

PACS numbers: 87.64.Ni; 42.62.Be
DOI: 10.1070/QE2002v032n11ABEH002329

Combination of fluorescence imaging and local spectrophotometry in fluorescence diagnostics of early cancer of larynx and bronchi

V.V. Sokolov, E.V. Filonenko, L.V. Telegina, N.N. Boulgakova, V.V. Smirnov

Abstract. **The results of comparative studies of autofluorescence and 5-ALA-induced fluorescence of protoporphyrin IX, used in the diagnostics of early cancer of larynx and bronchi, are presented. The autofluorescence and 5-ALA-induced fluorescence images of larynx and bronchial tissues are analysed during the endoscopic study. The method of local spectrophotometry is used to verify findings obtained from fluorescence images. It is shown that such a combined approach can be efficiently used to improve the diagnostics of precancer and early cancer, to detect a primary multiple tumours, as well as for the diagnostics of a residual tumour or an early recurrence after the endoscopic, surgery or X-ray treatment. The developed approach allows one to minimise the number of false-positive results and to reduce the number of biopsies, which are commonly used in the white-light bronchoscopy search for occult cancerous loci.**

Keywords: *laser medicine, fluorescence diagnostics, photosensitisers*.

1. Introduction

The fluorescence diagnostics (FD) of cancer is based on two effects: (i) the selective accumulation of photosensitisers in malignant neoplasms and their detection by characteristic laser-induced fluorescence and (ii) the difference in the intensity and spectra of the autofluorescence (endogenous fluorescence) of healthy and pathologic tissues excited by lasers in the visible or UV spectral ranges. The FD of tumours has been extensively developed in the last decades both in our country and abroad due to the use of different photosensitisers and advances in fibreoptics and optoelectronic instrumentation.

V.V. Sokolov, E.V. Filonenko, L.V. Telegina P.A. Hertsen Moscow Research Oncological Institute, Botkinskii per. 3, 125284 Moscow, Russia; Tel.: (095) 945 88 07; Fax: (095) 945 68 32;
e-mail: vik123@online.ru;
N.N. Boulgakova, V.V. Smirnov A.M. Prokhorov General Physics Institute, Russian Academy of Sciences, ul. Vavilova 38, 119991 Moscow, Russia; Tel./Fax: (095) 135 02 97;
e-mail: koker@kapella.gpi.ru

Received 23 May 2002
Kvantovaya Elektronika **32** (11) 963–969 (2002)
Translated by M.N. Sapozhnikov

2. Fluorescence diagnostics with the use of photosensitisers and endogenous fluorochromes

Clinical FD studies of cancer were initiated at P.A. Hertsen Moscow Research Oncological Institute in cooperation with A.M. Prokhorov General Physics Institute, RAS (Department of Optical Spectroscopy) in 1992, when first clinical tests of the method of photodynamic therapy (PDT) were allowed in Russia. At present, a considerable experience in the fluorescence detection of the early forms of malignant tumours of respiratory ways and digestive tract has been accumulated: over 1000 studies have been performed using such photosensitisers as Photoheme, Photosense, and Alasense [1–3].

Alasense (5-aminolevulinic acid, 5-ALA) proved to be most convenient for the FD. The administration of 5-ALA (which is a precursor in the synthesis of heme) to organism results in the selective accumulation of an endogenous photosensitiser protoporphyrin IX in cancerous cells [4]. The accumulation of protoporphyrin IX in the tumor occurs for several hours, and it remains in cells for 1–2 days. Upon excitation into one of the absorption bands in the blue or green spectral regions, protoporphyrin IX exhibits two intense fluorescence bands in the red region at 635 and 700 nm. This makes it possible to obtain a high contrast between the intensities of fluorescence of the tumour and a surrounding healthy tissue. This effect is employed in the 5-ALA-induced FD.

During the accumulation of experience in the field of the FD with selective photosensitisers, a number of restrictions in the application of this method in screening diagnostic tests have been revealed [5]. This rekindled interest in the study of the difference in the autofluorescence of malignant tumours and normal tissues excited by laser radiation without administration of photosensitisers to organism.

The autofluorescence spectrum of tissues in the visible range is determined by certain biological molecules (endogenous fluorochromes), which fluoresce at different wavelengths. Flavins, porphyrins, NADH, and some other fluorochromes exhibit fluorescence in the visible range upon excitation in the blue region [6].

The autofluorescence of malignant tumours of a human in the red spectral region was first described by Policard in 1924 [7]. He assigned the autofluorescence to endogenous porphyrins located in tumours. Modern studies have shown

that endogenous porphyrins can be accumulated in some types of malignant tumours at the late stages of their development, especially, during their necrosis and metastasis [8]. Lam et al. [9] have shown that pathologic loci at the stages of precancer and early cancer are characterised by a drastic decrease in the autofluorescence intensity in the visible range compared to fluorescence of surrounding healthy tissues.

At present, the most comprehensive data on clinical autofluorescence diagnostics have been obtained for lung cancer. The precancer pathology and early forms of bronchial cancer have, as a rule, small size (1 – 10 mm) and thickness (200 – 300 μm), so that the probability of their detection by the method of conventional white-light bronchoscopy is only ~ 36 %. A research group in Canada showed in 1992 [9] that local measurements of the autofluorescence of tissues excited at 442 nm enhance the sensitivity of the detection of early cancer during autofluorescence bronchoscopy up to 86 %.

To study the possibilities of this method, a LIFE System (Lung Imaging Fluorescence Endoscopy System, Xillix Technology Co. Canada) was developed to image the fluorescence of the surface of a bronchial mucosa. In 1994 – 1999, seven medical centres in the USA and Canada performed extensive clinical tests of autofluorescence bronchoscopy in the diagnostics of early lung cancer using this bronchoscope [10]. The autofluorescence images of bronchial tissues were detected in the spectral range from 480 to 800 nm by exciting fluorescence with a 442-nm He – Cd laser. After computer processing and normalisation of the video images, a pseudo-image was formed intended for diagnostic analysis. This method did not involve local spectral fluorescence measurements. The results of more than 1000 clinical studies performed with the help of the LIFE System are reported in the literature. It was shown that, while the white-light bronchoscopy is capable of detecting only about 40 % of early bronchial cancer, the autofluorescence bronchoscopy provides the detection of about 80 % [10 – 12].

The LIFE System was also used for the autofluorescence diagnostics of larynx cancer [13]. It was shown that autofluorescence diagnostics has a very high sensitivity (above 90 % of malignant tumours were detected). However, a great number of false-positive autofluorescence observations were noted, which were caused by chronic laryngitis accompanied by the development of cancer.

Therefore, the FD of larynx and bronchial cancer based on the analysis of the autofluorescence or 5-ALA-induced fluorescence images has a high sensitivity but is not specific enough. Despite the fact that many authors note the possibility to quantify the images obtained with the help of spectral fluorescence measurements, there are few papers where both approaches were realised [14].

In this study, we present the first results of the clinical application of a spectral fluorescence diagnostic setup in combination with fluorescence bronchoscopy. Thus, we combined two approaches, namely, the detection and analysis of fluorescence images (autofluorescence and 5-ALA-induced images) and the local spectrophotometry of biological tissues. We examined 37 patients from an oncology risk group with the aim of detecting occult forms of larynx and bronchial cancer. For ten patients, the FD was performed many times in the process of dynamic observation for one-two years.

The obtained results are, undoubtedly, preliminary because of a limited number of patients involved. However, they have shown a high correlation (more than 90 %) with the data of morphological analysis of a biopsy material, which demonstrates the reliability and efficiency of fluorescence methods for diagnostics of larynx and bronchial cancer.

3. Materials and methods

3.1 Equipment for fluorescence imaging

We detected fluorescence images in real time during laryngoscopy and bronchoscopy with the help of a D-Light/AF System flexible fluorescence bronchoscope (Karl Storz GmbH, Germany) [15], which allows endoscopic studies in white light, in the autofluorescence emission of tissues, and fluorescence of protoporphyrin IX excited by blue light in the regions 380 – 460 nm and 380 – 440 nm, respectively. The excitation source in this system was a xenon lamp with a set of light filters.

3.2 Equipment for recording fluorescence spectra

We performed the local spectrophotometry of mucosa of respiratory ways using a Spektr-Cluster fluorescence diagnostic setup developed at GPI, RAS jointly with MROI. This setup contains a fibreoptic device to deliver laser radiation and collect fluorescence emission, a spectrograph, a multichannel linear photodiode array, a PC, and lasers for excitation of fluorescence [16, 17]. In this paper, we used a 442-nm He – Cd laser because it provides efficient excitation of both tissue autofluorescence and fluorescence of induced protoporphyrin IX. At the same time, radiation of this laser is weakly absorbed by hemoglobin in the tissue.

To deliver laser radiation to the tissue and fluorescence emission to a photodetector, a Y-shaped ring catheter consisting of silica fibres is introduced into the biopsy channel of the endoscope. One of the ends of the catheter is a single fibre going from the laser to the tissue. Another end of the catheter represents a bundle of fibres going from the tissue to the spectrograph. At the third end of the catheter, which is located directly near the tissue, all the fibres are collected, the fibres for delivering fluorescence emission being located around the central fibre through which laser radiation is delivered. The spatial resolution during scanning of the tissue surface is determined by the diameter of a spot of diffusely scattered laser radiation and is ~ 1 mm, which allows one to determine the tumour boundary quite accurately. The fluorescence spectra are analysed with a diffraction grating polychromator with a reciprocal linear dispersion of 35 nm mm^{-1} in the spectral range from 350 to 800 nm. The spectra are detected with a multichannel photodetector and are processed with a PC. The spectral fluorescence diagnostic parameter D_f [18] calculated using a special algorithm, which allows the quantitative estimates and verification of fluorescence images, is displayed at the computer monitor in real time as well. It is defined as $D_f = I_{620-650}/I_{500-520}$, where $I_{620-650}$ and $I_{500-520}$ are the integrated intensities of fluorescence detected in the ranges 620 – 650 nm and 500 – 520 nm, respectively.

The step-by-step protocol of fluorescence detection was described in detail in paper [19]. To identify suspicious sites detected during fluorescence bronchoscopy, a laser radiation

spot was scanned along the surface of a suspicious site, its boundary, and surrounding healthy tissues. The detected spectra were displayed in real time on the computer monitor, and the diagnostic parameter D_f was monitored. All the spectra were recorded at the instant of contact of the catheter end with the surface of the mucosa. On average, about 30 fluorescence spectra were recorded during each examination and the parameter D_f was fixed for all the types of tissues studied. All fluorescence loci with abnormal average values of D_f were subjected to biopsy and morphological study.

It should be emphasised that it is very important to maintain invariable excitation conditions during *in vivo* measurements of fluorescence spectra. Therefore, a certain laser radiation power was rigorously maintained, which was measured at the end of the fibreoptic catheter, and each spectrum was detected when the catheter end was in contact with the tissue surface. This is especially important for the fluorescence studies of a tracheobronchial tree, when measurements performed tangentially to the tissue surface inevitably distort the intensity of spectra being detected.

4. Results and discussion

4.1 Autofluorescence

We observed the intense green autofluorescence of the normal larynx and bronchial tissues excited in the blue spectral region. At the same time, the intensity of autofluorescence of precancer and early cancer in the mucosa was substantially lower than that of the surrounding healthy mucosa. The data of local spectrophotometry in the 460 – 800-nm region confirmed drastic differences between the intensities of autofluorescence of healthy and pathological tissues (Fig. 1). Therefore, a typical autofluorescence image of tumours of a tracheobronchial tree detected in the endoscopic study is a dark spot against the bright green autofluorescence of a healthy tissue.

We observed for the first time a drastic decrease in the intensity of autofluorescence of tumours excited in the green region at 512 nm during clinical tests of the first domestic photosensitiser Photoheme in 1992 – 1994 by studying the selectivity of its accumulation in malignant neoplasms [20]. We performed spectral fluorescence clinical tests for more than 200 patients with differently localised tumours. A drastic decrease in the autofluorescence intensity of malignant tumours was observed for all patients, which allowed us to develop the endoscopic fluorescence method of malignant tumours of hollow internal organs [17].

Although the mechanisms of this effect are not studied in detail, we can make the following assumptions: first, the amount of fluorochromes in the tumour tissue is decreased and (or) their functional states, determined by the properties of redox processes, are changed; and second, the optical properties of the tumour tissue change due to an increase in the concentration of molecules strongly absorbing light in the visible spectral region [21]. It is known that the penetration depth of the UV radiation in tissues is 1 – 10 μm, whereas the penetration depth of green radiation is no more than 1 mm. The fluorescence study of the frozen slices of a bronchial tissue showed [22 – 24] that the epithelial layer of the bronchial mucosa (both in norm and for different pathologies) fluoresces very weakly, whereas the submucous layer exhibits bright fluorescence, although the fluorescence intensity of its surface layer is substantially greater than that of the underlying layer.

We assume that the decrease in the autofluorescence intensity of cancerous mucosa of larynx and bronchi is mainly caused by the thickening of epithelium in the precancer and early cancer loci. The theoretical calculations [22] of the autofluorescence intensity of a bronchial mucosa showed that, when the thickness of a respiratory epithelium increases from 50 to 200 μm, the autofluorescence intensity can decrease by a factor of 2.5. In actual practice, the bronchial epithelium thickens more than by 200 μm, 'quenching' in fact the intense fluorescence of the submucous layer, i.e., the mucosa loses its transparency in the site of the malignant tissue, and the autofluorescence image in this site is darkened.

Fig. 1* shows a typical example of the autofluorescence laryngoscopy of a patient suffering from chronic laryngitis. The white-light laryngoscopy images of both vocal cords are uniformly thickened, whereas the autofluorescence image of one of the vocal cords exhibits a dark spot against the background of homogeneous green fluorescence, which is caused by a drastic decrease in the autofluorescence intensity. Biopsy and a subsequent morphological analysis showed the presence of early cancer in this site.

An extremely low level of the intensity of autofluorescence of malignant tissues allowed us to detect X-ray-negative early central lung cancer in some patients, which was invisible in the white-light bronchoscopy. Fig. 2 shows that a tumor is observed in the autofluorescence emission as a darkened region against the background of bright green fluorescence on a normal bronchial mucosa. The data of local spectrophotometry showed that the autofluorescence intensities differed by factors from 5 to 10.

At the same time, autofluorescence studies also gave false-positive results. A decrease in the autofluorescence intensity was observed at the region of resected malignant tumours after endoscopic PDT, laser destruction, and surgery. We assume that false-positive autofluorescence is caused by a drastic change in the optical properties of the tissues (traumatisation, etc.) due to these operations.

4.2 5-ALA-induced fluorescence of protoporphyrin IX

At present in Russia (State Scientific Center of Russian Federation, Research Institute of Intermediate Products and Dyes, Moscow), a standard substance 5-ALA was synthesised by the original method and a new drug Alasense based on this substance was developed. We analysed fluorescence of healthy and pathologic tissues of larynx and bronchi during endoscopic examination performed 2 – 3 h after inhalation with Alasense.

2 – 3 h after inhalation with Alasense, the tumour of a vocal cord exhibited bright red fluorescence of induced protoporphyrin IX, whereas fluorescence of a healthy vocal cord remained green (autofluorescence) (Fig. 3). Our spectral study showed that the diagnostic parameter D_f equal to 0.3 in a healthy vocal cord increased up to 15.5 in the tumour site.

The visually homogeneous red fluorescence of induced protoporphyrin IX was also observed in the case of some chronic inflammatory processes. Upon scanning a laser radiation spot and monitoring the diagnostic parameter

*You can find colour images of Figs 1 – 4 free of charge on the web-site: http://www.turpion.org/specials/qe/2002/11/2329.pdf

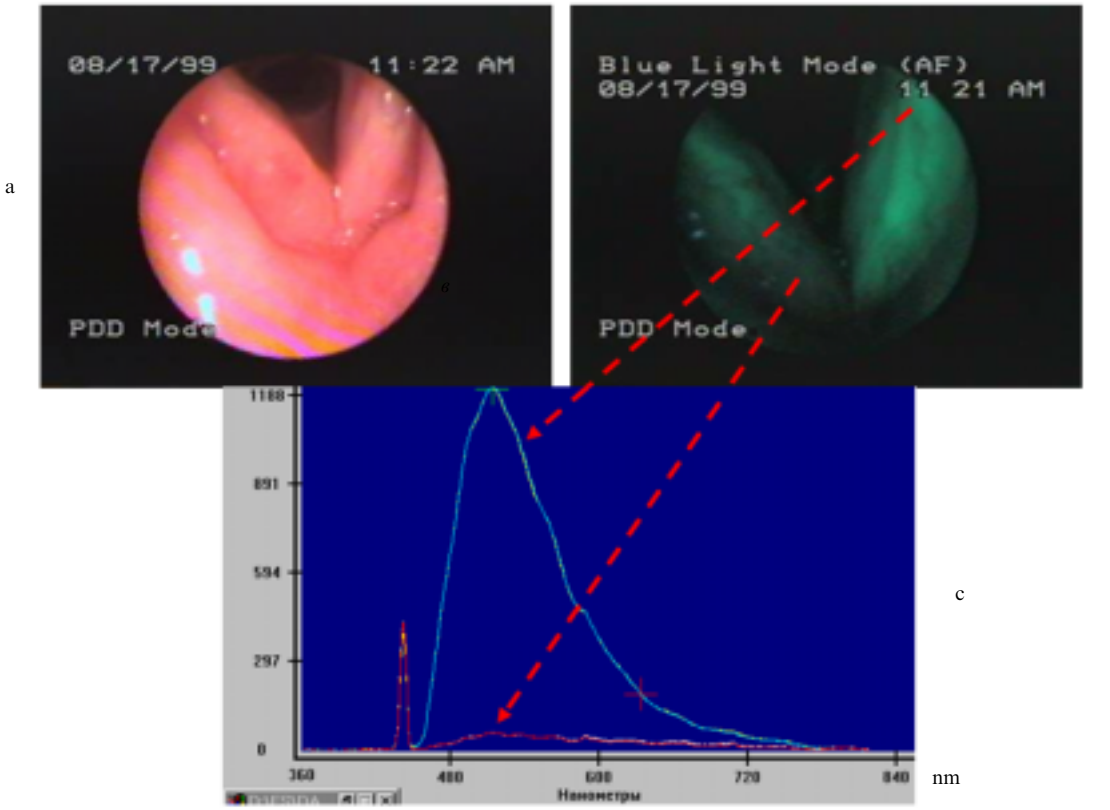

Figure 1. Autofluorescence laryngoscopy and local spectrophotometry of cancer of vocal cords *in situ* against the background of chronic hyperplastic laryngitis. The endoscopic (a) white-light and (b) autofluorescence images of the vocal cord, and (c) the autofluorescence spectra of the normal mucosa of the vocal cord and tumour excited at 442 nm. (Colour images of Figs 1–4 are available at: http://www.turpion.org/specials/qe/2002/11/2329.pdf.)

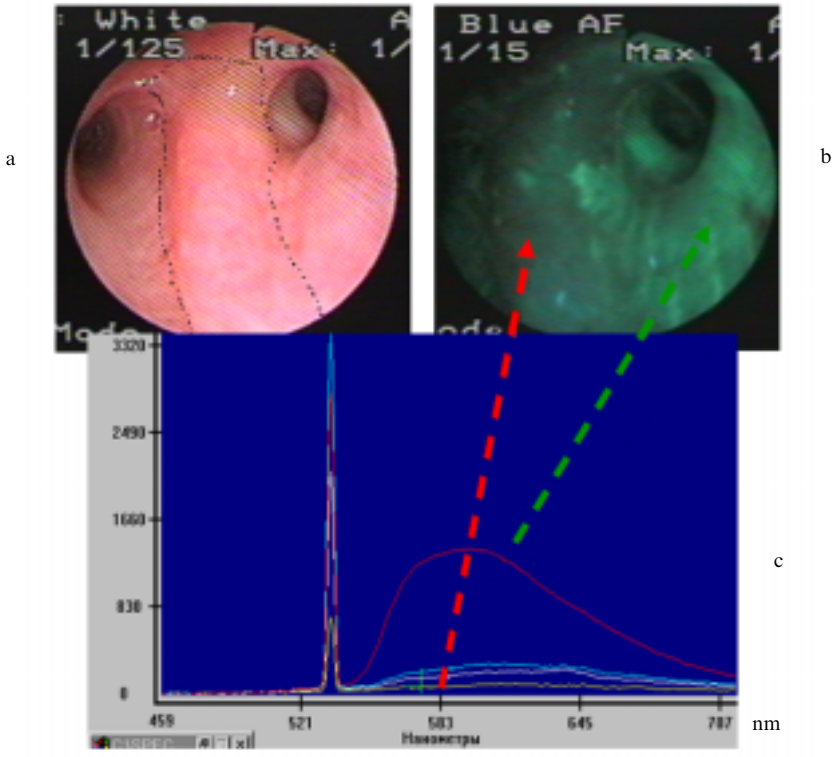

Figure 2. Autofluorescence bronchoscopy and local spectrophotometry of early X-ray-negative lung cancer (SCC RULB). The endoscopic (a) white-light and (b) autofluorescence images of the bronchus, and (c) the autofluorescence spectra of the normal mucosa of the bronchus and tumour excited at 442 nm.

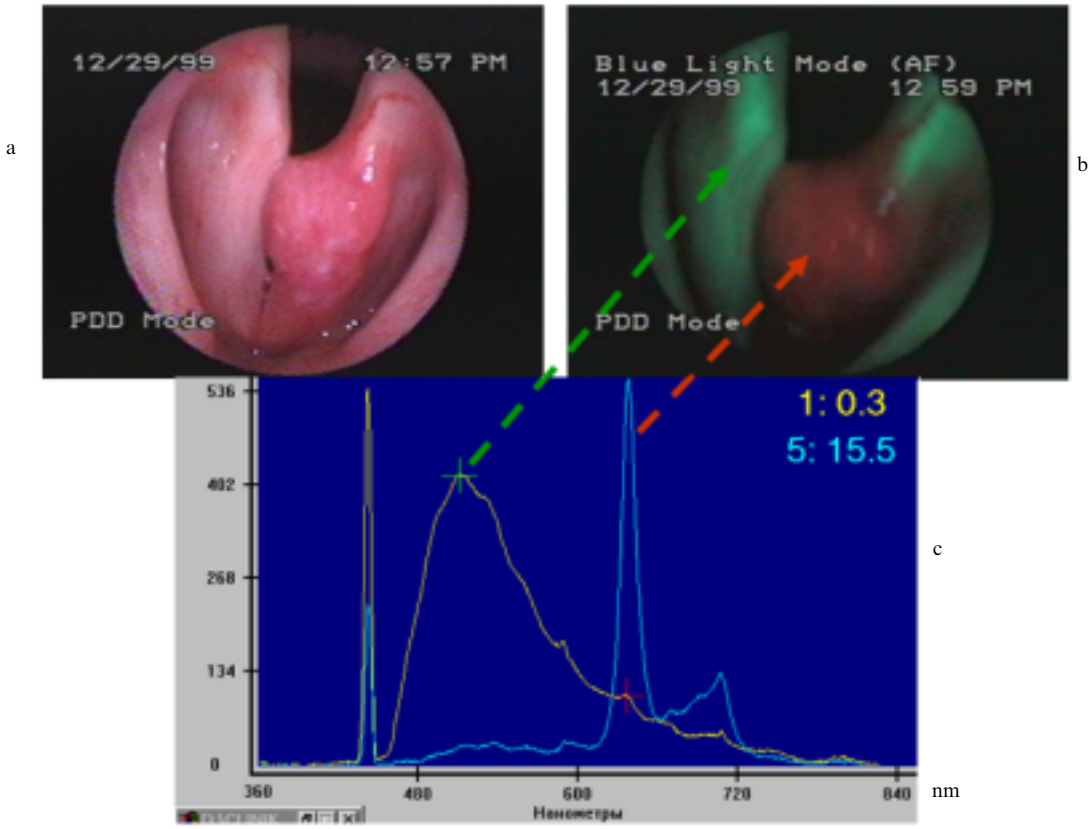

Figure 3. Fluorescence laryngoscopy and local spectrophotometry of cancer of the left vocal cord 2 h after inhalation with Alasense. The endoscopic (a) white-light and (b) fluorescence images of the vocal cord, and (c) the fluorescence spectra of the normal mucosa of the vocal cord ($D_f = 0.3$, spectrum No. 1) and tumour ($D_f = 15.5$, spectrum No. 5) excited at 442 nm.

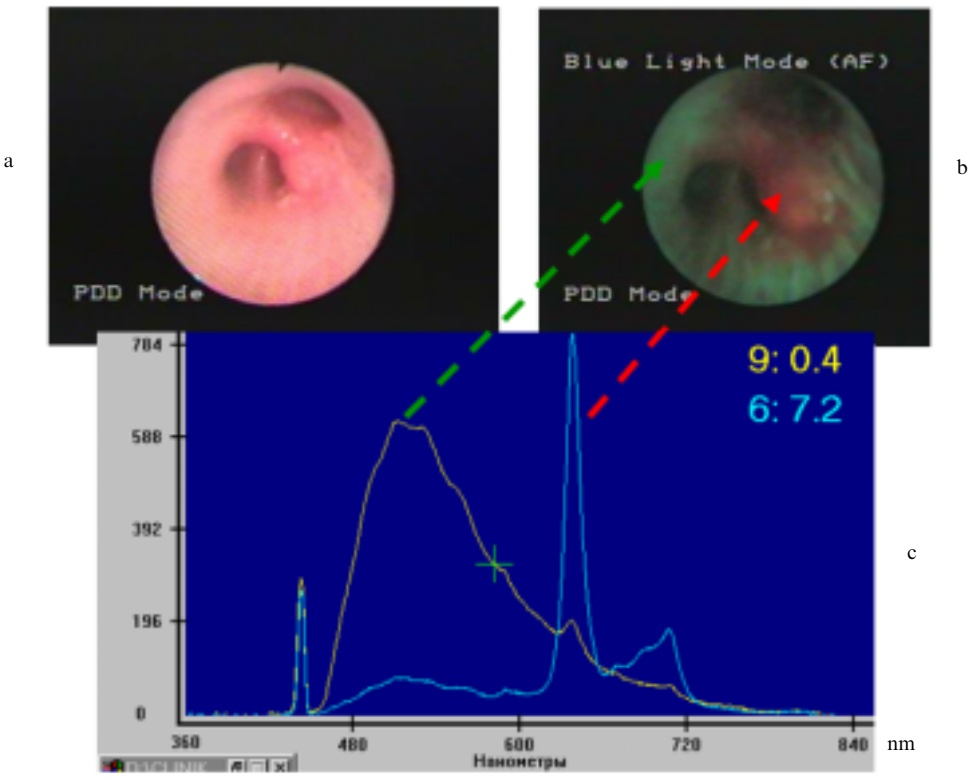

Figure 4. Fluorescence bronchoscopy and local spectrophotometry of early X-ray-negative lung cancer (SCC RULB) 2 h after inhalation with Alasense. The endoscopic (a) white-light and (b) fluorescence images of the bronchus, and (c) the autofluorescence spectra of the normal mucosa of the bronchus ($D_f = 0.4$, spectrum No. 9) and tumour ($D_f = 7.2$, spectrum No. 6) excited at 442 nm.

along the mucosa, the value of D_f in the region of red fluorescence is small on average, being 2.5–3, whereas $D_f = 0.3 - 0.5$ in a healthy tissue. However, for some patients with regions of homogeneous red fluorescence, characterised by averaged values of D_f, we observed separate sites where this value drastically increased up to 9–15. The morphological study of the biopsy material taken from these sites revealed early larynx cancer.

A typical image detected during fluorescence bronchoscopy with Alasense is the moderate red fluorescence of the tumor against the background of bright green autofluorescence of the surrounding healthy bronchial mucosa. In this case, $D_f = 0.4$ for the spectra of the normal bronchial mucous membrane; autofluorescence with a maximum at 520 nm being dominating. The induced red fluorescence of protoporphyrin IX is absent or very weak ($D_f \leqslant 0.9$). On the contrary, the autofluorescence intensity of the tumour is very low in the entire spectral range from 460 to 800 nm, whereas the fluorescence intensity of protoporphyrin IX is very high: $D_f = 7 - 15$. The fluorescence spectra are shown in Fig. 4.

We were especially interested in the possibility of the fluorescence detection of precancerous local alterations in bronchial mucosa, which cannot be revealed upon white-light bronchoscopy. The precancerous loci, developing against the background of chronic inflammatory processes, can be detected by the red fluorescence of induced protoporphyrin IX, whose intensity varies from weak to moderate. However, the diagnostic parameter D_f, which equals ~ 1.5 during chronic inflammatory processes, increases weakly (up to 2.5) in precancerous sites, not achieving the values typical of early bronchial cancer ($D_f = 7$ and more).

The fluorescence examination of a number of patients revealed early X-ray-negative lung cancers, which were not detected by white-light bronchoscopy and by other diagnostic methods (Fig. 4). During the fluorescence bronchoscopy of these patients inhomogeneous weak red fluorescence was detected. The measurements of spectra inside these fluorescent regions and the monitoring of the parameter D_f revealed suspicious sites with $D_f = 9$, which is substantially higher than average values in this region (1.5–2.5). The biopsy of the mucosa at these regions with a high diagnostic parameter and subsequent morphological analysis confirmed the presence of cancer.

The fluorescence bronchoscopy of one of the patients of this group detected four lung tumours. All these tumours exhibited very intense red fluorescence of induced protoporphyrin IX. The value of D_f in tumours varied from 7 to 9, whereas it was 0.6 for the normal mucosa (at a distance of 5 mm from tumours).

We also obtained false-positive results during the examination. The fluorescence of protoporphyrin IX was detected along the resection line of bronchus within 3–4 months after the surgery, which was determined by the inflammation of the mucosa and the growth of granulation. However, the diagnostic parameter D_f did not exceed 3.5 in the case of false-positive signals, which is much lower than the values observed for tumours. Therefore, the results of the method of local spectrophotometry developed by us correlate with the results of morphological analysis in the case of false-positive fluorescence as well.

As a whole, the results of the fluorescence bronchoscopy of 94 % of patients of this group were confirmed by the results of morphological analysis, which demonstrates a high sensitivity of the method being developed. We plan to study further the specificity of the method by examining a larger group of patients.

5. Conclusions

Thus, the approach developed by us showed that the analysis of fluorescence images (both autofluorescence and 5-ALA-induced images) in combination with local spectrophotometry substantially increases the reliability of guided biopsy at the 'fluorescent spot'. This combination improves the diagnostics of precancer and early cancer, a residual tumour or an early recurrence after previous treatment, and can be used for detecting a primary multiple malignancies. This approach makes it possible to minimise false-positive results and the number of biopsies that are usually required in the white-light bronchoscopy search for occult cancerous loci.

References

1. Sokolov V.V, Chissov V.I., Trakhtenberg A.K., Mamontov A.S., Frank G.A., et al. *Proc. SPIE Int. Soc. Opt. Eng.*, **2728**, 39 (1996).
2. Chissov V.I., Sokolov V.V., Filonenko E.V., et al. *Khirurgiya*, **5**, 37 (1995).
3. Zharkova N., Kozlov D., Smirnov V., Sokolov V., et al. *Proc. SPIE Int. Soc. Opt. Eng.*, **2325**, 400 (1994).
4. Ackerman G., Abels C., Baeumer W., et al. *Photochem. Photobiol. B: Biol.*, **47**, 121 (1998).
5. Baumgartner R., Huber R.M., Schulz H., et al. *Photochem. Photobiol.*, **36** (2), 169 (1996).
6. Karnaukhov V.N. *Tsitologiya*, **28** (4), 408 (1976).
7. Policard A. *CR Soc. Biol.*, **91**, 1423 (1924).
8. Stroka R., Baumgartner R., Buser A., et al. *Proc. SPIE Int. Soc. Opt. Eng.*, **1641**, 99 (1991).
9. Lam S., Hung J.Y., Kennedy S.M., et al. *Am. Rev. Respir. Dis.*, **146** (6), 1458 (1992).
10. Lam S., MacAulay C., LeRiche J.C., Palcic B. *Cancer*, **89** (11 Suppl.), 2468 (2000).
11. Kennedy T.C., Lam S., Hirsch F.R. *Oncologist*, **6** (3), 257 (2001).
12. Kurie J.M., Lee J.S., Morice R.C., et al. *J. Natl. Cancer. Inst.*, **90** (13), 991 (1998).
13. Delank W., Khanavkar B., Nakhosteen J.A., Stoll W. *Laryngoscope*, **110** (3 Pt.1), 368 (2000).
14. Pichler J., Stepp H., Baumgartner R., et al. *Lasermedizin*, **13**, 151 (1998).
15. Häußinger K., Stanzel F., Huber R.M., et al. *Diagnostic and Therapeutic Endoscopy*, **5**, 105 (1999).
16. Zharkova N., Kozlov D., Polivanov Yu., et al. *Proc. SPIE Int. Soc. Opt. Eng.*, **2328**, 196 (1994).
17. Chissov V.I., Sokolov V.V., Zharkova N.N., Filonenko E.V., Sukhin G.M., in *Materialy nauchno-prakticheskoi konferentsii Severo-zapadnogo regiona Rossii 'Lazernye i informatsionnye tekhnologii v meditsine XXI'* (Proceedings of Scientific and Practical Conference of the North-West Region of Russia on Laser and Information Technologies in Medicine XXI (St. Petersburg: Izd. State Scientific Center of Laser Medicine, 2001) p. 513.
18. Sokolov V.V., Zharkova N.N., Filonenko E.V. RF Patent No. 2129273 *'Sposob endoskopicheskoi fluorestsentnoi diagnostiki zlokachestvennykh opukholei polykh organov'* (Method of Endoscopic Fluorescence Diagnostics of Malignant Tumours of Hollow Organs) 26.11.1997.
19. Sokolov V.V., Zharkova N.N., Fabelinskii V.I., et al. *Diagnostika zlokachestvennykh opukholei na osnove fenomenov eksogennoi i endogennoi fluorestsentsii: posobie dlya vrachei* (Diagnostics of Malignant Tumours Using Exogenous and Endogenous Fluorescence: A Manual for Physicians) (Moscow, 2001).

20. Sokolov V., Chissov V., Filonenko E., et al. *Proc. SPIE Int. Soc. Opt. Eng.,* **2325**, 367 (1994).
21. Hung J., Lam S., LeRiche J. *Lasers Surg. Med,* **11**, 99 (1991).
22. Lam S., MacAulay C., LeRiche J. *Proc. SPIE Int. Soc. Opt. Eng.,* **2324**, 2 (1994).
23. Bottiroli G., Dal Fante M., Marchesini R., et al., in *Photodynamic Therapy and Biomedical Lasers* (Elsevier Science Publishers B.V., 1992) p.899.
24. Zonios G., Cothren R., Arendt J., et al. *Proc. SPIE Int. Soc. Opt. Eng.,* **2324**, 9 (1994).