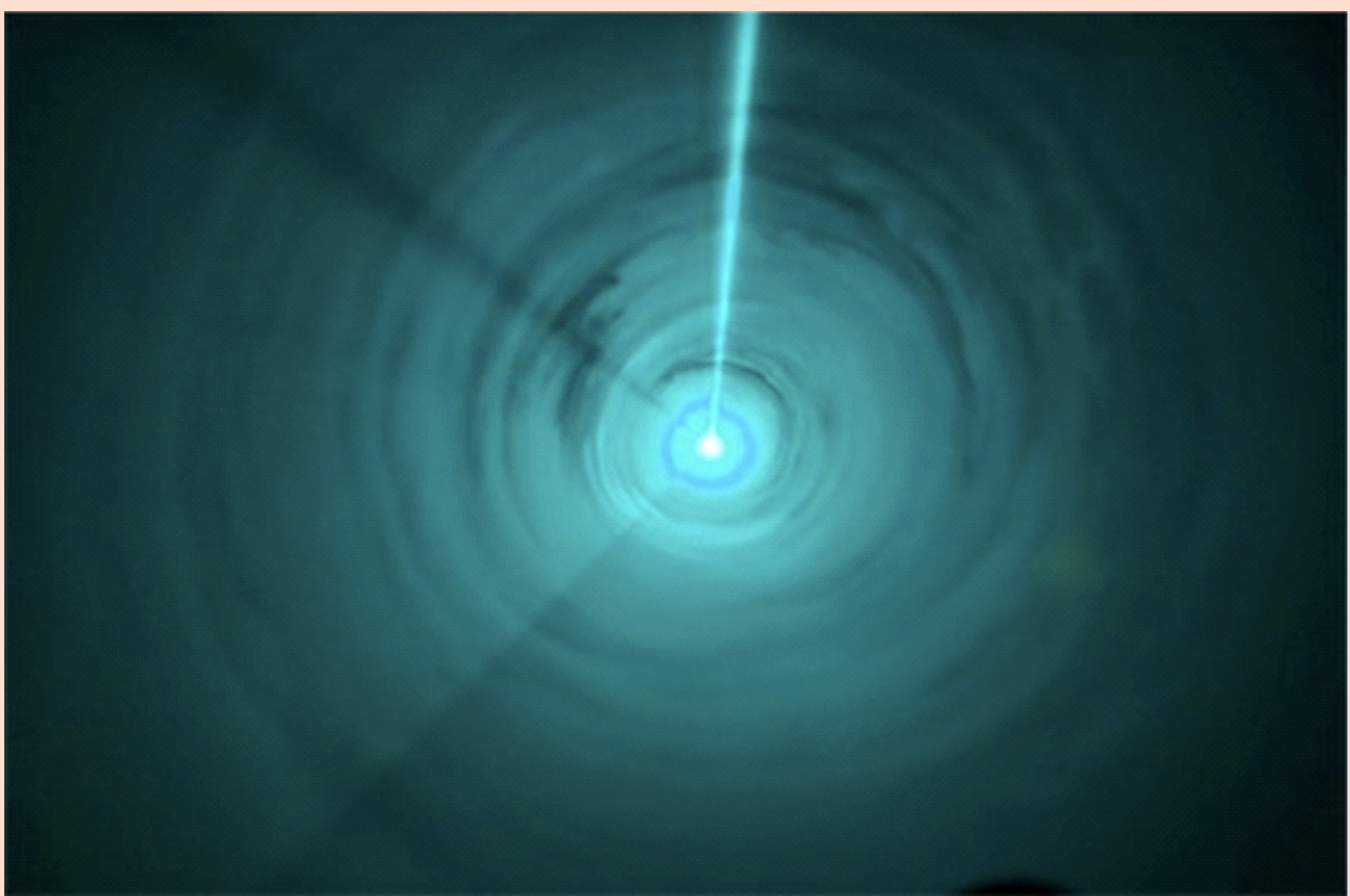


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Historical review of early researches on mitogenetic radiation: from discovery to cancer diagnostics

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Abstract. The review is devoted to the experimental studies of mitogenetic radiation (weak UV-chemiluminescence of biological objects) conducted between 1923 and 1948 years. In this period UV-radiation of various biological objects (so called inductors) and its influence on mitotic rate of other biological objects (detectors) were investigated very actively. Very promising results were obtained including the finding of the peptide tumor marker in blood, which was called cancer quencher due to its ability to quench UV-chemiluminescence of blood. Later these researches were interrupted and almost abandoned. The relevance of the scientific problems addressed in these works to this day is stated, the key experiments and the most valuable results obtained between 1923 and 1948 are described in details and subsequent researches are briefly traced up to the present. The prospects of future development are discussed. © 2018 Journal of Biomedical Photonics & Engineering.

Keywords: mitogenetic radiation; Gurwitsch radiation; mitogenetic effect; biochemiluminescence; biophotonics; UV-chemiluminescence; distant interaction; ultraweak photon emission; cancer diagnostics; cancer quencher.

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1 Introduction

It was almost a hundred years ago, when a distinguished biologist Alexander Gavrilovich Gurwitsch discovered an ability of one biological object (inductor) to influence a mitotic rate of another biological object (detector) by means of very weak UV-radiation [1, 2]. This phenomenon has been termed the mitogenetic effect, and the UV-radiation, which induced it, was referred to as the mitogenetic radiation.

Experiments of A.G. Gurwitsch attracted great attention of biologists, physicists, and chemists all over the world. First twenty five years after the discovery were the golden age of studies on mitogenetic radiation and mitogenetic effect. In the 1920s–1940s the mitogenetic effect was proved by more than a hundred of researchers in different countries and >700 works were published (see reviews of early works [3-6]). A.G. Gurwitsch was awarded with the highest scientific award of the USSR (Stalin Prize, 1941) and received 11 nominations for the Nobel Prize [7] for his works in this area.

Significant contributions were made by famous biologists, physicists, and chemists of that time. Nobel Prize winner D. Gabor with T. Reiter experimentally proved the acting factor of mitogenetic effect to be UV-radiation, they studied mitogenetic radiation of various bioobjects, wrote one of the first monographies on the subject [8]. The famous pupil of A.G. Gurwitsch and the founder of Pushchino scientific center, academician G.M. Frank made a number of pioneering experiments in this area: altogether with A.G. Gurwitsch he observed that the weakened UV-radiation from physical sources produces the same mitogenetic effect on biological detectors [9]; he studied the dependence of mitogenetic response of bioobjects from the wavelength and intensity of artificial UV-radiation (with academician Yu.B. Chariton and N.N. Kannegiesser) [10], analyzed spectra of mitogenetic radiation with biological detectors [11], proved an existence of UV-radiation of a number of biological objects and chemical reactions and

estimated its intensity with use of modified Geiger-Mueller counters (with S. Rodionow) [12-14], and made some other important discoveries in this area [5, 15-19]. Nobel Prize winner N.N. Semenov and academician Ya.B. Zeldovich put substantial corrections related to the chain reactions into the Gurwitsch's hypothesis of UV-photons generation [6, 20]. Well-known chemist R. Audubert demonstrated UV-chemiluminescence of a wide range of chemical reactions and biological objects with gas-discharge counters [21]. Academician S.I. Vavilov, who actively participated in scientific discussion on UV-chemiluminescence of bioobjects and chemical reactions [20], claimed the experiments of R. Audubert to be a conclusive evidence of this phenomenon [22]. Significant contributions into the researches of the mitogenetic radiation were made by the All-Union Institute of Experimental Medicine headed by A.G. Gurwitsch, laboratories of L.Ya. Blacher and B.S. Pesochensky (USSR), laboratories of O. Rahn (USA), L.K. Wolff (Netherlands), W.W. Siebert (Germany) and quite a few other reputable research teams and institutions. Many impressive findings were made in both basic and applied researches. For example, UV-chemiluminescence of a number of significant biochemical reactions was discovered and studied exactly with help of mitogenetic response of biological detectors, which were more sensitive than physical detectors of that time [23]. Broad study of mitogenetic radiation of blood in various diseases brought up the finding of the "cancer quencher", highly-specific substance appearing in blood in case of any malignant tumor and quenching UV-chemiluminescence [24]. It was shown that the cancer quencher was a peptide or a group of peptides with similar physical and chemical properties. The conclusion that the blood peptide can be used as a tumor marker was made on the basis of experiments on mitogenetic radiation of blood. It was a revolutionary statement for that time. Early cancer diagnostics based on the detection of cancer quencher

with use of mitogenetic effect was proved in clinical researches (specificity and sensitivity of the method >95%) [25-27].

After twenty five years of successful development of this research area the studies on mitogenetic radiation completely ceased by the late 40s: in Europe they were interrupted at the beginning of the World War II; the “Iron Curtain” strengthened the prejudice against this topic as to obscurantism of “Soviet science”; in the USSR, where this field of research had been developed the most extensively since the beginning, the researches were persecuted altogether with genetics after the decisions of the All-Union Academy of Agricultural Sciences in 1948, see details in [28]. Difficulties in physical detection of ultraweak UV-radiation also contributed to the bias against these researches: photoelectric elements and photographic plates had too low sensitivity to register mitogenetic radiation (for instance, [29, 30]), and the best modified gas-discharge counters registered it with rather low signal/noise ratio [12, 13, 21, 31-37].

After the complete stoppage of these researches for 8 years, the cell radiation attracted attention of scientists again in the middle 1950s due to the invention of photomultipliers. These studies mainly focused on the visible range, where the chemiluminescence intensity was much higher, see reviews [38-42]. At the same time, experiments with photomultipliers confidently proved an existence of weak UV-chemiluminescence of bioobjects and biochemical processes [43-46] as well as the estimates of its intensity and spectral range made with gas-discharge counters in the 1930s [47, 48]. One more surge in interest to the topic in the 1980-90s was initiated by rather beautiful hypothesis on ability of cells to radiate and highly-selectively detect radiation of extremely high coherence, “biophotons” [49]. This hypothesis has not got enough experimental proof till present [42, 50, 51], characteristic time of coherence of biological chemiluminescence in optical range seems to be no more than nanoseconds [52-54]. Lots of shallow popular scientific publications about this unverified hypothesis and its misrepresentation as an epoch-making discovery in mass media compromised the effect, which this hypothesis was trying to explain.

Although since the middle 1950s the researches somewhat resumed, however, they have been sporadic till now, moreover very few attempts were devoted to verification or progressive development of the scientific heritage of the 1920-40s on mitogenetic effect, for instance, Refs. [23, 46, 55]. Most of contemporary researches on phenomena of optical influence of biological objects on each other, do not attempt to clear whether they relate to UV-radiation, and their results can not be used for verification of old works on mitogenetic effect due to significant difference in experimental conditions [56-67]. Contemporary works are published mainly in English and available for the wide readership. They are clearly described in several reviews [28, 57, 68-72], and here we overview them rather briefly.

The aim of this review is to attract the interest of researchers to the key results of experiments on mitogenetic radiation made between 1923 and 1948 years. During this period a number of research teams headed by outstanding and well-known scientists carried out systematic massive large-scale researches, and, in contrary to the further works they were accompanied by active international discussion and multiple verifications of all the key experiments in different laboratories (see, for example, monographs [4, 5, 8, 24, 73, 74]). Here we review the publications on mitogenetic radiation mostly inaccessible to English readership. They were published in German and in Russian and usually one can not find them neither in libraries nor in the full-text databases, in particular, all the main publications on cancer diagnostics, which are of the most practical interest [24, 25].

Nowadays, researchers, who never have read any original experimental papers on the subject, usually share the common opinion, that the mitogenetic radiation have been proved to be a scientific fallacy of the beginning of the last century, a speculation based on very few occasional experiments. This opinion is as unreasonable as a statement, that all the old experiments on mitogenetic radiation can be trusted without up-to-date verification. Moreover, it is difficult to expect that the early attempts to explain these experiments would not require significant revision after almost a century of progress in biology and biochemistry. It also should be taken into account, that in 1923-1948 the experiments methodology (biological methods, statistical treatment of data etc.) was very different from the one used in our day. There were no recent attempts to verify any of these experiments with due attention to details and conditions described in original publications. At the same time, it is significant to mark a number of old experimental works, which were made at the leading edge of research techniques of that time, and their experimental methods and designs seem more convincing now, for instance, Refs. [10, 75-78]. The methods of mitogenetic effect observation were summed up and thoroughly analyzed in Refs. [3, 23], and in Ref. [28], we also recommend the critical review of some methods used in the first decade of experiments [79]. The majority of early works presented in our review were published with detailed protocols and descriptions of methods, and we consider their results worth of serious attention.

Study of scientific heritage of 1923-48 years on mitogenetic radiation, which was accumulated by prominent scientists and their laboratories or institutions, unbiased experimental verification of their results at the up-to-date level of validity are important both for basic science and for practical applications. Study of UV-chemiluminescence of cells is significant not only for better understanding of biochemical processes in them. As it is well known, the biological processes are controlled by complex regulatory systems: both species-specific chemical agents and “quorum-sensing factors”, highly universal “second messengers”,

as well as relatively recently discovered NH_3 , NO and CO_2 -mediated regulation. Thorough revision of old experiments opens the door for the enrichment of the theory of regulatory biological processes with a validation of mitogenetic radiation as one more signal agent or with disclosure of another factors, that caused consistent reproduction of the mitotic rate changes in thousands of independent experiments.

The cancer quencher seems the most promising finding for practical applications among the reviewed scientific results. It is surely the first substance suggested as a universal tumor marker [24], about 30 years before the alpha-fetoprotein. It should be noted that the cancer quencher is a blood peptide as well as the most of the tumor markers known by now. It was detected in blood long before any clinical sign of the cancer and was shown to be useful for a differentiation of malignant and non-malignant processes, estimation of efficacy of implemented therapy, timely detection of local recurrences and metastases [24, 25, 27]. Some clinics practiced such a cancer diagnostics at the admission of new patients long before the establishment of the final diagnoses and demonstrated specificity and sensitivity of this method above 95% [26].

We consider the relevance of problems addressed in researches on mitogenetic radiation and extensive experimental data require verification of these experiments at the up-to-date level, with judging not by public opinion, but by proofs and disproofs, as is customary in science. Confirmation of mitogenetic effect would open wide prospects in biology and medicine; unambiguous refutation would give a novel interpretation to the results of that time. In any case the further progress in this area is impossible without the detailed study of former researches with focus on methods and reproducibility.

2 The discovery and the first studies of the mitogenetic effect

The UV-radiation of biological objects was discovered in 1923 by an outstanding Russian biologist Alexander Gavrilovich Gurvitsch [1]. He made a series of experiments with onion (*Allium cepa*) roots and found that if a tip of one root (inductor) was directed to the meristem of the other root (detector) (Fig. 1), the meristem cells in the detector divided more intensively at the side the inductor root pointed to (Figs. 2, 3).

The phenomenon of distant influence on the mitotic rate was called “mitogenetic effect”. Besides the acceleration of the mitotic rate, the deceleration was also observed, but less commonly, for instance, in antagonistic bacteria [81].

The factor that caused the mitogenetic effect exhibited the properties of electromagnetic radiation (straight propagation, reflection, refraction in a quartz prism) [2, 8, 82]. Insertion of plates of materials with different transmittance ranges (mica, glass, gelatin, quartz, wood, etc.) between the inductor and the detector allowed researchers to attribute this radiation to

the UV range [1, 8]. The effect was observed when the quartz plate was inserted, but it disappeared with the glass plate. Later, the attribution of the mitogenetic radiation to UV-range was corroborated by the observation of the mitogenetic effect under the influence of weakened UV-radiation from physical sources (spark discharges, mercury lamps) [9, 10, 83] and UV-chemiluminescent reactions [13].

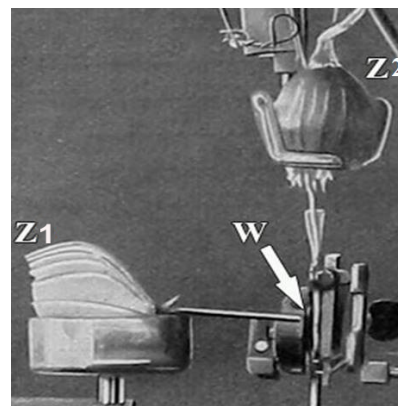


Fig. 1 Gurwitsch's experiment with onion: Z1 – inductor onion, W – the place of mitosis induction, Z2 – detector onion (reprinted with permission from Ref. [80]).

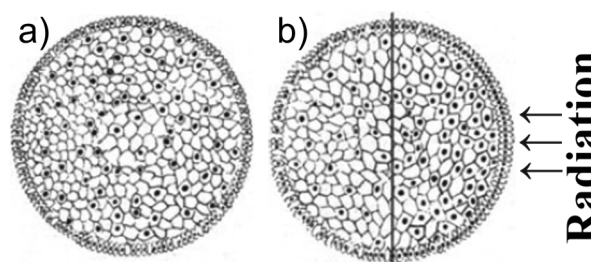


Fig. 2 Schematic drawings of onion meristem cross-sections: (a) – non-irradiated root, (b) – irradiated root (reprinted with permission from Ref. [8]).

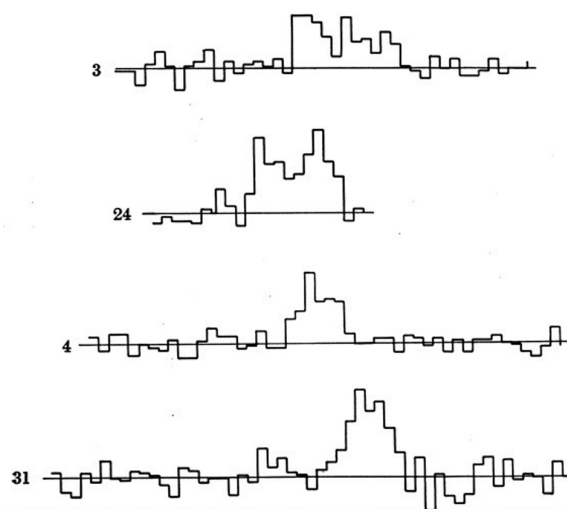


Fig. 3 The difference in the number of mitoses between the irradiated and non-irradiated sides of the root in serial cross-sections (reprinted with permission from Ref. [80]).

It was found that the phenomenon of mitogenetic effect was not species-specific, for instance, a frog muscle as an inductor and yeast fungi as a detector being representatives of different kingdoms demonstrated a clear mitogenetic effect [11]. Numerous experiments revealed a wide variety of inductors and detectors of mitogenetic effect, it was also found that certain biological objects were not inductors. The absence of species-specificity and the wide variety of inductors and detectors imply that the mechanisms underlying the mitogenetic effect appeared at the early stages of evolution.

The tissues of animals and plants, cell cultures and microorganisms as well as most biological objects under stressful conditions were proved to be sources of mitogenetic radiation.

Inductors

- Malignant tumors (the most active of the known inductors) [8, 73, 82, 84-88];
- Rapidly proliferating microorganisms [89-93];
- Tissues that A.G. Gurwitsch called "meristems in broad sense", i.e. tissues with mitotically active cells, regardless of the organism taxonomy [8, 87, 94];
- Resorptive and regenerating tissues [95-98];
- Tissue cultures [4, 99];
- Working muscles including the heart [4, 82];
- Excited neurons [100];
- The blood of young healthy humans [25, 101, 102];
- Embryos at certain stages (species-specific) [3-5, 91, 103, 104];
- Most biological objects under stressful conditions [4, 22];
- Physical sources of weak UV-radiation [10, 83];
- Some chemical reactions accompanied by UV-luminescence [3, 23].

Detectors

- Bacterial and yeast cultures in lag phase [75-77];
- "Aging" yeast cultures [89, 105]; probably, they meant cultures not in the stationary phase, but in the so-called "post-diauxic" phase prior to the true stationary G0 phase;
- Tissue cultures [106, 107];
- Plant meristems [1, 8];
- Corneal epithelium (e.g. corneas of tritons, frogs, rats [94]);
- Dividing embryonic cells (sea urchin eggs [90, 91], frog embryos [8], drosophila embryos [77, 108]).

The common features of the detectors were the spontaneous cell division (without any external influences), suboptimal growth conditions [4] (i.e. "ability to be stimulated" relative to the control) and "significant cell complex with adequate proximity of cells" (Ref. [4], p. 332).

Biological objects that are non-inductors of mitogenetic effect

- Non-growing or slowly growing cell cultures [3, 4];
- Internal organs [3];
- Blood of people with various diseases (cancer of different types [25-27, 101, 102], anemia, sepsis, pneumonia, scarlet fever, diabetes, etc. [4, 87, 109, 110]); blood of elderly or fatigued adults [4, 109, 111, 112].

The mitogenetic effect depended on the physiological state of the detector, the inductor and the regime of their interaction. It could manifest itself both in the acceleration of cell cycles, which occurred more often, and in deceleration [4, 81].

The mitogenetic response of the detector nonlinearly depended on the distance from the inductor and the exposure duration. There was some optimal range of exposure duration, which resulted in the maximal effect. This range depended on the inductor and detector and on the distance between them; both longer and shorter exposures reduced the effect.

In the first years after the discovery of the mitogenetic effect, the most common detectors were onion roots; the method of detection was described in details and well-established [113]. Later, the onion was replaced with yeast and bacteria detectors, for which it was easier to make a control group; moreover, it was possible to use them as detectors throughout the entire year unlike onion roots, which could be used as detectors only from the end of September to April. In addition, in bacteria and yeast, it was easier to quantify the change in the division rate by physical methods, for example, by measuring the optical density using a nephelometer [19] or a colorimeter [114] (nowadays the budding of yeast is more accurately and easier registered by the digital processing of microphotographs [115]). Methods of the yeast detection of mitogenetic radiation were described in details in [4, 89].

It should be noted that there were about 20 negative experimental papers on mitogenetic effect, for example, [116-118]. Counter-criticism [22, 23] ascribed these failures to the serious deviations from the known methods [22, 23], their methodological errors were analyzed in the recent review as well [28]. Negative works made up less than 3% of the total number of experimental works on mitogenetic effect.

3 Approaches to the registration of mitogenetic radiation

For a rather long time after the discovery of the mitogenetic effect the biological detectors remained the only possible means of registration of UV-radiation of biological objects. Physical detectors of that time were not sensitive enough, and that made some authors to doubt the existence of this radiation itself, for instance [29]. In fact, biological detectors had a range of significant drawbacks. Firstly, such methods of detection were very time-consuming. For example, one

registration of UV-radiation with onion detector required the counting of mitoses over microscopic fields of several thousands of cells. Secondly, experiments with biological detectors required very accurate operation and strict observation of procedures. Some authors failed to repeat the well-known experiments on mitogenetic effect due to the disregard of the methods of work with biological detectors (see details in Ref. [28]). Thirdly, variability of biological detectors themselves as well as variability of many environmental factors influencing their sensitivity made such method of registration of weak UV-radiation rather unstable. In fact, sometimes all the laboratories, which were well-experienced in experiments on mitogenetic effect, observed an inexplicable failure of biological detectors. For instance, A.G. Gurwitsch reported that sometimes the yeast cultures successfully used in his laboratory completely lost their sensitivity for several days without apparent reason, moreover, all the researchers observed this phenomenon simultaneously in independent experiments. In a few days or even weeks their ability to detect radiation sometimes restored also without any apparent changes in the yeast culture. Similar simultaneous failures of all the bacterial detectors in some days were observed by L.K. Wolff and G. Ras [119]; they found that the detection was still possible, but required a longer exposure. O. Rahn, W. Heinemann, and W. Siebert headed the laboratories, where thousands of successful experiments with biological detectors were conducted, and they also reported about such cases. A.G. Gurwitsch suggested that these failures of biological detectors were related to external radiofrequency electromagnetic fields, L. Wolff and G. Ras explained them by specific changes of biological detectors themselves, there were a number of other suggestions like climatic changes etc. [3]. Besides that, some biological detectors demonstrated a clear seasonal sensitivity, for instance, an onion worked as a detector only since the end of September till April.

All these drawbacks of biological detectors and significant interest to the Gurwitsch's discovery induced active attempts to find physical and chemical methods of registration of mitogenetic radiation. W. Stempell suggested an approach based on the influence of UV-radiation on the formation of Liesegang rings (concentric circles of precipitates that forms at slow diffusion of the drop of precipitant) [120] (as cited in Ref. [3]). This method seemed reliable for detection of UV-radiation from artificial sources, but the results for mitogenetic radiation from onion inducers were doubted, because of the influence of mustard oil evolved by onion on the Liesegang rings formation shown later [121]. The author took it into account in his further works [122, 123] (the last is as cited in Ref. [3]) and verified his first work (see Fig. 4). W. Stempell suggested one more detection method based on the accelerated decomposition of hydrogen peroxide under influence of mitogenetic radiation [124] (as cited in Ref. [3]). However, none of these methods was practiced widely, because of high sensitivity of

Liesegang rings formation and hydrogen peroxide decomposition to many different factors.

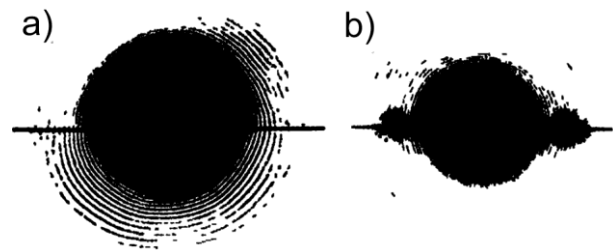


Fig. 4 Influence of mitogenetic radiation on Liesegang rings [123]. Onion pulp in metal tube with a slit (marked with a line) was used as a source of radiation. Liesegang rings were formed at precipitation of silver chromate in gelatine. a) exposure through cellophane, which is nontransparent for UV-radiation, b) exposure through a UV-transparent quartz plate (reprinted from Ref [3] with permission from E. Schweizerbart Science Publishers).

Later the method based on the flocculation of unstable colloidal solutions (sols of inorganic substances) was developed. Mitogenetic radiation accelerated the flocculation, the flocculation rate was characterized by a photoelectric differential turbidimeter [125, 126]. This method was used only in a very few researches.

O. Rahn in his review [3] wrote that almost all the early researchers of the mitogenetic radiation had made unsuccessful attempts to register it with photographic plates. Sensitivity of such detectors was not enough even at 90-days' exposure to regularly renewed yeast culture [29]. Photoelectric elements of that time were also not enough sensitive for mitogenetic radiation detection [10].

The gas-discharge counters were the first physical detectors that reliably registered UV-chemiluminescence of bioobjects. Various specially designed modifications of Geiger-Muller counters were used by B. Rajewsky [31-33], G. Frank and S. Rodionow [12, 13], W.W. Siebert and H. Seffert [34, 127], O. Glasser and H. Barth [35, 36], L. Grebe, A. Krost, L. Peukert [37] (as cited in Refs. [21, 23, 46]), R. Audubert [21] and others [3, 4, 23, 46]. The researchers registered radiation from working muscles, beating hearts, excited nerves, carcinomas, a wide range of redox reactions, "protein digestion" and many other biological inducers and chemical reactions. Besides that, no radiation was registered from the biological objects that earlier were found to be non-inductors of mitogenetic effect, for instance, muscles at rest. UV-component of radiation was demonstrated by comparison of measurement results for exposure through quartz and through glass or by introduction of monochromator between the inducer and the counter. Fig. 5 presents results for the sartorius muscle of the frog, which was stimulated electrically (special

experiments verified that the muscle motion did not influence the measurement results) [13].

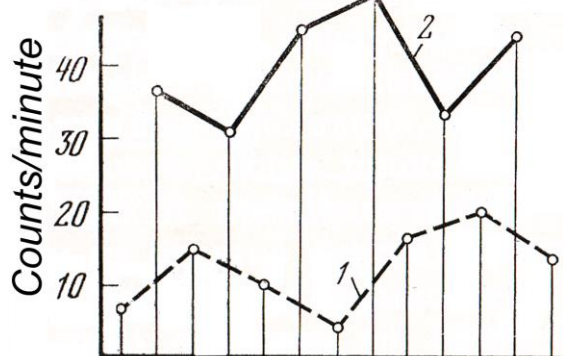


Fig. 5 Registration of mitogenetic radiation of the sartorius muscle of the frog, which was stimulated with electric current, with a gas-discharge counter: 1 – dark counts (resting muscle); 2 – signal counts (working muscle).

Gas-discharge counter registered radiation in intermittent periods of rest and work of the muscle (1 min. of rest, then 1 min. of regular electric stimulation of the muscle: stimulation 1–2.5 sec and pause 2–3 sec).

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Gas-discharge counters were rather imperfect devices for detection of such a weak radiation. Relative sensitivity of the counters made in one lot sometimes varied more than by an order of magnitude [36]. Registration of mitogenetic radiation required thorough selection of the best counters to get an acceptable signal/noise ratio. Besides that, it was necessary to test counters and replace them regularly because of desensitization.

In spite of all the drawbacks of these detectors and laboriousness of measurements the application of gas-discharge counters became an important step in the development of this field. In 1938 year at the conference of Faraday Society academician S.I. Vavilov summed up the results of these experiments as follows: «...emission of ultraviolet rays in many chemical reactions and biological processes is completely confirmed by usual physical methods. ...Wavelengths observed by Audubert belong to the same spectral range that was stated in Gurwitsch's laboratory...» [22].

Gas-discharge counters allowed researchers not only to prove an existence of mitogenetic radiation for a wide range of inductors, but also to estimate its intensity as $\sim 10\text{--}1000$ photons/cm² sec. The upper estimate was indirectly corroborated by negative experiment with counters of lower sensitivity. Later the intensity range measured with gas-discharge counters was verified with photomultipliers [43–48, 128–130].

4 Spectral analysis of mitogenetic radiation with biological detectors

The first attempt to analyze the spectrum of mitogenetic radiation was not successful [8]. Onion pulp was taken as an inductor, onion roots served as detectors, between them the quartz spectrograph was placed. Obtained spectra was significantly different from the spectra measured later in other laboratories [3, 4]. A number of mistakes in the experimental method was stated in Ref. [4], however the basic experimental setup was successfully used later on (see, for instance, Fig. 6(a)).

The first trustworthy spectra were obtained by G.M. Frank [11]. Experimental setup was similar to the spectrograph of that time with a set of agar blocks with yeast culture instead of a photographic plate as a detector (Fig. 6(a)). One detector block corresponded to the band of wavelengths down to 50 Å. Frog's muscle stimulated electrically was used as a source of radiation (as an inductor of mitogenetic effect). Results of three experiments are shown in Fig. 6(b).

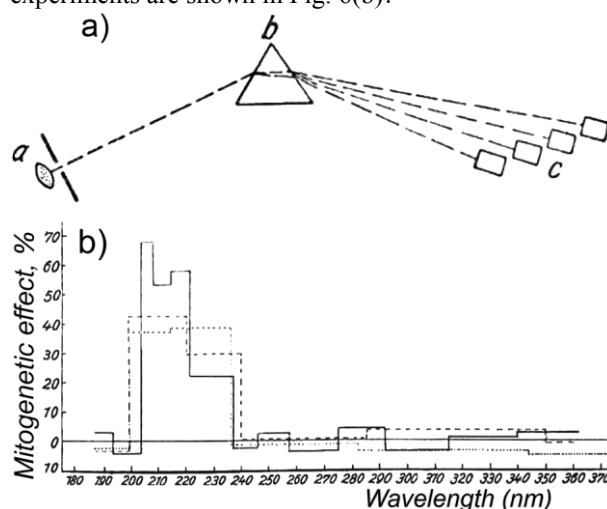


Fig. 6 Spectral analysis of mitogenetic radiation with biological detectors.

a) Experimental setup: (a) a sartorius muscle of the frog, (b) spectrograph (only a quartz prism and an optical slit are shown on the scheme), (c) agar blocks with yeast culture.

b) Three spectra of radiation from the electrically stimulated frog's sartorius. Mitogenetic effect is quantified as a change of budding index relative to the control yeast culture. Different step lengths correspond to the different width of yeast blocks.

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It was impossible to improve resolution significantly by the further miniaturization of yeast blocks. To get finer structure of spectra the bands of 10 Å were isolated out with monochromator [131]. The setup with monochromator required significant time consumption,

because the detectors had to be exposed one by one, usually not less than ten detectors per one band of wavelengths were exposed. Further improvements increased the spectral resolution up to 5 Å [132].

Obviously, the spectral analysis of UV-radiation with biological detectors was limited by the very low intensities and rather narrow spectral range of sensitivity of biological detectors. The mitogenetic spectra indicated only presence or absence of radiation within discrete spectral bands without any information about its intensity, thus they were significantly different from usual spectra obtained with physical devices. At the same time biological detectors showed an unsurpassed sensitivity to the weak UV-radiation. The spectral analysis of weak UV-radiation became one of the first practical applications of mitogenetic effect. Similar installations with biological detectors allowed the finding and study of UV-chemiluminescence of a wide range of biochemical and chemical reactions. Fig. 7 presents mitogenetic spectra of a number of enzymatic reactions – splitting of phosphocreatine, glucose and other [73].

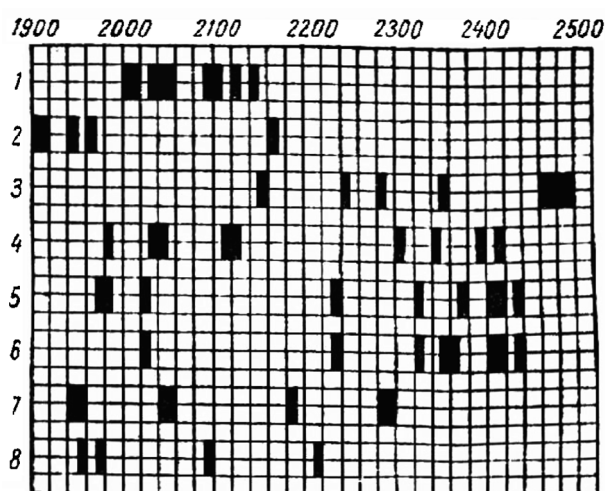


Fig. 7 Reference spectra of enzymatic cleavage reactions (reprinted with permission from Ref. [73]):

1 – phosphocreatine, 2 – glucose, 3 – nucleic acid and lecithin, 4 – peptides, 5 – maltose, 6 – sucrose, 7 – urea, 8 – lipids. The abscissa represents wavelengths in angstroms.

Comparison of the mitogenetic radiation spectra of various biological objects with the reference spectra of different biochemical processes allowed one to identify the processes causing mitogenetic radiation in any particular case. For instance, for long time A.G. Gurwitsch had been trying to explain unusual time dependence of mitogenetic radiation of the rabbit's eye during the starvation – it ceased completely and resumed after a week. The spectral mitogenetic analysis explained it easily: normal radiation was mainly due to glycolysis, which declines at starvation due to lack of glucose (after injections of glucose the “glycolytic” radiation restored very soon [73]), further starvation made proteolysis necessary for the life sustaining and

the radiation had typical proteolytic spectrum in this period [87]. Mitogenetic spectral analysis of tumors demonstrated that alive surface cells of tumor with active metabolism had glycolytic and nucleolytic (related to the splitting of nucleic acid by phosphatase) spectra, while internal necrotizing tissues were the sources of radiation typical for proteolysis [133]. Mitogenetic spectra of the maltose cleavage and starch cleavage were identical and different from the spectra of the sucrose cleavage. A.G. Gurwitsch concluded from these results that starch had the bonds between hexoses of the same type as in maltose and different from the ones in sucrose, mitogenetic analysis data led to some other findings related to the structures of biochemical substances [4].

Mitogenetic spectral analysis was a research method that provided lots of scientific information ahead of the time (see, for instance, chapters “Mitogenetic analysis of cancer cell” and “Mitogenetic analysis of nerve excitement” in the book [73]).

Radiation spectra of various biological inductors were attributed to rather general types of processes (glycolysis, oxidation, phosphorylation, proteolysis etc.). It was not only due to the lack of knowledge about the biochemical reactions, much more it was due to the limited resolution of mitogenetic spectra, which allowed analysis of the most general spectral features. Researchers of mitogenetic spectra often mentioned that the bands of 1 nm very probably contained unresolved lines.

In the 1930-40s years the mitogenetic spectral analysis was a unique instrument for non-invasive non-contacting study of biochemical processes in living organisms. As A.G. Gurwitsch pointed out in the monograph [4] “in contrary to any biochemical methods, the analysis is made not after but during the functioning”. This method was widely used for study of dynamic physiological processes: excitement of different nerves with various stimuli [134-136], muscle activity and other processes (see more about mitogenetic spectral analysis in monographs [4, 23, 73, 87]).

5 Physical parameters and specifics of mitogenetic radiation of biological origin

There are only the total intensity and the spectral range that were measured reliably enough among the parameters of mitogenetic radiation of bioobjects. Majority of biological inductors have spectral ranges within 190–260 nm [3, 4, 11, 134, 135, 137]. It should be noted, that there were no data on wavelengths below 190 nm because of the nontransparency of quartz used in prisms, cuvettes, windows of gas-discharge counters etc. Intensity of radiation varied within 10–1000 photons/cm² sec depending on the inducer and its physiological state. For instance, the muscles of frogs just taken from natural reservoir radiated with much higher intensity than after several months in aquarium [13]. The mitogenetic effect induced by UV-radiation

from artificial sources was studied in Refs. [10, 83]. The dependences of the mitogenetic response of the biological detector from the wavelength and intensity of incident radiation were estimated in Ref. [10]. The authors used aluminium, zinc, cadmium sparks and mercury arc lamp as sources of radiation. The attenuating filters lowered the intensity by several orders of magnitude, after them the large double quartz monochromator isolated discrete spectral bands, and then the detector (yeast culture) was placed. Mitogenetic response was observed within the range of 206–265 nm. Its dependence on the intensity had a maximum with damping of the effect by «high» doses of radiation, while the range of intensities producing mitogenetic effect did not change greatly with the wavelength (Fig. 8).

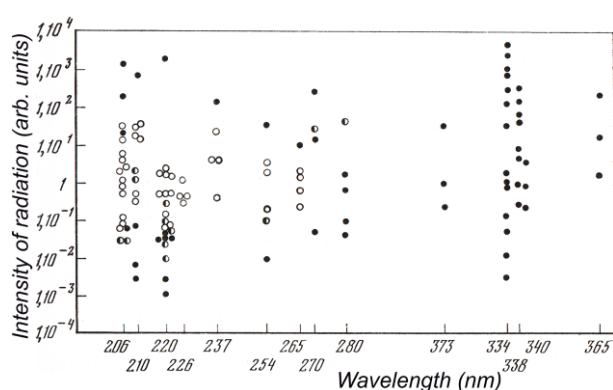


Fig. 8 Dependence of mitogenetic response of yeast detector on the intensity and wavelength of UV-radiation from artificial sources (aluminium, zinc, cadmium sparks and mercury arc lamp). The unit of intensity corresponds to the photoelectric current of $5 \cdot 10^{-14}$ A.

- – no mitogenetic response;
- – strong mitogenetic response;
- ◐ – weak mitogenetic response.

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Mitogenetic response of biodetectors required unexpectedly high intensity of artificial radiation [10]. First intensity estimations for natural mitogenetic radiation were published later [12, 13], however, by that time it had been known already that the radiation of bio-objects was so weak that it could be registered neither by photographic plates nor by usual photoelectric cells. Authors developed special highly-sensitive photoelectric installation, which detected 1/3 of the threshold intensity of artificial radiation producing mitogenetic effect, but it was not enough for registration of natural mitogenetic radiation [10]. Thus, the same mitogenetic response of yeast detector required much higher intensities of the radiation from artificial source than from biological inductor. It manifested some

specifics of UV-radiation of biological origin, which made biological detectors more sensitive to it than to the radiation from physical sources. Authors [10] related that to the high monochromaticity of the used physical sources and more complex spectra of biological inductors. They compared response of the yeast detector to the isolated spectral line (210 nm) and to an arbitrary set of spectral lines within the band of 205–215 nm. Detector demonstrated much higher sensitivity to the less monochromatic radiation: the mitogenetic response was observed at the intensity levels one-two orders of magnitude lower (about $6\text{--}7 \cdot 10^5$ photons/cm² sec) (see Fig. 9) than for monochromatic radiation, however, still orders of magnitude higher than the intensity of biological inductors [10].

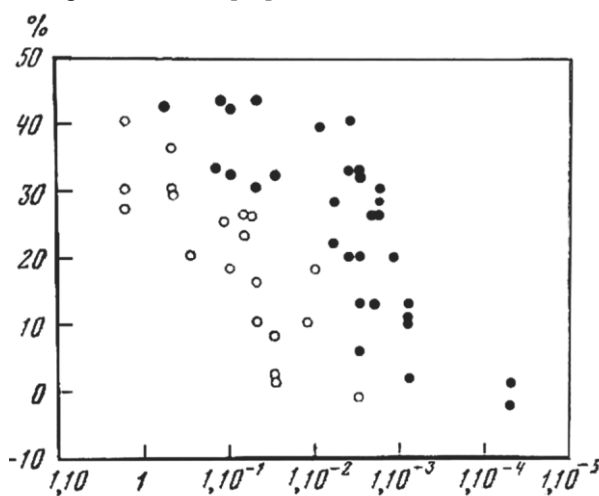


Fig. 9 Comparison of sensitivities of the yeast detector to the radiation of artificial UV-sources with higher and lower monochromaticities. Abscissa: intensities in relative units (the unit of intensity corresponds to the photoelectric current of $5 \cdot 10^{-14}$ A); ordinate: mitogenetic effect (%).

- – radiation of higher monochromaticity (spectral line of spark discharge 210 nm),
- – radiation of lower monochromaticity (arbitrary set of spectral lines within 205–215 nm).

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Researchers specially pointed out that they used a quite arbitrary set of spectral lines. They suggested that the threshold intensity probably could be lowered further if physical parameters of artificial radiation were similar to the ones of biological inductors. They mentioned that spectral composition, time dependence or other specific parameters may be of importance.

Higher sensitivity of biological detectors to the intermittent radiation was demonstrated earlier in experiments with yeast detectors and inductors [3, 4]. A rotating disk with one or more slits was placed between the detector and the inductor. Duration of exposure

periods, their frequency and total time of exposure depended on the rotation frequency, the widths and locations of slits. It was shown that the intermittent radiation allowed one to get a mitogenetic response at the shorter exposure time/the lower intensity than for the continuous radiation. For instance, a stable mitogenetic response was obtained after just 12.5–13 seconds of intermittent radiation with the frequency of interruption of 100–800 Hz, while it required 6–8 min of an exposure to continuous radiation. Thus, regular interruption lowered the duration of exposure in 30 times in comparison with continuous radiation. For continuous radiation the mitogenetic effect was observed at the inductor-detector distance of less than 3–4 cm, while regular interruptions of radiation increased the possible distance up to 15 cm (Refs. [4, 87]).

The experiments with slow approaching of yeast blocks also proved that the sensitivity of detector strongly depended on the time character of radiation influence. Yeast blocks were slowly moved from the distance, where the mitogenetic effect was not observed, to the distance, where the mitogenetic effect was stable in previous tests. Such a slow approaching corresponded to a gradual increase of the intensity of radiation. If the approaching was slow (>5–6 minutes), the detector did not show any response to mitogenetic radiation; at the faster approaching (<3 minutes) a stable mitogenetic effect was observed [4, 87].

Dependence of the sensitivity of biological detectors from polarization of radiation was also studied. Detectors were more sensitive to polarized radiation, i.e. mitogenetic effect was observed at the longer inductor-detector distances [138]. Problems of polarization and time dependence of natural mitogenetic radiation were studied experimentally in several experimental works, but they did not give an unambiguous answer. It may be related to the high variability of these parameters for different biological inductors and their dependence from a number of factors.

Parameters of natural mitogenetic radiation and mechanisms of its biological detection were studied not enough to explain a higher sensitivity to the natural mitogenetic radiation. Maybe it is not a single parameter, but a set of parameters that make this radiation so specific for biological detectors.

6 Blood radiation, cancer quencher and cancer diagnostics

Mitogenetic radiation of blood was studied quite widely, because blood was an easily accessible and reliable inducer of mitogenetic effect and at the same time it was commonly used for medical diagnostics. It was demonstrated, that the blood of healthy humans, various mammals, birds and amphibians, as well as the hemolymph of crabs and molluscs radiated quite well. H. Gesenius wrote: "Healthy blood never fails. If a failure occurs, it is time to test either the yeast or the apparatus" [139]. It was shown, that the blood radiation

in mammals was mainly related to glycolytic processes, whereas the blood of amphibians radiated due to oxidative processes [4, 24].

A detailed review of early studies of mitogenetic radiation of blood was presented in the second volume of the handbook of general hematology [140] (cited by Ref. [3]).

In various types of cancer diseases the blood radiation disappeared at the early stages of malignancy, long before the appearance of the first morphological signs of malignant growth, due to the UV-chemiluminescence quenching by a highly specific substance. This substance was called a cancer quencher. In cancer diseases the radiation of blood [101], urine [102] and corneal epithelium [4] ceased completely. In contrast, malignant tumors themselves were extremely active sources of mitogenetic radiation [4, 84].

Physical and chemical properties of the quencher were studied in detail, although its chemical composition remained unidentified. The quencher was found in the albumen-free blood fraction, it had negative electric charge, in solution it was stable enough but thermolabile; quencher diffused through the collodion membrane only under electrophoresis conditions, withstood drying at room temperature, it could be adsorbed on kaolin and washed out with an alkaline solution [24]. Researchers concluded that the cancer quencher was "a peptide having a predominance of the enol form of peptide bonds", threadlike structure and length about 3 nm [24]. The works on the cancer quencher were the first ones that claimed a possibility to use a blood peptide as a tumor marker. It cannot be excluded that the cancer quencher was not a single peptide but a whole group of similar peptides (maybe even specific for different types of cancer) with the active sites quenching UV-chemiluminescence. Active part of the cancer quencher was rather small, after careful hydrolysis in hydrochloric acid this substance diffused through collodion membrane without loss of the quenching properties.

It should be noted that the mitogenetic radiation of blood ceased in some physiological states (physical [111] and mental [112] fatigue, starvation [4], senility [111]) and in various diseases (pernicious anemia, leukemia, pneumonia, sepsis [87, 110, 140], chronic tonsillitis [141], diabetes, liver cirrhosis, etc. [4, 24]). It was found that in all cases not related to cancer diseases the blood radiation vanished due to the appearance of substances not quenching, but absorbing UV-radiation. These substances were called absorbers [24, 73]. Absorbers were found to be low molecular weight products of pathological metabolism, for instance, ketone bodies in diabetes. In contrast to absorbers the cancer quencher did not absorb UV-radiation significantly and did not change the UV-transmittance of thin layers of blood. Principal differences between the properties of the quencher and absorbers allowed to develop a method for the quencher detection in blood, which was based on the use of biological detectors of mitogenetic radiation.

The quencher was found only in the blood of humans and animals with malignant tumors or in precancerous states leading to them. It appeared at the very early stages of malignancy. The blood radiation disappeared 9-12 days after the tumor inoculation to healthy rats, while the tumors could be palpated only 28-32 days after the inoculation [142]. In experiments on mice, the cancer quencher appeared in the blood on the 1st–2nd day after carcinoma implantation and disappeared within 3-8 days after radical surgery, longer absence of blood radiation always followed by tumor recurrences [143, 144]. Experiments on mice with regular skin application of carcinogens showed that the quencher appeared in the blood at the still reversible stages of carcinogenesis, when papillomas grew on the bald patches [24-26]. If the carcinogen applications were stopped after the cancer quencher appearance, tumors developed only in mice with persistent quencher, other mice demonstrated local hair recovery, disappearance of papillomas and quencher. The quencher was detected in the blood of mice since 32nd–53rd day after the beginning of carcinogen applications and tumors were diagnosed only at day 191-222. Tumors were not formed in the mice, in which blood the quencher did not appear [25, 26].

Clinical studies of patients with different types of cancer demonstrated high accuracy of tumor diagnostics based on the cancer quencher (specificity and sensitivity >95%) and its successful application for the efficacy evaluation of implemented therapy [24-27, 73].

7 Research from the middle 50s to present

In the USA a general attitude to mitogenetic effect as a false phenomenon of “Soviet science” started forming as early as in the 30s and works on this topic were quite rare [3, 75, 76, 145-148]. Then the “Iron Curtain” strengthened this public opinion. In Europe active research in this area ceased at the beginning of World War II, in the USSR it was victimized along with genetics after the famous session of the All-Union Academy of Agricultural Sciences in 1948. There was practically no research done in this area from 1948 till the middle 50s.

Invention of photomultipliers stimulated a new wave of interest in radiation of biological objects. Ultraweak photon emission of plants was first detected by L. Colli and U. Facchini in 1954 [149]. In the USSR scientific groups of B.N. Tarusov and Yu.A. Vladimirov selected the best samples of photomultipliers at producing factories, designed liquid nitrogen cooling systems and achieved an extremely high sensitivity [150-152]. With such devices, they managed to detect ultraweak photon emission from animal tissues, which was much weaker than from plants [38, 151-153]. As B.N. Tarusov was occasionally remembering later, they had been “leafing through the books of A.G. Gurwitsch and repeating all experiments one after another”. Yet, the works of these authors were principally different from the original works on mitogenetic effect: the photomultipliers used by B.N. Tarusov et al. had much higher sensitivity to

visible light than to UV light, moreover, the studied bio-objects were placed inside “absolutely dark chambers”, which had to bring the mitogenetic UV-component of radiation to naught [3, 4], for more details see Ref. [28]. Thus, the radiation detected by these researchers had no relation to the mitogenetic effect.

At the same time the groups of A.A. Gurwitsch [43] and S.V. Konev [44], which continued studies with modified Geiger-Muller counters, started using photomultipliers as well. They demonstrated a reliable UV component in the ultraweak photon emission of yeast cultures and a number of different biological objects [154].

By the end of 60s the ultraweak photon emission research had focused on the visible component, because its intensity was much higher than ultraviolet one. These works laid the foundation of the presently well-investigated area of ultraweak bio-chemiluminescence (now mostly called ultraweak photon emission) [38-42, 155-157]. In the following years the mechanisms of ultraweak photon emission in the visible-range were found out, namely free radical recombination accompanying oxidative processes in lipid phase of membranes [38], and the reaction cascades involved in these processes were described in details [40, 158, 159]. It was shown that some diseases led to the changes of such ultraweak photon emission of various tissues, blood, urine etc. [160, 161], and ultraweak photon emission can be useful for medical diagnostics, including cancer diagnostics [162, 163].

The principal difference between the visible and the UV-components of ultraweak photon emission was incapability of the former to influence mitoses, and thus its irrelevance to mitogenetic effect. Nevertheless, the researches on visible ultraweak photon emission were important for the mitogenetic effect area, not only because the photomultiplier technique was developed, and the possible use of ultraweak photon emission for medical diagnostics was demonstrated. Molecular mechanisms of ultraweak photon emission, well investigated in this period, confirmed the free-radical hypothesis of photon generation, initially proposed by A.G. Gurwitsch on the basis of his study of the mitogenetic effect.

In the middle 60s, when the visible ultraweak photon emission was rather well-studied phenomenon, the group of T.I. Quickenden started a series of experiments on mitogenetic radiation. Their first attempts to detect UV ultraweak photon emission from yeast and bacterial cultures were unsuccessful [164] and the authors hastened to claim the mitogenetic radiation as a disproved misconception of the “Soviet science”. This work published in “Nature” well fitted in with the widespread opinion of those times about the “obscurantism” in the USSR, and had a significant negative influence on the general attitude to this field. Seven years later the authors significantly improved the experimental design (namely, they used a larger photomultiplier cathode, located the object closer to it, collected the askew radiation with mirrors etc.) and

managed to detect definite and reproducible UV component of ultraweak photon emission from suspensions of yeast cells under conditions of oxygen saturation [165]. In the following 20-year-long series of works, the authors investigated ultraweak photon emission from different microbial cultures both in visible and UV ranges [47, 48, 128-130, 165-169]. They definitely confirmed the results on the intensity of UV ultraweak photon emission, its spectrum and stage-specificity (i.e. appearance at certain stages of the culture growth) obtained in the 1930s–40s. This made the authors radically change their attitude toward the “early works” [166].

Two specific periods of photon emission were characteristic for growth of all investigated cultures: a small reproducible peak of ultraweak photon emission in the period of active growth (exponential and linear growth phase) and much more intensive, but highly variable radiation in the stationary phase (Fig. 10).

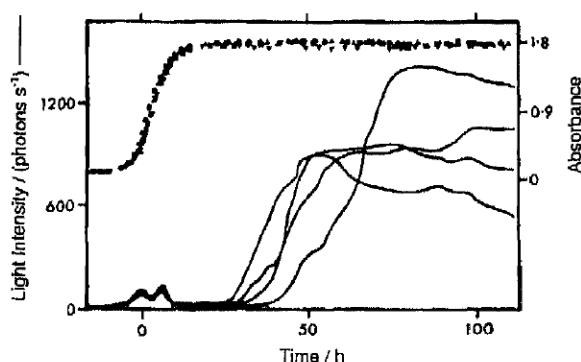


Fig. 10 Luminescence of *Candida utilis* (solid lines) and their growth dynamics (dots). 0 time point – time of half-maximal culture density. Reprinted from Ref. [48] with permission from John Wiley and Sons, Inc. © 1992 by John Wiley & Sons, Ltd.

During the period of active growth the ultraweak photon emission showed a definite UV component, 20–40% of total intensity of ultraweak photon emission, which was 10–100 photons/cm² sec (Fig. 11). The stationary-phase ultraweak photon emission had only visible component. The visible ultraweak photon emission (in both periods) corresponded to lipid peroxidation processes, increasing in respiratory-deficient cultures due to the lack of “physiological ways of oxygen utilization” [47, 48, 130]. The UV component was shown to be: (1) not connected to lipid peroxidation; (2) not cosmic-rays excited fluorescence; (3) oxygen-dependent; (4) not connected to a number of key biochemical processes [48].

The authors pointed out that UV component appeared at the same stages of growth as mitogenetic radiation, had the similar intensity, wavelength and other characteristics, and also suggested “oxidative side reactions associated with protein synthesis” as its possible source [48].

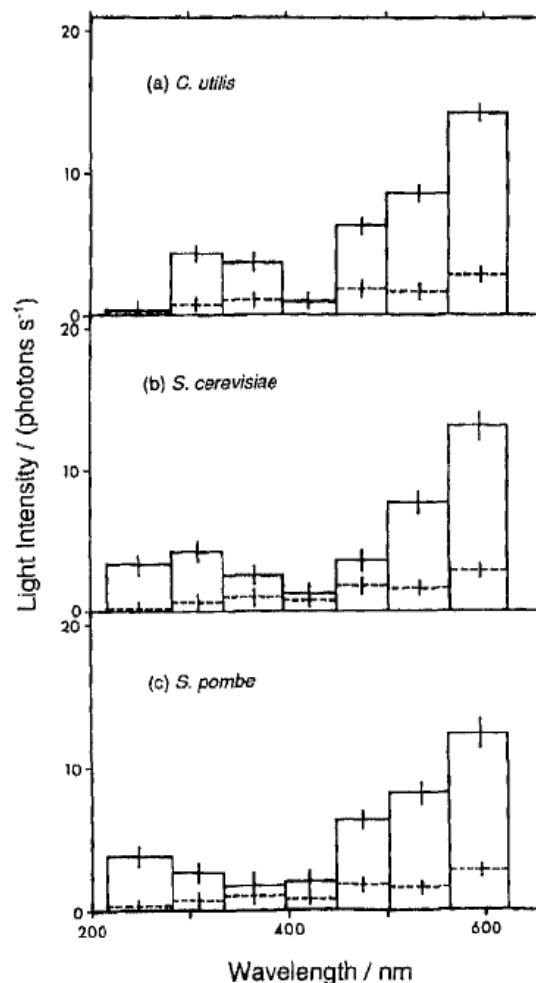


Fig. 11 Ultraweak photon emission spectra of various species in the linear-growth phase: (a) *C. utilis*, (b) *S. Cerevisiae*, (c) *S. pombe* (50% confidence intervals). The growth medium emission spectra are shown with dashed lines. Reprinted from Ref. [48] with permission from John Wiley and Sons, Inc. © 1992 by John Wiley & Sons, Ltd.

Yet these authors failed to observe the main (biological) mitogenetic effect [167, 170]. They thoroughly reproduced the conditions necessary for the culture-inducer to radiate, but violated a number of critically important requirements to the culture-detector, e.g. the recipient culture was in stationary (G0) phase, though stationary-phase cultures had been shown in earlier works to be absolutely insensitive to mitogenetic radiation [3, 4, 77, 78] (see analysis of other mistakes in [28]).

Another wave of interest to the problem of mitogenetic effect and distant biological interactions aroused in the 1980s–90s due to the activity of the International institute of biophysics in Germany, which was organized and headed by F.-A. Popp. It united research groups of 19 institutes from 13 countries in an international network, including a number of scientists from Russia (V.L. Voelkov, A.B. Burlakov, L.V. Belousov and their groups), Israel

(M.A. Lipkind), the Netherlands (R. and E. van Wijks), Italy (F. Musumeci, H. Niggli), China (J.J. Chang), India (R. Bajpai), etc. The scientific discussion of that time was mainly devoted to F.-A. Popp's beautiful hypothesis of specific quantum properties of biological ultraweak photon emission: abnormally high coherence, the so-called squeezed-state, etc. [171-174]. The researchers measured not as much spontaneous ultraweak photon emission of biological objects, as their photo-induced delayed luminescence. Based on different parameters of its dynamics, they calculated "degree of coherence" of the "inner electro-magnetic field of the object" [172]. Yet, none of their hypotheses has got an experimental proof by this time [42, 50, 51], while a huge number of popular "sensational" articles greatly compromised the whole research area.

Simultaneously, using both their own equipment and the (quite powerful) instrumentation of the International institute of biophysics, a number of authors were analyzing dynamics of spontaneous ultraweak photon emission from different biological objects: oocytes and embryos of fish, frogs [175, 176], cell and microbial cultures [63, 177], chicken eggs [54], human blood [178, 179] and simple aqueous systems [180]. The authors were searching for specific temporal order in ultraweak photon emission, which would be more likely to "suspect" in mitogenetic component than in the visible one. For this purpose they applied methods of digital processing of noisy signals (autocorrelation, Fourier analysis, wavelet, multifractal analysis, etc.) to the raw data from photomultipliers. Sometimes the authors claimed interesting regularities in ultraweak photon emission, however, they failed to show any parameters that would confidently and repeatedly distinguish the ultraweak photon emission of biological objects from corresponding controls. Wideband photomultipliers (~200–800 nm) with maximal sensitivity in the visible light were used in practically all these works, and (consequently) the objects were placed in very dark chambers, where mitogenetic effect could not be observed according to the "early works". Thus, as far as mitogenetic radiation was concerned, these attempts were practically hopeless.

The works on mitogenetic effect also stimulated investigations of possible distant (optical) interaction between microbial cultures [66, 68, 177, 181], cell cultures [60-62, 182-185], oocytes and embryos of fish [61, 62] and frogs [186]. Yet, none of the groups managed to obtain regularly reproducible results, that could be considered a solid proof of the phenomenon [57, 68, 70, 187]. It should be noted that all (!) these works on mitogenetic effect suffered from technical mistakes and did not stand up to scrutiny. All of these authors seem to have made their experiments without due technical equipment, financial support and scrupulousness, which (together with "delicacy" and methodical difficulty of observing the phenomenon [28], led to the low level of evidence in these works. The authors didn't try to reproduce the known results under previously established conditions and follow the

detailed methodical recommendations from "early works", but rather made attempts to get analogous results under conditions never investigated before (or even previously proven ineffective).

The problem of possible mechanisms of generation and detection of "mitogenetic signals" has been actively discussed since A.G. Gurwitsch's times up to these days [3, 4, 28, 50, 51, 69, 188]. The mechanisms of emission of UV photons are still unknown. It should be mentioned, that UV luminescence accompanying a number of branching-chain reactions (e.g. hydrogen peroxide + glycine) was studied in more details with photomultipliers and some specific features of these processes were found [43, 189, 190].

All basic works on cancer quencher were published only in Russian [24-27, 144, 191, 192] and remained almost unknown abroad. There were very few significant investigations related to quencher after the 1940s, and practically all of them were made in the group of A.A. Gurwitsch. The dissertation of L.I. Sneshko [193] on the role of spleen in the cancer quencher emergence described one of the most interesting findings of this period. If the spleen had been extirpated several days before the cancer induction, the cancer quencher was not secreted into blood during the following carcinogenesis, and mitogenetic radiation of blood did not change as well. V.F. Ereemeev investigated appearance of cancer quencher in liver of mice with implanted malignant tumors [55]. One of the chapters of the book [23] was devoted to the mitogenetic analysis of cancer cell biology and works on cancer quencher.

8 Conclusion

The review covers researches on the mitogenetic radiation made in 1923–1948 years.

Main achievements of this period:

- the mitogenetic effect was discovered;
- this effect was attributed to ultraweak UV-radiation, its spectral range and intensity were estimated;
- the mitogenetic effect was shown to be species-nonspecific;
- UV-chemiluminescence of a number of biochemical and chemical reactions was found out and their spectra were analyzed with use of biological detectors;
- mitogenetic radiation of various biological objects was attributed to the well-known biochemical processes;
- a highly-specific universal tumor marker, so called cancer quencher, was found in blood; it was shown to be a peptide (or group of similar peptides) quenching UV-chemiluminescence of blood.

Some valuable results had been proved later. Measurements with photomultipliers corroborated an existence of UV-chemiluminescence of bio-objects, its intensity and spectral range evaluations. Gurwitsch's hypothesis of photon generation in branching-chain reactions with recombination of free radicals was shown

to be correct for chemiluminescence in the visible region. Possibility to use a blood peptide as a highly-specific tumor marker was proclaimed 30 years before the alpha-fetoprotein and this statement was revolutionary for the 1930s. This conclusion was made exclusively on the basis of experiments with mitogenetic radiation of blood. An amazing correlation of the results of this cancer diagnostics for newly-admitted patients with their final diagnoses (specificity and sensitivity >95%) hardly could be explained by mere coincidence [24-27].

We do not claim all the results on mitogenetic radiation as postulates, however, there is no reason to consider them as falsification, mass misconception, or common systematic errors. These experiments were conducted in the leading laboratories of the world by distinguished scientists and their groups. All the main experiments had passed multiple verifications in different reputable laboratories. Negative works made up less than 3% of the total number of publications on mitogenetic effect; all of them had been refuted in response criticism with clear statement of errors. Before the World War II all the results on the subject were widely discussed at international level, supporting experimental data were quite massive, many publications included thorough descriptions of experimental methods and detailed protocols and they deserve serious attention. After the end of the 40s the attempts to reproduce the experiments on mitogenetic radiation were rather scarce. As far as we know, no significant researches on mitogenetic radiation were published in the last years. Recently, there were a number of papers devoted to the distant interaction of biological objects (for example, Ref. [67]), where the authors mentioned that maybe the observed effects were related to the mitogenetic radiation. However, they did not present any proof that the radiation underlying the effects belonged to UV range or stimulated mitoses. We strongly doubt that these effects related to mitogenetic radiation because of too long distance of interaction and some other reasons. In other works (for instance, Ref. [185]) the experimental conditions made the nature and the reproducibility of the effect doubtful, and they can not be attributed to the mitogenetic effect as well. We consider all these works irrelevant to the subject of this review.

The experimental verification of the results on mitogenetic radiation at the up-to-date level is possible and necessary in relation with basic science (for instance, for the clarification of possible role of UV-chemiluminescence in regulatory processes in cells and

multicellular organisms) and practical applications (prospects of an effective early-stage cancer diagnostics, optimization of biological processes with “mitogenetic factors” in R&D and industries).

Resumption of research on cancer quencher is especially important from the practical point of view: the identification of this peptide (or group of similar peptides), the finding of its role in carcinogenesis, verification of the described methods of the quencher extraction from blood and applicability of quencher detection to early screening of cancer, control of efficacy of implemented surgery/therapy, and timely detection of metastases. The most popular argument against the cancer quencher existence is the following one. If the experimental results on cancer quencher were true, then it would be widely used by now. At the same time the history of science demonstrates quite a few cases when true hypotheses and experiments were verified after decades and even centuries of oblivion. All the key experimental works on the cancer quencher were published in Russian [24-27, 144, 191-193] and remained almost unknown to the English readership, while in the USSR this scientific direction did not recover after the persecution in 1948 year.

Up-to-date methods of cytogenetic, cellular and biomolecular analysis, controlled environments, accurate control of biological states of inductors and detectors, spectroscopy, complex experimental design techniques and statistical tools allow to develop clear proofs or disproofs of early results on mitogenetic radiation. Verification of these results requires a thorough study of used experimental designs and methods with special attention to the details, which were examined in some “old works” [3, 4, 23, 145, 146, 194] and newly analyzed recently [28].

Substantial progress in biomolecular analysis, cell biology and biochemistry, physical methods and experimental techniques let us expect these undeservedly forgotten researches to be resumed at the up-to-date level.

This review is mainly grounded on the publications from the unique archive of scientific literature collected by A.G. Gurwitsch, his wife L.D. Gurwitsch, their daughter A.A. Gurwitsch and grandson L.V. Belousov.

Disclosures

All authors declare no conflict of interests for this manuscript and have no a financial interest in the materials used in the manuscript.

In vivo hyperspectral analysis of skin hemoglobin and melanin content for neoplasia detection

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Abstract. We present results of main skin chromophores (melanin and hemoglobin) optical analysis. Analysis of 91 *in vivo* skin tissues (50 benign and 41 malignant) was performed in visible spectral region with hyperspectral imaging technique. To assess the malignancy of skin tissues we proposed two methods for calculating the integral index of tissue optical density and performed a comparison of their effectiveness and effectiveness of physician survey. As the main diagnostic feature, we propose to use data of integral optical index dispersion from the studied tissue and healthy tissue area. The results of skin tissues classification with discriminant analysis are presented. The possibility of the proposed approaches application in the clinical practice is shown. © 2018 Journal of Biomedical Photonics & Engineering.

Keywords: Hyperspectral imaging; skin cancer; melanoma; basal cell carcinoma; skin chromophores.

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1 Introduction

Cancer is one of the most dangerous and yearly progressing diseases. According to the International Agency for Research on Cancer, about 14.1 million new cases of cancer have been detected throughout the world in 2012, and the estimated increase of new cancers by 2025 is up to 19.3 million cases due to the growth of the population and the increase in its average age [1]. Skin cancer is one of the most common forms of cancer [2]. For example, basal cell carcinoma of the skin is the most common type of cancer in the world and accounts for about 75% of all malignant skin formations.

Melanoma, on the contrary, has the lowest incidence rate in the world (9%) but ranks first in mortality (75%) among all forms of skin cancer [3].

Similar trends in cancer morbidity are also observed in Russia. For example, according to the Moscow Scientific Research Institute of Oncology named after P.A. Hertsen, the incidence of malignant neoplasms in Russia in 2015 was 589 341 cases. One of the main localizations in the general (both sexes) structure of the oncological morbidity is skin (14.2%) – the second most common oncological pathology in women and third in men [4]. The increase in the incidence of malignant skin

tumors from 2005 to 2015 is 31.47%, including a 34.84% increase in melanoma cases.

Taking into account the superficial localization of skin tumors the most convenient way of their early detection is a preventive examination. In the early detection of a tumor, the patient has a higher chance of recovery [5]. However, a visual examination of melanoma at an early stage is usually not enough, the accuracy of diagnosis of pigmented skin tumors in the early stage (especially for first-hand specialists) does not exceed 50% [4] due to the similarity of melanoma with a nevus.

Different types of skin biopsy do not apply since they are time-consuming and may lead to cosmetic injury [5]. In this regard, studies of many scientific groups are aimed at developing precisely non-invasive methods for primary diagnosis of skin oncological pathologies. The diagnostic efficiency of such methods as CT and MRI falls dramatically for small diameter tumors (less than 1.5 mm) due to insufficient spatial resolution [6]. In such cases, a more accurate assessment of the malignancy of skin formations can be obtained with the use of optical diagnostic methods, such as digital dermatoscopy [7], Raman spectroscopy [8, 9] and hyperspectral imaging [10].

Dermatoscopy provides the opportunity to enlarge the standard ABCD rule [11] or 7-point checklist [12] and involve additional criteria in suspicious skin tissues inspection. Thus, dermatoscopy opens up a new dimension on clinical morphology of pigmented skin lesions and enables well-trained physicians to improve their diagnostic accuracy up to 65% [13]. As this accuracy may be obtained only by a well-trained physician, hyperspectral imaging (HSI) technique may be used. This technique simultaneously allows for imaging of the pathology area and spectral data acquiring. The tissue image allows one to estimate tissue morphology and allows for finding the stage of tumor growth, while the spectral data allows one to make a conclusion about the chemical composition of the tested tissue area. For example, the quantitative change of melanin and hemoglobin indicates a high risk of tissue malignancy and leads to a discoloration of the skin, changes of the formation shape and appearance of atypical vascular and pigmentary patterns [14].

In current *in vivo* clinical trials, we tried to estimate applicability of integrated optical density indexes introduced in our previous preliminary studies [13] and calculated during HSI application in the visible wavelength range (450–750 nm) for areas of melanin and hemoglobin absorption. Analysis of optical indexes was performed for only neoplasm spectra and neoplasms and adjacent normal skin spectra to estimate the potential of normal skin examination during the neoplasm diagnosis. In contrast to earliest investigations on a smaller cohort of malignant neoplasms [15, 16] (less than 20 malignant tumors) our trial was performed on a cohort of 91 skin neoplasms, including 41 malignant tumors. It helped us to achieve reliable data about integral optical density indexes application in

clinical examination of skin cancers. The proposed approach was used to differentiate various skin malignant tumors from the benign. Malignant Melanoma (MM) and basal cell carcinoma (BCC) were studied in the malignant tumors group; in the benign diseases (Benign - BN) group keratomas, hemangiomas and nevi were studied.

2 Materials and methods

2.1 Experimental setup and spectra acquisition

To register the spectral images of skin we used an acousto-optical (AO) video spectrometer [17], its structural diagram is shown in Fig. 1.

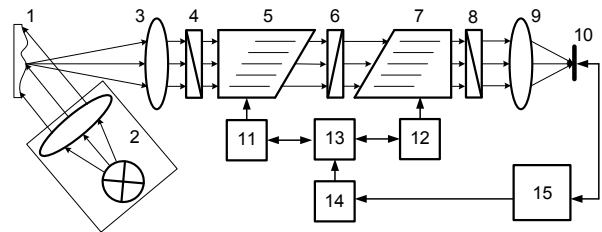


Fig. 1 Registration of skin hyperspectral images: 1 – skin sample, 2 – LED diode, 3, 9 – collection lenses, 5, 7 – AO cells, 4, 6, 8 – polarizers, 10 – camera, 11, 12 – high-frequency amplifiers 13 –high-frequency generator, 14 – controller, 15 – PC.

The broadband radiation of the LED source 2 reflected and scattered from the skin sample 1 is converted by an input lens 3 into a collimated beam and directed to the AO monochromator consisting of two AO cells 5 and 7 and three polarizers: an input 4, an intermediate 6 and output 8. A special feature of AO monochromator is double sequential filtering. It provides almost complete compensation of spatial and spectral image distortions caused by Bragg diffraction in a single anisotropic AO cell [15]. In the proposed scheme two identical AO cells 5 and 7 perform diffraction of ordinary and extraordinary waves. The second cell 7 is rotated around the first AO cell 5 in the diffraction plane by 180°. The polarization plane of the polarizer 6 is orthogonal to the plane of polarization of the identical polarizers 4 and 8. Light in AO cell 5 diffracts on the dynamic grating produced by the acoustic wave of a given frequency. As a result of diffraction, the radiation changes its linear polarization from the ordinary polarization to the extraordinary one. Similarly, repeated diffraction in the AO cell 7 occurs, which increases the contrast of the isolated spectral component with full compensation of linear and nonlinear distortion. Then, with the aid of the objective 9, the filtered light is focused on the matrix of the monochrome video camera 10. By varying the frequency of the acoustic wave the piezoelectrical excitation of the AO cells 5 and 7 occurs, and it is possible to reconstruct the diffraction grating period simultaneously in both AO cells. Thus it is possible to

Table 1 Summary of patients with skin tumors.

Lesion	Age	Gender		Location			
		Male	Female	chest/ stomach	neck/ head	limb	back
MM	38-78	7	15	2	3	8	9
BCC	37-85	8	11	3	9	3	4
BN	22-82	19	31	8	11	14	17

obtain the image of the object 1 at an arbitrary predetermined wavelength λ in the range 440–750 nm with a sufficiently high spectral resolution ($\delta\lambda = 2.5$ nm at $\lambda = 633$ nm) and spatial resolution of 0.14 mm.

2.2 Tissue samples

There were 91 patients with skin cancers (57 females and 34 males, all white, Caucasian, skin phototype I and II) enrolled in this study. A total of 19 BCC, 22 MM and 50 BN neoplasms (including 16 nevi, 5 papillomas, 4 hemangiomas, 7 different inflammatory diseases, 12 keratomas, 1 fibroma, 1 Bowen disease, 4 keratopapillomas) were studied. The detailed distribution of human skin lesions, including information about tumor locations, is provided in Table 1. Each neoplasm was examined by the oncodermatologist to get a medical specialist diagnosis. Every tumor study was accompanied by histological analysis to make a final diagnosis. The protocols of *in vivo* tissue diagnostics were approved by the ethical committee of Samara State Medical University. The Institutional Review Board of Samara National Research University approved the study protocols. This research adhered to the tenets set forth in the Declaration of Helsinki. Informed consent of each subject was obtained.

2.3 Spectra processing and data analysis

Typical normalized spectra and tumor images stabilization procedure may be found elsewhere [18, 19]. Generally, to obtain quantitative estimations of various human skin tissues backscattered spectra we used data about the spectral optical density of skin tissues. The diffuse backscattered reflectance from the skin in spatial point (x, y) may be characterized by optical density (OD) on the selected wavelength λ as:

$$OD(x, y, \lambda) = \lg \frac{I_o(x, y, \lambda)}{I(x, y, \lambda)}, \quad (1)$$

where $I_o(x, y, \lambda)$ is backscatter intensity from the background of the light source, $I(x, y, \lambda)$ is backscattered intensity from the tissue sample. While the exact determination of tumor type may be performed with OD analysis based on above spectral bands:

$$OD_i(x, y) = \frac{1}{\lambda_2 - \lambda_1} \int_{\lambda_1}^{\lambda_2} OD(x, y, \lambda) d\lambda, \quad (2)$$

where hemoglobin-related optical density OD_H defined for ($\lambda_1 = 530$ nm, $\lambda_2 = 570$ nm) and melanin-related OD_M – for ($\lambda_1 = 600$ nm, $\lambda_2 = 670$ nm). Further, we calculated mean OD_H and OD_M values for all studied tumors and healthy skin. Allocation of tumor area was performed on the basis of the approach presented in our previous studies and was performed based on image segmentation by Otsu method [19]. Healthy skin region was chosen near the tumor area with 1.5–2 cm indentation from the tumor and included approximately 1×1 cm area of healthy skin. Example of the hyperspectral image with tumor and healthy skin areas allocation as well as the distribution of OD_M coefficient may be found elsewhere [15].

Discrimination of tumors was performed based on two principles. The first principle took into account only raw values of OD_H and OD_M coefficients, while the second principle used data for the normal skin of the same patient for OD_H^N and OD_M^N coefficient as follows:

$$OD_i^N = \frac{OD_i^T}{OD_i^H}, \quad (3)$$

where OD_i^T and OD_i^H are OD_i coefficients calculated for one patient for tumor (index T) and healthy skin (index H) areas respectively.

Finally, we analyzed integral properties of skin for allocated tumor area and healthy skin area with an unbiased dispersion of OD_i and OD_i^N coefficients inside the area of interest:

$$\overline{OD}_i = \frac{1}{N-1} \sum_{n=1}^N (OD_n - \bar{M})^2, \quad (4)$$

where N is the number of pixels in the area of interest, OD_n is the OD (or normalized OD) in the n^{th} pixel of the area of interest, $\bar{M}$ is the mean value of OD_n inside the area of interest.

In skin tissues classification we utilized $\overline{OD}_i$ and OD_i^N coefficients. The choice of these coefficients was based on coefficients utility. While OD_i^N coefficients provide information about changes of chromophores content in the tumor in comparison to healthy skin of the same person, $\overline{OD}_i$ coefficients provide information

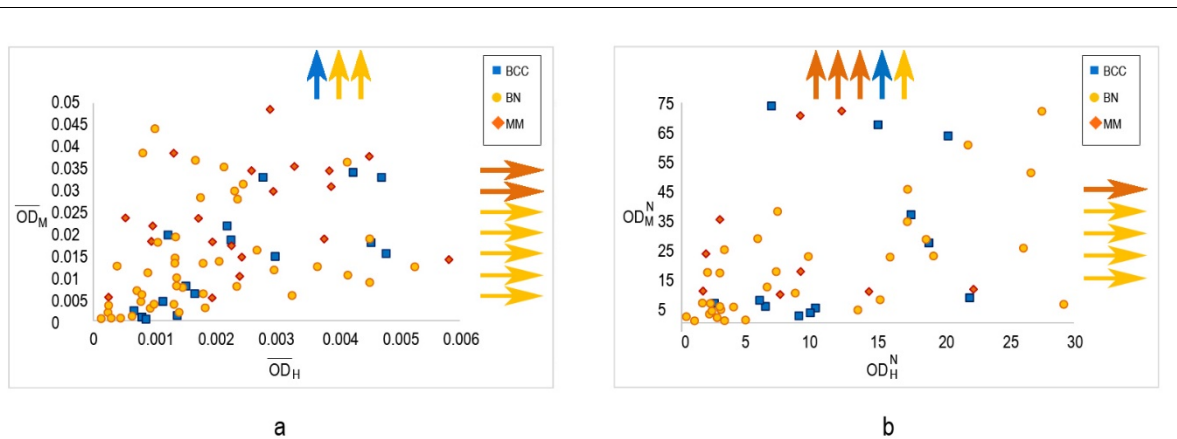


Fig. 2 Distribution of skin neoplasms on phase planes with $\overline{OD}_i$ (a) and OD_i^N (b) axes.

Table 2 Accuracy (%) of neoplasms classification

Classes	$\overline{OD}_H$	$\overline{OD}_M$	OD_H^N	OD_M^N	$\overline{OD}_H + \overline{OD}_M$	$OD_H^N + OD_M^N$	Physician
MM+BCC vs BN	61	71	65	60	76	78	51
MM vs BCC	54	59	66	59	68	79	56
MM vs BCC+BN	60	69	68	63	75	75	51

about mean values of chromophores concentration in the tumor area. Thus, these two approaches use different features of chromophores distributions and both may be useful in skin tissues classification.

$\overline{OD}_i$ and OD_i^N coefficients were used for tissue classification by discriminant analysis (DA). DA can separate two or more classes based on different statistical parameters of Gaussian distributions. The efficiency of the proposed approach is characterized by sensitivity and specificity and ability to select defined classes in different areas of the phase plane. The analysis of skin tissues data allocation was performed using quadratic DA classifiers [20]. In addition, data were analyzed using SPSS software version 23 (SPSS Inc., Chicago, IL, USA) with logistic regression, and a P-value of < 0.01 was considered to indicate statistically significant differences. Then, the plot of Sensitivity versus 1-Specificity (called receiver operating characteristic (ROC) curve) is built, and the area under the curve (AUC) was used as an effective criterion of skin tissues discrimination accuracy. This curve plays a central role in evaluating diagnostic ability of tests to discriminate the true state of subjects, finding the optimal cut off values, and comparing alternative diagnostic tasks when each task is performed on the same subject [21].

3 Results and discussion

Differentiation of skin neoplasms was performed with phase plane analysis. In the separation of phase plane classes axes of the plane are criterions of tissues

classification, and every tested sample is a point on the phase plane with coordinates corresponding to the criterion values for this tissue sample. Differentiation of BN pathologies from malignant neoplasms is possible with backscattered spectrum analysis in the visible region since it contains data on hemoglobin and melanin content in skin tissues. Fig. 2 demonstrates the joint application of $\overline{OD}_i$ and OD_i^N coefficients for skin tissues classification. These distributions may help classify neoplasms based on quadratic DA implementation. For example, the most accurate discrimination of MM and BCC is possible using 7.8 and 14.3 thresholds of OD_M^N and OD_H^N coefficients values. Most efficient malignant and benign tissues separation is possible using 0.016 and 0.0014 thresholds of $\overline{OD}_M$ and $\overline{OD}_H$ coefficients values. In addition, general information about neoplasms classification accuracy with proposed coefficients and oncological specialist collected in Table 2.

Analysis of Fig. 2 and Table 2 helps to reveal possibilities of HSI integral information application in skin neoplasms classification. Application of single $\overline{OD}_i$ or OD_i^N coefficient for melanin content leads to 60%–70% accuracy and these coefficients for hemoglobin content provides a slightly lower accuracy of 55%–65%. It is important to note, that accounting of normal healthy skin helps to improve accuracy for $\overline{OD}_H$ and decreases accuracy for $\overline{OD}_M$. These facts may be caused by inhomogeneity of melanin and hemoglobin distribution in the skin. While neoplasms have a heterogeneous distribution of hemoglobin and capillary network, normal skin has more homogeneous one [20],

and, thus, comparison of $\overline{OD}_H$ dispersion in neoplasm and normal tissue provides additional information for tissues discrimination. Melanin content of neoplasms frequently may be increased in comparison to healthy skin, but as every person have its own skin phenotype and unique level of melanin [22] it may be complicated to estimate differences in melanin content of neoplasm and healthy skin. That is why most likely in our study application of OD_M^N coefficient showed a rather low accuracy of skin neoplasms classification in comparison to $\overline{OD}_M$ comparison.

Application of coefficients combination ($\overline{OD}_H + \overline{OD}_M$ and $OD_H^N + OD_M^N$) helps to improve the accuracy of tissues classification by 5%–15% in comparison with single coefficient implementation. $OD_H^N + OD_M^N$ combination in comparison with $\overline{OD}_H + \overline{OD}_M$ pair shows almost the same rates of accuracy for cases of MM vs other neoplasms and malignant neoplasms vs BN classification but demonstrates 11% higher accuracy in case of MM vs BCC classification. That may be explained by OD_H^N coefficient usage as it shows high rates of tissues classification in comparison with unnormalized on healthy skin $\overline{OD}_H$ coefficient. Thus, we may try to combine normalized and unnormalized coefficients to achieve the highest performance of tissues classification.

In order to do this, we performed ROC analysis for the combination of proposed coefficients. Fig. 3 demonstrates ROC curves for different pairs of coefficients. One may see that that the best AUC values shows a combination of normalized and unnormalized coefficients. This fact proves that the best performance of HSI skin neoplasms study may be implemented with a joint analysis of pathology area and healthy skin. Combination of $OD_H^N + \overline{OD}_M$ and $\overline{OD}_H + \overline{OD}_M$ coefficients shows 0.693 and 0.686 AUC values respectively, while $OD_H^N + OD_M^N$ coefficients pair shows only 0.608 values. As higher AUC value the higher final accuracy of classification is, thus, unnormalized and combined approaches of neoplasm hemoglobin and melanin dispersion estimation may be implemented in clinical practice. Moreover, comparison of HSI analysis and physician survey accuracy presented in Table 2 shows that automatic analysis may significantly improve the accuracy of skin neoplasm detection. HSI analysis may be used during the first-hand examinations/ that suffer from very low specificity of malignant tumors detection [23].

Considering alternative optical methods of the proposed approach, one may say that the most frequently used today method is multispectral analysis with additional spectral ranges or native fluorescence analysis. For example, it was proposed to use the spectral region up to 1000 nm and analyze the content of NADH/ NAD+ [24, 25]. The total sensitivity and specificity for separation of MM from nevus and BCC were 97% and 96% respectively. However, this approach requires an additional light source (either a separate source for the near-IR or a white diode with a near-IR coating up to 1000 nm), as well as a camera

with a higher sensitivity (in comparison with CCD) in the 1000–1100 nm range, which significantly complicates the design of the multispectral camera. In the study by Nagaoka et al. [26] an index is proposed that describes the heterogeneity of the tumor, which is the analog of the color index in dermatoscopy. The sensitivity of the determination of melanoma was 90%, and specificity was 84%. However, it is important to note that described above results [24–26] were obtained on a small number of samples (only five–ten melanomas were included in the studied cohort).

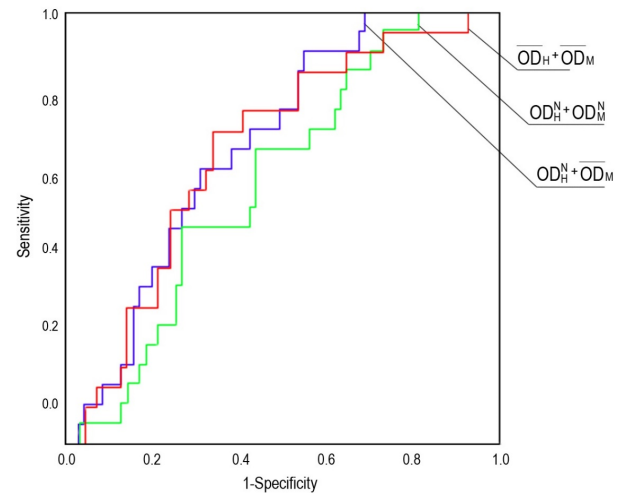


Fig. 3 ROC curves of MM vs BCC+BN classification with logistical regression for $OD_H^N + \overline{OD}_M$, $OD_H^N + OD_M^N$ and $\overline{OD}_H + \overline{OD}_M$ coefficients.

Evolution of the proposed technique may be based on the approach of spatial evaluation of diagnostic dermatoscopic signs, and for example, may be implemented with convolutional neural networks analysis [27]. The authors in Ref. [27] claimed maximum sensitivity and specificity of melanoma diagnosis at 90% and 85%, respectively. Therefore, image analysis approaches may be combined with the proposed technique to improve the overall accuracy in HSI skin cancer imaging.

4 Conclusions

The proposed approaches to skin tissues classification showed 68%–78 % accuracy. These values of accuracy are not quite high in comparison to 90% and higher accuracies of such optical approaches as Raman or fluorescent spectroscopies [8]. However, integral information about skin chromophores may significantly increase the accuracy of visual inspection by a dermatologist, as the proposed technique demonstrated 15%–20% higher accuracy in skin tumors classification. Wherein application of both $\overline{OD}_i$ and OD_i^N coefficients and their combination leads to the comparable accuracy in tumors classification. This fact proves that choice of visible range area (450–750 nm) for the analysis of the

chemical composition of skin may be useful in oncodermatological applications.

It should be noted, that in the proposed approach the processing of hyperspectral data is carried out in real time and does not require operator participation. In general, the simplicity of the equipment and the speed of processing results may allow for adapting the proposed method for tasks of mass screening of skin tumors. Moreover, in future works on the development of the proposed approach, we may increase the number of examined skin neoplasms samples and also combine the proposed technique with image pattern analysis methods.

Disclosures

All authors declare no conflict of interests for this manuscript and have no a financial interest in the materials used in the manuscript.

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Dermatoscopy software tool for *in vivo* automatic malignant lesions detection

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Abstract. Dermatoscopy is one of the most popular non-invasive methods of skin tumors diagnostics. Digital dermatoscopy allows one to perform automatic data processing and lesions classification that significantly increases diagnostics accuracy compared to general physicians. In this article, we propose a dermatoscopy tool equipped software automatic classifier of dermatoscopic data. Noise reduction and image histogram equalization were performed during the initial step of preprocessing. After this step, a feature-detection step was performed; the program finds region of interest and calculates Haar transform, linear binary patterns, and color-texture features in different color spaces (RGB, HSV and LAB) for both tumor and healthy skin areas. Finally, evaluated features are used for classification by using Support Vector Machines (SVM). This classifier has been trained and tested using 160 dermatoscopic images made with polarized backscattered light. The article shows data for two classes separation: malignant melanoma versus non-melanoma tumors and malignant versus benign lesions. Proposed approach has achieved sensitivity of 83% and specificity of 65% for melanoma versus non-melanoma classification and sensitivity of 61% and specificity of 60% for malignant versus benign lesion classification. Performed cross-validation ensures stability of the classifier. © 2018 Journal of Biomedical Photonics & Engineering.

Keywords: Classification; algorithm; cross-validation; image processing; dermatoscopy device.

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1 Introduction

In Russia in 2016, according to the overall statistics, skin has become the most frequent organ for malignant tumors allocation (14.2% out of all lesions, including 12.5% non-melanoma types) [1]. The most widespread tumor types include basal-cell carcinoma, squamous-cell carcinoma and malignant melanoma [2]. The key factor for better patients survivability consisted in early diagnostics, however, less than one third only of all tumors were diagnosed at the first stage in 2016 in Russia [1]. For this reason, there is a necessity to develop better diagnostic techniques or improve existing ones.

There are a number of different skin diagnostics methods exist. These methods have different nature and provide result of different effectiveness. For example, the sensitivity of a primary naked-eye examination by a general practitioner varies from 25 to 60% for a first time diagnosed melanoma [3]. This is the simplest diagnostics method, which does not require any equipment. Such examination is mandatory now, its accuracy is not high enough and, therefore, it is frequently complemented by instrumental methods.

A group of optical methods should be emphasized among the all instrumental methods. These techniques provide promising results in oncology, especially if

several methods have been used together [4]. Dermatoscopy is one of the simplest instrumental diagnostics methods and it may allow one to increase diagnostics accuracy significantly. Dermatoscopy relies on epidermis imaging with optical magnification followed by features analysis of pigmented lesions [3]. In the simplest case, dermatoscopy is performing visually by an oncologist, but a specialized program may be integrated into a dermatoscopy tool to evaluate necessary features [5, 6].

Dermatoscopy with ABCD analysis increases diagnostics sensitivity up to 78.4% and specificity up to 79.6% in melanoma vs non-melanoma case for trained physicians. Other analysis methods, such 7-point checklist, provide sensitivity up to 100% and specificity up to 69.8% in the same conditions [7]. One of the most successful results of automatic classification has been achieved by A. Esteva et al. [8]. They have achieved overall accuracy of 72.1% using Convolutional Neural Network (CNN) for three-way classification of benign, malignant and non-neoplastic lesions.

Other optical methods, such as confocal microscopy, Raman spectroscopy, hyperspectral and fluorescence diagnostics, optical coherent tomography (OCT) shows diagnostics accuracy of more than 80%. However, all of them share disadvantages of high cost and relative hardware complexity. Hyperspectral diagnostics allows one to analyze both morphological and spectral features of skin surface. Results of hyperspectral imaging diagnostics heavily depend on data processing methods, but many researchers report accuracy of malignant vs benign lesion classification to be more than 80% for melanoma detection [4, 9, 10]. Methods based on fluorescence diagnostics [11] and OCT [9, 12, 13] also show 80-90% accuracy for basal-cell carcinoma detection. Confocal microscopy [14, 15] and Raman spectroscopy [9, 11, 16] could result in more than 90% accuracy for various types of lesions detection, such as basal-cell carcinoma and malignant melanoma.

In addition, there are number of non-optical diagnostics methods, such as ultrasound or thermal imaging. Thermal imaging method is based on temperature difference between malignant and benign lesions. V. Anisimov [4] mentioned sensitivity of 90.5% and specificity of 80.2%. However, melanoma detection on early stages with dynamic thermal imaging is almost impossible [17] due to more significant temperature dependence on the blood flow. Thermal imaging diagnostics accuracy also depends on a lesion thickness [18].

Ultrasound methods allow one to determine local melanoma distribution, but thickness measurements could give erroneous results in some cases [3]. Melanoma versus nevi classification based on ultrasound data and using Support Vector Machines (SVM) classifier shows sensitivity of 85.8% and specificity of 79.6% [19].

The aim of this research is developing a dermatoscopy diagnostic tool with additional software SVM classifier. The tool allows us to control all

possible image acquisition parameters and makes it possible to eliminate any unwanted processing steps, which could unexpectedly influence classification algorithm. A prototype of the dermatoscopic tool and the software have been created. Clinical dermatoscopic examinations were also conducted and dermatoscopic data classification accuracy was estimated.

2 Materials and methods

2.1 Tool design

An optical design of the dermatoscopic tool prototype is presented in Fig. 1. This tool is based on a BASLER Ace 1920-25uc color camera. The camera produces 1920×1080 pixel images representing a Bayer (GB) mosaic for a 25×14 mm field of view on the surface of the skin. RGB images are constructed from the mosaic using linear interpolation. In terms of frame acquisition timing control, this camera has a rolling shutter, but a global reset release shutter mode has been enabled. Backlight LEDs are synchronized with the shutter in such a way that LEDs emit light only when all lines of the sensor are exposed. This shutter mode coupled with backlight synchronization effectively creates a global shutter effect in terms of input light. Integration time could be set independently for each type of backlight while all the other types of automatic acquisition control (i.e. gain, gamma, white balance corrections) are switched off.

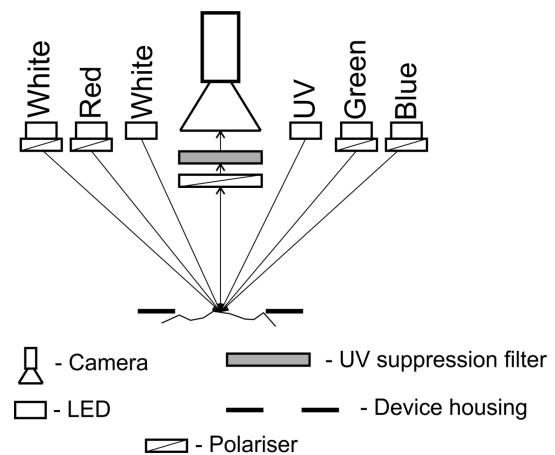


Fig. 1 Optical diagram of the dermatoscopic tool prototype.

Device uses several LEDs to illuminate skin area: 4 white LEDs FM-5630WDS-460W-R80 (2 of them are covered with a polarizer to visualize deeper skin layers); UV range LEDs LEUVA77V20RV00 (365nm) for skin autofluorescence analysis; three types of visible light LEDs CREE XPCReD-L1-0000-00301, CREE XRCGRN-L1-0000-00N01 and CREE XREBLU-L1-0000-00K01 to visualize skin in red (620nm peak), green (530nm peak) and blue (470nm peak) ranges respectively. For further 365nm excited autofluorescence analysis the radiation is filtered with

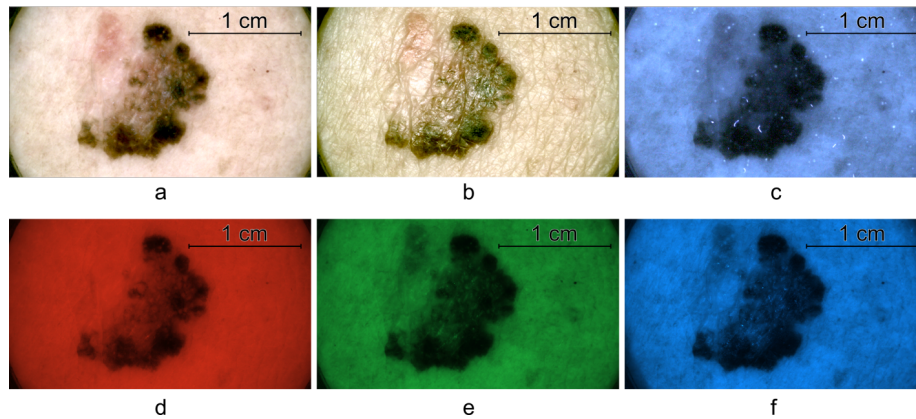


Fig. 2 Images made using different backscattered light (a-f): (a) White polarized light; (b) White non-polarized light; (c) Autofluorescence; (d) Red light; (e) Green light; (f) Blue light.

ThorLabs FGL435 (435nm) long-pass filter, which is always installed on the lens and non-removable for a user. All LEDs are switched in sequence synchronized with camera's frame acquisition. The current switching sequence is the following: polarized white light, not polarized white light, red light, green light, blue light and an ultraviolet autofluorescence.

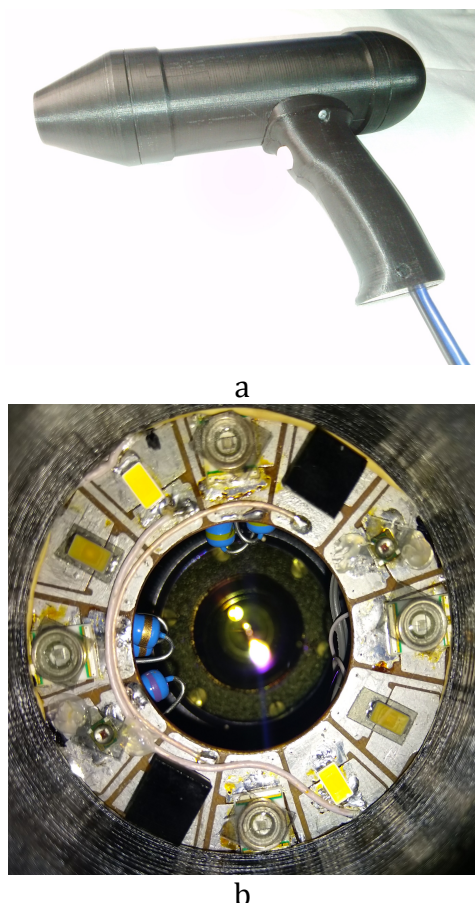


Fig. 3 The tool prototype used for data gathering. (a) General view of the designed dermatoscopy tool; (b) tool lighting ring and a camera lens.

The UV diode outputs up to 1W inside of 110° solid angle. Considering its placement at a distance of 75 mm from the device's window, the patient's skin UV exposure is 130J/m^2 for 1sec typical acquisition time that does not exceed ANSI maximum permissible exposure. All visible light LEDs output from 40 to 100Lm, which results in less than a 1J/m^2 exposure for a typical acquisition time of 0.1sec.

Linear polarization is used to filter out light directly reflected from skin surface, as it is necessary for deeper epidermis structure visualization and for light absorption estimation at different wavelengths in visible range. Linear polarization filter "ПФ-3" has been installed in the device. Examples of skin images obtained by the described above tool are presented in Fig. 2, the tool prototype itself is presented in Fig. 3a, while Fig. 3b shows the device backlighting LEDs ring. It could be noted that there are two LEDs of each type to provide more uniform backlight. A polarizing film is used for LEDs light polarization, while the ПФ-3 filter and a long-pass filter are installed permanently on the camera lens. The lens itself is placed behind the lighting ring looking down on a skin through the hole in the middle of it.

2.2 Automatic lesion detection

A proposed algorithm classifies tumors by using color and texture features evaluated from images with polarized white backlight (the closest approach to a usual dermatoscopy). Majority of this algorithm's steps were described in details in Ref. [20], but we are going to mention it here again in regard of this particular implementation for better representation of the whole system.

The algorithm (Fig. 4) consists of the following steps:

- 1) initial image processing;
- 2) region of interest (ROI) detection;
- 3) calculation of color and texture features;
- 4) SVM classifier training or using the pre-trained classifier to get the final diagnostic

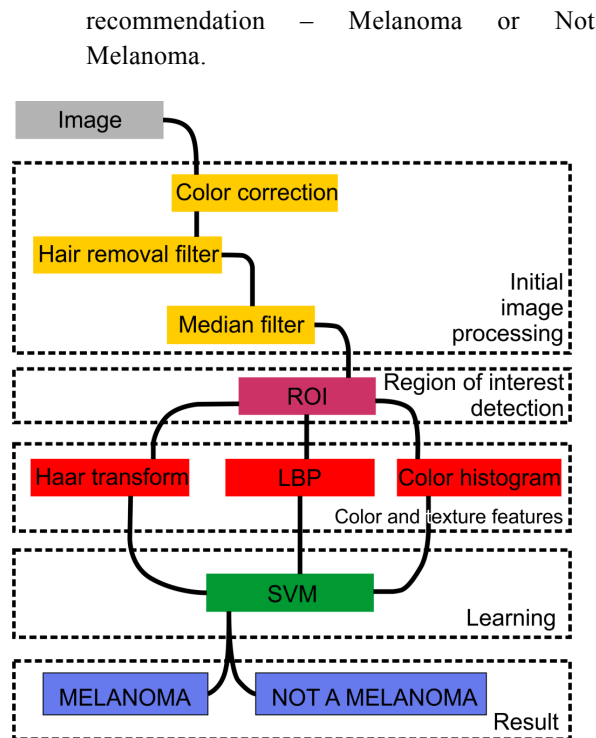


Fig. 4 Algorithm of automatic lesion detection.

Initial image processing consists of constant noise subtraction and histogram correction in order to increase image contrast and to use full available dynamic range. Fig. 5 demonstrates a histogram of the same image before and after this histogram processing. Histograms of values of all color channels together are shown here. Number of brightness levels is not equal due to the image color depth being converted from 48 (3 channels $\times$ 16 bits) to 24 (3 channels $\times$ 8 bits) bits on this step. Dynamic range of a resulting histogram may be controlled by a contrast setting while its horizontal shift may be controlled by a brightness setting. Described algorithm uses such properties that 1% of pixels occur to be beyond of the possible limits, this way dynamic range is fully used and “hot” pixel noise level is decreased.

Region of interest (ROI) detection. This step’s aim is to divide an image by an area related to lesion and another area that belong to healthy skin. K-means clustering algorithm [21] based on pixels’ brightness is applied to the image, that produces 40 (predefined feature) clusters to cover of possible values of brightness with minimal step. The whole brightness range is divided on sub-ranges according to the number of clusters, thus each sub-range represents one cluster. 10% of clusters with the least brightness are forcibly assigned to be related to a lesion, the rest of the clusters are assigned to represent surrounding healthy skin. Contrariwise, the image is spatially dividing by 2D blocks, and the amount of “lesion” pixels is calculated for each block. If there are more than 5% of such pixels, the block is marked as belonging to the ROI. The size of these blocks is determined in the following way: there are 20 blocks along the smallest image dimension, the

number of blocks along the largest dimension is calculated based on that the ROI blocks are squares.

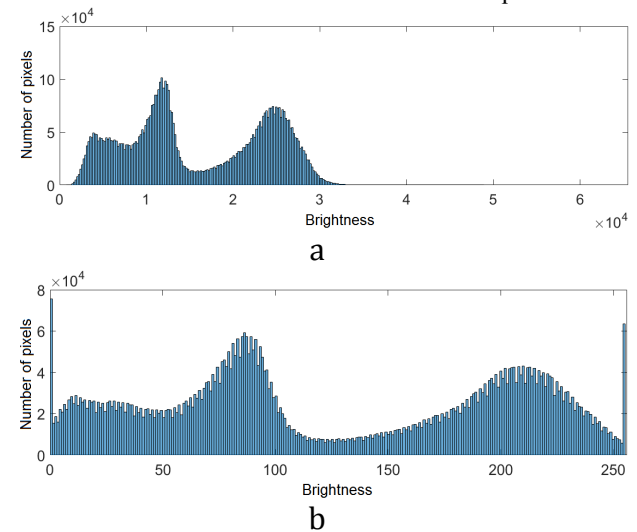


Fig. 5 Histograms of the initial image and of the image after processing. (a) Initial image histogram (3 channels, 16 bits); (b) processed image histogram (3 channels, 8 bits).

Color and texture features evaluation. Several approaches are being used on the features detection step. Result of each approach forms a part of the overall feature vector: Haar transform forms H_i vector, Local Binary Patterns approach forms L_i vector and color analysis defines C_i vector. These vectors are combined together in one vector later.

Haar transform

First, discrete three-layer Haar transform [22] is performed for each detected ROI block by using two filters: a high-pass and a low-pass filter. This is done here with the following algorithm:

- 1) These filters are applied to each row of the initial image resulting in two matrices: one contains approximated part and another contains fine details of the initial image.
- 2) This transform is then performed again for each column of these matrices, resulting in four total matrices, containing an approximated part and horizontal, vertical and diagonal details of the image.

Three layer Haar transform results 10 sub-images, as it is shown in Fig. 6 as an example. Haar transform is performed on a grayscale image created from the initial color one. A vector of mean value and dispersion of sub-images are calculated and combined in a feature vector for every ROI block. Thus, the number of feature vectors equals the number of ROI blocks for every image. ROI blocks are then clustered using k-means algorithm (10 clusters), and, finally, the histogram H_i for each image showing the distribution of the image feature vectors of clusters is calculated.

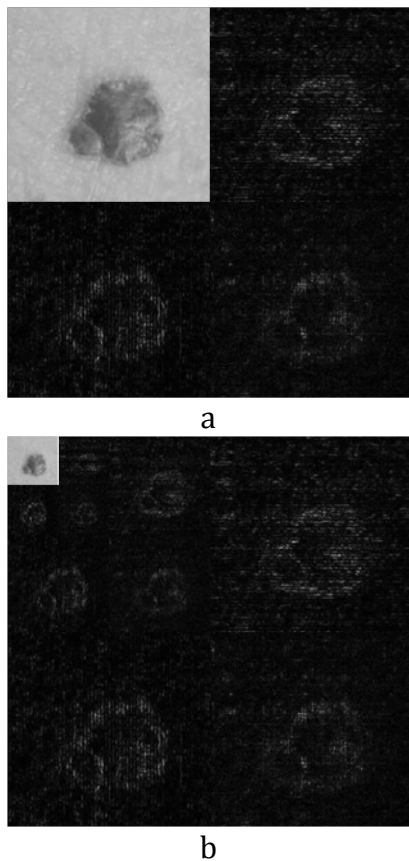


Fig. 6 Example of the Haar transform with different levels. (a) Result of the one layer Haar transform; (b) result of the three layer Haar transform.

Local Binary Patterns (LBP)

In addition to Haar transform, texture information is extracted using Local Binary Patterns (LBP). The main idea of LBP is to construct a p-bit number for every RGB image pixel by comparing its brightness with the brightness of p surrounding points using the following algorithm:

- 1) These points are located on a circle with the radius r , as it is shown in Fig. 7. Neighboring pixels' values are interpolated if the point is not exactly on any pixel;
- 2) brightness of the initial pixel is used as a threshold;
- 3) if a surrounding point value is more than the threshold, corresponding bit in the number is set to 1, and 0 otherwise.

The program uses two LBP algorithms with following parameters: $p = 16, r = 2$; $p = 24, r = 3$. The results are combined into a single 40-bit number. The resulting vector is normalized and this is what forms a vector L_i used for classification as a part of a general feature vector.

Color analysis

Color histograms are also used for the classification as a part of the features vector. Brightness range of every color channel is divided on P fixed intervals and

pixel brightness distribution is calculated for every channel in RGB, HSV and LAB color spaces in terms of these intervals. Different color spaces may be considered as different representations of the same data, which could improve classification results. The histograms' standard deviation and entropy values are also calculated and added to the histogram data to define a vector C_i .

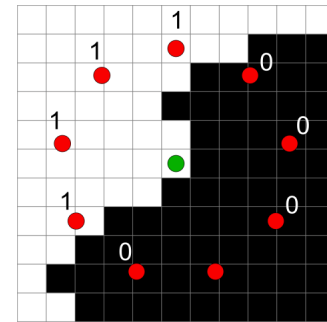


Fig. 7 LBP algorithm: an example of surrounding pixels selection and bilinear interpolation with central pixel as a threshold.

Comparative features

A comparative analysis method is used to improve classification of this classifier. All features are evaluated separately over the region of the lesion and over the region of the healthy skin (Fig. 8), and then the difference between these areas is calculated using Euclidean distance and cosine measure (Eq. (1) and Eq. (2)).

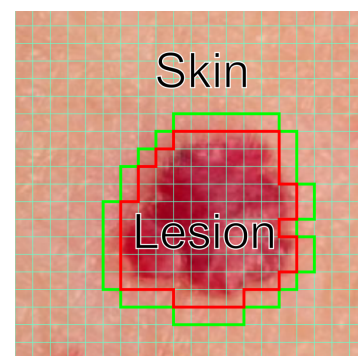


Fig. 8 Normal skin (green) and lesion (red) regions.

The Euclidean distance between the vectors $\bar{s}$ and $\bar{t}$, which are represented features vectors from Normal Skin and Tumor (Lesion) respectively (Fig. 8), is defined as

$$d_E(\bar{s}, \bar{t}) = \|\bar{s} - \bar{t}\|. \quad (1)$$

The cosine measure between the same vectors can be formalized as

$$d_{cos}(\bar{s}, \bar{t}) = \frac{\bar{s}^T \bar{t}}{\|\bar{s}\| \|\bar{t}\|}. \quad (2)$$

If this comparative mode is enabled, then, finally, exactly comparative features are considered. H_i , L_i and C_i vectors are replaced by corresponding comparative vectors without their size change.

Classification

Resulting features vectors from every method (H_i , L_i and C_i) are concatenated in a single vector for every image as shown in Fig. 9. Linear SVM method is used for classification [23].

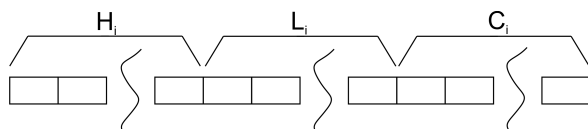


Fig. 9 Resulting vector concatenation.

Cross validation

Cross validation has been used to test the adequacy of the obtained classification model. One cross validation cycle includes dividing data into two parts, classifier training using one of the parts (training set) and validation using the other part (test set). Different cross-validation cycles are conducted with data divided differently and all results of all cycles are averaged to lower the final result deviation. We used this approach due to the low quantity of samples available. It allows us to estimate classifier's performance with various sizes of training and testing parts.

2.3 Skin tissue samples

Dermatoscopic images were gathered *in vivo* using the dermatoscopy tool in Samara Regional Clinical Oncological Dispensary. This survey was approved by ethical committee of Samara state medical university. Reference diagnostics results (Table 1) were obtained by histological analysis in each case (except Hemangioma, Keratoma, diagnoses of which have been concluded after visual inspection only). Histological analysis reviews lesion's microscopic features, such as: tissue type, stage, penetration of the lesion into the surrounding tissue. The extracted after surgical resection material was fixed in 10% neutral formalin. Serial sections were made (thickness up to 0.5mm), which were dyed with hematoxylin-eosin and picrofuchsin by Van Gieson method (Schiff reaction according to McManus).

3 Results

Two series of experiments have been performed in this study: classification of melanomas versus all other types, and classification of malignant lesions versus benign ones. The dataset was divided into two classes in each series: melanomas and non-melanomas (all remaining) in the first one; malignant (melanoma, basal-cell and squamous cell carcinoma) and benign (all remaining) in the second one. Classification of 36 melanomas and 37 non-melanomas dataset (Table 2)

result of 83.3% sensitivity and 70.3% specificity. In this case non-melanoma class consists of basal-cell carcinoma (18), nevus (12), keratopapilloma (7). With increased training dataset up to 205 samples, 169 of which are non-melanomas, classifier have predicted considerably worse result caused by properties of standard SVM algorithm settings. This is related to classes sizes being initially significantly unequal. In fact, a classifier is most efficient on classes having almost equal sizes. Therefore, presented results prove this condition.

Table 1 Tested skin samples.

Disease type	Number of tested samples
Fibroma	14
Keratopapilloma	12
Nevus	39
Melanocytic dysplasia	4
Keratoma	27
Benign pigmented lesion	1
Granulation	1
Inflammation	14
Hemangioma	12
Bowen's disease	1
Squamous cell carcinoma	2
Basal cell carcinoma	42
Melanoma	36

Classifying 80 malignant and 80 benign lesions classifier has got sensitivity of 63.3% and specificity of 61.3%. In this case, classes consisted of following: "malignant" class included melanomas (36), basal-cell (42) and squamous-cell (2) carcinomas; "benign" class included nevi (39), keratopapillomas (12), undetermined inflammations (14), hemangiomas (12), Bowen's disease (1), benign pigmented lesion (1), granulation (1).

Detailed results of the classification and cross-validation are presented in Tables 2-5.

Classifier remains its sensitivity above 70% and its specificity above 63% with mean values of 83% and 65% respectively during cross-validation for classification of melanomas versus non-melanomas, as it could be seen in Table 4. It signifies the classifier's potential operability with bigger data volumes, but class size equality remained condition. Cross-validation for "malignant" versus "benign" classification gives us

sensitivity of 60.9% and specificity of 59.7%. It should be noted that cross-validation results in case 90%/10% data splitting are not relevant because of too small test dataset (4 and 8 images in different cases) which depends a lot on the images themselves, so the results are mainly accidental in this case.

Table 2 Melanoma versus non-melanoma classification.

Number of melanomas	36
Number of non-melanomas	37
Sensitivity, %	83.3
Specificity, %	70.3
Tp	30
Fn	6
Fp	11
Tn	26

Tp - True positive, **Fn** - False negative, **Fp** - False positive, **Tn** - True negative

Table 3 Malignant versus benign classification results.

Number of malignant lesions	80
Number of benign lesions	80
Sensitivity, %	66.3
Specificity, %	61.3
Tp	53
Fn	27
Fp	31
Tn	49

Tp - True positive, **Fn** - False negative, **Fp** - False positive, **Tn** - True negative

4 Discussion

The classifier shows the best result with training on classes of similar sizes. The classifier has got sensitivity of 83.3% and specificity of 70.3% with melanoma versus non-melanoma classification and sensitivity of 66.3% and specificity of 61.3% with malignant versus benign tumors classification. These results could be improved by hybridization of the proposed algorithm with other optical non-invasive methods, and creation of a multimodal algorithm for optical data analysis.

Comparing the proposed system classification accuracy with other diagnostic methods efficiency, results of skin tumors classification by SVM of dermatoscopic images are comparable with accuracy of ultrasound diagnostics, thermal imaging diagnostics, hyperspectral imaging and ABCD diagnostics.

Delpueyo et al. [10] used multispectral imaging with LED backlight followed by feature detection and classification. The best achieved sensitivity for MM is 87.2% with specificity of 54.5%. Tomatis et al. [12] used multispectral imaging with CNN and achieved sensitivity of 80.4% and a specificity of 75.6% for

classification of melanoma vs non-melanoma. Pagnanelli et al. [7] report that dermatoscopy with ABCD analysis provides sensitivity up to 78.4% and specificity up to 79.6% in melanoma vs non-melanoma classification for trained non-experts.

Table 4 Melanoma versus non-melanoma cross-validation results.

Dataset		Sensiti vity	Specifi city	Tp	Fn	Fp	Tn
90/10	90% (32/33)	81.3%	63.6%	26	6	12	21
	10% (4/4)	65.6%	72.8%	21	11	9	24
80/20	80% (29/30)	86.2%	63.3%	25	4	11	19
	20% (7/7)	75.9%	70.0%	22	7	9	21
70/30	70% (25/26)	88.0%	69.2%	22	3	8	18
	30% (11/11)	88.0%	65.4%	22	3	9	17
60/40	60% (21/22)	95.3%	72.7%	20	1	6	16
	40% (15/15)	95.3%	63.6%	20	1	8	14
50/50	50% (18/19)	94.4%	68.4%	17	1	6	13
	50% (18/18)	72.2%	61.1%	13	5	7	11
100/0	(73/0)	83.3%	70.3%	30	6	11	26

Tp - True positive, **Fn** - False negative, **Fp** - False positive, **Tn** - True negative

Table 5 Malignant versus benign lesions cross-validation results.

Dataset		Sensitivity	Specificity	Tp	Fn	Fp	Tn
90/10	90% (72/72)	65.0%	65.0%	52	28	28	52
	10% (8/8)	58.8%	60.0%	47	33	32	48
80/20	80% (64/64)	65.0%	62.5%	52	28	30	50
	20% (16/16)	57.5%	60.0%	46	34	32	48
70/30	70% (56/56)	65.0%	61.3%	52	28	31	49
	30% (24/24)	55.0%	60.0%	44	36	32	48
60/40	60% (48/48)	67.5%	63.8%	54	26	29	51
	40% (32/32)	66.3%	57.5%	53	27	34	46
50/50	50% (40/40)	66.3%	66.3%	53	27	27	53
	50% (40/40)	65.0%	61.3%	52	28	31	49
100/0	(160/0)	66.3%	61.3%	53	27	31	49

Tp - True positive, **Fn** - False negative, **Fp** - False positive, **Tn** - True negative

However, classification accuracy of a presented method is usually less than accuracy of Raman spectroscopy, confocal microscopy, fluorescence diagnostics and OCT implementation. Jørgensen et al. [13] report an 81% success rate for classification of basal-cell carcinomas vs actinic keratosis using OCT data with machine learning approach. Confocal microscopy and classification using 6 criteria score results in 91.9% sensitivity and 69.3% specificity for finding melanoma, as reported by Pellacani et al. [14]. Zakharov et al. [16] implemented detection of melanoma using phase planes with Raman scattering data. This approach resulted in a sensitivity of 77.7%, and specificity of 87.8%. Kong et al. [11] also used Raman scattering in conjunction with autofluorescence, which was used to select points for Raman spectroscopy. This diagnostics approach based on a spectral classification model resulted in 100% sensitivity and 92% specificity.

Obtained results may be promising for primary diagnostics use, as the proposed system is pretty simple in comparison to Raman or OCT systems. Meanwhile, classification accuracy is considerably better than diagnostics accuracy of a general practitioner, estimated as 25%–60% [3].

5 Conclusion

We have implemented a dermatoscopic software system for *in vivo* automatic malignant lesion detection. This system utilizes SVM classifier coupled with the feature detection algorithm. First, the algorithm performs image

preprocessing followed with the Region of Interest detection. This is followed by Haar transform, LBP and color features extraction on a feature detection step. All extracted features are represented as a single vector, which is then used by an SVM classifier for training or a lesion classification.

We performed an experiment of lesion detection using data gathered by a custom designed multi-spectral dermatoscope, using only images taken with white polarized backlight.

The result shows the possibility of using proposed approach in clinics. Further enhancement in skin lesions classification with proposed system is possible by the proposed algorithm parameters tuning, usage of other channels data or better initial image processing. Neural network classification is also possible, including network pre-training on images from dermatoscopic databases and further final training using images made with proposed tool to lower variation of results [8]. There are also opportunities to hybridize the device with other optical modalities.

Disclosures

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