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Label-free discrimination of lung cancer cells through mueller matrix decomposition of diffuse reflectance imaging

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ABSTRACT

In this article, we explore the potential of an original label-free Near-Infrared (NIR) imaging technique, based on Mueller Matrix decomposition reflectance, for efficient detection and classification of histopathological samples of lung cancer cells.

Experimental results were acquired, processed, and analyzed by means of an accurate, fully-automated, auto-calibrated liquid-crystal NIR polarimetric imaging system, developed for real-time Mueller matrix analysis and optical characterization of target media. The polarimetric Figure-of-Merits (FOMs), estimated using Mueller matrix decomposition, as well as the statistics associated with the sixteen Mueller matrix elements of each lung cell sample indicate that enhanced discrimination among the samples can be achieved. Similarly, polarimetric Exploratory Data Analysis (pEDA), based on histograms obtained from diffuse reflectance polarimetric signals, has been used to determine if aberrations and/or changes in the spread of the histogram between different stages of lung cancer can be proved effective biomarkers for its progression and also discrimination among different lung pathologies.

The outcome of this study indicates that Mueller matrix formalism may be proved extremely useful in discriminating among healthy and malignant lung cells as well as differentiating among the different types of malignancies with high accuracy. As a result, it may contribute positively to the enhancement and implementation of the operational principles of the Whole Slide Imaging (WSI) field.

1. Introduction

In the year 2016, there were approximately 224,390 new diagnoses of lung cancer and 158,080 deaths attributed to the disease per the National Cancer Institute. There are two main types of lung cancer, small cell and non-small cell, of which the most common is non-small cell which makes up nearly 87% of diagnoses [1].

Early diagnosis of precancerous and malignant lesions is of the utmost importance for improving the current poor survival rate of patients with a variety of tumors. Squamous cell carcinoma of the central airway is thought of as a multistep process starting from a squamous metaplasia, which progresses to dysplasia, followed by Carcinoma In Situ (CIS), finally progressing to invasive cancer [2]. On the other hand, most peripheral tumors are adenocarcinomas or large cell carcinomas; because of their peripheral location, adenocarcinomas may not be caught early until they have developed extrathoracic metastases. For example, patients may show clinical signs of bone spread or intracranial metastatic disease. The first-line detection technique for lung cancer, like chest radiography (film or digital) fails to provide meaningful information that can be utilized towards the early detection of tumors. In recent publications, multi-year trials have revealed that low-dose spiral Computed Tomography (CT) can be a promising modality for lung cancer screening especially for heavy smokers [1,2]. However, even CT as well as other classical imaging techniques, even when computer-assisted, typically produce images attributed to the anatomy and structure of the tumor and surrounding tissue, instead of the physiology and pathology of the tumor itself. As a result, classical imaging techniques are less than ideal tools for cancer diagnosis and assessment.

Microscopic digital images can be static, dynamic (real-time robotic microscopy), or viewed after scanning of the glass slides

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(Whole Slide Digital Imaging (WSI) or virtual microscopy). WSI, which relates to the remote scanning of conventional glass slides to produce digital images, is the latest imaging modality appealing to the pathology departments worldwide. Integrative studies are a key catalyst factor in propelling the next phase of adoption. Barriers to adoption of WSI include technological limits, image quality, acquisition time, cost, digital slide storage, needs for high-throughput, regulatory barriers, ergonomics, and pathologists' reluctance for clinical validation and standardization [3]. On the other hand, the overall belief is that implementation of WSI in the healthcare industry would lead to increased efficiency, cost savings, and improved patient outcomes [4].

Optical imaging involves probing tissue with non-ionizing radiation in the visible and near-infrared region (400nm–1500 nm). Examining the optical properties of tissue reveals information that can potentially characterize diseases. It has the ability to provide both metabolic and anatomical information, and therefore the potential to enhance the detection process of early cancer [5–17]. An area of increasing interest in biomedical diagnostics is the high sensitivity of scattered polarized light to subtle alterations in tissue morphology, accompanied by high specificity and the rejection of background noise. Photon scattering from tissue is due to the presence of biological cells and connective medium and is dependent on the cell morphology and its surrounding. Most biological tissues are highly scattering at visible and near-infrared (NIR wavelengths), this causes quantitative interpretation of tissue spectroscopy and imaging to be difficult. Typically, diffused scattered light is often partially polarized to an extent that can be experimentally detected.

A method for selective detection of size-dependent scattering characteristics of epithelial cells in vivo based on polarized illumination and polarization sensitive detection of scattered light was presented in [13]. Findings of that study revealed that reflectance spectroscopy with polarized light can provide quantitative morphological information which could potentially be used for non-invasive detection of neoplastic changes.

In another study changes in light scatter were measured by combining both wavelength-dependent and polarization-dependent methods. This investigation revealed that the difference in scattering is attributed to the average dimension of the "scatterers", being a few tens of nanometers smaller in the healthy cells compared with the cancerous cells. This work also highlighted the significance of developing noninvasive, optical tissue diagnostic methods based on the sensitivity of wavelength-dependent and polarization-dependent light scattering measurements to cell morphology [14].

Recently, Giakos and coworkers pioneered the uses of NIR polarimetric detection and the principles of pEDA [25] towards the detection and characterization of unstained lung cancer tisues and cells through Label-free Near Infrared (NIR) Diffuse Polarimetric Reflectance Signatures [6], [19,20]; in addition, several designs of efficient lung cancer polarimetric imaging techniques and instrumentation were developed and presented [6], [18–20], [21–24], [28]. The paradigm for the NIR reflectance approach is that different types and stages of cancer cells are accompanied by changes in the overall biochemical composition of the tissue, along with well-known changes in cellular morphology and tissue architecture, which exhibit distinct optical signatures. These distinctions are observed via changes in the NIR polarimetric reflectance signatures. Polarimetric imaging can lead to the development of real time non-invasive diagnostic and therapy monitoring methods, improving both the detection of diseases, and treatment responses in early stages. It relies on the study of the polarization of the backscattered light, while offering distinct signatures related to structure, orientation, and target composition. In general, the polarization of the scattered light depends upon a number of geometrical and physical parameters, such as incident polarization state, shape, size, and concentration of the scatterer, metabolic and biochemical phenomena, related to the optical activity of the medium, as well as from the refractive indices of the scatterer and the surrounding medium. [6], [19,20], [24], [28], [29–33]. Optical polarimetry can be used as a key estimator for the identification, classification and discrimination of various media based on their optical properties. Polarimetry exhibits unique potential in providing high-contrast, high specificity images under high-dynamic range conditions in scattering media, while offering information related to the structure, composition, metabolic and chemical information [29–33]. On the other hand, pEDA was introduced in an effort to extract additional signatures from targets by integrating polarimetric principles with EDA [26]; for instance, it offers the opportunity to relate a physical process to discriminating polarimetric signatures by grouping and separating different parts of a histogram and thereafter to fit different statistical curves; as well as to interpret distributions of a polarimetric data set beyond formal modeling. Marotta expanded pEDA into the analysis, characterization, and discrimination of lung cancer tissues, by combining statistical analysis of histograms extracted from polarized signals [23]. From the clinical perspective, dedicated monoline lung cancer cell series were designed and cultured aimed at expanding the polarimetric study at cellular level [20]. Livanos, applied principles of pEDA analysis through use of the Welch's *t*-test and p-value statistics as a representative metric for discriminating distributions based on their mean and standard deviation [27].

Baba developed an automated polarimetric platform, using liquid crystal optical components [34,35]. The calibration of that system was tested against samples with known Mueller matrices such as air and linear polarizers. Later, the system was modified by Angela [36] to perform the Mueller matrix imaging for skin cancer detection using a 14 bit CCD camera as a detector. When normalized, the intensities of each Mueller matrix element indicated significant differences between cancerous and non-cancerous tissues. Another polarimetric platform was developed by Bueno which also utilized liquid crystal optical components for system calibration. Bueno's work required manual insertion of the optical components [37].

Petermann [21] and Shrestha [24] designed an automated polarimetric system aimed at the enhanced diagnosis of precancerous and cancerous lesions, as well as for the remote characterization of space resident objects. A complete description on the design, calibration and testing of the instrument is reported in [28]

The objective of the study is to present the experimental results of a novel detection characterization technique, based on label-free NIR polarimetric reflectance imaging for the classification of lung cancer histopathological samples from a tissue slide. NIR optical imaging is a newer technique that shows much promise for the earlier detection of many cancers and their subsequent characterization. The novelty of the study consists on the observation and analysis of inherent (label-free) NIR polarimetric reflectance signatures, using Mueller matrix formalism, as opposed to any external stains or labels used to treat the sample, of cellular components to aid classical cytopathology and histopathology. The outcome of this study would lead to the enhancement and implementation of the operational principles of Whole Sliding Imaging (WSI).

2. Theory

2.1. Mueller polarimetry

Mueller polarimetry is a matrix method for manipulating Stokes vectors and deals with the polarization of light. It is used for the analysis and study of a great variety of material samples in an

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