WIDE-FIELD LOW COHERENCE INTERFERENCE MICROSCOPY FOR QUANTITATIVE IMAGING OF BIOLOGICAL AND INDUSTRIAL OBJECTS

by

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Dedicated to,

My parents and all those people who try and fail

And then try again

CERTIFICATE

This is to certify that the thesis entitled **"WIDE-FIELD LOW COHERENCE INTERFERENCE MICROSCOPY FOR QUANTITATIVE IMAGING OF BIOLOGICAL AND INDUSTRIAL OBJECTS"** being submitted by **MR VISHAL SRIVASTAVA** to the **Instrument Design Development Center, Indian Institute of Technology Delhi** for the award of the degree of **DOCTOR OF PHILOSOPHY**. This thesis is a record of bona-fide work carried out by him under my guidance and supervision. In my opinion the thesis has reached the standards fulfilling the requirements for submission relating to the degree.

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

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ABSTRACT

During the last several decades investigations on the coherence properties of light field have been extensively done and it is well established that coherence is one of the fundamental property of light. Light source has two kind of coherences, i.e., spatial and temporal coherence. In the recent years both, spatial and temporal coherence properties of light source has received significant scientific interest in imaging, diagnostics, optical metrology, and optical instrumentation in a completely non-invasive manner. More recently, temporally low coherence light sources have been extensively used for quantitative imaging of biological and industrial objects. This is because most of the biological tissues are highly scattering multilayer structures, therefore, optical imaging techniques based on highly temporally coherent light sources, do not provide high resolution good quality images. High resolution, cross-sectional imaging of biological and industrial objects has been achieved using low coherence interferometry which is well known as optical coherence tomography (OCT). But most of OCT modalities provide 2D- or 3D-intensity based images and phase information is lacking. Further, the sub-cellular level imaging of biological cells can not be achieved by conventional fiber-optic based OCT systems. In order to obtain sub-cellular imaging and quantitative information, such as, refractive index (RI) and thickness, the quantitative phase microscopic techniques has been developed. The objective of the present thesis is to develop a wide-field low coherence interference microscopy for quantitative imaging of biological and industrial objects. The main purpose is to obtain sub-cellular level high-resolution imaging and quantitative information about the sample, such as, RI, thickness and height variations. The wide-field microscopy was developed to avoid lateral B-scan of the sample. It

is a noncontact, label-free optical microscopy, which has been increased interest as a non-mechanical scanning, high resolution *enface* imaging method to explore various applications in the field of biology, medicine and material science. Biological samples are dynamic in nature so quantitative imaging is still difficult with conventional WF-OCT. Single-shot WF-OCT is used to obtain quantitative imaging of dynamic samples. The images can be captured in real-time depending on the frame speed of the camera. High-resolution wide-field microscopy is used to obtain quantitative imaging of biological samples and industrial objects. Various studies conducted on *ex-vivo* specimens have demonstrated the high performance of WF-OCT. In conclusion, this thesis provides a deeper understanding of wide-field low coherence interference microscopy for quantitative imaging and three-dimensional reconstructions of biological and industrial objects.

Chapter I presents the literature survey about the low coherence interferometry. This chapter presents the basic principle of OCT, its classification along with a brief review of the past and recent developments in the field and their advantages and disadvantages. The chapter also provides the basic principle of wide-field OCT systems and its applications in several areas including biology and engineering. Various issues, such as, choice of a light source, axial and lateral-resolution, signal-to-noise ratio, performance and limitations of wide-field OCT are also presented. The chapter also emphasizes why quantitative phase imaging is important. A brief description about how WF-LCI is helpful in extracting the quantitative phase is presented.

In Chapter II a full-field Hilbert phase microscopy (HPM) using nearly common path low coherence off-axis interferometry for quantitative imaging of biological cells is reported. On-

axis interferometry requires minimum three phase shifted interferograms to extract the phase information. Most of the live biological samples are dynamic in nature and may change between the acquisition of the multiple interferograms or frames and also the phase noise may increase due to the sample fluctuations. For such cases the off-axis interferometry could be useful, because, we can obtain the quantitative phase information from single interferogram and this can be realized in real-time. In the present chapter, we describe a low coherence full-field Hilbert phase microscopic system based on Mirau-interferometric objective lens for quantitative imaging of biological cells. Spatial carrier frequency of interference fringes was increased by means of introducing tilt in one of the arm of the interferometer, thus making the system off-axis and single interferogram is recorded to reconstruct the phase map. Due to its single-shot in nature, the interferometric image acquisition time is limited only by the recording device which is useful in dynamic biological objects. The main advantages of the proposed system are it is completely non-mechanical scanning (because of full-field detection approach), ease of alignment; high stability because of it is nearly common-path geometry, compactness and single image acquisition for real-time imaging at high frequency. The present system is single shot imaging therefore, the image can be captured in real-time depending on the frame speed which is 15fps in present case.

Chapter III presents a quantitative phase imaging of human red blood cells (RBC's) using phase-shifting white light interference microscopy with colour fringe analysis technique. Blood analysis is useful diagnostic tool for physiological and biochemical states, such as disease detection, mineral content, drug effectiveness, and organ function. In the present chapter the phase shifting white light interference microscopy is used for quantitative phase

imaging and determination of wavelength-dependent refractive index of RBCs phase-shifted interferogram (PSI) are recorded by a color CCD camera and decomposed into Red (R), Green (G) and Blue (B) components digitally. There are two types of color CCD cameras, three-chip and a single chip color CCD camera. The three-chip color CCD system has higher color resolution as it registers red, green and blue interference fringe components pixel-by-pixel but it is costly. On the other hand, a single-chip color CCD produces images based on the Bayer color filter combination in which every pixel registers one color per pixel. Five frame phase-shifting methods were used and phase-shifted white light interferograms are recorded by a single-chip color CCD camera. Individual interferograms of R, G and B color are then separated from each phase-shifted interferogram and processed. From the reconstructed phase maps the refractive index for three wavelengths were calculated. The present technique does not require multiple color laser sources, spectral filters and dispersive optical elements to quantify wavelength dependent phase-maps and refractive index. It uses a low cost white light source, a conventional optical microscope, a nearly common path Mirau-interferometric objective lens and a low cost color CCD camera.

In chapter IV a high-resolution corneal topography and tomography of fish eye using wide-field white light interference microscopy is described. The conventional FF-OCT systems provide only amplitude images and phase information is not recovered. In this chapter, we find the topography of fish eye cornea using wide-field white light optical coherence tomographic (WF-WL-OCT) system with a coaxial and common path optical system based on Mirau interferometric objective lens. Simultaneous amplitude and phase images were obtained by means of recording 2D spatial interferograms at different depths. Multiple phase shifted interferograms were recorded with the help of a piezo-electric transducer (PZT), and

both the amplitude and the phase map of the interference fringe signals were reconstructed. From the reconstructed phase maps the corneal topography and hence the refractive index was determined and from amplitude images the cross-sectional image of fish cornea was reconstructed. However, the most important advantage is that topographic analysis can be done along with the high-quality cross-sectional imaging at a very low price and great ease of use compare to the other conventional OCT systems.

In chapter V a single shot Hilbert phase white light interference microscopy using colour fringe analysis for 3D surface profilometry of Si opto-electronic devices, i.e., Si-integrated circuits (Si-ICs) and Si-Solar cells has been reported. White light interferograms were recorded by a colour CCD camera and the interferogram is decomposed into three colors red, green and blue. Spatial carrier frequency of WL interferogram was increased sufficiently by means of introducing a tilt in the interferometer. Hilbert transform fringe analysis was used to reconstruct the phase map for red, green and blue colors from the single interferogram. 3D-step height map of Si-ICs and Si-solar cells was reconstructed at multiple wavelengths from a single interferogram. Experimental results were compared with Atomic Force Microscopy and found to be close to each other. The present technique is non-invasive, full-field and fast for the determination of surface roughness variation and morphological features of the objects at multiple wavelengths.

Chapter IV Tomographic and volumetric reconstruction of composite materials using full-field swept-source optical coherence tomography (FF-SS-OCT) has been demonstrated. The present chapter deals with the principle, experimental design and application of FF-SS-OCT system. The optical set-up consists of a swept-source system, a compact Mirau interferometer and an area detector. A brief characterization of SLD and AOTF and how a frequency-

tunable quasi-monochromatic source (swept-source) has been realized by scanning the wavelength of broad-band SLD using AOTF has been presented. The importance of composite material has increased tremendously in engineering and industrial applications. High performance, strength, stiffness and low weight are the attractive features which results in multidisciplinary applications. The FF-SS-OCT is based on a co-axial and common-path optical interferometric system based on Mirau objective lens. By means of sweeping the frequency of the broad-band light source, multiple spectral interferograms are recorded and stacked in the X-Y-λ axis and processed in the computer. Optically sectioned images of the composite material at different depths are reconstructed. From the spectral interference fringe signal the phase maps at various depths are also reconstructed. Due to it's common path configuration the present FF-SS-OCT system is highly stable and less sensitive to external vibrations and does not require lateral B-scan leading to large area measurement of the sample.

In chapter VII a common-path spectral-domain optical low coherence interferometric system for the measurement of small changes in refractive index of liquid crystal cell has been described. On applying the voltage to the cell, the LC molecules starts rotating resulting in small change in the RI of the cell. Small changes in the spectral modulations were observed as a function of voltage applied to the LC cell in steps. Hilbert transform fringe analysis was used for the extraction of the phase of the spectral interference fringe signal. The present system is common-path, yields a higher stability in measurement and compensates the optical path length perturbation and hence can be used in real time.

In chapter VIII a spatial coherence tomographic system for biological samples is described. The use of broad band light source requires dispersion compensation optics and high spatial

coherence requires speckle reduction mechanism. In the present chapter we describe FF-OCM using temporally highly coherent and spatially incoherent light source. Spatial coherence of the light source was reduced by generating multiple point sources with a combination of static diffuser and vibrating multi mode fiber bundle (MMFB). Due to low spatial coherence of the light source, high resolution of the system was achieved similar to that of conventional OCT systems using broad band light source i.e., temporally low coherent. The main advantage of the present technique is that it is nearly free from chromatic dispersion. The use of a high NA objective lens gives both high axial and transverse resolution. The speckles which arise due to the highly coherent laser source can be removed by using a vibrating MMFB. We have obtained both optically sectioned images, as well as the phase map of the multilayered structure and biological cells without mechanical scanning in experiment.

In chapter IX the main conclusion of the thesis and scope for the future is presented.

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