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# Diagnostics of the laser perforation of biological tissues by the method of autodyne detection of backscattered radiation

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***Abstract*. The method of autodyne detection of backscattered radiation is used to study the passage of $CO_2$ laser radiation through interfaces of model media and biological tissues *in vitro* during their laser perforation. It is shown that a stepwise change in the weighted mean frequency of the autodyne-signal power spectrum is a criterion for this passage in real time.**

***Keywords***: *laser radiation, perforation, interface between biological tissue layers, light scattering, autodyne detection.*

## 1. Introduction

Lasers are widely used in surgery. A human body and its organs can be considered as an assembly of tissue layers with various biophysical characteristics. Laser surgical operations allow one to remove (evaporate) only a certain tissue without damaging other tissues, to make a hole or a cut in tissues of one or several types, to interrupt a process in the case of an undesirable course of a surgical intervention, and to continue or terminate the evaporation of a tissue if specified conditions are attained. Such problems, which are associated with the determination of the moment of the radiation passage through interfaces between tissue layers, arise, in particular, upon removing malignant neoplasms and tumours on the skin surface (evaporation of a pathological tissue without damaging healthy tissues), during tissue perforation (e.g., during laser revascularisation of myocardium – producing a through hole), in operations on blood-saturated organs (when vessels may be damaged), and in other cases [1].

At present, the surgeon's eyesight, tactility, and experience usually serve as 'peripheral sensors' and a 'database' in the feedback loop during laser operations. However, such features of laser surgical interventions as their locality and a high-speed action substantially complicate this type of control. Therefore, a prompt diagnostics is required, which makes it possible to control real-time processes of laser evaporation of various tissues.

We proposed the method of autodyne detection of backscattered radiation [2] for studying processes occurring in the region of interaction of single-mode $CO_2$ laser radiation, which is most widely used in laser surgery, with biological tissues. In this method, a laser is simultaneously used as a source of a power action on biological tissues and a tool for diagnostics of the properties of their laser thermal ablation. Compared to the acoustooptical methods currently actively developed for these purposes, this diagnostics makes it possible to acquire information in real time directly from the region of laser irradiation. We showed earlier that the method of autodyne detection of backscattered radiation allows one to distinguish biological tissues during their laser ablation and to study the features of laser damage of real tissues as inhomogeneous multicomponent media [3, 4]. This paper presents the results of studies on determining the moment of the passage of $CO_2$ laser radiation through interfaces between model media and biological tissues *in vitro*.

## 2. Autodyne detection of backscattered radiation

The autodyne effect is observed when the radiation with a Doppler frequency shift backscattered by an external moving object enters a laser resonator and causes a modulation of the cw laser radiation and a modulation of the pump current. Apart from a wave at the fundamental frequency $\nu_0$, two waves with frequencies $\nu_0 + \Delta\nu$ and $\nu_0 - \Delta\nu$, where $\Delta\nu = \nu_0 2\upsilon/c$, $\upsilon$ and $c$ are the velocities of a particle and light, respectively, will exist in the laser resonator in this case. A mixing of these waves leads to a modulation of the power and frequency of laser radiation. As a result of mixing of light waves with different frequencies at a square-law IR photodetector, an electric signal modulated by the difference frequency is produced at the output. The signal represents beats with a spectrum unambiguously determined by the spectrum of the scattered light. By measuring the ac components of the output power or the pump current (the autodyne signal), we can determine the velocity of an object [5]. If the laser – target feedback is weak, the autodyne-signal power spectrum is the product of the backscattered radiation spectrum and the frequency response of the recording system (the laser operating in the autodyne regime). For lasers with relaxation oscillations, the frequency response has a resonance shape [5]. If the Doppler frequency shift falls

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within the resonance band, the autodyne signal appreciably exceeds the heterodyne signal for the same powers of the reference and signal radiations. In this case, the wave fronts of the incident and scattered radiations are matched automatically. The shape of the laser frequency response and its amplification characteristics are determined by such parameters as the photon lifetime in the resonator, the longitudinal relaxation time, the excess of the pump power over the lasing threshold, and the reflectivity of the output mirror [5]. When the radiation with a frequency detuned by $\Delta\nu$ with respect to the fundamental frequency enters the laser, the modulation depth of the laser output power is determined by the expression

$$M \equiv \frac{P_0}{\bar{P}} = 2[G(\Delta\nu)\beta]^{1/2},$$

where $P_0$ is the amplitude of ac power component; $\bar{P}$ is the mean output power; $\beta$ is the backscattering coefficient defined as the fraction of the scattered radiation power entering the laser aperture; and $G(\Delta\nu)$ is the frequency response of the recording system. For typical parameters of a cw single-mode $CO_2$ laser, the autodyne reception sensitivity reaches a maximum at $\Delta\nu_{max} = 0.4$ MHz, and the autodyne gain at the resonance amounts to $G(\Delta\nu_{max}) \sim 100$ [6]. This is important for applications, because one can use uncooled broadband HgCdTe photodetectors with a lower sensitivity. In case of intense radiation scattering, in addition to a component with a Doppler frequency shift, harmonics with frequencies $2\Delta\nu, 3\Delta\nu, ...$ may be present in the autodyne signal. The autodyne reception regime is linear when the harmonic intensities are much less than the intensity of the fundamental signal. As a rule, under typical conditions of focusing laser radiation on the surface of a material, the backscattering coefficient is $\beta \leqslant 10^{-4}$ at a solid angle of detection of $10^{-2}$ sr. Therefore, in our case, nonlinear distortions can be neglected. The sensitivity and frequency response of the recording system are determined from the Doppler response obtained in an experiment on light scattering by a rotating disk surface.

## 3. Autodyne signal recording and processing

Biological tissues of various types differ in their optical, thermal, and structural characteristics. During their laser ablation, an intense evaporation and carrying out of the substance mass occur due to phase transitions. A field of velocities of the destruction products, which depends on the structural features of tissues and the ablation regimes, forms in the ablation region. The products of destruction incorporate structural fragments, burnt tissue particles, and a vapour-droplet mixture, which move at different velocities and determine the properties of the Doppler signal depending on the tissue type [7].

Fig. 1 shows the scheme of our experimental setup for detecting backscattered radiation. The laser-ablation diagnostics using a Doppler backscattering signal consists in the revealing of the differences and specific features of the frequency response for biological tissues of various types [2, 6]. In the study of the tissue perforation, we measured the spectrum of a signal detected within a certain time window of duration $T$ rather than the spectrum of a signal detected during the entire irradiation time. We used a rectangular time window in the experiments.

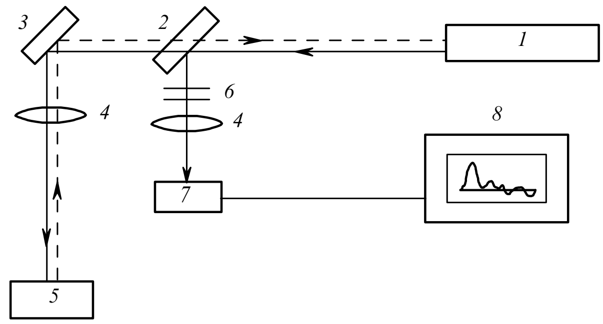


**Figure 1.** Schematic of autodyne detection of backscattered radiation: (*1*) single-frequency (single-mode) $CO_2$ laser; (*2*) beamsplitter; (*3*) mirror; (*4*) lenses; (*5*) sample of a biological tissue; (*6*) attenuators; (*7*) uncooled IR HgCdTe photodetector; (*8*) computer with a built-in ADC.

We characterised the spectrum within the time window selected by its power $P$ within the selected frequency range $\Delta\nu_{21} = \nu_2 - \nu_1$, which was calculated from the area of the spectrum in this spectral range, and the weighted mean frequency $\nu$ calculated as the centre of gravity of this spectral region. By shifting the time window, we obtained the time dependences of the power $P(t)$ and the mean frequency $\nu(t)$. If the true spectrum of the backscattered radiation is reconstructed from the known frequency response, the value of $P(t)$ will characterise the backscattered radiation power, for which the Doppler frequency shift lies between $\nu_1$ and $\nu_2$. If $P(t)$ is calculated for frequencies between $\nu_1 = 0$ and $\nu_2 = \tau_d/2$ ($\tau_d$ is the ADC sampling time), then it describes the total power of the backscattered radiation.

The signal was processed with an ADC built in the computer and a program, which represents a 16-bit Windows application based on the principle of a multidocument interface. The digitisation time for one point in our experiments was set equal to 320 or 640 ns ensuring the correct signal recording in the ranges of 0 – 1.56 and 0 – 0.781 MHz, respectively. Applying a fast Fourier transform to the signal in the time window of duration $T$ and shifting this window by a preset number of ADC samplings, we can determine the dynamics of the spectral characteristics $P(t)$ and $\nu(t)$. If the total number of ADC samplings is $N_0$ and the windows are shifted by $\Delta n$ points, then the functions $P(t)$ and $\nu(t)$ are plotted using $N = N_0/\Delta n$ values. The curves obtained in this way can be smoothed, the smoothing being efficient when $N$ is sufficiently large (at least 1000).

## 4. Experimental results

We studied the dynamics of the autodyne signal and related quantities (the Doppler spectrum, the area of the spectrum, and its weighted mean frequency) during laser perforation of layered materials and biomodels for various media and biological tissues *in vitro*. In the first experiments, we used an organic-glass plate 3 mm thick (as a tissue model) positioned over the water surface (as a blood model) as an object and for methodological measurements of a response of a structurised material to high-power laser radiation. A single-frequency 1-W cw $CO_2$ laser served as a radiation source [8]. The radiation power density at the plate surface was 1 kW cm$^{-2}$, and the exposure time was up to 500 ms.

The aim of these experiments was to reveal the features of autodyne signal formation during the perforation of holes in media containing substantially different components separated by the interface. We used a modified measuring circuit (see [9]) for recording a heterodyne signal without a shift of the reference-radiation frequency, which allowed us to compare it with the autodyne signal and to obtain 'true' (unaffected by the laser frequency response) backscattered radiation spectra.

The second series of experiments was performed using a single-mode $CO_2$ laser with an output power of up to 30 W operating in a single-pulse (100 ms) regime [10]. The laser power density at the surface of a biological tissue was 10–20 kW cm$^{-2}$. In these experiments, we simulated the perforation process for the following two-layer combinations: myocardium – blood, blood-vessel wall – blood, and skin – subcutaneous fat sellular tissue. Freshly prepared samples of pig tissues were used as objects exposed to laser irradiation. The recorded signals were processed using the procedures described above.

Fig. 2 shows time scans of an autodyne signal and its power obtained during perforation of the organic glass plate placed on the water surface. Three characteristic regions in the autodyne signal are observed during the perforation process: (1) a rapid decay of the signal power for a time $t_1$; (2) a region of quasi-stationary signal power $P_1$; and (3) a region of the signal power abruptly rising to a higher mean power $P_2$. The instant of time $t_0$ corresponds to the formation of a through hole in the organic glass plate and to the beginning of the interaction of radiation with water. When the laser radiation interacts with the organic glass and water the Doppler spectra also change. Fig. 3 shows the Doppler spectra measured using a scheme of heterodyne detection of backscattered radiation at different instants of time: Fig. 3a corresponds to region 1 (Fig. 2) of the autodyne signal (the onset of the radiation action on the organic glass), Fig. 3b corresponds to region 2, and Figs 3c and 3d correspond to the instant of time $t_0$ and to the action of radiation only on water, respectively. One can see that the Doppler spectrum in the latter case qualitatively differs from that recorded for the interaction of radiation with organic glass. In the case of such different model substances, the time dependence of the autodyne signal and its power spectrum clearly represent the dynamics of laser-beam passage through their interface.

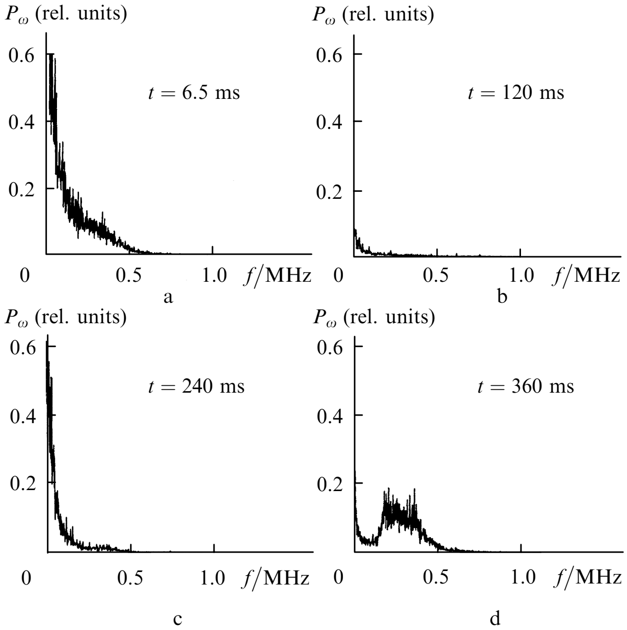


**Figure 3.** Spectra of the power $P_\omega$ obtained by heterodyne detection of backscattered radiation during the perforation of an organic glass plate on the water surface at different moments.

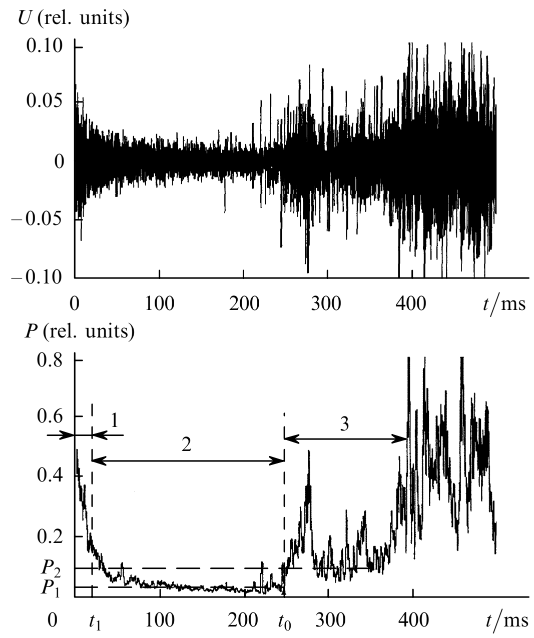


**Figure 2.** (a) Time scan of the autodyne signal $U$ and (b) the time dependence of the signal power $P$ during the perforation of an organic glass plate placed on the water surface.

Water is always present in soft biological tissues in the form of intracellular and intertissue liquids: its content is 70 % – 80 %, and the water content in these tissues differs slightly. For this reason, during laser ablation of biomodels containing soft tissues, blood, and skin – fat structures, other time dependences of the autodyne signal and its related quantities were observed. In particular, unlike model media containing substantially different components, the initial autodyne signal did not register the moment of the radiation passage through interfaces between soft tissues.

Figs 4 and 5 present the time dependence of the amplitude of the autodyne signal and its weighted mean frequency during laser perforation of biomodels. These dependencies were obtained using the following calculation parameters: $N_0 = 262144$, $\Delta n = 200$, and $T = 512 \times 0.32$ μs $= 163.84$ μs. The area of the spectrum and the weighted mean frequency were calculated within the range of Doppler frequency shifts of 18 – 736 kHz. The curves were smoothed by averaging the autodyne signal over 10 – 30 points. Compared to the model media, the passage through the interface is less pronounced, if it is registered by a change in the autodyne signal power. In this case, large amplitude fluctuations in the form of spikes take place, which are caused by an instability of the process of laser damage of tissues.

The radiation passage through the interface is observed as a change in the signal amplitude and frequency. In the

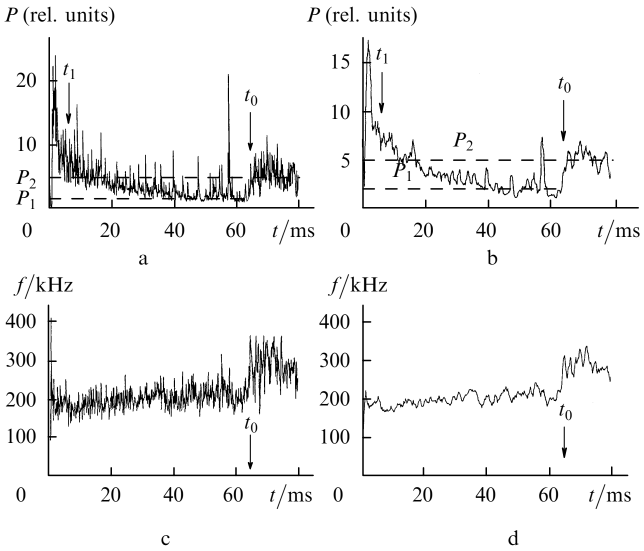


**Figure 4.** (a, b) Time dependences of the autodyne-signal power $P$ and (c, d) the weighted mean frequency $f$ of the signal spectrum obtained during the perforation of the myocardium – blood biomodel (the number of points in the sample is 512, the sample shift is 200 points) without (a, c) and with (b, d) averaging over 15 points. The radiation intensity is 20 kW cm$^{-2}$.

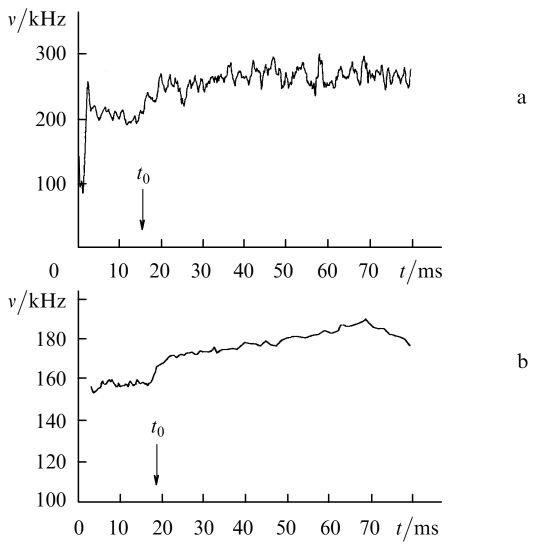


**Figure 5.** Time dependences of the weighted mean frequency $v$ of the signal spectrum obtained during the perforation of the (a) vessel wall – blood and (b) skin – fat biomodels (the number of points in the sample is 512, the sample shift is 200 points) with the averaging over (a) 15 and (b) 30 points. The radiation intensity is (a) 14 and (b) 10 kW cm$^{-2}$.

first case (Figs 4a, b), the degree to which this passage is pronounced strongly depends on the tissue type and thickness, as well as on the time resolution. The boundary of the radiation passage for the vessel wall – blood and skin – fat models is displayed more clearly when the digitisation time is decreased and the signal sampling is reduced. A stepwise change in the weighted mean frequency of the spectrum near the interface between tissues can serve as an efficient criterion for the radiation passage through the interface (Figs 4d and 5a,b).

## 5. Discussion

In our experiments, the autodyne detection was performed in a weak feedback regime, and the light scattering by moving fragments located in the illuminated region served as a source of autodyne signal. Moreover, the surface of the formed laser channel undergoes vibrations, which may result from the channel collapse (low-frequency vibrations) and from the development of small-scale surface waves, and can also be a signal source.

At the initial moment of the irradiation, the maximum contribution to the scattering signal is probably made by the surface (Fig. 6). In this case, the autodyne signal is maximal (see Figs 2 – 4), because the radiation is actually reflected by a flat boundary. As the ablation process develops, a decrease in the signal with increasing the channel depth is caused by the fact that the channel surface becomes curved. Under such conditions, the maximum contribution to the scattering intensity is due to the channel bottom, which is perpendicular to the laser beam axis. As the channel depth increases, the bottom area perpendicular to the beam decreases, and the angle $\alpha$ between the normal $\boldsymbol{n}$ to the side surface and the scattering direction, which is characterised by the wave vector $\boldsymbol{k}$, increases. As a result, the ratio between the surface-scattered and incident radiation powers decreases proportionally to $\cos\alpha$. The scattered radiation power significantly changes, when the channel depth is comparable to its transverse size, i.e., when $\alpha \sim 45^{\circ}$. One can see from Fig. 2 that the autodyne signal decreases by a factor of 2 at the instant $t_1 \approx 20$ ms. Taking into account that $t_0$ corresponds to the instant of perforation of a through hole in a 3-mm-thick sample and neglecting a nonuniformity of the channel deepening rate, we obtain that the instant $t_1$ corresponds to a channel depth of $\sim 200$ μm, which is of the same order of magnitude as the radius of the focused laser beam. A decrease in the signal with the channel deepening cannot be explained by the fact that the near-surface region is not included in the measured volume, because the longitudinal size of the caustic is $\Delta L \approx$

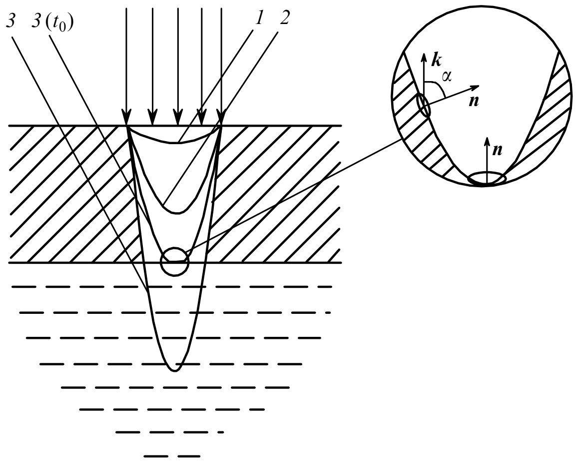


**Figure 6.** Model of different stages of laser radiation passage through an interface of media: (*1*) a small channel depth (the main contribution to the scattering intensity is made by the surface); (*2*) a deep channel (the scattering is determined only by the small bottom surface area); and (*3*) action on the liquid medium; $t_0$ is the instant of the formation of a through hole.

$\approx \lambda F^2/(\pi R^2) \approx 3 - 5$ mm, where $F = 120$ mm is the focal length of the lens and $R \sim 2$ mm is the beam radius at the laser output. At a sufficient penetration depth of the laser beam, the scattered radiation power is much lower than in the initial period and subsequently changes insignificantly. In this case, it cannot be asserted that the maximum contribution to the integrated scattering power is due to the surface.

The experimental results show that, at the initial stage of laser irradiation of organic glass and biological tissues, the scattered radiation spectra do not contain a separate Doppler component and are localised near low frequencies (Fig. 3a). This nature of a backscattering signal also confirms that the main contribution to the backscattering intensity in the initial period of the laser action is made by the surface, since, in its vibrations, its velocity continuously changes from zero to a certain maximum value and the spectrum is localised within the low-frequency region.

When radiation interacts with liquid media (water, blood), the nature of spectra is different. There is a separate Doppler component, and the low-frequency spectrum component is much weaker (Fig. 3d). This is determined by a different character of the radiation interaction with liquid media compared to solid media. The interaction of 10-μm radiation with an intensity of $>1$ kW cm$^{-2}$ with water is of unsteady-state character, which manifests itself in the form of a quasi-periodic sequence of microexplosions. Because blood contains $\sim 60\,\%$ of water, similar processes also occur in it, which is confirmed by our experimental results (e.g., in Fig. 4a).

As is known, a quasi-stationary vapour-gaseous channel forms under the action of continuous radiation on a liquid. The walls of such a channel are smooth, and, consequently, the backscattering from them is less intense. Therefore, as the channel deepens into the liquid, the separate Doppler component becomes determined predominantly by scattering from the ejected droplets, and in the case of ablation of biological tissues, it is determined by the scattering from the ejected destruction products representing a vapour – droplet mixture and particles of burnt and unburned tissues. Note that the characteristic Doppler frequency shifts of 100 – 400 kHz in the spectrum in Fig. 3d correspond to droplet motion velocities of 0.5 – 2 m s$^{-1}$ in the interaction regime. Analogous values were obtained in velocity measurements by the time-of-flight probing technique using a He – Ne laser. The contribution of the surface to the scattered component cannot be fully excluded, because the channel in the liquid may collaps from time to time [10].

When high-intensity laser radiation acts on soft water-containing tissues, an unsteady-state ejection of destruction products takes place, which is determined by the distribution of tissue water and structural features of the tissues [3]. This is manifested in the form of fluctuations of the autodyne signal (Fig. 2a) and related quantities (Fig. 4a). We observed a similar picture upon the laser ablation of other water-containing tissues [11], which confirms a significant role of processes similar to explosive water boiling [10].

A jump in the frequency response of the autodyne signal near the interface of tissue layers reaches 15 % – 25 % of the signal level detected upon the ablation of the first layer. Depending on the biophysical properties of tissue layers, the characteristic signal fluctuations caused by an instability of the ablation process can be comparable with the magnitude of this jump. In order to detect the moment of radiation passage through the interface between media in real time, these fluctuations should be smoothed by selecting optimal parameters of the autodyne signal processing (selection of the averaging method and temporal resolution). In our experiments, the autodyne signal averaging over 10 – 30 points was sufficient for smoothing fluctuations and selecting the jump of the weighted mean frequency that arises upon the passage of the laser beam through the interfaces between tissue layers. This passage is most clearly pronounced as a stepwise change in the weighted mean frequency (Figs 4d and 5a,b), while the autodyne signal power (Fig. 4a) is characterised by spike-type fluctuations, which complicate the determination of the temporal boundary of the radiation passage at the instant $t_0$ in real time.

Note that the passage through the vessel wall – blood interface averaged over 15 points (Fig. 5a) is less pronounced compared to the myocardium – blood passage. The tissue processing parameters should be evidently optimised for different types of tissues and the intensity ranges corresponding to them. For example, the passage can be made more distinct by increasing the number of averaging points, as was done for the passage through the skin – fat interface (Fig. 5b). On the other hand, it can also be detected by the averaged derivative $\mathrm{d}v/\mathrm{d}t$, which should have a maximum in the region of the passage through the interface.

Thus, our experiments have shown that the interaction of $CO_2$ laser radiation with various biological tissues is nonstationary and manifests itself in changes in various spectral characteristics of the scattered radiation. The revealing of different types and specific features of the frequency response of the autodyne signal and its related quantities makes it possible to determine the instant of the laser radiation passage through an interface of biological tissue layers in real time. A stepwise change in the weighted mean frequency of the autodyne-signal power spectrum near the tissue interface serves as a criterion for the passage instant.

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