# Effect of spatial coherence of light on the photoregulation processes in cells

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The effect of the statistical properties of light on the value of the photoinduced reaction of the biological objects, which differ in the morphological and physiological characteristics, the optical properties, and the size of cells, was studied. The fruit of apple trees, the pollen of cherries, the microcuttings of blackberries in vitro, and the spores and the mycelium of fungi were irradiated by quasimonochromatic light fluxes with identical energy parameters but different values of coherence length and radius of correlation. In all cases, the greatest stimulation effect occurred when the cells completely fit in the volume of the coherence of the field, while both temporal and spatial coherence have a significant and mathematically certain impact on the physiological activity of cells. It was concluded that not only the spectral, but also the statistical (coherent) properties of the acting light play an important role in the photoregulation process.

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## I. INTRODUCTION

Short-term exposure to quasimonochromatic light fluxes of certain spectral bands can significantly enhance the functional activity of different living organisms—from bacteria to humans. This effect has reliable experimental confirmation but the lack of scientific justification. Depending on the interpretation of the data, conflicting points of view are expressed regarding the mechanism of the observed phenomenon. Most controversial was the question about the role of the coherence of optical radiation in the photoregulation processes. In a number of studies, it is argued that for biological objects the coherence of light is insignificant [1–5]. The reason for this was a similar stimulation effect of the laser radiation, which was called "coherent," and the nonlaser (from thermal, gas-discharge, or fluorescent sources), called "incoherent," These definitions should not be considered valid, since the radiation of the nonlaser sources was cut to the spectrally narrow beam (to produce radiation, similar to a laser one by its optical parameters) with the monochromators and the aperture diaphragms. In this case, the light has a sufficiently high spatial and temporal coherence, although smaller than that for the laser beam [6]. A number of other works, also on the basis of experimental data, show that the stimulation effect is higher for a more coherent light [7–9].

Almost all of these publications pay attention only to the spectral properties of the radiation, such as "broadband light" and "narrow-band light" [10,11], i.e., to the temporal coherence. The influence of the spatial coherence on the functioning of living organisms was left out of the discussion. This concept is used only in the analysis of light diffusion and speckle pattern formation in biological tissues [12,13]. However, it is the spatial coherence that may be the key to understanding the role of the statistical properties of light in the photoregulation processes, due to its independence of the linewidth $\Delta\lambda$, unlike the temporal coherence. The present work is devoted to this issue.

## II. MATERIALS AND METHODS

Living organisms, which differ in their organization, morphological parameters, and optical characteristics, were used to reveal the similarity of photoinduced reactions. Accordingly, four series of experiments were carried out. The potential abilities to photoinduced reactions and optimal modes of light exposure were recognized in preparatory experiments with laser irradiation. In each series of experiments radiation sources formed light fluxes with different coherence, but the same power characteristics (mean wavelength, power density, and exposure).

In the first series the biological model was the fruit of the apple tree. The fruit was irradiated with quasimonochromatic light of high or low coherence within 60 s at the light power density of $4\,\mathrm{W/m^2}$. After irradiation the fruit was kept in storage at a temperature of $+4\,^{\circ}\mathrm{C}$. Each variant and the control consisted of 100–120 fruits, divided into four groups (replications). In 160, 190, and 220 days of storage the proportion of affected fetuses was calculated according to the criterion of visible symptoms of microbial diseases.

In the second series, the pollen of plum was used. Prior to irradiation, it was applied on the surface of a nutrient medium containing 0.8% agar, 15% sucrose, and 0.001% boric acid. The irradiation was carried out for 16, 32, 64, 128, and 256 s at the light power density of $0.7\,\mathrm{W/m^2}$. After irradiation the preparations with pollen were placed in moist chambers, where they were kept at a temperature of $+28\,^{\circ}\mathrm{C}$ for 24 h. Further pollen was inactivated by chloroform and the proportion of germinated pollen grains was determined with a microscope. Each variant consisted of six preparations; each preparation was viewed over ten fields of view.

A third series of experiments was carried out with blackberry microcuttings. They were cultivated on a nutrient

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Murashige and Skoog medium [14] containing 1 mg/l of 6-benzylaminopurine, 0.1 mg/l of $\beta$-indole-3-butyric acid, and 1 mg/l of gibberellic acid. Before irradiation, the duration of the dark phase was 16 h. Natural illumination in the area of exposure was 1 lx. The duration of exposure was 480 s; power density, 0.3 $W/m^2$. After irradiation the microcuttings were cultured in a phytotron for 50 days at a temperature of $+22 \pm 1$ °C, daylight duration of 16 h, and illumination of 2000 lx. In each variant of the experiment, 28 microcuttings were used. The number of shoots formed on each cutting (net reproduction) and their length were taken into account.

A fourth series was conducted on colonies of the fungus *Fusarium microcera*. Colonies were cultivated on potato-glucose agar containing 1% glucose and 1% agar. The irradiation was carried out in 24 h after sowing of mycelium into a nutrient medium. The duration of exposure was 240 s; power density, 1 $W/m^2$. After 6 days of cultivation in the dark at a temperature of $22 \pm 1$°C the volume of the colonies was calculated by measuring their area and thickness.

In the first series for radiation a single-mode He-Ne laser (632.8 nm) and a thermal light source were used. The necessary radiation flux density was formed by means of a microlens and Fourier filter with a diameter of 35–40 $\mu$m. The latter has eliminated the highest spatial frequencies arising in an optical path due to diffraction noise. For the last three series only the thermal source was used. It was a high-temperature filament lamp with an infrared optical filter. The stream of radiation passed also through one of the interferential optical filters which had different values of width of transmission spectrum, but identical wavelengths in a maximum of a spectral band ($\lambda_{max} = 633 \pm 1$ nm). Transmission spectra of the infrared and interferential optical filters were measured with an Analytik Jena Specord 250 Plus spectrophotometer (Germany) with an accuracy of 0.5 nm and then $L_{coh}$ of the thermal source was calculated by a formula $L_{coh} = \lambda^2_{max}/\Delta\lambda$ [15], where $\Delta\lambda$ is a half-height linewidth. The aperture diaphragm defining the angular size of the thermal light source, was placed in the center of the beam behind the filters. This optical scheme (Fig. 1) formed a quasimonochromatic spatially limited wave with a relatively uniform intensity distribution over the front. In this case, the module of normalized transverse correlation function of the field $\gamma$ between two points $\boldsymbol{r}_1, \boldsymbol{r}_2$ can be written as [16] $\gamma(s) = 2|J_1(kas/z)/(kas/z)|$, where $s = |r_1 - r_2|$, $J_1(kas/z)$ is the Bessel function, $k = 2\pi/\lambda$ is the wave vector, $2a$ is a linear aperture of the source, and $z$ is a distance from the radiation source to the object. The first zero of $\gamma$ is at $kas/z = 3.83$, and the correlation length in this case will be $r_{cor} = 0.61\,\lambda z/a$. The power and the power density of the radiation were detected by a VEGA Ophir (Israel) power meter and an IMO-2N calorimetric meter ("Standard," Russia). Specific statistical parameters of irradiation are given in the description of each experiment. At the diagrams and histograms the average values and errors of the mean are specified.

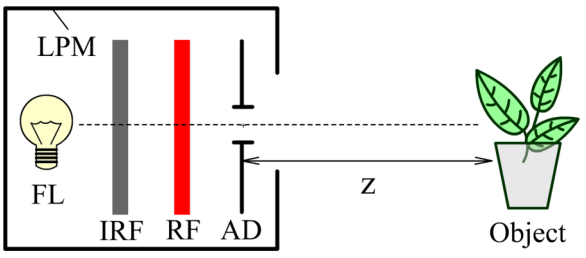



FIG. 1. Scheme of the experiment with a thermal light source. LPM is a light-proof mantle, FL is high-temperature filament lamp, IRF is infrared filter, RF is red interference filter, and AD is aperture diaphragm with a round hole of the diameter of $2a$.

## III. RESULTS AND DISCUSSION

In the first series of experiments the fruit of an apple tree with pathogenic fungi spores located on their surface were used. The response of such a dynamic system varies depending on the spatial and temporal coherence of the light. The radiation of the thermal source ($L_{coh}$ and $r_{cor} \approx 8-10\,\mu$m) increased fruit damage, indicating preferential stimulation of relatively small cells of "parasite" (at the time of exposure not exceeding 10 μm). The laser light ($L_{coh}$ and $r_{cor} > 1000\,\mu$m), on the contrary, decreased fruit disease, which could be due to increased functional activity (immune response) for significantly larger cells of the host (reaching into the epidermal and parenchymal tissues of the fruit 40–50 μm) as well. This picture remains stable in the postradiation period (Fig. 2). Comparing the maximum dimensions of the cells with the characteristic parameters and $L_{coh}$ and $r_{cor}$, it was concluded that the greatest photoinduced reaction occurs in the cells that fit entirely in a volume of the coherence of the field [6,17].

The coherence length of a quasimonochromatic beam is related to its linewidth as $L_{coh} = (\lambda_{max})^2/\Delta\lambda$. Therefore, experiments with different values of $L_{coh}$ cannot result in an unambiguous conclusion, whether the spectral or the correlation properties of radiation affect the change of photoinduced reactions of living organisms. In order to avoid contradictions, in the next series of experiments, the statistical degree of order of the luminous flux from the thermal source was changed by means of the spatial coherence while preserving the temporal one. In the pollen of a plum (pollen grains of size 40–60 $\mu$m) it was shown that at a constant value of $L_{coh} = 32\,\mu$m and certain durations of irradiation, stimulation effect is reliably

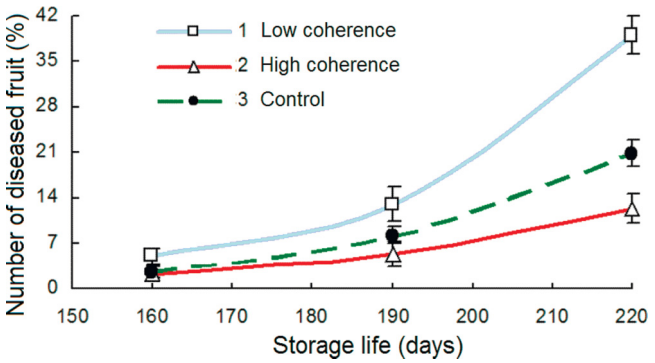



FIG. 2. Dynamics of disease of apples exposed before laying on storage by (1) low-coherent or (2) high-coherent light. (3) is control (nonirradiated fruit). Duration of exposure is 60 s; power density is 4 $W/m^2$.

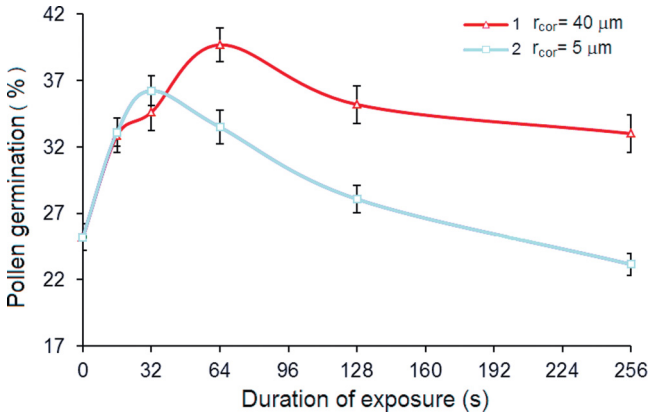
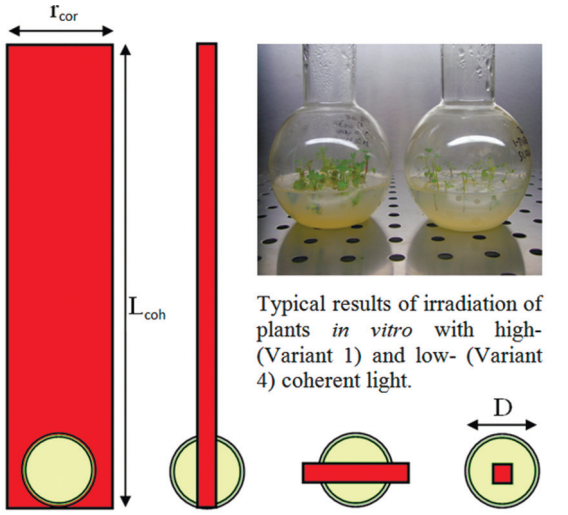



FIG. 3. Dependence of plum pollen germination on the duration of exposure of quasimonochromatic light from thermal sources with the same temporal coherence ($L_{coh} = 32\,\mu$m) and different spatial coherence: (1) $r_{cor} = 40\,\mu$m, (2) $r_{cor} = 5\,\mu$m. Power density is 0.7 W/m$^2$. Control (without irradiation) corresponds to the point of zero exposure duration.




| Variant 1 | Variant 2 | Variant 3 | Variant 4 |
|---|---|---|---|
| $L_{coh}$ = 135 μm, $r_{cor}$ = 30 μm | $L_{coh}$ = 135 μm, $r_{cor}$ = 5 μm | $L_{coh}$ = 5 μm, $r_{cor}$ = 30 μm | $L_{coh}$ = 5 μm, $r_{cor}$ = 5 μm |
| D = 18 μm | D = 18 μm | D = 18 μm | D = 18 μm |
| $K_{st}$ = 2.03 | $K_{st}$ = 1.35 | $K_{st}$ = 1.34 | $K_{st}$ = 1.00 |
| $\alpha < 0.001$ | $\alpha = 0.22$ | $\alpha = 0.14$ | $\alpha = 0.99$ |

FIG. 5. Stimulation coefficient $K_{st}$ in plant tissue culture at different ratios between the cell size $D$ and $L_{coh}$, $r_{cor}$ of exciting light. The volume of the coherence of the light field is represented as a rectangular projection with sides $L_{coh}$ and $r_{cor}$. The size of the cells is represented as a circle of diameter $D$, and the statistical evaluation of the differences with the control as a value of $\alpha$ (probability of the null hypothesis).

distinguished (probability of the null hypothesis $\alpha < 0.001$) for beams with a correlation radius of 40 $\mu$m rather than for beams with a correlation radius of 5 $\mu$m (Fig. 3). Consequently, the spatial coherence of the light beam is also able to affect the photoinduced reaction of cells.

As another biological model we used the blackberry explants (microcuttings), irradiated with quasimonochromatic radiation ($\lambda_{max} = 633 \pm 1$ nm) from the thermal source. By using interference filters and aperture diaphragms four light fluxes with identical power density but various statistical parameters were formed. The greatest stimulation effect was observed in the first variant of the experiment (Fig. 4) for $L_{coh} = 135\,\mu$m and $r_{cor} = 30\,\mu$m. In this case, almost all cells (average cell size $D = 18 \pm 0.3\,\mu$m) fit in the coherence volume of the light field, i.e., $L_{coh}$ and $r_{cor} > D$. At the same coherence length, but with a correlation radius of 5 $\mu$m (variant 2), the stimulation effect was reliably ($\alpha < 0.05$) lower. A similar picture was observed for the coherence length of 5 μm (variants 3 and 4). The stimulation effect of two fluxes with correlation radii 5 μm and 30 $\mu$m differs approximately by a factor of 1.5, but was noticeably lower than that in variant 1. This is probably due to the fact that cells only partially fit in the volume of coherence of the field.

Comparing stimulation coefficients $K_{st}$ (ratio of the values of representative property in the experiment and control) for various types of radiation (Fig. 5), one can conclude that the stimulation effect does not depend on the absolute values of $L_{coh}$ and $r_{cor}$, but on their correspondence with the cell size. The greater the part of the cell that is placed in the quasimonochromatic beam coherence volume, the more pronounced is the photoinduced reaction. It does not matter which of the $L_{coh}$ or $r_{cor}$ parameters is limiting, i.e., is less than $D$. This is evidenced by the close values of the growth

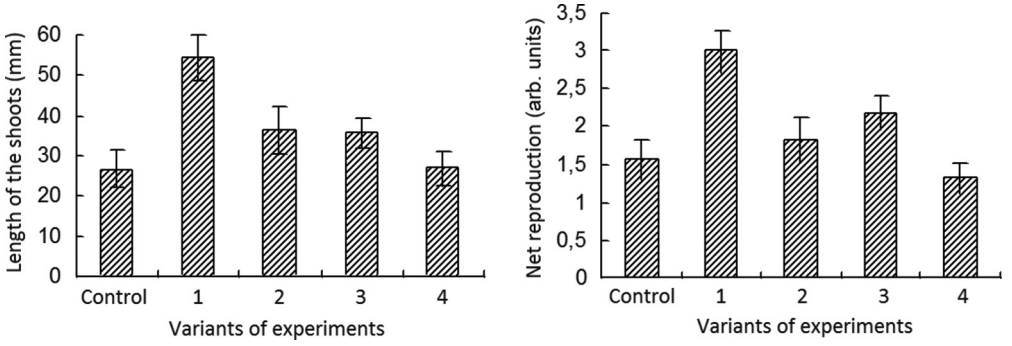



FIG. 4. Effect of quasimonochromatic light of different spatial and temporal coherence in the development of the blackberry explants, cultivated *in vitro*: (1) $L_{coh} = 135\,\mu$m, $r_{cor} = 30\,\mu$m; (2) $L_{coh} = 135\,\mu$m, $r_{cor} = 5\,\mu$m; (3) $L_{coh} = 5\,\mu$m, $r_{cor} = 30\,\mu$m; (4) $L_{co} = 5\,\mu$m, $r_{cor} = 5\,\mu$m. Control, without irradiation.


FIG. 6. Effect of coherence of quasimonochromatic light in the development of the fungus *Fusarium microcera* colonies infected with the bacterium *Pseudomonas syringae*: (1) $L_{coh} = 135\,\mu$m, $r_{cor} = 18\,\mu$m; (2) $L_{coh} = 135\,\mu$m, $r_{cor} = 5\,\mu$m. Control, without irradiation.

coefficients in variants 2 and 3 (Fig. 5), for which both coherence length and the radius of correlation were changed, but a part of the cell entering the area of phase correlation of the photon ensemble remained constant.

The reaction of the fungus *F. microcera* (Wollenw.) Bilai on the irradiation by red quasimonochromatic light with high and low coherence was the same as in plants. Maximum growth of the colonies occurred for the light beam with $L_{coh} = 135\,\mu$m and $r_{cor} = 18\,\mu$m, i.e., when the cells (average cell size is $11 \pm 0.5\,\mu$m) completely fit in the volume of coherence of the field. The growth value at $L_{coh} = 4\,\mu$m and $r_{cor} = 5\,\mu$m was 1.6 times lower. The change in the radius of correlation from 18 to 5 μm at a fixed value of the coherence length of 135 $\mu$m reduced the photoinduced reaction of the fungus cells as well (Fig. 6).

In all of the considered organisms we observed an increase in the functional activity after short-term exposure to quasimonochromatic radiation. Taking into account the spectral range of the radiation, it is possible to suggest the excitation of a plant photoregulatory system [18,19]. In this case, the acceptor of photons is the family of phytochromes—proteins with a chromophoric group similar in structure to phycobilins. A characteristic feature of these molecules is the ability of *cis-trans*-isomerization of the chromophore. Under the action of red light the reversible photoconversion of the phytochrome to the physiologically active form occurs, which increases the intensity of various intracellular processes, up to the gene expression [20,21]. Far-red light leads to their inhibition. The mechanism of transduction of the light signal into a chemical one and its further transformations are well studied [18–21]; however, the primary photophysical processes are not considered so deeply.

The above results show that the analysis of photoregulated processes should not be limited to assessing the intensity, duration, and wavelength of the acting light. One should take into account its statistical characteristics. This follows from the fact that at the same energy parameters, including the width of the spectral lines, the change in the spatial coherence of the field significantly affects the value of the photoinduced reaction of different organisms (Figs. 3–6). Individual molecules of chromoproteids are unable to distinguish the correlation properties of radiation. Therefore, some supramolecular system that performs the functions of a phase detector must exist in the cells. Most likely such a system is a biological membrane associated with the chromoproteidss. For example, the phytochromes form protein-membrane complexes, which change the properties of a lipid bilayer under the action of light, in particular lipid bilayer permeability [18,19]. In the biological membranes and biopolymers cooperative and coherent processes can occur [22–25]. One proof of this is the generation of coherent photons by living organisms [26,27]. Assuming that the entire membrane pool of cells participates in the evaluation of the statistical properties of radiation, the previously set pattern becomes clear: other conditions being equal, the photoregulatory reactions are most pronounced when a cell is completely within the coherence volume of the field [6,17,28]. Then the size of the cells $D$ can be taken as the discrimination threshold of the correlation properties of radiation inherent in biological organisms, i.e., a kind of biological measure of coherence.

## IV. CONCLUSIONS

In a four types of organisms it was shown that the photoinduced reaction of various living organisms depends on the statistical properties of the light acting on them. It is most pronounced in the case when each cell fits completely in a volume of coherence of the field of the quasimonochromatic light beam. This condition is necessary but not sufficient. The wavelength should match the action spectrum of photoregulatory systems and the cell itself should be competent, i.e., be able to increase its functional activity.

The change in spatial coherence of light significantly influenced the photoregulatory processes of living organisms of various levels of organization. At this, the spectrum of quasimonochromatic light flux (linewidth $\Delta\lambda$) remained strictly constant. Therefore, not only spectral, but also correlation properties of light radiation are important for the biological system functioning. This conclusion results in a critical attitude towards the established ideas about the primary mechanisms of photoregulatory processes. In the description of the chromoproteids excitation by photons only the energy parameters of the laser were concerned, while the statistical ones were ignored. This is insufficient in view of the obtained results. There is a necessity of finding some sort of cooperative system (phase detector), responsible for the recognition of coherent properties of radiation.

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