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Diagnosis of colon cancer using frequency domain fluorescence imaging technique

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Abstract

Early detection and treatment of colon cancer has been associated with better disease prognosis. Conventional and reported optical techniques have limitations in detecting early stages of colon cancer growth. In this paper, a homodyne signal processing assisted frequency domain (FD) fluorescence imaging methodology is proposed for the early diagnosis of colon cancer. Simulated phantom tissues representing the biopsy samples at different stages of colon cancer growth are prepared and used for the imaging study. Selective imaging of healthy and diseased sites simulated in the samples was achieved even for fluorescence emissions having close lifetimes and wavelength values. Possible extension of the methodology for in vivo investigations is also discussed.

Keywords: Frequency domain imaging; Fluorescence lifetime; Colon cancer; Phantom colon tissue

1. Introduction

Colon cancer is a major form of cancer afflicting people across the globe. The American Cancer Society estimated that about 145,000 cases of colorectal cancer would be diagnosed and about 56,000 people would die from the disease in 2005 alone [1]. For better disease prognosis, it is always favorable to detect colorectal cancer at its early stage [2]. However, the detection of colon cancer often requires examining the suspected site in vivo. Although cancer can often be accurately diagnosed using techniques like biopsy, it is a surgical and hence an invasive technique. Constant monitoring and diagnosis based on invasive techniques requires the patient to undergo surgical incisions every time he/she undergoes a routine check-up. Furthermore, biopsies are generally obtained blindly at set locations or guided by visual impression of mucosal abnormalities even when endoscopic procedures are employed [3]. Fecal Occult Blood Test is another means of colon cancer diagnosis. It detects the presence of blood in stool samples to diagnose cancer [4]. However, blood in the stool maybe present due to other gastric disorders or wounds. Thus, this technique is not confirmatory. In this context, non-invasive techniques for colon cancer detection show their supremacy. They are expected to facilitate and encourage routine check-ups among patients and allow doctors to perform more guided confirmatory biopsies.

Generally, invasive imaging techniques have been employed for colon cancer detection, which include virtual colonoscopy [5], barium X-ray [6], Computed Tomography (CT) [7], Positron Emission Tomography [8] and Magnetic Resonance Imaging [9]. However, all these imaging techniques suffer from the problem of missing polyps or low percentage of abnormality detection.

Among the non-invasive optical techniques currently being investigated for colon cancer detection, fluorescence spectroscopy has shown great promise. It is well known that the colon tissue contains naturally occurring macromolecules that exhibit fluorescence when exposed to light. These fluorophore sources include connective tissues (collagen, elastin), cellular metabolism related coenzymes

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(reduced Aicotinamide Adenine Dinucleotide (NADH), Favin Adenine Dinucleotide (FAD), and Flavin Mononucleotide (FMN)), aromatic amino acids (tryptophan, tyrosine, phenylalanine), by-products of heme biosynthesis (porphyrins) and lipopigments (lipofuscin, ceroids) [10]. Fluorescence spectroscopy has been widely applied for detection of morphological changes taking place in tissues. Hanlon et al. [11] reported the successful application of fluorescence spectroscopy in the in vitro detection of Alzheimer's disease. Grosenick et al. [12] successfully used time-domain fluorescence spectroscopy for mammography and imaging and got promising results.

Several researchers have reported work in the fluorescence based colon cancer detection using excitation lights of different wavelengths [13–16]. Detection of difference in the fluorescence intensity from a normal tissue and an adenomatous polyp has been proposed for cancer detection [17–20]. However, in such cases, the accuracy can be affected due to the positioning and other errors attributed to the intensity fluctuations [21]. Also, the fluorescence emission wavelength difference based approach reported in literatures was about 10 nm, which is at relatively advanced stage of growth [22–24]. In this context, a more accurate optical technique that is capable of imaging and differentiating a cancerous growth site at its very early stage is thus needed. Here comes the significance of imaging technique, which is based on fluorescence lifetime differences instead of the intensity variations, to overcome the problem.

In the early stages of colon cancer, only minor biochemical changes take place in the tissues. Due to these changes, the difference in the cellular fluorescence lifetime will be small between normal and early cancerous cells. It was reported that the typical lifetime difference between benign and cancerous tissues at initial stage of growth is only around 0.4–0.6 ns [25]. It shows that the differentiation between normal and early stages of cancer growth can be carried out by the subnanosecond fluorescence lifetime difference.

Lifetime based imaging has already been reported in the context of selective imaging of fingerprints deposited on fluorescing backgrounds [26,27]. In such methods, suppression of the background fluorescence was achieved based on the small lifetime difference between the fingerprint fluorescence and the background fluorescence. Hence, such lifetime based imaging technique can be applied for the early diagnosis of colon cancer.

In this paper, homodyne signal processing assisted FD technique has been applied in the context of imaging and differentiation of healthy and diseased colon tissues. Subnanosecond fluorescence lifetime difference between the tissues at early stages of growth is used for the characterization of the abnormalities. Phantom colon tissue samples were stained with common exogenous fluorophores to simulate various scenarios of cancer growths such as early, intermediate and advanced stages based on their lifetime values.

This technique can be employed to suppress the fluorescence emission from one of the tissue sites enabling the other to be imaged. This selective imaging of tissues with subnanosecond resolution in fluorescence lifetime will form a platform for differentiating normal and unhealthy tissues at an early stage of cancer growth.

2. Theory

Generally, sample is excited with an intensity-modulated light in FD imaging technique. Due to the time lag between absorption and emission, the emission is delayed in time relative to the modulated excitation. At each modulation frequency, this delay is described as the phase shift (ϕ). The phase difference 'ϕ' and the modulation amplitude factor 'm' are related to the modulation frequency 'ω' and lifetime 'τ' of fluorescence in the following way [28]:

$$\tan\phi = \omega\tau \tag{1}$$

and

$$m = (1+\omega^2\tau^2)^{-1/2} \tag{2}$$

In FD imaging technique, the fluorescent samples excited at same modulation frequency will have different phase shifts and demodulation factors depending upon their lifetime values. Based on the relations in Eqs. (1) and (2), fluorophores having lifetimes in nanosecond range have to be excited with high modulation frequency, typically in the order of tens of megahertz to obtain relatively large phase shift values. At such high modulation frequency, processing of weak and high-frequency signal is difficult. In this context, homodyne signal processing technique is employed for the easier processing of the high-frequency signals as well as to increase the signal to noise ratio.

Let the modulated excitation signal be $I(t)$ such that,

$$I(t) = A[1 + m_{ex}\sin(\omega t)] \tag{3}$$

'$I(t)$' is the total excitation intensity at time 't 'and 'ω' is the angular frequency, which is equal to $2\pi f$, where 'f' is the linear frequency. 'A' is the DC component of the excitation signal and 'm_{ex}' is the modulation depth.

Upon excited by such an intensity-modulated signal, the resulting modulated fluorescence emissions from the sample is given by,

$$F(r,t) = \begin{cases} A'(r_c)[1 + m_{ex}m_c\sin(\omega t - \phi_c)], & \text{when } r = r_c \\ A'(r_n)[1 + m_{ex}m_n\sin(\omega t - \phi_n)], & \text{when } r = r_n \end{cases} \tag{4}$$

Here, the subscripts 'c' represents 'cancerous' lesions and 'n' denotes 'normal' tissue, while 'r_c' and 'r_n' denotes the different locations in the tissue corresponding to cancerous and normal sites. Also, 'm_c' and 'm_n' represent the modulation amplitude factors of the fluorescence emission from the cancerous and normal tissues respectively.