Optical Ptychography Imaging

Summer Project

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Abstract

This report details a 10 week summer project carried out in Research Complex at Harwell (RCaH) Oxfordshire; a collaboration with Prof Ian Robinson's group specialising on "Phase Modulation Technology for X-ray imaging". The main goal of this project was to develop a new kind of phase contrast, structured illumination optical microscope. It relies on a recently developed imaging technique known as ptychography, a coherent diffraction imaging technique. Far-field ptychography has proven to be successful and this project has been aimed to test a variation of it, near field ptychography. The principle is to take a coherent light source; a low power solid state laser and focus the light on a sample. The near field diffraction pattern of the sample is collected using CCD sensor of the latest technology. The technique works by overlapping these recorded diffraction patterns of defocused samples and then using an iterative algorithm to generate a reconstruction of the objects transmission function. This method allows to acquire the phase definition of the object as well as magnitude, and is thus ideal for imaging clear cells which are difficult to see using traditional microscopes. The response of the microscope is calibrated by scanning a known test object first. The setup has been designed to be "user" friendly and can be delivered to RCaH to be used by the biology groups working in the complex. Upon the completion of the project the instrument has been able to perform successful scans of objects although some further calibrations are required to achieve perfect results. The setup is relatively inexpensive and also readily available set-up compared to X-ray phase tomography, and hence the technique may have an interesting future for several applications.

Contents

1. Introduction

This project was mainly aimed on the development of a near field ptychography instrument. Near field ptychography is a variation of far field ptychography that has been tested and proved to be a useful technique for imaging in the field of biology. A completely new setup has been installed for near field ptychography during this summer project.

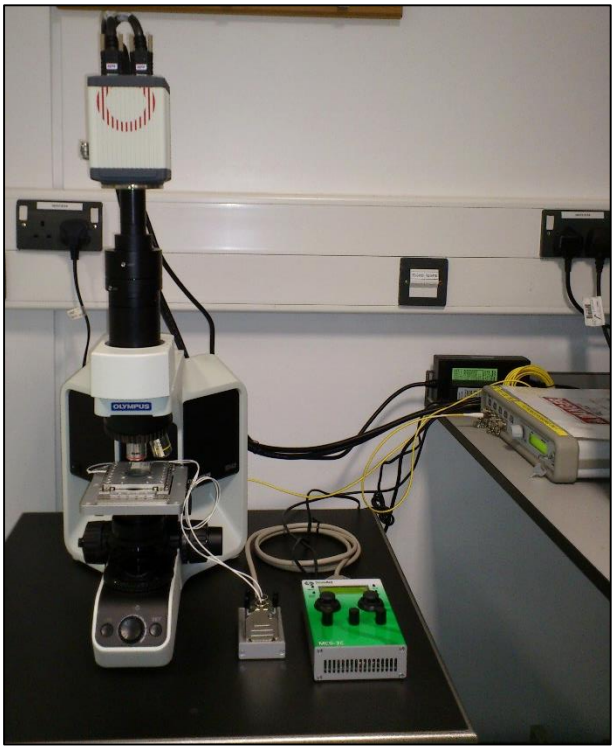

Figure 1 – Near-Field Ptychography Setup

The setup is integrated in a standard confocal microscope, consisting of a solid state laser, an X-Y stage and a CMOS camera. The laser is connected to the back of the microscope with a fibre and the beam is focused on the sample using a collimator and a condenser beneath the stage. An iris is present in the microscope body which helps to control the probe size (diameter of the laser beam). The X-Y stage height and hence the samples height can be adjusted using a knob mechanism to focus the sample. The camera is attached to the microscope body using C-mount adapters and tubes. All the hardware components are interfaced via a computer using a Matlab program written specifically for this apparatus.

2. Ptychography

2.1. Phase

Phase is an important parameter in imaging systems. For example when imaging clear cells, phase definition helps to visualise the sample when the magnitude information may not be so useful. In fact phase information can be much more important than information provided my magnitude.

This can be shown by Parseval's theorem which states: *the mean-square value on one side of the Fourier transform is proportional to the mean-square value.* A smaller root mean error in the image in real space would be introduced by a change in magnitude in frequency domain compared to a change of the same amount in phase in frequency domain (figure below) [1].

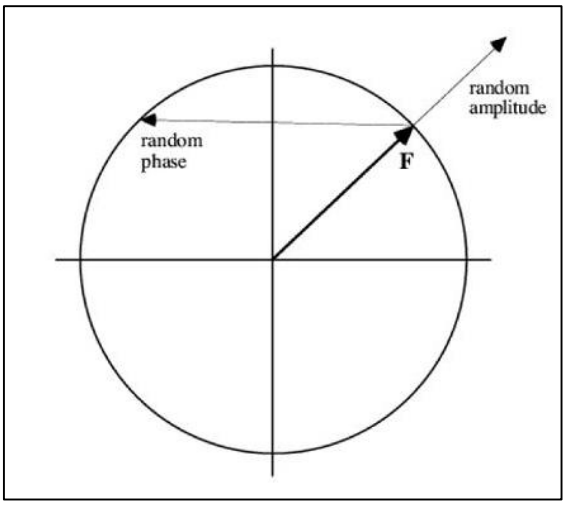

Figure 2 – Alterations to Image to changes in Phase and Magnitude[1]

2.2. Ptychography

Ptychography, from the Greek word "Ptycho" relates to the folding of the diffractions via the convolution of the Fourier transform of an illumination function in the plane of the object[2]. The object function $o(x)$ of a thin (2D) object and the illumination function $p(x)$ can be multiplied to retrieve the transfer function.

$$\psi_f(x) = p(x) \times o(x) \qquad (2.1)$$

Convolution theorem at Fraunhofer plane results in Fourier transform of $\psi_f(x,y)$ to be:

$$\psi_f(u) = P(u) * O(u) \qquad (2.2)$$

where $P(u)$ and $O(u)$ are the Fourier transformed $p(x)$ and $o(x)$ and the convolution operator $*$ is defined as:

$$P(u) * O(u) = \iint P(u) \times O(u-U)\, dU \qquad (2.3)$$

However the problem is to solve the convolution equation as in practice only the intensity for $p(x)$ and $o(x)$ in real space can be measured by the detector and thus all the phase information is lost.

This problem can be eliminated by sampling the object at many different positions with the probe in such a way that the positions partially overlap each other. An algorithm written specifically for this method makes an initial guess of the transmission function and iteratively improves this guess by applying Fourier transforms back and forth. The algorithm also applies constraints to match the intensity of the diffraction pattern to the recorded intensities and that the transmission function must be consistent in all overlapping regions with all of the recorded diffraction patterns. In addition the

algorithm works to enhance the illumination function so a good estimate of the illumination function $P(x)$ is necessary instead of a perfect knowledge.

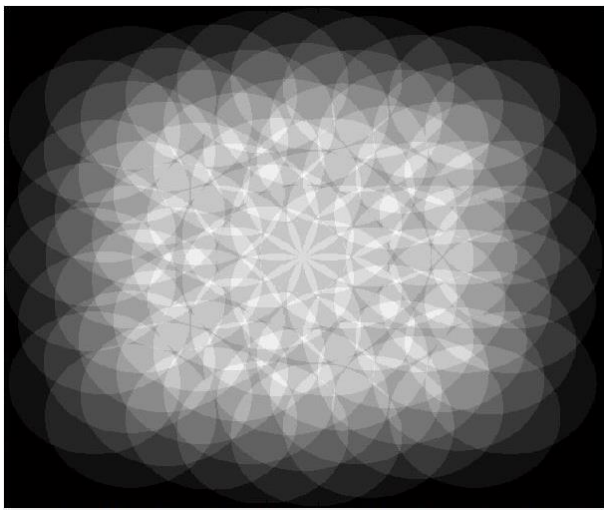

Figure 3 - An illustration of the overlap map of a scan

The above figure shows how a scan is performed and the different positions that the probe illuminates the sample, each producing a diffraction pattern which is measured. In practice the method works by capturing a circular array of out of focused images in a close range so that the images have an overlapping region. Three images of different exposures are captured to effectively enhance the dynamic range of the camera. The algorithm then reconstructs the magnitude and phase image of the object. The instrument response is calibrated by scanning a known object, such as a pinhole of known diameter to determine the probe size accurately.

This technique is quite robust and far field ptychography has already been tested to be very successful technique[2]. Near field ptychography has also shown promising results with some degree of fine adjustments to achieve great results. One major disadvantage of this technique is the time required to perform a scan. To perform a complete scan to reconstruct a single image, usually 81 diffraction patterns needs to be recorded and sometimes even more than 200. This restricts some applications of this technique. It is also necessary to keep the system stable to avoid vibrations that may distort the reconstruction process.

3. Hardware and Software Setup

The final apparatus of the phase contrast microscope has been placed on an isolated air cushioned table in the laboratory to ensure maximum stability and minimise vibrations. The entire setup has been organised in a way to enhance user friendliness and provide an accessible way to perform scans of the samples. A program has been written to interface with the hardware and provide the user with full control over the scan.

3.1. Hardware

The entire setup has been assembled from beginning using the key components as they arrived through shipment. The main body of the phase contrast microscope is the BX43 Manual

manufactured by OLYMPUS[3]. Ptychography requires a coherent light source hence the LED lamp in the microscope has been replaced with a solid state laser source.

The laser is the MCLS1 from Thorlabs which consists of 4 laser channels at different wavelengths and power from which one of the channel is used having a wavelength of 638nm and power of 14.2 mW[4]. Although power output from the required intensity during operation is very low ~1 mW, however radiation precautions and laser training has been taken before operation. A fibre connects the laser to the back of the microscope.

Figure 4 - Olympus BX43[3]

Figure 5 - Thorlabs MCLS1[4]

The microscope stage has been replaced with a precision controlled linear stage from SmarAct[5]. The stage has been custom ordered to fit the microscope body. A separate controller controls the stage[6]. The stage has two channels with linear sensors while vertical movement has to be manually done using knobs.

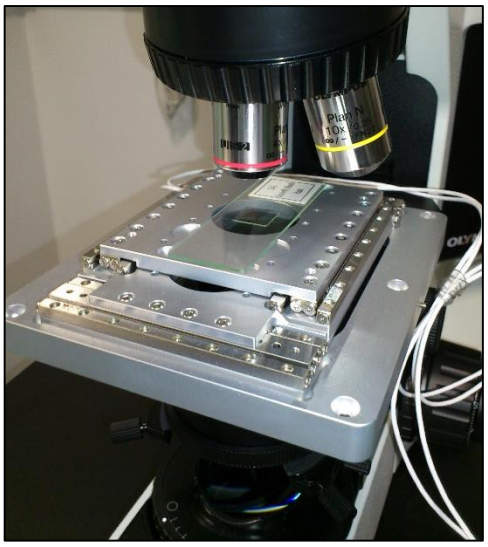

Figure 6 - SmarAct Stage

Figure 7 - SmarAct MCS Controller

Finally the eyepiece of the microscope is replaced with a CMOS digital camera, the ORCA-Flash4.0 V2 C11440-22CU by Hamamatsu[7]. The camera offers superior noise performance and fast data acquisition. A frame grabber connects to the camera via two camera links and the computer to allow 100 frames per second data capture[8].

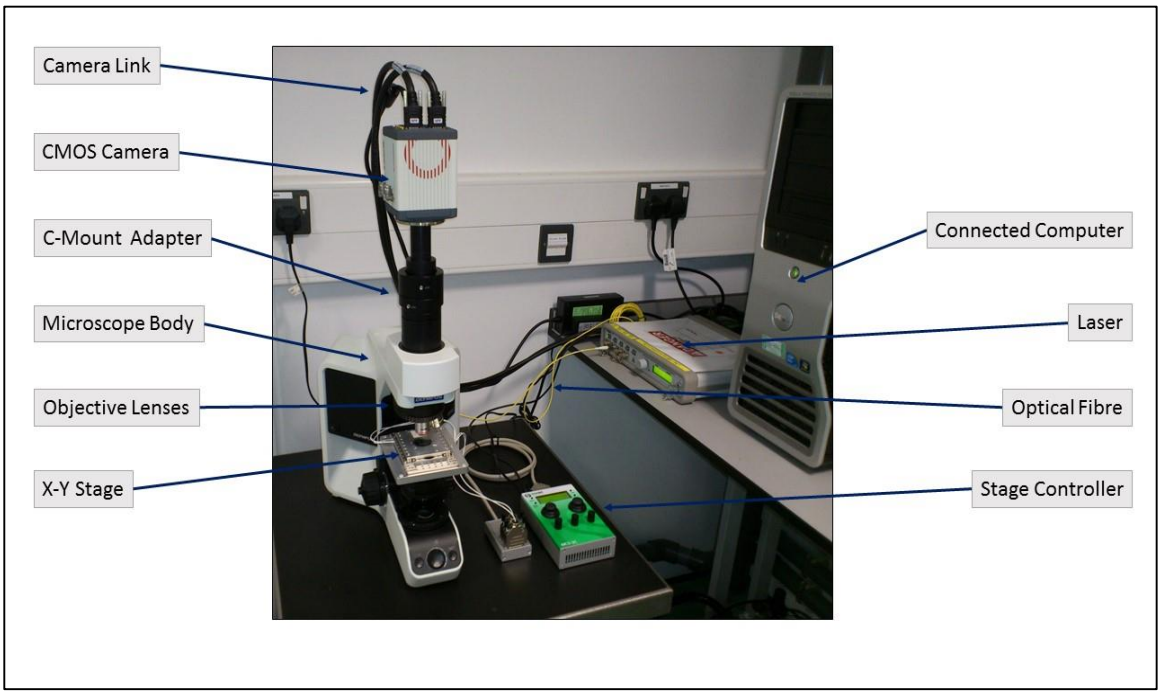

Figure 8 - Hardware Setup

The diagram below shows a detailed demonstration of the setup. The collimator helps to achieve a parallel beam and the probe size is controlled via the iris. The condenser focuses the beam on to the sample which then is picked up by the objectives through to the camera. The C-mount adapters for the camera also houses multiple lenses to provide magnification. The image is first focused on to the camera and then a known height tube C-mount adapter is inserted to defocus the image.

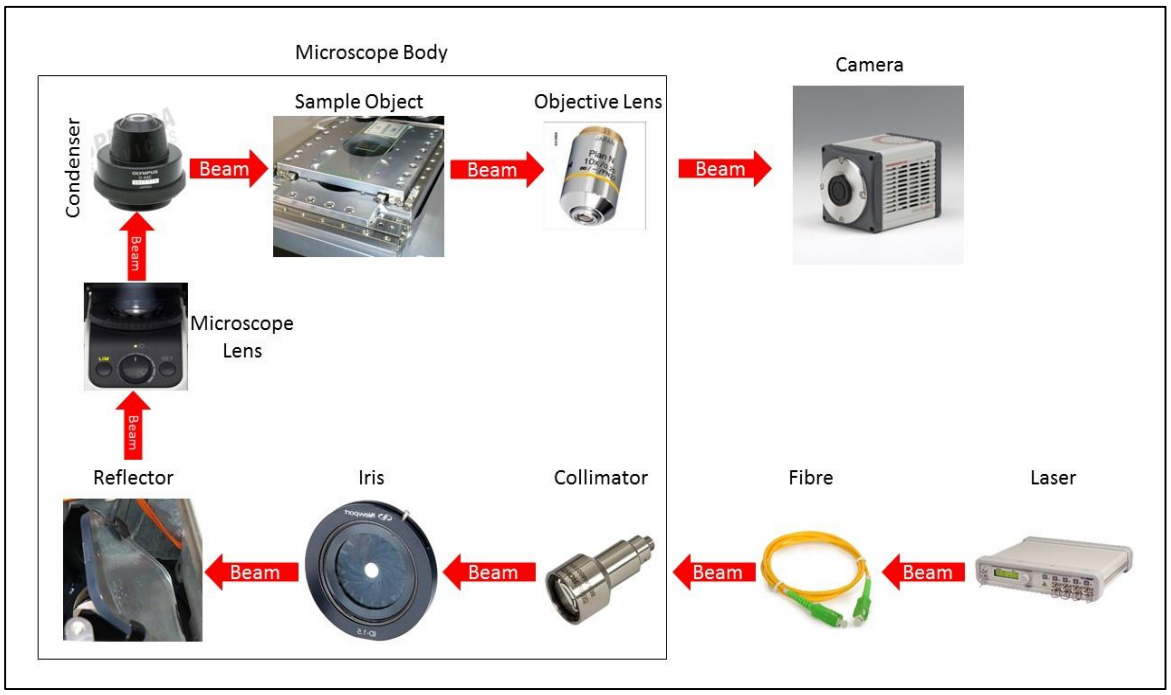

Figure 9 - Detailed demonstration of hardware

All the hardware components are linked to a single computer controlled by a program discussed in the next section.

3.2. Software

Most of the time for this project has been spent in developing the Matlab software for the new hardware to acquire data. An interface for the stage and the camera had to be created and then needed to be integrated to work together in synchronisation. The program can also control the laser, stage and the camera individually with adequate controls for each hardware available. The reconstruction program also had to be adjusted work with the new setup.

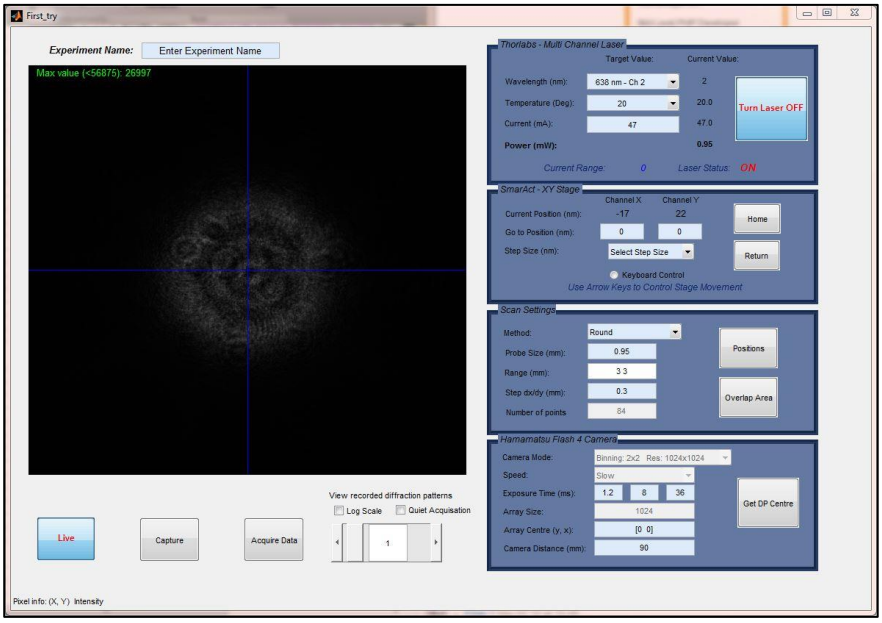

Figure 10 - Screenshot of program for data acquisition

To perform a scan, the user must first configure the hardware and insert the sample to be scanned within the stage. The program can then be initialised through Matlab. The user can then find the area of scan by controlling the laser, stage and camera settings. The default settings for the scan and hardware are pre-set but can be adjusted by the user. To find the area, the laser first needs to be turned on and the drive current can be adjusted according to the power required. The camera live feed needs to be enabled to view the sample, while the capture functionality allows to acquire snapshots. Stage control can be gained by pressing "Keyboard Control", then using the keyboard arrow keys to control the stage and the "+/-" keys to change step size.

Once the region of interest is set, the user can simply proceed to perform the scan or adjust the scan settings. The scan usually takes around a couple of minutes to perform but may vary significantly depending on the number of points selected to scan. The scan procedure is quite straightforward, the stage travels to the predefined co-ordinates set by the program settings while the laser in kept illuminated. The camera then simply captures three images using three exposures at those coordinates and saves them. This procedure is of course done in a synchronous order and repeated at every point. The reconstruction program then reconstructs the image of the sample.

3.3. Other Attempted Procedures

A smaller and evenly illuminated probe yields better results when scanning an object. It was attempted to achieve the best probe size and illumination possible. Hence extra lenses using rails and tube components were also inserted to attempt to enhance the probe illumination. However the extra lenses added little to no benefit as ultimately the required probe size is very small, of which the collimator does a decent job of producing a parallel beam. Also including the lenses was not very practical as the microscope had to be opened every time a change had to be made. Hence they were removed from the final setup.

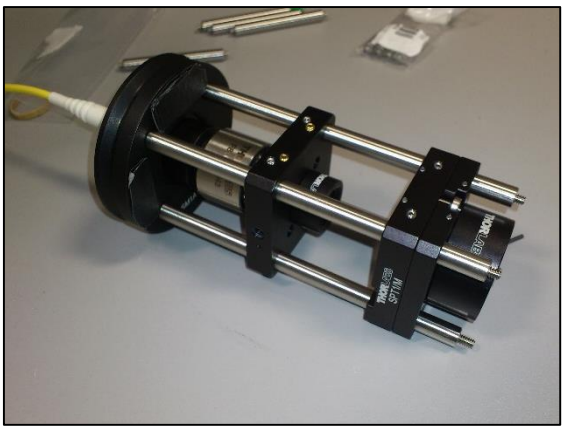

Figure 11 – Extra lens using rails to enhance beam

Figure 12 - The lenses inside the microscope

Also the beam needs to be centred with the camera and aligned horizontally to ensure successful results. Since the manufacturer design of the microscope included an LED light source, the laser does not fit into the microscope perfectly. Care has to be taken when attaching the laser to the microscope for alignment. A metal reinforcement has been designed which allows fixing the laser to the microscope easily while maintains a tight and stable fit.

4. Results

After successfully installing the hardware setup and writing the software code some of the samples were scanned. As with most new setup the equipment needed some calibration and several scans were performed until the reconstruction algorithm and the equipment were in harmony and produced acceptable sample images.

As mentioned in theory, the calibration is performed with a pinhole (known object specification) which allows to gain a good knowledge of the probe size. The images below show both phase and magnitude images of the object (pinhole) and the probe.

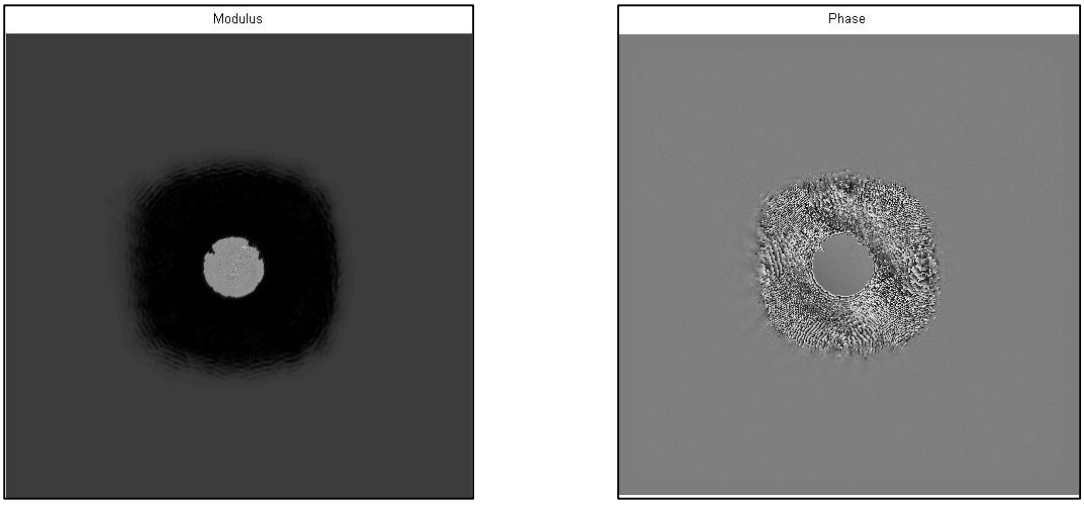

Figure 13 - Magnitude and phase images of pinhole as object

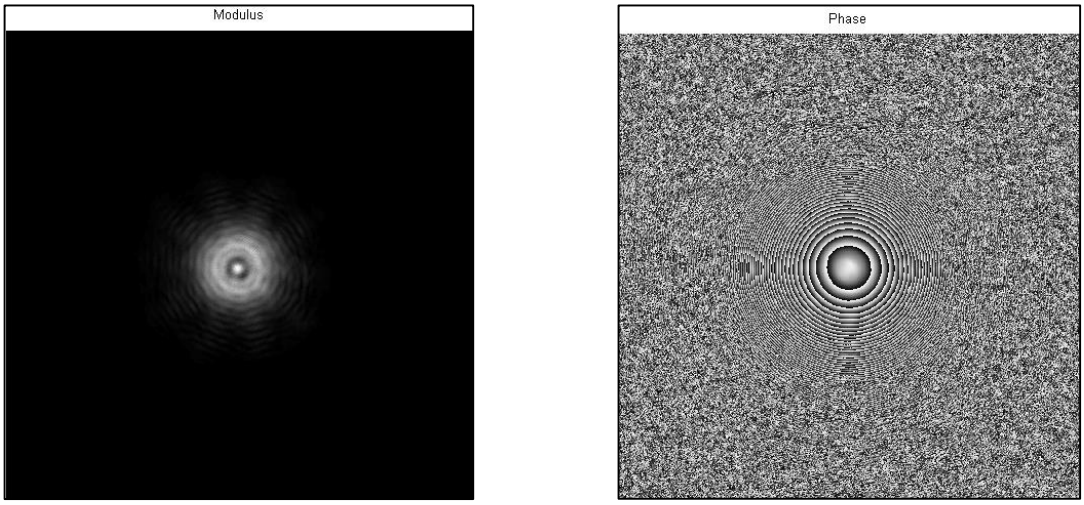

Figure 14 - Magnitude and phase image of laser probe

A generic lens cleaning tissue structure has very strong contrast features and suffices as a very good sample[9]. The reconstruction object images are presented below retrieved from a tissue scan.

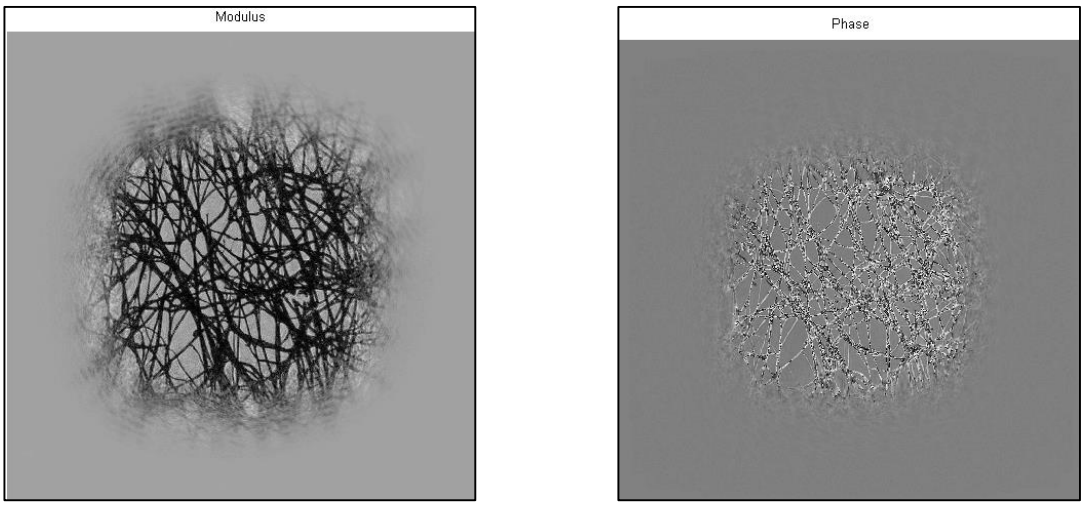

Figure 15 - Magnitude and phase images of the tissue structure

Chromosome samples that were present in the laboratory facility were also scanned. The images below show the scans of clear chromosome samples and it can be seen that the phase images are quite useful in imaging of clear samples.

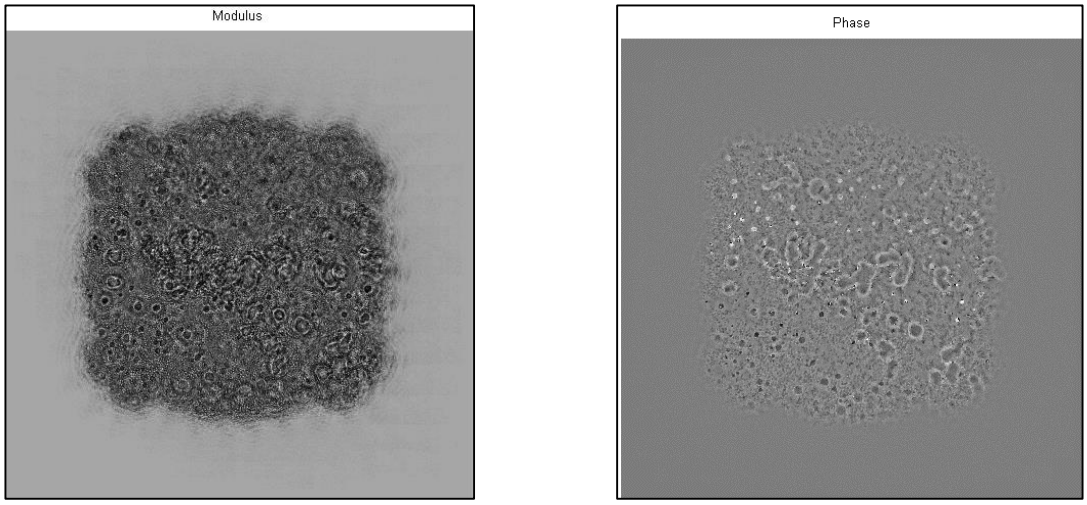

Figure 16 - Scan of chromosome samples – YOR4

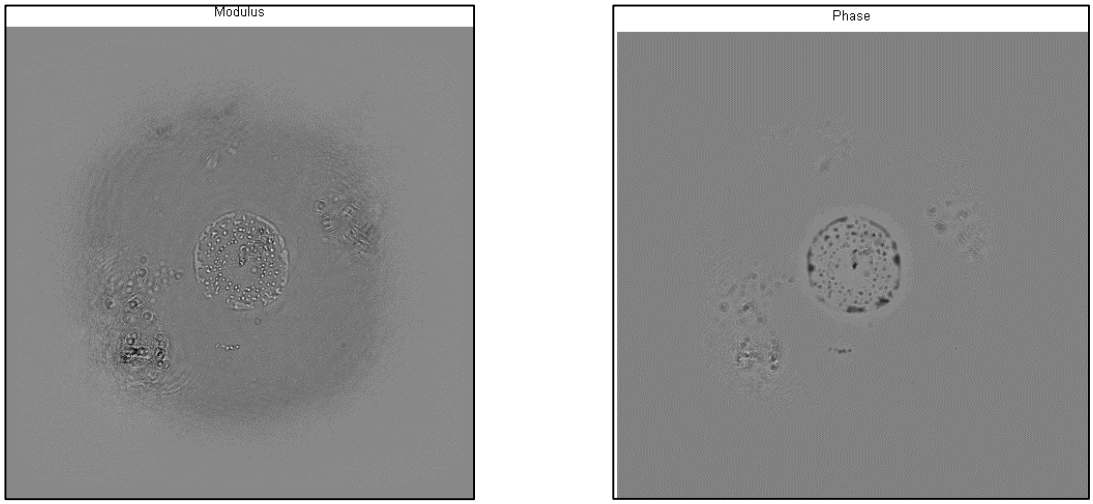

Figure 17 - Scan of chromosome samples – YOR9

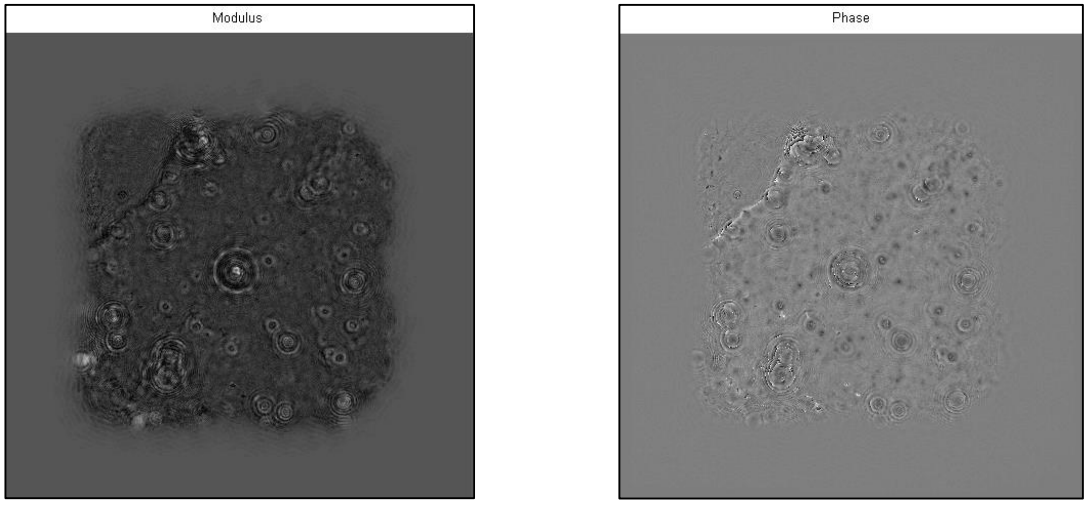

Figure 18 - Scan of chromosome samples – DAPI Stained

5. Conclusion

At the time of writing this report the instrument was working and was capable of producing scans of samples in a relatively short amount of time. However additional calibration and adjustment needs to be made to the instrument to yield better results as there are so many parameters to experiment with when performing a scan, all of which could not be done during the scope of this project. It was observed that the computer would get stuck sometimes during the scan, which may occur due to glitches in the software program which can later be ironed out through debugging.

For future developments, the water cooling kit can be installed to the camera to provide better noise performance. The current condenser lens can be replaced with one having a different numerical aperture which would allow the probe size to be smaller and hence enhancing the resolution of the final image.

Overall this project has been quite challenging but at the same time rather enjoyable. The time spent in the Harwell Science Campus has been great, working with a phenomenal team on a very compelling research area. Thorough research, practical and programming experience has been gained and it has been a fantastic summer experience.

6. References

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