Application of Lasers in the Diagnosis of Diseased Tissues

Chia Teck Chee

This research project reports investigations of light propagation in the tissue by Monte Carlo modelling, laser-induced autofluorescence (LIAF) spectroscopy and the dynamics of autofluorescence decay in normal and tumour human colonic tissues. Two instrumentation systems were developed to advance this research. A theoretical model was developed to further improve the understanding of macroscopic spectroscopic measurements.

A laser-based fibre-optic spectrofluorimetric (FSF) system was developed to study the steady-state and time-resolved autofluorescence spectroscopy of in vitro human colonic tissues using visible excitation wavelengths ranging from 420 to 670 nm. Several distinctive features of macroscopic spectral properties of normal and tumour tissues were found and analysed in detail. The fluorescence lifetimes of abnormal colonic tissues were found to be shorter than those of normal tissues, suggesting the occurrence of an enhancement of non-radiative relaxation as tissue progresses from normal to the pre-malignant and the malignant states. A diagnostic algorithm based on the ratio of autofluoreseence intensities at 550 nm to that at 630 or 680 nm was found to be able to distinguish colonic tumours from normal tissues with a high degree of sensitivity and specificity. This diagnostic criterion may form a basis for a new surveillance technique (guided biopsy) for patients with tumour colonic tissues.

The fibre-optic spectrofluorimetric system was extended to study the photobleaching process of autofluorescence in normal and tumour human colonic tissues in vitro during continuous exposure to laser light. The effects of exposure time and intensity on the behaviour of the autofluorescence decays were investigated in detail. The dynamics of autofluorescence photobleaching in colonic tissues were found to fallow a double exponential function, with the fast decay term corresponding to mucosa photobleaching, and a slow decay term representing the submucosa photobleaching from deeper tissue layers.

A rnicrospectrophotometric (MSP) system was developed to further investigate microscopic spectral properties and images of autofluorescence emissions in human colonic tissues under the excitation of laser light at 442 nm. The microscopic properties on histological structures, fluorophores micro-distributions and intrinsic fluorescence spectra in individual tissue layers in the colon were obtained. The differences in the microscopic characteristics of normal and tumour tissues were investigated on a microscopic level. The data on intrinsic fluorescence microscopy and imaging and histological Organisation of colonic tissue provide a basis for the explanation of macroscopic autofluorescence in vitro or in vivo.

A five-layer colon optical model (epithelium, lamina propria, muscularis mucosa, submucosa, muscularis propria) was developed based on the data obtained, namely, (a) the histological structures of tissues, (b) the microscopic fluorophore distributions in the tissue, (c) the intrinsic fluorescence spectra. The model also uses (d) the published optical parameters of colonic tissue. Using this theoretical model, the fractional contribution of the submucosal tissue in the colon to total measured autofluorescence signal was found to be 17%, which was very close to the pre-exponential coefficient value of 0.178-0.169 obtained in photobleaching experiments. Moreover, the autofluorescence spectrum was also reconstructed theoretically according to the new model. This simulated autofluorescence spectrum agrees well with the experimental autofluorescence spectra. The good agreement between the experimental and the theoretical results indicates that the quantitative relationships between microscopic properties of colonic tissue and the macroscopic autofluorescence measurements have been established by this theoretical modelling.

The results of this project provide a solid foundation for understanding the macroscopic and microscopic spectral

characteristics of laser-induced autofluorescence of human colonic tissues, and elucidating the effects of tissue optics on the macroscopic autofluorescence measurements. Moreover, the results are also useful to aid in the interpretation of the underlying mechanisms by which the laser-induced autofluorescence (LIAF) spectroscopy is used to differentiate tumour tissues from normal tissues. This study 'forms the foundation for developing an optimum algorithm for the implementation of a LIAF system for colonic endoscopy. Further research on the endoscopic autofluorescence imaging of colonic tissues is now in progress in our laboratory. The results from the investigations have been used to upgrade the Biophysics (Hons) module in areas concerning laser applications in medicine. The experimentation systems of the project will provide practical activities for MSc (Biophysics) course as well as for Advanced Postgraduate Diploma in Life Science course.

Team Members: Chia Tek Chee (Principal Investigator), Diong Cheong Hoong, Huang Zhiwei, Lim Tuan Kay, Francis Seow Choen

*This study was **supported by the Academic Research Fund (AcRF)***