Quantitative Phase Imaging Microscopy with Multi-Wavelength Optical Phase Unwrapping

by

Nilanthi Warnasooriya

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Department of Physics
College of Arts and Sciences
University of South Florida

Major Professor: Myung K. Kim, Ph.D.
Srikanth Hariharan, Ph.D.
Chun-Min Lo, Ph.D.
Sarath Witanachchi, Ph.D.

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Dedication

To my parents.
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Quantitative Phase Imaging Microscopy with Multi-wavelength Optical Phase Unwrapping

Nilanthi Warnasooriya

ABSTRACT

This dissertation presents a quantitative phase imaging microscopy technique that combines phase-shifting interferometry with multi-wavelength optical phase unwrapping. The technique consists of a Michelson-type interferometer illuminated with any of three types of light sources; light emitting diodes, laser diodes and a ring dye laser. Interference images are obtained by using a 4-frame phase shifting method, and are combined to calculate the phase of the object surface. The $2\pi$ ambiguities are removed by repeating the experiment combining two and three different wavelengths, which yields phase images of effective wavelength much longer than the original. The resulting image is a profile of the object surface with a height resolution of several nanometers and range of several microns. To our knowledge, this is the first time that a three wavelength optical phase unwrapping method with no amplified phase noise has been presented for full-frame phase images.

The results presented here are divided into three main categories based on the source of illumination; light emitting diodes, laser diodes and a ring dye laser. Results for
both two-wavelength optical unwrapping and three-wavelength optical unwrapping techniques are demonstrated.

The interferographic images using broadband sources such as light emitting diodes are significantly less affected by coherent noise compared to images obtained using lasers. Our results show that the three wavelength optical phase unwrapping can also be effectively applied to unwrap phase images obtained using coherent light sources such as lasers and laser diodes, without amplifying phase noise in the final phase image.

We have successfully shown that our multi-wavelength phase-shifting technique extends the range free of $2\pi$ ambiguities in the phase map without using conventional computation intensive phase unwrapping methods. This phase imaging technique can be used to measure physical thickness or height of both biological and other microscopic samples, with nanometer axial resolution. An added advantage of the multi-wavelength optical phase unwrapping technique is that the beat wavelength can be tailored to match height variations of specific samples.
CHAPTER 1

INTRODUCTION

1.1. Optical Microscopy

The technique of using a single lens or a series of lenses to magnify features of an object is known as microscopy. The main goal of a microscope is to resolve and magnify minute details in a specimen so that they are visible to the human eye. It is not known when the first microscope was invented; a single lens has been used as a magnifying device for hundreds of years. The first compound microscope was built in 1590 by two Dutch eyeglass makers; Hans Jansen and Zacharias Jansen. Two well known users of single lens and compound microscopes are Anton Leeuwenhoek and Robert Hooke. Anton Leeuwenhoek (1632-1723), later known as the “father of microscopy”, used a single convex lens to study samples placed on an adjustable needle. He was the first to observe and describe microorganisms. Robert Hooke (1635-1703) used a simple compound microscope consisting of two lenses – one lens as the objective and the other as the eyepiece. He wrote the book Micrographia, describing his observations of microscopic structures using the term “cell” for the first time and confirming Leeuwenhoek’s observations of living organisms in water.
During the 18\textsuperscript{th} and 19\textsuperscript{th} centuries, compound microscopes rapidly gained popularity and were improved with the new knowledge in optics. In the 1730’s Chester More Hall combined a convex lens made of crown glass with a concave lens made of Flint glass and invented the achromatic lens to solve chromatic aberrations. However, he did not publish his invention. In 1759, a telescope maker named John Dolland used Hall’s idea to make an achromatic lens and obtained a patent. Another major milestone of microscopy is the solution to spherical aberration. In 1830, Joseph Jackson Lister mathematically showed how to minimize spherical aberration of an optical system. Microscopes with Lister-corrected lenses were produced a few years later. In 1873, Ernst Abbe demonstrated a formula that gives the resolving power of a microscope. According to Abbe’s formula, the minimum resolved distance is related to the wavelength of the light and the ‘numerical aperture’. The numerical aperture is a combination of the refractive index of the medium and the half angle of the cone of light that enters the lens. In other words, Abbe’s formula states that in order to get the maximum resolution, the cone of light that enters the lens must be maximum. In 1893, August Koehler developed an illumination method that improves the resolution of the microscope by using an evenly illuminated field of view. Today the method is known as “Koehler illumination” and is used by all modern microscope users.

1.1.1 Modern Optical Microscopy

Modern optical microscopes have surpassed 19\textsuperscript{th} century microscopes in numerous ways. They are equipped with advanced lenses that minimize aberrations, high numerical apertures for better resolution, digital image acquisition and processing.
methods and ergonomical designs. Different techniques such as scanning microscopy, confocal microscopy, fluorescence microscopy, phase contrast microscopy and digital holographic microscopy have been used in biomedical, engineering and materials science fields to investigate biological cells, micrometer scale electronic and mechanical devices and nanometer scale polymer composites. Each technique has advantages and unique features that are suitable for specific applications. For example, when studying a living organism, a phase contrast microscope or digital holographic microscope can be used without a sample preparation, while a scanning electron microscope or a fluorescence microscope needs a vacuum or staining.

1.2 Phase Contrast Microscopy

In microscopy, specimens can be divided in to two main categories; amplitude objects and phase objects. Amplitude objects absorb light to create sufficient intensity contrast to be observed with bright field microscopy. If the specimen doesn’t absorb enough light, it is not visible to the human eye. Phase objects do not absorb light to produce visible intensity contrast. Since the human eye is capable of seeing only color or intensity differences, phase changes have to be converted to intensity changes.

Phase contrast microscopy converts phase changes into amplitude changes. The technique was first introduced by Fritz Zernike in 1942. There are several phase contrast microscopy techniques; Zernike phase contrast (ZPC), differential interference contrast (DIC), Hoffman modulation contrast (HMC) etc.
1.2.1 Zernike Phase Contrast Microscopy

Zernike phase contrast microscopy, introduced by Fritz Zernike [1, 2], converts changes in phase into corresponding changes in amplitude by using a phase plate. It uses common path interferometry, where a partially coherent light beam passes through the specimen. The light that is diffracted due to phase variations of the specimen, and the light that passes without diffraction are then focused to form a phase contrast image of the specimen. A schematic diagram of Zernike phase contrast microscope is shown in Figure (1.1).

![Figure 1.1: Zernike phase contrast microscope.](image)

```latex
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{zernike_phase_contrast_microscope.png}
\caption{Zernike phase contrast microscope.}
\end{figure}
```
1.2.2 Normaski Differential Interference Contrast Microscopy

In 1950's George Normaski modified the conventional Wollaston prism used in differential interference contrast microscope so that it can be placed outside the objective [3]. The Normaski DIC microscope converts refractive index changes of the sample into amplitude changes by splitting a polarized beam into two perpendicularly polarized beams less than a micrometer apart. The phase of the two beams changes after passing through the sample, due to variations of the refraction index. The two beams are recombined using a Wollaston (Normaski) prism. The recombined beam is sent through an analyzer where it passes only the circularly and elliptically polarized light beams to form an image of the specimen. A schematic diagram of Normaski differential interference contrast microscope is shown in Figure (1.2).

![Figure 1.2: Normaski differential interference contrast microscope.](image)
1.2.3 Hoffman Modulation Contrast Microscopy

In 1975, Robert Hoffman invented a new phase contrast technique, named Hoffman modulation microscopy [4]. HMC microscopy converts optical phase gradients into amplitude using a spatial filter called “modulator”, which has three light-passing zones, a slit plate and a circular polarizer. Different specimen thicknesses deflect the light to different modulator zones thus controlling the contrast of the image. A schematic diagram of Hoffman modulation contrast microscope is shown in Figure (1.3).

![Figure 1.3: Hoffman modulation contrast microscope.](image)
1.3 Interference Microscopy

In interference microscopy, an image is formed by the interference of two light beams; one beam reflected from (or transmitted through) the specimen, and one beam reflected from a plane mirror known as reference mirror.

In 1887, Albert Michelson and Edward Morley invented a device that uses the interference of light to measure the difference in velocity of two perpendicular light beams [5]. Today, this device is known as the Michelson interferometer and is extensively used in a range of interference microscopy techniques.

Most quantitative microscope systems are based on the Michelson interferometer or the Mach-Zehnder interferometer or their variations.

1.3.1 Michelson Interferometer

In the basic Michelson interferometer, a light beam is divided into two beams of equal intensity and these two beams illuminate two mirrors. A compensator is used so that light beams on both arms travel the same optical path lengths. The two reflected beams are then combined again to form interference fringes. In the Michelson type microscope, the object to be analyzed is placed in one arm, replacing one of the mirrors. The phase information of the object can be extracted from interferograms.
A variation of the Michelson interferometer, where two microscope objectives are used to obtain higher magnification is known as the Linnik interferometer. The objectives are placed in front of the object and of the reference mirror. This configuration is widely used in modern microscopy systems to acquire desired magnifications.

The Michelson type configurations are best suitable for studying reflective and semi-transparent samples. For fully transparent samples a Mach-Zehnder interferometer can be used.

### 1.3.3 Mach-Zehnder Interferometer

The Mach-Zehnder interferometer, invented by Ernst Mach and Ludwig Zehnder, uses two beam splitters (or two half silver mirrors) and two regular mirrors to divide the light beam into two paths and then recombines them to produce interference. The sample
can be placed in one of the arms so that the light beam can traverse once through the sample.

**1.4 Phase-shifting Interference Microscopy**

The basis of the phase-shifting interference microscope (PSIM) is a phase-shifting interferometer (PSI), where the phase difference between two beams of the interferometer is changed in equal steps. At each step, the intensity of the interference pattern is recorded and then the interferograms are combined to obtain the phase map of the object. Phase shifting can be done by using several different techniques as described in Chapter 2. Depending on the researcher’s needs, PSI can be built in a Michelson, Linnik or Mach-Zehnder configuration.

**1.5 Phase Unwrapping**

Each fringe in an interferogram represents an area of data ranging from 0 to $2\pi$. Therefore, the final phase map obtained from a series of interferograms also contains $2\pi$ ambiguities. Such phase maps are called ‘wrapped’ phase maps, and are needed to be ‘unwrapped’ by removing $2\pi$ ambiguities. Figure (1.5) shows a phase map with $2\pi$ discontinuities where the black represents phase value of zero and the white represents phase value of $2\pi$. 
Once these $2\pi$ ambiguities are removed, a continuous surface profile of the test object can be obtained. Such a surface profile provides height information of surface features. Generally, phase unwrapping is done by using numerical algorithms. Most of these numerical algorithms are computationally intensive and can fail when there are irregularities in the test object.

### 1.5.1 Multi-wavelength Phase Unwrapping

Two or more wavelengths can be used to extend the range free of $2\pi$ ambiguities in the phase map, thus avoiding the need for phase unwrapping. In two-wavelength phase unwrapping, two wavelengths are used to produce a longer wavelength called the ‘beat wavelength’. For two wavelengths $\lambda_1$ and $\lambda_2$, the beat wavelength $\Lambda_{12}$ is defined by

$$\Lambda_{12} = \frac{\lambda_1 \lambda_2}{|\lambda_1 - \lambda_2|}.$$  

By choosing closer wavelengths, the beat wavelength can be extended to suit the surface heights of the object. One problem of the two-wavelength phase unwrapping method is noise amplification. The phase noise in each single wavelength
phase map is amplified by a factor equal to the magnification of the wavelengths. This problem has been successfully addressed in this thesis and the multi-wavelength phase unwrapping has been extended to three wavelengths without noise amplification.

1.6 Light Sources

The source of light is a very important part of any microscopy technique. An ideal light source for a microscope should provide a constant luminosity without voltage drop variances. It should be small enough to fit in a microscope, cost effective and should have a long life span.

In this experiment, light emitting diodes (LED), laser diodes (LD) and a ring dye laser are used as light sources. Specifications of each light source are described in detail in Chapter 3.

1.7 Research Contributions

The microscope is an indispensable tool in many areas of modern science. The need for microscopy techniques that provide high magnification and resolution has become vital with the progress of nano technology and bio-medical fields. Studying properties and characteristics of new compositions, cells and tissues has been easier with the development of phase imaging microscope techniques. As the use for phase imaging techniques increases, so does the need for simple, user friendly phase unwrapping methods.

For the first time, to our knowledge, we have presented full-frame phase images by using a three wavelength optical phase unwrapping method with no amplified phase noise. We present unwrapped phase images using both two-wavelength and three-
wavelength phase unwrapping methods. The experimental setup is a widely known Michelson type phase shifting interferometer, but the use of incoherent light sources (light emitting diodes) and low coherent light sources (laser diodes) provides significantly less speckle noise than laser light sources. The added advantages are reduced apparatus dimensions, low cost and ease of operation. Such qualities are important factors of a user friendly microscope system.

We have successfully shown that our multi-wavelength phase-shifting technique extends the range free of \(2\pi\) ambiguities in the phase map without using conventional computational intensive phase unwrapping methods. This phase imaging technique can be used to measure physical thickness or height of samples, with nanometer axial resolution.

1.8 Thesis Organization

This thesis consists of 8 chapters and 2 appendices. Chapter 1 provides a general introduction of phase imaging and phase unwrapping techniques included in the study. Chapter 2 consists of basic concepts and principles of phase shifting interferography describing interference of two light beams, the principle of phase shifting interference microscopy, phase shifting methods, 3 and 4 frame phase shifting algorithms and examples of single wavelength phase images. In Chapter 3 the experimental setup is described beginning with the interferometer. It also describes the camera and image acquisition process, computer programs used in the experiment and properties of the light sources. Chapter 4 presents multi-wavelength phase shifting interference microscopy beginning with numerical algorithms for phase unwrapping and then describing multi-
CHAPTER 2

PHASE SHIFTING INTERFEROGRAPHY

This chapter presents several fundamental principles of optics. The first section describes principles of interference of light waves which is the base of this experiment. Then Section (2.2) presents the principle of phase shifting interference microscopy- a technique that utilizes both interference phenomenon and phase shifting for imaging. Section (2.3) describes different phase shifting methods.

2.1 Interference

Interference is the superposition of two or more electromagnetic waves. In optics, this means addition of two or more light waves. Since light is a type of electromagnetic wave, the electric field $\overline{E}$ at a point $(x, y, z)$ can be given as

$$\overline{E}(x, y, z, t) = A(x, y, z, t)e^{i\phi(x, y, z, t)}$$  \hspace{1cm} (2.1)$$

Here $A$ is the amplitude and $\phi$ is the phase of the light wave. For linearly polarized light, in which the electric vector of the light vibrates only in one plane, the electric field can be written as
\( \vec{E}(x, y, z, t) = A(x, y, z)e^{i[\omega t - \phi(x, y, z)]}, \) where \( \omega \) is the average frequency. \( \text{(2.2)} \)

The intensity of the field can be obtained by time averaging the modulus value of square of the field \( E \).

\[ I(x, y, z) = \langle |E(x, y, z)|^2 \rangle \quad \text{(2.3)} \]

When two waves \( E_1 \) and \( E_2 \) interfere, the resultant intensity \( I(x, y, z, t) \) is equal to

\[ I(x, y, z, t) = \langle |E_1|^2 \rangle + \langle |E_2|^2 \rangle + \langle E_1 \cdot E_2^* \rangle + \langle E_1^* \cdot E_2 \rangle \quad \text{or} \]

\[ I(x, y, z, t) = I_1 + I_2 + \langle E_1 \cdot E_2^* \rangle + \langle E_1^* \cdot E_2 \rangle \quad \text{(2.4)} \]

For two linearly polarized waves using Eq. (2.2), the resultant intensity is given by

\[ I(x, y, z, t) = I_1 + I_2 + 2(A_1 \cdot A_2) \cos \left\{ (\omega_1 - \omega_2) t - \left[ \phi_1(x, y, z) - \phi_2(x, y, z) \right] \right\} \quad \text{(2.5)} \]

If two linear polarizations are parallel, \( A_1 \cdot A_2 = \sqrt{I_1 I_2} \)

Then

\[ I(x, y, z, t) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos \left\{ (\omega_1 - \omega_2) t - \left[ \phi_1(x, y, z) - \phi_2(x, y, z) \right] \right\} \quad \text{(2.6)} \]

If both waves have the same angular frequency, \( \omega_1 = \omega_2 \)

Then

\[ I(x, y, z, t) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos \left[ \phi_1(x, y, z) - \phi_2(x, y, z) \right] \quad \text{(2.7)} \]

15
\[ I(x, y, z, t) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(\Delta \phi) \]  

(2.8)

Here \( \Delta \phi \) is the phase difference.

Phase difference or phase shift between two light beams can therefore be determined by interfering the two beams. If one of the beams passes through an object or reflected from an object, that beam carries the information of the object. By combining this beam with a reference beam, one can determine the phase shift of the two beams due to the object.

2.2 Coherence

The ability of light beams to interfere is called ‘coherence’. Coherence is the correlation of phase at two points separated in time or space in the two interfering beams.

2.2.1 Temporal Coherence

Temporal coherence is a measurement of how well a wave interferes with itself at different times. Consider two points in the two interfering waves. The maximum distance between the two points while maintaining a constant phase difference is called temporal coherence length \( l_c \). The time required for waves to travel the coherence length is called coherence time \( \tau_c \). The temporal coherence length depends on spectral band width and wavelength of the light source. In the two beam interferometry, interference fringes can
only be seen when the optical path difference between reference and object arms are
within the coherence length $l_c$.

2.2.2 Spatial Coherence

Spatial coherence is a measurement of how well two points on the same
wavefront can interfere, and is defined by the mutual correlation of different points of the
same wavefront. In other words, it describes the correlation between wave fronts at
different points in space. The spatial coherence depends on both the geometry of the
interferometer and the properties of the light source. A misaligned interferometer can
cause wavefront mismatch, thus allowing low spatial coherence and therefore poor
interference visibility. An incandescent bulb, in which each point of the filament acts as
an individual light source, is an example of an incoherent light source.

2.3 Principle of Phase Shifting Interference Microscopy

Phase shifting interference microscopy is a widely used technique for quantitative
measurements of microscopic objects. It can be used for both transparent and reflective
samples without special sample preparation [6-12]. Different phase shift methods can be
used in an interferometer setup to produce a phase shift between the object beam and the
reference beam. Typically, phase difference between two beams is changed in steps and
intensity values of the interference beam at each step are recorded. The phase difference
between object beam and reference beam can be calculated by combining such intensity
values.
2.4 Phase Shifting Methods

Phase shifting can be used in any two beam interferometer and the phase can be shifted in many different ways [13]. Several such methods, including the method used in our research, are described below.

2.4.1 Moving the Reference Mirror

In this method, the reference mirror is moved by using a piezoelectric transducer (PZT). When the PZT moves the position of the reference mirror, it produces a phase change. This is shown in Figure (2.1a).

2.4.2 Using a Tilted Glass Plate

In this method, a phase shift is introduced by placing a glass plate in the path of the collimated light beam as shown in Figure (2.1b). When the glass plate makes an angle $\theta_1$ with the optical axis, then the phase shift $\phi$ is given by $\phi = \frac{t}{k} (n \cos(\theta_2) - \cos(\theta_1))$

Where, $t$ is the thickness of the glass plate, $n$ is refractive index of the glass, $k = \frac{2\pi}{\lambda}$, $\theta_1$ and $\theta_2$ are angles between the normal to the plate and the light rays outside and inside the plate respectively. The optical path difference can be increased by increasing the angle $\theta_1$.

2.4.3 Using a Phase Plate

In this method, phase shift is introduced by a rotating phase plate. When a circularly polarized light beam passes the half wave phase plate as shown in Figure (2.1c),
the direction of polarization is reversed. When the plate is rotated by an angle $\theta$, then the phase change is given by $\phi = 2\theta$.

Figure 2.1: Several methods of phase shifting. (a) using a moving mirror; (b) using a tilted glass plate; (c) using a half wave plate.

2.5 Phase Shifting Algorithms

Once a phase shift is introduced to the experimental setup using one of above mentioned methods, a phase shifting algorithm is needed to determine the relative phase between the reference mirror and the object. There are several phase shifting algorithms, and three-frame phase shifting algorithm requires the minimum number of frames. The general expression for the intensity of interference fringes can be given as
\[ I = I_B + I_m (\phi + \phi_f) \] 

(2.1)

where \( I_B \) is the background intensity, \( I_m \) is fringe modulation intensity, \( \phi \) is the relative phase between the object and the reference mirror and \( \phi_f \) is the phase shift.

### 2.5.1 Three-frame Phase Shifting Algorithm

At minimum, three intensity values are needed at phase interval of 90°. If intensity values of the interference beam are recorded at 0°, 90° and 180° phase intervals, Equation (2.1) can be written as

\[
\begin{align*}
I_0 &= I_B + I_m \cos \phi \\
I_{\pi/2} &= I_B + I_m \cos(\phi + \pi/2) = I_B - I_m \sin \phi \\
I_{\pi} &= I_B + I_m \cos(\phi + \pi) = I_B - I_m \cos \phi
\end{align*}
\]

(2.2)

From Equations (2.2), the phase \( \phi \) can be calculated as

\[
\phi = \tan^{-1} \left[ \frac{-I_0 + 2I_{\pi/2} - I_{\pi}}{I_0 - I_{\pi}} \right]
\]

(2.3)

### 2.5.2 Four-frame phase shifting algorithm

In this experiment, four step phase shifting algorithm is applied to the interference microscope. Though the minimum number of intensity values needed for phase calculation is three, even a small error in measurements can cause a large phase error.

Taking four intensity measurements can reduce this effect. In order to acquire phase images, the Michelson interferometer is used as the experimental setup as shown in Figure (2.2).
Figure 2.2: Experimental setup. A standard Michelson-type interferometer consists of the reference arm and the object arm.

The Intensity $I(x,y)$ of the light captured by CCD can be written as:

$$
I(x,y) = I_O(x,y) + I_B(x,y) + I_R(x,y) + 2\sqrt{I_O(x,y)I_R(x,y)} \cos[\phi_i + \phi(x,y)]
$$

(2.4)

Here $I_O(x,y)$ is the part of the beam reflected by the object that is coherent with respect to $I_R(x,y)$, the intensity of the beam reflected by the reference mirror. $I_B(x,y)$ is part of reflection from the object that is incoherent with respect to the reference – i.e. outside the coherence length. $\phi(x,y)$ is the relative phase between the object and the reference mirror and $\phi_i$ is the phase shift introduced by moving the reference mirror by quarter wavelength intervals. The reference mirror is mounted on a piezo transducer and is dithered by applying a ramp waveform. Four images are acquired at $\phi_i = 0, \pi / 2, \pi$
and $3\pi/2$. Intensity distributions corresponding to the four images can be given as follows:

\[
I_0 = I_O + I_B + I_R + 2\sqrt{I_O I_R} \cos \phi \\
I_{\pi/2} = I_O + I_B + I_R - 2\sqrt{I_O I_R} \sin \phi \\
I_\pi = I_O + I_B + I_R - 2\sqrt{I_O I_R} \cos \phi \\
I_{3\pi/2} = I_O + I_B + I_R + 2\sqrt{I_O I_R} \sin \phi
\] (2.5)

The phase map of the object is given by:

\[
\phi = \tan^{-1}\left(\frac{I_{3\pi/2} - I_{\pi/2}}{I_0 - I_\pi}\right)
\] (2.6)

And the amplitude of the image of the object is given by:

\[
I_O = \frac{(I_0 - I_\pi)^2 + (I_{\pi/2} - I_{3\pi/2})^2}{16I_R}
\] (2.7)

Once a phase profile of a specimen is obtained, it can be used to determine the height profile of the specimen.

The optical path difference (OPD) between the object wave and reference wave is given by

\[
OPD = \frac{\lambda \phi}{2\pi}
\] (2.8)
Here $\lambda$ is the wavelength of the light beam and $\phi$ is the relative phase between the object and reference mirror. In the given Michelson type interferometer, the height profile of the object is half the OPD because the light travels towards the object, reflects and travels back. Therefore, the height profile $h$ is related to the phase $\phi$ as follows.

$$h = \frac{1}{2} \left( \frac{\lambda}{2\pi} \right) \phi \quad (2.9)$$

The reference mirror is dithered by a distance equal to the wavelength of the light source, by supplying a ramp wave from the signal generator. Four images are acquired when the distance $= \lambda/4, \lambda/2, 3\lambda/4 \lambda/4$ and $\lambda$. The accuracy of these images can be easily tested by acquiring an additional image so that the first image is equal to the fifth image as shown in Figure (2.3). The difference of phase values of the first image and the fifth image must be zero and this is also checked to verify the accuracy of the procedure. The intensity distributions of the first and the fifth frames are plotted in the same graph to check the accuracy of phase shifting. If the two distributions coincide as shown in Figure (2.4), they have the same phase value.

**Figure (2.3):** Five image frames acquired when the shift of reference mirror is 0, $\lambda/4, \lambda/2, 3\lambda/4 \lambda/4$ and $\lambda$.
Figure 2.4: An interferogram (left) and intensity distribution of the first image frame (I=0) and the fifth image frame (I = 2\pi) (right). If two distributions coincide the first and fifth frames have the same phase value.

2.6 Moving the Reference Mirror

Reference mirror has to be dithered in specific amount so that five images can be captured within one ramp cycle. The applied ramp waveform is shown in Figure (2.5).

The CCD camera captures 30 frames per second. Here a green light with 530 nm wavelength is used for an example. A LabVIEW program is used to capture 5 intensity images, each at quarter wavelength intervals, and then use first four images to calculate the final phase image. The program is shown in Appendix A.
A Stanford Research System Model DS345 30 MHz Synthesized Function Generator is used as the voltage supply. The function output is connected to the piezoelectric transducer, which is glued to a plane mirror. This mirror is used as the reference mirror. The sync output of the generator is connected to the computer so that the LabVIEW program and voltage signal are synchronized. Once the LabVIEW program is started, it provides a trigger signal. The program is written so that the first image is captured five frames after the trigger signal and next four images are captured at 11th, 16th, 21st and 26th frames by skipping 4 frames at a time. This is shown in Figure (2.6).
Since each image is captured at the 5th frame and they should be at quarter wavelength intervals,

5 frames = \( \frac{\lambda}{4} = 0.53\mu m /4 \)

1 frame = \( 0.53\mu m /20 = 0.0265\mu m \)

Time for 1 frame = 1/30 seconds

Length per second = 0.0265 \( \mu m \)/frame x 30 frames/second = 0.795 \( \mu m \)/second

1.8 V needs to travel one wavelength. (This is by observation of fringes)

\[ \frac{1.8V}{0.53\mu m} \times 0.795 \mu m/second = 2.7V/second \]

If voltage need is \( V \),

\[ \frac{V}{1 \text{ second}} = 2.7 \text{ V/second} \]

\[ V = 2.7 \text{ Volts} \]

Therefore, for 0.53 \( \mu m \) wavelength, 2.7 Volts should be given to the reference mirror, in order to dither it by quarter wavelength intervals.

Using the above method, reference mirror can be dithered by quarter wavelength intervals for different wavelengths. Figure (2.7) shows a sample of interferogram. In the interferograms, optical path length between two fringes is \( \lambda \) and this is equal to a physical distance of \( \lambda/2 \) because of the round trip of light in reflection mode.
Figure 2.7: An interferogram. The fringes appear when the object beam and the reference beam interfere. The optical path length between two fringes is equal to one wavelength.

2.7 Phase Shifting Interference Microscopy Using Single Wavelength

The experimental setup is shown in Figure (2.2). A standard 1951 USAF resolution target is used as the object. Elements 2-6 of group 7 are imaged. The image size is 314 by 264 pixels and 100 μm by 120 μm. After obtaining four interferograms at quarter wavelength intervals, they are combined according to Equation (2.5). Figure (2.8) shows the final phase image using green LED (\( \lambda = 550.18 \text{ nm} \)) and the cross section of the surface profile.
Figure 2.8: Wrapped phase image of a resolution target (left) and its surface profile along the dotted line (right). The light source is a light emitting diode of ($\lambda = 550.18$ nm).
CHAPTER 3

EXPERIMENTAL SETUP

This chapter describes the experimental setup and computer software used to acquire data in detail. The first section explains how each part of the interferometer setup works. Section (3.2) is a description of the camera and image acquisition. Section (3.3) describes the computer software used to acquire and analyze phase images. These programs are shown in Appendix (A). Section (3.4) presents characteristics of light sources used in the experiment.

3.1 Interferometer

The experimental setup is shown in the Figure (3.1). A set of light emitting diodes (driven by i-Xitanium™ LED electronic driver) illuminates a Michelson type interferometer. The light is collimated by lens L1 and then linearly polarized by the polarizer P.
Figure 3.1: Experimental setup. MO, microscope objective; L1, collimating lens; L2, focusing lens; P, polarizer; PBS, polarized beam splitter; QW1,QW2, quarter-wave plates; A, analyzer; OBJ, object; REF, reference mirror; PZT, piezo-electric transducer; CCD, charged-coupled device.
Figure 3.2: Unpolarized light coming from the LED is linearly polarized along the transmission axis of polarizer P.

The polarized beam splitter PBS splits the incoming beam into an S-polarized (polarization plane is perpendicular to polarization axis) ray and a P-polarized (polarization plane is parallel to polarization axis) ray. This is shown in Figure (3.3). S-polarized beam is reflected at the PBS to illuminate the sample object OBJ and P-polarized beam is transmitted through PBS to illuminate the reference mirror REF. When S-polarized light passes through quarter wave plate QW1, phase changes by 90° and it becomes circularly polarized. After reflecting at the Mirror and going through another 90° phase shift at QW1, the light becomes P-polarized. This change from S-polarization to P-polarization avoids light traveling back to LED and directs all reflected light to the CCD. Similarly, P-polarized light illuminating the REF changes to S-polarized light and travels to the CCD. At the analyzer A, the two S-polarized and P-polarized light beams are changed into a common polarization state so that interference can occur on the CCD plane. This is shown in Figure (3.3).
Figure 3.3: Effect of quarter wave plates and polarizer-analyzer combination on incoming light.

The polarizer-analyzer pair also allows continuous variation of the relative intensity between the two arms. In order to acquire images with high resolution, two 20X microscope objectives are placed in front of the object and the reference mirror. The reference mirror is mounted on a piezo-electric transducer (PZT). A function generator supplies a ramp signal to the PZT to dither the reference mirror by a distance of one wavelength. Images are recorded at quarter wavelength intervals.
3.2 Camera and Image Acquisition

Images acquired by the CCD are sent to an image acquisition board (National Instruments IMAQ PCI™-1407) installed in the computer. The CCD (Charged Coupled Device) used in the experiment is Sony XC-ST50 black & white camera module. It has a 6.4 mm × 4.8 mm sensing area, 768 × 494 pixels with 8.4 μm × 9.8 μm pixel size.

3.3 Computer and Programs

Intel Pentium® 4 CPU 2.80 GHz computer with Microsoft Windows XP Professional Version 2002 is used in the experiment. Image analysis and calculations are done by using LabVIEW programs.

The first LabVIEW program used in the experiment records interference images at quarter wavelength intervals and calculates the final phase image of the object being imaged. For each color LED, a final phase image is produced and saved in the computer.

Another LabVIEW program is used to combine single wavelength phase images to produce an unwrapped phase image. This program consists of optical phase unwrapping based on both two wavelength and three wavelength phase unwrapping methods.

These programs are shown in Appendix A.
3.4 Light Sources

Three types of light sources are used in the experiment; light emitting diodes, laser diodes and ring dye laser. Light emitting diodes have very small coherence length and are categorized as an incoherent light source. Laser diodes have a larger coherence length compared to that of light emitting diodes, but still smaller than the coherence length of ring dye laser.

3.4.1 Light Emitting Diodes

Light emitting diodes (LED) have been used as in interferometric light sources in order to reduce the speckle noise inherent to lasers [14-17]. Interference of coherent waves produces speckle noise, which limits the phase map’s information. Since a LED’s coherence length is in the micron range, speckle noise is greatly reduced. LEDs also cost much less than lasers, are easy to use and replace and can reduce overall apparatus dimensions. The LEDs used in this experiment are Luxeon™ Emitter diodes from Lumileds Lighting LLC. All of the LEDs used herein have a Lambertian (high dome) radiation pattern. And their spectra are shown in Figure (3.4). These spectra were taken with Ocean Optics SD-1000 fiber optics spectrometer.
Figure 3.4: A light emitting diode used in the experiment.

Figure 3.5: Spectra of light emitting diodes.

The peak wavelengths, luminous flux, calculated and measured coherence lengths for the red, amber and green LEDs used in this experiment are shown in Table (3.1). The calculated coherence length of a light source is given by $\lambda_c = (2\ln2/\pi)(\bar{\lambda}^2/\Delta\lambda)$, where $\bar{\lambda}$ is the mean wavelength and $\Delta\lambda$ is the full width half maximum (FWHM) of Gaussian
spectrum [18]. The coherence length was directly measured here by counting the number of fringes in the interference of the tilted mirror object.

**Table 3.1**: Characteristics of LEDs. Luminous flux values are at 350 mA, Junction Temperature $T_J = 25^\circ C$ [19]

<table>
<thead>
<tr>
<th>Color</th>
<th>Luminous Flux $\Phi$ (lm) [14]</th>
<th>Peak Wavelength $\lambda$ (nm)</th>
<th>Spectral Width (nm)</th>
<th>Calculated Coherence Length ($\mu m$)</th>
<th>Measured Coherence Length ($\mu m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>44</td>
<td>$653.83 \pm 0.07$</td>
<td>$27.24 \pm 0.15$</td>
<td>$6.91 \pm 0.04$</td>
<td>$9.15 \pm 2.45$</td>
</tr>
<tr>
<td>Red-Orange</td>
<td>55</td>
<td>$643.42 \pm 0.07$</td>
<td>$23.21 \pm 0.14$</td>
<td>$7.85 \pm 0.05$</td>
<td>$10.29 \pm 2.57$</td>
</tr>
<tr>
<td>Amber</td>
<td>36</td>
<td>$603.48 \pm 0.03$</td>
<td>$17.53 \pm 0.05$</td>
<td>$9.14 \pm 0.03$</td>
<td>$10.86 \pm 2.56$</td>
</tr>
<tr>
<td>Green</td>
<td>25</td>
<td>$550.18 \pm 0.09$</td>
<td>$38.93 \pm 0.19$</td>
<td>$3.42 \pm 0.02$</td>
<td>$3.85 \pm 1.46$</td>
</tr>
</tbody>
</table>

### 3.4.2 Laser Diodes

Laser diodes (LD) have been widely used as a light source in interferometry due to their frequency tunability, smaller size and cost compared to those of lasers [20-24]. They have shorter coherence lengths, typically few centimeters, compared to coherence length of lasers. The laser diodes used in this experiment have following properties as shown in Table (3.2). Measured wavelengths are obtained from laser diode spectra. Spectra are shown in Figure (3.5).
Figure 3.6: A laser diode used in the experiment.

Figure 3.7: Spectra of laser diodes.
Table 3.2: Characteristics of laser diodes.

<table>
<thead>
<tr>
<th>Name</th>
<th>Power (mW)</th>
<th>Manufacturer’s typical wavelength (nm)</th>
<th>Measured wavelength $\lambda$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatronic LDM145</td>
<td>1</td>
<td>633</td>
<td>$636.89 \pm 0.01$</td>
</tr>
<tr>
<td>DL3148-025 Red Laser Diode</td>
<td>5</td>
<td>635</td>
<td>$653.22 \pm 0.01$</td>
</tr>
<tr>
<td>DL3147-060 Red Laser Diode</td>
<td>7</td>
<td>650</td>
<td>$659.77 \pm 0.01$</td>
</tr>
<tr>
<td>HL6724MG AlGaInP Laser Diode</td>
<td>5</td>
<td>670</td>
<td>$677.81 \pm 0.01$</td>
</tr>
</tbody>
</table>

3.4.3 Ring Dye Laser

The ring dye laser used in the experiment is Coherent CR-699 Ring Dye Laser and uses Rhodamine 6G (R6G) dye. It is pumped by Spectra-Physics Millenia V diode pumped solid state (DPSS) laser with an output of 5 W, 532 nm light. With R6G dye, the ring laser has a scan range of 560 nm - 625 nm. The beam diameter is 0.75 mm [25].

Figure 3.8: The ring dye laser used in the experiment.
CHAPTER 4

MULTIWAVELENGTH PHASE SHIFTING INTERFERENCE MICROSCOPY

Multi wavelength phase shifting interference microscopy is a technique that combines phase shifting interference microscopy and optical phase unwrapping. Phase shifting methods were described in the previous chapter. In this chapter phase unwrapping methods are described in detail.

When the optical depth of an object is greater than the wavelength, the phase image contains $2\pi$ discontinuities. Therefore, phase data has to be unwrapped from $(-\pi, +\pi)$ or $(0, 2\pi)$ interval to $(-\infty, +\infty)$ interval before one can obtain unambiguous optical thickness profile. Phase unwrapping is not difficult when there is no noise present in the phase map and the absolute value of the phase difference between adjacent phase samples is less than $\pi$.

4.1 Numerical Algorithms for Phase Unwrapping

In basic phase unwrapping method, phase image is divided to horizontal lines and these lines are unwrapped separately by scanning pixels and adding an offset to each pixel. At each discontinuity a $2\pi$ offset is added or subtracted. After all horizontal lines are unwrapped, they are connected vertically and the unwrapping process is done along vertical lines. There are many phase unwrapping methods to remove $2\pi$ discontinuities.
and most can be categorized into two types; path-dependent methods and path-independent methods [26]. Path-dependent methods detect positions of edges and phase discontinuities in images and use this information to calculate phase offset values. In path-independent methods, areas that can cause errors in unwrapping are identified and eliminated before the unwrapping process starts.

In 1994 Ghiglia and Romero used a least squares integration method with phase unwrapping [27]. In this method, which is known as least squares integration of phase gradient method, the phase gradient is obtained as wrapped phase differences along two perpendicular directions and the gradient field is least squares integrated to obtain continuous phase. However, this method is not effective for phase maps with high noise [28].

P. G. Charette and I. W. Hunter proposed a robust phase unwrapping method for phase images with high noise content [26]. The basic concept behind this method is to identify contiguous areas that are not on or close to a fringe boundary by locally fitting planes to the phase data. Then these areas are phase shifted with respect to one another by multiples of $2\pi$ to unwrap the phase.

Software algorithms that exist for detecting and removing $2\pi$ discontinuities are mostly computational-intensive and prone to errors when the phase profile is noisy or when the object has irregularities. Multi-wavelength phase unwrapping is an easy method that can be used to eliminate $2\pi$ ambiguities in phase maps without such problems.
4.2 Multi-Wavelength Phase Unwrapping

The basis of multi-wavelength phase unwrapping method is the idea of beat wavelength. For two wavelengths \( \lambda_1 \) and \( \lambda_2 \), the beat wavelength \( \Lambda_{12} \) is defined as

\[
\Lambda_{12} = \frac{\lambda_1 \lambda_2}{|\lambda_1 - \lambda_2|}
\]  

(4.1)

For years it has been known that a longer wavelength light source produces fewer fringes over a given object than will a short wavelength light source, thus reducing the number of \( 2\pi \) ambiguities in the phase image. However, the drawback is the need of infrared light sources instead of visible light sources. J. C. Wyant has shown that two wavelengths of visible region can be used in the context of holography to produce a longer beat wavelength [29]. Using various pairs of wavelengths from an Argon and HeNe lasers an aspheric optic element was tested. First, a hologram of the test target was obtained by using a wavelength \( \lambda_1 \). Then the hologram was processed and placed at the original position of the interferometer and illuminated by a second wavelength \( \lambda_2 \). The resultant interferogram was identical to the interferogram that would have been illuminated by a light source of \( \Lambda_{12} \); the beat wavelength of wavelengths \( \lambda_1 \) and \( \lambda_2 \).

The term ‘two-wavelength interferometry’ was first used by C. Polhemus in a paper where he introduced a two-wavelength technique for interferometric testing [30]. In the method of static interferometry, a fringe pattern obtained with a light source of wavelength \( \lambda_1 \) is recorded and replaced into the system as a moiré reference mask. Then the light source is replaced by one with wavelength \( \lambda_2 \). The resultant moiré fringe pattern is identical to the fringe pattern that would have been obtained with a light source of \( \Lambda_{12} \).
Polhemus modified the method to apply in real time systems. In the method of dynamic interferometry, light sources of $\lambda_1$ and $\lambda_2$ are operated simultaneously in the interferometer setup, giving a resultant fringe pattern of $\Lambda_{12}$.

In 1984, two-wavelength phase shifting interferometry was introduced as an optical phase unwrapping method [31]. In this method, phase shifting interferometry and two-wavelength interferometry were combined to extend the phase measurement range of single-wavelength phase shifting interferometry. Two methods were introduced to solve $2\pi$ ambiguities by using two-wavelength phase shifting interferometer. In the first method, two sets of wrapped phase data were obtained with wavelengths $\lambda_1$ and $\lambda_2$. The data is then used to calculate the phase difference between pixels for beat wavelength $\Lambda_{12}$. Then all phase difference values are integrated to calculate the relative surface height of the test object. In the second method, two phase maps of different wavelengths are used to produce a phase map of beat wavelength. The beat wavelength phase map is then used as a reference to correct $2\pi$ ambiguities in the single wavelength phase map. Both methods were used to measure 1-D surface heights. Two-wavelength phase shifting interferometry has also been used to obtain three dimensional contour maps of aspheric surfaces with an accuracy of $\Lambda_{12}/100$ [32]. However, a disadvantage of this optical phase unwrapping is that the phase noise in each wavelength is magnified by a factor equal to the magnification of the wavelengths. This problem has been addressed by J. Gass, A. Dakoff and M. K. Kim [33] In the context of digital holography, two phase maps of wavelengths $\lambda_1$ and $\lambda_2$ are used to produce a phase map called “coarse map” with beat wavelength $\Lambda_{12}$. Then one of the single wavelength phase maps is used to reduce the
amplified phase noise of coarse map. The resultant ‘fine map’ has noise similar to single wavelength phase map, with a larger axial range free of $2\pi$ ambiguities.

The two-wavelength phase unwrapping method has been extended to multiple wavelengths; enabling measurements of steep surfaces without software phase unwrapping. A hierarchical phase unwrapping algorithm that chooses a minimum number of wavelengths to increase the accuracy of optical unwrapping has been introduced by C. Wagner, W. Osten and S. Seebacher [34]. The basic principle of this method is to start with a larger beat wavelength. Then a systematic reduction of beat wavelengths is used to improve the accuracy of the measurement while the information of the preceding measurements is used to eliminate $2\pi$ ambiguities. A similar version of hierarchical phase unwrapping has been presented by U. Schnars and W. Jueptner [35], however it has not been used experimentally. Three-wavelength phase unwrapping algorithms have been introduced in both interferometry and digital holography enabling measurements of steep surfaces without software phase unwrapping [9-10, 36-39]. However, while the principle of multi-wave phase unwrapping has been known in interferometry, known applications have been confined to optical profilers with raster-scanned pointwise interferometry. Other than recent digital holography experiments [33, 38, 39], multi-wave phase unwrapping has not yet been applied to full-frame phase images in interferometry.
4.3 Principle of Two-Wavelength Optical Phase Unwrapping

When an object is imaged by using a wavelength smaller than the object’s height, the phase image contains $2\pi$ discontinuities, as shown in Figure (4.1).

![Figure 4.1: Phase Vs Distance. $2\pi$ ambiguities occur when the distance is a multiple of the wavelength.](image)

From Figure (4.1) it is clear that there are many distance values for a given phase value. In order to obtain unambiguous optical thickness profile, we need to have only one $z$ distance for a given phase. These $2\pi$ discontinuities are eliminated by using multi-wavelength optical phase unwrapping method. For the $m^{th}$ wavelength $\lambda_m$, the surface profile $Z_m$ of an object is related to the phase difference $\phi_m$ as follows;

$$Z_m = \frac{\lambda_m \phi_m}{2\pi} \quad (4.2)$$

It is apparent that unambiguous range of $Z$ can be increased by using a longer $\lambda$.

Consider two single wavelength phase maps $\phi_1$ and $\phi_2$ with wavelengths $\lambda_1 = 530 \text{ nm}$ and $\lambda_2 = 470 \text{ nm}$ respectively. The beat wavelength $\Lambda_{12}$ for $\lambda_1$ and $\lambda_2$ is given
by \( \Lambda_{12} = \frac{\lambda_1 \lambda_2}{|\lambda_1 - \lambda_2|} = 4.151 \mu m \). The \( \Lambda_{12} \) can be increased by choosing closer values of \( \lambda_1 \) and \( \lambda_2 \). The phase map for \( \Lambda_{12} \) is obtained by subtracting one single wavelength phase map from the other and then adding \( 2\pi \) whenever the resultant value is less than zero. This phase map is called “coarse map” \( \phi_{12} \). The surface profile for coarse map \( \phi_{12} \) is given by \( Z_{12} = \Lambda_{12} \phi_{12}/2\pi \). However, the phase noise in each single wavelength phase map is magnified by the same factor as the magnification of wavelengths. In the two-wavelength optical phase unwrapping method introduced by J. Gass et al. [33], the phase noise is reduced by using the following steps.

First, the surface profile \( Z_{12} \) is divided into integer multiples of a single wavelength, say \( \lambda_1 \). Then, the result is added to the single wavelength surface profile \( Z_1 \). This significantly reduces the phase noise in the coarse map. However, at the boundaries of wavelength intervals \( \lambda_1 \), the noise of the single wavelength phase map appears as spikes. These spikes can be removed by comparing the result with the coarse map surface profile \( Z_{12} \). If the difference is more than half of \( \lambda_1 \), addition or subtraction of one \( \lambda_1 \) depending on the sign of the difference removes the spikes. The final result ‘fine map’ has a noise level equal to that of single wavelength surface profile. If a single wavelength phase map \( \phi_m \) contains a phase noise of \( 2\pi\varepsilon_m \), the two-wavelength phase unwrapping method works properly for \( \varepsilon_m < \frac{\lambda_m}{4\Lambda_{12}} \). Using a larger beat wavelength reduces the maximum noise limit.
4.4 Two-Wavelength Optical Phase Unwrapping Simulations

Following simulations were done for green (530 nm) and blue (470 nm) wavelengths with $\varepsilon_1 = \varepsilon_2 = 0.02$. Each step is shown in Figure (4.2). The object is a tilted mirror with a height 5 $\mu$m as shown in Figure (4.2a). Surface profiles of green and blue wavelengths are shown in Figures (4.2b) and (4.2c). Their phase maps are shown in Figures (4.2d) and (4.2e). Figure (4.2f) shows $\phi_1 - \phi_2$; subtraction of two phase maps.

Adding $2\pi$ whenever the result is less than zero produces the “coarse map” $\phi_{12}$, as shown in Figure (4.2g). The surface profile of coarse map is shown in Figure (4.2h).

Coarse map has a longer axial range without $2\pi$ ambiguities and the range is equal to the beat wavelength 4.15 $\mu$m.
Figure 4.2: Simulation of two-wavelength optical phase unwrapping. (a) the slanted plane of height $h = 5 \, \mu m$; (b) surface profile $Z_1(x)$ for $\lambda_1=0.53 \, \mu m$; (c) surface profile $Z_2(x)$ for $\lambda_2=0.47 \, \mu m$; (d) phase map $\phi_1(x)$ for $\lambda_1=530 \, nm$; (e) phase map $\phi_2(x)$ for $\lambda_2=470nm$; (f) subtraction of two phase maps $\phi_{12}(x) = \phi_1 - \phi_2$; (g) phase map $\phi_{12}(x)$; (h) coarse map $Z_{12}(x)$ with beat wavelength $\Lambda_{12} = 4.15 \mu m$. 
4.4.1 Noise Reduction of Coarse Map

Once coarse map $Z_{12}(x)$ is obtained, noise has to be reduced. Figure (4.3) shows simulation results of noise reduction. Figure (4.3a) is surface profile of coarse map. Figure (4.3b) shows the result after coarse map was divided by integer multiples of $\lambda_1$. In Figure (4.3c), the surface profile of $\lambda_1$ is added to the result of Figure (4.3b). Now, Figure (4.3c) is compared with the surface profile of coarse map $Z_{12}$ by subtracting Figure (4.3c) from $Z_{12}$ and the result is shown in Figure (4.3d). If the difference is more than half of $\lambda_1$, one $\lambda_1$ is added or subtracted depending on the sign of the difference as shown in Figure (4.3e). The final result ‘fine map’ is obtained by adding the result to Figure (4.3c).
Figure 4.3: Noise reduction for two-wavelength phase unwrapping. (a) coarse map $Z_{12}(x)$; (b) coarse map $Z_{12}(x)$ is divided into integer multiples of $\lambda_1$; (c) $Z_1(x)$ is pasted on result (b); (d) comparison of (c) with coarse map; (e) adding or subtracting $\lambda_1$ to $Z(c)$; (f) fine map.
Figure (4.4) shows phase noise in coarse map and fine map. For $\lambda_1 = 530$ nm and $\lambda_2 = 470$ nm, the maximum noise limit is $\varepsilon_m \sim 3.2\%$. Using a larger beat wavelength reduces the maximum noise limit.

![Figure 4.4: Phase noise in coarse map and fine map. (a) noise in the coarse map; (b) noise in the fine map.](image)

4.5 Principle of Three-Wavelength Optical Phase Unwrapping

The advantage of three wavelength phase unwrapping method is that the beat wavelength can be increased without reducing the maximum noise limit. Suppose the three chosen wavelengths are $\lambda_1 = 625$ nm, $\lambda_2 = 590$ nm, and $\lambda_3 = 530$ nm. The first two wavelengths give beat wavelength $\Lambda_{12} = 10.53$ $\mu$m. Instead of using the surface profile $Z_{12}$ of $\Lambda_{12} = 10.53$ $\mu$m, which has a high noise, an identical surface profile can be produced by using surface profiles $Z_{13}$ and $Z_{23}$ with beat wavelengths $\Lambda_{13} = 3.49$ $\mu$m and $\Lambda_{23} = 5.21$ $\mu$m. The resultant “coarse map of coarse maps” $\phi_{13-23}$ with surface profile $Z_{13-23}$ also has the same beat wavelength $\Lambda_{13-23} = \Lambda_{13}\Lambda_{23}/|\Lambda_{13} - \Lambda_{23}| = 10.53$ $\mu$m.
The noise reduction is done as follows. In the first step, the quantity of integer multiples of $\Lambda_{13}$ present in the range $Z_{13-23}$ is calculated. The result $Z(a)$ is given by

$$Z(a) = \text{int} \left[ \frac{Z_{13-23}}{\Lambda_{13}} \right] \Lambda_{13} \quad (4.3)$$

In the next step, the result is added to the surface profile $Z_{13}$

$$Z(b) = Z(a) + Z_{13} \quad (4.4)$$

The resultant map $Z(b)$ is then compared with $Z_{13-23}$. If the difference $Z(c)$ is more than half of $\Lambda_{13}$, one $\Lambda_{13}$ is added or subtracted depending on the sign difference.

$$Z(d) = \begin{cases} 
Z(c) + \Lambda_{13} & \text{if } Z(c) > \Lambda_{13} / 2 \\
Z(c) & \text{if } -\Lambda_{13} / 2 \leq Z(c) \leq \Lambda_{13} / 2 \\
Z(c) - \Lambda_{13} & \text{if } Z(c) < -\Lambda_{13} / 2 
\end{cases} \quad (4.5)$$

The resultant surface profile $Z(d)$ is called “intermediate fine map” and has significantly reduced noise. Any remaining noise is due to the noise in the phase map $\phi_{13}$. The remaining noise in $Z(d)$ is reduced by using a single wavelength, say $\lambda_1$. First, the intermediate fine map $Z(d)$ is divided into integer multiples of $\lambda_1$.

$$Z(e) = \text{Int} \left[ \frac{Z(d)}{\lambda_1} \right] \lambda_1 \quad (4.6)$$

Then the result is added to the single wavelength surface profile $Z_1$.

$$Z(f) = Z(e) + Z_1 \quad (4.7)$$
The resultant map $Z(f)$ is then compared with $Z_1$. If the difference is more than half of $\lambda_1$, one $\lambda_1$ is added or subtracted depending on the sign difference. The noise in the final map is equal to the noise in the single wavelength surface profile $Z_1$. The maximum noise level $\varepsilon_m$ in the single wavelength phase map for the three wavelength phase imaging to work is given by the smaller value of $\Lambda_{13}/4\Lambda_{12} \sim 8.3\%$ or $\lambda_1/4\Lambda_{13} \sim 4.5\%$. Therefore, the three wavelength phase unwrapping method increases the beat wavelength without magnifying the noise in the final phase map.

### 4.6 Three-Wavelength Optical Phase Unwrapping Simulations

Simulations were done for $\lambda_1 = 625$ nm, $\lambda_2 = 590$ nm and $\lambda_3 = 530$ nm wavelengths with $\varepsilon_1 = \varepsilon_2 = \varepsilon_3 = 0.04$ as shown in Figure (4.5). The object is a tilted mirror with a height 12 $\mu$m as shown in Figure (4.5a). Surface profiles of $\lambda_1 = 625$ nm, $\lambda_2 = 590$ nm and $\lambda_3 = 530$ nm wavelengths are shown in Figures (4.5b), (4.5c) and (4.5d). The surface profile of coarse map of $\lambda_1 = 625$ nm, $\lambda_2 = 590$ nm with beat wavelength $\Lambda_{12} = 10.53$ $\mu$m is shown in Figure (4.5e). The fine map $Z_{12}$ produced by following the procedure described in section (4.4.1) is shown in Figure (4.5f). The phase noise in the fine map $Z_{12}$ is too large.
Figure 4.5: Simulation of three-wavelength optical phase unwrapping. (a) the slanted plane of height 12 μm; (b) surface profile Z₁ for $\lambda₁ = 625$ nm; (c) surface profile Z₂ for $\lambda₂ = 590$ nm; (d) surface profile Z₃ for $\lambda₂ = 530$ nm; (e) coarse map $Z'_{12}$ with beat wavelength $\Lambda_{12} = 10.53$ μm; (f) fine map $Z_{12}$. 
Figure (4.6) shows the next steps of simulations of the three wavelength optical unwrapping method. Surface profiles $Z_{13}$ and $Z_{23}$ with beat wavelengths $\Lambda_{13} = 3.49 \ \mu m$ and $\Lambda_{23} = 5.21 \ \mu m$ are shown in Figure (4.6a) and Figure (4.6b). The surface profile of “coarse map of coarse maps” with beat wavelength $\Lambda_{13-23} = 10.53 \ \mu m$ is shown in Figure (4.6d). Figures (4.6e) and (4.6f) show ‘intermediate fine map’ and the final fine map after applying the noise reduction.
**Figure 4.6:** Continuation of three-wavelength optical phase unwrapping simulations.

(a) coarse map \( Z'_{13} \) with beat wavelength \( \Lambda_{12} = 3.49 \ \mu m \); (b) coarse map \( Z'_{23} \) with beat wavelength \( \Lambda_{32} = 5.21 \ \mu m \); (c) ‘coarse map of coarse maps’ with the beat wavelength \( 10.53 \ \mu m \) ; (d) ‘intermediate fine map’ \( Z''_{13-23} \) where \( Z'_{13} \) is pasted on \( Z'_{13-23} \) \((=Z'_{12})\); (e)

Final fine map \( Z_{13-23} \) where \( Z_1 \) is pasted on \( Z''_{13-23} \)
Figure 4.7: Noise reduction in three-wavelength optical phase unwrapping. (a) noise in coarse map of coarse maps $Z_{13-23}$; (b) noise in intermediate fine map; (c) noise in final fine map.

Figure (4.7) shows how the three wavelength optical phase unwrapping method effectively reduces phase noise in the final phase map. All three graphs are normalized for better comparison.
CHAPTER 5

MULTI-WAVELENGTH OPTICAL PHASE UNWRAPPING USING LIGHT EMITTING DIODES

This chapter presents results of multi-wavelength optical phase unwrapping (OPU) using light emitting diodes. First section presents results of two-wavelength optical phase unwrapping and the second section presents results of three-wavelength optical phase unwrapping. Subsections of each section present different samples describing image size, wavelengths used for unwrapping process and noise in the phase image.

All samples shown in this chapter are imaged with 20X microscope objectives in reference and object arms of the experimental setup.

5.1 Two-Wavelength Optical Phase Unwrapping with Light Emitting Diodes

In this section two light emitting diodes of different wavelengths are used to obtain a larger beat wavelength. A phase image is obtained using each wavelength and then two phase images are combined to produce a coarse map and a fine map as described in the Chapter 4. The samples used in the experiment are; a resolution target, onion cells, a micro electro-mechanical system (MEMS), a transmission electron microscope (TEM) grid and cheek cells.
5.1.1 Resolution Target

The sample is a USAF 1951 resolution target in reflection mode. It consists of vacuum deposited chromium bars on a 1.5 mm thickness of soda lime glass substrate. The dimensions are 2"×2". Bars are organized in groups and elements. Each group consists of 6 elements and each element consists of 3 horizontal and 3 vertical equally spaced bars. Table (5.1) shows the specification table of USAF 1951 resolution target.

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Element 0</th>
<th>Element 1</th>
<th>Element 2</th>
<th>Element 3</th>
<th>Element 4</th>
<th>Element 5</th>
<th>Element 6</th>
<th>Element 7</th>
</tr>
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<td>4.00</td>
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<td>16.00</td>
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<td>64.0</td>
<td>128.0</td>
</tr>
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<td>2.24</td>
<td>4.49</td>
<td>8.98</td>
<td>17.95</td>
<td>36.0</td>
<td>71.8</td>
<td>144.0</td>
</tr>
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<td>1.26</td>
<td>2.52</td>
<td>5.04</td>
<td>10.10</td>
<td>20.16</td>
<td>40.3</td>
<td>80.6</td>
<td>161.0</td>
</tr>
<tr>
<td>4</td>
<td>1.41</td>
<td>2.83</td>
<td>5.66</td>
<td>11.30</td>
<td>22.62</td>
<td>45.3</td>
<td>90.5</td>
<td>181.0</td>
</tr>
<tr>
<td>5</td>
<td>1.59</td>
<td>3.17</td>
<td>6.35</td>
<td>12.70</td>
<td>25.39</td>
<td>50.8</td>
<td>102.0</td>
<td>203.0</td>
</tr>
<tr>
<td>6</td>
<td>1.78</td>
<td>3.56</td>
<td>7.13</td>
<td>14.30</td>
<td>28.50</td>
<td>57.0</td>
<td>114.0</td>
<td>228.0</td>
</tr>
</tbody>
</table>

Red and green light emitting diodes with wavelengths $\lambda_1 = 653.83$ nm and $\lambda_2 = 550.18$ nm are used to obtain a beat wavelength $\Lambda_{12}$ of 3.47 μm. Image area is 0.1 mm × 0.12 mm.
Figure 5.1: Resolution target. The direct image is of the elements of group 7.

The experimental results for two-wavelength optical phase unwrapping are shown in Figure (5.2). Red ($\lambda_1 = 653.83$ nm) and green ($\lambda_2 = 550.18$ nm) LEDs are used as the two wavelengths. The beat wavelength $\Lambda_{12} = 3.47$ $\mu$m. Figure (5.2a) and (5.2b) show single wavelength phase maps $\phi_1$ and $\phi_2$ with $\lambda_1 = 653.83$ nm and $\lambda_2 = 550.18$ nm respectively. The coarse map $\phi_{12}$ with $\Lambda_{12} = 3.47$ $\mu$m is shown in Figure (5.2c) and the final phase map with reduced noise is shown in Figure (5.2d). Figure (5.2e) shows the 3-D rendering of final phase map.

Figure (5.3) shows cross sections of phase maps along the lines shown in Figure (5.2) and the phase noise in the chosen regions. Figure (5.3a) is a cross section of single wavelength phase map with $\lambda_1 = 653.83$ nm and Figure (5.3b) is a cross section of coarse map with $\Lambda_{12} = 3.47$ $\mu$m. A cross section of the fine map with reduced noise is shown in Figure (5.3c). For maps (a), (b) and (c), the vertical axis is 4 $\mu$m. The root mean square (rms) noise of the single wavelength phase map is 4.65 nm. This is shown in Figure (5.3d). Figure (5.3e) shows the noise in coarse map. The root mean square (rms) noise is 42.89 nm. Final fine phase map with reduced noise is shown in Figure (5.3f). The rms phase noise in the final phase map is 7.16 nm.
Figure 5.2: Results of two-wavelength optical unwrapping for resolution target. (a) phase map with $\lambda_1 = 653.83$ nm; (b) phase map with $\lambda_2 = 550.18$ nm; (c) coarse map with beat wavelength $\Lambda_{12} = 3.5 \mu$m; (d) fine map with reduced noise; (e) 3-D rendering of (d).
Figure 5.3: Surface profiles of resolution target. (a) single wavelength surface profile; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of single wavelength phase map in the region between the two markers in plot (a). Rms noise is 4.65 nm; (e) noise of coarse map in the area shown in plot (b). Rms noise is 42.89 nm; (f) noise of final unwrapped phase map in the area shown in (c). Rms noise is 7.16 nm.
5.1.2 Onion Cells

A fresh layer of onion skin is placed on a plane mirror and used as the object. Red and amber light emitting diodes with wavelengths $\lambda_1 = 653.83$ nm and $\lambda_2 = 603.48$ nm are used to obtain an beat wavelength $\Lambda$ of 7.84 $\mu$m. Onion cells are rectangular in shape and the size ranges from 250 $\mu$m to 400 $\mu$m. Figure (5.4) shows an interferogram of a sample of onion cells.

Figure 5.4: Interferogram of onion cells.

Figure (5.5) shows the experimental results of two-wavelength optical phase unwrapping of onion cells. Images are of a 184 $\mu$m $\times$ 184 $\mu$m area. Figure (5.5a) and (5.5b) show single wavelength phase maps $\phi_1$ and $\phi_2$ with $\lambda_1 = 653.83$ nm and $\lambda_2 = 603.48$ nm respectively. The coarse map $\phi_{12}$ with $\Lambda_{12} = 7.84$ $\mu$m is shown in Figure (5.5c) and the final phase map with reduced noise is shown in Figure (5.5d). Figure (5.5e) shows the 3-D rendering of final phase map.
Figure (5.6) shows cross sections of phase maps along the lines shown in Figure (5.5) and the phase noise in the chosen regions. Figure (5.6a) is a cross section of single wavelength phase map with $\lambda_1 = 653.83$ nm and Figure (5.6b) is a cross section of coarse map with $\Lambda_{12} = 7.84$ $\mu$m. A cross section of the fine map with reduced noise is shown in Figure (5.6c). For maps (a), (b) and (c), the vertical axis is 8 $\mu$m. The root mean square (rms) noise of the single wavelength phase map is 9.04 nm. This is shown in Figure (5.6d). Figure (5.6e) shows the noise in coarse map. The root mean square (rms) noise is 139.04 nm. Final fine phase map with reduced noise is shown in Figure (5.6f). The rms phase noise in the final phase map is 9.04 nm.
Figure 5.5: Results of two-wavelength optical unwrapping for onion cells. Images are of a 184 μm × 184 μm area; (a) phase map with $\lambda_1 = 653.83$ nm; (b) phase map with $\lambda_2 = 603.48$ nm; (c) coarse map with beat wavelength $\Lambda_{12} = 7.84 \mu$m; (d) fine map with reduced noise; (e) 3-D rendering of (d).
Figure 5.6: Surface profiles of onion cells. (a) single wavelength surface profile with $\lambda_i = 653.83$ nm; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of single wavelength phase map in the region between the two markers in plot (a). Rms noise is 9.04 nm; (e) noise of coarse map in the area shown in plot (b). Rms noise is 139.04 nm; (f) noise of final unwrapped phase map in the area shown in (c). Rms noise is 9.04 nm.
5.1.3 Micro-Electro-Mechanical System (MEMS)

The object here in Figure (5.7) is a micro-electrode array biosensor. It consists of 16 gold electrodes on a Pyrex glass substrate. The center is a 125μm diameter circle with an approximate thickness of 2μm [40]. The center of the device was imaged and the experimental results for two-wavelength optical phase unwrapping are shown in Figure (5.8). Red (λ₁ = 653.83 nm) and green (λ₂ = 550.18 nm) LEDs are used as the two wavelengths. The beat wavelength Λ₁₂ = 3.47 μm. Images are of a 184 μm × 184 μm area. Figure (5.8a) shows a single wavelength phase map φ₁ with λ₁ = 653.83 nm. The coarse map φ₁₂ with Λ₁₂ = 3.47 μm is shown in Figure (5.8b) and the final phase map with reduced noise is shown in Figure (5.8c). Figure (5.9) shows cross sections of phase maps along the lines shown in Figure (5.8) and the phase noise in the chosen regions. Figure (5.9a) is a cross section of single wavelength phase map with λ₁ = 653.83 nm and Figure (5.9b) is a cross section of coarse map with Λ₁₂ = 3.47 μm. A cross section of the fine map with reduced noise is shown in Figure (5.9c). For maps (a), (b) and (c), the vertical axis is 4 μm. The root mean square (rms) noise of the coarse map is 43.27 nm. This is shown in Figure (5.9d). Figure (5.9e) shows the reduced noise in fine phase map. Since the center of the MEMS device has a curvature, a paraboloid is fitted to the data. The red dotted line is the best-fit parabolic curve. After subtracting the curvature from the data, the Figure (5.9f) shows the corrected phase noise of 10.29 nm.
Figure 5.7: micro-electrode array bio sensor.

Figure 5.8: Results of two-wavelength optical phase unwrapping for biosensor. (a) single wavelength phase map; (b) two-wavelength coarse map; (c) two-wavelength fine map with reduced noise.
Figure 5.9: Surface profiles of biosensor. (a) single wavelength surface profile; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of the coarse map in the region between the two markers in plot (b). Rms noise is 43.27 nm; (e) noise of final unwrapped phase map in the area shown in (c). Red dotted line is the best fit parabolic curvature and black solid line is data; (f) corrected phase noise of the unwrapped phase map, after subtracting the curvature of the object. Rms noise is 10.29 nm.
5.1.4 Transmission Electron Microscope (TEM) Grids

The object here is a transmission electron microscope (TEM) grid with a hexagonal 150 mesh pattern. The grid is made of copper and the diameter is 3.05 mm diameter with a solid border. Typical hole width is 130 μm and bar width is 35 μm.

![TEM Grid Image](image)

**Figure 5.10**: TEM grids. Image is taken by National DC2-156-S Digital Microscope. The length of the green line is 125.7 μm.

Figure (5.11) shows the results of two-wavelength optical phase unwrapping using red and green light emitting diodes with wavelengths $\lambda_1 = 653.83$ nm and $\lambda_2 = 550.18$ nm are used to obtain a beat wavelength $\Lambda_{12}$ of 3.47 μm. Images are of a 184 μm × 184 μm area.

Figure (5.11a) shows a single wavelength phase map $\phi_1$ with $\lambda_1 = 653.83$ nm. The coarse map $\phi_{12}$ with $\Lambda_{12} = 3.47$ μm is shown in Figure (5.11b) and the final phase map with reduced noise is shown in Figure (5.11c). Figure (5.12) shows cross sections of phase maps along the lines shown in Figure (5.11) and the phase noise in the chosen regions. Figure (5.12a) is a cross section of single wavelength phase map with $\lambda_1 =
653.83 nm and Figure (5.12b) is a cross section of coarse map with \( \Lambda_{12} = 3.47 \mu \text{m} \). A cross section of the fine map with reduced noise is shown in Figure (5.12c). For maps (a), (b) and (c), the vertical axis is 4 \( \mu \text{m} \). The root mean square (rms) noise of the coarse map is 99.01 nm. This is shown in Figure (5.12e). Figure (5.12f) shows the reduced noise in fine phase map. The rms noise is 10.88 nm.

**Figure 5.11:** Results of two-wavelength optical phase unwrapping for TEM grids. (a) single wavelength phase map; (b) two- wavelength coarse map; (c) two-wavelength fine map with reduced noise.
Figure 5.12: Surface profiles of TEM grids. (a) single wavelength surface profile with $\lambda_i = 653.83\ \text{nm}$; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of single wavelength phase map in the region between the two markers in plot (a). Rms noise is 10.88 nm; (e) noise of coarse map in the area shown in plot (b). Rms noise is 99.01 nm; (f) noise of final unwrapped phase map in the area shown in (c). Rms noise is 10.88 nm.
5.1.5 Cheek Cells

In this section experimental results of two-wavelength optical phase unwrapping using a sample of basal mucosa (commonly known as cheek cells) are presented.

![Cheek Cell Image]

Figure 5.13: Direct image of a cheek cell.

Red and green light emitting diodes with wavelengths \( \lambda_1 = 653.83 \text{ nm} \) and \( \lambda_2 = 550.18 \text{ nm} \) are used to obtain an beat wavelength \( \Lambda \) of 3.5 \( \mu \text{m} \). Image size is 66.9 \( \mu \text{m} \times 77.8 \mu \text{m} \) with 233 \times 271 pixels. Experimental results of two-wavelength optical unwrapping are shown in Figure (5.14). Figure (5.14a) shows a single wavelength phase map \( \phi_1 \) with \( \lambda_1 = 653.83 \text{ nm} \). The coarse map \( \phi_{12} \) with \( \Lambda_{12} = 3.47 \mu \text{m} \) is shown in Figure (5.14b) and the final phase map with reduced noise is shown in Figure (5.14c). Figure (5.14d) shows the 3-D rendering of final phase map.
Figure 5.14: Results of two-wavelength optical unwrapping for cheek cell. (a) phase map with $\lambda_1 = 653.83$ nm; (b) phase map with $\lambda_2 = 550.18$ nm; (c) coarse map with beat wavelength $\Lambda_{12} = 3.47\mu m$; (d) fine map with reduced noise; (e) 3-D rendering of (d).
5.2 Three-Wavelength Optical Phase Unwrapping with Light Emitting Diodes

In three-wavelength optical phase unwrapping, three light emitting diodes of different wavelengths are used. Here the beat wavelength $\Lambda$ is increased by choosing closer wavelengths, and the third wavelength is used to reduce the noise in phase maps. The samples used in the experiment are; a resolution target, a micro electro-mechanical system (MEMS), a transmission electron microscope (TEM) grid and cheek cells.

5.2.1 Resolution Target

The object here is the same resolution target used in section (5.1.1). Red ($\lambda_1 = 653.83$ nm), amber ($\lambda_2 = 603.48$ nm) and green ($\lambda_3 = 550.18$ nm) LEDs are used as the three wavelengths. The beat wavelength $\Lambda_{13-23} = 7.84$ µm. Figure (5.15) shows the experimental results of three-wavelength optical phase unwrapping. Image area is 0.1 mm × 0.12 mm. Figure (5.15a) shows a single wavelength phase map $\phi_1$ with $\lambda_1 = 653.83$ nm. The coarse map $\phi_{12}$ with $\Lambda_{12} = 7.84$ µm is shown in Figure (5.15b) and the final phase map with reduced noise is shown in Figure (5.15c).

![Figure 5.15: Results of three-wavelength OPU for resolution target. (a) single wavelength phase map; (b) three-wavelength coarse map; (c) three-wavelength fine map with reduced noise.](image)
Cross section of each phase map is taken along the lines shown in Figure (5.15). These cross sections and phase noise of coarse and fine maps are shown in Figure (5.16). Figures (5.16a) - (5.16c) show surface profiles of single wavelength phase map, coarse map and fine map respectively. According to Figure (5.16c), the thickness of the vertical bars of group 7 element 4 is ~ 70 nm. Vertical axis for each map is 7 μm. Figure (5.16d) shows 144.6 nm rms noise of the coarse map. The reduced phase noise in the fine map is 3.98 nm as shown in Figure (5.16e).
Figure 5.16: Surface profiles resolution target. (a) single wavelength surface profile; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of coarse map in the area shown in (b). Rms noise is 144.6 nm; (e) noise of final unwrapped phase map in the area shown in (c). Rms noise is 3.98 nm.
5.2.2 Onion Cells

The object here is the same onion cell sample used in section (5.1.2). Red (\(\lambda_1 = 653.83\) nm), amber (\(\lambda_2 = 603.48\) nm) and green (\(\lambda_3 = 550.18\) nm) LEDs are used as the three wavelengths. The beat wavelength \(\Lambda_{13-23} = 7.84\) \(\mu\)m. Image area is 184 \(\mu\)m \(\times\) 184 \(\mu\)m. Figure (5.17a) is the single wavelength phase map with \(\lambda_1 = 653.83\) nm. The three wavelength coarse map is shown in Figure (5.17b) with a beat wavelength \(\Lambda_{13-23} = 7.84\) \(\mu\)m. The final fine map with reduced noise is shown in Figure (5.17c).

Figure 5.17: Results of three-wavelength OPU for onion cells. (a) single wavelength phase map; (b) three-wavelength coarse map; (c) three-wavelength fine map with reduced noise.

Figure (5.18) shows cross sections of phase maps along the lines shown in Figure (5.17) and the phase noise in the chosen regions. Figure (5.18a) is a cross section of single wavelength phase map with \(\lambda_1 = 653.83\) nm and Figure (5.18b) is a cross section of coarse map with \(\Lambda_{12} = 7.84\) \(\mu\)m. A cross section of the fine map with reduced noise is shown in Figure (5.18c). For maps (a), (b) and (c), the vertical axis is 8 \(\mu\)m. The root mean square (rms) noise in the selected region of the coarse map is 349.67 nm. This is
shown in Figure (5.18d). Figure (5.18e) shows the reduced noise in fine phase map. The rms noise is 31.43 nm.
**Figure 5.18**: Surface profiles of onion cells. (a) single wavelength surface profile with $\lambda_i = 653.83$ nm; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of coarse map in the area shown in plot (b). Rms noise is 349.67 nm; (e) noise of final unwrapped phase map in the area shown in (c). Rms noise is 31.43 nm.
5.2.3 Micro-Electro Mechanical System (MEMS) Biosensor

The experimental results for three-wavelength optical phase unwrapping are shown in Figure (5.19). Red ($\lambda_1 = 653.83$ nm), amber ($\lambda_2 = 603.48$ nm) and green ($\lambda_3 = 550.18$ nm) LEDs are used as the three wavelengths. The beat wavelength $\Lambda_{13-23} = 7.84 \mu$m. The object is the center of the same micro-electrode array bio-sensor used in section (5.1.3). Images are of a 184 $\mu$m × 184 $\mu$m area. Figure (5.19a) is the single wavelength phase map with $\lambda_1 = 653.83$ nm. The three wavelength coarse map is shown in Figure (5.19b) with an beat wavelength $\Lambda_{13-23} = 7.84 \mu$m. The final fine map with reduced noise is shown in Figure (5.19c).

Figure 5.19: Results of three-wavelength OPU for biosensor. (a) single wavelength phase map; (b) three-wavelength coarse map; (c) three- wavelength fine map with reduced noise.

Cross section of each phase map is taken along the lines shown in Figure (5.19). These cross sections and phase noise of coarse and fine maps are shown in Figure (5.20). Figures (5.20a)-(5.20c) show surface profiles of single wavelength phase map, coarse map and fine map respectively. Vertical axis for each map is 11 $\mu$m. Figure (5.20d) shows 105.79 nm rms noise of the coarse map. Because of the curvature of the object
surface, a paraboloid is fitted with the final fine map data. This is shown in Figure (5.20e).

The black line is data and the red dotted line shows the best-fit parabolic curve.

Corrected phase noise in the fine map is 4.78 nm, which is shown in Figure (5.20f).
Figure 5.20: Surface profiles of biosensor. (a) single wavelength surface profile; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of coarse map in the area shown in (b). rms noise is 105.79 nm; (e) noise of final unwrapped phase map in the area shown in (c). Red dotted line is the best fit parabolic curvature and black solid line is data; (f) Final noise of the unwrapped phase map, after subtracting the curvature of the object. Rms noise is 4.78 nm.
5.2.4 Transmission Electron Microscope (TEM) Grids

In this section, experimental results of three wavelength optical unwrapping are presented with the same TEM grid used in section (5.1.4). Three wavelengths are \( \lambda_1 = 653.83 \text{ nm} \), \( \lambda_2 = 603.48 \text{ nm} \), and \( \lambda_3 = 550.18 \text{ nm} \). The beat wavelength is 7.84 \( \mu \text{m} \). Images are of a 184 \( \mu \text{m} \times 184 \mu \text{m} \) area. Figure (5.21a) is the single wavelength phase map with \( \lambda_1 = 653.83 \text{ nm} \). The three wavelength coarse map is shown in Figure (5.21b) with a beat wavelength \( \Lambda_{13-23} = 7.84 \mu \text{m} \). The final fine map with reduced noise is shown in Figure (5.21c).

![Figure 5.21](image)

**Figure 5.21**: Results of three-wavelength OPU for TEM grids. (a) single wavelength phase map; (b) three-wavelength coarse map; (c) three-wavelength fine map with reduced noise.

Cross section of each phase map is taken along the lines shown in Figure (5.21). These cross sections and phase noise of coarse and fine maps are shown in Figure (5.22). Figures (5.22a)-(5.22c) show surface profiles of single wavelength phase map, coarse map and fine map respectively. Vertical axis for each map is 7 \( \mu \text{m} \). Figure (5.22d) shows 32.26 nm rms noise in the single wavelength phase map. Figure (5.22e) shows 303.77 nm
rms noise of the unwrapped coarse map. Final phase map with reduced phase noise is shown in Figure (5.22f). The reduced rms noise in the final phase map is 32.11 nm
Figure 5.22: Surface profiles of TEM grids. (a) single wavelength surface profile with $\lambda_i = 653.83$ nm; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of single wavelength phase map in the region between the two markers in plot (a). Rms noise is 32.26 nm; (e) noise of coarse map in the area shown in plot (b). Rms noise is 303.77 nm; (f) noise of final unwrapped phase map in the area shown in (c). Rms noise is 32.11 nm.
5.2.5 Cheek Cells

In this section experimental results of three-wavelength optical phase unwrapping using a sample of basal mucosa (commonly known as cheek cells) are presented with $\lambda_1 = 653.83$ nm, $\lambda_2 = 603.48$ nm and $\lambda_3 = 550.18$ nm. The beat wavelength is 7.84 $\mu$m. Image size is 66.9 $\mu$m $\times$ 77.8 $\mu$m with 233 $\times$ 271 pixels. Figure (5.23a) is the single wavelength phase map with $\lambda_1 = 653.83$ nm. The three wavelength coarse map is shown in Figure (5.23b) with an beat wavelength $\Lambda_{13-23} = 7.84$ $\mu$m. The final fine map with reduced noise is shown in Figure (5.23c). Figure (5.23d) shows the 3-D rendering of the final fine map.
Figure 5.23: Results of three-wavelength OPU for cheek cells. (a) phase map with $\lambda_1 = 653.83$ nm; (b) coarse map with beat wavelength $\Lambda_{13-23} = 7.84\mu$m; (c) fine map with reduced noise; (d) 3-D rendering of (c).
5.3 Discussion and Conclusions

Both two wavelength and three wavelength optical phase unwrapping techniques can be successfully used to unwrap phase images obtained using light emitting diodes. Since the coherence length of light emitting diodes is in micro meter range, images are free of speckle noise. However, the height profiles are limited by the coherence length and the features that extend beyond coherence length appear as noise. Therefore, light emitting diodes are most suitable for imaging objects with features smaller than the coherence length of the diode. For objects with larger features, laser diodes or lasers can be used as described in next chapters.
This chapter presents results of multi-wavelength optical phase unwrapping using laser diodes. Diode were chosen so that the combination of diodes provides a much larger beat wavelength that that of LEDs. First section presents results of two-wavelength optical phase unwrapping and the second section presents results of three-wavelength optical phase unwrapping. Subsections of each section present different samples describing image size and wavelengths used for unwrapping process.

6.1 Two-Wavelength Optical Phase Unwrapping with Laser Diodes

In this section experimental results of two-wavelength optical phase unwrapping (OPU) using a sample of basal mucosa (commonly known as cheek cells) are presented with $\lambda_1 = 679.68$ nm and $\lambda_2 = 660.86$ nm. The beat wavelength $\Lambda_{13-23}$ is 23.87 $\mu$m. Image size is 92.42 $\mu$m and 448 pixels per side. Figure (6.1a) is the single wavelength phase map with $\lambda_1 = 653.83$ nm. The three wavelength coarse map is shown in Figure (6.1b) with a beat wavelength $\Lambda_{13-23} = 23.87$ $\mu$m. The final fine map with reduced noise is shown in Figure (6.1c). Figure (6.1d) shows the 3-D rendering of final fine map.
Figure 6.1: Results of two-wavelength OPU for cheek cells. (a) phase map with $\lambda_1 = 679.68$ nm; (b) coarse map with beat wavelength $\Lambda_{12} = 23.87 \mu$ m; (c) fine map with reduced noise; (d) 3-D rendering of (c).
6.2 Three-wavelength Optical Phase Unwrapping with Laser Diodes

In three-wavelength optical phase unwrapping, three light emitting diodes of different wavelengths are used. Here the beat wavelength $\Lambda$ is increased by choosing closer wavelengths, and the third wavelength is used to reduce the noise in phase maps. The samples used in the experiment are; a micro electro-mechanical system (MEMS), and a sample of LP grooves.

6.2.1 Micro Electro Mechanical (MEM) Biosensor

The object here is a micro-electrode array biosensor. It consists of 16 gold electrodes on a Pyrex glass substrate. The center is a 125\(\mu\)m diameter circle with an approximate thickness of 2\(\mu\)m [40]. The unwrapped phase map shows the grainy surface of electrodes. The three wavelengths are $\lambda_1 = 677.81$ nm, $\lambda_2 = 639.37$ nm and $\lambda_3 = 636.89$ nm with a beat wavelength of $\Lambda_{13-23} = 11.27 \mu m$. Figure (6.2) shows the results of three-wavelength optical phase unwrapping. Figure (6.2a) is the single wavelength phase map with $\lambda_1 = 677.81$ nm. The three wavelength coarse map is shown in Figure (6.2b) with a beat wavelength $\Lambda_{13-23} = 11.27 \mu m$. The final fine map with reduced noise is shown in Figure (6.2c). Figure (6.2d) shows the 3-D rendering of final fine map.

Cross section of each phase map is taken along the lines shown in Figure (6.2). These cross sections and phase noise of coarse and fine maps are shown in Figure (6.3). Figures (6.3a)-(6.3c) show surface profiles of single wavelength phase map, coarse map and fine map respectively. Vertical axis for each map is 14 \(\mu\)m. Figure (6.3d) shows 326.9 nm rms noise of the unwrapped coarse map. Final phase map with reduced phase
noise is shown in Figure (6.3e). The reduced rms noise in the final phase map is 257.08 nm.
Figure 6.2: Three-wavelength OPU of MEMS sensor. Image size is 86.6 μm and 300 pixels per side. (a) a single wavelength phase map with \( \lambda = 677.81 \, \text{nm} \); (b) Coarse map of coarse map produced by \( \lambda_1 = 677.81 \, \text{nm} \), \( \lambda_2 = 639.37 \, \text{nm} \) and \( \lambda_3 = 636.89 \, \text{nm} \) with \( \Lambda_{13-23} = 11.27 \, \mu \text{m} \); (c) Final fine map with reduced noise, (d) 3-D rendering of (c).
Figure 6.3: Surface profiles of MEMS sensor. (a) single wavelength surface profile; (b) surface profile of coarse map; (c) surface profile of final unwrapped phase map with reduced noise; (d) noise of coarse map in the area shown in (b). rms noise is 326.9 nm; (e) noise of final unwrapped phase map in the area shown in (c). Rms noise is 257.08 nm.
6.2.2 Long Playing (LP) Record Grooves

The object is a piece of 33 1/3 r.p.m. long playing (LP) record. For 33 1/3 r.p.m.
records the typical width at the top of the groove ranges from 25.4 $\mu$m to 76.2 $\mu$m and
the groove spacing is 84 $\mu$m - 127 $\mu$m [41]. The sample is coated with a layer of 200 nm
Aluminum for better reflectivity. The three wavelengths are $\lambda_1 = 677.81$ nm, $\lambda_2 = 659.77$

nm and $\lambda_3 = 636.89$ nm with a beat wavelength of $\Lambda_{13-23} = 24.77$ $\mu$m. Figure (6.4) shows
the results of three-wavelength optical phase unwrapping. Image size is 147 $\mu$m × 110

$\mu$m, 640 × 480 pixels. Figures (6.4a)-(6.4c) show single wavelength phase map with $\lambda_1 =

677.81$ nm, coarse map of coarse map with beat wavelength $\Lambda_{13-23} = 24.77 \mu$m and the
final fine map with reduced noise. Figure (6.4d) is the cross section taken along the line
shown in Figure (6.4b). The rms noise in the region shown is 1.9 $\mu$m. Figure (6.4e)
shows the cross section along the line shown in Figure (6.4c). The rms noise in the region
is 0.6 $\mu$m.
Figure 6.4: Three-wavelength OPU of LP grooves. Image size is $147\ \mu \text{m} \times 110\ \mu \text{m}$, $640 \times 480$ pixels. (a) a single wavelength phase map with $\lambda = 677.81\ \text{nm}$; (b) Coarse map of coarse map with $\Lambda_{1-2} = 24.77\ \mu \text{m}$; (c) Final fine map with reduced noise; (d) surface profile of coarse map; (e) surface profile of final unwrapped phase map with reduced noise; (e) noise of coarse map in the area shown in (b). rms noise is $1.9\ \mu \text{m}$; (f) surface profile of final unwrapped phase map in the area shown in (c); (g) final noise of the unwrapped phase map. Rms noise is $0.6\ \mu \text{m}$. 
6.3 Discussion and Conclusions

Laser diodes can be effectively used as a light source for multi-wavelength optical phase unwrapping. Small size and low price compared to those of lasers are advantages of laser diodes. The coherence length of laser diodes is larger than that of light emitting diodes and therefore laser diodes can be used to image objects that cannot be successfully used with light emitting diodes.
This chapter presents experimental results of three-wavelength optical phase unwrapping obtained with a ring dye laser. With ring dye laser there are more wavelength choices and the uncertainty of wavelength measurements is 0.1 nm. Cheek cells and aluminum coated LP grooves are used as the samples.

### 7.1 Cheek Cells

Using wavelengths $\lambda_1 = 579$ nm, $\lambda_2 = 577$ nm and $\lambda_3 = 574$ nm, a cheek cell is imaged. The beat wavelength is $167 \mu m$. Image size is $102 \mu m$ and 448 pixels per side.


**Figure 7.1**: Three-wavelength OPU of cheek cells. Image size is 102 $\mu m$ and 448 pixels per side. (a) direct image of cheek cell; (b) a single wavelength phase map ($\lambda = 579$ nm); (c) coarse map of coarse map produced by $\lambda_1 = 579$ nm, $\lambda_2 = 577$ nm and $\lambda_3 = 574$ nm with $\Lambda_{13-23} = 167.04$ $\mu m$; (d) Final fine map with reduced noise; (e) 3-D rendering of (d).
7.2 LP Record Grooves

The object is a piece of 331/3 r.p.m. LP record, coated with 200 nm Aluminum layer. For 331/3 r.p.m. records the typical width at the top of the groove ranges from 25.4 μm to 76.2 μm and the groove spacing is 84 μm - 127 μm [41]. The three wavelengths used for optical unwrapping process is \( \lambda_1 = 577 \text{ nm} \), \( \lambda_2 = 575 \text{ nm} \) and \( \lambda_3 = 570 \text{ nm} \) with a beat wavelength of 166 μm. Figure (7.2a) is the single wavelength phase map with \( \lambda_1 = 577 \text{ nm} \). The three wavelength coarse map is shown in Figure (7.2b) with beat wavelength \( \Lambda_{13-23} = 166 \mu m \). The final fine map with reduced noise is shown in Figure (7.2c). Figure (7.2d) is the 3-D rendering of final fine map. In the final unwrapped phase map, the width of the top of the groove is measured along the line shown in Figure (7.2d). The measured width is 44 μm.

Cross sections and phase noise of coarse and fine maps are shown in Figure (7.3). Figure (7.3a) is the unwrapped coarse map and Figure (7.3b) is the final fine map with reduced noise. Figure (7.3c) is the surface profile of coarse map along the line shown in Figure (7.3a). The rms noise in coarse map in the area shown in (a) is 2.12 μm and this is shown in Figure (7.3d). Figure (7.3e) shows the surface profile of fine map along the line shown in (b). The groove depth \( h = 18 \mu m \). Figure (7.3f) shows the noise of the fine map in the selected area. Rms noise is 1.36 μm.
Figure 7.2: Three-wavelength OPU of LP record grooves. Image size is 102 μm and 448 pixels per side. (a) a single wavelength phase map with $\lambda = 577$ nm; (b) Coarse map of coarse map produced by $\lambda_1 = 577$ nm, $\lambda_2 = 575$ nm and $\lambda_3 = 570$ nm with $\Lambda_{13-23} = 165.89$ μm; (c) Final fine map with reduced noise; (d) 3-D rendering of (c). The groove width is 44 μm.
Figure 7.3: Surface profiles of LP record grooves. (a) Coarse map of coarse map with $\Lambda_{13-23} = 165.89 \ \mu$m; (b) final fine map with reduced noise; (c) surface profile of coarse map along the line shown in (a); (d) noise in coarse map in the area shown in (a). Rms noise is 2.12 $\mu$m; (e) surface profile of fine map along the line shown in (b). The groove depth $h = 18 \ \mu$m; (f) noise of the fine map in the area shown in (b). Rms noise is 1.36 $\mu$m.
7.3 Discussion and Conclusions

Effectiveness of multi-wavelength optical phase unwrapping using a ring dye laser as the light source is shown. The technique is a valuable tool for imaging both biological samples and other microscopic samples.
CONCLUSIONS AND FUTURE WORK

In this research we have successfully demonstrated the effectiveness of the multi-wavelength optical unwrapping method. To our knowledge this is the first time that three wavelengths have been used for phase unwrapping without increasing phase noise. Conventional software unwrapping methods fail when there is high phase noise and also cannot be used for objects with irregularities. The multi-wavelength optical phase unwrapping method is free of such problems. Software unwrapping algorithms can take more than ten minutes to unwrap phase images. This is a disadvantage when one needs to study live samples in real time or near–real time. The multi-wavelength optical unwrapping method is significantly faster than software algorithms and can be effectively used to study live samples in real time. Another advantage is that the optical phase unwrapping method is free of complex algorithms and needs less user intervention.

The advantage of three wavelength optical phase unwrapping over two wavelength optical phase unwrapping is that, the use of three wavelengths increases the beat wavelength without increasing phase noise in the final unwrapped phase image. The ability to extend the beat wavelength is important because this allows studying samples with height variations of hundred micrometers or more.
Multi-wavelength optical phase unwrapping method can be used successfully with any type of light source; incoherent or coherent. This provides the user a greater freedom of choosing a light source suitable for sample features and research goals. Incoherent light sources such as light emitting diodes reduce the speckle noise inherent to lasers. However, light emitting diodes are available only in several different wavelengths. Therefore, wavelength combinations that produce large beat wavelengths are limited. Because of small coherence lengths of light emitting diodes, imaging phase profiles of samples with features larger than the coherence range is not possible. In this case, laser diodes which have larger coherence lengths than that of light emitting diodes can be used. Both light emitting diodes and laser diodes are small in size and provide reduced apparatus dimensions. This is an ideal feature for a compact, portable microscope system. If larger beat wavelengths are needed, a ring dye laser can be used to extend the beat wavelength to more than hundred micrometers. The three wavelength optical unwrapping method successfully eliminates $2\pi$ ambiguities while reducing phase noise in the final unwrapped phase profile to the order of several nanometers, regardless of the light source.

The technique can be optimized by minimizing possible errors in the phase shifting procedure and by modifying the experimental setup with a horizontal sample mount and a color CCD. Here we suggest several modifications to the setup.

In the phase shifting interferometry, the accuracy of phase shifting depends on the phase shifting device. In this experiment, a function generator is used to send a ramp waveform to the PZT mounted reference mirror. The amplitude of the waveform is calculated according to the wavelength of the light source, so that the reference mirror is
moved by quarter-wavelength intervals. The practical phase shift can be different from
the theoretical value because of several factors; accuracy of the function generator and
PZT, air turbulences and vibrations of the setup. Many methods have been introduced to
compensate the phase shift error [42-47]. Kinnstaetter’s method is based on a Lissajous
figure formed by using the interference pattern. The Lissajous figure is used to detect the
accuracy of the calibration of the phase shifter and the phase steps, the non-linear
characteristics of detectors and mechanical vibrations [42]. Many error compensating
methods use averaging techniques where more than four interferograms are used to
calculate the final phase profile [44, 46].

In our experiment five frames, each 90° apart are used and the intensity profiles of
the first and the fifth frames are checked to determine the accuracy of phase shifting. If
the PZT is calibrated well enough, the first and the fifth frames should coincide. The
breadboard on which the experimental setup built is also placed on inner tubes to
minimize vibration effects. Furthermore, the entire setup is covered while measurements
are taken to reduce the effect of air currents.

The setup can be further improved by using a horizontal sample holder instead of
the current vertical once. This facilitates using samples in the setup easier. A compact
version of the setup is also needed to make it portable. At present the desired wavelength
is chosen by moving the light sources. Only one light source can be turned on at a time
since stray light can adversely affect the interference. Moving light sources can cause
collimating and focusing errors if not done carefully. Three fiber optic cables as shown in
Figure (8.1) can be used to change the wavelengths efficiently. This also reduces the time
that is needed to acquire single wavelength phase profiles since changing wavelengths can be done by simply turning off two unwanted light sources.

![Figure 8.1: Modified setup with fiber optic cables.](image)

The Michelson-type setup is most suitable for reflective samples or transparent samples mounted on a reflective surface. The technique can also be easily used in a Mach-Zehnder type setup, which is more suitable for transparent samples.

The images presented in this study have not been subjected to image enhancing techniques, except for pseudo color rendering of 3-dimensional phase images, since our goal was to demonstrate the effectiveness of multi-wavelength optical phase unwrapping technique. If user wishes image enhancing techniques can be applied to improve the quality of phase images once they are unwrapped.
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APEENDICES
APPENDIX A

COMPUTER PROGRAMS

A.1 LabView Program for Quadrature Phase Shifting

When the PZT is dithered by supplying a ramp waveform from the function generator, this LabView program also receives a signal from the function generator and starts capturing 5 frames as describes in the section (2.5). Then it combines the frames to calculate the final phase image of the object.
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Quadrature Phase Image - AAA.vi
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To save phase images.

External trigger 0

Trigger start of acquisition

Interface IMAQ Init.vi

Acquire images

IMAQ Image To Array.vi

IMAQ ImageToArr.Y 0

Acquire images

IMAQ Sequence.vi

IMAQ Sequence.vi

IMAQ ImageToArr.Y 0

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Quadrature Phase Image- AAA.vi
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A.2 LabView Program for Two-Wavelength Optical Phase Unwrapping

After obtaining two wrapped phase images, each with a different wavelength, they are combined in this program to produce the final unwrapped phase image with a larger beat wavelength. The method is described in the section (4.3).
A.3 LabView Program for Three-Wavelength Optical Phase Unwrapping

After obtaining three wrapped phase images, each with a different wavelength, they are combined in this program to produce the final unwrapped phase image with a larger beat wavelength. The method is described in the section (4.5).
APPENDIX B

LIST OF ACCOMPLISHMENTS

Peer Review Journals


Conference Presentations


About the Author

Nilanthi Warnasooriya grew up in Colombo, Sri Lanka. She received a Bachelor of Science degree in Physics from the University of Colombo, Sri Lanka in 2000 and a Master of Science degree in Physics with High Energy Nuclear Physics from Creighton University, Nebraska, USA in 2003. In Fall 2003 she entered the PhD program in Applied Physics at the University of South Florida, USA.

Nilanthi joined Digital Holography & Microscopy Laboratory at the Department of Physics, USF for her PhD research under Professor M. K. Kim and has presented her work at many conferences including The International Society for Optical Engineering (SPIE), The Conference on Lasers and Electro-Optics (CLEO) and topical meetings of Optical Society of America (OSA). She has submitted her work to peer reviewed journal Optics Express. She completed an industrial practicum at Varioptic SA, Lyon, France.

Nilanthi currently resides in Tampa and enjoys traveling, reading and gardening as pastimes.