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Detection of Colorectal Diseased Tissues Using Laser Induced Autofluorescence Spectroscopy

Chia Teck Chee

Colorectal cancer is currently the second leading cause of cancer mortality in Singapore after lung cancer for males and *after* breast cancer for females. The possibility of survival from colorectal cancer is closely related to the clinical and pathological stages of the disease during diagnosis. However, most deaths can be prevented by early detection. Thus, further research and development on novel strategies for early, noninvasive or micro-invasive detection can greatly

alleviate the incidence of mortality from colorectal cancer in the future. Research studies have demonstrated that fluorescence spectroscopy can prwide highly sensitive and cast-effective diagnosis of early malignant diseases.

Study of the Intensity Ratio from the Characteristics of Auto-fluorescence Spectra of the Human **Colorectal Tissue**
Autofluorescence is widely investigated as a sensitive method in early diagnosis of diseased tissue, but most measurements of tissue autofluorescence were performed with only a single excitation wavelength. Our work aimed to optimize the excitation wavelength or emission wavelength bands for fluorescence spectroscopy for better clinical diagnostic accuracy. The autofluorescence spectra of colorectal tissue were studied over a wide excitation range (350nm-600nm). The excitation 350 nm and 470 nm were identified as the effective excitation wavelength for inducing tissue autofluorescence. The ratio of emission peak intensity at 470 nm versus 610 nm excited by 350 nm and the ratio of emission peak intensity at 510 nm versus 61Onm excited by 470 nm were found to be good indicators for distinguishing normal and diseased tissue. Statistical t-test showed significantly difference at the level of $p = 0.05$. The observed peaks of the fluorescence spectra were also compared with some well know tissue fluorophores likely to be found in gastrointestinal tissue. It is believed that the fluorescence of normal colonic tissue was dominated by lamina proprial and submucosal fluorescence, with the submucosa emitting stronger fluorescence by a rough factor of eight. It seems *that* the peak around 610 nm in cancerous spectra may primarily be attributed to porphyrin. The peaks around 470 nm and 510 nm in normal colorectal tissue may be assigned to NADH and FAD. The intensity of emission peaks around 460 and 510 nm could reflect the metabolic activity of the tissue. The findings confirmed that autofluorescence technique is a potential diagnostic tool for tissue analysis in the future.

Autofluorescence Spectral Changes in the Colonic **Mucosa** of the Rat During **Colorectal** Cancer Formation
A noninvasive in *vivo* laser-induced autofluarescence (LIAF) technique was applied to investigate the in vivo autofluorescence spectral changes in the colonic mucosa of the rat during early colorectal cancer formation. In order to induce the tumor model in the colon and rectum, each rat in the experiment group was subcutaneous injected 1mg/kg azoxymethane (AOM) once a week for two weeks This method has been proved to be effective in inducing colorectal cancer in rats at the Singapore General Hospital. The study was therefore performed on in *vivo* normal and tumor rat colorectal modeb using 442 nm wavelengths excitation light. The fluorescence intensity was markedly reduced in tumor tissues compared with normal tissues. The intensity ratio at the first and second wave peak was used accurately to discriminate normal colon and rectal tumors. Sixteen weeks after AOM injection, the tumor is observable under the white light using the choledochoscope. However *the* autofluorescence spectra changes were already detectable by our system by the 12th week giving a much earlier detection time of diseased tissue than conventional detection by white light. Furthermore, based on the change in the average intensity ratio and statistical analysis of the ratio, we were able to confirm that the rat colorectal mucosa structure drastically changed in morphology during cancer formation. Moreover, we found that there was significant increase in the subsequent intensity ratio as the rat colorectal tumor progressed. Histopathological studies were also performed on the tumor tissue from experimental rats at different stages during tumor formation. Histopathology cutting sections demonstrated that the marked tumors are adenoma. AOM induced rat colon-rectum tumors display many features of the human disease. Therefore the results on the rats provided a potentially useful model to study the role of the AF spectra in colorectal cancer diagnosis. Moreover, we believe that we can use this spectral ratio algorithm to detect earlier colorectal carcinoma during the clinical colonoscopy for patients.

Detection of Colorectal Dysplasia in vivo at Colonoscopy Using Laser Induced autofluorescence spectroscpy
Under the NIE-SGH collaborated research project on the "Detection of colorectal dysplasia in vivo at colonoscopy using laser induced autofluorescence spectroscopy", a total number of 73 patients were involved. The duration of the study was about six months. The project has just been completed. The main system, microspectrophotometer, was designed, developed and set-up in 1998 and the compieted system, laser-fibre-endoscope microspectrophotometer system for *in-vivo* measurement of laser-induced auto-fluorescence in human colorectal track was set up in 2000. The results were very encouraging. In the study, auto-fluorescence spectral intensity ratio of characteristic peaks was used to distinguish dysplasia tissue from the normal tissues. The results provide an experiment support for the use of LIAF technique as a new technique in the early in-vivo detection of colorectal dysplasia. The most important contribution of this project was the confirmation that in-vivo auto-fluorescence based endoscopy had great potential to provide clinicians with the powerful spectral analysis techniques to detect colorectal cancer as well as its location on the colorectal track at the earlier stage. The main advantages of our approach are: (a) the measurements are safe and non-invasive, and (b) it provides real-time spectral analysis during clinical examination. The system used and the approach adopted for in-vivo clinical colorectal examination was the first time introduced in Singapore.

Implications of the research
The results from the studies have been used to update/upgrade the Biophysics (hons) module in the areas of applications of Physics in medicine. The experimental systems of the investigations will provide practical projects for MSc (Biophysics) course as well as for Advanced Postgraduate Diploma in life Science course.

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