In-vitro diagnosis of human uterine malignancy using N_2 laser-induced autofluorescence spectroscopy

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Results of an *in-vitro* study of N_2 laser excited autofluorescence from cancerous and adjoining normal human uterine tissues are presented. Discrimination functions based on multivariate linear regression analysis of the spectra could discriminate between the two tissue types with sensitivity and specificity values towards cancer of 100% when the cancerous site exhibited red fluorescence band and around 90% when cancerous site did not show red fluorescence.

LASER-INDUCED fluorescence (LIF) from native tissues (autofluorescence) is being actively investigated for its potential use in cancer diagnostics[1]. This promising technique offers several important advantages like a very high intrinsic sensitivity, suitability for detecting small superficial tumours not detectable by other techniques such as X-ray diagnostics and the use of non-ionizing radiation which avoids the risks associated with ionizing radiation. Further, the diagnostics can be done in near real time and *in situ* without any tissue removal. Moreover, tissue diagnostics by this technique can be easily automated, facilitating use by less skilled personnel and mass screening. Extensive studies have therefore been carried out using human tissues, removed at surgery or at biopsy, and induced tumours in animals to evaluate the potential of this technique for discriminating cancerous as well as precancerous tissues from normal. The results have been very encouraging and are motivating considerable efforts to realize the *in vivo* diagnostic potential of the technique[2,3].

In this article we present results of our *in vitro* studies on N_2 laser excited autofluorescence from pathologically confirmed cancerous and adjoining normal human uterine tissues. A step wise multivariate linear regression (MVLR) analysis was used to quantify the observed spectral differences and form a discrimination function on the basis of which the tissue could be classified as normal or cancerous. Sensitivity and specificity values towards cancer of 100% were obtained when the cancerous site exhibited red fluorescence band and around 90% for cases where the cancerous site did not show any red fluorescence.

Experimental procedure

Pathologically characterized full thickness tissue samples were obtained from Choithram Hospital and Research Centre, Indore, from six patients with uterine cancer, immediately after resection at surgery or at biopsy. The tissue samples were stored in ice until study. The spectroscopic experiments were performed within 4 to 24 h of tissue removal. For the experiments the specimens were thawed to room temperature, kept moist with buffered saline solution (pH 7.4) and mounted on a rectangular quartz slide for LIF studies paying attention to the orientation of the sample.

LIF spectra of the tissue samples were recorded using the experimental set-up shown in Figure 1. The excitation source used was a home-built pulsed N_2 laser emitting 7 ns pulses with a repetition rate of 10 Hz and a pulse energy of 200 μJ. The laser beam was coupled to a quartz optical fiber (core diameter 400 μm) via a dichroic mirror which reflected N_2 laser radiation and transmitted longer wavelength fluorescence output. Typical energies delivered to a tissue sample were 40–50 μJ. No sample photobleaching was observed at these energies. The power of the N_2 laser was monitored by a beam-splitter-photodiode combination and the fluorescence from the tissue kept in contact with the fiber was collected by the same fiber and imaged on the entrance slit of a scanning monochromator. The wavelength dispersed light at the exit slit of the monochromator was detected by a photomultiplier tube (Hamamatsu R406) detector. A microprocessor-based system developed in-house was

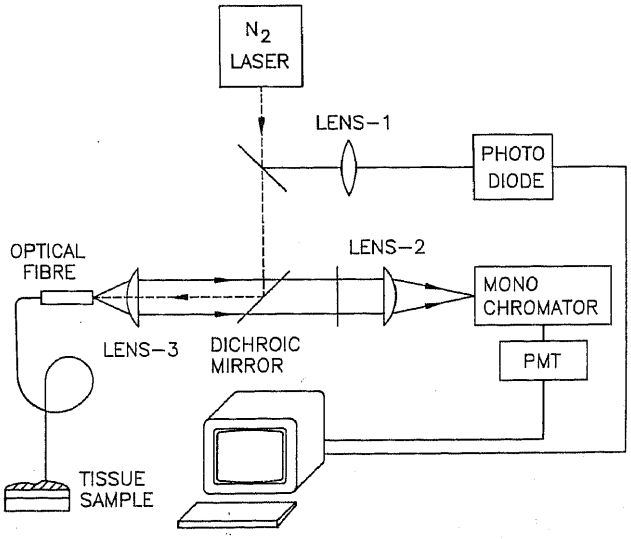

Figure 1. Schematic diagram of the experimental set-up for autofluorescence spectroscopy of tissues.