Laser Induced Fluorescence Spectroscopy

The LIFS technique is used widely in research for a variety of analytical applications, from interrogation of plasma plumes in Laser Induced Breakdown Spectroscopy (LIBS), to determination of cancerous tissues, to fluorescence spectroscopy of single molecules.

LIF is one of the most sensitive approaches available for analytical purposes. It is relatively easy to implement, phenomenological straightforward and well investigated, and largely non-invasive which can be imperative for biomedical application.

The core instrumental requirements of LIFS, consist of a laser excitation source, focusing and collection optics (lenses/fibre optics) a spectrometer and a sensitive spectroscopic CCD detector. Andor 16-bit, highly sensitive spectroscopic CCDs have been successfully employed in a broad range of LIFS approaches. In addition, it is entirely feasible to make use of an ICCD detector, opening up the availability to perform time-resolved LIFS, thus accessing the temporal dimension as well as the spectral. For example, a compact LIFS system for clinical purposes has been developed by researchers at the Lund Institute of Technology, Sweden, offering the ability to perform non-invasive interrogations of clinical tissue, such as colonoscopy, via an external optical fibre probe. Time-resolved measurements may be accessed to monitor the relative intensity of normal vs. tumour tissue fluorescence, at time delays beyond the lifetime of generic tissue autofluorescence, providing increased contrast for tumour demarcation.

Compact System for Clinical Recordings of Laser-Induced Fluorescence Spectra

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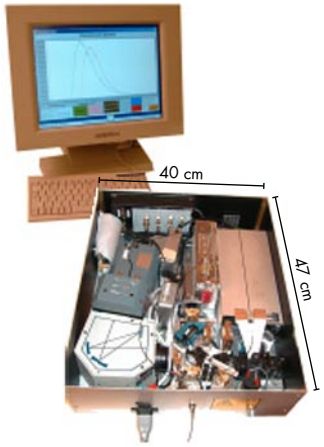
Abstract

A compact point-measuring fluorosensor, fully adapted to clinical studies, is described. Two different excitation wavelengths can be used. Both time resolved and time integrated spectra can be recorded. In addition, a white light halogen lamp can be used to investigate the diffuse reflectance.

The controlling software has been designed to be user-friendly, and can also easily be modified for different applications.

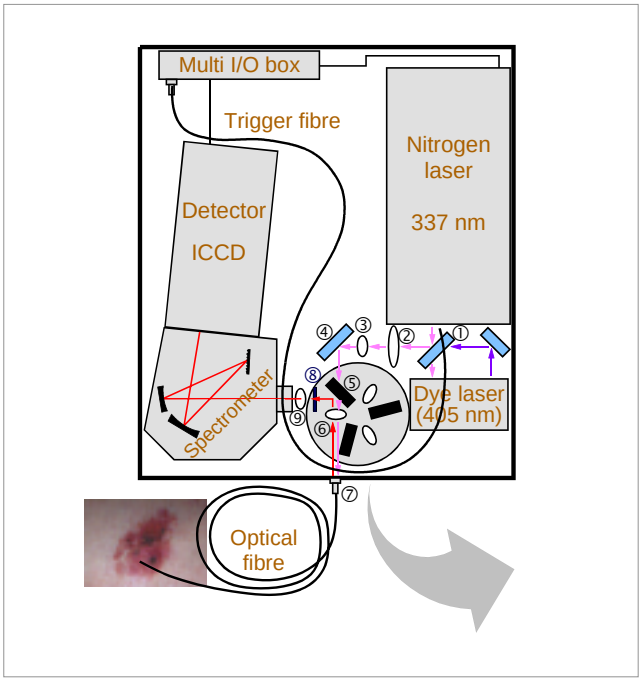


Overview

All the optics and electronics are packed into a compact box (47x40x21 cm^3). A panel-PC is used to control the acquisition and to store the data. The user-interface can be operated via the touch screen.

1. Light sources

The excitation light is provided by a small sealed-off nitrogen laser alone (337 nm) or in combination with a dye laser (4,4'-diphenyl-stilbene DPS, 405 nm). The light is delivered in 3 ns pulses at about 15 Hz. A motorised flip-in mirror (1) is used to switch between the two light sources.



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2. Optics

The excitation light is transformed in a telescope (lenses 2 and 3), passes through a beam-splitter (5), and is focused onto the fibre port (7). The fluorescence emission returns through the same fibre port, and is reflected towards the detection system by the beam-splitter. A glass filter (8) is used to prevent scattered laser light from entering the spectrometer.

3. Probe

A single 600 µm fused silica optical fibre is used as a probe. The distal end of the fibre is held in light contact with the tissue under investigation. The excitation light is absorbed in a small volume close to the tip. A fraction of the fluorescence emission is collected by the fibre and is guided back to the detection system.

The pulse energy of the excitation light is on the order of 1 µJ, and usually spectra are accumulated from 20 laser pulses.

4. Detection system

The fluorescence emission focused onto the 100 µm-wide entrance slit of a 125 mm spectrometer. A thermoelectrically cooled, imageintensified CCD-camera is used to record the spectra up to about 800 nm with a spectral resolution of about 2 nm.

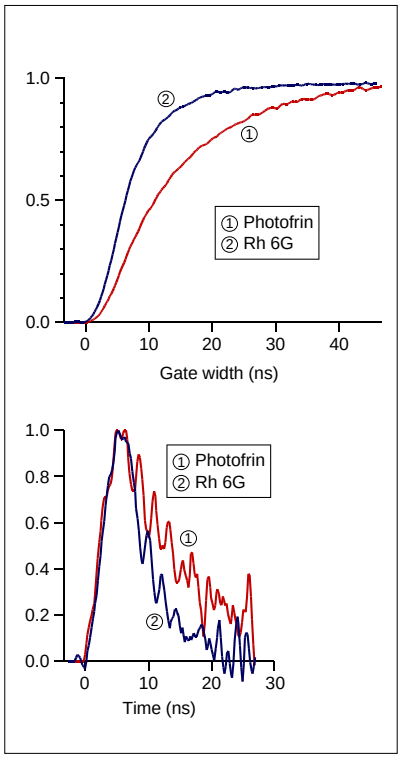
Andor's ICCD camera as used in this technique

For time-integrated spectra, the gatewidth of the intensifier is about 100 ns and the gate is synchronised with the laser. Thus, the ambient light is suppressed by approximately 10^5.

Results & Discussion...

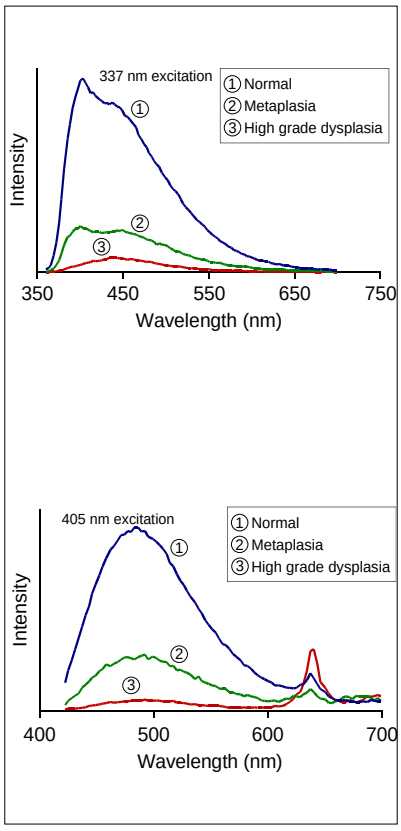
System performance

Fluorescence spectra recorded from the skin using gain-setting 6 (115 counts per photoelectron) resulted in signal-to-noise ratios at about 25 to 30 for the two excitation wavelengths. The noise in a typical background was of the order of 150 counts, yielding a dynamic range of the system of approximately 500.



Time-resolved investigations

An external delay generator must be used for short gating times and to change the delay of the gate. In the upper curve, the intensity is shown as a function of gate width for Photofrin and Rhodamine 6G. These curves are then differentiated to yield the decay curves shown to the right. The time resolution of the system is approximately 4 ns.



Colonoscopy

Example of time-integrated fluorescence spectra from measurements of normal tissue, metaplasia, and high grade dysplasia in the colon, following excitation at 337 nm (upper curve) and 405 nm (lower curve). The patient had received ∂-aminolevulinic acid (ALA) at a dose of 5 mg per kg body weight prior to the investigation. ALA is converted to the fluorescent tumour marker protoporphyrin IX, which is responsible for the peak at about 635 nm in the right panel.

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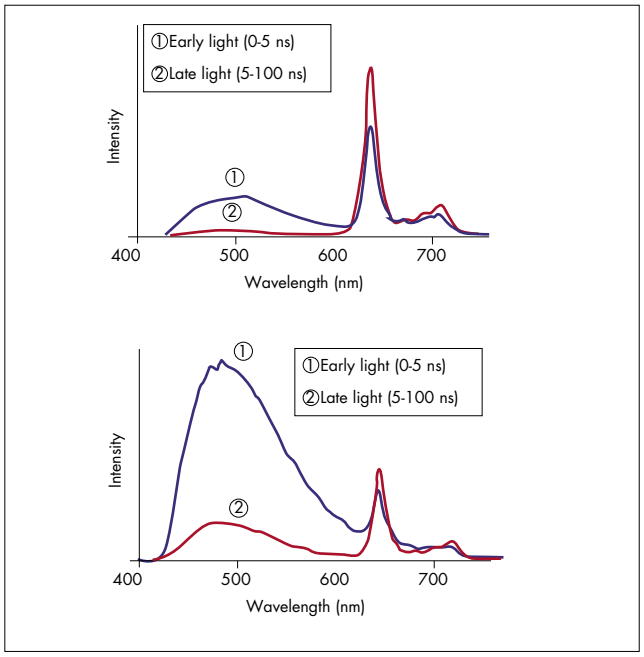
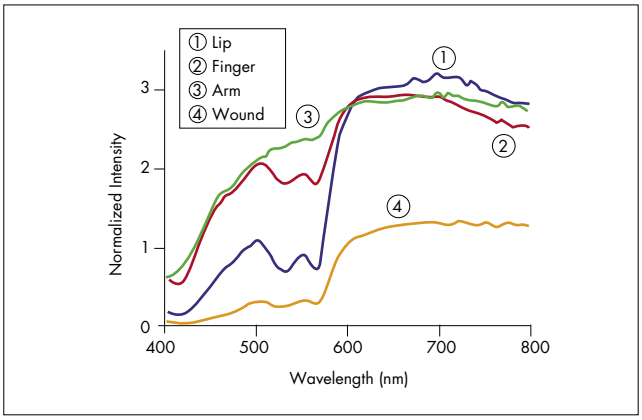
Dermatology

Spectra recorded from a basal cell carcinoma (upper panel) and the adjacent normal skin (lower panel) following 4 h topical application of ALA. The differences in the early and late light show that the fluorescent tumour marker (peaks at 635 and 705 nm) has a longer lifetime compared to the tissue autofluorescence (about 490 nm). This can be used for increased contrast for tumour demarcation. Excitation wavelength is 405 nm.



Diffuse reflectance

Diffuse reflectance spectra recorded from a white light halogen lamp, using one optical fibre for light delivery and one for detection. The fibres were 1 mm apart (centre-to-centre). The curves are normalised to a reflectance standard.



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