

MULTISPECTRAL QUANTITATIVE PHASE AND POLARIZATION MICROSCOPE IMAGING FOR BIOMEDICAL APPLICATIONS

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by

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Submitted

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



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Dedicated to,

My parents

CERTIFICATE

This is to certify that the thesis entitled, “**MULTISPECTRAL QUANTITATIVE PHASE AND POLARIZATION MICROSCOPE IMAGING FOR BIOMEDICAL APPLICATIONS**” submitted by **Ms. PRIYANKA MANN** to the Department of Physics, Indian Institute of Technology Delhi for the award of the degree **DOCTOR OF PHILOSOPHY**. This thesis is a record of bonafide research work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements, which to our knowledge have reached the requisite standard for the submission of this thesis.

The results contained in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

Date:

Place: Delhi, India

Prof. Dalip Singh Mehta

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ABSTRACT

The biomedical research is experiencing a rapid growth with increased demand in diagnostics and advancements in the biomedicine industry. Most of the biological specimens, such as red blood cells, HeLa cells, MG63, human mesenchymal stem cell lines, etc., are transparent under visible light illumination. Although conventional optical microscopes are proficient in visualizing micron-size specimens, but they often fail to provide high-contrast images of transparent specimens, offering only the qualitative details. To overcome this limitation, various imaging techniques have been developed to improve the visibility and provide both the qualitative and quantitative information about the specimens. Quantitative phase microscopy (QPM) has emerged as a non-contact, label-free optical technique capable of offering quantitative insights of the samples. Its applications span diverse fields, such as, life sciences, material science, electronics, and photonics. The underlying principle of quantitative phase imaging (QPI) involves wavefront distortion. When plane wavefronts of incident light interacts with the sample, they undergo deformation due to the refractive index variations across the specimen. This deformation, known as the phase delay, is encoded in the resulting interferograms via the interference between the sample and the reference beams which is recorded on the charged couple device (CCD) camera plane. QPM facilitates the visualization of various biological cells and tissues and aids in determining essential biophysical parameters like morphology, refractive index, cell dry mass, etc. Additionally, considering the birefringent nature of the biological specimens, it becomes necessary to explore their optical anisotropic properties. This can be accomplished through polarization microscopic imaging integrated with a multispectral concept, enabling the extraction of diverse polarization parameters.

In the first part of the thesis, we proposed a Mirau-based white light phase shifting interferometer designed as a whole slide scanner and phase profiler. This system aims to determine both the qualitative and quantitative information over a large field of view. The lateral translation of the sample slide was executed such that there exists a correlation between adjacent frames, and these frames were stitched together using correlation functions. The final stitched image has a field-of-view of 0.24×1.14 mm which can be extended further with high resolution ~ 0.8 μm . Circular Hough transform (CHT) algorithm was implemented for cell counting, and five-step phase-shifting algorithm was employed to retrieve the phase profiles over a large field of view. This methodology

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was employed to investigate the difference between normal and anemic erythrocytes, revealing significant changes in anemic cells compared to normal cells.

To address the wavelength-dependent refractive index variations in biological specimens, a wavelength tunable light source utilizing a supercontinuum source and acousto-optic tunable filter (AOTF) based multispectral digital holographic microscopy (DHM) system was developed. This system was employed for multispectral quantitative phase imaging of biological cells across a wide range of wavelengths. The refractive index was assessed at each wavelength, offering a multispectral response of the cell lines in the visible range from 510 to 650 nm with a sampling size of 10 nm. Since smoothly varying surfaces (biological specimens) will not suffer from phase ambiguity as we can trace the deformation of fringes accordingly. However, when it comes to step height measurements, they suffer from phase ambiguities.

To address these ambiguities and the bulkiness and expense of the aforementioned tunable light source, in the next work a compact and cost-effective multispectral light-emitting diode (LED) based tunable light source was developed for 3-D surface profilometry. This was achieved using synthetic wavelength scanning interferometry with multiple-color LEDs with extended range of height measurement. This system offers an extended range of height measurements, from sub-wavelength to tens of wavelength orders ($0.63\text{ }\mu\text{m}$ up to $4.8\text{ }\mu\text{m}$). Notably, it eliminates the need for expensive multiple-color filters or any wavelength scanning mechanism, along with a broadband light source.

Further, to investigate the interaction of polarized light with biological specimens, we presented a Stokes imaging-based multispectral polarization based polarimetric imaging of oral tissue samples. This was accomplished using a polarization microscope system with a broadband light source and polarization-based parameters, such as the degree of polarization, angle of the fast axis, retardation, and birefringence has been retrieved. The polarimetric properties of oral tissue at different stages of cancer were analysed, revealing significant changes from normal to pre-cancerous lesions to cancerous stages in birefringence quantification as $(1.7\pm0.1)\times10^{-3}$, $(2.5\pm0.2)\times10^{-3}$ and $(3.3\pm0.2)\times10^{-3}$ respectively.

Abstract

Finally, we proposed a polarization sensitive non-interferometric based quantitative phase microscope system to achieve birefringence mapping of oral tissue. The birefringence measurement system is employed by integrating a polarization microscope with a phase retrieval algorithm based on the transport of intensity equation. In this context, polarization-sensitive phase retrieval was performed for different stages of oral tissue samples, with potential applications in classification problems.

The present thesis will discuss improvements, advantages, challenges, and developments in label-free multispectral quantitative phase and polarization imaging techniques and their applications majorly for biological specimens and industrial samples as well for the betterment of the field.

सार

बायोमेडिसिन उद्योग में निदान और प्रगति की बढ़ती मांग के साथ बायोमेडिकल अनुसंधान तेजी से विकास का अनुभव कर रहा है। अधिकांश जैविक नमूने, जैसे कि लाल रक्त कोशिकाएं, हेला कोशिकाएं, एमजी63, मानव मेसेनकाइमल स्टेम सेल लाइनें, आदि, दृश्य प्रकाश रोशनी के तहत पारदर्शी होते हैं। यद्यपि पारंपरिक ऑप्टिकल माइक्रोस्कोप माइक्रोन-आकार के नमूनों को देखने में कुशल हैं, लेकिन वे अक्सर पारदर्शी नमूनों की उच्च-विपरीत छवियां प्रदान करने में विफल रहते हैं, केवल गुणात्मक विवरण प्रदान करते हैं। इस सीमा को पार करने के लिए, दृश्यता में सुधार करने और नमूनों के बारे में गुणात्मक और मात्रात्मक दोनों जानकारी प्रदान करने के लिए विभिन्न इमेजिंग तकनीकों का विकास किया गया है। मात्रात्मक चरण माइक्रोस्कोपी (क्यूपीएम) एक गैर-संपर्क, लेबल-मुक्त ऑप्टिकल तकनीक के रूप में उभरी है जो नमूनों की मात्रात्मक अंतर्दृष्टि प्रदान करने में सक्षम है। इसके अनुप्रयोग विभिन्न क्षेत्रों में फैले हुए हैं, जैसे, जीवन विज्ञान, सामग्री विज्ञान, इलेक्ट्रॉनिक्स और फोटोनिक्स। मात्रात्मक चरण इमेजिंग (क्यूपीआई) के अंतर्निहित सिद्धांत में वेवफ्रंट विरूपण शामिल है। जब आपतित प्रकाश के समतल तरंगाग्र नमूने के साथ अंतःक्रिया करते हैं, तो वे पूरे नमूने में अपवर्तक सूचकांक भिन्नता के कारण विरूपण से गुजरते हैं। यह विकृति, जिसे चरण विलंब के रूप में जाना जाता है, नमूना और संदर्भ बीम के बीच हस्तक्षेप के माध्यम से परिणामी इंटरफेरोग्राम में एन्कोड किया गया है जो चार्ज युगल डिवाइस (सीसीडी) कैमरा विमान पर दर्ज किया गया है। क्यूपीएम विभिन्न जैविक कोशिकाओं और ऊतकों के दृश्य की सुविधा प्रदान करता है और आकृति विज्ञान, अपवर्तक सूचकांक, कोशिका शुष्क द्रव्यमान आदि जैसे आवश्यक जैव-भौतिकीय मापदंडों को निर्धारित करने में सहायता करता है। इसके अतिरिक्त, जैविक नमूनों की द्विअर्थी प्रकृति को ध्यान में रखते हुए, उनके ऑप्टिकल अनिसोट्रोपिक गुणों का पता लगाना आवश्यक हो जाता है। इसे मल्टीस्पेक्ट्रल अवधारणा के साथ एकीकृत ध्रुवीकरण सूक्ष्म इमेजिंग के माध्यम से पूरा किया जा सकता है, जो विविध ध्रुवीकरण मापदंडों के निष्कर्षण को सक्षम बनाता है।

थीसिस के पहले भाग में, हमने एक मिराऊ-आधारित सफेद प्रकाश चरण शिफ्टिंग इंटरफेरोमीटर का प्रस्ताव रखा, जिसे संपूर्ण स्लाइड स्कैनर और चरण प्रोफाइलर के रूप में डिज़ाइन किया गया था। इस प्रणाली का लक्ष्य दृश्य के बड़े क्षेत्र पर गुणात्मक और मात्रात्मक दोनों जानकारी निर्धारित करना है। नमूना स्लाइड का पार्श्व अनुवाद इस तरह निष्पादित किया गया था कि आसन्न फ्रेमों के बीच एक सहसंबंध मौजूद था, और इन फ्रेमों को सहसंबंध कार्यों का उपयोग करके एक साथ सिला गया था। अंतिम सिलाई वाली छवि का फ़ील्ड-ऑफ़-व्यू 0.24×1.14 मिमी है जिसे उच्च रिज़ॉल्यूशन $\sim 0.8 \mu\text{m}$ के साथ आगे बढ़ाया जा सकता है। सेल गिनती के लिए सर्कुलर हफ़ ट्रांसफॉर्म (सीएचटी) एल्गोरिदम लागू किया गया था, और दृश्य के बड़े क्षेत्र पर चरण प्रोफाइल को पुनः प्राप्त करने के लिए पांच-चरण चरण-शिफ्टिंग एल्गोरिदम नियोजित किया गया था। इस पद्धति का उपयोग सामान्य और एनेमिक एरिथ्रोसाइट्स के

बीच अंतर की जांच करने के लिए किया गया था, जिससे सामान्य कोशिकाओं की तुलना में एनेमिक कोशिकाओं में महत्वपूर्ण परिवर्तन सामने आए।

जैविक नमूनों में तरंग दैर्ध्य-निर्भर अपवर्तक सूचकांक भिन्नताओं को संबोधित करने के लिए, एक सुपरकॉन्टिनम स्रोत और एकाँस्टो-ऑप्टिक ट्यून करने योग्य फ़िल्टर (एओटीएफ) आधारित मल्टीस्पेक्ट्रल डिजिटल होलोग्राफिक माइक्रोस्कोपी (डीएचएम) प्रणाली का उपयोग करते हुए एक तरंग दैर्ध्य ट्यून करने योग्य प्रकाश स्रोत विकसित किया गया था। इस प्रणाली को तरंग दैर्ध्य की एक विस्तृत श्रृंखला में जैविक कोशिकाओं की मल्टीस्पेक्ट्रल मात्रात्मक चरण इमेजिंग के लिए नियोजित किया गया था। प्रत्येक तरंग दैर्ध्य पर अपवर्तक सूचकांक का मूल्यांकन किया गया था, जो 10 एनएम के नमूने के आकार के साथ 510 से 650 एनएम तक दृश्य सीमा में सेल लाइनों की बहुस्पेक्ट्रल प्रतिक्रिया की पेशकश करता है। चूंकि सुचारू रूप से बदलती सतहें (जैविक नमूने) चरण अस्पष्टता से ग्रस्त नहीं होंगी क्योंकि हम तदनुसार फ़िजों के विरूपण का पता लगा सकते हैं। हालाँकि, जब चरण की ऊँचाई मापने की बात आती है, तो वे चरण अस्पष्टताओं से ग्रस्त होते हैं।

इन अस्पष्टताओं और उपरोक्त ट्यून करने योग्य प्रकाश स्रोत की भारीपन और व्यय को संबोधित करने के लिए, अगले कार्य में 3-डी सतह प्रोफिलोमेट्री के लिए एक कॉम्पैक्ट और लागत प्रभावी मल्टीस्पेक्ट्रल प्रकाश उत्सर्जक डायोड (एलईडी) आधारित ट्यून करने योग्य प्रकाश स्रोत विकसित किया गया था। ऊँचाई माप की विस्तारित सीमा के साथ बहु-रंग एलईडी के साथ सिंथेटिक तरंग दैर्ध्य स्कैनिंग इंटरफेरोमेट्री का उपयोग करके इसे हासिल किया गया था। यह प्रणाली उप-तरंग दैर्ध्य से लेकर दसियों तरंग दैर्ध्य ऑर्डर (0.63 μm से 4.8 μm तक) ऊँचाई माप की एक विस्तारित श्रृंखला प्रदान करती है। विशेष रूप से, यह ब्रॉडबैंड प्रकाश स्रोत के साथ-साथ महंगे बहु-रंग फिल्टर या किसी तरंग दैर्ध्य स्कैनिंग तंत्र की आवश्यकता को समाप्त करता है।

इसके अलावा, जैविक नमूनों के साथ ध्रुवीकृत प्रकाश की परस्पर क्रिया की जांच करने के लिए, हमने मौखिक ऊतक नमूनों की स्टोक्स इमेजिंग-आधारित मल्टीस्पेक्ट्रल ध्रुवीकरण आधारित पोलारिमेट्रिक इमेजिंग प्रस्तुत की। यह एक ब्रॉडबैंड प्रकाश स्रोत और ध्रुवीकरण-आधारित मापदंडों के साथ एक ध्रुवीकरण माइक्रोस्कोप प्रणाली का उपयोग करके पूरा किया गया था, जैसे कि ध्रुवीकरण की डिग्री, तेज अक्ष का कोण, मंदता और द्विभाजन को पुनः प्राप्त किया गया है। कैंसर के विभिन्न चरणों में मौखिक ऊतक के पोलारिमेट्रिक गुणों का विश्लेषण किया गया, जिसमें सामान्य से पूर्व-कैंसर वाले घावों से लेकर कैंसर के चरणों में बायरफ़्रींगेंस मात्रा निर्धारण में महत्वपूर्ण परिवर्तन सामने आए $(1.7 \pm 0.1) \times 10^{-3}$, $(2.5 \pm 0.2) \times 10^{-3}$ और $(3.3 \pm 0.2) \times 10^{-3}$ क्रमशः।

अंत में, हमने मौखिक ऊतक के द्विअर्थी मानचित्रण को प्राप्त करने के लिए एक ध्रुवीकरण संवेदनशील गैर-इंटरफेरोमेट्रिक आधारित मात्रात्मक चरण माइक्रोस्कोप प्रणाली का प्रस्ताव दिया। तीव्रता समीकरण के परिवहन के

आधार पर एक चरण पुनर्प्राप्ति एल्गोरिथ्म के साथ एक ध्रुवीकरण माइक्रोस्कोप को एकीकृत करके द्विअर्थी माप प्रणाली को नियोजित किया जाता है। इस संदर्भ में, वर्गीकरण समस्याओं में संभावित अनुप्रयोगों के साथ, मौखिक ऊतक नमूनों के विभिन्न चरणों के लिए ध्रुवीकरण-संवेदनशील चरण पुनर्प्राप्ति की गई थी।

वर्तमान थीसिस लेबल-मुक्त मल्टीस्पेक्ट्रल मात्रात्मक चरण और ध्रुवीकरण इमेजिंग तकनीकों में सुधार, फायदे, चुनौतियों और विकास और मुख्य रूप से जैविक नमूनों और औद्योगिक नमूनों के साथ-साथ क्षेत्र की बेहतरी के लिए उनके अनुप्रयोगों पर चर्चा करेगी।

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