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Light sources and diagnostic systems currently used in photodynamic therapy

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The work on photodiagnosis and photodynamic therapy focuses on therapeutic light sources and diagnostic systems applicable to detection of fluorophores in an organism as well as to the monitoring of photodynamic therapy (PDT) parameters. Optoelectronic devices employed in this method are being still improved and adapted for application in clinics. The paper presents currently available light sources used for treatment of various human organs and new designs of photodiagnostic apparatus. Also, our own designs of imaging systems and fibre fluorescence analysers are shown.

1. Introduction

The development of the photodynamic method for tumour photodiagnosis (PDD) and therapy (PDT) was observed in the early 1970's. Since 1988, the third stage of clinical investigations in the U.S.A., Canada, Japan, and in the European countries has been continued. The investigations connected with the PDT method are carried out concerning the synthesis and application of new photosensitisers, design of new light sources, radiation dosimetry in tissues, mechanisms of chemical reaction, and its application in clinics.

Tumours treatment consists in selective oxidation of biological material of a tumour tissue by a singlet oxygen or radical forms. Such forms are generated due to molecular oxygen solved in cells, exogeneously introduced photosensitiser better accumulated in the diseased tissues than in healthy ones, and the delivered light of adequate power and wavelength. Such a therapeutic method enables selective destruction of tumour tissues, simultaneously protecting the healthy ones. It is used for treatment of tumours of skin, urogenital tracts, lungs, oesophagus gullet, tongue, pharynx, stomach, intestines, and urinary bladder. The tumour diagnosis is indirect and relies on localisation of photosensitisers absorbed in tumours tissues using fluorometric methods.

Sometimes interstitial and oral introduction of photosensitiser into an organism is applied, depending on tumour type and photosensitiser kind. Finally, cytostatic effect depends mainly on photosensitising properties of a dye and its ability of retention in tumour tissues, the amount of delivered light, and the stage of tissue oxygenation. After

the intravenous injection, all tissues absorb photosensitiser, but different amounts of it are accumulated or eliminated. From majority of healthy tissues it is eliminated after several hours, but in tumour ones it remains for several days. The mechanism of selective accumulation of dyes has not been completely explained yet.

The PDT method for tumour treatment, in comparison with traditional ones (surgery, irradiation, chemotherapy), is much more selective, however, it has many constraints that limit its intensive development and clinical applications. First of all, this method requires optical instruments that are very expensive and difficult to use, *i.e.*, lasers and highly-sensitive cameras for fluorescence imaging. The method also causes a side effect, *i.e.*, high skin sensitivity to light exposure which lasts for six weeks after introducting a photosensitiser into an organism and causes many problems of laser light dosimetry in a biological tissue because of the strong light diffusion.

The paper presents current development of optoelectronic devices employed in PDT method and description of future trends of their further improvement.

2. Light sources

The ideal PDT light source should generate the light of sufficient and adequate wavelength that should be tunable to conveniently deliver light to a tissue. Simultaneously, such a source should be reliable, affordable, and "clinic-friendly" [1].

For majority of clinical applications with currently used photosensitisers, the required light dose is about 50–200 J/cm^2 with power density in the range of 50–150 mW/cm^2. For effective treatment of 15–20 minutes irradiation, adequate light source should be of total power of at least 1 W. For small lesions, up to 2 cm^2, the sources with power of 200–500 mW are sufficient. We deal with such areas in majority of clinical cases. For large diseased areas (lungs, urinary bladder, large skin lesions), light sources of 1.5–3 W power are necessary.

Generally, the sources applied can be divided into two categories – laser sources and non-laser ones.

2.1. Laser sources

Lasers commonly used in clinical applications of PDT method can be divided, with respect to operation mode, into pulse lasers and continuous work ones (CW lasers). The first group includes EDL-excimer dye laser, CVDL-copper vapour dye laser, dye laser pumped with II harmonic YAG:Nd (KTP/532) and gold-vapour laser. CW lasers are argon dye laser (ADL) pumped dye lasers, ion lasers: (Ar-Kr lasers), gaseous lasers (He-Ne), and semiconductor lasers [2].

Until quite lately, Ar pumped dye lasers with the power of 25–50 W were used in the U.S.A. and Western Europe as standard laser systems (Spectra Physics, Coherent, Meditec firm). The output power of red light at the fibre end is up to 2–3 W. Also dye lasers pumped with II harmonic Nd:YAG laser (Multilase Dye 600, Technomet Int. Laseroscope), copper laser (Cooper Laser Sonics), and excimer (Hamamatsu Photonics) ones were used [3]. Dye lasers are tunable ones and they can be used for

various photosensitisers. However, they are troublesome to use and very expensive ($150000–200000). The cheapest source is He-Ne laser (632.8 nm). However, maximum output powers of He-Ne lasers are only of about 100 mW for laser tube of 2 m in length. Also the systems including several shorter tubes are used (*e.g.*, 20 mW of 80 cm in length) in which powers of particular laser beams are added up to the value of 100–200 W using the system of prisms or focusing lens [4]. Kr^+ laser, like Ar laser, belongs to ion lasers and it generates several wavelengths, the most intensive of them are 406.7, 461.9, 647.1, and 676.5 nm.

In PDT method, Kr^+ lasers are used in diagnostic systems ($\lambda = 406.7$ nm) and in therapeutic systems ($\lambda = 647.1$ and 676.5 nm) for excitation of II generation photosensitisers (Purlytin, Foscan, Lutex, Visudyna). The output powers of commercial gold-vapour lasers, generating radiation at 627.8 nm, are up to 10 W with water-cooling and up to 1.5 W with air cooling. However, the lifetime of a discharge tube of such lasers is short, *i.e.*, 300 hours after each filling with metal.

For many years, development of PDT method has been connected with semiconductor lasers. In 1999, SDL firm developed a diode source generating the wavelength of 630, 635, and 670 nm with power of over 2 W at the fibre output, which was the crucial moment for the sources used in PDT method. Earlier, diode sources generating the wavelength of 650–690 nm, adequate for II generation photosensitisers (Spectra Diode Lab and Applied Optronics Sources), were developed. At present, the price of diode sources is not higher than $40000 and it is expected to be decreasing. Diomed and Biolitec firms offer commercial systems employing semiconductor diodes (Fig. 1). These sources are commonly used instead of Ar-dye lasers or KTP-dye lasers. The advantage of tunable lasers lies in the possibility of tuning or selecting light frequency for various photosensitisers. Diode lasers are characterised by significantly narrower band of tuning that is mainly dependent on temperature and in comparison

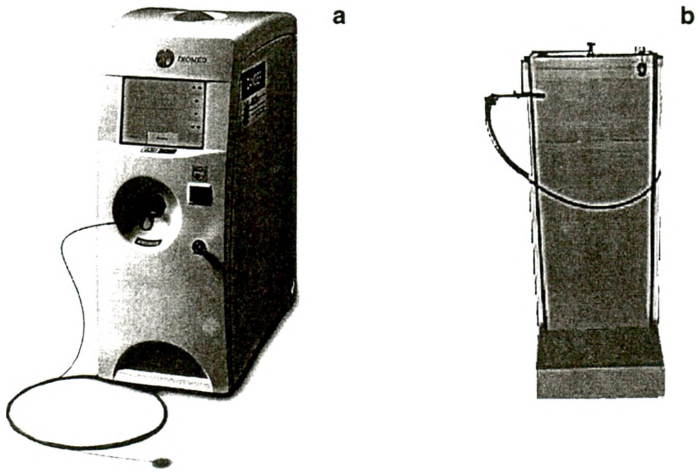

Fig. 1. Laser sources for PDT method: semiconductor laser, 630 nm (2 W), Diomed (**a**); He-Ne laser, 633 nm (200 mW) (**b**).

with dye lasers it can be accepted that they emit radiation at one constant frequency. Important advantages of diode sources are also their high efficiency and low weight, small dimensions, and easy service and transport.

Semiconductor lasers are easily excited with electric current and they show high quantum yield (high efficiency of conversion of electric energy into a radiant one), high reliability, and durability.

The basic drawback of radiation emitted by semiconductor lasers is the higher divergence of a beam compared with other lasers. It results from the shape and transverse dimensions of a generating p-n junction. Oval shape of transverse distribution of radiation of these lasers requires correction with special optical systems. Semiconductor lasers can generate radiation within visible range (635, 652–690 nm), near IR (780–960 nm), and mid IR (up to 12 μm). Figure 1 shows exemples of applications of laser sources.

2.2. Non-laser sources

Two main non-laser sources are known. The light of high-brightness lamps is filtered for specific bands of wavelengths and can be configured either for direct-beam illumination or fibre-optic delivery. Such sources have sufficient power and their wavelengths can be easily changed.

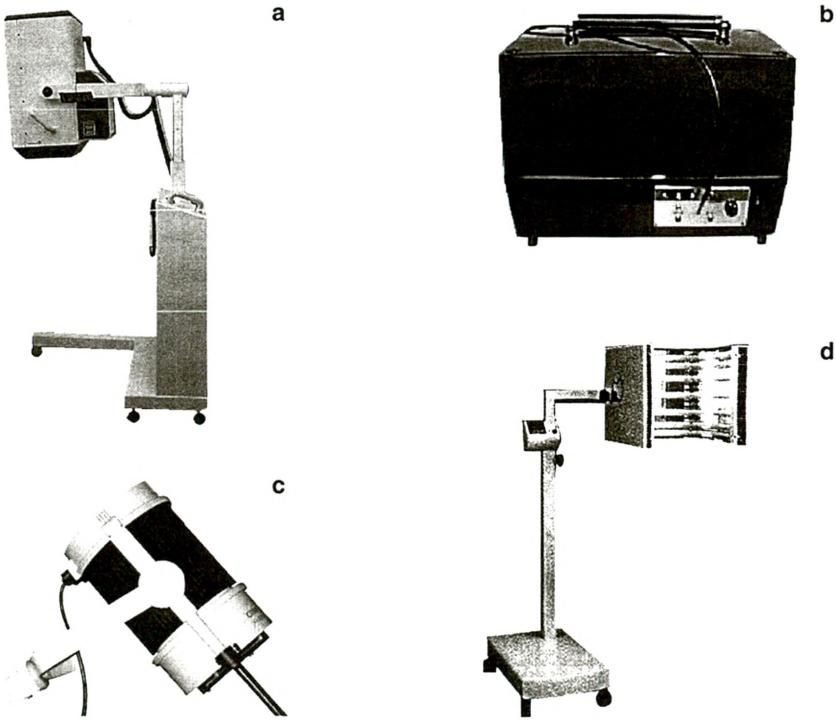

Fig. 2. Non-laser sources for PDT: Waldmann (**a**), xenon arc (**b**), Cosmetico (**c**), fluorescent lamp system for superficial PDT of scalp and face (Dusa) (**d**).

In dermatology, non-coherent light sources are commonly used, *i.e.*, halogen, xenon, metal halogen, lithium and iodine lamps. These lamps deliver red light of the power 500–1000 mW with the filter half-width of 25 nm. They are cheap (up to $ 10000), easy to use, and reliable. At the Institute of Optoelectronics, Military Academy of Technology, Poland, several illuminators were designed, including xenon, light emitting diode (LED), halogen, and metal halogen ones. The light emitted by xenon arc (Fig. 2**b**) is in the spectral rage of UV, visible, and near infrared. For relatively high attenuation of the light, that is, out of the useful bands, an adequate system of light filtration is required. A quartz lens focusing quasi-parallel light beam at the input of liquid fibre is situated next to the set of filters, which ensures proper fixing of an aperture. A diameter of the core of fluid fibre is 3 mm and its outer diameter is 5 mm. One of the known metal halogen sources used in dermatology is Waldmann system (Fig. 2**a**). The total output power of the light in the spectral range of 600–700 nm is 1300 mW and maximum power density is 240 mW/cm^2. A similar illuminator, but of smaller dimensions, was constructed in co-operation with Cosmetico firm. It includes exchangeable filters and is used for tumour diagnosis and therapy (Fig. 2**c**). Another source designed for specific clinical applications is a blue fluorescent lamp for treatment of actinic keratosis of scalp and face (Fig. 2**d**).

Alternatively, the arrays of LEDs can provide surface illumination with adequate power and bandwith and their shapes can be changed for particular applications. The value of a wavelength is constant.

For superficial illumination, the illuminator is constructed with electro-luminescence diode matrix and it has been developed for many years in several research centres. Due to stability of its operation and low price this illuminator is treated as promising source of red light for application in surgery.

Two types of LED illuminator were constructed. One of them has the output power of 1200 mW and maximum power density 50 mW/cm^2. The system consists of 500 diodes placed on a spherical cap, which ensures maximum focusing of 5 cm beam, so it can be used for illumination of large areas.

LED illuminators in which light enters the fibre of 5 mm in diameter, are used in gynaecology and laryngology. To introduce the light into a fibre, a new solution was applied. 250 diodes were spaced on a plate surface and in front of them a focusing lens was placed (Fig. 3). Radiation was incident on a mirror-lens and after reflection it was focused on a lens situated at the fibre input. Such a construction ensures correction of calculated optical system. The maximum power obtained at the fibre output was 200 mW. It is a source convenient for use in the treatment of lesions with the area of up to 5 cm^2.

LED sources have also been used in intraoperative applications, with the source at the distal end of an applicator or even interstitially.

Recently, also novel and low cost designs of lamps have been introduced that are based on high-pressure lithium-iodide or neon large-arc lamps providing very high continuous power level in a relatively narrow wavelength band.

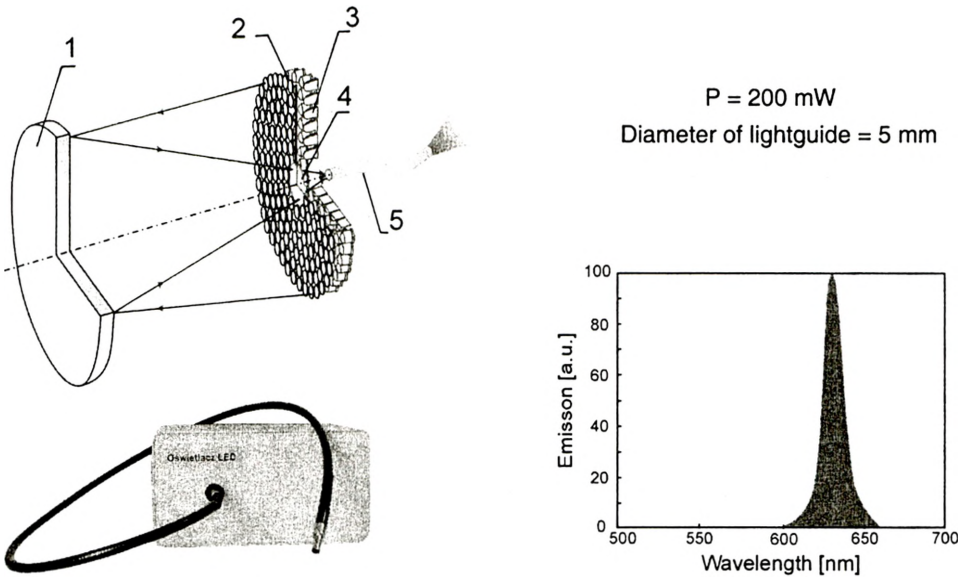

Fig. 3. LED array source: 1 – mirror-lens, 2 – matrix of collimating lenses, 3 – matrix of diodes, 4 – collimator, 5 – light guide.

With the increasing variety of sources, an important issue is their photobiological equivalence. A given fluence delivered from broadband sources may be significantly less effective than that from a laser with monochromatic light at the peak photosensitiser activation spectrum (Fig. 4). Effective light absorption coefficient can be expressed as:

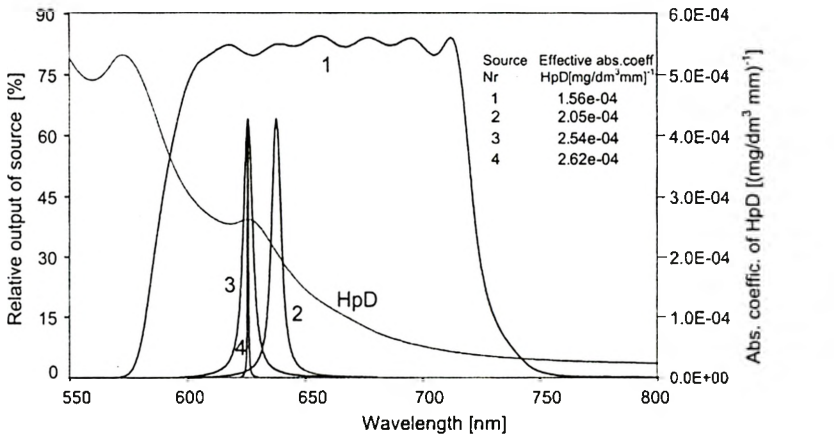

Fig. 4. Absorption band hematoporphyrin derivatives (HpD) used for therapy, emission characteristics of various light sources and calculated effective absorption coefficient.

$$\varepsilon_\lambda(s) = \frac{\int_{\lambda_1}^{\lambda_2} \varepsilon(\lambda) I(\lambda) \mathrm{d}\lambda}{\int_{\lambda_1}^{\lambda_2} I(\lambda) \mathrm{d}\lambda}$$

where $\varepsilon(\lambda)$ is the absorption coefficient of a photosensitiser, $\varepsilon_s(\lambda)$ is the effective absorption coefficient, and $I(\lambda)$ is the light intensity.

On the other hand, a high fluence rate of pulsed laser sources may also make the same total light fluence less effective. The factors causing this effect are: decrease in oxygen concentration, saturation of the ground-state photosensitiser absorption, transient photobleaching or even two-photon excitation.

3. General characteristics of detection systems

The intensity of fluorescence of excited biological tissues is low because of relatively low quantum yields of fluorescence of chromophores as well as limited quantity of excitation light which can cause photobleaching and photodynamic effects. Thus, the equipment used should be of high sensitivity and there must be good conditions for background elimination. The easiest way to obtain this is to light up the tissue exclusively with the excitation light, which will be carefully separated in the emission spectra. Another way is to modulate fluorescence signals in time with temporal background filtration. The method of time-resolved fluorescence eliminates influence of background [5]. Biological material is excited with pulsed lasers of a bandwidth of every single nanosecond and fluorescence is collected with very short gating times.

Detection systems are still being improved together with technological progress. In general, they can be divided into the systems for registration of fluorescence spectra from one point or for imaging of fluorescence areas. The spectrum of the tissue *in vivo* may be obtained in spectrofluorometer with the introduced optical fibre sensor used for fluorescence excitation and collection or using linear photodiode array for emission measurement, simultaneously for the whole spectrum. To distinguish the tissues, based on the spectra, a method of differential fluorescence is applied, *e.g.*, defining ratios at two selected wavelengths. It enables us to eliminate such factors as intensity of light excitation, measurement geometry, loss of signals on the elements of apparatus.

The CCD intensified camera itself, or in connection with image intensifiers, records images. In addition, to compile an image of two different wavelengths the filter wheel or a system of two cameras working at the same time is used. An image can be divided into a few colourful images using optical system.

The present technology makes it possible to connect conventional imaging and spectroscopy [6]. Spectral bio-imaging is a powerful method for measuring the

spectrum of light in every element of two-dimensional image (each pixel of an array detector contains a diagnostic spectrum). This new method is particularly useful for spectral identification of multiple fluorophores, detection of micro-environmental changes in sub-cellular compartments. The advantages of spectral bio-imaging are: measurements of the whole spectrum, separation and mapping of many spectrally overlapping fluorescent probes, ability to eliminate undesirable background luminescence, spectral identification of multiple fluorophores and their relative concentration in single measurements [7]. The spectra cube TM method combines Fourier spectroscopy with a CCD array detector application. The fluorescence light emitted by a sample is collected in the fore-optics and enters the Sagnac interferometer. The real image is focused on the CCD array, yielding the spectrum at each pixel [7].

Such a sophisticated apparatus is not required in every case of fluorophores location. When porphyrins of concentration of several mg/kg of the body are used, their accumulation in the skin malignancies can be observed with a single CCD camera with an image intensifier and an appropriate red filter. The areas of tumour tissues and surrounding healthy tissue were simultaneously illuminated with blue radiation (titanium laser) and the signal from illuminated areas was registered by CCD camera in red band. The image analysis makes it possible to quantitatively compare background fluorescence intensity with the intensity of endogenous photosensitiser fluorescence.

4. Review of diagnostic apparatus

The first research works on location of the lung tumours using hematoporphyrin derivatives (HpD) and mercury-arc lamp were taken at the Mayo Clinic (USA) in 1960 [8]. Research on application of lasers for diagnostics of early tumours was started by PROFIO *et al.* [9]. Today, several teams all over the world apply the laser-induced fluorescence method. Many versions of the systems for emission spectra measurement or fluorescence areas were built. Several sources of violet light were used in the systems, *i.e.*, high-pressure mercury lamps, xenon lamp with the filter systems or nitrogen-dye, titanium, helium-cadmium, (442 nm), krypton (407 nm), argon (488 nm, 514 nm), helium-neon (633 nm) lasers.

Most of currently used diagnostic systems are based on registration and analysis of the whole fluorescence spectrum. For instance, such a solution has been taken advantage of at the Lund University of Technology Clinic [5]. The excitation light from the pulsed nitrogen-dye laser (337 nm or 405 nm) is focused into a 600-μm thick optical fibre. The fluorescence light is captured by the same fibre and separated from the excitation light by a dichroic mirror and then focused onto the slit of the multichannel analyser system.

The aim of our work was designing, performance, and clinical testing of the sets intended for diagnostics of tumours of skin and inner organs. For excitation of endogenous and exogenous fluorophores emission, the simple lasers, reliable in use, were applied. These were helium-cadmium lasers (He-Cd, 442 nm), helium-neon laser

(He-Ne, 632.8 nm), Nd:YAG laser generating II harmonic (532 nm) and blue semiconductor lasers (10–25 mW). Selection of these sources should enable precise detection of exogenous photosensitisers of the 1st and 2nd generation and autofluorescence changes in tumour tissues without administration of endogenous photosensitisers. Proper choice of various wavelengths within the range of porphyrin excitation depends on varying penetration of light into different tissues. For tumours localised under mucous membrane, blue radiation is used for excitation and for deeply localised diseased changes much more adequate is radiation of longer wavelength.

General scheme of the sets built is shown in Fig. 5. In fluorescence spectrometers, the detection system is polychromator with an array of detectors. A laser source equipped with power supply, diaphragm, adapter of fibre input of diameter 180 μm, and fibre sensor (catheter) is applied. Catheter consists of a concentric fibre for introduction of laser light and six fibres for fluorescence light receiving. A laser radiation is scattered on tissue and fluorescence light is incident on a polichromator slit. Laser light is a convenient reference signal by means of which fluorescence intensity can be normalised, depending on intensity of excited light and measurement geometry. This light is reflected from the object under investigation and incident on a detector being 10^4 times weakened. The ratio of the surface of laser emission band and fluorescent object is dependent only on intensity of fluorophore concentration. The light, after splitting on a polychromator, reaches detector array consisting of

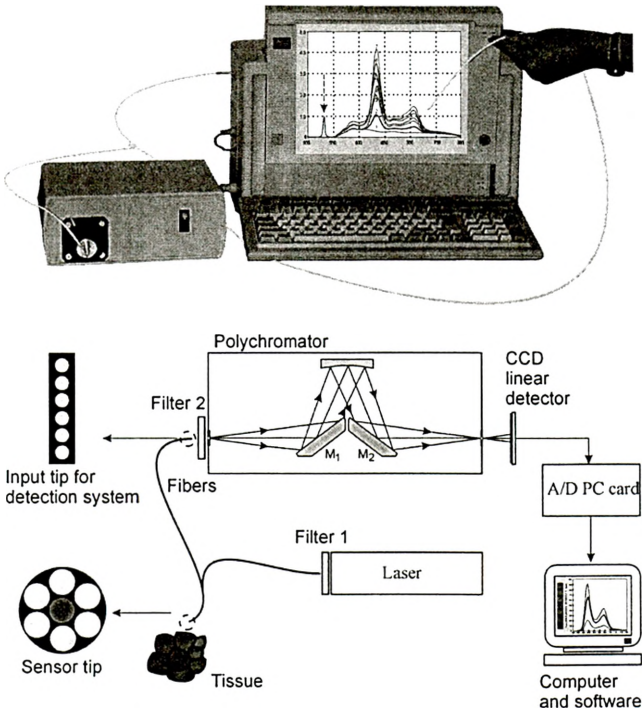

Fig. 5. Setup and view of fiberoptic fluorescence spectrometer.

1024 elements. The fibre spectrometer of the BIOSPEC firm (Russia), installed on the computer card (ISA) with software controlling measurements and spectra processing, makes it possible to simultaneously record the whole spectral range of the analysed fluorescence spectrum. The measurements can also be performed inside a human organism. Fibres are constructed so that they can be positioned in endoscopes. Small dimensions of the sets and their easy exploitation enable their transport and operation in medical clinics.

Imaging systems fulfil fundamental role in clinical diagnosis. They can detect both endogenous and exogenous fluorophores.

A comparison of autofluorescence, induced by the He-Cd laser (442 nm) in green and red part of the spectrum, was carried out by the research staff of the British Columbia Centre Agency in collaboration with the Xillix Technology Corporation in Vancouver [10], [11] to give rise to LIFE (lung induced fluorescence endoscopy) system (Fig. 5). The system consists of a typical fibreoptic bronchoscope, He-Cd laser (442 nm, 150 mW), two image-intensified CCD image cameras with green and red filters, and a computer with an imaging card. A decrease in autofluorescence in "green" and smaller in "red" regions of the spectrum was observed. The imaging card simultaneously captures the fluorescence images of the same tissue area. These images are then combined and processed by the imaging card using a specially developed algorithm for distinguishing between healthy and tumour tissue.

Recently, the clinical research group in Lausanne in collaboration with Richard Wolf Endoskope GmbH have developed fluorescence device for detection of bronchial tumours [12]. The detection system includes one sensitive black-and-white CCD camera. Excitation of fluorescence is achieved with a filtered 300 W xenon lamp in violet-blue range. Two images are created on the chip, one for "green" image and second for "red" one. A decrease in autofluorescence is observed in green image and a smaller decrease in red region of the spectrum. After separation into two channels, a video chip delays the corresponding two video signals. The delay is different for the two channels, so superimposition of the images is possible [8].

The first solution of early tumour diagnostics in urology has been implemented at the Urology Clinic in Munich [13]. The protoporphyrins IX (PPIX accumulation) in lesion tissues are synthesised by introducing the water solution of the 5-amino-levulinic acid (3%) to the urinary bladder. The acid is a precursor of the PPIX synthesis. The light of a krypton laser (407–413 nm, coherent) has been introduced to the 500-μm-fibre optic with the double-cone ending ensuring homogeneous illumination of the bladder surface. For registration of fluorescence a special colour video camera was used (Storz, Tuttlingen, Germany). At present, Storz firm designed very efficient xenon source (D-light) with a blue filter for fluorescence imaging. This system is commonly used for PPIX detection. It is based on colour contrast fluorescence imaging. Monochrome fluorescence observation of PPIX in a region of 600–700 nm does not ensure high contrast. However, combination with the reemission image (blue) and the autofluorescence (green) provide reliable information on the existence and localisation of the tumour [14].

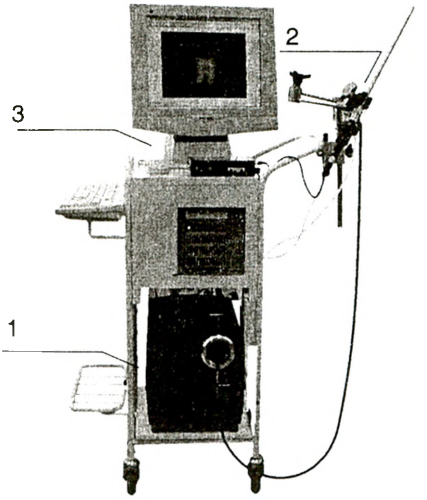

Fig. 6. Equipment for fluorescent imaging (GIN-1): 1 – xenon arc lamp (405 nm, 180 mW; 630 nm, 500 mW), 2 – colour CCD camera and endoscope, 3 – PC and software.

Similar system for gynaecological photodiagnosis was built at the Institute of Optoelectronic, Military Academy of Technology, Poland (Fig. 6). Also other methods of selective separation of porphyrin area were tested.

Separation of the background signal from the endogenous dyes was performed by the Italian group [15] using measurement of the time-gated fluorescence method. The fluorescence derived from endogenous porphyrins is characterised by a lifetime shorter than 6 ns, when the lifetimes of the introduced exogenous hematoporphyrins are longer, *i.e.*, about 15 ns. The measurement of fluorescence signal with an appropriate delay eliminates the background and enforces contrast between the healthy and tumour tissues. The light source used in the method is a pulse dye laser (395 nm, lifetime of a pulse <1 ns, repetition rate 50 Hz) pumped by a nitrogen laser. The fluorescence image is obtained using the SIT camera connected to an image intensifier, synchronised with the laser pulse. After laser excitation pulse with the duration of 1 ns, the CCD camera is opened after 10 ns and fluorescence area appears only from the introduced porphyrin. Minimum time of the intensifier opening, after the laser pulse, is 5 ns.

5. Conclusions

The review showed how many optoelectronic devices are used for PDT method. Application of pulse lasers, CW lasers, and various lamps makes it difficult to standardise the method which, for majority of applications, is still an experimental one. The basic factor limiting standardisation of devices are their high prices. At present, a tendency is clearly observed towards application of semiconductor lasers, excluding dermatology where lamp sources are most frequently used. Further decrease in prices of semiconductor lasers should enable us to solve the problem of therapeutic

source application in the near future as well as standardisation of devices and conditions of the experiments carried out. An important role in PDT method is played by diagnosis resulting in monitoring of a photosensitiser before and after irradiation. Photodynamic therapy is often repeated many times because there is no information of actual concentration of photosensitiser in a tissue. Kinetics of accumulation and extraction of compounds is decisive for selection of adequate irradiation time after photosensitiser application. It is also very important to check photochemical distribution of dyes under influence of radiation and regulation of therapeutic light intensity. Fluorescence analysers appear to be ideal tools for checking actual concentration of photosensitisers. Due to their low prices they should be included in the standard equipment of each team carrying out treatment with PDT methods.

References

[1] WILSON B.C., Photodynamics News **1** (1998), 6.
[2] MIERCZYK Z., KWAŚNY M., *Laser system for tumors diagnostics and theraphy used in photodynamic method*, [In] *Photodynamic Method for Tumor Diagnostics and Therapy*, [Ed.] A. Graczyk, Bellona, Warszawa 1999, pp. 244–299 (in Polish).
[3] KONAKA C., KATO H., HAYATA Y., Lasers in Medical Science **2** (1987), 17.
[4] KWAŚNY M., MIERCZYK Z., GIETKA A., GRACZYK A., DOMANIECKI J., Proc. SPIE **3188** (1997), 48.
[5] ANDERSSON-ENGELS S., JOHANSSON J., SVANBERG K., SVANBERG S., Photochem. Photobiol. **53** (1991), 807.
[6] TREADO P.J., Laser Focus World **10** (1995), 75.
[7] GARINI Y., KATZIR N., CABIB D., BUCKWALD R.A., *Spectral bio-imaging* [In] *Fluorescence Imaging Spectroscopy and Microscopy*, [Ed.] Xue Feng Wang, Brian Herman, Chemical Analysis Series Vol. 137, Wiley, New York 1996, 87–124.
[8] SANDERSON D.R., FONTANA R.S., LIPSON R.L., BALDES E.J., Cancer **30** (1972), 1368.
[9] PROFIO A.E., DOIRON D.R., KING E.G., Med. Phys. **6** (1979), 523.
[10] LAM S.C., MACAULAY C., LERICHE J., IKEDA N., PALCIC B., Proc. SPIE **2324** (1995), 2.
[11] LAM S., MACAULAY C., HUNG J., LERICHE J., PROFIO A.E., PALCIC B., J. Thorac. Cardiovasc. Surg. **105** (1993), 1035.
[12] GOUJON D., ZELLWEGER M., VAN DEN BERGH H., WAGNIERES G., Photodynamic News **3** (2000), 11.
[13] STEPP H., BAUMGARTNER R., BEYER W., KNUECHEL R., RICK K., STEINBACH P., STEPP H.G., KRIEGMAIR M., Proc. SPIE **2324** (1995), 20.
[14] STEPP H., Photodynamic News **3** (1998), 2.
[15] CUBBEDU R., CANTI G., TARONI P., VALENTINI G., [In] *Potodynamic Therapy and Biomedical Lasers*, Excerpta Medica, Amsterdam 1992, pp. 882–892.

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