Coherent optical tomography of microscopic inhomogeneities in biological tissues

V. M. Gelikonov, G. V. Gelikonov, R. V. Kuranov, K. I. Pravdenko, A. M. Sergeev, F. I. Fel'dshteĭn, Ya. I. Khanin, and D. V. Shabanov
Institute of Applied Physics, Russian Academy of Sciences, 603600 Nizhniĭ Novgorod, Russia

N. D. Gladkova, N. K. Nikulin, G. A. Petrova, and V. V. Pochinko
Nizhniĭ Novgorod Medical University

(Submitted 16 December 1994)
Pis'ma Zh. Eksp. Teor. Fiz. **61**, No. 2, 149–153 (25 January 1995)

A method has been developed for constructing images of microscopic objects hidden in optically turbid media through the use of superluminescence sources in the IR frequency range with a coherence time $\leqslant 30$ fs. The method involves detecting a low-intensity coherent component of the reflected light. The method has been used for diagnostics of soft human tissues at depths up to 1.5 mm with a spatial resolution as good as 10 μm. Optical tomograms of pathological states of biologic al tissues have been recorded for the first time. © *1995 American Institute of Physics.*

Progress in the field of ultrashort pulses of optical radiation is opening up opportunities for studying physical objects at temporal and spatial scales previously inaccessible. One of the most interesting and unexpected applications of the method of detecting signals at a femtosecond-range time resolution involves constructing images in turbid media through temporal selection of the unscattered (or weakly scattered) component of the probe radiation, which carries information about optical inhomogeneities of the medium. In the method of coherent optical tomography,[1] this selection is achieved because of preservation of the coherence properties of the weakly scattered photons transmitted through the medium[2] or reflected from it.[3–5] The spatial resolution of the method is determined by the coherence time of the signal and is thus ~ 10 μm in the range of tens of femtoseconds. This resolving power is clearly of interest for observing the microstructure of objects hidden in strongly scattering media, primarily, microscopic inhomogeneities of biological tissues.

In this letter we are reporting experiments on the construction of images of biological tissues *in vivo* and *in vitro* in the near-IR range ($\lambda \sim 0.83$ μm) at depths up to 1.5 mm at a resolution as good as 10 μm. To the best of our knowledge, these are the first optical tomograms to be obtained of pathological states of human tissues (including tumors).

In the near-IR region ($0.83 \leqslant \lambda \leqslant 1.3$ μm), soft biological tissues are optically turbid media which absorb the radiation weakly. Because of the complex spatial structure of the scatterers and their substantial variation in size (there are dimensions both greater than and smaller than the wavelength), there is as yet no generally accepted microscopic model for the optical properties of biological tissues. Macroscopic coefficients found experimentally are ordinarily used for descriptions.[6] The attenuation length due to photon scattering, l_s, is usually few hundreds of microns, while the absorption length is longer

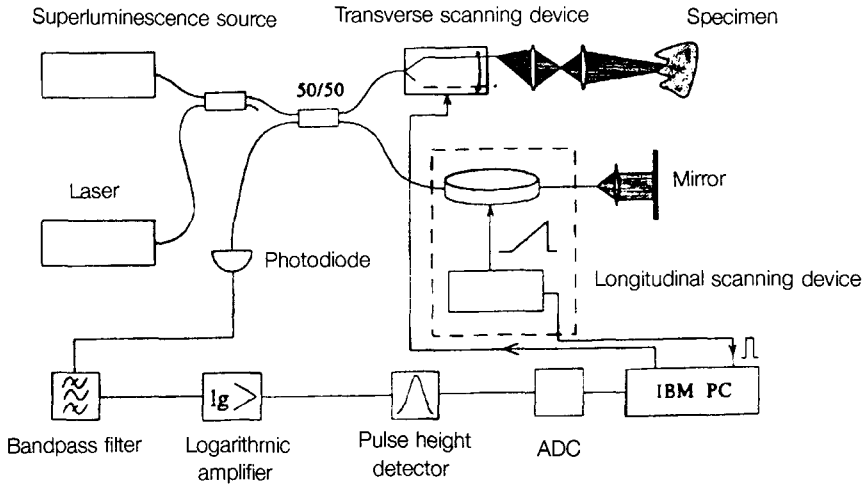

FIG. 1. Layout of the coherent optical tomograph.

by at least an order of magnitude. The attenuation of the unscattered component of the light wave over a path a few millimeters long thus reaches 100 dB. Detecting this unscattered component against the background of the strongly scattered component is the key to the attainment of optical images.

The experimental apparatus which we used to obtain images of the tissues (Fig. 1) consists of a single-mode fiber Michelson interferometer with a superluminescent semiconductor diode ($\lambda = 0.83$ μm, $\Delta\lambda = 30$ nm, radiation power of 30 μW at the surface of the specimen). The specimen is placed in one arm of the interferometer, where it acts as a reflecting object. The optical length of the other arm (the reference arm) is scanned at a constant linear velocity V. The interference signal at the Doppler frequency $f = 2V/\lambda$ is proportional to the reflection coefficient of the optical inhomogeneity inside the sample for the unscattered component. The position of this inhomogeneity is determined by the equality of the optical path lengths traversed by the interfering light beams. The spatial resolution in the longitudinal direction (into the sample) is obviously the same as the coherence length (~ 10 μm). Scanning in the transverse direction is achieved with the help of a optomechanical system which moves the focal spot of the probe radiation along the surface of the sample. The transverse resolution of the location process is determined by the radius of the focal spot. In the experiments, the latter radius is usually $a < 20$ μm; it is chosen in accordance with the condition that the Rayleigh length of the waist, $2n\pi a^2/\lambda$ (where $n \approx 1.35$ is a typical refractive index of biological tissue), be no smaller than the longitudinal size of the region being probed. In order to resolve in detail regions of the images which are of interest it is necessary to reduce a and arrange the corresponding decrease in the longitudinal scanning range. The received interference signal goes through stages of analog and digital processing. The subsequent visualization of the signal makes it possible to obtain, in real time, 2D images of the field of the reflection

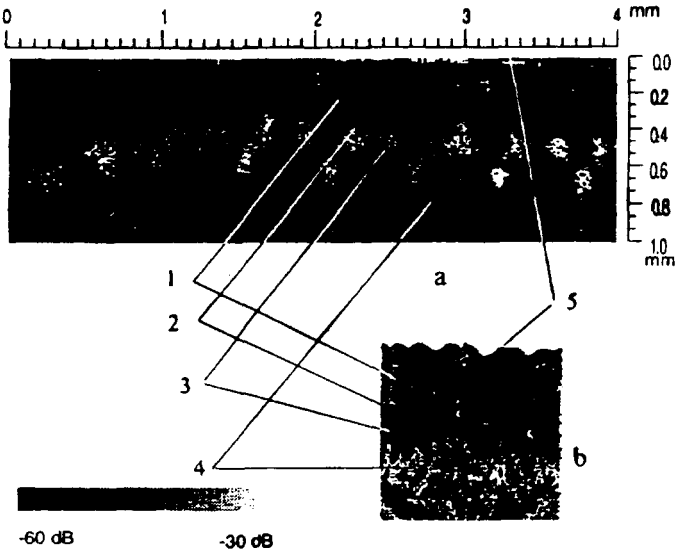

FIG. 2. Tomogram (a) and histological pattern (b) (Ref. 7) of the skin of a healthy finger. 1—Horny layer; 2—lucid layer; 3—granular, spiny, and basal layers; 4—papillary layer of demal layer; 5—surface of skin

coefficient of the coherent component of the light. We call these images "optical tomograms." Depending on the method used for the longitudinal scanning (piezooptic or mechanical), the time taken to record a tomogram of a region with dimensions of 4 $\times$ 1.5 mm at a resolution of 15 μm is between 5 and 20 s.

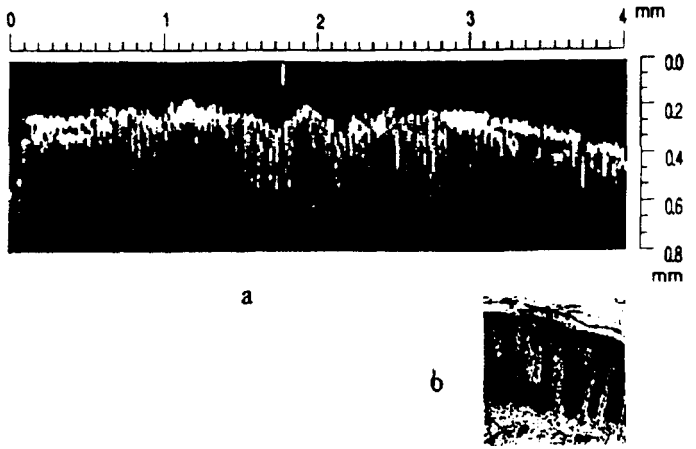

FIG. 3. Tomogram (a) and histological pattern (b) (Ref. 8) of tissue with psoriasis.

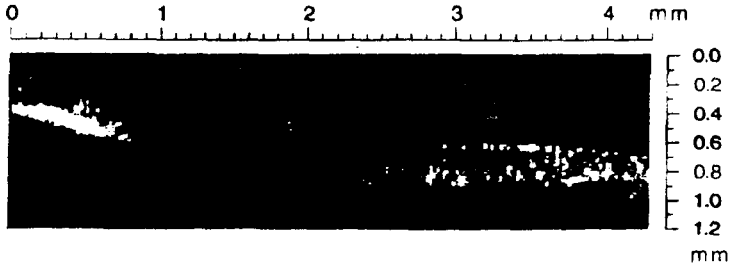

FIG. 4. Lens (at right) and edge of the iris (left) of an eye with a cataract.

Figures 2–5 show optical tomograms of various specimens. The vertical coordinate corresponds to a change in the optical length of the reference arm of the interferometer. The scale here must be normalized to the refractive index in order to find geometric lengths inside the tissues. Figures 2a and 3a are images of *in vivo* healthy skin (the pad of a finger) and diseased skin (the dorsal side of a hand with psoriasis) in accordance with the scale shown for visualizing the reflected signal. Comparison with typical histological images of similar tissue specimens (Figs. 2b and 3b) demonstrates unambiguously that these are successful IR tomograms of biological tissues.

Figure 4 shows a tomogram of part of an eye (the lens and the iris) with a large cataract. The 3D small-scale structure of optical inhomogeneities which lead to the clouding of the lens is visualized here. Figure 5 shows a tomogram of an *in vitro* boundary of a tumor region and healthy tissue of the rectum of a patient recorded a few hours after an operation to remove the cancerous region. In Fig. 5, the tumor tissue has a smaller backscattering coefficient, so one can unambiguously determine the boundary of this region and the nature of the growth of the tumor into normal epithelial tissue.

In addition to revealing the geometric dimensions and positions of inhomogeneities, these tomograms make it possible to determine optical characteristics of tissues, such as an average scattering characteristic. For example, if there are no large-scale inhomoge-

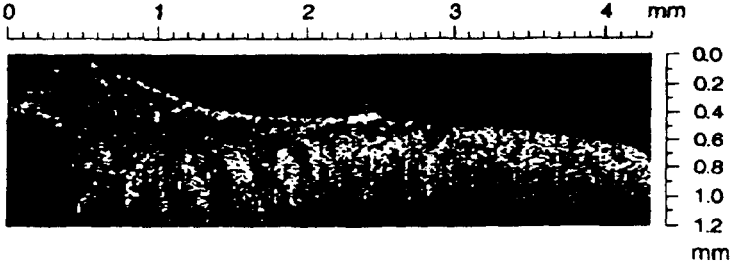

FIG. 5. Tomogram of a region of an *in vitro* rectum. A healthy section is at the right; a cancerous tumor is at the left.

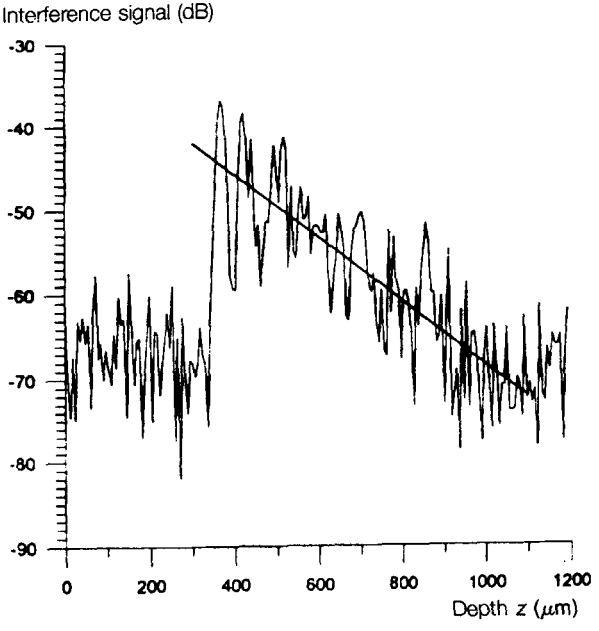

FIG. 6. Attenuation of the signal along depth and exponential approximation of the attenuation.

neities of the medium in the localization zone, the attenuation of the interference signal along depth can be approximated quite well by an exponential function (Fig. 6). Taking the refractive index to be 1.35, we find an attenuation length $\sim$ 170 μm.

In summary, the first results of experiments carried out on an apparatus for coherent optical tomography have yielded information on the microstructure of biological tissues—information not accessible by other methods for locating microscopic inhomogeneities, including x-ray and ultrasonic tomography. This new method would seem to have unprecedented possibilities for nondestructive diagnostics of biological specimens. It should find use in future medial research.

We wish to thank L. S. Dolin for useful discussions. This study had financial support from the Russian Fund for Fundamental Research and the Nizhniĭ Novgorod Center for Development of Science-Rich Technologies.

[1] D. Huang *et al.*, Science **254**, 1178 (1991).
[2] M. R. Hee *et al.*, Opt. Lett. **18**, 950 (1993).
[3] E. A. Swanson *et al.*, Opt. Lett. **18**, 1864 (1993).
[4] J. M. Shmitt *et al.*, in *Advances in Optical Imaging and Photon Migration, Technical Digest, 1994* (Optical Society of America, Washington DC, 1994), p. 190.
[5] A. Sergeev *et al.*, in *Biomedical Optoelectronic Devices and Systems II*, Proc. SPIE **2328**, 144 (1994).
[6] B. C. Wilson and S. L. Jacques, IEEE J. Quantum Electron. **26**, 2186 (1990).
[7] I. V. Almazov and L. S. Sutulov, *Atlas of Histology and Embryology* [in Russian] (Meditsina, Moscow, 1978).
[8] G. M. Tsvetkova and V. N. Mordovtsev, *Pathomorphological Diagnostics of Skin Diseases*[in Russian] (Meditsina, Moscow, 1986).

Translated by D. Parsons