

**DIGITAL HOLOGRAPHY AND
HOLOGRAPHIC OPTICAL ELEMENTS IN
QUANTITATIVE CELL IMAGING AND
TEMPERATURE MEASUREMENT**

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**CENTRE FOR SENSORS, INSTRUMENTATION AND
CYBER PHYSICAL SYSTEM ENGINEERING**

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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Submitted

**in fulfillment of the requirements of the degree of Doctor of Philosophy
to the**



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*Dedicated To My
'Guru'*

CERTIFICATE

This is to certify that the thesis entitled “**DIGITAL HOLOGRAPHY AND HOLOGRAPHIC OPTICAL ELEMENTS IN QUANTITATIVE CELL IMAGING AND TEMPERATURE MEASUREMENT**” being submitted by **Mr. VIVEK RASTOGI** to the **INDIAN INSTITUTE OF TECHNOLOGY DELHI** for the award of the degree of “**DOCTOR OF PHILOSOPHY**”, is a record of the authentic research work carried out by him under our supervision and guidance. He has fulfilled all the requirements for submission of this thesis, which to the best of our knowledge has reached the required standard.

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

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Date:

Vivek Rastogi

ABSTRACT

Optical measurement techniques have evolved from research to commercial instrumentation over the last few decades. Optical measurement techniques are non-contact type and non-intrusive in nature and do not disrupt the process or mechanism being investigated/analyzed. Optical measurement methods have been employed in the field of science, engineering, aerospace, industrial production and also in biomedical applications for almost a century. However, various additional advancements have broadened its scope and efficiency. The invention of the lasers was the most significant breakthrough of these new advancements. Many of the limits imposed by traditional light sources have been addressed by the development of lasers, allowing for the development of several novel interferometric techniques. Holography introduced a new dimension to optical measuring techniques that take advantage of lasers' unique characteristics such as monochromaticity, coherence, stability, directionality, and high output power, particularly the extended coherence of the laser light source. Owing to improvements in faster semiconductors, electronics and digital computing power, digital holography has witnessed a remarkable advancement in the last decade.

A new paradigm in general imaging applications has emerged with the development of digital holography (DH). In today's world, DH is a board area with several applications in microscopy, biomedicine, manufacturing, commercial electronics, augmented and virtual reality, entertainment, defense, and security etc. Due to its non-contact, non-intrusive, and label-free nature, DH has emerged as a valuable tool in microscopy and metrology applications. The major advantage of DH is that it provides both amplitude as well as phase information of the object wavefront. Conventional microscopic techniques provide good contrast high-resolution amplitude/intensity images of the objects. However, in

conventional optical microscopic methods phase information of the object is absent. Digital holographic interferometry (DHI) based microscopic systems have the significant advantage that they provide phase information. This will facilitate the user to acquire necessary quantitative information, such as refractive index, cell thickness profile, cell morphology etc. DHI provides both qualitative and quantitative information of the object under test using optical recording and numerical reconstruction techniques. Two main advantages of DHI based microscopy system make it different from other microscopic techniques. First, the reconstructed image of the object under test can be made to focus on different planes of the object by numerical reconstruction without using optomechanical adjustment, and second, in digital holographic interferometric microscopy distortions due to aberration in the optical system are avoided by the interferometric comparison of the reconstructed phase with and without the object under study. Also, in DHI, the interference phase can be determined by subtracting the phase of the individual object wavefront reconstructed in its different states without generating the phase shifting interferograms. Thus, DHI eliminates additional efforts to compute the interference phase. These features of DHI increases the measurement flexibility and makes the measurement process faster, enabling DHI to be implemented in almost real-time. DHI has been widely used in metrology applications such as for the measurement of temperature in laminar free convective flow of water, measurement of natural convective heat transfer coefficient along the surface of a heated wire, effect of magnetic field on temperature profile of gaseous flames, to measure dynamic flow field, measurement of refractive index, temperature, displacement, vibration, tilt, slope, contouring, diffusion coefficient etc. DHI is a burgeoning field with applications in metrology, biomedical imaging, three-dimensional imaging, and three-dimensional display systems. However, recording and displaying a 3D picture of an object are just a small fraction of holography. The holographic optical element

(HOE) is a type of optical diffracting element that can incorporate a variety of features onto a single transparent thin film or plastic. The most significant benefit of HOEs is their ability to simultaneously include several different optical functions on a single element. The ability to measure the deformations and change in the index of refraction of transparent objects at the microscopic level makes digital holographic interferometry a prominent tool in metrology and biomedical application.

In this thesis, a volume phase holographic optical element based DHI system is designed and used for quantitative cell imaging and temperature measurement. In addition to this, a holo-shear lens based lateral shear interferometer is used to investigate the temperature profile and temperature stability of micro diffusion flame under the influence of the magnetic field. This thesis is organized into six chapters.

Chapter 1 provides a brief introduction of optical measurement techniques and their application in microscopy and metrology, as well as a brief description of conventional holography and digital holography. This chapter also covers the detailed description of digital hologram recording and various reconstruction processes. The brief outline of the research work presented in this thesis is also summarized in this chapter.

Chapter 2 presents label-free quantitative cell imaging using digital holographic interferometry. In this chapter, a conventional Mach-Zehnder configuration based digital holographic microscopic (DHM) system is demonstrated for non-destructive, label-free, in-vitro imaging of small microorganism *Escherichia coli* (*E. coli*) bacteria. Also, a volume phase holographic grating (VPHG) based DHI system is introduced for quantitative phase imaging of microscopic biological cells such as human red blood cells, white blood cells, platelets, and micro-sized *Staphylococcus aureus* (*S. aureus*) bacteria cells.

Chapter 3 presents a volume phase holographic optical element based digital holographic interferometer, which is used for the measurement of temperature, temperature profile and temperature fluctuations inside the macro and micro candle flames. The proposed system is simple, compact, requires lesser optical components, and is easy to use.

Chapter 4 introduces a non-contact temperature measurement of human hand skin using volume phase holographic optical element based digital holographic interferometer. In this system, a volume phase holographic optical element based DHI system has been used in a lensless Fourier transform (LLFT) configuration.

Chapter 5 presents a holo-shear lens-based interferometer used to investigate the effect of gradient magnetic fields (i.e., upward decreasing and upward increasing), uniform magnetic field on the temperature, and temperature profile of wick stabilized micro diffusion flame created from the candle. The holo-shear lens-based interferometer can be used to study other sensitive phenomena such as thermal and chemical quenching in microflames.

Chapter 6 concludes the work presented in the thesis and suggests some ideas for further investigations.

सार

पिछले कुछ दशकों में ऑप्टिकल माप तकनीक अनुसंधान से वाणिज्यिक उपकरण तक विकसित हुई है। ऑप्टिकल माप तकनीक गैर-संपर्क तथा गैर-हस्तक्षेप प्रकार वाली होती हैं और जांच/विश्लेषण की जा रही प्रक्रिया या तंत्र को बाधित नहीं करती हैं। ऑप्टिकल माप विधियों को विज्ञान, इंजीनियरिंग, एयरोस्पेस, औद्योगिक उत्पादन के क्षेत्र में और बायोमेडिकल अनुप्रयोगों में भी लगभग एक सदी से नियोजित किया गया है। हालांकि, विभिन्न अतिरिक्त प्रगति ने इसके दायरे और दक्षता को विस्तृत किया है। लेजर का आविष्कार इन नई प्रगति की सबसे महत्वपूर्ण सफलता थी। लेजर के विकास ने पारंपरिक प्रकाश स्रोतों की कई सीमाओं का हल प्रदान किया, जिससे कई नई इंटरफेरोमेट्रिक तकनीकों के विकास की अनुमति मिलती है। होलोग्राफी ने ऑप्टिकल माप तकनीकों के लिए एक नया आयाम पेश किया जो लेजर की अनूठी विशेषताओं जैसे कि मोनोक्रोमैटिकिटी, कोहरेन्स, स्थिरता, दिशात्मकता और उच्च आउटपुट पावर, विशेष रूप से लेजर प्रकाश स्रोत के विस्तारित कोहरेन्स का लाभ उठाती है। तेज अर्धचालकों, इलेक्ट्रॉनिक्स और डिजिटल कंप्यूटिंग शक्ति में सुधार के कारण, पिछले दशक में डिजिटल होलोग्राफी में उल्लेखनीय प्रगति हुई है।

डिजिटल होलोग्राफी (डीएच) के विकास के साथ सामान्य इमेजिंग अनुप्रयोगों में एक नया प्रतिमान उभरा है। आज के विश्व में, डीएच एक विस्तृत क्षेत्र है जिसके उपयोग माइक्रोस्कोपी, बायोमेडिसिन, मैनुफैक्चरिंग, वाणिज्यिक इलेक्ट्रॉनिक्स, ऑगमेंटेड और आभासी वास्तविकता, मनोरंजन, रक्षा और सुरक्षा आदि में पाए जाते हैं। अपने गैर-संपर्क, गैर-हस्तक्षेप और लेबल-मुक्त प्रकृति के कारण, डीएच माइक्रोस्कोपी और मेट्रोलॉजी अनुप्रयोगों में एक मूल्यवान उपकरण के रूप में उभरा है। डीएच का प्रमुख लाभ यह है कि यह ऑब्जेक्ट वेवफ्रंट के एम्प्लीट्यूड और साथ ही फेज़ दोनों की जानकारी प्रदान करता है। पारंपरिक माइक्रोस्कोपिक तकनीकें वस्तुओं की अच्छी कंट्रास्ट वाली उच्च-रिज़ॉल्यूशन एम्प्लीट्यूड/तीव्रता वाले चित्र प्रदान करती हैं। तथापि, पारंपरिक ऑप्टिकल माइक्रोस्कोपिक तकनीकों में वस्तु के फेज़ की जानकारी अनुपस्थित होती है। डिजिटल होलोग्राफिक इंटरफेरोमेट्री (डीएचआई) पर आधारित माइक्रोस्कोपिक प्रणालियों का महत्वपूर्ण लाभ यह है कि वे फेज़ की जानकारी प्रदान करती हैं। यह उपयोगकर्ता को आवश्यक परिमाणात्मक जानकारी प्राप्त करने की

सुविधा प्रदान करेगा, जैसे कि अपवर्तक सूचकांक, सेल थिकनेस प्रोफाइल, सेल आकृति इत्यादि डीएचआई ऑप्टिकल रिकॉर्डिंग और संख्यात्मक पुनर्निर्माण तकनीकों का उपयोग करके परीक्षण के तहत वस्तु की गुणात्मक और परिमाणात्मक दोनों जानकारी प्रदान करता है। डीएचआई आधारित माइक्रोस्कोपी प्रणाली के दो मुख्य लाभ इसे अन्य माइक्रोस्कोपी तकनीकों से अलग बनाते हैं। सबसे पहले, परीक्षण के तहत वस्तु की पुनर्निर्मित छवि को ऑप्टोमैकेनिकल समायोजन का उपयोग किए बिना संख्यात्मक पुनर्निर्माण द्वारा वस्तु के विभिन्न तलों पर फोकस करने के लिए बनाया जा सकता है, और दूसरा, डिजिटल होलोग्राफिक इंटरफेरोमेट्रिक माइक्रोस्कोपी में अध्ययन के तहत वस्तु के साथ और उसके बिना पुनर्निर्मित फेज की इंटरफेरोमेट्रिक तुलना का प्रयोग करके ऑप्टिकल सिस्टम में उपस्थित विपथन के कारण विकृतियों को दूर किया जाता है। इसके साथ ही, डीएचआई में, फेज स्थानांतरण इंटरफेरोग्राम उत्पन्न किए बिना अपनी अलग-अलग स्थितियों में पुनर्निर्मित व्यक्तिगत ऑब्जेक्ट वेवफ्रंट के फेज को घटाकर इंटरफेरेंस फेज को प्राप्त किया जा सकता है। इस प्रकार, डीएचआई इंटरफेरेंस फेज की गणना के लिए अतिरिक्त प्रयासों को समाप्त करता है। डीएचआई की ये विशेषताएं माप के लचीलेपन को बढ़ाती हैं और माप प्रक्रिया को तेज बनाती हैं, जिससे डीएचआई को लगभग वास्तविक समय में लागू किया जा सकता है। डीएचआई को व्यापक रूप से मेट्रोलॉजी अनुप्रयोगों में उपयोग किया जाता है जैसे कि पानी के लामिनार मुक्त संवहन प्रवाह में तापमान की माप, गर्म तार की सतह पर प्राकृतिक संवहन ऊष्मा हस्तांतरण गुणांक की माप, गैसीय ज्वालाओं के तापमान प्रोफाइल पर चुंबकीय क्षेत्र का प्रभाव, गतिशील प्रवाह क्षेत्र, अपवर्तक सूचकांक, तापमान, विस्थापन, कंपन, झुकाव, स्लोप, कंटूरिंग, प्रसार गुणांक इत्यादि। डीएचआई मेट्रोलॉजी, बायोमेडिकल इमेजिंग, ग्री-डायमेशनल इमेजिंग और ग्री-डायमेशनल डिस्प्ले सिस्टम अनुप्रयोगों में एक उभरता हुआ क्षेत्र है। हालांकि, किसी वस्तु की ग्री-डायमेशनल तस्वीर को रिकॉर्ड करना और प्रदर्शित करना होलोग्राफी का एक छोटा सा अंश है। होलोग्राफिक ऑप्टिकल एलिमेंट (एचओई) एक प्रकार का ऑप्टिकल विवर्तन एलिमेंट है जो एक पारदर्शी पतली फिल्म या प्लास्टिक पर विभिन्न प्रकार की विशेषताओं को समाविष्ट कर सकता है। एचओई का सबसे महत्वपूर्ण लाभ एक ही एलिमेंट पर कई अलग-अलग ऑप्टिकल कार्यों को एक साथ समाविष्ट करने की उनकी क्षमता है। सूक्ष्म स्तर पर पारदर्शी वस्तुओं में विकृति और

अपवर्तन के सूचकांक परिवर्तन को मापने की क्षमता डिजिटल होलोग्राफिक इंटरफेरोमेट्री को मेट्रोलॉजी और बायोमेडिकल एप्लिकेशन में एक प्रमुख उपकरण बनाती है।

इस शोध प्रबंध में, वॉल्यूम फेज़ होलोग्राफिक ऑप्टिकल एलिमेंट आधारित डीएचआई सिस्टम को परिमाणात्मक सेल इमेजिंग तथा तापमान माप के लिए डिज़ाइन और उपयोग किया गया है। इसके अलावा, चुंबकीय क्षेत्र के प्रभाव में सूक्ष्म प्रसार लौ के तापमान प्रोफाइल और तापमान स्थिरता की जांच के लिए एक होलो-शीयर लेंस आधारित लेटरल शियर इंटरफेरोमीटर का उपयोग किया गया है। यह शोध प्रबंध छह अध्यायों में व्यवस्थित है।

अध्याय १ माइक्रोस्कोपी और मेट्रोलॉजी में ऑप्टिकल माप तकनीकों और उनके अनुप्रयोगों का एक संक्षिप्त परिचय प्रदान करता है, साथ ही पारंपरिक होलोग्राफी और डिजिटल होलोग्राफी का संक्षिप्त विवरण प्रदान करता है। इस अध्याय में डिजिटल होलोग्राम रिकॉर्डिंग और विभिन्न पुनर्निर्माण प्रक्रियाओं का विस्तृत विवरण भी शामिल है। इस शोध प्रबंध में प्रस्तुत शोध कार्य की संक्षिप्त रूपरेखा भी इसी अध्याय में दी गई है।

अध्याय २ डिजिटल होलोग्राफिक इंटरफेरोमेट्री का उपयोग करके लेबल-मुक्त परिमाणात्मक सेल इमेजिंग प्रस्तुत करता है। इस अध्याय में, एक पारंपरिक मेक-ज़ेन्डर कॉन्फ़िगरेशन आधारित डिजिटल होलोग्राफिक माइक्रोस्कोपिक (डीएचएम) प्रणाली को छोटे सूक्ष्मजीव एशेरिकिया कोलाए (ई० कोलाए) बैक्टीरिया के गैर-विनाशकारी, लेबल-मुक्त, इन-विट्रो इमेजिंग के लिए प्रदर्शित किया गया है। इसके अलावा, मानव लाल रक्त कोशिकाओं, सफेद रक्त कोशिकाओं, प्लेटलेट्स, और सूक्ष्म आकार के स्टैफिलोकॉकस ऑरियस (एस० ऑरियस) बैक्टीरिया सेल जैसे सूक्ष्म जैविक कोशिकाओं की परिमाणात्मक फेज़ इमेजिंग के लिए वॉल्यूम फेज़ होलोग्राफिक ग्रेटिंग (वीपीएचजी) आधारित डीएचआई प्रणाली को प्रस्तुत किया गया है।

अध्याय ३ एक वॉल्यूम फेज़ होलोग्राफिक ऑप्टिकल एलिमेंट आधारित डिजिटल होलोग्राफिक इंटरफेरोमीटर प्रस्तुत करता है, जिसका उपयोग मैक्रो और माइक्रो कैंडल प्लेम के अंदर तापमान, तापमान प्रोफाइल और तापमान में उतार-चढ़ाव के

माप के लिए किया गया है। प्रस्तावित प्रणाली सरल, कॉम्पैक्ट है, इसमें कम ऑप्टिकल पुरजों की आवश्यकता है, और इसका उपयोग करना आसान है।

अध्याय ४ वॉल्यूम फेज़ होलोग्राफिक ऑप्टिकल एलिमेंट आधारित डिजिटल होलोग्राफिक इंटरफेरोमीटर का उपयोग करके मानव हाथ की त्वचा के गैर-संपर्क तापमान माप का परिचय देता है। इस प्रणाली में, एक लेंसलेस फूरियर ट्रांसफॉर्म (एलएलएफटी) कॉन्फ़िगरेशन में वॉल्यूम फेज़ होलोग्राफिक ऑप्टिकल एलिमेंट आधारित डीएचआई सिस्टम का उपयोग किया गया है।

अध्याय ५ एक होलो-शीयर लेंस-आधारित इंटरफेरोमीटर प्रस्तुत करता है जिसका उपयोग करते हुए मोमबत्ती से निर्मित विक-स्थिरीकृत माइक्रो प्रसार फ्लेम में तापमान तथा तापमान प्रोफाइल पर ग्रेडिएंट चुंबकीय क्षेत्रों (अर्थात्, ऊपर की ओर घटते और ऊपर की ओर बढ़ते हुए), एक समान चुंबकीय क्षेत्र के प्रभाव की जांच के लिए किया गया है। होलो-शीयर लेंस-आधारित इंटरफेरोमीटर का उपयोग अन्य संवेदनशील घटनाओं जैसे कि माइक्रोफ्लेम में थर्मल और रासायनिक शमन के अध्ययन के लिए किया जा सकता है।

अध्याय ६ शोध प्रबंध में प्रस्तुत कार्य का निष्कर्ष देता है और आगे की जांच के लिए कुछ विचार सुझाता है।

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LIST OF ABBREVIATIONS

Abbreviation	Definition
DHI	Digital Holographic Interferometry
DH	Digital Holography
DHM	Digital Holographic Microscopy
LLFT	Lensless Fourier Transform
CCD	Charge-Couple Device
CMOS	Complementary Metal-Oxide Semiconductor
DCG	Dichromated Gelatin
HOE	Holographic Optical Element
VPHG	Volume Phase Holographic Grating
MO	Microscope Objective
PH	Pin Hole
CL	Collimator
BS	Beam Splitter
SF	Spatial Filter
<i>Re</i>	Real Function
<i>Im</i>	Imaginary Function
CP	Central Peak
LP	Left Peak
RP	Right Peak
FFT	Fast Fourier Transform
IFFT	Inverse Fast Fourier Transform

QPI	Quantitative Phase Imaging
DIC	Differential Interference Contrast
TTP	Thrombotic Thrombocytopenic Purpura
HUS	Hemolytic Uremic Syndrome
SIM	Structure Illumination Microscopy
MPM	Multiphoton Microscopy
CARS	Coherent Anti-Stokes Raman Scattering
LB	Luria Broth
DI	Deionized
SD	Standard Deviation
OPD	Optical Path Difference
ROI	Region of Interest