Faculty of Engineering

USE OF LASER FOR NON-CONTACT MEASUREMENT OF MICRO-COMPONENTS, INSECTS, AND BIOLOGICAL SPECIMENS

Hillary Assan Anak Liam

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Final Year Project Report	
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……………………………………. ……………………..

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(Supervisor)

USE OF LASER FOR NON-CONTACT MEASUREMENT OF MICRO-COMPONENTS, INSECTS, AND BIOLOGICAL SPECIMENS

HILLARY ASSAN ANAK LIAM

A dissertation submitted in partial fulfillment

of the requirement for the degree of

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Dedicated to my beloved family and friends

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ABSTRACT

The use of conventional techniques such micrometer screw gauge or vernier caliper are not suitable for the measurement of micro-components, insects, and biological specimens. Besides, these techniques involve the device come into contact with the specimens thus increases the probability of the specimens being deformed due to the external force exerted by the measuring device. For this reason, a non-contact measuring device is going to be developed and perform a measurement using four samples which are fishing line, hair, goose hair, and a red ant. Two experiments will be done with these four samples. First experiment is for the calibration of the developed device using a fishing line as a reference sample whereas the other three samples will be used for second experiment which is a real experiment. The developed device will work in such a way that a laser is the source of light which will be reflected by two reflecting mirrors with a specimen is being put in between those mirrors. The laser beam will pass through the specimen and will display an image on a screen known as fringe pattern. This image will then process using computer software (MATLAB). Then, the results will be tabulated and discussed.

ABSTRAK

Pengunaan teknik konvensional seperti “micrometer screw gauge” ataupun “vernier caliper”adalah tidak sesuai untuk pengukuran komponen micro, serangga, dan juga spesimen biologi. Lagipun, teknik ini melibatkan ia bersentuhan dengan spesimen dan akan menyebabkan spesimen mengalami deformasi yang disebabkan oleh tekanan daripada alat ukuran tersebut. Disebabkan ini, alat ukuran yang tidak melibatkan sentuhan akan dicipta dan melakukan pengukuran mengunnakan empat sampel atau spesimen iaitu tali pancing, rambut, bulu roma, dan semut merah. Dua eksperimen akan dilakukan menggunakan keemat-empat sampel tersebut. Pertama adalah eksperimen untuk melakukan kalibrasi alat ukuran yang dicipta menggunakan tali pancing sebagai sampel utama manakala tiga sampel yang lain akan digunakan untuk eksperimen kedua. Alat ukuran yang dicipta ini adalah di mana laser digunakan sebagai cahaya dan akan dipantul menggunakan dua cermin pemantul manakala spesimen akan diletakkan di antara dua cermin tersebut. Pancaran cahaya laser tersebut akan melalui spesimen yang digunakan dan akan memaparkan sebuah imej pada skrin yang dikenali sebagai corak pinggir. Kemudian, imej tersebut akan diproses menggunakan peranti komputer (MATLAB). Selepas itu, keputusan yang diperolehi akan dipaparkan dan dibincangkan.

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LIST OF ABBREVIATIONS

LSCM	-	Laser Scanning Confocal Microscopy
FTP	-	Fourier Transform Profilometry
DH	-	Digital Holography
DHM	-	Digital Holographic Microscopy
QPI	-	Quantitative Phase Imaging
HT	-	Hilbert Transformation
CCD	-	Charge Coupled Device
2D	-	2-Dimensional
3D	-	3-Dimensional
MATLAB	-	Matrix Laboratory
CAD	-	Computer Aided Design
AutoCAD	-	Automatic Computer Aided Design
MMA	-	Methacrylate Monomer
LINPACK	-	Linear System Package
EISPACK	-	Eigen System Package
LTE	-	Long Term Evolution
FT	-	Fourier Transform
FPP	-	Fringe Projection Profilometry

CHAPTER 1

INTRODUCTION

1.1 Overview

All living things can be divided into two main groups which are the Animal Kingdom and the Plant Kingdom. In the Animal Kingdom (Kingdom Animalia), there are many different kinds of species and they are classified using a specific system for grouping animals into smaller and smaller groups.

The Animal Kingdom is first divided into groups called “phyla” (singular: phylum). The phyla are then divided into “classes”, the classes into “orders,” and so on until we reach to the smallest division called “species”. Scientists often used this method to classify various types of animals. In the Animal Kingdom, there are 15 phyla of animals and each phylum consists of animals that have a combination of characteristics that the animals in other phyla do not possess.

Insects are classified as Phylum Arthropoda because they have characteristics such as jointed legs, segmented bodies, and a tough or hard outer covering that also serves as their skeleton. In addition, they are the Arthropoda that belong to Insecta Class and have three legs, a segmented body that is divided into three parts which are head, thorax, and abdomen, a pair of antenna, and usually wings.

One obvious example is bees. They are flying insects that closely related to wasps and ants and known for their role in pollination and in the case of best-known bee species, the European honey bee, for producing honey and beeswax. For instance, in pollination especially for producing honey, bees play an important role of maintaining the natural plant communities and ensuring the seeds production in most flowering plants. They use their long, tube-like tongues as a straw to draw the nectar out of the flowers and store it inside their stomachs and carry it to the beehive. Bees belong to the Order Hymenoptera and Superfamily Apoidea which presently known as Clade Anthophila with seven recognized families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae, Stenotritidae) for known species of bees.

Another example is mosquitoes and they belong to the Order Diptera and Family Culicidae (Superfamily : Culicoidea). They are different even though they appear to be the same if seen with naked eyes. One of the factors that made human being think like that is due to their size which is very small. Moreover, mosquitoes are often being connected to cause infectious diseases towards the host especially human because mosquitoes feed on human blood by piercing the their skin using its tube-like mouthparts while simultaneously transmitting some harmful injections such as Malaria, Yellow Fever, Dengue, Chikungunya, Zika Virus, and other kind of related diseases. In a worst case, the side effects from the harmful injections of the mosquitoes may cause death. For instances, Malaria is one of the most serious diseases with a serious socioeconomic impact with 300 to 500 million people affected and causes 1 to 2 million deaths (Ramirez, Garver, & Dimopoulos, 2009).

In conclusion, the morphological structures of the insects allow them to fulfill their role which in turn may contribute to positive or negative impacts. Their morphological structures are very small in size and cannot be measured by using conventional measuring devices such as micrometer screw gauge and vernier caliper. For example, when biologists want to conduct an experiment that requires them to study the morphological characterization of the insects such as the legs, wings, antenna or any other parts of the insects, they will need a non-contact measuring device to help them without affecting the specimen itself. So, in this research, it is hope that by developing a measuring device that is inexpensive and portable, the gap of wanting to perform an automatic measurement of insect morphology without affecting it physically can be bridged.

1.2 Problem Statement

The use of standard micrometer screw gauge and vernier caliper are not suitable to be applied in the size measurements of micro-components, insects, biological specimens. The main problem involving the size measurement taken by these devices is that, the specimens or samples will easily be deformed when subjected to an external pressure or force. In other words, the shape and size of the insects will change which in turn may lead to an inaccurate reading. Furthermore, the changes will be depending on the amount of pressure or force applied during measurement process.

Like any other conventional measuring devices, micrometer screw gauge and vernier caliper also have their limitations which is its sensitivity or the minimum size that can be measured. If the size of the insect is too small, there will be no reading and further analysis cannot be done because these devices have reached its limit.

Based on this reasons, a non-contact measuring device is going to be developed in order to perform automatic size measurement of insects, . In addition to that, the aim is to develop an inexpensive and portable measuring device that is not limited to laboratory environment.

1.3 Objectives

a) To develop an inexpensive portable non-contact measuring device using a laser source.
b) To compare measurement results of known objects acquired using a micrometer screw gauge and developed device.
c) To perform automatic size measurement and micro-components, insects, and biological specimens.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The term laser is an acronym for “light amplification by stimulated emission of radiation” which emits light through a process of optical amplification. In other words, it is a device that produces a beam of monochromatic (single) light by stimulated emission of photons from excited atoms or molecules. Different from normal light, laser contains only one wavelength or one specific color which is determined by the amount of energy released when the excited electrons drops to a lower orbit.

In this chapter, a brief overview of the existing non-contact measurement techniques will be provided which can be found in the previous studies that were proposed by the researchers. Furthermore, the discussion will mention about the strengths and weaknesses of the proposed techniques and a little bit of explanation as a way to point out the gaps that can be bridged during this research or in the near future. Furthermore, the discussion will be related to image processing and Fourier Analysis.

2.2 Past Researches

2.2.1 Principles and Practices of Laser Scanning Confocal Microscopy

Laser Scanning Confocal Microscopy (LSCM) is one of the tools that can be used in three dimensional (3D) size measurements of micro-components and morphological characterizations of living insects. According to Paddock (2000), the major application of LSCM is in the biomedical sciences especially for imaging of fixed or living tissues labeled with one or more fluorescent probes; molecules that absorb light of a specific wavelength and emit light of different wavelength. The author also stated that, LSCM produced an image by scanning one or more focused beam of light (laser) across the specimen and this process was called optical section, which makes imaging of living specimens easier, thus enable the automated collection of 3D data in the form of Z-series and improved the images of multiple labeled specimens. However, the LSCM could be wobbled and distorted due to the movement of the biological specimens which then resulting in the loss of resolution in the image itself. In addition, it is not possible to perform microinjection of fluorescently labeled probes when the specimen or sample is moving.

2.2.2 Whole Insect and Mammalian Embryo Imaging with Confocal Microscopy: Morphology and Apoptosis

Furthermore, according to Zucker (2006), the fixation and clearing methods have been enhanced to allow the optical sectioning through the embryos with the thickness approaching 1mm (z-axis). Zucker (2006) managed to do it by staining the live embryo (LysoTracker), followed by fixation (aldehyde) and clearing (benzyl alcohol/BABB). Besides, LSCM system was incorporated with a Leica inverted DIRMB microscope and an Omnichrome argon Krypton laser that emits three different wavelengths which are 488 nm, 568 nm, and 647 nm. To reveal the morphological structures of the embryo or insects, the glut auto-fluorescence were excited by 488 nm excitation. Besides, the images of the tissues were observed as they were sequentially excited with a 488 nm laser (green fluorescent) and then excited with a 568 nm laser (red fluorescent). Consequently, the overlapping of laser emission caused by two different fluorescent were eliminated.

2.2.3 Fourier Transform Profilometry Using a Binary Area Modulation Technique

Fourier Transform Profilometry technique was proposed by Lohry and Zhang (2012). They mentioned that it was difficult to eliminate third-order harmonics using the squared binary defocusing technique without affecting the fringe quality. This is why the author had proposed new technique to achieve high-quality three dimensional measurements in order to eliminate third-order harmonics by modulating the squared binary structured patterns. Beside, this technique modulates the binary structured pattern in x-direction and y-direction which in turn completely eliminates the third-order harmonics even when the fringe is dense. In addition, since the fringe is dense, all higher order harmonics can easily be suppressed by slightly defocusing. However, the success was heavily relied on the carrier fringes because the measurement will be erratic if the carrier fringes are non-sinusoidal. Moreover, only pattern with a fringe period of 12 pixels can completely eliminate the third-order harmonics compared to the binary pattern with fringe period of 16 pixels.

2.2.4 Single Shot High Resolution Digital Holography

Digital Holography (DH) was one of the important imaging modality with wide range of applications. Photographic film was replaced by digital array detectors to be DH's recording medium, and most of the current methods of image reconstruction were still the same as the physical hologram reconstruction process (Khare et al., 2013). However, a new computational approach for image recovery was needed to overcome the limitations of DH such as the pixel size of digital array detectors, image resolution in off-axis DH, and minimum reference beam angle condition. Even though DC and cross terms had been overlapping with each other, the authors were able to describe new computational approach for high quality image recovery from a single hologram frame. So, an experiment was done in order to test the single-shot image recovery in Digital Holography using Mach-Zehnder configuration. However, the authors also mentioned that the removal of most of the dc and twin image technique depends on the presumption that the dc and cross terms in the hologram were well separated in the Fourier dominion or depends upon the multiple hologram frames as in phase-shifting methods.

2.2.5 Accurate Single-Shot Quantitative Phase Imaging of Biological Specimens with Telecentric Digial Holographic Microscopy

The technique proposed by Doblas et al., (2014) used a telecentric imaging system in Digital Holographic Microscopy (DHM) to study biological specimens. The recorded fringe pattern allows the recovery of the phase changes which contain the refractive index and thickness of the specimen thus making the measurement accuracy determinant in the different applications where DHM can be utilized or applied. Moreover, the authors also stated that the regular required numerical compensation approaches are no longer needed since there was no curvature phase distortions presented in the recorded holograms. On the other hand, the accuracy of Quantitative Phase Imaging (QPI) depends upon the recording and computational reconstruction of complex object wave. Moreover, its measurement could be affected if the remaining phase factor was found even after applying different methods to have it removed.

2.2.6 3D Surface Imaging by Multi-Frequency Phase Shift Profilometry with Angle and Pattern Modeling for System Calibration

Wang (2016) presents three dimensional (3D) surface imaging by using multi-frequency phase shift profilometry. In addition, in order to decrease noises that are caused by the optical irregularity and external influences and to determine the phase map, the combination of four shifted phases with three high carrier frequencies are used which in turn generate analytical solutions. According to Wang (2016), this technique has higher measurement accuracy and measurement efficiency compared to other non-contact three dimensional imaging techniques. In addition, the author also mentioned that the technique was able to provide information regarding the depth of the object or specimen which leads to the breaking of the restriction of traditional cameras and sensors that could only acquire 2D images. However, this technique also has its limitations. Its measurement accuracy is still remarkably affected by the noise and optical irregularity in the camera and projector which in turn lead to the use of multiple frequencies to cipher multiple phases and then unwrapped them to obtain the final phase to suppress the noise. Besides, there is a possibility of the measurement accuracy to be affected by external factors such as uneven light beam, non-perfect image intensity distributions and non-perfect image processing algorithms.

2.2.7 Single Frame digital Fringe Projection Profilometry for 3D Surface Shape Measurement

Kumar, Somasundaram, Kothiyal, and Mohan (2013) proposed the use of Hilbert transformation method for measuring the 3D surface shape. This method only requires one fringe pattern for phase extraction for the information regarding surface height or depth variations of the object thus reducing calculation time. In addition, the method was capable of conducting an automated measurement at video frame rate. The authors also stated that it was easier to implement Hilbert Transformation (HT) method in digital fringe projection technique because the technique allows the projection of linear fringes on to the object surface for the phase evaluation. On the other hand, the phase evaluation of the object surface cannot be done directly using HT method because it would result in an ambiguous phase that need further image processing to remove the ambiguity. Besides, the sensitivity of the digital fringe projection system is depending on the angle between the axes of the projector and the CCD (Charge Coupled Device) camera.

2.2.8 Flexible Real-Time Natural 2D Colour and 3D Shape Measurement

The method by Ou et al. (2013) suggested that 2D colour and 3D shape measurement can be captured simultaneously in real time. However, this method requires two cameras in which one is used to perform 3D shape measurement (using near infrared camera/projector pair) while the other one is used to capture 2D colour images solely illuminated by ambient lights. Even so, the flexibility of the dual-camera can be improved by a simple calibration method that could quickly determine the mapping from the colour camera to the 3D captured geometry. The authors further mentioned that the existing real-time 3D shape measurement systems only produced non-nature texture (having an illumination other than ambient lights) that induces shadow realated issues. Meaning that, the texture was not captured without the directional projection light.