ENSC 476/895 - Biophotonics
Lab 3 - Quantitative Phase Microscopy

August 11, 2016
Lestley A. Gabo - 301170055
Pasang Sherpa - 301153625
Amandeep Singh Mand - 301139878

Purpose

The objective of this lab is to gain hands on experience with interferometry for quantitative measurements of phase microscopy. In this lab, we will be retrieving quantitative phase information from a transparent object with high accuracy and low noise as depicted by Hilbert Phase Microscopy. During this lab, we should be able to compare our experimental measurements with the expected values of measurements. We will align the imaging optics (objective lens and tube lens), place a sample at the focus, and acquire images at the detector with and without interferometric fringes.

Methods

We were given an optical fibre sample as the phase object for this experiment. To acquire our data, we had to align a free-space Mach-Zehnder Interferometer (MZI) by aligning the objective lens and tube lens so that the light can be re-coupled to the CCD camera to get fringes through the beam expander by proper alignment .This data is obtained by using a high speed CCD camera which is connected to a computer via blue ethernet cable. We are able to control and obtain our data using the software which showed real time changes of the data as it happened. This helped us obtain the best possible image while aligning the objective lens, tube lens and sample.

A laser that operates in the 633nm wavelength was used as a light source. Therefore, the laser light was red in colour and we used a shutter to decrease the intensity of the laser so that it was not too dangerous. The lenses we were given had 10mm and 100mm focal length where 10mm focal length lens is used as an objective lens and 100mm focal length lens is used as a tube lens. Figure 1 below shows the initial setup we were provided.

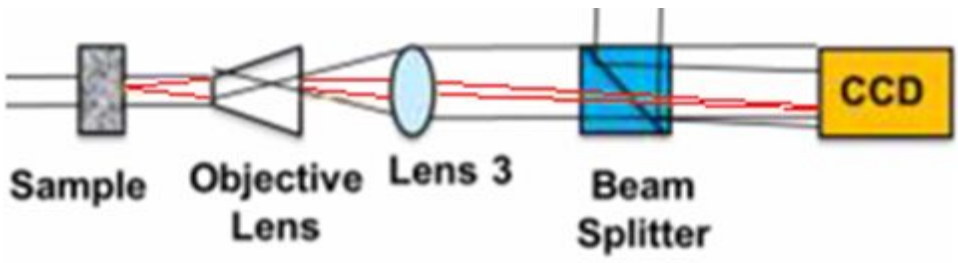

Figure 1: Initial setup provided for the lab

Initially, we made sure that the we got a uniform field of the beam (parallel/collimated) at different distance to help us get precise fringe patterns

throughout the experiment. The setup of our beam expander with the configured distances is shown in Figure 2 below.

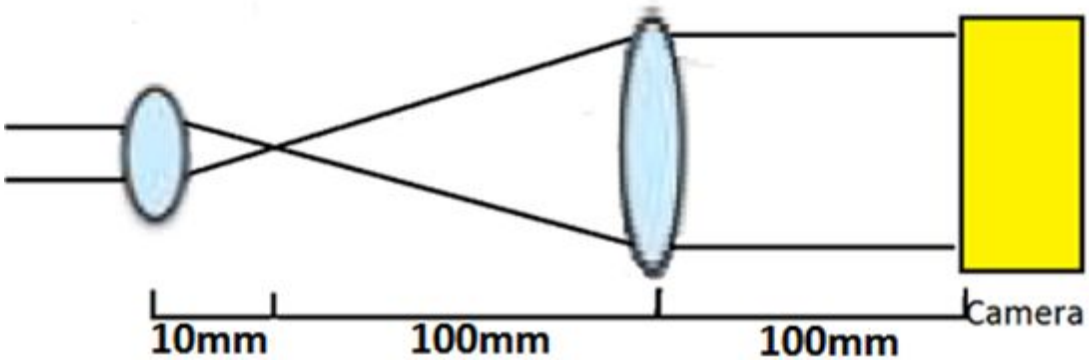

Figure 2: Beam expander setup

We ignored the beam splitter and made the 100mm tube lens focus on the camera and since we wanted a beam expander the objective lens was 110mm distance away from the tube lens. This setup creates a beam expander because the objective lens has a 10mm focal point.

After we constructed the beam expander, we placed the phase object sample at the appropriate distance to focus it on the CCD camera. Figure 3 below shows our final experimental setup with distances properly configured between all the lenses, sample and the CCD camera detector.

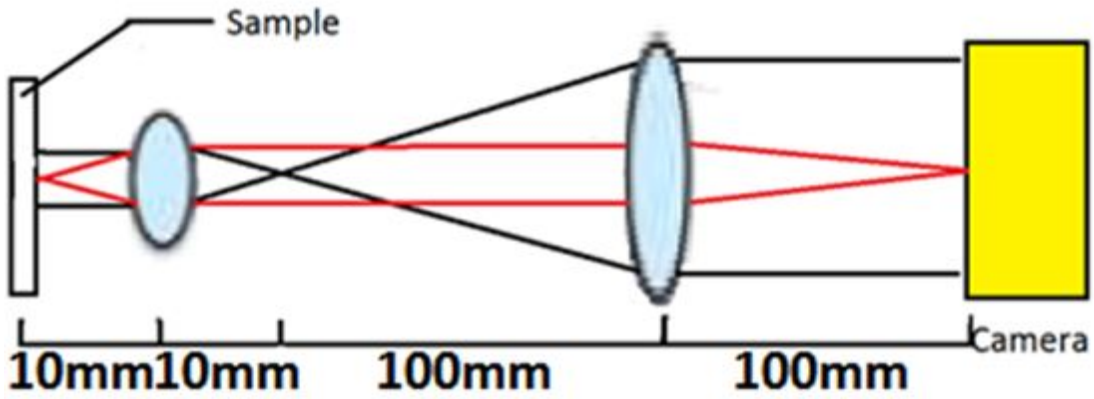

Figure 3: Final setup with the phase object (sample)

Results

Figure 4 below shows the image of the optical fibre with the reference arm blocked when it is focused on the camera. Once the beam expander is setup, we put the sample in front of the objective lens at 10mm and adjusted the distance until we got the focused image on the monitor.

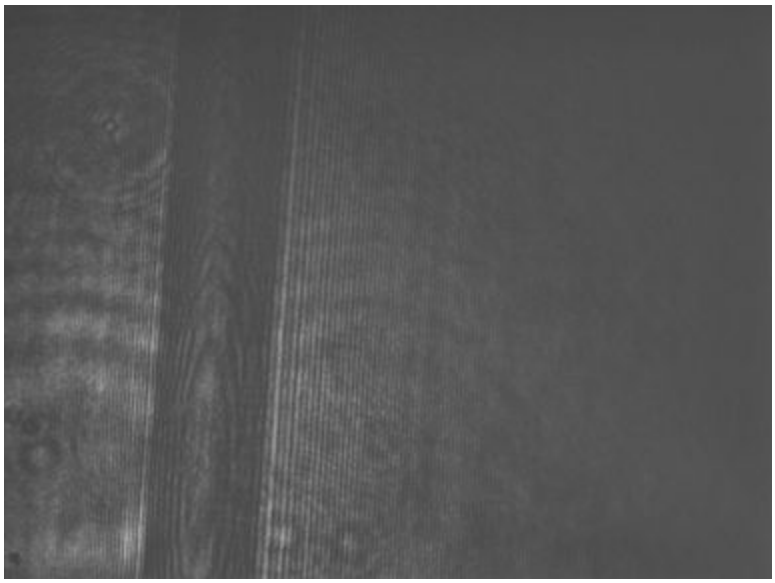

Figure 4:Image of fibre with the reference arm blocked

Once we acquired a focused image of the fiber with reference arm blocked, we un-blocked the reference arm and observed the interferometric image. Figure 5 below shows the image acquired with the reference arm unblocked. By changing the angle of incoming laser we were able to change the interferometric pattern.

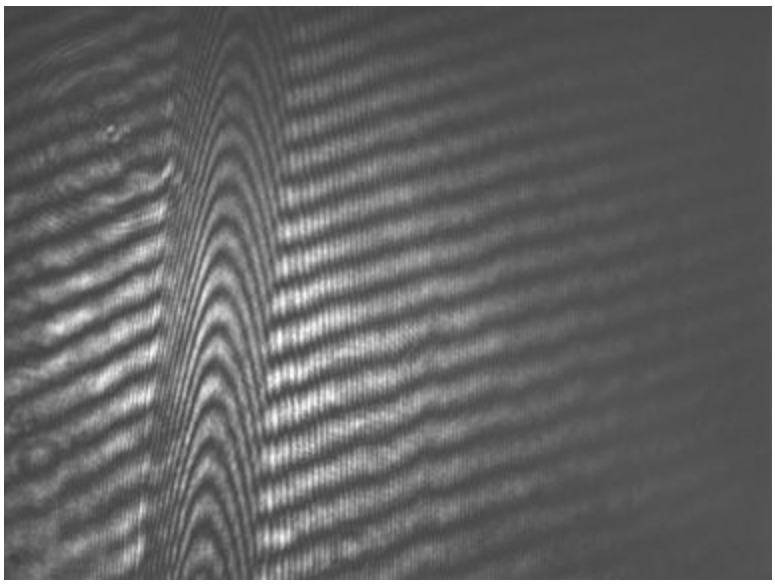

Figure 5:Image of fibre with the reference arm unblocked

As expected, we can see the interference in the image due to the optical path length difference between the light through the sample and the recombined light from the reference arm. This shows that the light passing through the phase object accumulates additional phase resulting in the change of fringe pattern in comparison to the blocked image from Figure 4.

Signal Processing and Discussion:

We used the MATLAB algorithm provided by the TA to perform signal processing of the acquired image. We extracted a line of the interferometric image and created the unwrapped phase profile of the image. This was achieved by the HPM technique that allowed us to retrieve the full-field-phase image from a single spatial interferogram recording. Figure 6 below shows our sample image, the Hilbert Transform Real/Imaginary and the phase profile of the interferogram.

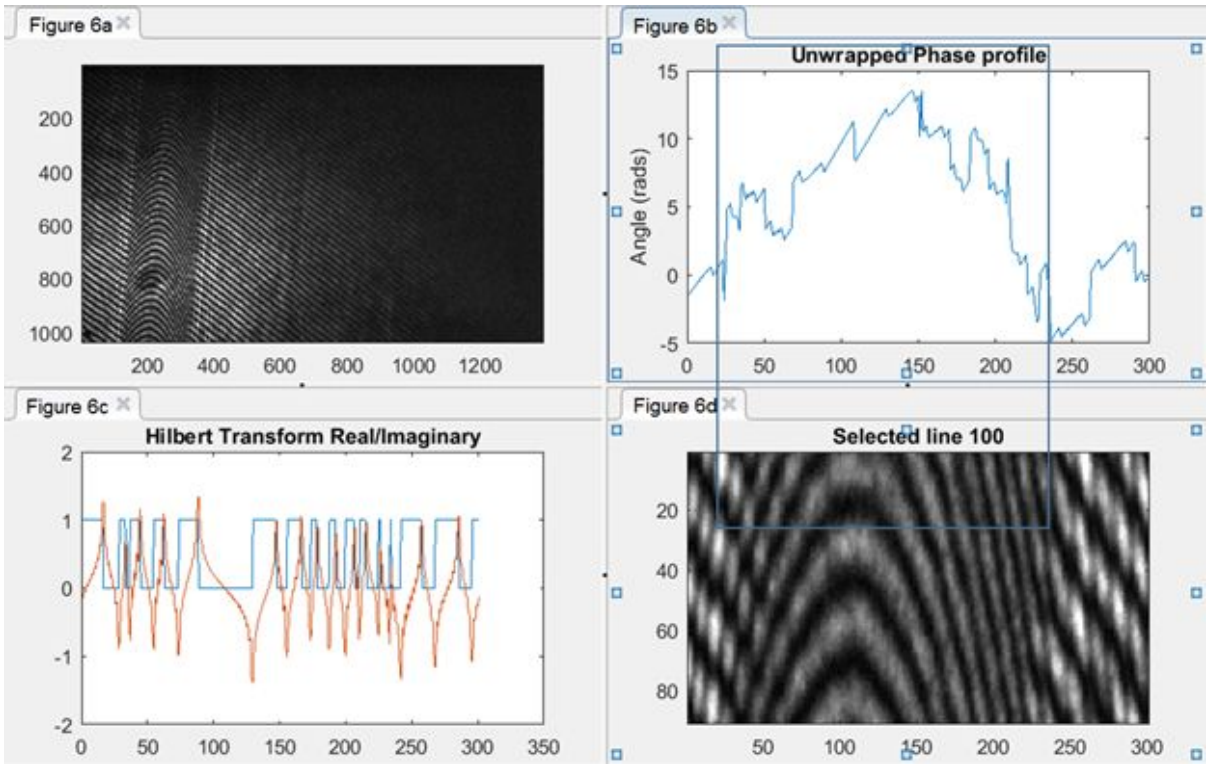

Figure 6:Phase profile and Hilbert Transform real/imaginary of the interferogram

Figure 6a is the image acquired of the sample and Figure 6d is a zoomed in version of Figure 6a. This zoomed in version makes it easier to get the unwrapped phase profile shown in Figure 6b.

Looking at Figure 6c above, we can see the sinusoidal square wave pattern in blue and the Hilbert transform of the real-valued signal in red. This sinusoidal pattern represents the carrier frequency and the observed distortion (phase change) due to the change in the optical path length from phase object relative to the background. From the sinusoidal pattern, we can observe higher pulse frequency between 20 - 70 and then lower pulse frequency between 70 - 150 continued by higher pulse frequency after that. As observed, we can see that Figure 6b above shows the

expected parabolic pattern which represents the theoretical fit and the cross section corresponding to the physical fibre.

The HPM technique also helped us to see the negative phase shift in the fringe pattern. Most importantly, it can help us find the instantaneous magnitude and phase of the original interferogram.

Doing the calculations were not required as per instructor's guidance, however below are the governing equations of the whole system. The optical path length is given by the following formula.

$$OPL = \frac{\lambda\varphi}{2\pi} \quad [1]$$

The *OPL* represents Optical Path Length, λ represents the wavelength of the source, and, φ represents the phase accumulation. Total Irradiance describes the spatially varying irradiance at the CCD camera. The equation for Total Irradiance equation is:

$$I(x) = I_R + I_S(x) + 2[I_R I_S(x)]^{1/2} cos[qx + \varphi(x)] \quad [2]$$

One of the important observation is that the $I_S(x)$ is weakly dependant on the x and also that I_R is constant. This gives an approximation to $I(x)$ with $I_R + I_S(x)$ as the D.C. term and the sinusoidal term $2[I_R I_S(x)]^{1/2} cos[qx + \varphi(x)]$ as the A.C. term. The A.C. term can then be isolated using high pass filter and Fourier transform. With the Hilbert transform of the A.C. term (concept of complex envelope) we can obtain the total phase of the interferogram using the following formula:

$$\varphi(x) = arctan\,[\,Im[z(x)]/Re[z(x)] \quad [3]$$

With the total phase above, we can obtain the desired $\varphi(x)$ by simply subtracting *qx* (reference blocked) from the $\varphi(x)$ (Unblocked phase). This will give us the quantitative phase image of the optical fibre. Then by extracting the phase difference $\varphi(x)$, we can successfully obtain the optical thickness and this was the main goal of our lab.

Conclusion

We were able to tell that our imaging optics were aligned properly because of the interferometric fringes we could see from the fibre sample. This was achieved by comparing the reference arm laser light to be the same size as the beam expanded sample arm laser light. The comparison was done by blocking the reference arm and then unblocking it. As expected, we saw on the real time data the interference fringes due to the optical path length difference once we summed up the reference

arm and the beam expanded sample arm. The MATLAB algorithm processed the image data to enable us to see the unwrapped phase profile and the Hilbert Transform. We concluded that our acquired data was good because the unwrapped phase profile (Figure 6b) of the zoomed in image (Figure 6d) forms a parabolic pattern which is the theoretical fit.