Mishra and Prof. Anurag Srivastava from All India Institute of Medical Sciences (AIIMS) New Delhi for their collaborative spirit, expertise, and dedication. Their contributions and shared enthusiasm have enriched this thesis, elevating it to new dimensions. Their constant discussion about the real-time challenges in cancer screening and diagnosis served as a catalyst, inspiring me to intensify efforts and innovate novel ideas and devices poised for a seamless transition from the laboratory to practical field applications.

I would like to express my deepest appreciation to my thesis committee members, Prof. Kedar Khare, Prof. A.K. Shukla, and Prof. Deepak Joshi, for their insightful comments and encouragement and also for the technical questions that encouraged me to widen my research from various perspectives. I acknowledge them for their constructive feedback, critical evaluation, and valuable suggestions during the progress report presentations that significantly enhanced the quality and depth of this work.

I will be very thankful to Prof. Balpreet Singh Ahluwalia for supporting me during the three-month internship program at UiT The Arctic University of Norway. He has always been very supportive and has given me the liberty to work on multiple projects without any objection. I acknowledge Prof. Balpreet Singh Ahluwalia for the scientific discussions across various projects, which have significantly influenced my thinking, proving instrumental in shaping my thought process and proving invaluable towards the successful completion of the proposed thesis.

Acknowledgements

I highly thank Dr. Veena Singh, Dr. Shilpa Tayal, and Dr. Ankit Butola for their mentorship throughout this journey. Their exemplary work ethic and dedication have set a benchmark, inspiring me to strive for excellence in this thesis. I am indebted to them for fostering a collaborative and supportive work environment. Their encouragement and willingness to share ideas have created a conducive atmosphere, propelling the progress of this thesis.

I am also grateful for the collaborative support received at IIT Delhi and UiT Tromsø. I am incredibly thankful to Dr. Azeem Ahmad and Dr. Vishesh Dubey for their valuable technical inputs during my internship. I am also thankful to Prof. Kristin Anderson (Department of Medical Biology, Tromsø, Norway) for providing the biological samples and sharing her biological expertise to complete the work.

I would like to thank my fellow lab mates, Anand Kumar, Himanshu Joshi, Sathi Das, Anuj Saxena, Anjika Kumari, Meenakshi, and Sachin Dhawan, for the stimulating scientific and social discussions, for the sleepless nights, and for all the fun we have had in the last five years. I would like to thank Priyanka Mann for her unwavering support, camaraderie, and shared enthusiasm throughout this academic journey have been invaluable. Her friendship has made both the lab and life infinitely more fulfilling. I would like to express my heartfelt gratitude to Sunil Bhatt for his unwavering encouragement and unwavering support, which have been the bedrock upon which this thesis stands. His patience during long hours of work, understanding during moments of stress, and constant belief in me have been the driving force behind the completion of this academic milestone. Furthermore, I extend heartfelt gratitude to Manisha “Airy” for her unwavering support throughout my Ph.D. journey. Her consistent emotional support proved invaluable, especially during the most challenging times, serving as a source of encouragement that propelled me toward completing the thesis. Also, I wish to acknowledge my friends Manas Pant, Deepak Raikwal, Deekshya Raj, Rimpi Bhandari, and Ashish Khanna. Their unconditional support has been essential in these years. Their inspirational thoughts, kindness, and loving nature are completely insane.

Lastly, and most importantly, the primary source of my life energy is my family. I would like to thank my grandparents for believing in me, which has given me the strength to complete this thesis. I would like to thank my parents for understanding me; they always support me in walking further and thinking beyond the shell. I would like to thank my brother Saurav Thapa for always supporting me during my hardship on my Ph.D. journey. I would like to thank my cousins, Diksha Thapa, Gaurav Thapa, Pawan Thapa, Kanchan Thapa, Sweta Thapa, and

Acknowledgements

Krishna, for making my vacation special during this journey and for their emotional support. There are no words to convey love and gratitude to my whole family. They have given up many things for me to be at IITD, cherished with me every moment, and supported me whenever I needed it. I am eternally grateful for their love and support.

I would like to thank CSIR, India, for funding the work carried out in this thesis. Last but not least, I would like to thank THE ALMIGHTY!

Pramila Thapa

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 ± 6 nm, 3 ± 4.36 nm, and 1.33 ± 0.47 nm 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 $4.38 \mu\text{m}$ and a spectral resolution of 0.5nm , encompassing a field of view measuring $1.44 \times 2.55 \text{ mm}^2$. 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.

सार

पारंपरिक कैंसर स्क्रीनिंग विधियां आमतौर पर बड़े शहरी अस्पतालों तक ही सीमित हैं, जो खर्च, समय की खपत और सीमित पहुंच से संबंधित चुनौतियां पेश करती हैं। मल्टी-मोडल ऑप्टिकल इमेजिंग और स्पेक्ट्रोस्कोपिक उपकरणों जैसे तेज़ और विश्वसनीय स्क्रीनिंग टूल को लागू करने से स्वास्थ्य देखभाल लागत को कम करने और बीमारियों के निदान में संवेदनशीलता और सटीकता बढ़ाने का वादा किया गया है। ये गैर संपर्क या कम आपत्तिजनक तरीके, जो दृश्य और नजदीकी दृश्य विद्युत तरंग सीमाओं के भीतर कार्य करते हैं, समूहित रूप से "ऑप्टिकल बायोप्सी" कहे जाते हैं, वास्तविक समय में और सटीक कैंसर निदान प्रदान करते हैं। इस थीसिस का उद्देश्य पोर्टेबल, बहु-वर्णीय, बहु-प्रकारी उपकरणों जैसे ऑटो-फ्लोरोसेंस (एएफ) इमेजिंग, फ्लोरोसेंस (एफएल) इमेजिंग और स्पेक्ट्रोस्कोपी, और माइक्रो-एंडोस्कोपी-आधारित पॉइंट-ऑफ-केयर उपकरण विकसित करना है। एफ और एफएल तकनीकें उच्च विशिष्टता और संवेदनशीलता प्रदर्शित करती हैं; मानव ऊतकों के आणविक स्तर से सीधे जुड़े होने के कारण, इन्हें कैंसर की पहचान या निदान के लिए एक मात्रात्मक उपकरण के रूप में उपयोग किया जा सकता है। ये प्रगतियाँ मुँह और स्तन कैंसर की जांच, निदान, उपचार और रोकथाम में क्रांतिकारी बदलाव ला सकती हैं, जिससे त्वरित और अधिक कुशल स्वास्थ्य देखभाल के अवसर मिलेंगे।

सबसे पहले, हम ट्यूमर हटाने के दौरान ट्यूमर मार्जिन के भीतर इनवेसिव डक्टल कार्सिनोमा (आईडीसी) का पता लगाने के लिए पोर्टेबल, लागत प्रभावी और उपयोगकर्ता के अनुकूल एफएल-आधारित स्मार्टफोन इमेजिंग और स्पेक्ट्रोस्कोपिक उपकरणों के विकास का प्रस्ताव करते हैं। ये मल्टी-मोडल उपकरण आणविक-स्तर की एफएल संवेदनशीलता का लाभ उठाते हैं, जो सामान्य, कैंसरयुक्त और सीमांत ऊतकों में अलग-अलग व्यवहार प्रदर्शित करते हैं। इंट्रा-ऑपरेटिव स्तन कैंसर प्रक्रियाओं के दौरान एक साथ एफएल इमेजिंग और स्पेक्ट्रोस्कोपी को नियोजित करने से ट्यूमर मार्जिन की पहचान करने और ट्यूमर और स्वस्थ ऊतकों के बीच अंतर करने में काफी फायदे होते हैं। हमारे अवलोकन उल्लेखनीय वर्णक्रमीय परिवर्तनों को उजागर करते हैं, जैसे कि लाल-शिफ्ट, पूर्ण-चौड़ाई आधा अधिकतम (एफडब्ल्यूएचएम) में भिन्नता, और बढ़ी हुई तीव्रता, सामान्य ऊतक से ट्यूमर केंद्र की ओर प्रगति। सिद्धांत घटक विश्लेषण (पीसीए) का उपयोग 93% की सटीकता, 75% की संवेदनशीलता और 92.8% की विशिष्टता के साथ आक्रामक डक्टल कार्सिनोमा के वर्गीकरण के लिए किया जाता है। हमने सामान्य ऊतक के संबंध में आईडीसी के लिए औसत 6.17 ± 1.66 नैनोमीटर रेड-शिफ्ट प्राप्त किया। एफएल-आधारित स्मार्टफोन इमेजिंग और स्पेक्ट्रोस्कोपिक उपकरणों के विकास के बाद, इन उपकरणों ने प्रारंभिक चरण के स्तन कैंसर फाइब्रोएडीनोमा का निदान करने की क्षमता भी प्रदर्शित की है। उसके लिए, हमने फाइब्रोएडीनोमा, आईडीसी और सामान्य ऊतक का विश्लेषण और वर्गीकरण किया है। सामान्य (कुल दक्षता) की तुलना में आईडीसी और फाइब्रोएडीनोमा के लिए संवेदनशीलता, विशिष्टता, सकारात्मक पूर्वानुमानित मूल्य, नकारात्मक पूर्वानुमानित मूल्य और समग्र नैदानिक सटीकता क्रमशः 78.6%, 90%, 91.6%, 75% और 95% है। इसके अतिरिक्त, हमने सामान्य ऊतक से फाइब्रोएडीनोमा के लिए एक लाल-शिफ्ट भी देखा, जो 4.96 ± 2.61 नैनोमीटर है। इस अध्ययन में कुल 11 आईडीसी और 10 फाइब्रोएडीनोमा रोगी शामिल हैं।

इसके अलावा, हम तेजी से और वास्तविक समय में मौखिक कैंसर की जांच के लिए मल्टी-मोडल एफ और एफएल इमेजिंग और स्पेक्ट्रोस्कोपिक (एमएएफ-आईएस) स्मार्टफोन-आधारित सिस्टम के विकास की रिपोर्ट करते हैं। एमएएफ-आईएस प्रणाली स्वदेशी रूप से विकसित की गई है और कम लागत वाली, सुविधाजनक, गैर-संपर्क, गैर-आक्रामक और आसानी से संचालित होने वाली डिवाइस होने के फायदे प्रदान करती है जिसे कम संसाधन वाली सेटिंग्स सहित अस्पतालों में नियोजित किया जा सकता है। इस अध्ययन में, हम विकसित उपकरणों का

उपयोग करके 28 ओएससीसी और 15 मौखिक संभावित घातक विकारों (ओपीएमडी), यानी, उपकला डिसप्लेसिया और मौखिक सबम्यूकोस फाइब्रोसिस वाले 43 व्यक्तियों के परिणामों की रिपोर्ट करते हैं। हमने शारीरिक (इन-विवो) में फ्लोरेसेंस उत्सर्जन स्पेक्ट्रम में एक लाल-शिफ्ट का अवलोकन किया। हमने सामान्य की तुलना में ओएससीसी, एपिथेलियल डिसप्लेसिया और ओरल सबम्यूकोस फाइब्रोसिस के मामले में क्रमशः 7.72 ± 6 नैनोमीटर, 3 ± 4.36 नैनोमीटर और 1.33 ± 0.47 नैनोमीटर का रेड-शिफ्ट पाया। परिणामों की तुलना हिस्टोपैथोलॉजी से की गई और उन्हें सुसंगत पाया गया। इसके अलावा, एमएफ-आईएस प्रणाली एकल पद्धति का उपयोग करने वाले उपकरणों की तुलना में उच्च सटीकता और संवेदनशीलता के साथ वास्तविक समय में परिणाम प्रदान करती है। हमारा सिस्टम रोगियों की कम संख्या (ओएससीसी के 28 रोगियों) के साथ भी क्रमशः 100% और 94.7% की संवेदनशीलता और विशिष्टता के साथ 97% की सटीकता प्राप्त कर सकता है। प्रस्तावित एमएफ-आईएस डिवाइस में भविष्य में मौखिक कैंसर की तेजी से जांच और निदान की काफी संभावनाएं हैं।

स्तन और मौखिक कैंसर के लिए स्मार्टफोन-आधारित इमेजिंग और स्पेक्ट्रोस्कोपिक उपकरणों के विकास के बाद, जीवित ऊतकों के भीतर और इन-विवो मोड में सूक्ष्म स्तर पर कैंसर की प्रगति में वास्तविक समय के सेलुलर परिवर्तनों का निरीक्षण करने की आवश्यकता उत्पन्न होती है। जबकि मौजूदा माइक्रो-एंडोस्कोप इस उद्देश्य को पूरा करते हैं, उनकी जटिलता और भारीपन उनकी कार्यक्षमता को केवल इमेजिंग तक सीमित कर देती है। इसे संबोधित करते हुए, हमने एक माइक्रो-स्पेक्ट्रो-एंडोस्कोप (एमएसई) विकसित किया, जो कॉम्पैक्ट और उपयोगकर्ता के अनुकूल है, जो इमेजिंग और स्पेक्ट्रोस्कोपिक दोनों तौर-तरीकों में काम करता है। एमएसई माइक्रो-एंडोस्कोपी के लिए एक ग्रेडेड इंडेक्स (जीआरआईएन) रॉड-लेंस का उपयोग करता है और स्पेक्युलर प्रतिबिंबों को खत्म करने के लिए तिरछी रोशनी का उपयोग करता है। विशेष रूप से, एमएसई 4.38 माइक्रॉन का स्थानिक रिज़ॉल्यूशन और 0.05 नैनोमीटर का वर्णक्रमीय रिज़ॉल्यूशन प्राप्त करता है, जिसमें 1.44×2.55 मिमी² मापने वाला दृश्य क्षेत्र शामिल होता है। एफ और एफएल तकनीकों का उपयोग करते हुए, हमारा एमएसई ओएससीसी, मौखिक डिसप्लेसिया और सामान्य ऊतकों की जांच करता है। विश्लेषण से इन मामलों में महत्वपूर्ण स्पेक्ट्रम पैरामीटर बदलावों के साथ-साथ विशिष्ट एफएल कंट्रास्ट साधनों का पता चलता है, विशेष रूप से ओएससीसी और मौखिक डिसप्लेसिया परिदृश्यों में देखा गया है।

एमएसई का एफएल मोड में आवेदन करने के बाद, हमने विकसित MSE में एफ को एकीकृत करने का कार्य किया। इस एकीकरण ने हमें ल्यूपस नेफ्रिटिस (एलएन) की जांच करने में सक्षम बनाया, जो एक ऑटोइम्यून बीमारी है जो प्रोटीनुरिया द्वारा विशेषता है। तीन चरणों में एमआरएल स्ट्रेन से किडनी के ऊतकों का उपयोग करते हुए: युवा, एंटीबॉडी-पॉजिटिव और प्रोटीनुरिया से पीड़ित, हमने एफ स्पेक्ट्रोस्कोपी का उपयोग किया। हमारे निष्कर्षों ने इन तीन मामलों में अलग-अलग बदलावों का खुलासा किया, जो विशेष रूप से हिस्टोपैथोलॉजिकल टिप्पणियों के साथ संरेखित थे। एमएसई ने एफ और एफएल मोड में अपनी दोहरी-मोडैलिटी, इमेजिंग और स्पेक्ट्रोस्कोपी के साथ, अपनी बहुमुखी नैदानिक क्षमता का प्रदर्शन करते हुए, मौखिक कैंसर और प्रोटीनुरिया से पीड़ित गुर्दे के नमूनों की प्रभावी ढंग से जांच की है।

अंत में, हमने मौखिक कैंसर के ऊतकों के साथ फ्लोरेसेंस सोडियम नमक (एफएसएस) की टैगिंग से संबंधित एक व्यापक परीक्षण किया, जिसका उपयोग पहले हमारे पिछले शोध में एफएल तकनीक के लिए किया गया था। इस अध्ययन में एफएसएस के साथ मौखिक कैंसर की बाह्य-शरीरी और इन-विवो दोनों जांचों को शामिल किया गया, जिससे हमारे मुँह कैंसर की पहचान और विश्लेषण के संदर्भ में इसके अनुप्रयोग और व्यवहार की समझ में विस्तार हुआ।

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ABBREVIATION

AF- Autofluorescence

AUC- Area Under the Curve

FAD-Flavin Adenine Dinucleotide

FL- Fluorescence

FSS- Fluorescein Sodium Salt

FWHM- Full Width Half Maximum

GRIN- Graded Index

ICG-Invasive Ductal Carcinoma

IDC-Invasive Ductal Carcinoma

LN- Lupus Nephritis

MAF-IS- Multimodal Autofluorescence Fluorescence Imaging and Spectroscopic

MRL- Murphy Roth large

MSE- Micro-Spectro-Endoscope

NADH- Nicotinamide Adenine Dinucleotide

OPMD- Oral Potentially Malignant Disorder

OSCC- Oral Squamous Cell Carcinoma

PCA- Principal Component Analysis

ROI- Region of Interest

SVM- Support Vector Machine