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High-sensitive synchronous detection technique for early fluorescence diagnosis of carcinoma tissue

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The visual inspection of human cancer is sometimes insufficient for exact diagnosis. For that reason, a synchronous detection apparatus for fluorescence diagnosis has been designed and constructed. This apparatus is very sensitive and enables both the direct and endoscopic observation of various human tissues as well as quantitative optical measurements. Its operation is described in this paper.

1. Introduction

Photodynamic therapy (PDT) and fluorescence endoscopy are relatively new methods of detection and treatment of various types of human malignancy. The major advantage of these methods is their high sensitivity and selectivity. PDT has its greatest importance in early stages of the disease [1]–[3].

Early detection of precancerous and cancerous stages is important for the treatment and further development of the patient's health. It plays significant role in lowering the cancer mortality. As it is known, only 10–15% of patients suffering from the lung cancer and being treated by traditional diagnostic methods usually survive for not more than five years. Whenever the fluorescence diagnostics can be used more than 90% of these patients are reported to be fully curable [4]. The fluorescence diagnostic result depends on the efficacy of the apparatus used. Many of such apparatus have been designed but only few of them can be used in medical practice [5], [6]. Among the basic requirements concerning the fluorescence diagnostic apparatus are small dimensions, low power consumption, flexibility, movability, and easy handling and maintenance without the necessity of employing specially experienced persons as it is required for ion lasers. These conditions could

probably be satisfied in the future using semiconductor and optoelectronic technologies.

The main purpose of this paper is to describe the fluorescence diagnostic apparatus operating on the principle of the synchronous detection technique.

2. Fluorescence diagnostics and therapy

The fluorescence diagnostics is based on observation of fluorescence emitted by cancerous tissue. The fluorescence therapy is based on destructive process of the malignant cells, caused by the singlet oxygen.

As photosensitizers, derivatives of hematoporphyrin (Photofrin, Photosan) or 5-aminolevulinic acid (ALA) are used. Both the high-power ion lasers (Argon-, Krypton-, Day-, and Ti:Sapphire lasers), as well as the noncoherent optical lamps (Xe-, HgXe-, and Kr-Arc-lamps) are used as optical sources.

As a rule, the short wave emitting sources are used for the fluorescence diagnostics, while the long wave light is used in photodynamic therapy. Short wavelengths penetrate less deeply into the tissue.

3. Diagnostic apparatus

The fluorescence diagnostic apparatus contains the following basic parts: a) the optical source, b) the detection system, and c) the components ensuring the processing, delivery and distribution of the optical signals. The block diagram of the set-up using the lock-in amplifier is schematically shown in Fig. 1. A version in Fig. **1a** is suitable for diagnostic investigations of the surface of the body, for example, the skin, while a version in Fig. **1b**, containing the endoscope, can be used for both the surface and hollow organs (bronchus, stomach, intestine and bladder). The endoscopic version is less sensitive due to lower fluorescence caused by higher optical attenuation in the endoscope. In Figure **1a**, the light going out from the optical source (OS) is filtered by a set of optical filters (F1), enters the diagnostic tube (DC), and irradiates the tumour (TU). The fluorescence light excited in the tumour enters back into the tube, is filtered by optical filter (F2), and falls on the lock-in amplifier detector (D). The computer (C) and the recorder (R) are connected to the detector. The version in Fig. **1b** includes the endoscope (E) which enables investigation of hollow organs. The telescope (T) serves for direct eye observation and better localization of inspected tumours.

3.1. Optical source

The most important components of this part are the optical source and the set of input filters.

As the optical source we use the Xenon lamp. It is the special high-intensity narrow-beam short-arc Cermax lamp (producer ILC Technology, USA) with a fixed rugged internal parabolic reflector ensuring the maximum optical power to be launched into the diagnostic tube. The lamp has single-crystal sapphire windows

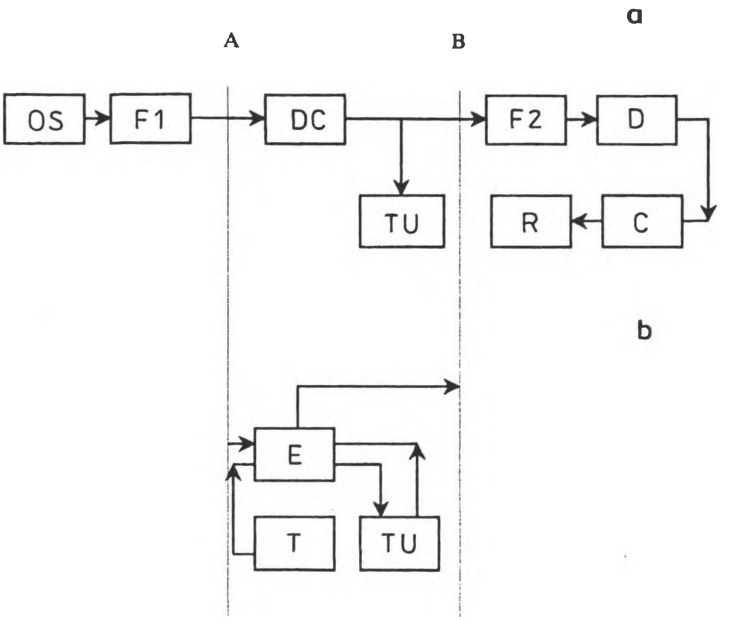

Fig. 1. Experimental set-up for the fluorescence diagnosis of carcinoma tumours. **a** – version with the diagnostic tube, **b** – version with the endoscope. OS – optical source, F1 – set of optical filters, DC – diagnostic tube, TU – tumour, F2 – optical filter, D – lock-in amplifier detector, C – computer, R – recorder, T – telescope, E – endoscope

with high transmission in the ultraviolet wavelength region necessary for the fluorescence diagnosis. The infrared rejection filter eliminates the infrared light from the optical beam which could overheat the tumour tissue. The nominal parameters of the lamp are: the operating power 180–320 watt, electrical current 10–22 A d.c., electrical voltage 13–16 V, and the ignition voltage 23 kV. The nominal radiant optical output power in the ultraviolet wavelength range is 2.6–6.6 watt.

The set of input filters enables generation of sufficient fluorescence of photosensitizer used for the diagnosis and suppression of undesirable optical signals, for example, the autofluorescence. These signals could penetrate into the photodetector and lower the optical detection efficiency. The optimal fluorescence excitation wavelength for the most photosensitizers lies in the vicinity of 400 nm. As excitation filters we used the interference filter of the Preciosa Crytur, Ltd., Turnov, and the short-wave-pass filter LCLS-450-F of the Laser Components GmbH, Olching, Germany.

3.2. Lock-in amplifier detector

The synchronous detection system based on sampling lock-in amplifier is the key part of the fluorescence diagnostic apparatus. It facilitates fully automatic and very sensitive predefined spectral measurements of intensity of optical signals as well as other optical measurements, for example, measurement of absorption, reflectance

and others. Our version is composed of several parts which can be arranged according to the immediate laboratory requirements.

The block diagram of the synchronous detection system is shown in Fig. 2. It contains the following main parts: the chopper (CH), the optical detector with a preamplifier (OD), the synchronization-detection card (C1), and the synchronization-circuit card (C2).

Fig. 2. Lock-in amplifier detector (see box D in Fig. 1): Ch – chopper, DC – diagnostic tube, OD – optical detector, C1 – synchronization-detection card, C2 – synchronization-circuit card

The synchronized detection is the main operating principle of this system. The optical beam measured is periodically modulated by a mechanical chopper which is phase-controlled by signals from the synchronization-circuit card. This card is connected with the card of the synchronization detector which enables the processing of two signals, one from the optical detector and the other from the preamplifier. Both cards can be placed in any computer. The optical detector head is connected with the computer by means of an electrical cable. We use the interference filters for filtration of input optical signals in the wavelength range 620–680 nm.

The synchronous detection system is fully automatized and very sensitive apparatus which eliminates the disturbing optical signals. Its basic operation parameters are: the dynamic range 125 dB (from 10 dBm to −115 dBm for Si detector), and the resolution 0.001 dB in the wavelength range 250–2000 nm.

3.3. Endoscope

In our detection system, a modified version of the Olympus endoscope, type GIF-Q, was used. The block diagram is given in Fig. 3. It includes the head section with lenses, prisms, mechanical controls of brightness and sharpness, the light transmitting cable (with two illumination channels, one imaging channel and one measuring channel), and the view section with lenses and a swivel-prism for side inspection of inner parts of the hollow organs. Our modification consists in construction of a new channel, the measuring channel, by use of special optical fibres.

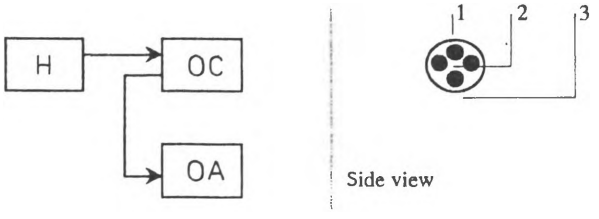

Fig. 3. Block diagram of the endoscope. H – head, OC – optical cable, 1 – viewing channel, 2 – illuminating channels, 3 – measuring channel, OA – optical analyser

Our modified endoscope version enables, besides the direct visual observation, the measurement of the tumour fluorescence light intensity.

3.4. Diagnostic tube

Several types of diagnostic tubes were designed and constructed [7], [8]. We describe our final version. It was checked by practical experimental measurements and was evaluated to be convenient for fluorescence diagnostics.

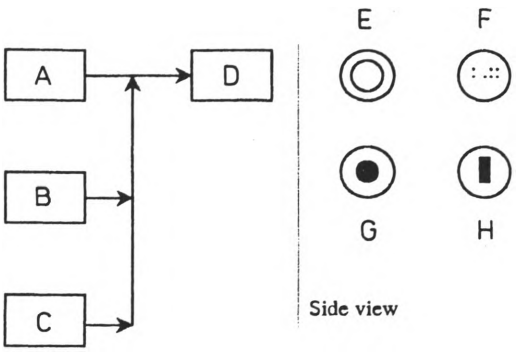

Fig. 4. Diagnostic tube. A — entrance connector, B and C — measuring connectors, D — tube collector, E, F, G, H — end profiles of fibre configurations

The diagram of the tube is shown in Fig. 4. The key part is the end section D, which from the point of view of the need of collecting the maximum amount of the fluorescence light from the observed malignant tissue, is a very difficult task to be solved. Only a restricted number of optical fibres can be used due to the rectangular and very narrow profile of the entrance slit of the measuring optical analyser or detecting monochromator. The side view shows several possible end configurations of the tube connectors.

4. Testing experiments

Figure 5 shows the fluorescence emission calibration curve of the photosensitizer $TPPS_4$. The basic set-up in Fig. 1**b** with the endoscope and the optical analyser (not present in this figure) was used in this measurement.

The fluorescence emissions of several filter papers are shown in Fig. 6. This fluorescence was excited by ultraviolet light, while the emission light was detected at the wavelength of 630 nm. The set-up shown in Fig. 1**a** was used in this experiment.

5. Conclusions

The diagnostic device for reflecting fluorometry was constructed. The fluorescence diagnosis was investigated in both the theoretical and experimental respects. The diagnostic system described above is very sensitive and gives good quantitative

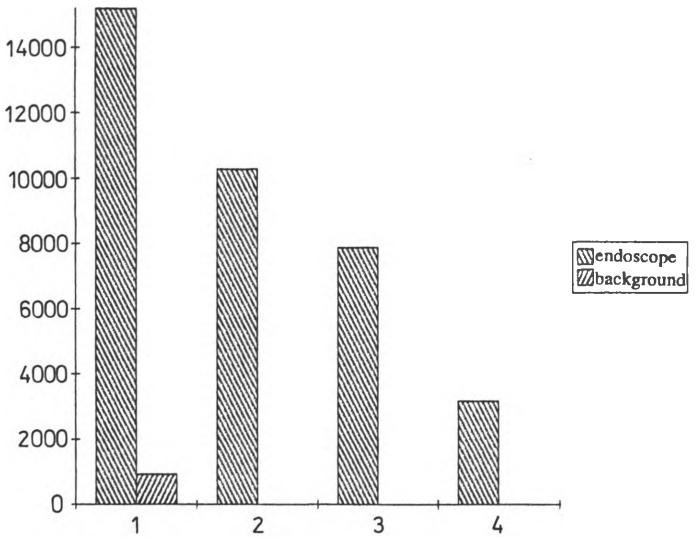

Fig. 5. Emission fluorescence of the photosensitizer $TPPS_4$. Concentrations (μg/ml): 1 — 24.7, 2 — 12.3, 3 — 6.5, 4 — 3.1. The set-up with the endoscope shown in Fig. 1 was used. Fluorescence is given in arbitrary units

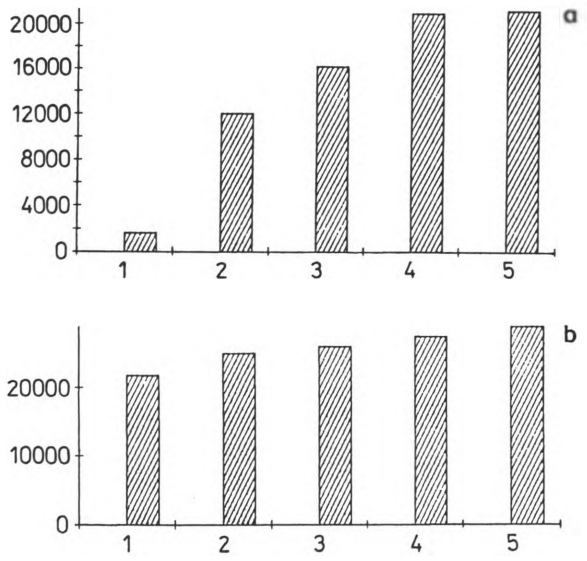

Fig. 6. Natural fluorescence of several background papers. **a:** 1 — black paper, 2 — copy paper, 3 — filtration paper, 4 — printer paper, 5 — wrapping paper; **b:** 1, 2 — note paper, 2, 3 — writing paper, 4 — copy paper, 5 — glue paper. Fluorescence is given in arbitrary units

fluorescence measurements needed in fluorescence diagnostics of malignant tissues. It can be used for detection of concerous and precancerous tissues as well as for various optical and biochemical measurements made in laboratory or on the patients. Success has been achieved in dermatology. In spite of its above-mentioned advan-

tages, further improvements are desirable to be made, especially with respect to its medical use. The following improvements are considered: a faster computer, the changing of the computer software in such a way that the complete desirable data would be immediately produced and processed more rapidly and more effectively than by the contemporary version.

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