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Compact fluorosensor based on a continuous-wave violet diode laser, an integrated spectrometer and fiber-optic sampling

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A compact fluorosensor with a fiber-optic measurement probe was developed, employing a continuous-wave violet diode laser as an exciting source and an integrated digital spectrometer for the monitoring of fluorescence signatures. Results from measurements on vegetation and human premalignant skin lesions are reported, illustrating the potential of the instrument.

1. INTRODUCTION

Laser-induced fluorescence is a powerful technique for non-intrusive, real-time diagnostics in a multitude of contexts. Solids and liquids, and complex compounds exhibit a broad-band fluorescence distribution devoid of sharp spectral features due to rotational quenching [1]. Fluorescence monitoring can be performed on samples *in situ*, e.g. in a laboratory or an operating room, through a microscope [2] or remotely using a fluorescence LIDAR system [3,4]. It can be applied in point monitoring [5] or in imaging applications [6,7]. Applications include diagnostics of human malignant tumors [8,9] and atherosclerotic disease [10], vegetation and water monitoring [11,12], analytical chemistry techniques such as HPLC and capillary electrophoresis [13], and forensic sciences [14].

Fluorescence diagnostics has been largely simplified through the availability of CCD detectors for direct read out of the spectrum obtained in the image plane of a spectrometer. With a short-pulse ultra-violet excitation laser, a gated and intensified CCD detector can be employed for efficient suppression of ambient light. Our group has developed several systems of this kind, which have been employed in medical and environmental monitoring. Original systems [15] were quite bulky, whereas later developments resulted in more portable equipment [16]. In the present paper we report on the construction and utilization of a highly compact fiber-optic fluorosensor of the size 22 x 13 x 8 cm³. This achievement became possible through two interesting recent developments. First, violet and blue continuous-wave lasers have become commercially available at a power level of 5 mW [17]. Second, compact integrated spectrometer units with CCD read out are now available providing unprecedented convenience for spectral assessment.

This paper is organized as follows: Section 2 describes the construction of the fluorosensor; section 3 gives examples from vegetation and human tumour diagnostics; and section 4 provides suggestions for further developments.

2. FLUOROSENSOR SET-UP

The general lay-out of the new instrument is demonstrated in Fig. 1(a) and photographs of the system are presented in Figs 1(b) and 1(c). As a light source for inducing fluorescence we use a violet diode laser with a nominal operating wavelength of 396 nm (Nichia NLHV500). We note, that below 400 nm the eye safety regulations are relaxed by a factor of about 1000 since the cornea no longer transmits the radiation to be focused on the fovea. It is particularly convenient to be able to operate at these sufficiently short excitation wavelengths. The diode laser is driven by a power-supply (Wavelength Electronics LDD200-3M) with a 9 volt battery as input for decoupling from possible net transients, which can be detrimental to diode lasers. The diode laser is placed in a tube together with a collimating lens (Geltech C230TM-A). The output light is "cleaned up" for broadband spontaneous emission using a narrow-band interference filter (CVI F25-400-4-0.5). The violet radiation is focused by a fiber-port lens assembly (Optics for Research PAF-SMA-6-NUV-Z) into a 600 μ m diameter optical fiber. Before entering the fiber the light beam is reflected off an appropriate dichroic beamsplitter (CVI). Fluorescence is induced in an object placed in contact with the distal tip of the fiber, where about 1.2 mW of laser power is available. Stokes-shifted fluorescence light is conducted back through the fiber and is focused by a fiber port into a short fiber which is connected to the spectrometer (Ocean Optics S2000) equipped with a 100 μ m slit. Elastically backscattered diode laser light is effectively blocked by a Schott GG420 colored glass cut-off

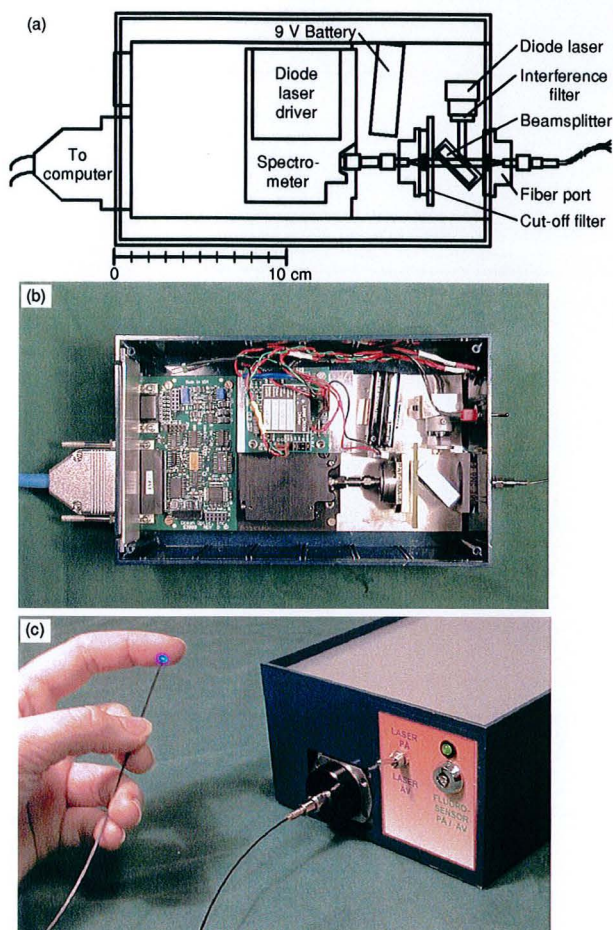


Figure 1. (a) Schematic lay-out of the compact fluorosensor. (b) Photograph of the system interior. (c) Photograph of the system inducing skin fluorescence.

filter placed behind the dichroic. The grating (600 lines/mm) disperses the light and a spectral region of about 330 - 1000 nm is captured on the 2048 element CCD detector. A spectral resolution of about 5 nm is obtained, which is fully adequate for environmental and medical monitoring.

The wavelength scale of the spectrometer is calibrated using a mercury/argon lamp (Ocean Optics HG-1). A spectrum from the calibration lamp is shown in Fig. 2(a) illustrating the resolution of the system. Since the exciting light is transmitted through the same fiber as the one used for the detection, fluorescence from the fiber itself constitutes a background. A background spectrum can be obtained without a sample in place and subtracted from spectra collected from samples. The level of fiber fluorescence is shown in Fig. 2(b). A spectrum for a solution of Rhodamine 6G (10 mg/60 ml ethylene glycol), observed through a 1 mm thick quartz window is shown in Fig. 2(c). In order to obtain a flat intensity response over the full spectrum a calibration is performed using a 200 W calibrated quartz tungsten/halogen lamp (Oriel 63355) with a known spectral profile. The reference spectrum is recorded

through the optical fiber. By dividing the true spectral intensity by the recorded intensity a multiplicative correction curve is obtained which when multiplied by a recorded spectrum yields a standardized spectrum for the case of a "white-light" spectral response. The experimental spectral correction curve is given as Fig. 2(d). All spectra presented in the present paper were handled with Microsoft Excel software after transfer from the spectrometer. All spectra shown below are spectrally corrected.

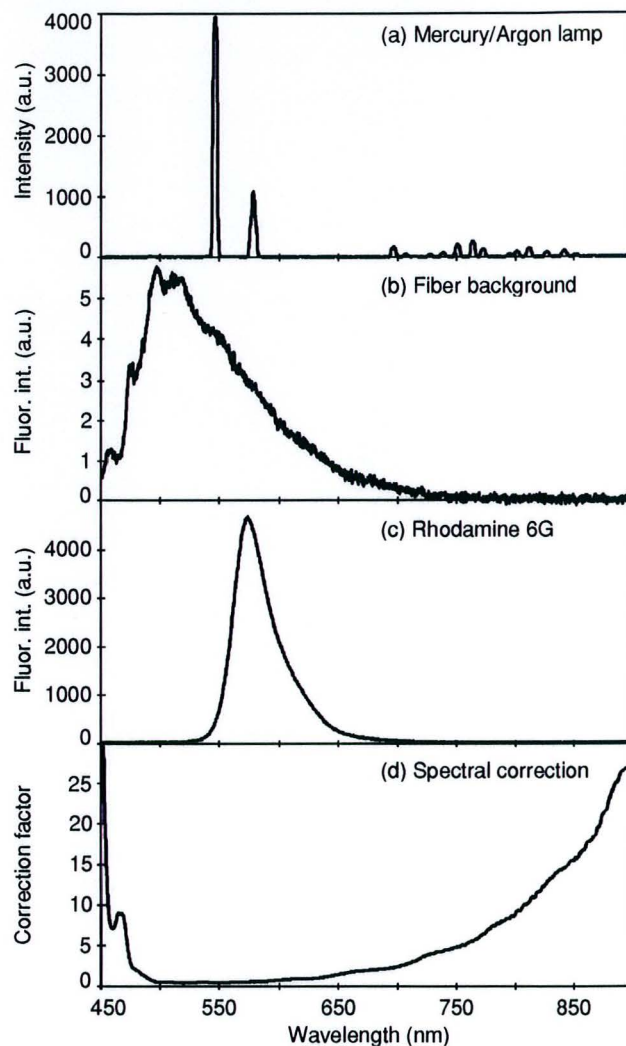


Figure 2. (a) Recording of the emission spectrum of a mercury/argon lamp (integration time 10 ms). (b) Laser-induced intrinsic fiber fluorescence level to be subtracted from recorded spectra (intensity scaled for 10 ms integration time). (c) Fluorescence spectrum from a Rhodamine 6G solution (integration time 10 ms). (d) Spectral correction curve.

Since the laser source is not pulsed and the detection is not gated in contrast to more complex systems [15,16], there is a risk that ambient light may add an undesired background. However, since the fiber is put in contact with the specimen under study, the ambient light is effectively blocked from entering the fiber in most cases. Under special

circumstances (observing weak fluorescence) a reduction of the ambient light or a local shadow formation at the measuring site might be required. In indoor work with luminescent lamp illumination, the presence of mercury lines in a recorded spectrum would indicate problems due to ambient light.

3. MEASUREMENTS

We have used the new compact instrument in test measurements in two fields extensively studied at our department: vegetation monitoring and human malignant tumor diagnostics.

A. Plant fluorescence

Green vegetation exhibits characteristic fluorescence peaks in the near infrared spectral region. Chlorophyll *a* is effectively excited in the blue and red spectral regions yielding a dual-band spectral profile with peaks at about 685 and 740 nm [18]. The first peak is situated at a wavelength where the chlorophyll pigment still absorbs light. This means, that when the chlorophyll contents in a leaf increases the first peak cannot rise at the same rate as the second one. Thus, the intensity ratio $I(740 \text{ nm})/I(685 \text{ nm})$ is related to the chlorophyll contents, and after calibration this ratio can be used for chlorophyll concentration assessment. Fluorescence recordings from a beech leaf exhibiting fully green as well as yellow and

brown sections, due to senescence, are shown in Fig. 3. The CCD integration time was 1 sec. Experimentally we find the intensity ratios 0.59, 0.28, and 0.17 for the green, yellow and brown regions of the leaf front side, respectively. The corresponding values for the back side are 0.54, 0.19, and 0.17, respectively.

B. Tumor fluorescence

Human tissue exhibits a bluish fluorescence when excited with a violet source. Molecules contributing to the fluorescence include elastin, collagen, NADH and carotene [10]. Chemical compounds, called sensitizers, are selectively retained in malignant tumours following systemic, oral or topical administration. Sensitizers, including porphyrins, phthalocyanines and chlorines, exhibit characteristic fluorescence signatures in the near infrared spectrum which can serve for clinical tumour diagnostics [8,15]. By irradiation of red light the same compounds can also mediate tumor cell necrosis through selective singlet oxygen release (photodynamic therapy [19], PDT). A particularly interesting sensitizer is protoporphyrin IX, which is synthesized at a higher rate in malignant tumours after administration of δ -amino levulinic acid [20,21]. The synthesis of protoporphyrin IX can be readily seen through its characteristic fluorescence peaks at 635 and 690 nm, which can be used for tumour identification and demarcation [21].

Fluorescence is routinely used as a diagnostic method in connection with photodynamic tumor therapy at the Lund

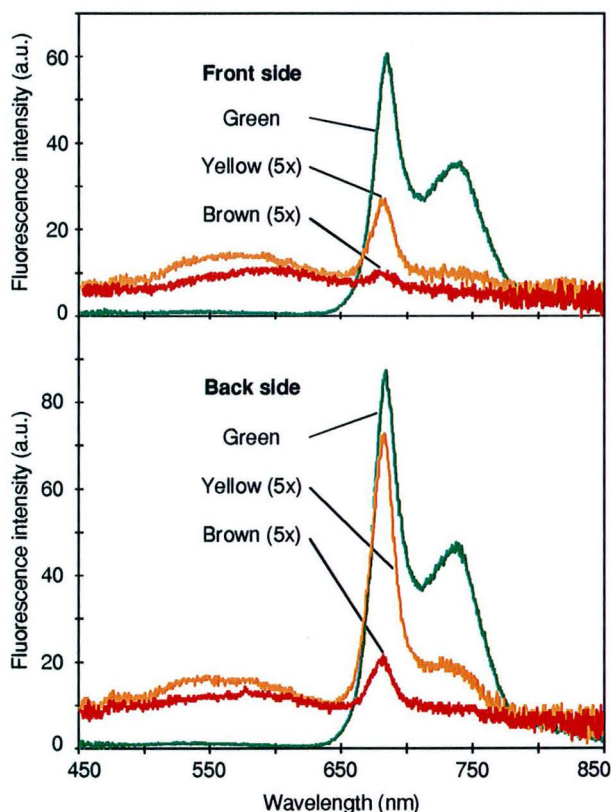


Figure 3. Spectra from different parts of a beech leaf.

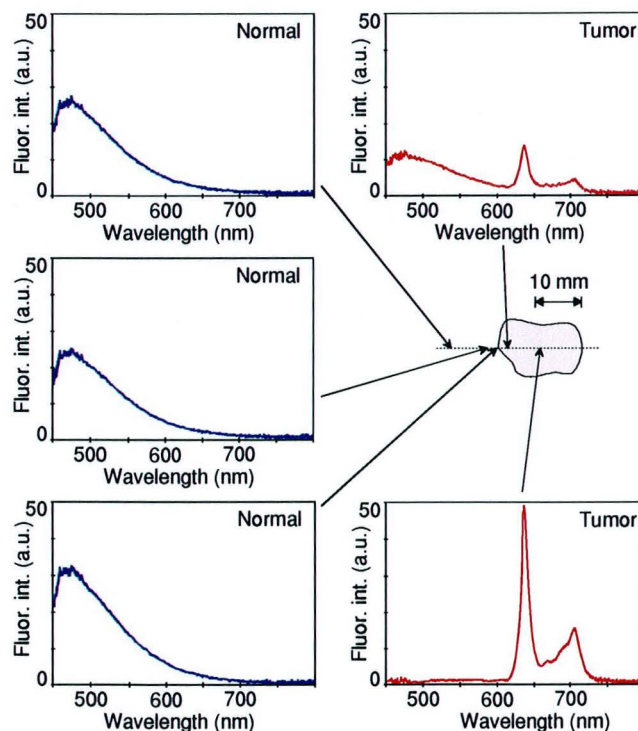


Figure 4. Fluorescence spectra recorded in a scan across an actinic keratosis, sensitized by a cream containing δ -amino levulinic acid. Recordings before photodynamic therapy are shown.

Laser Centre. The compact fiber-optic fluorosensor described here was used in connection with the treatment of a 74 year old male patient presenting with actinic keratosis, a premalignant skin disease. The tumors were prepared with a 20 % ALA cream which was left covered by occlusion pads during a 6 hour protoporphyrin IX build-up period. The pads and the cream were then removed. Fluorescence spectra (Figs. 4 and 5) were recorded in scans across the tumors before and after PDT. Each spectrum was obtained with a 400 ms integration time. In Fig. 4 the characteristic build-up of protoporphyrin IX in the tumor is clearly illustrated. The decrease of the blue fluorescence in the tumor is also a well-known phenomenon [7,8]. Fig. 5 illustrates the strong photobleaching of the protoporphyrin IX induced by the photodynamic treatment, a phenomenon that can be used for dosimetry.

4. DISCUSSION

A compact fiber-optic fluorosensor was constructed and employed in vegetation and human skin monitoring. The system performance is similar to what is obtained from much larger and more complex fluorosensors. Ambient light influence is the major draw-back in the present

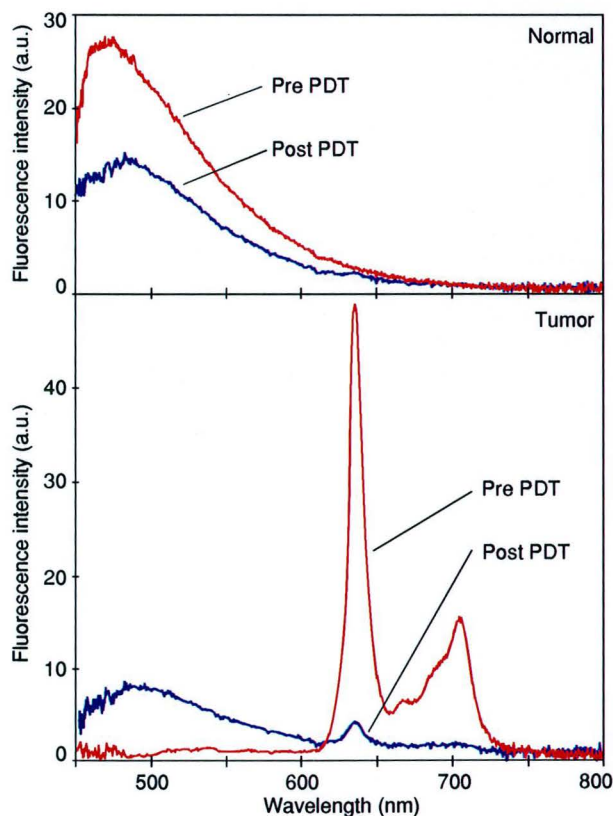


Figure 5. Fluorescence recordings for actinic keratosis and normal surrounding skin before and after photodynamic therapy.

version of the system, sometimes requiring a reduction of the background light. Presently, an ordinary personal computer was used for the data processing. Clearly, a laptop computer, all-battery power and efficient soft-ware routines for on-line evaluation of diagnostic information will further enhance the attractiveness of fluorosensors of this kind.

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