Proc. Estonian Acad. Sci. Phys. Math., 1991, 40, N 3, 233—238
https://doi.org/10.3176/phys.math.1991.3.12

УДК 535.372:621.375.826:616—006.04

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LASER-EXCITED AUTOLUMINESCENCE OF SOME MALIGNANT TISSUES

(Presented by K. K. Rebane)

The autoluminescence spectra of some malignant versus normal human tissues (rectum, stomach) have been recorded under different laser excitation wavelengths (337, 488, 514.5 or 633 nm), at 300 and 77 K. The spectra of malignant and normal tissues of the same anatomical origin appear to be rather similar, the most notable differences can be observed in the case of 337 nm excitation by the nitrogen laser.

Since the pioneering works in the early seventies [1, 2], the method of photodynamic therapy (PDT) of cancer has been taken under extensive experimental investigation (see e. g. the reviews in [3, 4]), and has become quite widely rooted in clinical practice by now. The PDT procedure starts with injecting of a certain tumor-selective agent (most usually the so-called hematoporphyrin derivative) into patient's organism. After some time, the concentration of the injected substance in the malignant tissue appears to be much higher than that in the normal ones. The subsequent laser irradiation within the absorption band of injected molecules initiates a sequence of (photo)chemical reactions which ultimately lead to a selective destruction of malignant cells.

The tumor-sensitivity of some compounds has also caught attention in connection with the cancer diagnostics, and, historically, even earlier [5] than the PDT itself was introduced. The corresponding techniques based on the detection of the fluorescence of extraneous agents are now well developed [6, 7]. At the same time, an alternative diagnostic possibility — comparison of the autoluminescence spectra of normal and malignant tissues — has been rather sparsely studied, if to collate with the huge number of works dealing with the PDT approach. It should be emphasized that the injection of alien compounds can generally cause inadvisable side-effects in the patient's organism and it has been shown that the PDT does so indeed [8]. Therefore any possibility to extract relevant information for cancer diagnostics without the application of extraneous substances undoubtedly remains of particular interest.

We have undertaken a systematic study of the autoluminescence spectra of some malignant versus the corresponding normal tissues at different laser excitation wavelengths, sample temperatures, and delay times between the surgical sample secession and recording its spectrum. The preliminary results of the work are presented in this report.

The round slices (4 mm in diameter and 2 mm thick) of human rectum and stomach tissues taken from malignant and adjacent normal regions during oncosurgical operations, served as samples in our experiments. The luminescence was recorded 2—20 hours after surgery, in the meantime the samples were kept on ice. As far as the available number of independent stomach samples (3) has been smaller than that of the rectum ones (8), only the spectra of the latter are pictured below. It should be noted that the luminescence spectra of both malignant and normal rectum

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slices appeared to be well reproducible for various surgical cases and the figures given below represent just a random choice from the spectra obtained from our first experiments.

The excitation sources used for luminescence measurements were the following: (a) an ILGI-503 pulsed (100 Hz) nitrogen laser, 337 nm; (b) a LGN-402 CW argon ion laser, 488 or 514.5 nm; (c) a LG-79 CW helium-neon laser, 633 nm. The excitation intensity on sample surface was kept at about 1 mW/mm² in all cases.

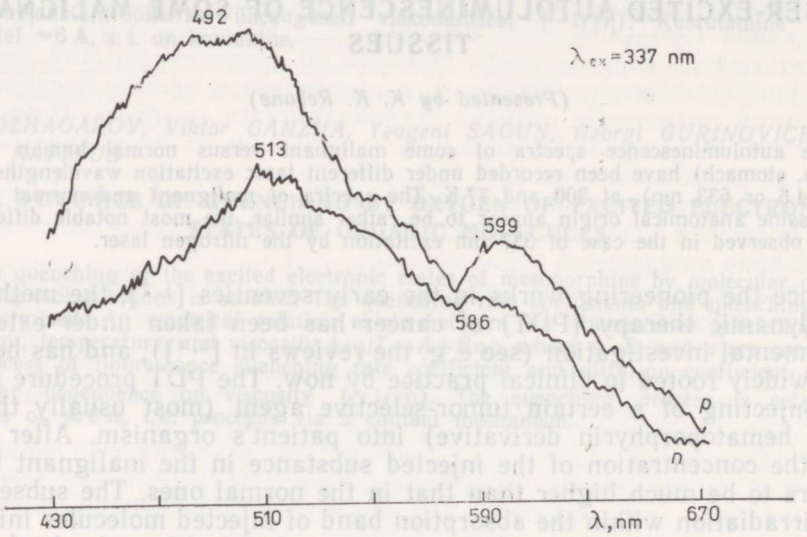

Fig. 1. Luminescence spectra of malignant (p) and normal (n) tissues of human rectum, recorded 2 hours after oncosurgery. Nitrogen laser excitation, $T=300$ K.

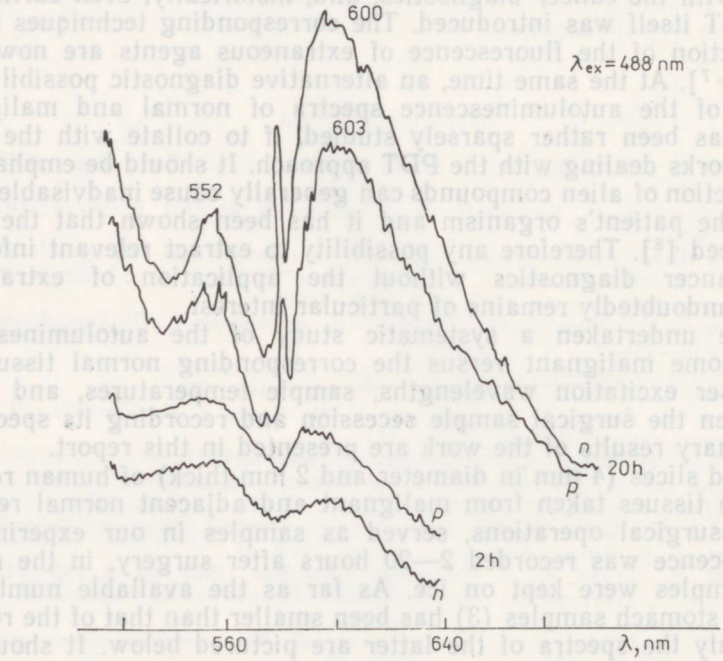

Fig. 2. Luminescence spectra of malignant (p) and normal (n) tissues of human rectum, recorded 2 (lower pair of spectra) and 20 hours (upper pair of spectra) after oncosurgery. Argon ion laser excitation, $T=300$ K.

Luminescence spectra were recorded under 45° geometry via a DFS-12 spectrometer (4 Å/mm) with FEU-106 photomultiplier in the photon counting mode. The spectra were collected by Nokia LP 4050 multichannel analyzer operating in the multiscaler or pulseheight-analysis mode, depending on the character of the excitation source (CW or pulsed).

The luminescing sample temperatures were 300 K or 77 K, in the latter case the samples were immersed into liquid nitrogen in a quartz cryostat.

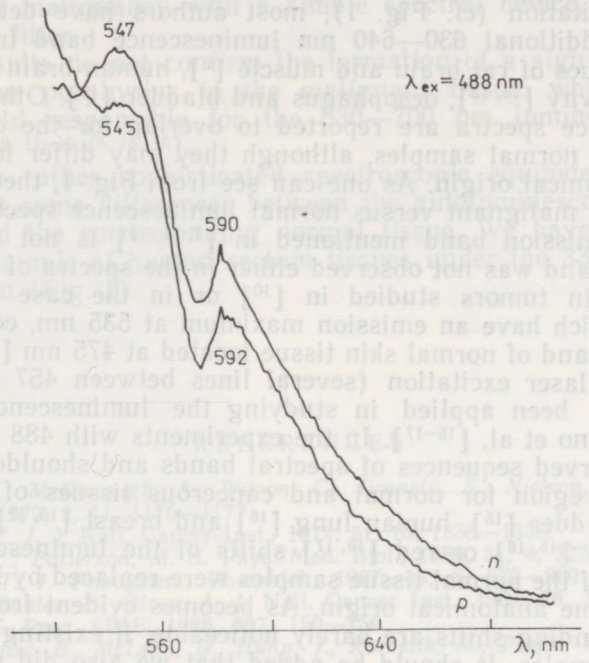

Fig. 3. Luminescence spectra of malignant (p) and normal (n) tissues of human rectum, recorded 2 hours after oncosurgery. Argon ion laser excitation, $T=77$ K.

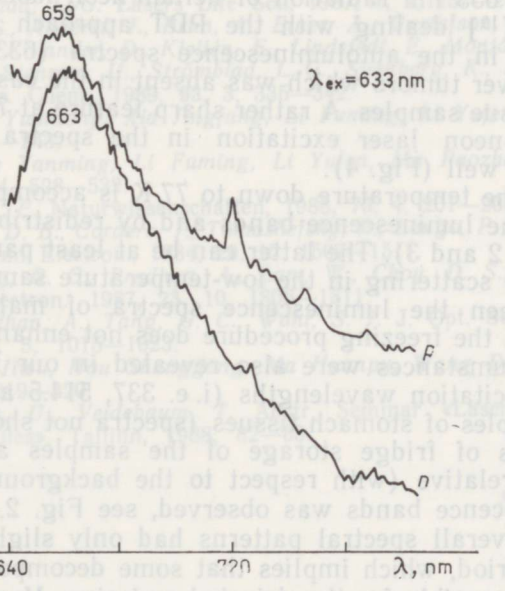

Fig. 4. Luminescence spectra of malignant (p) and normal (n) tissues of human rectum, recorded 2 hours after oncosurgery. Helium-neon laser excitation, $T=77$ K.

Examples of the luminescence spectra of malignant and normal human rectum tissues measured under the described experimental conditions are shown in Fig. 1—4. A comparison of these spectra with those obtained (at room temperature) by other authors [9–18] can not be carried out unambiguously, mainly due to the different types of tissues used in these works. Still, a short review seems to be appropriate to outline some context for our results.

When using a 337 nm N_2-laser [9–11] or a $\approx$365 nm non-laser source [12–14] for excitation (cf. Fig. 1), most authors have detected an appearance of additional 630—640 nm luminescence band in the case of malignant tissues of rat brain and muscle [9], human brain [11], stomach [12, 13], oral cavity [12, 13], oesophagus and bladder [13]. Other features of the luminescence spectra are reported to overlap for the corresponding malignant and normal samples, although they may differ for samples of different anatomical origin. As one can see from Fig. 1, there are certain changes in our malignant versus normal luminescence spectra of tissues, but the red emission band mentioned in [9, 11–13] is not revealed. The 630—640 nm band was not observed either in the spectra of various types of human brain tumors studied in [10] or in the case of malignant melanomas which have an emission maximum at 535 nm, contrary to the luminescence band of normal skin tissue located at 475 nm [14].

The argon laser excitation (several lines between 457 and 515 nm) has repeatedly been applied in studying the luminescence of various tumors by Alfano et al. [15–17]. In the experiments with 488 nm excitation they have observed sequences of spectral bands and shoulders within the 515—640 nm region for normal and cancerous tissues of rat prostate, kidney and bladder [15], human lung [16] and breast [16, 17]. At that, the 3—10 nm blue [15, 16] or red [16, 17] shifts of the luminescence maxima were detected if the normal tissue samples were replaced by the malignant ones of the same anatomical origin. As becomes evident from Fig. 2 and 3, the corresponding shifts are barely noticeable, if existing at all, in the case of our samples. It should be added that we also did not detect the pair of tumor-characteristic luminescence bands at 630 and 690 nm reported in [18] to occur under 450 nm excitation.

We could not find any previous reference concerning tumor emissions excited by the 633 nm radiation of helium-neon laser. During an earlier investigation [19] dealing with the PDT approach we had observed a 760 nm band in the autoluminescence spectra (633 nm excitation) of certain mice liver tumors which was absent in the case of the corresponding normal tissue samples. A rather sharp feature at 720 nm is observable under helium-neon laser excitation in the spectra of human rectum malignancy as well (Fig. 4).

Lowering the temperature down to 77 K is accompanied by a $\lesssim$10 nm blue shift of the luminescence bands and by redistribution of their intensities (cf. Fig. 2 and 3). The latter can be at least partially caused by the increased light scattering in the low-temperature samples. As for the differences between the luminescence spectra of malignant and normal rectum tissues, the freezing procedure does not enhance these, see Fig. 3. The same circumstances were also revealed in our measurements while using other excitation wavelengths (i. e. 337, 514.5 and 633 nm) as well as on the samples of stomach tissues (spectra not shown).

After hours of fridge storage of the samples an increase of both absolute and relative (with respect to the background level) intensities of the luminescence bands was observed, see Fig. 2. It must be pointed out that the overall spectral patterns had only slightly changed during the storage period, which implies that some decomposition products may have been responsible for the detected emission. Moreover, the evolution of the spectra during the interval between surgery and spectroscopy seems

to be identical for both the malignant and normal tissue. If true, these observations can seriously affect the hope to find a simple and unambiguous way for using the autoluminescence of tissues in cancer diagnostics.

Summarizing the results of the present work one can draw the following conclusions.

1. The overall shapes of the autoluminescence spectra of malignant and normal tissues of human rectum are similar enough to diminish a hope to detect malignancy with a simple spectral device, e. g. with the common glass filter.

2. Our results do not confirm the formation of a significant amount of endogeneous porphyrins in the malignant tissue, which have been tentatively held responsible for the 630—700 nm luminescence bands observed in [9, 11–13, 15, 16, 18].

3. When rather sophisticated spectroscopic equipment is applied, one can detect some differences between the autoluminescence spectra of malignant and the corresponding normal tissue. We have demonstrated this on the example of human rectum tissues under the 337 nm nitrogen laser excitation (Fig. 1).

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Received
March 6, 1991

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MÕNEDE PAHALOOMULISTE KUDEDE AUTOLUMINESTSENTS LASERERGASTUSEL

On mõõdetud mõnede pahaloomuliste ja vastavate normaalsete inimkudede (pärasool, magu) autoluminestsentsi spektrid erinevatel laserergastuse lainepikkustel (337, 488, 514,5 või 633 nm) temperatuuril 300 ja 77 K. Sama anatoomilise päritoluga pahaloomuliste ja normaalsete kudede spektrid osutusid üsna sarnaseks, märkimisväärseimad erinevused ilmnesid ergastamisel 337 nm lämmastiklaseriga.

Юло ПАРТС, Райво ТАМКИВИ, Маргус ЛЭПНЕР

АВТОЛЮМИНЕСЦЕНЦИЯ НЕКОТОРЫХ ЗЛОКАЧЕСТВЕННЫХ ТКАНЕЙ ПОД ЛАЗЕРНЫМ ВОЗБУЖДЕНИЕМ

Измерены спектры автолюминесценции некоторых злокачественных и нормальных тканей органов человека (прямая кишка, желудок) под лазерным возбуждением на разных длинах волн (337, 488, 514,5 или 633 нм) при 300 и 77 К. Спектры люминесценции опухолевых и нормальных тканей одной и той же анатомической природы оказались весьма схожими. Наибольшие различия наблюдались при 337 нм в случае возбуждения азотным лазером.