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On the ability of cells to distinguish the coherence of optical radiation

A.V. Budagovsky

***Abstract*. The role of coherent optical radiation in photoregulatory processes caused by chemiluminescence of living cells is discussed. The effect of low and highly coherent quasi-monochromatic light on a dynamic 'host – parasite' system is studied. It is shown that plant organisms can distinguish the statistical order of irradiation. A significant increase in the functional activity was observed only for cells that were completely located within the coherence volume of the electromagnetic field. It is concluded that the cell size in living organisms is the discrimination threshold of the statistical properties of radiation and may serve as a specific biological measure of coherence.**

Keywords: *photoregulation, coherent radiation, coherence volume, photobiological processes.*

1. Introduction

Many biochemical reactions are initiated by photons or accompanied by emission in the visible spectral region. Photobiological process play an important role in different control chains, including gene expression [1 – 3]. Cells have specials photoreceptors such as phytochrome (PC), cryptochrome (CrC), cytochrome (CC), rhodopsin, etc. whose excitation leads to the activation of various regulatory systems in organism. The examples are bacterial phototaxis, photomorphogenesis and photoperiodicity of plants, as well as retinal processes of higher animals. Biochemiluminescence – an extremely weak emission of cells caused by their vital functions is also observed in the visible region [4 – 6].

A number of facts show that not only sunlight but also the intrinsic emission of living organisms can be involved in photoregulatory processes [7 – 13]. However, the mechanism of such a regulatory channel in biosystems remains unclear. First of all this is due to an extremely low intensity of chemiluminescence of cells. Against the intense stochastic background produced by a natural light, weak signals can be reliably detected only when their coherence is high enough [14]. Therefore, the necessary condition for transmitting regulatory signals by using endogenous emission is the statistical order of this emission.

It has been predicted theoretically [15 – 20] and confirmed experimentally [16, 21, 22] that under the action of stochastic factors biopolymers in condensed phase can form cooperative excited states relaxing with emission of coherent photons. Low-intensity light fluxes with a high statistical order were detected during the establishment of communication relations between chemically isolated organisms [11]. The coherent component of luminescence of leaves of different plants was detected against the natural illuminance background [17, 23, 24]. It was also shown that an irregular phase screen reducing the spatial coherence of emission placed in the channel of optical communication between chemically isolated biosystems prevents the intercellular interaction [25]. This suggests that a light flux performing communication has a certain statistical order.

One can assume that cells communicating regulatory signals by means of chemiluminescence should not only generate coherent emission but also distinguish the degree of its statistical order. In this paper, an attempt is made to prove the existence of this property in living organisms.

2. Analytic section

The importance of coherence of light in photobiological processes has been pointed out in a number of papers [26 – 30]. The experiments of Devyatkov and his co-authors showed that 'according to the amplitude of the electrophysiological reaction, the efficiency of polarised coherent light was higher by a factor of 1.5 than that of polarised incoherent light and by a factor of 1.7 higher than that of nonpolarised incoherent light' [31] (p. 146). There also exists the opposite point of view, denying the ability of cells to react to the coherence of irradiation [32 – 35]. The arguments presented in these papers are based on a comparison of the biological action of laser and thermal or gas-discharge radiation sources. A narrow spectral line of width $\Delta\lambda = 8 - 14$ nm with a maximum at the laser wavelength was separated from the emission spectra of non-laser sources by using interference filters, diffraction gratings, etc. Such radiation was called 'monochromatic incoherent radiation' [36, 37], 'incoherent red light' ($\Delta\lambda = 8.5$ nm [33], $\Delta\lambda = 14$ nm [32, 35]), 'incoherent blue light' ($\Delta\lambda = 28$ nm [38]), and 'narrowband red light' ($\Delta\lambda = 14$ nm [39]), while the laser radiation was called 'coherent'. Such definitions and methodical approaches

A.V. Budagovsky I.V. Michurin All-Russian Research Institute of Fruit Crops Genetics and Breeding, 393770 Michurinsk-10, Tambov region, Russia; e-mail: Budagovsky@mail.ru

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based on them became quite popular in biophysical studies. Due to the similarity of photostimulation effects produced by radiation sources of these two types, it was concluded that: 'The results of the experimental studies in which the action of coherent and incoherent light on biological objects was correctly compared show that the coherence of light is indeed insignificant' [35] (p. 370). However, this conclusion cannot be considered justified because it follows from the assumption that the quasi-monochromatic radiation of non-laser sources is incoherent. To interpret experimental data correctly, it is necessary to pass from qualitative to quantitative estimates.

The fields of different radiation sources (laser, thermal, gas-discharge, etc.) formed by optical systems have although substantially different but quite certain statistical orders. The statistical order can be estimated quantitatively by using the coherence volume $V_{\mathrm{coh},}$ which represents a part of space where the phase correlation of a photon ensemble is preserved. The Wiener–Khinchin theorem gives the relations: $\tau_{\mathrm{coh}}\Delta\omega \sim 1$ and $r_{\mathrm{cor}}\Delta\Theta \sim \lambda_0$ required for the calculation of V_{coh}, where τ_{coh} and r_{cor} are the coherence time and the correlation radius of a light beam (characteristic values of the argument of the coherence function); $\Delta\omega$ and $\Delta\Theta$ are the widths of the temporal and spatial emission spectra; and λ_0 is the effective wavelength. (The relations are written with accuracy to constant factors, which depend on the type of spectral distributions.)

Because the conditions of the field factorisation [14, 40] are fulfilled for the above-mentioned experiments, the correlation radius is independent of the correlation time. Therefore, it is possible to consider the spatial and temporal coherence independently. In this case, V_{coh} can be written as the product of the coherence area $A_{\mathrm{coh}} = \pi r_{\mathrm{cor}}^2 \sim \pi(\lambda_0/\Delta\Theta)^2$ by the coherence length $L_{\mathrm{coh}} = c\tau_{\mathrm{coh}} \sim \lambda_0^2/\Delta\lambda$, so that $V_{\mathrm{coh}} = A_{\mathrm{coh}}L_{\mathrm{coh}} \sim \pi(\lambda_0/\Delta\Theta)^2\lambda_0^2/\Delta\lambda$, where $\Delta\lambda$ is the wavelength range corresponding to the frequency range $\Delta\omega$ and c is the speed of light in vacuum. Therefore, the higher monochromaticity of radiation and the narrower its spatial frequency spectrum, the larger is the coherence volume V_{coh} of this radiation.

Quasi-monochromatic radiation from non-laser radiation sources was obtained in almost all papers [32, 33, 35–39] discussed here by using grating monochromators such as an MDR-2. In this case, the optical system had the following typical parameters: the entrance linear aperture was $2a = 4$ mm, the optical path was $z \approx 2$ m, the linear dispersion of the grating monochromator was 2 nm mm^{-1}, the effective wavelength was $\lambda_0 = 633$ nm, and the linewidth was $\Delta\lambda = 14$ nm. Assuming that we deal with a spatially restricted wave with a comparatively homogeneous intensity distribution over its front, we will define the modulus of the transverse correlation function as $\gamma(s) = 2|J_1(kas/z)(kas/z)^{-1}|$, where $J_1(kas/z)$ is the Bessel function; $k = 2\pi/\lambda$ is the wave number; $s = |\boldsymbol{r}_1 - \boldsymbol{r}_2|$; and z is the distance between the radiation source and object [41]. The first zero of the function $\gamma(s)$ is located at $kas/z = 3.83$, i.e., when $s = 0.61\lambda z/a$. This condition $r_{\mathrm{cor}} = s = 0.61\lambda z/a$ if $\gamma(s) = 0$] can be considered as the definition of the correlation radius of a spatially restricted light wave with the homogeneous intensity distribution. For such optical systems, we have typically $r_{\mathrm{cor}} \gg L_{\mathrm{coh}}$. Simple calculations show that the correlation radius is 386 μm and the coherence length is 29 μm. The phase correlation of a photon ensemble will be preserved in the field volume $V_{\mathrm{coh}} \approx \pi\times$ (386 mm)$^2 \times 29$ μm. When a DFS-24 grating monochromator ($z \approx 6$ m) is used, the correlation radius exceeds 1 mm. Such radiation can be in no way considered incoherent. The example of radiation with a low statistical order is scattered sunlight. Its coherence volume is six orders of magnitude lower than that in experiments considered above.

The coherence length for gas lasers is quite large; however, the correlation radius can be even smaller than for a collimated light beam from a thermal source. For a multimode laser with spherical resonator mirrors, $r_{\mathrm{cor}} = (\sqrt{3}r_z)/N^{1/2}$, where r_z is the characteristic radius of the beam and N is the number of transverse modes [14]. For $r_z = 1$ mm and $N \sim 10^2$, the correlation radius is 173 μm. The degree of the temporal and spatial coherence can be even lower. The calculations show that there are no fundamental differences between the correlation properties of light from thermal and laser radiation sources. The difference appears only in the higher orders of coherence. However, according to the experimental data reported in [32–39], this difference has no effect on reactions proceeding in biological systems.

The results obtained in the papers considered above give no way of making an unambiguous conclusion. Similar biological effects produced by laser and monochromatic radiation from a thermal source can be explained either by the insensitivity of irradiated cells to the coherence of light or by the fact that coherence was sufficient in both cases for producing simulation. To solve this problem, additional experimental studies should be performed.

3. Experiment

3.1 Materials and the method

A dynamic host–parasite system in which cells of different size interacted by the mechanism of induced immunity was selected for irradiation. The system was *Malus domestica Borkh* apple fruits with spores of pathogenic fungus *Penicillium expansum Link, Botrytis cinera Pers., Mucor racemosus Fres.*, etc. These infected fruits were briefly exposed to quasi-monochromatic light with high or low coherence. A helium–neon laser ($\Delta\lambda/\lambda_0 \ll 10^{-3}$) without linear polarisation was used as a highly coherent radiation source. An incandescent lamp with a set of optical filters and collimating optics ($\Delta\lambda/\lambda_0 \approx 10^{-1}$; $T_{\mathrm{c}} = 2700$ K) was used as a low-coherent radiation source. The energy parameters of quasi-monochromatic beams were set equal; however, their statistical characteristics were substantially different (Table 1). The radiation power was measured with an IMO-2H colorimeter (Etalon, Russia) with an accuracy of 6 %. The transmission coefficients of optical filters were measured with a SF-26 spectrophotometer (LOMO, Russia). The irradiation parameters were measured and calculated by standard methods [14, 42].

Fruits were irradiated at a temperature of +18 °C and a background illuminance of 30–40 lx produced by scattered sunlight. Control fruits were in the same conditions but carefully isolated from monochromatic radiation. The average size of the host cells (epidermal and parenchymal tissues of fruits) during irradiation was $D_{\mathrm{h}} = 40 - 50$ μm and that of the parasite cells (fungus spores) was $D_{\mathrm{p}} = 3 - 8$ μm. The amount of damaged fruits was determined after 70 days of storage at a temperature of +4 °C and the

Table 1. Parameters of radiation sources.

Radiation source	Energy parameters			Statistical parameters	
	Wavelength/nm	Radiation intensity/W m^{-2}	Exposure time/s	Coherence length/μm	Correlation radius/μm
He – Ne laser	632.8	4	5, 10, 20, 30 40, 60, 120	> 1000	> 1000
Incandescent lamp with a monochromator	633	4	5, 10, 20 30, 40, 120	5 – 8	8 – 10

Note: Background illuminance is 30 – 40 lx; for this illuminance, $L_{coh} < 1$ μm, and $r_{cor} < 1$ μm.

relative humidity 90 %. The criterion of damage was the presence of symptoms of fungus diseases [43].

3.2 Results and discussion

The irradiation by quasi-monochromatic light from both sources considerably affected the state of the host – parasite system. The reaction of organisms had a nonlinear, multimodal dependence on the exposure time [44]. The responses of the system to low and highly coherent light were opposite (Fig. 1). Laser treatment during optimal exposure times reduced the sick rate of fruits by a factor of 2 – 3 compared to the control. On the contrary, upon irradiation by an incandescent lamp, the sick rate approximately doubled. It was shown earlier that highly coherent laser radiation enhanced the functional activity of both host and parasite cells [45]. One can assume that laser stimulation of the protective reaction of the host upon the interaction between such cells prevents the development of a pathogenic process and fruits are damaged weaker than in the control [curve (*1*)]. A different situation observed upon irradiation by light from a thermal source [curve (*2*)] demonstrates the enhanced activity of only parasite cells. The experimental results showed that the reaction of plant cells to quasi-monochromatic radiation depends on its coherence. This confirms the initial assumption that living cells are capable of distinguishing the degree of statistical ordering of light.

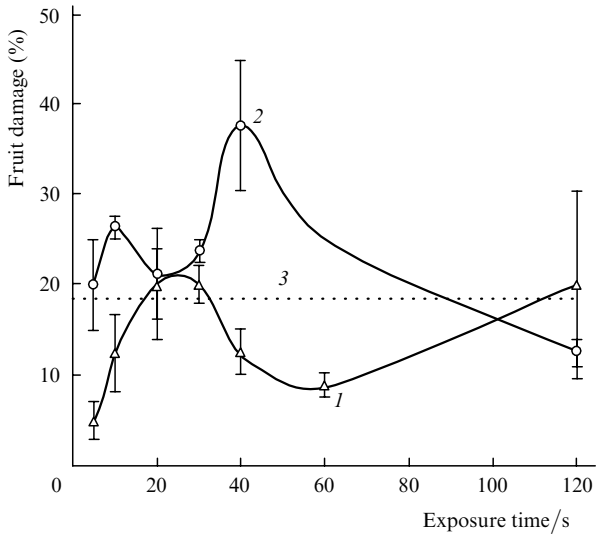

Figure 1. Response of the dynamic host – parasite system to optical radiation with different coherence: irradiation by highly coherent light from a helium – neon laser ($r_{cor}, L_{coh} > D_p, D_h$, both components of the system are stimulated) (*1*); irradiation by quasi-monochromatic light from an incandescent lamp ($D_p \leqslant r_{cor}, L_{coh} < D_h$, only parasite cells are stimulated) (*2*); background illuminance ($r_{cor}, L_{coh} \ll D_p, D_h$, control) (*3*).

A comparison of cell sizes in the host – parasite system with the statistical parameters of the field (Fig. 2) leads to the conclusion that the functional activity of cells completely located within the coherence volume V_{coh} of irradiating light is enhanced to a greater extent. Upon laser irradiation, this condition is fulfilled for both components of the system. When quasi-monochromatic light from the incandescent lamp is used, only smaller parasite cells can be completely accommodated within the coherence volume, which results in the enhancement of the functional activity only for these cells. This means that the size of the detected region of phase correlations of the field is specified by the maximum size D of a cell, and the condition for distinguishing a coherent signal has the form $L_{coh}, r_{cor} \geqslant D$. Then, the parameter D, serving as a discrimination threshold for statistical properties of radiation, can be treated as a biological measure of the optical radiation coherence.

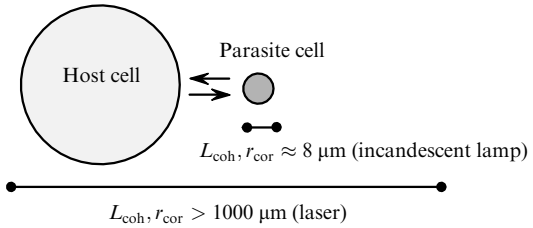

Figure 2. Schematic image of the sizes of cells interacting in the dynamic host – parasite system and their relation with the coherence parameters of fields from two radiation sources.

It is interesting to analyse from this position the results of photobiological experiments in which the action of laser and thermal sources with close emission spectra were compared (Table 2). The studies were performed for bacteria *E.coli*, lymphocytes of human blood, *HeLa* cell cultures, and other objects. In all cases studied, quasi-monochromatic irradiation in the spectral range from 400 to 950 nm enhanced the functional activity of biosystems only when the condition $L_{coh} \geqslant D$ was fulfilled, irrespective of whether the laser or thermal source was used. In the case of low coherence ($L_{coh} < D$) [28, 44], no effects typical of highly coherence radiation were observed. Therefore, bacterial and animal cells, as well as plant cells can distinguish the coherence of optical radiation. This is indirectly confirmed by the conclusion made in [53] (p. 61) based on the analysis of the Russian and foreign literature: 'therapeutic effect is produced to a greater extent by optical radiation with the spectral bandwidth smaller than 15 – 20 nm; when the spectral bandwidth is smaller

Table 2. Response of cells to quasi-monochromatic radiation with different coherence.

Irradiated object	Radiation source	Wavelength/nm	Coherence length/μm	Cell size/μm	Photostimulation	References
E.coli	Ar laser	454.0	> 1000	2	+	[38, 46–49]
	He–Ne laser	632.8	> 1000	2	+	
	Ga–As laser	890	> 100	2	+	
	Superluminescent diode	950	36	2	+	
	Incandescent lamp	454	15	2	+	
		560	22	2	+	
		622	27	2	+	
		633	29	2	+	
		750	7	2	+	
Lymphocytes of human blood	He–Ne laser	632.8	> 1000	8	+	[33, 50, 51]
	Xenon lamp	415	20	8	+	
		550	36	8	+	
		633	47	8	+	
Culture of fibroblast cells of animals	Second harmonic of a neodymium laser	534	> 1000	20	+	[33, 36, 37, 50]
	He–Ne laser	632.8	> 1000	20	+	
	Mercury lamp	540	29	20	+	
		630	40	20	+	
	Xenon lamp	415	20	20	+	
		550	36	20	+	
		633	47	20	+	
	Incandescent lamp	760	21	20	+	
Culture of *HeLa* cells	He–Ne laser	632.8	> 1000	25	+	[32, 46]
	Incandescent lamp	620	27	25	+	
		633	29	25	+	
		760	41	25	+	
Human myocardium	He–Ne laser	632.8	> 1000	50	+	[28]
	Halogen lamp	633	3	50	–	
Plant cells	He–Ne laser	632.8	> 1000	40	+	[44]
	Incandescent lamp	633	8	40	–	
				5	+	
	Ga–As laser	885	78	15	+	[52]

Note: '+' – photostimulation, '–' – absence of photostimulation.

than 5–10 nm, a further narrowing of the spectrum does not enhance the effect'. Indeed, for the bandwidth of 15–20 nm, the coherence length for red light often used in experiments will be of the order of 20–30 μm, which is not smaller than the size of most animal cells. A further narrowing of the spectral band down to 5–10 nm provides the coherence length (40–80 μm) that is excessive (according to the D criterion) in fact for all cells. It was shown above that for optical schemes used in most experiments discussed here, $r_{cor} \geqslant L_{coh}$, i.e., r_{cor} is not a limiting parameter. For some papers, it is impossible to calculate the correlation radius from the data presented in methodical sections. However, the results reported in these papers are in good agreement with other experiments, which suggests that the spatial coherence is high enough. We can assume that in these cases, a cell is also completely located inside the coherence volume when the condition $L_{coh} \geqslant D$ is fulfilled.

The discrimination threshold (biological measure) of coherence by the maximum cell size shows that the entire volume of the cell takes part in the estimate of statistical properties of radiation. We can assume that a phase detector of the corresponding cell size is the membrane pool of the cell. The primary photon acceptors in such a detector are chromoproteins associated with a lipid bilayer. It seems that their excitation by sufficiently coherent light (according to the D criterion) increases the probability of cooperative processes in biological membranes, resulting in a discrete (trigger) change in their regulatory functions. As a result, the biological activity of coherent radiation can be high enough for using weak light fluxes in cells for communication. This model does not contradict to the known biological properties of biological membranes [1, 54] and agrees with the concepts of Frohlich [55, 56] and Devyatkov and co-authors [57, 58] about cooperative and coherent processes in cellular structures.

The presence of a stochastic noise additionally restricts the parameter D: the cell size should be greater than the coherence length and correlation radius of radiation pro-

ducing natural background, i.e., $D > L^*_{coh}, r^*_{cor}$. The coherence length and correlation radius of scattered light from thermal sources, in particular, sunlight are comparable with the wavelength: $L^*_{coh}, r^*_{cor} \approx \hbar c/k_B T \sim \lambda$, where $\hbar$ is Planck's constant; k_B is the Boltzmann constant; and T is the absolute temperature of a radiation source [14]. The values of L^*_{coh} and r^*_{cor} are smaller than the size of most cells and, hence, the relation $L_{coh}, r_{cor} \geqslant D > L^*_{coh}, r^*_{cor}$ is readily fulfilled for quasi-monochromatic radiation acting against the natural illuminance background.

The requirement of the coherence normalisation according to the parameter D does not exclude the fulfilment of other conditions for the operation of photoregulatory systems. The radiation wavelength should correspond to the absorption spectrum of the corresponding acceptor, for example, PC, CrC, CC, and a cell itself should be competent, i.e., it should response to a stimulus.

The ability of living organisms to generate coherent radiation and detect it against the uncorrelated noise background corresponds to the necessary conditions of cellular regulation involving chemiluminescence. For the normal operation of such a communication channel, a medium where the signal propagates should not affect considerably statistical properties of the signal. This was assumed impossible because of a high heterogeneity of cellular and subcellular structures [59, 60].

It was shown earlier that quasi-monochromatic radiation propagated through several cellular layers preserved its coherence, which could be detected with a phase detector [61]. When different probe radiation sources were used (a 632.8-nm helium–neon laser, a 650-nm semiconductor laser, and a 640-nm LED), the same effect was observed: the spatial coherence of radiation scattered by tissues of a healthy organism was higher than in the case of pathology. For example, upon irradiation of healthy lettuce leaves by a helium–neon laser, the value of $\gamma(s)$ was 0.39 ± 0.01, in the case of a weak development of infection, this value decreased to 0.24 ± 0.02, and it further deceases down to 0.16 ± 0.01 when the tissue damage was visually noticeable. Coherence changes similarly under the action of other destabilising factors (critical temperatures, deficit of mineral nutrition, pesticides, virus and fungus diseases, etc.) [61]. Therefore, a plant tissue is a phase screen whose stochastic properties depend on the organism state.

The correlation radius and, therefore, the field coherence volume will change during the propagation of a coherent wave through a plant tissue in different functional states. In [61], the radiation of a single-mode helium–neon laser was used with the spatial intensity distribution $I(r) = I_0 \exp(-2r^2/r_z^2)$. In this case, the modulus of the normalised correlation function of a scattered beam has the form [14]

$$\gamma(s) = \exp\left[-\frac{1}{2}\left(\frac{kas}{2z}\right)^2\right],$$

where a is the beam radius at the exit from the tissue. Then, the correlation radius at the e^{-2} level is $r_{cor} = 2\lambda z/\pi a$. This means that the decrease in $\gamma(s)$ from 0.39 to 0.16 during the development of a pathogenic process is caused by the broadening of the angular spectrum of the beam and the decrease in its correlation radius by a factor of 1.4. As a result, the discrimination condition $L_{coh}, r_{cor} \geqslant D$ for a coherent optical signal can be violated, and cells located outside the damaged region of the cell will cease to response to this signal as a regulatory (stimulating) factor. It is this effect that was found after the artificial introduction of a stochastic factor (a random phase screen) into the optical communication channel of interacting cells [25]. In a healthy (active) tissue, an optical signal propagates with a lower loss of its statistical ordering, which is demonstrated by a higher degree of the spatial coherence of scattered probe radiation. The phase correlation in a light wave is observed during its propagation through a tissue of a few millimetres in thickness (Fig. 3), which corresponds to tens and hundreds of cellular layers. (The degree of spatial coherence was estimated with a polarisation interferometer for a fixed value of s [14].) It seems that a change in the structure of issues in biological organisms caused by their activity modulates the properties of the optical communication channel, thereby affecting the regulatory process. Thus, another (optical) relation between the structure and functions of biosystems exists.

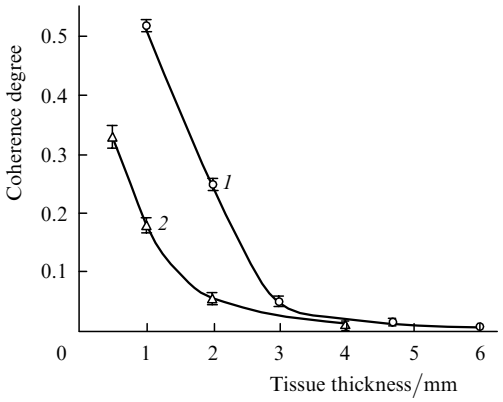

Figure 3. Change in the degree of spatial coherence of a probe laser beam scattered by the tissue of fruits of different thickness; (*1*) apple; (*2*) cucumber.

4. Conclusions

The results obtained in the study show that the conditions required for the participation of chemiluminescence in photoregulatory processes can be realised in biological systems. First of all, this is due to the ability of cells to discriminate the coherence of optical radiation. Contradictions existing in this connection are removed when experimental results are analysed by describing quantitatively the statistical properties of quasi-monochromatic radiation (coherent or incoherent) instead of the qualitative description. The coherence of radiation can be estimated with the help of the characteristic parameters L_{coh} and r_{cor} of the field. In experiments reported in the literature, a considerable change in the functional activity of biosystems was observed when a cell was completely located within the coherence volume, i.e., when the condition $L_{coh}, r_{cor} \geqslant D$ was fulfilled. The cell size D can be treated as the discrimination threshold of radiation coherence inherent in biological organisms.

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