Multi-spectral polarimetry imaging for early gastric cancer diagnosis

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**MULTI-SPECTRAL POLARIMETRY IMAGING FOR
EARLY GASTRIC CANCER DIAGNOSIS**

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EARLY GASTRIC CANCER DIAGNOSIS

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Abstract

Gastric cancer, which develops from the lining of stomach, is the fifth most severe disease worldwide. It leads to significant number of death each year. The early diagnosis of gastric cancer can dramatically increase the survival rate within five years. Thus the investigation on the early diagnosis of gastric cancer is of paramount importance. Polarimetry imaging, a technique utilizing the polarization properties of biological samples, is of great potential to diagnose cancer. This technique is able to provide quantitative information about tissue morphology and structural properties noninvasively. Thus, it is worth investigating the diagnosis of gastric cancer using polarimetry imaging.

This dissertation presents several studies in the development of polarimetry imaging techniques for gastric cancer diagnosis in order to approach the eventual goal of the detection of early gastric cancer *in vivo* in real time.

The background about gastric cancer, polarimetry imaging and polarimetry is first introduced. Then the comparison between cancer and normal gastric samples were performed at different wavelengths based on different polarization properties (retardance, diattenuation, depolarization, linear retardance, linear diattenuation, linear depolarization, circular retardance and circular depolarization) of samples together with all possible combinations. It was observed that all the polarization parameters except diattenuation and circular retardance demonstrated significant differences between cancer and normal samples for 4 μm gastric samples.

Moreover, it was found that wavelength did not play a critical role in improving the diagnostic accuracy.

To further acquire more detailed information, we measured samples under 20X objective lens at only one wavelength 632 nm. Besides cancer and normal glands, other samples including intestinal metaplasia and dysplasia were also included. The data processing was conducted at the both macroscopic and microscopic level for different regions of interest ranging from 1×1 to 640×512 . The results demonstrated the feasibility of performing “digital staining” using polarimetry imaging.

While these results showed the great potential of polarimetry imaging in classifying gastric samples especially between cancer and normal, it is quite time consuming using the above systems and the equipment cannot be incorporated into a commercial endoscope. To address this problem, we proposed a snapshot polarimetry imaging method and the algorithm to enable all measurements in real time. The system has been tested on standard samples such as air and polarizers. The potential reasons for disagreement between experimental and theoretical results were discussed and future directions were pointed out.

In summary, we have demonstrated that polarimetry imaging is of great potential to classify gastric samples at both the macroscopic and microscopic levels, especially for distinguishing between cancer and normal tissue samples. Snapshot polarimetry imaging is worth further investigation to achieve real time polarimetry imaging for clinical uses.

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Chapter 1: Overview

Gastric cancer, which develops from the lining of the stomach, is one of the most severe diseases worldwide. The early diagnosis of gastric cancer can dramatically increase the survival rate to over 90% within five years [1, 2]. Thus the investigation on the diagnosis of early gastric cancer is of paramount importance. Polarimetry imaging, a technique measuring the polarization properties of biological samples, has demonstrated great potential in cancer diagnosis. This noninvasive technique is able to provide quantitative information about tissue morphology and structural properties, which is correlated to the disease status of a given tissue. This dissertation presents several studies in the development of polarimetry imaging techniques for gastric cancer diagnosis in a hope to achieve the goal of detecting early gastric cancer *in vivo* in real time in the long term.

In chapter 2, the background of gastric cancer and the current techniques for cancer detection are introduced in addition to the principle and state of the art of polarimetry imaging. While polarized light is modeled by Stokes vector, the polarization properties of a tissue sample can be fully characterized by Mueller matrix. Both Stokes vector and Mueller matrix will be explained in details in combination with polarized light propagation.

In Chapter 3, the roles of polarization parameters and wavelength choices on the differentiation between cancer and normal gastric samples were investigated. The comparison between cancer and normal gastric samples were performed at a

range of wavelengths based on different polarization properties (retardance, diattenuation, depolarization, linear retardance, linear diattenuation, linear depolarization, circular retardance and circular depolarization) of samples together with all their possible combinations. It was observed that all the polarization parameters except diattenuation and circular retardance demonstrated significant differences between cancer and normal samples for 4- μm gastric samples with a significance level of 0.05. Besides, it was found that wavelength did not play a critical role in diagnostic accuracy. It should be noted that samples were measured under 2X objective lens and data processing was performed at the macroscopic level, in which the average polarization properties of the entire image was used to represent each sample.

In Chapter 4, the potential of Mueller matrix polarimetry imaging was investigated for gastric cancer diagnosis. In this study, we focused on the measurements of samples under 20X objective lens to achieve the microscopic resolution at only 632 nm. Besides cancer and normal glands, intestinal metaplasia and dysplasia were also included in sample classification to be more realistic. Data processing was conducted under both the macroscopic and microscopic levels for a range of regions of interest (ROI) sizes from 1×1 to 640×512 pixels to evaluate the effect of ROI size on diagnosis. Besides polarization parameters and all their combinations, we also investigated Mueller matrix elements and their principal components scores for classifying samples into three categories—i.e., cancer, dysplasia and normal/intestinal metaplasia. To improve the accuracy of

classification, we developed a strategy of two-step classification, which was more effective than the traditional one-step classification.

The results in Chapter 3 and Chapter 4 show the great potential of polarimetry in diagnosing gastric samples *ex vivo*. However, traditional polarimetry measurements is very time timing especially in an imaging setup. Because 16 images need to be recorded for each sample corresponding to different polarization states in the modules of light delivery and detection, it cannot be easily incorporated into a commercial endoscope to detect gastric cancer *in vivo*.

We proposed in Chapter 5 a snapshot polarimetry imaging technique to perform polarimetry measurements in real time and spectral images can be reconstructed using a previously developed algorithm. This system has been set up and used to measure reference samples such as air and polarizers. Due to the insufficient spectral resolution of the spectrometer available, experimental measurements do not exactly match theoretical prediction but the trends agree well with each other. We will continue to explore this technique once a spectrometer with high spectral resolution is available.

In Chapter 6, we summarized the findings in all above studies and discussed the future directions of polarimetry imaging.

Chapter 2: Introduction

In this Chapter, we first introduce the background of gastric cancer, which includes the clinical significance, the symptoms and the different stages and types of gastric cancer. Then the current detection techniques for gastric cancer are listed. At the end, the principle and background of polarimetry imaging are described in details, which is the focus in this study.

2.1 Introduction to gastric cancer

Gastric cancer or stomach cancer refers to cancer arising from some part of the stomach [1-6]. It is the fifth most common cancer in terms of occurrence rate and the second most common cause of cancer deaths around the world [1-6]. More than 700,000 persons in the world die from gastric cancer annually and each year around 870,000 new cases of gastric cancer are identified worldwide [5]. The high mortality rate is partly attributed to the fact that it has often been too late when a patient with gastric cancer is definitively diagnosed. As a matter of fact, this disease could be cured by resection alone if it is diagnosed at an early stage [2]. Therefore, the early diagnosis of gastric cancer followed by optimal treatment is important for increasing the survival rate [7].

Normally, the development of gastric cancer starts from intestinal metaplasia or gastric atrophy and go through the intermediate step of dysplasia [8]. Dysplasia refers to the abnormal development of tissue. It is the indication of an early neoplastic process consisting of the increased number of immature cells and the

corresponding decrease in both the number and location of mature cells. Dysplasia can be contrasted with intestinal metaplasia where one type of mature cells were replaced by another type of mature, differentiated cells [9]. Gastric atrophy is the chronic inflammation process of the stomach mucosa, which can lead to the loss of gastric glandular cells and replacement by fibrous components or intestinal tissues [10]. Sometimes, gastric cancer might develop from gastric adenomatous polyps representing a dysplastic epithelium that arises in raised lesion [8].

Gastric cancer either has few noticeable symptoms or only produces non-specific symptoms at its early stage. The cancer, more often than not, has developed into a later stage when the symptoms become noticeable. This is one major reason that the early diagnosis rate is poor. Gastric cancer can cause weakness or bloating of the stomach after meals, pain in the upper abdominal abdomen and sometimes nausea and vomiting of blood etc. [11]. There are many possible causes of gastric cancer such as the infection of *Helicobacter pyloric* bacteria that accounts for 65%-80% of gastric cancer, smoking, genetic components and dietary factors like salted food, smoked food or pickled vegetables [11].

Gastric cancer has five stages in total. In stage 0, cancer cells are limited only to the innermost lining of the stomach, which is curable by endoscopic mucosal resection. In stage 1, cancer penetrates into the second or third layer of the stomach or nearby lymph nodes. In stage 2, cancer penetrates into more distant lymph nodes and the second layer or even all four layers but it does not go into the muscle

layer of the stomach. Then in stage 3, cancer develops into more distant lymph nodes and the third layer, or even all the four layers and nearby tissues. In the most severe stage, i.e., stage 4, cancer has spread to other parts of the body or nearby tissues and distant lymph nodes by metastasis. It is quite possible to cure in stages 0-2 but not for stages 3-4 [12, 13].

Histologically, gastric carcinoma can be segregated into two major types. One is named as the intestinal type which is characterized by the presence of gland forming mitotically columnar cells with enlarged nuclei, with accumulation of mucin in the malignant glands and without much intracellular mucin accumulation. The other is the diffuse type in which cells generally have smaller and more uniform nuclei and shape in comparison to the first type [3, 14]. The morphological findings can be directly used in histopathology. Moreover, they lead to numerous other changes in polarization properties which could be used for the diagnosis of stomach cancer using polarimetry imaging. For instance, fibrous structures may become disorganized when a normal stomach tissue develops into cancer, which leads to the change in polarization parameters such as retardance. Similarly, changes in nuclei size and its distribution may cause alteration in other polarization parameters like depolarization. This would then allow for cancer diagnosis by measuring changes in polarization parameters from normal to cancerous stomach samples [15].

2.2 Current technologies available for gastric cancer diagnosis

Several technologies are currently available in the clinical setting for gastric cancer diagnosis as briefly surveyed below with their limitations.

Gastrosopic exam, the examination of the inside of the gullet, stomach and duodenum. This exam is performed using a fiber-optic instrument that passes through the mouth to see whether there are any damages to the lining of the esophagus or stomach, and whether there exist any ulcers in the stomach or duodenum. This exam is usually conducted under white light but is limited when technical factors prevent the comprehensive observation of the antrum and lead to the misinterpretation of “pyloric closure”. This occurs to the proximal true pylorus. Distal antral lesion may be hidden in this situation [16].

Roentgenogram, the photography of internal structures made by X-ray that passes through the body to generate a showdown image on a sensitized film, which is also called roentgenograph or X-ray imaging. The value of Roentgenogram is remarkably enhanced by using contrast materials such as barium to make structures visible on the film after the discovery of X-rays in 1895. However, X-ray is ionizing thus can cause damage to human body.

Computed tomography (CT), a medical imaging procedure using computer-processed X-ray images to get tomographic images of specific regions of body. These images can be used for the purposes of diagnostics or therapy in a variety of medical disciplines.

Optical coherence tomography (OCT), an optical signal acquiring and

processing technique which typically employs near-infrared light that can penetrate into a scattering medium to capture micrometer-resolution, three dimensional images. Unfortunately OCT cannot image well for aortic ostial lesions because it can only penetrate about 2-3mm. This means that it requires the use of additional contrast agents to work in some cases but may not work in other cases [17].

Endoscopy, it typically refers to looking inside the body for medical purposes by directly inserting an endoscope to the organ, which allows the examination of the interior of a hollow cavity or organ. Endoscopy also means using a borescope for visualization when the direct line of-sight observation is not feasible.

Currently, the routine diagnostic methods include gastroscopic exam, barium roentgenogram, computed tomography, optical coherence tomography and white light endoscopy etc. [18] Among all the techniques, white light endoscopy is the major noninvasive modality and various methods have been proposed to improve it [19-23]. It was shown that narrow band imaging (NBI) can help visualize mucosa surface and vasculature [20, 24, 25] while autofluorescence imaging (AFI) can enhance the sensitivity of optical diagnosis [26, 27]. NBI and AFI have been incorporated into commercial endoscopes to help improve diagnostic accuracy [21, 23]. The combination of white light imaging, autofluorescence imaging and narrow band imaging prove to be feasible to detect superficial gastric neoplasia [23]. The NBI and AFI improve white light endoscopy by taking advantages of the absorption and fluorescence properties of tissues that are altered when normal tissues develop into dysplasia. However, these two techniques also have their own

limitations. They can produce high false positive rate when benign pathologies such as inflammatory changes exist [28]. They did not fully exploit the potential of light properties in probing tissue structural changes as explained in the next section.

These technique have their advantages and disadvantages. Polarimetry imaging is found to be of great potential in cancer diagnosis due to the finding that this technique can measure morphological changes of tissue structures by looking at the changes in polarization properties when a normal tissue undergoes structural changes due to diseases such as dysplasia and cancer. Our study will investigate the potential of this technique on cancer diagnosis.

2.3 Polarimetry imaging

2.3.1 Polarimetry

Polarimetry refers to the science of measuring the state of polarization of light and the retarding, depolarizing and diattenuating properties of materials [29].

Polarized light has played important roles in many applications. It helps to map the valence band electronic structure of graphite [30], to understand the chirality of biological molecules [31], to reveal material anisotropy [32], to detect faults in heat seals of food packages [33], to explain the three dimensional characteristics of chemical bonds, to detect micrometer sized particles with endoscope [34], to characterize collagen orientation in human dermis [35] and to

differentiate between normal and abnormal biological tissues [36]. Traditional polarimetry, pursued in a variety of practical applications, is suitable for clear media application and surface studies.

Recently, polarimetric methods have attracted much attention in biomedical applications. It was used by Sankaran et al. [37] to investigate the propagation of polarized light in biological tissues. They used Stokes polarimetry to study how linearly and circularly polarized light is depolarized by scatter structures within tissues. This study indicated that linearly and circularly polarized light are depolarized differently depending on tissue structures. Sokolov et al. [38] showed the sensitive changes of reflectance spectra with polarized light caused by cellular and nuclear morphology. Circularly polarized light was used by Wu et al. [39] to illuminate rat tail tissues and strong depolarization by intervertebral discs and soft tissue regions of rat tails was observed. Their group also found that different contrasts of tissue structures could be obtained with the degree of linear polarization (DOLP) and the degree of circular polarization (DOCP) images. Ambirajam et al. [40] reached the conclusion that the degree of polarization of the diffuse light field depends on the optical thickness of the turbid media and scatterers' size using Monte Carlo simulations.

2.3.2 Stokes vector and Mueller matrix

A Stokes vector, frequently denoted by S , is a four-element vector that characterizes the intensity and polarization of a light beam [42].

$$S = \begin{bmatrix} I \\ Q \\ U \\ V \end{bmatrix} = \begin{bmatrix} IH + IV \\ IH - IV \\ IP - IM \\ IR - IL \end{bmatrix} \quad (2.1)$$

in which I is the total intensity of light, Q is the difference of intensity between horizontally (IH) and vertically linearly polarized light (IV), U is the difference of intensity between $+45^\circ$ linearly polarized (IP) and 135° linearly polarized light (IM) and V is the difference of intensity between right circularly (IR) and left circularly polarized light (IL).

A Stokes vector contains information about both the unpolarized and polarized portions of light. Therefore, Eq. (2.1) can be divided into the unpolarized portion S_0 and the polarized portion S_1 as shown below:

$$S = S_0 + S_1 = \begin{bmatrix} I - \sqrt{Q^2 + U^2 + V^2} \\ Q \\ U \\ V \end{bmatrix} + \begin{bmatrix} \sqrt{Q^2 + U^2 + V^2} \\ Q \\ U \\ V \end{bmatrix} \quad (2.2)$$

Mueller matrix, which is frequently denoted by M is a 4×4 real matrix, represents the transfer function of the Stokes vector during light propagation through a medium which changes the polarization state [42]. This can be explained with the following matrix product:

$$S_{out} = M_{sample} \times S_{in} \quad (2.3)$$

The Stokes vector of light change from S_{in} to S_{out} after interaction with the sample whose polarization properties can be completely characterized by the Mueller matrix of the sample M_{sample} [43, 44]. All the polarization altering properties of the

sample can be derived by decomposing the Muller matrix [45]. These polarization parameters include retardance (R), which includes linear retardance (also named birefringence: δ) and circular retardance (also called optical rotation: ψ), depolarization (Δ) and diattenuation (D) etc. The definitions of these properties and how they are correlated with tissue parameters are described below.

Retardance – phase shift between two orthogonal polarizations of the light. It is related to structural proteins, collagen, elastin and other fibrous tissues [4, 36, 46].

Linear retardance – retardance arises due to the difference in phase between orthogonal linear polarization states, e.g. between vertical and horizontal, or between 45° and 135° linearly polarized light.

Circular retardance – retardance arises due to the difference in phase between right circularly polarized (RCP) and left circularly polarized (LCP) light.

Depolarization – a process coupling polarized light into unpolarized light. It is mainly determined by scattering related to cellular density, cell concentration, nuclei size and distribution etc. [5, 22].

Diattenuation – attenuation difference between orthogonal polarization states (either linearly or circularly polarization states). It is also induced mainly by scattering [5, 22].

Meanwhile, it is worth mentioning that in the case when circular polarization state is not considered, the Stokes vector can be described as a vector with only the first three elements:

$$S = \begin{bmatrix} I \\ Q \\ U \end{bmatrix} \quad (2.4)$$

Correspondingly, the Mueller matrix becomes a 3×3 matrix which only describes how linearly polarized light or the linearly polarized components of a light change after interaction with a medium. The advantage of studying 3×3 Mueller matrix over 4×4 Mueller matrix of a medium is that it only requires the input of linearly polarized light that can be achieved with only one polarizer. The system required for reconstructing 3×3 Mueller matrix is simpler. However, it cannot fully describe how the polarization states are changed by a medium due to the loss of information due to circular polarized components.

2.3.3 *Polarimetry imaging*

Polarimetry imaging has emerged as a new technique with important research applications in medicine and biology [47]. It is a non-invasive technique which can provide plenty of quantitative information about tissue structures [48]. Various components of tissues can be investigated by correlating them with the polarization parameters derived from Mueller matrix. Several methods have been proposed to utilize polarization parameters for tissue characterization.

In the first group of methods, polarization was used as a gating mechanism to discriminate multiply scattered light from singly scattered light, thus improving the resolution of tissue images and its underlying structures [46, 48]. Demos et al. [49] proved that practically this gating technique was able to discriminate forward propagating light from diffusive light within 100 ps. Jacques et al. [50] used

polarization ratio (Pol) images to diagnose skin pathology with high resolution as shown in Fig. 2.1, the normal and polarized images of squamous cell carcinoma are shown in the left and right respectively. Freckle and pigmented nevus in superficial tissues were also discriminated using this method [51]. Similarly, Anastasiadou

Figure 2. 1 Images of squamous cell carcinoma. Left: normal image, right: polarized (Pol) image (Image adapted from Ref. [50]).

et al. [52] used this method (DOP technique) to detect cervical cancer, the results of which were compared with that from classical colposcopy. They concluded that this method was able to identify normal and cancerous cervical tissue. This technique was also adopted by some other groups and turned out to be an effective method to identify normal and diseased tissues in different organs [53-55].

Further, Sokolov et al. [38] used this method to study the response of reflectance spectroscopy to cellular and nuclear morphology. It was shown that quantitative morphological information such as changes in nuclei size could be provided by reflectance spectroscopy.

In the second groups of methods, the Stokes vectors of light were measured

and analyzed for tissue studies. Wu et al. [39] calculated the linear degree of polarization and circular degree of polarization using measured Stokes vectors for rat samples. They found that the contrasts of images between structures and adjacent tissue layers were affected by the depth of tissues and the detection geometry. Moreover, the incident circularly polarized light was strongly depolarized by the intervertebral discs and soft tissue regions of rat tails. Sankaran et al. [37] compared the depolarization of linearly polarized light and circularly polarized light propagating through biological tissues using Stokes vector measurements. It turned out that linearly and circularly polarized light was depolarized differently according to tissue structures. In addition, the degree of polarization was plotted as a function of I_{out} / I_{in} (I_{out} is the total output light intensity from the sample while I_{in} is the total light intensity incident on the sample) to compare the depolarization of the state of polarization relative to the attenuation of light intensity. This indicates situations where polarized light was preferred over unpolarized light. For example, polarized light could image deeper into blood than unpolarized light because the depolarization rate is slower than the light attenuation rate. Studinski et al. [56] investigated how polarized light interacts with tissues and tissue-like media in the exact backscattering direction by modulating the incident polarized light to detect the first term of Stokes vector in various situations. They found that a large proportion of light polarization was preserved along this direction in both phantom and samples. Guo et al. [57] studied the effects of turbid chiral media in various directions around scattering samples

using a refined linear stokes polarimeter in which the principle was similar to that of Ryan et al's system [56]. It was found that, the backward geometry might be more preferable to detect glucose in highly turbid media, and that the refractive index matching effect dominates the optical rotation induced by glucose over the chiral nature of the solute. Ghassemi et al. [58] developed an out-of-plane Stokes imaging polarimeter for early skin cancer diagnosis. This system was able to obtain information regarding skin roughness by the decomposition of Stokes vector into its polarized and unpolarized components. This method, in which tissue roughness was derived by analyzing Stokes vectors for early cancer detection, was also used by Lemaillet et al [1]. They developed a hemispherical imaging Stokes polarimeter to assess the roughness of the epidermis and monitor skin cancer by illuminating the samples at different angles and calculating the degree of polarization correspondingly.

In the third group of methods, the full Muller matrix was measured and processed to derive a full set of polarization parameters. This allows for noninvasive tissue diagnosis and for treatment or prognosis assessment [46, 48, 59]. There have been substantial theoretical [60-63] and experimental [36, 42, 43, 64, 65] efforts on Mueller matrix based polarimetry imaging. Baba et al. [66] developed and calibrated an automated Mueller matrix polarization imaging system. Twietmeyer et al. [67] proposed an effective optimization method for Mueller matrix measurements in the presence of errors. Hielscher et al. [68] proved that the 16 elements of Mueller matrix were able to completely describe the

polarization properties of a highly scattering medium in the diffuse backscattering mode by observing the intensity patterns backscattered from the media. Golnik et al. [69] presented several practical data processing methods for Stoke vector and Mueller matrix components including statistical analysis, autocorrelation, Fourier transform, à trous decomposition and multiscale entropy. Lu et al. [62] presented a useful algorithm for effective polar decomposition of Mueller matrix to deduce polarization parameters. The polar decomposition method has been used by many researchers [36, 43, 70, 71]. Ghosh et al. [72] validated the polar decomposition method on measurements from biological samples such as stem cells and rat myocardium samples.

Besides, Wood et al. [42] presented the first *in vivo* demonstration of a Mueller matrix decomposition method for the polarization-based characterization of tissues. Smith et al. [65] characterized various dermatological diseases using Mueller matrix polarimetry imaging. Their preliminary results at 633nm showed that malignant moles depolarized less than surrounding tissues and lupus lesions had rapidly varying retardance orientation. Baldwin et al. [73] suggested that normal, benign mole and cancerous lesions could potentially be differentiated in some Mueller matrix elements. Significant differences between different tissue types were observed in Mueller matrix elements M_{22} , M_{33} and M_{44} . Liu et al. [4] developed a polarization-based optical imaging system to measure the Mueller matrix of rat skins and tissue phantoms simulating *vivo* melanoma. It was demonstrated that structures within tissues could be differentiated by the

decomposition of Mueller matrix and reconstruction of displacement and strain from polarization images. The strain-based measurement and reconstruction methods were able to enhance contrasts between tissues and structures with polarization dependent biomechanical properties. Chung et al. [71] used a high-speed polarimetry system to image oral precancer on hamster cheek pouches and observed significant differences in depolarization images and retardance images, obtained by the polar decomposition of Mueller matrix, between normal and precancerous tissues. They concluded that cancerous lesion depolarized light less significantly than normal tissues and the retardance values within cancers were also smaller than that for normal tissues. Similarly, it was found by Shukla et al. [74] using Mueller matrix decomposition that depolarization power was sensitive to morphological changes between normal and dysplastic states in epithelial cervical tissue while changes in the stromal region were revealed in retardance values.

In addition, Antonelli et al. [75] interpreted experimental Muller matrix images from colon tissues with the help of Monte Carlo modeling. They observed smaller depolarization power from tumorous parts at early cancerous samples in comparison to healthy ones and found that the colon tissues depolarized circularly polarized light more than linearly polarized light. Laude-Boulesteix et al. [76] presented a multi-spectral imaging system based on a liquid crystal tunable filter. This system was used to measure the Mueller matrix of samples from hepatic biopsies at three wavelengths 500 nm, 550 nm and 650 nm and good contrasts in

the decomposition images were obtained. Recently, Manhas et al. [77] measured 3×3 Mueller matrix of healthy and cancerous tissues from human oral cavity and breast at wavelengths ranging from 350 nm to 550 nm. They found that normal oral cavity tissues had higher values in linear depolarization, linear retardance and diattenuation compared to cancerous ones, which was contrary to the trends for breast tissues. Pierangelo et al. [64] also used multi-spectral Mueller matrix imaging to stage human colon cancer. They found that mucus-free tumorous tissues with high cellular density had lower depolarization powers while healthy serosa had higher depolarization power. Besides, an improved contrast for the staging of human colon cancer were observed using multi-spectral Muller imaging. These studies confirm the potential of polarimetry imaging for the quantitative analysis of tissue sections.

Polarimetry imaging has been proved to be of great potential in biomedical application. In our first study, we focused on the investigation of the application of this technique on gastric cancer diagnosis and the roles of wavelength choice on diagnosis was also investigated, which was described in the following Chapter.

Chapter 3: Roles of polarization properties and wavelength choice on differentiation between *ex vivo* normal and cancerous gastric samples

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3.1 Introduction

Several morphological changes, which includes enlarged nuclei and cell size and changes in nuclei density and cell distribution [78] can occur when biological tissues develop into cancer. These changes are reflected in tissue polarization properties which can be measured by polarimetry techniques [46]. Specifically, polarization properties, such as retardance, depolarization and diattenuation, can be derived by performing polar decomposition of the Mueller matrices measured from tissues [71, 79]. It is worth noting that, Swami et al [80] proposed that linear polarization properties, including linear polarization, linear retardance and linear diattenuation, as well as circular retardance can be reconstructed from 3×3 Mueller matrix, which correspond to the first nine elements of 4×4 full Mueller matrix, under the assumption that the depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector. However, other circular polarization properties such as circular

depolarization and circular diattenuation cannot be derived. Compared to other optical techniques based on different contrasts such as fluorescence spectroscopy and imaging [81-83], polarimetry imaging has great advantages in signal strength and sensitivity to cellular structures. While the former advantage enables fast data acquisition, the latter one facilitates the characterization of structural changes in tissues. Differences in polarization parameters have been frequently observed between malignant and normal tissues. Smith et al. [65] characterized various dermatological diseases using Mueller matrix polarimetry imaging. Their preliminary results at 633nm showed that both lupus lesion and malignant moles could be identified by polarimetric measurement. Baldwin et al. [73] suggested that normal, benign mole and cancerous lesions could be potentially differentiated by multiple Mueller matrix elements. Chung et al. [71] used a high-speed polarimetry system to image oral precancer on hamster cheek pouches and observed considerable differences in depolarization images and retardance images between normal and precancerous tissues. Similarly, it was found by Shukla et al. [74] that depolarization power was sensitive to morphological changes from normal to dysplastic state in epithelial cervical tissue while changes in stromal region were revealed in retardance values.

Due to the potential of polarimetry imaging in cancer diagnosis, various approaches have been proposed to acquire data rapidly and derive polarimetry parameters. Manhas et al. [84] developed a system to obtain 3×3 Mueller matrix and demonstrated that the values of all linear polarization parameters except linear

depolarization, i.e., linear retardance and linear diattenuation, in normal oral cavity tissues were higher than cancerous tissues at wavelengths ranging from 400 nm to 550 nm. This observation was the opposite of that in breast tissues based on their results. Pierangelo et al. [64] observed enhanced contrasts for staging human colon and distinguishing between various histological variants of tumor by looking at depolarization and M22 from multi-spectral Mueller matrices. Laude-Boulesteix et al. [76] built a Mueller polarimetric imaging system with liquid crystals and observed differences in polarization properties including retardance, diattenuation and depolarization among different wavelengths in a hepatic tissue sample. J. Soni et al. [85] built a spectral Mueller matrix polarimetric system for both fluorescence and elastic scattering measurements recently, in which strong diattenuation were observed in the connective region of a tissue sample from cervical precancer. Despite a large number of publications in this field, none of the above reports about polarimetry imaging have compared the potential of circular polarization properties, including circular retardance and circular depolarization with that of linear polarization properties to find out whether it is necessary to acquire 4×4 Mueller matrix instead of 3×3 Mueller matrix that can only be used to extract linear properties or an even smaller matrix at multiple wavelengths. Since adding circular polarization into a Mueller matrix imaging system could induce significant complexity and cost, the question is worth systematic investigation. Moreover, the influence of light wavelength choice on optical diagnosis in polarimetry imaging has not been studied systematically for gastric tissues.

In this chapter, we investigate the relative importance of both linear and circular polarization parameters, derived from 4×4 Mueller matrix measurements, in discriminating cancer from normal gastric tissues over the visible spectral region from 470 nm to 632 nm. Moreover, the diagnostic value of each wavelength is compared against each other. Our results answer the questions proposed in the previous paragraph, which will guide the design of a portable polarimetry imaging system for *in vivo* examination of gastric tissues. It should be noted that the novelty of this paper is not about the development of a multi-spectral polarimetry imaging system, but rather the investigation on the importance of linear and circular polarization parameters and wavelength choices using the imaging system.

3.2 Materials and Methods

3.2.1 Materials and instrument

A total of 46 tissue samples, obtained from 40 patients, were examined in this study, in which 26 normal gastric samples were obtained as endoscopic biopsies while 20 gastric cancer samples were from gastrectomy. All patients had to sign a written consent form before their tissue samples were used in this study. For multiple normal samples from one single patient, the values of polarization parameters were averaged to represent only one sample in classification. This procedure yielded a total of 20 sets of data for normal samples and 20 sets of data for cancer samples, one set for each patient. Each sample was fixed using 10%-formalin solution before it was embedded in paraffin. Then two 4- μ m vertical

sections immediately next to each other were made in the sample. One tissue section was routinely stained with Hematoxylin and Eosin (H&E) to generate a pathological report. The other tissue section was not stained, which was placed on a microscope slide without a cover slip for polarimetry measurements.

The schematic of the multi-spectral Mueller matrix polarimetry system used in this study is shown in Fig. 3.1, in which all optical components are mounted in a commercial microscope (Ti-U, Nikon, Tokyo, Japan). The illumination light from a 100-W Halogen lamp is polarized by a polarization State Generator (PSG) that consists of a linear polarizer (Model no. 47213, Edmund Optics, Barrington, NJ, US), mounted in a filter wheel (HF110, Prior Scientific instrument, Fulbourn, Cambridge, UK), followed by a nematic liquid crystal variable retarder (LCR-1-VIS, Thorlabs, Newton, New Jersey, US). After passing through the sample, the transmitted light is analyzed by a Polarization State Analyzer (PSA) with the same optical components but in reverse order. The Mueller matrix images are recorded on a CCD (DS-Qi1Mc, Nikon, Tokyo, Japan) with a resolution of 640×512 pixels.

Figure 3. 1 Schematic of the polarimetry imaging system to record 4×4 Mueller

matrix at multiple wavelengths.

A total of 9 band pass filters with central wavelengths of 470, 488, 508, 532, 550, 568, 589, 610, 632 nm (Product no. 65144, 65147, 65151, 65155, 65159, 65160, 65162, 65164 and 65166, Edmund Optics, Barrington, NJ, US) with a bandwidth of around 10 nm were mounted in a filter wheel to enable multi-spectral imaging. The light spot focused on the sample is about 3 mm in diameter under 2X objective lens. In most normal samples obtained from biopsy, the entire area of specimens can be covered in the field of view. For each cancer sample obtained from surgery with a larger size, repeated measurements were made at multiple locations and results were averaged to represent the entire sample, which was to take into account large heterogeneity in cancer samples.

3.2.2 *Imaging method*

Both PSG and PSA can generate the following polarization states by switching between the linear polarizers with different orientations and adjusting the retardance of LCVR, which include horizontal polarization (Stokes vector $H: [1 \ 1 \ 0 \ 0]^T$), vertical polarization ($V: [1 \ -1 \ 0 \ 0]^T$), $+45^\circ$ linear polarization ($P: [1 \ 0 \ 1 \ 0]^T$) and right circular polarization ($R: [1 \ 0 \ 0 \ 1]^T$). Sixteen images corresponding to HH, HV, HP, HR, VH, VV, VP, VR, PH, PV, PP, PR, RH, RV, RP and RR, in which the two letters indicate the polarization states of the PSG and the PSA respectively, were recorded at nine wavelengths. After background subtraction, the acquired images were used to reconstruct the Mueller matrix [43], which were then

decomposed to extract polarization parameters using polar decomposition [71].

Each tissue sample was fixed on a microscope slide during measurements. To minimize the uncertainty in the Mueller matrix of a sample due to the microscope slide and the system components, the following calibration was performed for every tissue sample. For each sample fixed on a slide, one blank location of the slide was measured first and the slide's Mueller matrix (M_b) was reconstructed. Then the sample location on the slide was measured and the Mueller matrix of the sample on top of the slide (M_{b+s}) was reconstructed. Finally the Mueller matrix of the sample on top of the slide was multiplied by the inversion of the slide's Mueller matrix to yield the true Mueller matrix of the sample (M_s) in which the effects of the slide and system throughput have been removed. Mathematically, this process can be denoted as

$$M_s = M_{b+s} \times M_b^{-1} \quad (3.1)$$

We validated the experimental setup (Fig. 3.1) and the approach of matrix inversion in the previous paragraph by measuring the Mueller matrix of a quarter-wave plate, a half-wave plate and a linear polarizer with known polarization properties prior to imaging gastric tissue samples. These standard samples were put on top of a microscope slide and the effects of slides were removed after measurements. The results of these measurements are shown at the beginning of the next section, which suggests that the experimental setup works well.

3.2.3 Reconstruction of Mueller matrix

During experiments, 16 different combinations of PSG and PSA are used to record 16 images as shown in above section for reconstructing Mueller matrix images. Eq. (3.2) shows the reconstruction method [4, 43]:

$$\mathbf{M} = \begin{bmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{bmatrix} = \begin{bmatrix} HH + HV + VH + VV & HH + HV - VH - VV & 2PH + 2PV - m_{00} & 2RH + 2RV - m_{00} \\ HH - HV + VH - VV & HH - HV - VH + VV & 2PH - 2PV - m_{10} & 2RH - 2RV - m_{10} \\ 2HP + 2VP - m_{00} & 2HP - 2VP - m_{01} & 4PP - 2PH - 2PV - m_{20} & 4RP - 2RH - 2RV - m_{20} \\ 2HR + 2VR - m_{00} & 2HR - 2VR - m_{01} & 4PR - 2PH - 2PV - m_{30} & 4RR - 2RH - 2RV - m_{30} \end{bmatrix} \quad (3.2)$$

H: linearly polarized in the horizontal direction, V: linearly polarized in the vertical direction, P: linearly polarized in 45° relative to the horizontal direction, R: circularly polarized in clock-wise direction. In this table, the first letter represents polarization state for generator PSG while the second denotes polarization state for analyzer PSA.

3.2.4 Decomposition of Mueller matrix

The Mueller matrix of a depolarizing sample can be expressed as the product of three 4×4 matrices: the depolarization matrix (M_{Δ}), the retardance matrix (M_R)

and the diattenuation matrix (M_d). The procedure is briefly described below [60, 62, 71]:

The Mueller matrix (M) is decomposed in the following order using the polar decomposition method [62] which has been used as a robust mathematical tool to interpret the polarization properties of a medium as illustrated and applied in several other papers [60, 64, 71, 74]:

$$M = \begin{bmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{bmatrix} \quad (3.3)$$

$$M = M_{\Delta} \times M_R \times M_d \quad (3.4)$$

The diattenuation (D) value is derived from the elements on the first row of the matrix using the following equation [62]:

$$D = \frac{1}{m_{00}} \sqrt{m_{01}^2 + m_{02}^2 + m_{03}^2} \quad (3.5)$$

Then, the diattenuation property is removed by multiplying Mueller matrix (M) by the inversion of diattenuation matrix (M_d) to get M' [62].

$$M' = M_{\Delta} M_R = M M_d^{-1} \quad (3.6)$$

These above matrices have the following forms [62]:

$$M_{\Delta} = \begin{bmatrix} 1 & \vec{0}^T \\ \vec{P}_{\Delta} & m_{\Delta} \end{bmatrix}, M_R = \begin{bmatrix} 1 & \vec{0}^T \\ \vec{0} & m_R \end{bmatrix}, M' = \begin{bmatrix} 1 & \vec{0}^T \\ \vec{P}_{\Delta} & m' \end{bmatrix} \quad (3.7)$$

where, m_{Δ} , m_R and m' are the 3×3 sub-matrices of M_{Δ} , M_R and M' respectively.

$m' = m_{\Delta} \times m_R$ and m_{Δ} can be computed by [62]:

$$m_{\Delta} = \pm \left[m'(m')^T + (\sqrt{\lambda_1 \lambda_2} + \sqrt{\lambda_2 \lambda_3} + \sqrt{\lambda_3 \lambda_1}) I \right]^{-1} \times \left[(\sqrt{\lambda_1} + \sqrt{\lambda_2} + \sqrt{\lambda_3}) m'(m')^T + \sqrt{\lambda_1 \lambda_2 \lambda_3} I \right] \quad (3.8)$$

where λ_1 , λ_2 and λ_3 are the eigenvalues of $m'(m')^T$. Noted that if the determinant of m' is positive, we choose the positive sign on the right side of the above equation, Otherwise, the negative sign is used. Further, the depolarization power (Δ) and the sub-matrix of retardance matrix (m_R) can be calculated [62]:

$$\Delta = 1 - \frac{|trace(m_{\Delta})|}{3} \quad (3.9)$$

$$m_R = m_{\Delta}^{-1} m' \quad (3.10)$$

This enables one to calculate the retardance matrix M_R according to Eq. (3.7).

Finally, the retardance (R), linear retardance (δ) and circular retardance (φ) are calculated as follows [60, 86]:

$$R = \cos^{-1} \left[\frac{trace(M_R)}{2} \right] - 1 \quad (3.11)$$

$$\delta = \cos^{-1} (\{ [M_R(2,2) + M_R(3,3)]^2 + [M_R(2,3) - M_R(3,2)]^2 \}^{1/2} - 1) \quad (3.12)$$

$$\varphi = \frac{1}{2} \tan^{-1} \left[\frac{M_R(2,3) - M_R(3,2)}{M_R(2,2) + M_R(3,3)} \right] \quad (3.13)$$

According to the original Mueller matrix M , the linear degree of polarization (LDOP) and circular degree of polarization (CDOP) are obtained as [54]:

$$LDOP = \frac{m_{10} + m_{11}}{m_{00} + m_{01}} \quad (3.14)$$

$$CDOP = \frac{m_{30} + m_{33}}{m_{00} + m_{03}} \quad (3.15)$$

in which LDOP describes to what degree the linearly polarized light preserves its polarization state after interaction with sample while CDOP denotes to what degree the circular polarization of light is preserved. Then linear depolarization (LD) and circular depolarization (CD) can be computed as

$$LD = 1 - LDOP \quad (3.16)$$

$$CD = 1 - CDOP \quad (3.17)$$

3.3 Results

The normalized Mueller matrix of a horizontal linear polarizer at different wavelengths and the retardance of a quarter-wave plate and a half-wave plate are shown below in Fig. 3.2(a) and 3.2(b), respectively. In Fig. 3.2(a), the differences between experimental measurements and theoretical prediction for the linear polarizer are smaller than 0.06 when all elements are divided by m_{00} at the corresponding wavelength. Similarly as shown in Fig. 3. 2(b), the measured retardance value differs from the reference value given by the manufacturer by less than 2% for both the half-wave plate and the quarter-wave plate. The small discrepancies could be attributed to the finite bandwidth of the band pass filter at each wavelength and the slight variation in the retardance of the LCVR due to temperature fluctuation. The reference retardance values of two wave plates at 470

nm and 488 nm are not available from the manufacturer thus not shown Fig. 3.2(b).

The excellent agreement in the Mueller matrix of the linear polarizer between experimental measurements and theoretical prediction and the agreement in the retardance values of the wave plates between experimental measurements and reference values provided by manufacturers demonstrate that the Mueller matrix imaging system and data analysis methods work well. In addition, the standard deviations of retardance, linear retardance, depolarization, linear depolarization, and circular depolarization values for gastric samples measured in different days are more than four times larger than those for those standard samples such as air, polarizer and retarders. In contrast, the standard deviations of diattenuation and circular retardance for the standard samples are only about twice those for gastric samples. This observation suggests that the major portion of the variances in the former five parameter values of tissue samples is contributed by tissue samples while a large portion of the variances in the latter two parameter values of tissue samples is contributed by the system, which will influence data interpretation as discussed later.

Figure 3. 2 (a) Mueller matrix elements of a horizontal linear polarizer (experimental and theoretical values) and (b) retardance values of a half-wave plate (left) and a quarter-wave plate (right) at nine different wavelengths (experimentally measured values and reference values provided by the manufacturer) ranging from 470 nm to 632 nm. In Fig. 3.2(a), the vertical axis represents the value of each Mueller matrix element divided by m_{00} .

Figure 3.3 shows the following derived polarization parameters, i.e., (a) retardance, (b) diattenuation and (c) depolarization of normal and cancerous gastric samples at nine wavelengths from 470 nm to 632 nm. The average retardance of normal samples is smaller than that of cancer samples. There is no overlap in the error bars between normal samples and cancerous samples at all nine wavelengths, which suggests that retardance could be used to effectively differentiate normal and cancer samples. In contrast, there are significant overlaps in the error bars for diattenuation and visible overlaps in those for depolarization between normal and cancer samples at all wavelengths.

Figure 3. 3 Polarization parameters including the (a) retardance, (b) diattenuation, (c) depolarization of normal (■) and cancerous (●) gastric samples at

nine different wavelengths ranging from 470 nm to 632 nm.

To further identify the contributions from linearly and circularly polarized light, linear retardance and circular retardance are plotted as a function of wavelength as shown in Fig. 3.4. The linear retardance value is similar to the retardance value at each wavelength, implying that linear retardance is the main contributor of retardance in gastric tissues. Linear retardance also shows a similarly significant difference between normal and cancerous samples. In contrast, circular retardance is much smaller and the overlaps in error bars between normal tissues and cancer are significant, which suggests potentially insignificant diagnostic value. Linear depolarization and circular depolarization are also plotted as a function of wavelength as shown in Fig. 3.5. The general trend, i.e., the depolarization of normal samples is smaller than that of cancer samples, is consistent for both linear and circular depolarization. The subtle difference is that the overlaps in the error bars of linear depolarization between normal and cancer samples appear to be smaller than circular depolarization, which suggests that linear depolarization likely differentiate between them more effectively than circular depolarization.

Figure 3. 4 (a) Linear retardance and (b) circular retardance of normal (■) and cancerous (●) gastric samples at nine different wavelengths ranging from 470 nm to 632 nm.

Figure 3. 5 (a) Linear depolarization and (b) circular depolarization of normal (■) and cancerous (●) gastric samples at nine different wavelengths ranging from 470 nm to 632 nm.

To quantitatively investigate the diagnostic values of these polarization parameters over the spectral range, linear discriminant analysis (LDA) was performed to differentiate cancer samples from normal samples at all nine wavelengths.

Figure 3.6(a) shows that the classification using one single polarization parameter yields the decreasing overall accuracy in the order of retardance, depolarization and diattenuation at most wavelengths. The observation that diattenuation yields the lowest accuracy could be attributed to the highly overlapping error bars between cancer and normal samples as shown in Fig. 3.3(b). This could be related to the previous calculation that the ratio of the standard

deviations in the diattenuation values of gastric samples to that of the optical components is only around two. Therefore, a significant portion of variance in the measured diattenuation values of gastric samples comes from the system thus not reliable for classification. Figure 3.6(b) indicates that linear retardance always yields higher overall accuracy compared to circular retardance, which validates the prediction made earlier according to Fig. 3.3. Figure 3.6(c) shows that linear depolarization yields slightly higher overall accuracy at some wavelengths but lower overall accuracy at other wavelengths compared to circular depolarization.

Figure 3. 6 LDA classification accuracy (overall accuracy) for (a) retardance, diattenuation and depolarization (b) linear retardance and circular retardance (c) linear depolarization and circular depolarization at nine different wavelengths ranging from 470 nm to 632 nm.

To find the optimal combination of polarization parameters for classification, a Wilcoxon rank sum test was performed at each wavelength for all parameters to decide which parameters should be used for combination. Table 3.1 presents the p-values calculated from the Wilcoxon test. With a significance level of 0.05, it was found that most parameters except diattenuation and circular retardance show significant differences between normal and cancerous gastric samples at all wavelengths, which agrees well with the low classification accuracy of these two parameters shown in Fig. 3.6.

Then all the possible combinations of these polarization parameters except diattenuation and circular retardance were used for classification based on the results of Wilcoxon rank sum tests. Table 3.2 shows the best combinations including a different number of polarization parameters at all wavelengths. It was found that the combination of linear depolarization (LD) and linear retardance (δ) shows the highest overall accuracy (95.00%) among all the possible combinations of two parameters. This combination also demonstrates considerable improvement in overall accuracy compared to that for either linear retardance (82.50%) or linear depolarization (77.50%) alone.

Table 3. 1 *P*-values obtained from Wilcoxon rank-sum tests for polarization parameters at nine wavelengths for differentiation between normal and cancer samples.

Polarization parameters							
λ (nm)	<i>R</i>	Dia	Δ	δ	φ	LD	CD
470	3.50E-06	0.0385	3.07 E-06	2.69 E-06	0.0154	3.07 E-06	4.68 E-05
488	4.54 E-06	0.6359	3.50 E-06	4.54 E-06	0.2085	4.54 E-06	7.58 E-06
508	5.87 E-06	0.0098	1.81 E-06	5.17 E-06	0.1479	5.17 E-06	2.92 E-05
532	5.17 E-06	0.0003	9.75 E-06	6.67 E-06	0.0640	1.25 E-05	3.71 E-05
550	3.50 E-06	0.5428	2.04 E-06	2.36 E-06	0.0207	5.87 E-06	9.28 E-05
568	5.17 E-06	0.0071	1.81 E-06	4.54 E-06	0.0315	5.17 E-06	4.68 E-05
589	2.69 E-06	0.8817	3.71 E-06	2.69 E-06	0.0020	5.17 E-06	1.79 E-04
610	5.17 E-06	0.2085	5.25 E-06	5.17 E-06	0.0294	6.67 E-06	2.75 E-04
632	3.07 E-06	0.0256	1.44 E-04	3.50 E-06	0.0179	7.58 E-06	2.00 E-04

Note: *R* – Retardance, Dia – Diattenuation, Δ – Depolarization, δ – Linear retardance, φ – Circular retardance, LD – Linear depolarization, CD – Circular depolarization. The entries with bold font indicate p-values larger than 0.05, which implies insignificant difference between normal and cancer samples at a significance level of 0.05.

Table 3. 2 Accuracy of classification using the combination of two, three, four and five parameters at all wavelengths.

Number of parameters	Best combination	Overall accuracy	Sensitivity	Specificity
2	LD+ δ	95.00%	95.00%	95.00%
3	LD+ δ + <i>R</i>	90.00%	95.00%	85.00%

4	LD+ δ +R+CD	90.00%	90.00%	90.00%
5	LD+ δ +R+CD+ Δ	90.00%	85.00%	95.00%

3.4 Discussion

In this study, we built a multi-spectral Mueller matrix imaging system, which was used to measure unstained gastric tissue sections from 470 nm to 632 nm. A full set of tissue polarization parameters were derived from measurements, which were then used to discriminate gastric cancer from normal samples in an attempt to investigate the individual roles of linear and circular polarization properties and the effect of wavelength choice on classification accuracy. Several interesting findings were made as discussed below.

It is noted in Fig. 3.3(a) and (b) that error bars for the retardance and diattenuation of normal samples are slightly larger than those for cancer samples. The trend is the opposite for depolarization as shown in Fig. 3.3(c), for which the error bars for cancer samples are much larger. The higher variation in the retardance of normal samples could be possibly attributed to the higher variation in the types of tissue components such as fibrous stroma and various glands responsible for retardance [46, 74]. When normal gastric tissues become malignant there are both architectural and cytological changes. The glands invade the mucosa and become disorganized, which then develop into cancers. In the process, the nucleocytoplasmic ratio increases and nuclei become vesicular and variable in size and shape. In addition, the tumor associated stroma in cancer samples shows

modification at both histological and molecular level. The fibroblasts become “activated”, which are termed as “cancer associated fibroblasts”. Proliferation of endothelial cells and increased number of inflammatory cells may also be observed in the tumor associated stroma. The above mentioned stromal changes help form a microenvironment that allows the growth of the tumor and increased angiogenesis and metastasis. This matches the previous reports [78, 87] that the malignant changes in the basal membrane include the alteration in the structures and ratios between various components such as collagen and fibronectin, and that there are cross-linked fibrin accumulated in tumor fibrous stroma. This could be the major reason for larger retardance and linear retardance values in cancerous samples compared to normal samples. Typical H&E images of both stained normal and cancer gastric samples taken under 10X objective lens are shown below in Fig. 3.7. It can be observed that gastric glands become more disorganized and there are more disorganized fibrous structures in the cancer sample compared to the normal one. Moreover, these components in normal samples are less consistent than that in cancerous samples due to the fact that normal samples obtained from biopsy procedures were much smaller than cancer samples obtained from surgery procedures. Thus fewer measurements were thus performed for averaging from normal samples compared to cancer samples, which potentially resulted in larger error bars in the retardance of normal samples. As for the trend for depolarization, nuclei size and nuclei density, which are known to increase from normal samples to cancer samples as mentioned above, are less consistent in cancerous samples

due to variance in the size of tumors for different patients, [88] which is also based on pathologist's observation and report from National University Hospital. This inconsistency should contribute to higher variances for cancerous samples in depolarization (Fig. 3.3), linear depolarization and circular depolarization (Fig. 3.5) that are tightly related to nuclei size and density [46, 74]. In addition, it could be observed from Fig. 3.5 that the values of circular depolarization are significantly higher than those of linear depolarization. This could be due to the fact that circularly polarized light was strongly affected by paraffin wax used to fix samples. The measurement of paraffin wax alone demonstrates significant circular depolarization for a wax layer of 4 μm (result not shown). However, we believe that the existence of wax does not affect our conclusion because the effect of wax was equally present in both normal and cancer samples. Besides, the higher retardance and depolarization in cancer could partially be attributed to stronger scattering resulting from enlarged nuclei and larger nuclei density [74]. The small mean values and large error bars of diattenuation and circular retardance were observed for gastric samples in this study, which may suggest that most tissue components in thin tissue sections are not strongly birefringent for circularly polarized light and not strongly diattenuating.

Figure 3. 7 H&E images for normal (left) and cancer (right) gastric samples under 10X objective lens. In the left image, the arrow points to a normal gastric gland. In the right image, the top arrow points to a malignant gland while the other two point to intervening disorganized fibrous stroma.

In Table 3.2, which shows the accuracy of classification using the combination of different parameters, it is interesting to observe that the overall accuracy does not improve as the number of parameters combined increases. Linear retardance and linear depolarization are included in all best combinations. This implies that these two parameters, which can be extracted from 3×3 Mueller matrix, are likely most important for clinical classification. Retardance is the third additional parameter in the best combination of three parameters while circular depolarization shows up in the best combination of four parameters. The derivation of both retardance and circular depolarization requires 4×4 Mueller matrix.

To investigate the effect of wavelength choice on classification accuracy, all the possible combinations of the nine wavelengths with the number of wavelengths ranging from one to nine were evaluated for all the individual parameters (data not shown due to the large space required) except diattenuation and circular retardance

that have been shown unable to effectively distinguish between normal and cancer samples according to Table 3.1. It was found that short wavelengths including 470 nm and 488 nm appear most frequently in the best combinations for depolarization and linear depolarization but the differences in classification accuracy between the optimal combination and other combinations are small. Moreover, all the combinations of wavelengths regardless of the number of wavelengths show nearly identical classification accuracy for retardance and linear retardance. Interestingly, it is also observed from Fig. 3.6 that the overall classification accuracy does not change significantly from one wavelength to another or to the combination of all wavelengths. This observation suggests that wavelength might not be a critical factor in terms of classification accuracy in this particular setup in which tissue samples were 4 μm in thickness only. It should be aware that this trend could change when tissue samples are thicker, in which case a reflection setup is required and the light path would be longer. A greater path length would result in larger retardance and diattenuation. The subsequent multiple scattering could increase depolarization. For a similar reason, the polarization properties at a shorter wavelength, at which case light scattering is usually stronger and light path is longer, may yield significantly different accuracy from those at a longer wavelength.

Due to the scarcity of polarization properties of gastric tissue samples in the literature, we had to compare our findings with those for other types of tissue samples from the literature as shown in Table 3.3. It is observed that our results for

gastric samples are consistent with those for cervical or breast samples in depolarization and linear retardance but disagree with those for oral and colon samples in retardance, diattenuation, depolarization, linear retardance and linear depolarization. This is possibly related to the differences in the polarimetry imaging configuration and the biological structure of tissue samples from one study to another. For example, the measured polarimetry signal could be sensitive to different biological structures in each type of tissue samples if the configuration is arbitrarily sensitive to a fixed depth as the biological structure can vary with the tissue type. This suggests the importance of studying the polarization properties of individual cell types, such as those in foveolar glands, pyloric glands and oxyntic glands, rather than normal or cancer samples in general. Compared to other tissue studies, this study yields a comprehensive collection of polarization parameters, which will provide a reference for subsequent studies in the future.

Table 3. 3 Comparison in the polarization properties between the results of this study and those from the literature.

Polarization parameters	Gastric sample (this study, normal vs. cancer)	Cervical sample (Normal vs. precancer) [74]	Breast sample (normal vs. cancer) [84]	Oral tissue (normal vs. cancer) [84]	Oral tissue (normal vs. precancer) [71]	Colon sample (normal vs. cancer) [75]
Retardance (R)	$R_n < R_c$	$R_n > R_n$	NA	NA	$R > R$	NA
Diattenuation (Dia)	$Dia_n \approx Dia_c$	NA	$Dia_n < Dia_c$	$Dia_n > Dia_c$	NA	NA
Depolarization (Δ)	$\Delta_n < \Delta_c$	$\Delta_n < \Delta_p$	NA	NA	$\Delta_n > \Delta_p$	$\Delta_n > \Delta_c$
Linear retardance ($\mathcal{S}$)	$\delta_n < \delta_c$	NA	$\delta_n < \delta_c$	$\delta_n > \delta_c$	NA	NA
Circular retardance (φ)	$\varphi_n \approx \varphi_c$	NA	NA	NA	NA	NA

Linear Depolarization (LD)	$LD_n < LD_c$	NA	$LD_n < LD_c$	$LD_n > LD_c$	NA	NA
Circular Depolarization (CD)	$CD_n < CD_c$	NA	NA	NA	NA	NA

Note: the subscripts with “n”, “c”, and “p” indicate normal, cancer and precancer samples. “NA” infers that relevant data is “Not Available”.

3.5 Conclusion

A multi-spectral 4×4 Mueller matrix imaging system was used to investigate *ex vivo* gastric tissue section samples that were not stained. There were significant differences in most polarization parameters, including retardance, depolarization, linear retardance, linear depolarization and circular depolarization, between normal and cancer gastric samples. The combination of linear depolarization and linear retardance yields the highest accuracy in sample classification. When the depolarization of linearly polarized light due to scattering is independent of the orientation angle of the incident linear polarization vector, the derivation of linear polarization properties will require only 3×3 Mueller matrix, which would significantly reduce the complexity of the polarimetry imaging system. When additional parameters are needed to complement the two linear polarization parameters, retardance, circular depolarization and depolarization can be included in classification in the order of preference. However, these additional parameters would require the measurement of 4×4 Mueller matrix. In addition, it appears that wavelength is not a critical factor in terms of classification accuracy for thin tissue sections in this study.

The potential of polarimetry imaging at the macroscopic level for gastric cancer diagnosis has been proved in this Chapter. The data obtained yields information about the overall category of a given tissue sample. However, a typical pathological report in biopsy analysis will offer much more details about a sample such as all involved cell types and the distribution. Such information can be only acquired in a microscopic study, which will be the focus in the next Chapter.

Chapter 4: Investigation of the potential of Mueller matrix polarimetry imaging for digital staining

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4.1 Introduction

Hematoxylin and Eosin (H&E) staining and other staining techniques are commonly used in the clinical setting to stain tissue components such as nuclei and cytoplasmic components for pathologists to diagnose and grade the severity of a disease based on the color, shape, degree of staining and pattern of stains. However, this procedure is time consuming and it requires intensive sample preparation by a well-trained pathologist with rich experience to achieve optimal diagnosis. Such requirements are not always available, for example, in low resource regions. Digital staining has been proposed to complement these procedures to lower down these requirements. Digital staining utilizes digital processing techniques to transform the image of an unstained sample to its stained image counterpart. It can not only enhance the contrast between the background and objects of interest, but also mark the sample's unique property that may help in diagnosis [89]. Digital staining has attracted great attention from researchers due to

several advantages. First, digital staining provides quantitative information of tissue structures and components for diagnosis and reduces the hands-on time of histopathologists. Second, this technique could be much cheaper than the existing chemical staining methods due to its label-free trait. Furthermore, it does not destroy the biological sample and therefore the same sample slide could be analyzed by different digital staining protocols. Finally, it is harmless to users in contrast to toxic chemical stains used in regular staining [90].

Several researchers have proposed different techniques for digital staining. Amrania et al. [91] developed a mid-infrared imaging method to augment the H&E tissue staining protocol, which was able to generate false-color computer images with information on both tissue morphology and distributions of the nuclear-to-cytoplasmic ratio. Pinky et al. [92] separated different histological components such as nucleus, cytoplasm, red blood cells and the white region from kidney specimens using a digital staining method in which the linear mapping of spectral transmittance values and further classification based on transmittance values for different tissue components were conducted. Tanji et al. [93] digitally stained each intracellular structures such as nuclei, cytoplasm and erythrocytes without chemical staining process by using a digital staining method based on stimulated Raman scattering and statistical machine learning. Yoichi et al. [94] combined the high-speed stimulated Raman scattering with multivariate analysis including independent component analysis and principal component analysis to perform digital imaging on tumor-grafted mouse tissues. They observed the

different shapes and components from cancerous and noncancerous regions from the pancreas and liver. Bautista et al. [95] performed the classification of H&E stained tissue components and the transformation of their transmittance spectra to Masson's trichrome (MT) stained counterparts to do digital staining. They demonstrated that multi-spectral imaging was able to perform digital staining in the pathological context from liver specimens. Bini et al. [96] used spectral analysis for digital staining to simulate histology-like appearance using confocal mosaicing microscopy on *ex vivo* human skin samples. Fluorescence mosaics were used to stain nuclei pink and reflectance mosaics to stain cellular cytoplasm and dermis pink in their digital staining process. While these studies showed that digital staining can transform an unstained slide to a digital staining image, no one has looked into the possibility of perform digital staining to different tissue regions according to the pathological status on the microscopic level for gastric cancer diagnosis to our best knowledge. Furthermore, although polarimetry imaging has been used to differentiate cancer samples from normal gastric samples previously, this technique has not been utilized to identify specific regions according to the pathological status in a tissue sample.

Polarimetry imaging has been studied for medical diagnosis due to the great potential of this technique such as reflecting morphological changes in tissue and its stronger signal in comparison to other techniques like fluorescence spectroscopy [46, 82, 97]. The morphological changes of tissues from normal state to cancer, such as the increase in nuclei size and density and the disorganization in

various glands, can be reflected in the increase or decrease in the values of polarization parameters including retardance, diattenuation and depolarization [15, 46, 78]. The polarization parameters can be derived by polar decomposition of the full 4×4 Mueller matrix of a biological sample or its sub-matrix, i.e., the 3×3 Mueller matrix which consists of the first 9 elements of the 4×4 matrix. The former can be used to extract all the polarization parameters including retardance, diattenuation, depolarization, linear retardance, circular retardance, linear depolarization and circular depolarization while the latter can only be used to derive circular retardance and linear properties including linear retardance, linear diattenuation, linear depolarization [54, 60, 62, 80].

Based on literature review, we can conclude that there have been significant achievements in this field, especially in the application of this technique for disease diagnosis or tissue identification. Specifically, Chung et al. [71] stated that depolarization and retardance images are potential for tissue boundary identification and the differentiation between normal and cancerous tissues. They observed that precancers are less depolarizing than normal tissues and the values in retardance for precancerous tissues are generally smaller than the surrounding normal ones. Baldwin et al. [73] observed that there are marked differences in the M_{22} , M_{33} and M_{44} elements of Mueller matrix between normal, mole and cancer tissues from Sinclair Swine. Smith et al. [65] distinguished between normal, lupus lesion and malignant moles by polarimetric measurements at 633 nm wavelength. Antonelli et al. [75] concluded that healthy tissues depolarize light more than

cancerous ones and that circular depolarization is always higher than linear depolarization for both normal and cancerous samples based on measurements on colon samples using Mueller imaging polarimeter. Pierangelo et al. [64] stated that multi-spectral polarimetry imaging may enhance contrast for the identification of various types of cancers and staging the advancement and penetration of cancers by looking at M_{22} images of colon tissues at wavelengths from 500 nm to 700 nm. Shukla et al. [74] found that in epithelial cervical tissue depolarization power is sensitive to morphological changes from normal to dysplasia while changes in stromal region can be revealed in retardance values. J. Soni et al. [85] developed a spectral Mueller matrix polarimetry imaging system for the measurements of fluorescence and elastic scattering measurements and observed strong diattenuation in the connective region of a tissue sample from cervical precancer. Laude-Boulesteix et al. [76] built a polarimetric system with liquid crystals, in which significant differences were observed in retardance, diattenuation and depolarization among different wavelengths from 500 nm to 700 nm in a hepatic tissue sample. Recently, Martin et al. [98] investigated the response of pig skin to irradiation by looking at the changes in values of polarization parameters including retardance, diattenuation and depolarization at different levels of irradiation. Pierangelo et al. [99] explored the diagnosis of residual cancer of rectum after neoadjuvant treatment using a multi-spectral polarimetry system operated from 500 nm to 700 nm. They observed that there are obvious contrasts between the footprints of cancer and surrounding rectum tissues from patients after neoadjuvant

treatment and that the footprints of initial tumorous regions depolarizes less than the surrounding healthy ones when even a very small volume fraction of a residual cancer is present. Qi et al. [100] designed a narrow-band 3×3 Mueller matrix polarimetric imaging endoscopy and used it to distinguish different tissues based on their composition and structure by looking at polarization images at various wavelengths in rat samples. Our group [15] used a multi-spectral system operated from 488 nm to 632 nm to investigate the differences in polarization parameters between normal and cancer gastric samples using an objective lens with low magnification, in which the data from all the pixels in a sample were averaged to represent the entire sample thus the spatial resolution was poor. We observed significant differences in most parameters including retardance, depolarization, linear retardance, linear depolarization and circular depolarization between normal and cancer gastric samples and found that the combination of linear depolarization and linear retardance shows the best overall classification accuracy for gastric samples, and wavelength might not be a critical factor for the diagnosis in terms of $4\text{-}\mu\text{m}$ gastric samples at transmission mode.

While there are so many studies about the differentiation of normal and cancer/dysplasia in the literature, polarimetry imaging has not been investigated for differentiating more tissue categories, for example, cancer, dysplasia and normal glands, simultaneously on the microscopic level for the purpose of digital staining to our knowledge. The primary potential reason that polarimetry imaging has not been used to classify tissue regions could be that, it is time consuming and

labor intensive for a pathologist to demarcate tissue regions in various categories especially for dysplasia which is quite rare in stained tissue slides. Moreover, it is difficult to find the same tissue regions in the unstained slides as those in the stained slides. We overcome this problem by working with a dedicated pathologist closely and creating a clever way to coregister the regions in the stained slides with those in the corresponding unstained slides. We have not seen any previous report using the principal components derived from Mueller matrix elements for tissue differentiation as performed in this study. Such a study will help evaluate the potential of polarimetry imaging in digital staining, which will provide an alternative effective tool for digital staining if successful.

In this Chapter, we investigate the possibility of using polarimetry imaging to distinguish among gastric cancer, dysplasia and intestinal metaplasia (IM)/normal glands on the microscopic level, in which the data measured from every pixel in a sample were analyzed individually or in a region of interest (ROI) to investigate the potential of non-invasive diagnosis using polarimetry images with high spatial resolution. This work can be treated as the extension of the previous digital staining technology. Similar methods have been called digital pathology earlier [101, 102]. An effective two-step classification scheme is developed to improve the classification accuracy. Furthermore, the tissue classification on the microscopic level for a series of sizes of region of interest is explored for the purpose of digital staining. The resulting digital staining images in comparison to the color images of stained slides provide information to address the above

question about the potential of polarimetry imaging for digital staining. The possible ways to improve the accuracy are also proposed. It should be noted that the novelty of this study is not about the development of new techniques or instrumentation but about the investigation of the possibility to use polarimetry imaging for digital staining/pathology; therefore, all Muller matrix data are obtained using a classical optical setup that are slow but reliable.

4.2 Materials and Methods

4.2.1 Sample preparation

A total of 84 gastric samples from 59 patients were examined in this study, of which 20 were cancer samples, 15 were dysplasia samples, 22 were intestinal metaplasia samples and 27 were from normal glands such as foveolar gland, fundic gland and pyloric gland. All samples were fixed by 10%-formalin solution before embedded into paraffin for tissue sections. Then two 4- μ m vertical sections immediately next to each other were obtained in each sample, one used for Hematoxylin and Eosin (H&E) staining and the other unstained for polarimetry measurements. The stained slide was then used by a pathologist for generating the pathological report. A surgical pen was used on the blank side of the slide to mark regions of different cell types, including cancer, dysplasia and IM/normal glands to facilitate the search for these cell types in the corresponding unstained slides. All the identified tissue types were measured by a customized polarimetry imaging system under 20X objective lens as described below. After polarimetry imaging measurements, the unstained slide was stained for the pathologist to confirm the

tissue types in all marked areas.

4.2.2 Mueller matrix imaging system and measurements

Figure 3.1 shows the schematic of the polarimetry imaging system in this study to measure Mueller matrix elements, in which all the optics are mounted on a commercial microscope (Ti-U, Nikon, Tokyo, Japan). The light for illumination first passes through a band pass filter (Product no. 65166, Edmund Optics, Barrington, NJ, US) for wavelength selection, then gets polarized by a Polarization State Generator (PSG) consisting of a linear polarizer (Model no. 47213, Edmund Optics, Barrington, NJ, US) in a filter wheel (HF110, Prior Scientific instrument, Fulbourn, Cambridge, UK), followed by a nematic liquid crystal variable retarder (LCR-1-VIS, Thorlabs, Newton, New Jersey, US). After transmitting through the sample, the output light is analyzed by a Polarization State Analyzer (PSA) which consists of the same optical components as in the PSG but mounted in the reverse order. The resulting image is recorded on a monochromatic CCD (DS-Qi1Mc, Nikon, Tokyo, Japan) with a resolution of 640×512 pixels.

This configuration is similar to that in our previous study [15]. One difference is that polarimetry images were acquired at 632 nm only in this study by using an optical filter with a central wavelength at 632 nm and a 10-nm bandwidth (Product no. 65166, Edmund Optics, Barrington, NJ, US). The other difference is that the imaging measurement was performed under 20X objective lens. The reason that the images were taken only at 632 nm is that the measurement error of the system was small at this wavelength. Moreover, there was no significant difference with

varying wavelength in terms of classification accuracy for 4- μm thick samples as seen in the previous study.

Both PSG and PSA can generate all required polarization states including horizontal polarization (Stokes vector $H:[1 \ 1 \ 0 \ 0]^T$), vertical polarization ($V:[1 \ -1 \ 0 \ 0]^T$), $+45^\circ$ linear polarization ($P:[1 \ 0 \ 1 \ 0]^T$) and right circular polarization ($R:[1 \ 0 \ 0 \ 1]^T$). Sixteen images were recorded during the measurements with polarization states including HH, HV, HP, HR, VH, VV, VP, VR, PH, PV, PP, PR, RH, RV, RP and RR, in which the former letter represents the polarization state of the PSG and the latter one indicates the polarization state of the PSA. After background subtraction, these images were used to reconstruct the Mueller matrix elements at each pixel [43], which was followed by Mueller matrix decomposition for the extraction of polarization parameters [71].

The experimental setup (Fig. 3.1) was validated by measuring a quarter-wave plate, a half-wave plate and a linear polarizer with known polarization properties at 632 nm prior to measuring tissue samples. The results of the validation are shown in Fig. 4.2 in the Results section.

4.2.3 Data processing for sample classification and digital staining

According to the pathologist, it is nearly impossible to differentiate various tissue regions in unstained samples with white light bright field microscopy because the details of tissue structures are extremely obscure in this configuration. In this study we intend to demarcate various tissue regions by digitally analyzing

polarimetry imaging measurements including Mueller matrix elements, principal components cores derived out of these elements and polarization parameters, in each region of interest taken from unstained samples. This is essentially a digital staining technique.

The polarimetry data were processed as described below. The 16 Mueller matrix elements were calculated from the measured polarimetry data first. The seven polarization parameters including retardance (R), linear retardance (LR), circular retardance (CR), diattenuation (Dia), depolarization (Dep), linear depolarization (LD) and circular depolarization (CD) were then derived from the Mueller matrix elements at each pixel of the sample. Finally, Mueller matrix elements were aligned in one row as $[m_{00} \ m_{01} \ m_{02} \ m_{03} \ m_{10} \ m_{11} \ m_{12} \ m_{13} \ m_{20} \ m_{21} \ m_{22} \ m_{23} \ m_{30} \ m_{31} \ m_{32} \ m_{33}]$ to form vectors, from which principal component analysis (PCA) was performed and principal component (PC) scores were calculated. After all three sets of data, i.e., Mueller matrix elements, PC scores out of these elements and the polarization parameters were derived, Linear Discriminant Analysis (LDA) was performed for each set of data sequentially. Note that there were a total of 16 Mueller matrix elements, a total of 16 PC scores and 7 polarization parameters for each region of interest. The actual number of variables used in LDA depended on how many of these showed significant differences in the Wilcoxon Rank Sum test as shown in Fig. 4.1. The LDA yields the pathological state of each region of interest in a sample, which will be labeled by the assigned color in the final diagnostic image.

4.2.3.1 Sample classification of cancer and normal glands

To confirm the consistency in sample measurements and data analysis and the repeatability in classification accuracy, the data measured from all the pixels in each tissue sample pathologically classified as cancer and normal glands were first averaged to represent the entire sample and analyzed using three procedures as shown in Fig. 4.1. Polarization parameters, Mueller matrix elements and PC scores were explored for classification to identify optimal quantities yielding the highest accuracy. In Procedure 1, classification was performed using selected Mueller matrix elements directly. The Mueller matrix elements were first aligned in a row for each sample. Then the Wilcoxon rank sum test was performed to evaluate the differences in all the elements between the cancer and normal glands. Next, all the possible combination of those Mueller matrix elements with p values less than 0.05 were selected to do linear discriminant analysis (LDA) classification to find the optimal combination. In Procedure 2, classification was performed using selected polarization parameters derived from Mueller matrix elements. The polar decomposition method [62] was used in derivation and all parameters were individually evaluated in a Wilcoxon rank sum test for the difference between cancer and normal glands. The combinations of those parameters that yielded p-values smaller than 0.05 in the Wilcoxon Rank Sum test were retained for linear discriminant analysis (LDA). This step was the same as what we did in the previous study [15]. In Procedure 3, classification was performed using selected

principal component (PC) scores that were calculated from Mueller matrix elements. Principal Component Analysis (PCA) was first performed on a series of values that were formed by all Mueller matrix elements (16) aligned in a row for each sample. The Wilcoxon rank sum test was performed first to find the resulting PC scores showing significant differences between cancer and normal glands. Finally, All possible combinations of the first four PC scores showing p-values smaller than 0.05 were used to conduct LDA to identify the optimal combination. The reason that we only chose first 4 PC scores is that they account for 99.99% accumulated variance in the data thus sufficient for classification. In addition to Mueller matrix elements and the corresponding PC scores, polarization parameters extracted from Mueller matrix were also used in classification because these parameters can be correlated to the morphological features of samples [46]. The results of sample classification using the optimal polarization parameters, Mueller matrix elements PC scores are shown in Fig. 4.3. Note that Procedure 2 was performed in the same manner as in the previous study [15] thus the result can be compared with the previous results for cross validation.

Figure 4. 1 Three procedures of data analysis using Mueller matrix elements, polarization parameters and PC scores derived from Mueller matrix elements.

4.2.3.2 Sample classification of cancer and normal glands

For digital staining, we initially aimed to distinguish among the four tissue categories in terms of pathology, including cancer, dysplasia, IM and normal glands. But it was found that there was no quantity, in any Mueller matrix element, polarization parameter or principal component score calculated from Mueller matrix elements, that demonstrated a statistically significant difference (with a p-value smaller than 0.05) between IM and normal glands. This is possible because IM rarely leads to changes in nuclei size or density which significantly contributes to changes in polarization properties, which is confirmed by the pathologist. So we

decided to combine IM and normal glands into one tissue category, i.e., IM/normal glands. This regrouping is clinically meaningful because the association of IM with gastric carcinoma is supposed to be weaker than dysplasia. There are three types of IM, i.e., type I, II and III. While type I IM has almost no association with gastric cancer, type III IM has the strongest association with gastric cancer among all IM. The association of type II IM with gastric cancer is controversial. Routinely the subtyping of IM is not done, therefore it may be possible that the cases classified as IM in our cohort could be anyone of the three types, which on average has much weaker association with the progression to gastric cancer than dysplasia [103-107].

In this study, we first performed the traditional one-step classification between the three groups of tissue regions, i.e., cancer, dysplasia and IM/normal glands, using three sets of data, i.e., polarization parameters, PC scores and Mueller matrix elements. For every parameter in each set of these data, the Wilcoxon rank sum test was first performed to evaluate the differences in the parameter between every pair of the three groups of tissue regions. All possible combinations of those parameters that yielded p-values smaller than 0.05 were used to conduct LDA for classification of three groups in a single step.

4.2.3.3 Two-step classification and investigation of region of interest (ROI) size

To improve the accuracy of digital staining for classifying three categories of tissue samples, i.e., cancer, dysplasia and IM/normal samples, we developed a two-step classification scheme. In the first step, binary classification is conducted

to discriminate between the high-risk group (cancer and dysplasia) and the low-risk group (IM/normal glands) using all data. While in the second step, the second binary classification is conducted to discriminate between cancer and dysplasia within the high-risk group using the data from cancer and dysplasia samples only. The two-step classification scheme is applied in all three procedures as described in Section 4.2.3.1 to identify optimal quantities for classification. There are two reasons to perform two-step classification in addition to one-step classification. The first reason is that the division of high-risk and low-risk groups in patients is clinically meaningful. The second reason is that this scheme could potentially improve the overall accuracy because each of the two steps with a smaller number of groups can be optimized more easily.

The effect of the size of ROI on both classification accuracy and tissue structure visualization is also investigated. Note that each ROI represents one resolvable element in the digital staining image so a large ROI would yield an image with poor spatial resolution. In each ROI, the polarization measurements of all pixels will be averaged to represent the ROI. On one hand, averaging within each ROI could reduce the influence of measurement noise on deduced polarization parameters. On the other hand, a large ROI would yield low spatial resolution. A series of ROI sizes are explored to generate images with a range of spatial resolutions from the microscopic level to the macroscopic level, which included 640×512 pixels (135689.011 $\mu\text{m}^2/\text{pixel}$), 64×64 pixels (1696.113 $\mu\text{m}^2/\text{pixel}$), 16×16 pixels (106.007 $\mu\text{m}^2/\text{pixel}$), 8×8pixels (26.502 $\mu\text{m}^2/\text{pixel}$), 4×4

pixels ($6.625 \mu\text{m}^2/\text{pixel}$), 2×2 pixels ($1.656 \mu\text{m}^2/\text{pixel}$) and 1×1 pixel ($0.414 \mu\text{m}^2/\text{pixel}$). To clarify, an ROI size of 640×512 pixels means that the entire image is treated as one data point in classification since this is also the image size. In contrast, an ROI size of 1×1 pixel means that every single pixel in an image is treated as one data point in classification. The comparison between the one-step and two-step classification schemes for different ROI sizes is shown in Fig. 4.4.

4.2.3.4 *Digital staining*

To obtain both the digital staining image from an unstained slide and the corresponding stained slide image, the following steps were performed. First, the stained slide is observed under white light in the customized system to identify the desired region close to the edge of the tissue sample. The edge of the stained sample image is marked on the screen. Then the unstained slide will replace the stained slide and the edge of the unstained sample will be used to match that of the stained sample image by visual observation. Finally, polarimetry imaging is performed on the desired region once the edge is matched. Because it is difficult to precisely identify a tissue region in the middle of an unstained slide corresponding to the same region in the stained slide cut next to it due to the lack of salient features in the unstained slide, only the tissue regions close to the edge of the sample were selected, in which it is relatively easier to identify the shape of the corresponding region in the unstained slide. It should be highlighted that the pathological states of the unstained samples were stained after polarimetry measurements and checked again by the pathologist to confirm the initial

judgment.

It is found that an ROI size of 8×8 pixels appears to be a good tradeoff between the classification accuracy and the spatial resolution. The tissue category of every ROI in a digital staining image is determined using the two-step classification scheme based on Mueller matrix elements data, which is then displayed as a unique color assigned to the category. The white light image of the stained tissue slide cut immediately to the unstained slide from the same tissue sample is shown with the digital staining image side by side as in Fig. 4.5 to facilitate the comparison. It is noted that paraffin wax is assigned as an additional category because it is present in all slides.

4.3 Results

Commercial polarization optics were measured by the Mueller matrix imaging system described in the Methods section for system validation. Figure 4.2(a) shows the Mueller matrix elements of a horizontal linear polarizer, in which each element has been divided by m_{00} . Fig. 4.2(b) compares the reference retardance values and the corresponding experimentally measured values of a quarter-wave plate and a half-wave plate both at 632 nm. In Fig. 4.2(a), the expected values of the Mueller matrix elements are 0 except m_{00} , m_{01} , m_{10} and m_{11} whose values are 1. Therefore Fig. 4.2(a) indicates good agreement between experimental measurements and theoretical prediction for the linear polarizer, in which the largest difference is around 0.06. Similarly, it can be seen from Fig. 4.2(b) that the measured retardance value differs from the reference value given by

the manufacturer by less than 0.6% for both the quarter-wave plate and the half-wave plate. The excellent agreement between experimental results and the reference/theoretical values in Fig. 4.2 demonstrates that our imaging system can record Muller matrix images with high accuracy.

(a)

(b)

Figure 4. 2 (a) Measured values of Mueller matrix elements for a horizontal linear polarizer at 632 nm, in which the vertical axis represents the value of each Mueller matrix element divided by m00. (b) Retardance values of a quarter-wave plate (left) and a half-wave plate (right) at 632 nm. The reference values are provided by the manufacturer.

Figure 4.3 shows the overall classification accuracy for differentiating between cancer and normal glands using different parameters. The result from the previous study is included for comparison. It can be seen that the overall classification accuracy of this study is generally comparable to our previous study

[15] in spite of

Figure 4. 3 Overall classification accuracy for differentiating between cancer

samples and normal gland samples 1) in the previous study [13] using the combination of linear retardance (LR) and linear depolarization (LD), 2) in this study using LR and LD, 3) in this study using selected Mueller matrix elements and 4) in this study using selected principal components (PC) scores.

slight variation for different parameters. It is worth mentioning that the highest classification accuracy using polarization parameters in this study is achieved for the combination of linear retardance and linear depolarization, which is the same combination as in the previous study. The moderate difference in the classification accuracy can be due to the differences in the number of samples and the change in the objective lens from 2X to 20X. Selected PC scores (PC 1, PC 3 and PC 4) yield the highest accuracy for the differentiation of cancer from normal glands in this study, which suggests the great diagnostic power of PCA. Selected Mueller matrix elements (m_{01} , m_{02} , m_{03} and m_{10}) yield the accuracy slightly higher than that for the combination of linear retardance and linear depolarization.

Figure 4.4 shows the optimal overall classification accuracy for differentiation among the three pathological states including cancer, dysplasia and the joint set of IM/normal glands using both one-step and two-step methods when the size of the ROI is varied. It should be pointed out that the highest overall accuracy for both one-step classification and two-step classification were achieved using selected Mueller matrix elements. It can be seen from Fig. 4.4 that the classification accuracy goes down as the size of ROI decreases. Moreover, the

accuracy of the two-step classification is always higher than that of the one-step classification. Meanwhile, we also examined the variance of polarimetry parameters for different ROI sizes. In general, a larger ROI size yields a smaller variance. The variances for retardance, linear retardance, depolarization, linear depolarization and circular depolarization are much smaller than those for circular retardance and diattenuation. This is consistent with our result in the earlier study [15].

Figure 4. 4 The optimal overall classification accuracy for differentiation among the three pathological states including cancer, dysplasia, IM/normal glands using the one-step and two-step methods for various sizes of region of interest (ROI) including 640×512, 16×16, 4×4, 8×8 , and 1×1 pixels based on Mueller matrix elements.

The best overall accuracy for the differentiation among cancer, dysplasia and IM/normal glands as shown in Fig. 4.4 is significantly lower than that for the differentiation between cancer and normal glands as shown in Fig. 4.3. To investigate the source of such a difference in the classification accuracy, the confusion matrix in the case of an ROI of 540×612 pixels using the two-step method was shown below in Table 4.1. It can be seen that the tissue regions of IM/normal glands are classified most accurately while dysplasia regions are classified least accurately. A total of 15 dysplasia regions are almost equally classified as cancer, dysplasia or IM/normal glands. Out of totally 20 cancer regions, 5 are classified as dysplasia and 1 as IM/normal glands. This makes sense because cancer is closer to dysplasia in terms of the disease progression stage. This general trend in misclassification for other ROI sizes and for the one-step method, for which the data are not shown, is similar to Table 4.1.

Table 4. 1 Confusion matrix for the two-step classification with an ROI size of 640×512 pixels. The row header indicates the actual tissue type and the column header indicates the classification result based on Mueller matrix elements.

Classified Actual	Cancer	Dysplasia	Normal glands/IM	Total number	Accuracy (%)
Cancer	14	5	1	20	70.0
Dysplasia	6	4	5	15	26.7
Normal glands/IM	1	3	45	49	91.8

To facilitate the observation of digital staining results obtained using the polarimetry imaging technique, Figure 4.5 shows the original H&E images (top left) and the digital staining images (top right) for samples pathologically classified as (a) cancer and (b) normal foveolar glands. In the process of generating the digital staining images, the ROI size was 8×8 pixels and the two-step classification based on selected Mueller matrix elements was used. The reason why we chose ROI to be 8×8 pixels is because it is the tradeoff between classification accuracy and visualization of morphological features among those ROIs. The stained images and digital staining images were acquired from the two slides, one stained and the other unstained, cut from the same gastric tissue specimen in positions immediately next to each other. Values of each Mueller matrix element in one ROI were averaged to represent the ROI. In this manner every image was composed of several ROIs. Then each ROI was classified to cancer (red), dysplasia (yellow), IM/normal glands (light blue) and paraffin wax (dark blue). Figure 4.5 shows that the shapes in the stained image and the corresponding digital staining image match each other quite well. In Fig. 4.5(a), which was pathologically determined to be a cancer sample, the digital staining image shows the lower right corner to be mainly the cancer region, which changes to a mixture of dysplasia and IM/normal glands in the middle region. In Fig. 4.5(b), which was pathologically determined to be a normal gland sample, the majority of the ROIs were classified as IM/normal glands. However, a small portion of ROIs between glands were misclassified as cancer or dysplasia. In both Fig. 4.5(a) and Fig. 4.5(b), the ROIs classified as

dysplasia were stochastically distributed between the cancer and IM/normal glands regions, which may be related to the fact that dysplasia was not well differentiated from two other types according to Table 4.1. In both images, the areas marked with black solid lines by the pathologist are mainly either inflammatory cells or stroma, which were frequently misclassified as either dysplasia or cancer. Paraffin wax seemed to be well recognized by the digital staining procedure based on polarimetry imaging.

To facilitate the interpretation of digital staining images, the linear retardance images (bottom left) and linear depolarization images (bottom right) are also shown in Fig. 4.5 for both cancer and normal samples. Linear retardance and linear depolarization were chosen because they demonstrated the best classification accuracy among all the possible combinations of two polarization parameters, as shown in our previous study [15]. It can be seen from Fig. 4.5 that the general features of both linear retardance (bottom left) and linear depolarization (bottom right) images are quite similar to the original H&E images (top left) in both normal and cancer samples. The area of paraffin wax has a larger value than tissue areas in both linear retardance and linear depolarization. It is interesting to see that the boundary between the cancer region and the IM/normal region shown in the digital staining image of Fig. 4.5(a) mimics that between the high-value region and the low-value region in the linear retardance image, which suggests that the selected Mueller matrix elements used to obtain the digital staining image may be strongly related to linear retardance. This is not true for the depolarization image. In

contrast, both linear retardance and linear depolarization images show boundaries similar to the digital staining image in Fig. 4.5(b). The images of Mueller matrix elements are not shown due to the limited space.

Figure 4. 5 The original stained images (top left), the digital staining images (top

right), linear retardance images (bottom left) and linear depolarization images (bottom right) for (a) cancer (b) normal foveolar glands. The size of each ROI in the above digital staining images was 8×8 while the size of each ROI in the polarization parameter images was 1×1 .

4.4 Discussion

In this study, we first validated our system by comparing the Mueller matrix elements of horizontal linear polarizer and the retardance values of wave plates at 632 nm with their expected values. The comparison in the classification accuracy between cancer and normal samples with our previous study demonstrates the repeatability of our experiments. The biological basis of using polarimetry imaging to distinguish among normal glands/IM, dysplasia and cancer lies in the morphological changes [8, 15, 46], including nuclei size/density and collagen density, from normal samples to dysplasia and then to cancer, can lead to the variation in polarization properties, Mueller matrix elements and the corresponding PC scores. For example, retardance reflects the birefringence property of tissue structures contributed by mainly structural proteins such as collagen and it is known structural proteins often break down in carcinogenesis [15, 46, 78].

It was found that there was no quantity, in any Mueller matrix element, polarization parameter or principal component score calculated from Mueller matrix elements or the combination of them, that demonstrated a statistically significant difference (with a p-value smaller than 0.05) between IM and normal

glands. The potential reason for this finding may be due to the fact that IM is the change from a fully differentiated cell type to another cell type that implies adaptation to environmental stimuli, which is potentially reversible. Unlike dysplastic or cancerous changes, there might not be much alteration in the cellular structure of IM leading to the detectable changes in polarization properties such as nuclei size and density or nucleo-cytoplasmic ratio [15, 103, 104].

Figure 4.3 shows that selected PC scores yield the highest accuracy when only comparing cancer and IM/normal glands among all available data sets including polarization parameters, Mueller matrix elements and PC scores. This could be explained by the fact that totally 11 out of 16 Mueller matrix elements show significant differences between cancer and IM/normal glands at a significance level of 0.05. PC scores can take full advantage of all discriminators since each PC score is related to the combination of these Mueller matrix elements. In contrast, selected Mueller matrix elements yield higher accuracy than selected PC scores in two-step classification of cancer, dysplasia and IM/normal glands in Fig. 4.4. This difference could be explained as follows. Only four Mueller matrix elements show significant differences when comparing any two tissue types out of cancer, dysplasia, and IM/normal glands, which means there are only four effective discriminators. Therefore, PCA may dilute the differentiating power in these four elements because most Mueller matrix elements used when forming the principle components are not effective discriminators.

Different parameters including polarization parameters, PC scores and

Mueller matrix elements were employed for classification using both the one-step and two-step method. It can be observed from Fig. 4.4 that the two-step classification method we developed yields higher accuracy than the traditional one-step method. The reason is that the two-step method takes full advantage of the most effective discriminators in each step with fewer tissue types while a tradeoff has to be made in the choice of discriminators to maintain the best overall accuracy for all tissue types in the one-step method. Moreover, the classification accuracy drops with the decrease in the ROI size. This is because the averaging operation in each smaller ROI reduces noise less effectively than a larger ROI and the noise in the data can degrade classification. On one hand, the averaging of all parameters from different pathological tissue regions can reduce the contrast from a cancer sample to a normal sample, which may degrade the accuracy. On the other hand, averaging minimizes the variation in the same pathological tissue region, which may improve the accuracy. Because there is usually one dominant pathological type in a sample and many ROIs belong to the dominant type, the latter factor may play a larger role, which would result in better accuracy for a large ROI size. The latter observation suggests that it is difficult to achieve high accuracy and high spatial resolution at the same time. When the ROI size is smaller, the visualization of the detailed structures in the tissue sample is better; however, the accuracy of classification will be worse. The optimal choice of the ROI size is the tradeoff between the visualization outcome and the classification accuracy. In this study it is found that an ROI size of 8×8 pixels appears as a reasonable

choice under 20X magnification as shown in Fig. 4.5.

In Table 4.1, the classification accuracy for dysplasia is much lower than those for cancer and IM /normal glands. It can be seen that 6 out of 15 dysplasia samples are misclassified as cancer while 5 of them are misclassified as IM/normal glands. This is the observation of this study that the diagnostic accuracy for dysplasia is only 26.7%. One potential reason can be that our dysplasia sample contains a mixture of high grade and low grade dysplasia. In total, there were only one high grade dysplasia and one low grade dysplasia that are pathologically confirmed and the grade of dysplasia was not mentioned in the pathological reports for other dysplasia samples. Based on these two samples, the values of Mueller matrix elements for high grade dysplasia are quite close to those of cancer while the values of low grade dysplasia are close to those of IM & normal glands, even though the average value for all dysplasia samples falls in between those for cancer and IM /normal glands. Therefore, it may be worth exploring parameters optimized for the differentiation between cancer and high-grade dysplasia and that between low-grade dysplasia and IM /normal glands and developing a multi-step classification scheme to improve the classification accuracy for dysplasia samples in the future. Another potential way to improve the accuracy of dysplasia sample classification is to use thicker fresh samples not processed by paraffin wax. This is because a larger sample thickness increases the difference in polarization parameters such as depolarization and retardance. Moreover, a fresh sample may enable small polarization property differences between dysplasia samples and

other samples to stand out because of the absence of paraffin wax, whose retardance and depolarization values are much larger than the tissue sample according to our measurements thus may dominate over such small differences.

The digital staining images for an ROI size of 8×8 pixels as in Fig. 4.5 show boundaries between the tissue region and paraffin wax resembling the corresponding stained images. In the cancer sample as shown in Fig. 4.5(a), the major portion of the cancer region (unmarked regions with stained cells) in the sample is classified correctly as shown in red. In the normal gland slide as shown in Fig. 4.5 (b), the major portions of the foveolar glands are correctly classified as IM/normal glands. Overall, it can be seen that the major regions in the samples are correctly classified in spite of misclassification in a few small regions. It is interesting to see that the left marked area in Fig. 4.5(a) consisted of most inflammatory cells and a small portion of stroma are mainly classified as IM/normal or dysplasia while the right marked stroma area is classified as cancer. In Fig. 4.5(b), the small marked area containing mostly stroma and a small portion of inflammatory cells are classified as dysplasia. This could be partially explained by the fact that stroma is a highly birefringent structure with large retardance, which is similar to the change from normal tissue to cancer [15]. The increase in birefringence has been found common when a normal tissue turns to cancer [15, 46], which could be the consequence of high scattering due to enlarged nuclei and nuclei density [15, 46]. Therefore it makes sense that stroma could be misdiagnosed as cancer. This finding suggests that another technique may be

necessary to help differentiate cancer from stroma in the future development of polarimetry imaging for digital staining. For inflammatory cells, their morphological features can be similar to or quite different from normal cells due to many factors. For example, the nuclei size and density of inflammatory cells could be smaller, equal to or bigger than normal cells. So it is likely that some inflammatory cells are classified as normal cells while others as dysplasia. This observation could also contribute to the pixelated patterns of dysplasia and cancer ROIs in Fig. 4.5(a) and 4.5(b) because inflammatory cells and stroma are randomly distributed in the spaces between glands or normal cells in both cancer and normal gland samples thus causing misclassification. The pixelated pattern would become less obvious when each ROI size increases since the contribution from the surrounding tissue will dominate in the overall polarimetry properties so the pixelated pattern will average out.

Based on Fig. 4.5, it can be seen that digital staining based on Mueller matrix imaging is possible. The accuracy of classification in digital staining using polarimetry imaging on the microscopic level is varied from 75% to 56% for the discrimination of cancer, dysplasia and IM/normal tissue regions depending on the ROI size according to Fig. 4.4. Compared to other techniques that have been used for digital staining such as Raman spectroscopy [93], polarimetry imaging has great advantages in terms of cost effectiveness and signal strength. With advances in polarimetry imaging and the integration of the spectral reconstruction technique

[108], it is possible to realize snapshot polarimetry imaging, which could further speed up the technique for clinical uses.

4.5 Conclusion

In this Chapter, we explored the potential of polarimetry imaging for tissue diagnosis at the microscopic level, i.e., digital staining, by performing one-step and two-step classification using polarization parameters, PC scores and Mueller matrix elements, which, instead of the development of an clinical optical instrument, is the novelty and focus of this study. It was observed that the classification accuracy varies significantly with the choice of the datasets used. Therefore it is important to evaluate the use of all Mueller matrix elements, polarization parameters and PC scores as well as their respective combinations to find the optimal dataset for classification when the measurement condition or sample types change. Moreover, it was found that two-step classification yielded higher diagnostic accuracy than one-step classification and that the accuracy of classification using Mueller matrix elements was higher than that using PC scores and polarization parameters. It is worth highlighting that Mueller matrix images with a lower spatial resolution generated higher diagnostic accuracy while those with a higher spatial resolution provided more details on morphological features. Although the accuracy for dysplasia classification was lower than cancer and intestinal dysplasia/normal glands, it may be improved by applying multi-step classification and using thicker fresh tissue samples. The potential of polarimetry

imaging in digital staining shown in this study and its cost effectiveness compared to other techniques suggest that this direction is worth further investigation.

The results in this Chapter and Chapter 3 show the great potential of polarimetry in diagnosing gastric samples *ex vivo*. However, traditional polarimetry measurements is very time timing especially in an imaging setup. Because 16 images need to be recorded for each sample corresponding to different polarization states in the modules of light delivery and detection, it cannot be easily incorporated into a commercial endoscope to detect gastric cancer *in vivo*. In Chapter 5, we proposed a snapshot polarimetry imaging technique to perform polarimetry measurements in real time and the algorithm to process the data.

Chapter 5: Multi-spectral snapshot polarimetry imaging for gastric cancer detection

5.1 Introduction

Chapters 3 and 4 demonstrate that polarimetry image has great potential in diagnosing gastric cancer and classifying gastric samples according to pathological status at both the macroscopic and microscope levels. However, the system is limited by its measurement speed because it needs to record 16 images in each sample and several band pass filters need to be used to record Muller matrix images at different wavelengths. Although we have proved that wavelength choices have no significant effect on diagnostic accuracy for 4- μm thin samples in the transmission mode, we are not sure whether wavelength could play a more important role in diagnosis in thicker tissue samples.

In this chapter, we intend to overcome the limitations by proposing a snapshot polarimetry system which will be able to record data required to reconstruct Mueller matrix images at various wavelengths ranging from 400-700 nm in real time. The proposed system will be used in reflection mode for thicker samples. The successful development of the snapshot Mueller matrix polarimetry imaging system and its application will be an important progress towards the clinical use of polarimetry imaging for early cancer diagnosis. It will guide the design of an endoscopic polarimeter that can assist with detecting early gastric cancer quickly and accurately *in vivo*.

In previous study, we have proved that polarimetry imaging works well in the

transmission mode for thin samples. However, the reflection mode will be preferable in the clinical setting. The feasibility of performing polarimetry imaging in the reflection mode will be studied using MC modeling in Section 5.2.

Then in Section 5.3, we will describe the principle of the proposed snapshot system and the key algorithm of spectral reconstruction.

The initial results obtained from the benchtop snapshot system, which still works in the transmission mode for the convenience of validation, was compared with reference results in Section 5.4.

It should be noted that this chapter is focused on the initial investigation of the snapshot imaging technique and discussion on the future improvement due to time limit in project development.

Various snapshot spectropolarimeters have been reported [109, 110]. Savart polariscopes [111], disperser [112], polarization gratings [113, 114] and calcite plates [110] were used respectively by different groups to develop snapshot systems that can generate data to reconstruct the Stokes vector of an output light beam or the Mueller matrix of a given sample in real time. Specifically for the measurement of Stokes vector of light, Oka et al. [115] proposed a snapshot imaging polarimeter using a pair of Savart plates which required no active or mechanical components to control polarization and was able to determine two dimensional distributions of all parameters related to the state of polarization from a single image. Sabatke et al. [112] combined channeled spectropolarimetry with computed tomographic imaging spectrometry to develop a snapshot imaging

Stokes spectropolarimeter that can be implemented without moving parts. The proposed system employed a computer-generated holographic disperser to obtain the dispersed images of a target scene as well as both spatial and spectral information. Hagen et al. [116] proposed a visible-spectrum snapshot polarimeter on the basis of a computed tomographic imaging spectrometer. The system is able to perform Stokes vector polarimetry at a reduced spectral resolution at around 10 nm. Cao et al. [111] proposed a snapshot polarimeter using two modified Savart polariscopes which created a geometric optical path difference between the extraordinary ray and extraordinary rays of incident polarized light. This system was able to demonstrate two times higher frequency in Fourier domain than using traditional Savart polariscopes, which significantly improved the signal-to-noise ratio and the spatial resolution. Besides, the proposed system had the advantages in simplicity, compactness, and the function of snapshot compared to the conventional system. Aumiller et al. [117] developed two snapshot spectropolarimetry systems based on computed tomographic imaging spectrometry. One was operated in the visible spectrum and the other in the infrared spectrum. Both systems had no moving parts and did not require scanning thus could record data without artifacts that are normally associated with scanning spectropolarimetry.

As for the measurements of Mueller matrix which is the property of a sample, Kudenov et al. [113] presented an imaging spectropolarimeter using polarization gratings. The polarization information was encoded in the spectrum which could

be reconstructed in Fourier domain by obtaining the peaks at various locations. This system was able to acquire spectral and polarization information simultaneously at a high resolution and in a simple and compact way. Dubreuil et al. [110] presented a Mueller matrix polarimeter based on wavelength polarization coding and the feasibility of this technique was tested on the vacuum and a linear polarizer. Besides, Dubreuil et al. [118] demonstrated a two-channel snapshot polarimeter by separating two orthogonally polarized beams using a Wollaston prism. This system enhanced accuracy for real time measurements by reducing influences from random noise and systematic errors, which was an improvement compared with the previous system stated above [110]. Recently, Le Gratiot et al. [119] proposed a snapshot polarimeter in the reflection mode to perform fast polarimetry imaging by use of spectral coding of polarization. This system allowed Mueller matrix measurements at a repetition speed of 100 kHz, which could be implemented in a laser scanning microscope to conduct multimodal imaging. Silva et al. [120] used the snapshot polarimeter developed by Dubreuil et al. [110] to investigate the layer dynamics of a conventional surface-stabilized ferroelectric liquid crystal (SSFLC). The snapshot polarimeter was used to perform several measurements on SSFLC samples and a link was established between the liquid crystal director of SSFLC directors and the polarization properties of layered structures. Babilotte et al. [121] also used Dubreuil et al. 's system [110] to measure the birefringence properties of hepatic tissues. They identified the disease stage of liver samples by deriving at the retardance and depolarization properties

extracted from Mueller matrix. Nevertheless, results at multiples wavelengths were not demonstrated and it was not systematically studied how different polarization parameters vary as the tissue structure changes. Plenty of reports on snapshot polarimetry imaging have been presented, but the reconstructed Mueller matrices at multiple wavelengths were never demonstrated. Moreover, all the above systems can only measure one single location at a time. In summary, none of the above snapshot systems are able to generate imaging data to reconstruct Mueller matrix data at multiple wavelengths at many pixels simultaneously and it has not been studied how polarization properties vary with wavelength in thick gastric samples.

Thus, we propose to develop a snapshot Mueller matrix polarimetry imaging system to fill those above mentioned gaps. The system will be able to acquire images in one snapshot to reconstruct Mueller matrix at multiple pixels. The system will then be used to systematically study how polarization properties change from normal gastric tissue to cancer at wavelengths ranging from 400 nm to 700 nm.

5.2 Feasibility study of polarimetry imaging in the reflection mode by Monte Carlo modeling

The study of polarimetry imaging in reflection mode has been done by many. Chung et al. [71] diagnosed oral precancer using polarimetry imaging in reflection mode and demonstrated the potential of polarimetry imaging for early oral cancer diagnosis. Baldwin et al. [73] showed 90% sensitivity and specificity for cancer

detection by classifying among normal, benign moles and cancerous lesion in Sinclair Swine using polarimetry imaging in reflection mode. Liu et al. [4] developed a polarimetry system in the reflection mode and used it to identify the malignant lesion boundaries. Smith et al. [122] also investigated the polarization properties of skin tissues in reflection mode. Recently, Le Gratiot et al. [119] proposed a snapshot polarimeter in the reflection mode to perform fast polarimetry imaging by use of spectral coding of polarization. All these studies experimentally proved the feasibility of polarimetry imaging in reflection mode for various types of samples. In this section, we further validated the feasibility in theory for gastric samples using Monte Carlo simulation.

The introduction to Monte Carlo modeling of polarized light propagation and the results of Monte Carlo simulations are shown in this Section.

5.2.1 Principle of Monte Carlo modeling of polarized light transport

The Monte Carlo method is frequently used to model the light propagation inside tissues. This method can guide the design of experimental system for optical measurements on biological tissues. The parameters can be modeled such as the illumination and collection angles of light signal, light distribution in tissues and the way how light interacts with tissues such as scattering and absorption.

The trajectory of a photon can be calculated by simulating its ‘random walk’ [123] while step size is determined by a probability distribution sampling which is the function of scattering and absorption coefficients. The Mie scattering is approximated using the Henyey-Greenstein phase function to obtain the deflection

angle at each step. Refraction, transmission and reflection are allowed, which are determined by certain physical laws.

A number of input parameters including light wavelengths, sample thickness and optical properties can be specified. The output data could include those that can be measured like transmission and reflectance and those that cannot be measured like light distribution.

Normally, a large number of photons are launched to ensure the convergence of this 'random work' process. The simulation may take several hours to a couple of days depending on the number of photons launched, the dimension and optical properties of tissues.

The above principle of a standard Monte Carlo simulation is revised to incorporate the polarization state of a sample [124]. After launching the photons, the initial reference frame and Stokes vector are selected and defined such as defining X-Z plane as the horizontal linear polarization state. Then the photon moves governed by the scattering and absorption properties similar to unpolarized light with a difference that a scattering matrix including the combinations of elements of Stokes vector has to be used to determine the polarization properties of the scattered light. The polarization state after the scattering event is the result of the multiplication of the incident Stokes vector and the scattering matrix. The scattering angle and the azimuth angle in the scattering plane are chosen using rejecting method. This is achieved with a rotational matrix. The reference coordinate system is adjusted after scattering using the rotational matrix. Therefore,

the new Stokes vector after changing the reference frame is the multiplication of scattering matrix with the product of the rotational matrix and the Stokes vector, which in principle is the same for each scattering event. Finally, two more rotations are performed to return the Stokes vector to the meridian plane and to put the reference frame of the photon in the reference frame of the detector respectively when the photon reaches the boundary. This is all about the change of polarization state during propagation.

The incident light with four different polarization states were generated to illuminate the sample, which are H (horizontal linear polarization), V (vertical linear polarization), P (45° linear polarization) and R (right circular polarization), respectively, similar to that in Chapter 3. After simulations, the light intensities of the output images (HI, HQ, HU, HV, VI, VQ, VU, VV, PI, PQ, PU, PV, RI, RQ, RU and RV) were obtained. The former letters (H, V, P and R) correspond to the polarization state of incident light while the latter letters (I, Q, U and V) represent the elements of the output Stokes vector as shown in Eq. (2.1). It should be noted that the purpose of this simulation is to find out the signal strength in the reflection mode. It should be noted that these 16 quantities are sufficient to reconstruct the full Mueller matrix.

A public-domain code was used for the purpose of simulations [125] as described above. The following simulation parameters were set in the Monte Carlo simulations. Since the absorption coefficients for gastric samples the visible spectrum are similar [126], only one wavelength at 632 nm was used in the

simulation. The scattering coefficient and absorption coefficient were set as 45 cm^{-1} and 2 cm^{-1} respectively, which are representative of gastric cancer [126]. The mean radius of scatterers in cells including mitochondria, organelles and nuclei from gastric sample is estimated to be $0.6 \text{ }\mu\text{m}$ after approximating human cells as spheres [127], which was used as the scatterer size in the code. A sample thickness of 5 cm was set, for which most photons cannot transmit. The number of photons launched was 1 million.

5.2.2 Monte Carlo simulation results

In this simulation, light with four different polarization states was incident on the samples including H (horizontal linear polarization), V (vertical linear polarization), P (45° linear polarization) and R (right circular polarization). After simulation, the light intensities of the output images (HI, HQ, HU, HV, VI, VQ, VU, VV, PI, PQ, PU, PV, RI, RQ, RU and RV) were obtained. The area covered by these images was around 9 mm^2 , which was almost the same size as that of the field of view covered by the CCD camera under 2X objective lens in our real system. Next, the total number of photons of each image was calculated by adding the numbers at all pixels together, which was then divided by the total number of photons (1 million) incident on the samples. Eventually, we found that the ratio between the number of output photons to that of input photons was generally over 22% for all images.

Based on the observation above, we are expected to collect over $220 \text{ }\mu\text{W}$ light intensity given an incident light power of 1 mW . This means that a light power of

$6.7 \times 10^{-4} \mu\text{w}$ could be detected at each pixel in our CCD (DS-Qi1Mc, Nikon, Tokyo, Japan) with a resolution of 640×512 pixels which only requires a much lower power of $4 \times 10^{-12} \mu\text{w}$ to achieve a signal to noise ratio of 1. Therefore, the output signal strength is sufficient to be detected for a commercial CCD. This means that the output images required for the reconstruction of Mueller matrix could be recorded effectively in reflection mode. In summary, based on the Monte Carlo simulation results, we found that the polarimetry system we are developing can work well in the reflection mode in terms of signal strength.

5.3 Schematic of system setup and method of data processing

5.3.1 System setup

A snapshot Mueller matrix imaging system will be developed to acquire data in a snapshot for the reconstruction of Muller matrix images at multiple wavelengths. The system configuration is shown in Fig. 5.1. Broadband light from a white light source (400 – 700 nm) will pass through a horizontal polarizer first. After the two calcite plates serving as retarders with a thickness of e between which an angle of 45 degrees is formed. Encoded by the combination of the first polarizer and the two calcite plates, the light will contain spectrally varying polarization states. This light with various polarization components will then illuminate a sample perpendicularly. Backscattered light from the tissue sample will be detected obliquely to avoid most specularly reflected light, which will go through another two calcite plates with a thickness of $5e$ with their orientations

shown in Fig. 5.1. The two calcite plates together with the vertical polarizer will decode the output light that has interacted with the sample so that the Mueller matrix elements can be separated in the Fourier domain, which is necessary for subsequent data analysis. This particular combination of calcite plates will enable significant convenience in data processing as explained later in the section of data analysis [128]. Then the light will be picked up by a color CCD to acquire light intensity in the Red, Green and Blue channels. The RGB values at each pixel will be used to construct both spectral information using Wiener estimation and to reconstruct Mueller matrix elements using Fourier transform as described below.

Figure 5. 1 Schematic of snapshot Mueller matrix polarimetry system

5.3.2 Data processing

5.3.2.1 Wiener estimation for the reconstruction of spectral images

The goal of this step is to reconstruct a diffuse reflectance spectrum from RGB values at each pixel in terms of the minimum-mean square error (MMSE) criterion, which is achieved by using Wiener estimation as described in [129]. Its principle is briefly described as follows.

The response of the CCD camera g for the i -th color filter at position (x, y) is described as [129]

$$g_i(x, y) = \int t_i(\lambda) R(\lambda) P(\lambda) C(\lambda) f(x, y; \lambda) d\lambda, \quad i=1, 2, 3 \quad (5.1)$$

where $t_i(\lambda)$, $R(\lambda)$, $P(\lambda)$, $C(\lambda)$ and $f(x, y; \lambda)$ respectively denote the spectral transmittance of the i -th filter of the CCD, the spectral irradiance of the light source, the total transmittance of all the optics including two polarizers and two retarders, the spectral sensitivity of the CCD camera and the spectral reflectance of the samples examined. It should be noted that the background of the recorded images is assumed having been subtracted from raw images.

For convenience, each spectrum is sampled with l wavelengths and the variables involved in Eq. (5.1) can be expressed by a vector or matrix. So Eq. (5.1) can be written as [129]

$$\vec{g}(x, y) = T' R P C \bullet \vec{f}(x, y) \quad (5.2)$$

where $[\cdot]'$ depicts matrix transpose. The size and meaning of each elements in the above equation are as follows: g : 3×1 column vector presenting the

camera response; T : a $l \times 3$ matrix in which each row represents the transmittance of a certain wavelength for each filter; $\vec{f}(x, y)$: a $l \times 1$ vector representing the spectral response of the sample at pixel position (x, y) ; R , P and C are $l \times l$ matrices respectively representing the spectral radiance of incident light, spectral transmittance of all the optics together and the spectral sensitivity of the CCD camera.

Hereafter omitting the (x, y) , the equation can be rewritten as

$$\vec{g} = H\vec{f} \quad (5.3)$$

where $H \equiv T^tRPC$ with $3 \times l$ elements.

The inversion of Eq. (5.3) yields the estimation of the original spectrum, indicated by $\hat{f}$, using the observed data $\vec{g}$ [129]

$$\hat{f} = W\vec{g} \quad (5.4)$$

Here, the explicit form of Wiener estimation matrix w is given as [129]

$$W = R_{ff}^{-1} H^t (H R_{ff}^{-1} H^t)^{-1} \quad (5.5)$$

where R_{ff} is the correlation matrix given by [129]

$$R_{ff} = \langle ff^t \rangle \quad (5.6)$$

in which $\langle \bullet \rangle$ denotes the ensemble average.

Wiener estimation will yield a diffuse reflectance spectrum for each pixel. Because the polarization change is encoded in wavelength, the response in different polarization at a given wavelength is equivalent to the spectral response around that wavelength, which will be further processed to estimate Mueller matrix elements.

5.3.2.1 A Fourier transform based method to estimate Mueller matrix elements at a range of wavelengths

The goal of this step is to estimate Mueller matrix elements at a range of wavelengths from the spectral images reconstructed in the previous step. To achieve that, it is necessary to model the polarization change from the incident light to the detected light as shown below [110].

The retardance values of the four calcite plates are assumed to be $\phi, \phi, 5\phi$, 5ϕ , respectively at wavelength λ , according to the equation [110]

$$\phi = (2\pi e \Delta n) / \lambda \quad (5.7)$$

where Δn denotes the birefringence of the plate and e is the thickness of two thinner plates.

Let $P(\theta)$ be the Mueller matrix of the polarizer with an orientation angle θ and $R(\phi, \theta)$ be the Mueller matrix of the retarder with a retardance of ϕ and an orientation angle of θ . According to Stokes-Mueller formalism, we obtain [110]

$$\vec{S}_{out} = [P(90)][R(5\phi, 45)][R(5\phi, 0)][M][R(\phi, 0)][R(\phi, 45)][P(0)]\vec{S}_{in} \quad (5.8)$$

where $\vec{S}_{in}$ is the Stokes vector of the incident light and $\vec{S}_{out}$ is the Stokes vector of the detected light. Assuming that the incident light is totally unpolarized, the Stokes vector of the incident light and the Mueller matrices of all related elements are:

$$\vec{S}_{in} = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad P(0) = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$\begin{aligned}
R(\phi, 45) &= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(\phi) & 0 & -\sin(\phi) \\ 0 & 0 & 1 & 0 \\ 0 & \sin(\phi) & 0 & \cos(\phi) \end{bmatrix}, & R(\phi, 0) &= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & \cos(\phi) & \sin(\phi) \\ 0 & 0 & -\sin(\phi) & \cos(\phi) \end{bmatrix} \\
R(5\phi, 45) &= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(5\phi) & 0 & -\sin(5\phi) \\ 0 & 0 & 1 & 0 \\ 0 & \sin(5\phi) & 0 & \cos(5\phi) \end{bmatrix}, & R(5\phi, 0) &= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & \cos(5\phi) & \sin(5\phi) \\ 0 & 0 & -\sin(5\phi) & \cos(5\phi) \end{bmatrix} \\
P(90) &= \begin{bmatrix} 1 & -1 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \tag{5.9}
\end{aligned}$$

As an ordinary CCD is insensitive to the polarization state, the intensity at a single pixel in an image captured by such a CCD is only the first element of the Stokes Vector. When considering light in a narrow band with a bandwidth $\Delta\lambda \approx 15nm$ can be expressed by plugging Eq. (5.9) into Eq. (5.8) and keep the first element of $\vec{S}_{out}$, as shown in the equation below [110].

$$\begin{aligned}
I(\lambda) = \frac{1}{16} [& 8m_{00} + 4m_{02} - 4m_{20} - 2m_{22} + (8m_{01} - 4m_{21}) \cos(\phi) - (4m_{02} - 2m_{22}) \cos(2\phi) + \\
& 2m_{12} \cos(3\phi) - 4m_{11} \cos(4\phi) - (8m_{10} + 4m_{12}) \cos(5\phi) - 4m_{11} \cos(6\phi) + 2m_{12} \cos(7\phi) - \\
& (m_{22} - m_{23}) \cos(8\phi) + 2m_{21} \cos(9\phi) + (4m_{20} + 2m_{22}) \cos(10\phi) + 2m_{21} \cos(11\phi) - \\
& (m_{22} + m_{23}) \cos(12\phi) + (4m_{03} - 2m_{23}) \sin(2\phi) + 2m_{13} \sin(3\phi) - 2m_{13} \sin(7\phi) - \\
& (m_{23} + m_{32}) \sin(8\phi) + 2m_{31} \sin(9\phi) + (4m_{30} + 2m_{32}) \sin(10\phi) + 2m_{31} \sin(11\phi) + \\
& (m_{23} - m_{32}) \sin(12\phi)] \tag{5.10}
\end{aligned}$$

where m_{ij} ($i, j = 0, 1, 2, 3$) are 16 elements of the Mueller matrix for a given sample.

Then the retardance of the calcite plates is approximated to the first order using Taylor expansion at wavelength λ_0 , which can be any wavelength between 400 nm and 700 nm, i.e.,

$$\phi \approx \phi_0 + f_0 \lambda \quad (5.11)$$

in which f_0 is the fundamental frequency, i.e., the value of the first-order derivative of ϕ at wavelength λ_0 [110].

$$f_0 = -2\pi e \Delta n / (\lambda_0)^2 \quad (5.12)$$

where Δn is the difference in the refractive index between the extraordinary and ordinary axes of the calcite plate. As ϕ_0 and f_0 are constants, $I(\lambda)$ is the function of the combinations of various sine and cosine functions with different frequencies and each frequency the integer times of its fundamental frequency f_0 . The Fourier transform of the above equation can create 12 peaks whose real and imaginary magnitudes are the combinations of the Mueller matrix elements as shown in Table 5.1 [110].

Table 5. 1 Magnitudes of the real and imaginary parts of the peaks after Fourier transform

Real part		Imaginary part	
Frequency	Magnitude ($\times 32$)	Frequency	Magnitude ($\times 32$)
0	$16m_{00} + 8m_{02} - 8m_{20} - 4m_{22}$	0	0
f_0	$8m_{01} - 4m_{21}$	f_0	0
$2f_0$	$-4m_{02} + 2m_{22}$	$2f_0$	$4m_{03} - 2m_{23}$
$3f_0$	$2m_{12}$	$3f_0$	$2m_{13}$
$4f_0$	$-4m_{11}$	$4f_0$	0
$5f_0$	$-8m_{10} - 4m_{12}$	$5f_0$	0
$6f_0$	$-4m_{11}$	$6f_0$	0
$7f_0$	$2m_{12}$	$7f_0$	$-2m_{13}$
$8f_0$	$-m_{22} + m_{33}$	$8f_0$	$-m_{23} - m_{32}$
$9f_0$	$2m_{21}$	$9f_0$	$2m_{31}$
$10f_0$	$4m_{20} + 2m_{22}$	$10f_0$	$4m_{30} + 2m_{32}$
$11f_0$	$2m_{21}$	$11f_0$	$2m_{31}$
$12f_0$	$-m_{22} - m_{33}$	$12f_0$	$m_{23} - m_{32}$

Based on table 5.1, there will be in total 19 equations, among which 16 equations are independent. Thus 16 Mueller matrices can be derived from these equations as shown in Table 5.2 below.

Table 5. 2 Method of calculating Mueller matrix from the magnitudes in table 5.1.

Note: F_n corresponds to the magnitude of the n-th peak in the real part and IF_n corresponds to the magnitude of the n-th peak in the imaginary part as shown in Table 5.1.

$$(1) \quad m_{00} = (F_0 + 4m_{22} + 8m_{20} - 8m_{02}) / 16$$

$$(2) \quad m_{01} = (F_1 + 4m_{21}) / 8$$

$$(3) \quad m_{02} = (F_2 - 2m_{22}) / (-4)$$

$$(4) \quad m_{03} = (IF_2 + 2m_{23}) / 4$$

$$(5) \quad m_{10} = (F_5 + 4m_{12}) / (-8)$$

$$(6) \quad m_{11} = F_4 / (-4)$$

$$(7) \quad m_{12} = F_3 / 2$$

$$(8) \quad m_{13} = IF_7 / (-2)$$

$$(9) \quad m_{20} = (F_{10} - 2m_{22}) / 4$$

$$(10) \quad m_{21} = F_9 / 2$$

$$(11) \quad m_{22} = (F_8 + F_{12}) / (-2)$$

$$(12) \quad m_{23} = (IF_8 - IF_{12}) / (-2)$$

$$(13) \quad m_{30} = (IF_{10} - 2m_{32}) / 4$$

$$(14) \quad m_{31} = IF_9 / 2$$

$$(15) \quad m_{32} = (IF_8 + IF_{12}) / (-2)$$

$$(16) \quad m_{33} = (F_8 - F_{12}) / 2$$

For the white light covering 400 – 700 nm, the entire spectrum will be

divided into 20 narrow bands with 15-nm bandwidth. The Mueller matrix elements with the bandwidth are assumed to be approximately constants. Therefore, the above method can be used to estimate Mueller matrix elements in all 20 narrow bands at all pixels from the snapshot images in Red, Green and Blue channels.

5.4 Preliminary results

5.4.1 Preliminary system

The proposed multi-spectral snapshot system upgraded from the existing system developed by Dubreuil et.al [110] by replacing spectrometer with CCD. To validate the system with our optics such as calcite plates and spectrometer, we first tested it in transmission mode as shown in Fig. 5.2. The data processing method was the same as shown in Table 5.1 and Table 5.2. Noted that there was no need to reconstruct spectral images as the detector was a spectrometer in this step.

Figure 5. 2 Snapshot polarimetry system in transmission mode

5.4.2 Preliminary results

As the principle for different wavelengths is the same, only results at 632 nm are shown in this Section. The first step is to measure the light source spectrum using the system in Fig. 5.2 in which four calcite plates were removed, which was used to correct the wavelength dependence of the light source and polarizers. As the light intensity output from two orthogonal linear polarizers was close to zero, the second vertical polarizer was rotated to the horizontal orientation to measure the spectrum from the light source. This is reasonable because the influences of two linear polarizers on the transmittance of unpolarized light are nearly identical and their wavelength dependence is not affected by orientation as shown in Fig. 5.3. It can be seen that after passing through either single polarizer (orientated from 0 to 180 degrees) or two polarizers (angle rotated from 0 to 80 degrees between them), the normalized output spectra are nearly identical. In Fig. 5.3, all curves generally agree with each other for both single polarizer and two polarizers at different angles in spite of minor variation, which could be due to the slight change in the transmittance spectra of polarizers with angle.

Figure 5. 3 (a) Light spectra after passing through a single polarizer oriented at 0 degree, 80 degrees and 160 degrees and (b) Light spectra after passing through two polarizers forming an angle between them at 0 degree, 35 degree and 70 degree.

Figure 5.4 (a) and 5.4 (b) show the theoretical and the experimental spectra of the snapshot system for air at 632 nm after calibration. It can be seen from the figure that the theoretical curve obviously does not match well with experimental curve for air. However, the experimental results in Reference [110] matched quite well with the theoretical ones, in which the same setup was used except a spectrometer with higher spectral resolution and a different wavelength (829 nm). Compared with the spectrometer used in Reference. [110] that has a spectral resolution of 0.02 nm, the best spectral resolution of our spectrometer is only 0.056 nm, which might be the potential reason of the mismatch.

(a)

(b)

Figure 5. 4 (a) Theoretical curve and (b) experimental spectra of the snapshot system for air at 632 nm.

5.4.2.1 Convolution by the system response

Due to the obvious difference in spectral resolution, the convolution of system response with the theoretical curve was next performed to obtain convoluted curves which were compared with experimental curve for further calibration. The system response was measured using 632 nm laser as shown in

Fig. 5.5.

Figure 5. 5 System response for laser at 632 nm

Figure 5.6 (a), 5.6 (b) and 5.6 (c) show the comparison between theoretically convoluted curves and experimental curves for air , horizontal linear polarizer and 45^0 polarizer. It can be observed from Fig. 5.6 that there are good match for the general shapes of air, linear polarizer and 45^0 linear polarizer between theoretically convoluted curves and experimental curves. However, the periods between experimental and convoluted curves are different for them, which is what we need to address in the next step.

(c)

Figure 5. 6 Theoretically convoluted spectra and experimental spectra for (a) air, (b) horizontal linear polarizer and (c) 45° linear polarizer.

5.4.2.2 Period calibration

The reason behind the mismatch in the period is the mismatch between the refractive index Δn (the difference in the refractive index between the extraordinary and ordinary axes of the calcite plate as stated in Eq. (5.12) which is

obtained by interpolation and the data provided by manufacturer. The errors in this parameter can propagate to that in the estimated retardance of the calcite plate calculated using Eq. 5.12, thus leading to the error in the period of the theoretical spectrum estimated using Eq. 5.10 in comparison to the experimental value. It should be mentioned that the refractive index provided by manufacturer ranging from 200 to 1200 nm at an increment of as large as 200 nm, which leads to the error in the interpolated results used to calculate f_0 that has direct effect on the period.

To account for the mismatch, the refractive index was calibrated according to the period of the experimental spectrum. The periods at multiple wavelengths in experimentally measured spectra were first calculated according to the segment of measured spectra at different central wavelengths, based on which the refractive indices at various wavelengths were deduced using Eq. (5.10), Eq. (5.11) and Eq. (5.12). The calibrated refractive index was then used to calibrate the theoretical spectrum of different components. Figure 5.7 shows the comparison of theoretically convoluted spectra for which the period are calibrated and the experimental curves for (a) air, (b) horizontal linear polarizer and (c) 45° linear polarizer. The experimental and theoretical spectra were compared after shifting the experimental ones to match the peak location with the theoretical ones. It can be seen that the calibrated theoretical spectra match well with the experimental ones after the step of period calibration. This observation proves the agreement between the theory and the experiment to a large extent in spite of the poor spectral

resolution. Besides, it is worth mentioning that the match in the features especially in the magnitude for air is still poorer than that in polarizers even after period calibration. This could be attributed to the denser peaks in the spectrum of air that cannot be captured adequately due to the poorer spectral resolution of the spectrometer as well as the higher number of peaks with small magnitude that might be difficult to capture and easy to be affected by nearby high peaks. For example, the number of both high peaks and small peaks in the air's spectrum is almost twice as many as that in the horizontal polarizer and there are almost no small peaks in the 45^0 linear polarizer even though the number of peaks of it is comparable to that of air. Based on the observation, we believe that for a sample with a dense spectrum, a spectrometer with higher spectral resolution will be required to capture detailed features. Due to the low spectral resolution of the spectrometer, the peaks magnitude as stated in Table 5.2 cannot be accurately reconstructed by Fourier transform. Thus it is necessary and feasible to perform further measurements using a spectrometer with high spectral resolution to proceed to this study. It should be also noted that we have modeled the effects of alignment of calcite plates on the spectrum, it was found that minor misalignment only affects the magnitudes of the spectrum but not on the period. Therefore, the slight mismatch in the magnitude for both air and polarizers should be due to imperfect alignment besides the low spectral resolution as discussed above.

(a)

(b)

(c)

Figure 5. 7 Theoretically convoluted spectra after period calibration and experimental spectra for (a) air, (b) horizontal linear polarizer and (c) 45^0 linear polarizer.

5.5 Conclusion

In this chapter, we proposed a multi-spectral snapshot polarimetry imaging system and an algorithm to reconstruct spectral images from RGB images. The system is capable of recording RGB images in real time. The spectral images reconstructed will then be used to reconstruct full Mueller matrices at multiple wavelengths for all the pixels. Due to the limitation in the spectral resolution of our system, we are currently not able to reconstruct Mueller matrix with high accuracy, but we anticipate that this approach is feasible if a spectrometer with higher spectral resolution is available based on the good agreement in experimental and theoretical spectra for polarizers.

Chapter 6: Conclusions and Future Directions

6.1 Conclusions

This dissertation presents a series of studies on the progress of early gastric cancer detection using multi-spectral polarimetry imaging method. We first introduce the background of gastric cancer and polarimetry imaging as well as the related concepts such as Mueller matrix and Stokes Vector. In Chapter 3, we investigate the effectiveness of all the polarization parameters and their combinations in the differentiation between cancer and normal gastric samples at macroscopic level at different wavelengths. It was found that the combination of linear retardance and linear depolarization demonstrate optimal diagnostic accuracy and wavelengths might not be a critical factor in classification for thin gastric sample. Chapter 4 describes the potential of Mueller matrix imaging for digital staining by processing data from macroscopic to microscopic level at various regions of interest. Besides, three ways of classification using both one-step and two-step classification are well explained based on polarization parameters, Mueller matrix elements and PC scores respectively. It was observed that two-step classification yielded higher classification accuracy than the traditional one-step classification and that pixel classification based on Mueller matrix elements yielded higher accuracy than that based on polarization parameters and derived principal components. Moreover, Mueller matrix images with a lower spatial resolution generated higher classification accuracy but those with a higher spatial resolution revealed more morphological details. Inspired by

the effectiveness of polarimetry imaging on classification among gastric tissues in various pathological status, in Chapter 5, we further extend our polarimetry imaging to snapshot polarimetry method which dramatically increases the detection speed, guiding the development of endoscope. In this Chapter, the multi-spectral snapshot system is proposed together with the algorithm for the reconstruction of Muller matrices at multiple wavelengths for each pixel. Although no good results are showcased in this chapter, the feasibility and promising future of this technique is well presented based on the existing data.

6.2 Future Direction

As explained in Chapter 5, snapshot imaging is a promising method for the future development of endoscope. Limited by the spectral resolution of our spectrometer, we could not fully explore the potential of the proposed snapshot system in tissue measurements. In the future, we hope to use a spectrometer with higher spectral resolution to further develop this technique for multi-spectral snapshot polarimetry imaging. First, we will reconstruct the Mueller matrices of standard samples such as air, polarizer and retarder at multiple wavelengths based on the RGB images measured by the transmission system as shown in Fig. 5.2. In this step, the entire spectrum ranging from 400-700 nm will be recorded for different samples. Then, the narrow band spectrum centered at various wavelengths will be cut from the original spectrum, which will be used to reconstruct Mueller matrix at each wavelength using the algorithm stated in

Chapter 5. These reconstructed Mueller matrices will then be compared with the theoretical Mueller matrix of each standard samples.

In the second step, the system will be used to measure gastric samples using the similar method as in the first step. Simultaneously, those samples will also be measured using our previous system as shown in Chapter 3 in the transmission mode. The Mueller matrices reconstructed from measurements by the snapshot system in the transmission mode will be compared with those obtained from our previous system in order to validate the effectiveness of this system in measuring real samples.

After validating the system using samples in the transmission mode, the system will finally be applied to thick and fresh samples in the reflection mode. As stated in our previous chapters, gastric samples protected by paraffin wax can have different polarization properties compared to fresh samples. Therefore, a study involving thick fresh samples measured in the reflection mode may offer new insight into the clinical value of polarimetry imaging.

The data processing method will be similar to that in Chapter 4, which will consist of three steps. Polarization parameters extracted from Mueller matrices, Mueller matrix elements and their principal components scores will be used as discriminants to perform LDA classification. The optimal parameters or their combinations will be identified in this process. Meanwhile, both one-step and two-step classification will be conducted for comparison. If the accuracy of this technique will be superior to that of the standard procedure in the conventional

endoscopy [5], it will justify the further development of this technique so that it can be incorporated into a commercial endoscope for the future clinical study.

In summary, the proposed snapshot polarimetry imaging system is of great potential in achieving real-time imaging and being adopted clinically for gastric cancer diagnosis. However, more clinical studies in both the transmission and reflection modes need to be performed to validate its effectiveness as well as improving diagnostic accuracy before it could be utilized clinically.

Authors publications

Academic Journals

1. **W. Wang**, L. G. Lim, S. Srivastava, J. S. B. Yan, A. Shabbir, and Q. Liu, 'Roles of linear and circular polarization properties and effect of wavelength choice on differentiation between *ex vivo* normal and cancerous gastric samples', J Biomed Opt. **19**(04), 046020-046020 (2014)
2. **W. Wang**, L. G. Lim, S. Srivastava, J. S. B. Yan, A. Shabbir, and Q. Liu, 'Investigation of Mueller matrix imaging for digital staining', J Biophotonics, **9999**, (2015)

Conferences and Presentations

1. **W. Wang** et al., 'Roles of linear and circular polarization properties and effect of wavelength choice on differentiation between *ex vivo* normal and cancerous gastric samples' in *Digestive Disease Week in Gastroenterology*, vol 146, Chicago, S-523 (2014)
2. **W. Wang** et al., 'Investigation of Mueller matrix imaging for digital staining' in *SPIE photonics West in Optical Diagnostics and Sensing XV: Toward Point-of-Care Diagnostics*, San Francisco, 9332-28 (2015)

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