

Scientific session of the Division of General Physics and Astronomy, Academy of Sciences of the USSR (24–25 March 1982)

Usp. Fiz. Nauk 138, 321–328 (October 1982)

PACS numbers: 01.10.Fv

A joint scientific session on the Division of General Physics and Astronomy and the Division of Nuclear Physics of the USSR Academy of Sciences was held on March 24 and 25, 1982 at the P. N. Lebedev Physics Institute. The following papers were presented:

March 24

1. Yu. E. Nesterikhin, S. G. Rautian, and M. I. Shtokman, Selective laser modification of macromolecules.

2. V. F. Kitaeva, A. S. Zolot'ko, and N. N. Sobolev,

Yu. E. Nesterikhin, S. G. Rautian, and M. I. Shtokman. *Selective laser modification of macromolecules.* Studies of the selective interaction of laser radiation with macromolecules have been in progress at the Institute of Automation and Electrometry, Siberian Division, USSR Academy of Sciences since 1978. A note^{1,2} published in 1979 reported discovery of the phenomenon of nonlinear laser cutting (NLC) of DNA—the splitting of double-filament DNA molecules into shorter fragments under laser irradiation. New kinetic effects were predicted and observed on the basis of this phenomenon: light-induced diffusion (LID) of DNA³ and laser electrophoresis (LEP) of DNA.⁴ Applications of phenomena in this group, both those that have already been confirmed by experiment and some that appear theoretically likely, have also been discussed.

Selectivity in the interaction of laser radiation with macromolecules (DNA, proteins, etc.) is highly interesting from the standpoint of the optics and physics of these molecules and for biophysics. At the same time, selective optical methods would appear promising for applications in biochemistry and molecular biology. One can discuss selectivity both by molecule types (intermolecular selectivity) and by position within a molecule (intramolecular selectivity). It is difficult to attain selectivity because the absorption spectra of the macromolecules, e.g., DNA and proteins, are broad in solutions and overlap strongly; there is also strong overlapping of the spectra of the various monomeric elements in the macromolecule.

A general approach to securing selectivity was proposed in Refs. 1, 2, and 5. The idea was to use an impurity center—a dye molecule attached to the macromolecule, e.g., by covalent or hydrogen bonding, etc. Sufficiently longwave, for example soft UV, radiation is

Self-focusing of laser radiation in the case of Fredericks transition.

3. V. I. Belinicher, V. K. Malinovskii, and V. I. Sturman, A new class of photogalvanic effects in solids.

March 25

4. A. M. Baldin, Spin effects in inclusive processes and the quark plasma.

5. É. V. Shuryak, The quark-gluon plasma.

We publish below brief contents of three of the papers.

not directly absorbed by the macromolecule, but is subject to quasiresonant absorption by the dye. Under laser irradiation with a (pulse) intensity of ~ 100 MW/cm², the dye molecule successively absorbs two quanta of light and transfers to a higher singlet state with an excitation energy of 6–8 eV, which corresponds to the hard UV or VUV region. This energy is then transferred to the macromolecule within a radius of ~ 5 Å around the dye molecule in a nonradiative quasiresonant transition.⁵ In the case of DNA, diffusion of the excitation does not strongly delocalize it.⁶ We note that a similar approach, one that also uses a dye molecule as a selective and nonlinear-optical element, was proposed by a group of Italian investigators⁷ simultaneously with Refs. 1, 2.

Intermolecular selectivity is easiest to bring about. In particular, the dye 8-methoxypsoralene (8-MOP), which was used in Refs. 1–4, binds selectively to DNA, but not to RNA and not to proteins.⁸ It is also possible to plant the dye at a predetermined position within the DNA molecule (address it to that position). This can be done, for example, by complementary addressing,⁹ which is based on the selectivity of Watson-Crick hydrogen bonds. Other approaches to dye addressing are also possible.^{5,6}

An energy of 6–8 eV transferred to the macromolecule is sufficient for effective modification, and specifically for bond rupture. Breaks in the chain of the macromolecule that result in its fragmentation are distinctive from the standpoint of physical properties. Such breaks do indeed change the coefficients of diffusion and electrophoretic mobility of the molecules, while other forms of damage have no significant influence on these characteristics. Theory predicts a strong dependence of the cutting probability on the irradiation parameters.²

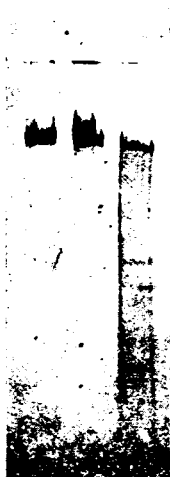


FIG. 1. Result of gel electrophoresis of DNA from phage T7.^{1,2} Radiation dose 60 J/cm^2 . Tracks (from right to left): first, intensity of light $I \approx 200 \text{ MW/cm}^2$; second, $I \approx 0.1 \text{ MW/cm}^2$; third, $I \approx 200 \text{ MW/cm}^2$ in absence of dye.

In an experiment, fragmentation of DNA (the NLC effect) was detected by gel electrophoresis (Fig. 1^{1,2}). The long band in Fig. 1, which is absent in the control, is due to the short molecules formed by NLC, which have high mobility in the gel.

If NLR is done in a limited volume of solution, a spatial redistribution of the density of the DNA will be observed. It appears because the cut molecules, which have higher diffusion coefficients, leave the irradiated volume more quickly than intact molecules from contiguous regions can penetrate into it. As a result, a negative DNA density peak appears in the irradiated volume, and the DNA contents rise in neighboring regions (LID of the DNA^{3,4}). This structure can be seen in Fig. 2⁴; it agrees quite well with the theory.⁶ The coefficient of diffusion of the original DNA molecules, the dependence of the diffusion coefficient on the length of the molecule, and the quantum yield of ruptures can be determined very accurately from such an experiment (cf. Refs. 4, 6, 10). Temperature effects and striction forces have been eliminated as causes of DNA LID by running a control experiment with a different dye.³

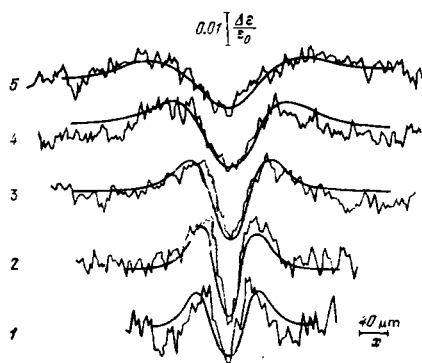


FIG. 2. Series of space curves of optical density ($\lambda = 256 \text{ nm}$) of DNA during LID. The smooth curves are from theory. Field 2 V/cm . Curves 1–5 were registered 2, 5, 10, 30, and 65 min after the end of 3 minutes of laser irradiation.

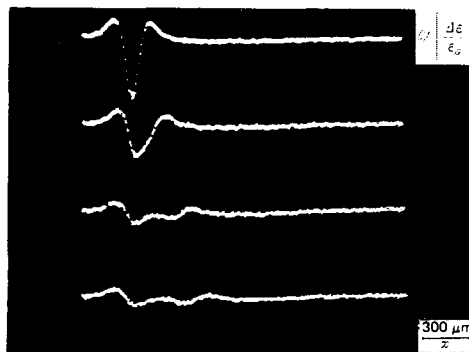


FIG. 3. Space curves of optical density in LEP of mixture of DNAs from phages T7 and λ (field 2 V/cm). The curves (top to bottom) were registered 0, 2, 4, and 6 min after the end of irradiation.

When an electric field was applied to the solution in the course of the experiment described above, electrophoretic drift resulted in separation of the cut and intact molecules, as manifested in a characteristic spatial distribution of DNA optical density (LEP effect^{4,6}). In the case of a polydisperse original solution, the optical-density valley is split into components the number of which is equal to the number of DNA species in the solution (Fig. 3⁴, two DNA species). The LEP effect can be used to analyze mixtures of DNAs. With comparable resolution, it reduces the measuring time by two orders of magnitude as compared to the gel electrophoresis that is ordinarily used, which requires hours or tens of hours.

NLC selectivity can be used to cut DNA (or RNA) at a predetermined point (by the complementary addressing technique) in the process of preparing recombinant DNA. Another possible application is in modifying the nuclei of living cells with the object of controlled mutagenesis, study of radiation sensitivity, etc.

Cutting intraviral DNA (RNA) results in inactivation of viruses without affecting their protein membranes, as is necessary for the production of effective vaccines. The selectivity of NLC of intraviral DNA has already been used to investigate DNA packing.¹¹ NLC of proteins can, in principle, be used to investigate the structure of enzyme active centers. The authors thank D. G. Knorre for stimulating discussions.

¹M. I. Shtokman and A. I. Parkhomenko, in: *Trudy 6-ĭ Vavilovskoi konferentsii po nelineinoi optike* (Proceedings of Sixth Vavilov Conference on Nonlinear Optics). Part 2, Novosibirsk, 1979, p. 85.

²A. I. Parkhomenko, S. G. Rautian, and M. I. Shtokman, *Dokl. Akad. Nauk SSSR* **250**, 225 (1980).

³A. L. Kozionov, S. Yu. Novozhilov, V. E. Soloboev, and M. I. Shtokman, *Pis'ma Zh. Eksp. Teor. Fiz.* **31**, 606 (1980) [*JETP Lett.* **31**, 570 (1980)].

⁴L. Z. Benimetskaya, A. L. Kozionov, S. Yu. Novozhilov, and M. I. Shtokman, in: *Trudy 7-ĭ Vavilovskoi konferentsii po nelineinoi optike* (Proceedings of Seventh Vavilov Conference on Nonlinear Optics). Novosibirsk, 1981.

⁵M. I. Stockmann, *Phys. Lett. Ser. A* **76**, 191 (1980).

⁶S. G. Rautian and M. I. Shtokman, Collection cited in [4].

⁷A. Andreoni, C. A. Sacchi, and O. Svelto, in: *Chemical and Biochemical Applications of Lasers*. Academic Press,

New York, 1979.

⁸J. E. Hearst, *Ann. Rev. Biophys. and Bioeng.* **10**, 69 (1981).

⁹L. Z. Benimetskaya, L. M. Gerasimova, N. I. Grineva, and G. G. Karpova, *Mol. Biologiya* **12**, 988 (1978).

¹⁰A. L. Kozionov, S. Yu. Novozhilov, V. E. Soloboev, and M. I. Shtokman, *Avtometriya* **6**, 73 (1981).

¹¹M. A. Shurdov, A. V. Shishaev, A. P. Sadovskiĭ, and G. P. Kishchenko, Collection cited in [4]; *Biofizika* (1982).
