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Alterations of absorption coefficients of tissue water as a result of the heating under the IR FEL radiation with different wavelengths

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ABSTRACT

The effect of temperature dependent shift of water absorption band, known for pure water, has been examined, for the first time, for tissue water, using the IR Free Electron Laser radiation. Cooling kinetics of cartilage and cornea irradiated was measured with a fluorimeter. We have modified the computation algorithm to calculate the optical properties from these measurements more precisely. Temperature dependence of the absorption coefficient of tissue water is studied, for both sides of water absorption bands at 3.0 and 6.1 μm. It is shown that cooling kinetics for samples irradiated with small laser intensity is the same, for both wavelengths of each pair: 6.2 and 6.0; 6.35 and 5.92; 3.22 and 2.81; 3.15 and 2.87 μm. For high laser intensity, the cooling curves are differ, for above wavelengths. From cooling kinetics curves we have calculated the values of absorption coefficient and their alterations for above wavelengths. We have modified the computation algorithm taking into account the real FTIR spectra of the tissue, the effect of water evaporation from the tissue, and specific characteristics of the IR detector used. It is shown that absorption coefficient may increase or decrease depending on laser wavelength and fluence, and that the water absorption bands have a tendency to shift under laser heating. The IR absorption spectra of cartilage and cornea have been measured by the FTIR spectrometer. The limitation and possible errors of two techniques used have been discussed.

Keywords: Pulsed photo-thermal radiometry, IR absorption, biological tissue, FTIR spectra, IR detector

1. INTRODUCTION

The light absorption coefficient is one of the most essential characteristics of laser tissues interaction. It is known that position and amplitude of the 3-μm absorption line of pure water is temperature dependent[1-3]. Similar behavior has been noted, for other absorption lines of pure water[4,5]. It is not obvious that this effect will be true for water in a biological tissue since the water-water intermolecular interaction differ from the water-organic compound intermolecular interaction, and various forms of water (pure, «free» and «bound» water in cartilaginous tissue) show different FTIR absorption spectra[6]. FTIR spectroscopy has been also used for study the resorption of water from the collagen films[7].

It is difficult to use FTIR to measure the absorption of a biological tissue in the course of laser irradiation when temperature grows fast enough. The method of pulsed photo-thermal radiometry (PPTR) is used for this goal since 1987[8,9] and later on. The typical computational algorithm for the determining of absorption from the experimental data has been discussed in[10].

This method of calculation is based on several approximations. First of all, it is suggested, that all thermo-physical and optical properties of the measured sample do not change with time and distance from the surface irradiated. The second simplification is connected with the linearization of the math problem, i.e. it is assumed a linear dependence of the measured signal on the

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temperature increment. The measured signal is proportional to the integral $\int_0^\infty dx\, \mathbf{T(x,t)}\ \mathbf{exp}\ (-\alpha \mathbf{x})$, where α is the approximating value (or the averaged value) of IR absorption of a sample in the band of sensitivity of the IR detector and Δ **T(x,t)** is coordinate **(x)** and time **(t)** dependent temperature increment caused by a laser pulse.

The aim of this paper is to study the alterations in absorption spectra of tissue water under laser heating. The Free Electron Laser (FEL) has been used as a laser source since the alteration in tissue absorption can be small, and it is of importance to use similar conditions for different laser wavelengths. We have used PPTR technique to measure the kinetics of laser cooling and have modified the computation algorithm to calculate optical properties from these measurements more precisely.

2. MATERIALS AND METHODS

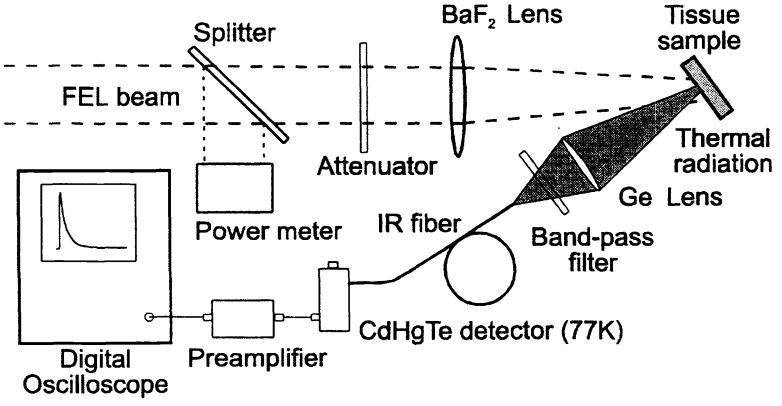


Fig.1. The scheme of experimental setup for radiometric measure of the kinetic of thermal fluorescence from FEL irradiated samples

Figure 1 shows the scheme of the experimental setup used. The Free Electron Laser (FEL) at Vanderbilt University (Nashville, Tennessee) allowed us to tune the wavelength of laser beam in 2.0-9.0 μm spectra region. The temporal structure of a superpulse of the laser beam is a train of pulses of 1ps in duration following in 350ps from each other during about 6μs[11]. The typical energy of superpulses used in our experiments was 20-30mJ. The density of laser energy has been varied by the insertion of the additional IR filters.

The laser beam was formed with a BaF_2 lens and directed to a sample surface at the angle of 45°. The IR thermal radiation emitted from the tissue sample was collected by a Ge-lens and transported with an IR optical fiber to the surface of sensitive HgCdTe crystall of an IR detector which was kept at the temperature of liquid Nitrogen. The frequency bandwidth of the broadband preamplifier of the HgCdTe detector is in the region from 50 Hz to 500 kHz, and its spectrum sensitivity is known as about 6 – 14 μm (Fig.2). To avoid the direct action of the laser light onto the IR detector the band-pass filter (transmission region >7.0μm) was used. Nevertheless, part of the laser radiation could effect on the detector during the length of a superpulse. We measured the time dependence of the PPTR signal **S(t)** taking into the consideration only the data for time after 6 μs from the maximum of the signal.

Fresh bull nasal septum and eyes were used to prepare samples of hyaline cartilage and cornea. Separated nasal septum and eyes were placed between gauze sponges soaked in 9% saline and stored at 2 - 4 ^{0}C in a closed box. The samples for investigation were prepared for several minutes before experiment. The samples were fastened in a special holder. Several cartilage samples (2.4x5x25 mm) were prepared for FTIR spectra measurements. Every place of the sample was used only for one irradiation procedure. A spectrometer Bruker IFS 66V has been used for the examination of the ATR-FTIR spectra.

The PPTR signal was measured for 111 samples of cartilage and for 35 samples of cornea. All wavelengths used for the irradiation occur near the strong absorption line of the water $\lambda \approx$ **3 μm** and $\lambda \approx$ **6.1 μm,** specifically it have been used the wavelengths **6.45, 6.35, 6.29, 6.20, 6.09, 6.00, 5.92** and **3.22, 3.15, 3.00, 2.87, 2.82, 2.81** μm. These wavelengths were selected since they are located symmetrically relative to the maximums of the absorption lines **6.1 and 2.9** μm and are correlated with the approximately initial value of absorption. The energy density of the laser pulse **E** is varied from **0.02** to **0.14 J/cm^2**. Each experiment has been repeated from three to five times.

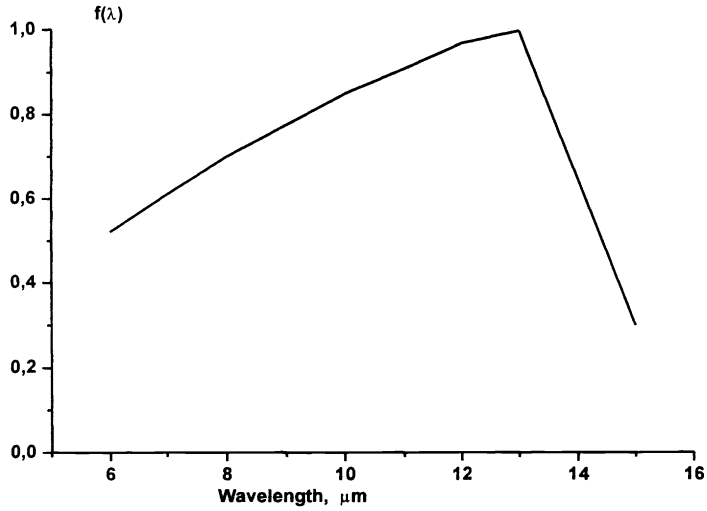


Fig.2. The spectra of sensitivity of the HgCdTe detector.

3. EXPERIMENTAL RESULTS

The time dependencies of the radiometric signal from cartilage irradiated by the FEL are shown on the Figs 3 and 4 for varios laser wavelengths and intensities. Curves 1 and 2 represent higher laser energy and higher temperature than those for curves 3 and 4. The IR absorption spectra of cartilage and of cornea measured by the FTIR spectrometer are shown on the Fig.4. We used selected pairs of wavelength, near 3 and 6 μm water absorption bands, for which the absorption coefficients were initially almost the same for each pair (curves 3 and 4). When increasing laser energy, the curves 1 and 2 gather spread, and the sequence of the curves changes. These data clearly shows that absorption coefficient of tissue water change is temperature dependent. The detailed calculations will be presented bellow.

4. CALCULATION OF THE ABSORPTION COEFFICIENT

When the signal was measured by a specific device it is necessary to take into account its imperfection. The formula for the signal neglected by the real transient response of the preamplifier of the detector. The real transient response of this device for the low frequency may be written as: $\mathbf{h(t)=\theta(t)\ exp(-t/\tau_R)}$. Here $\tau_R \approx \mathbf{16.2 \pm 5.7\ ms}$ is the characteristic time of the decreasing of a signal at the output of the amplifier measured at the constant temperature. The input signal of the amplifier Z(t) and the output signal S(t) recorded by the oscilloscope (3) may be written as[12]:

$$\mathbf{S(t)=Z(t)\ h(0)} + \int_0^\infty \mathbf{Z(t-\tau)\ h'(\tau)d\tau} \qquad (1).$$

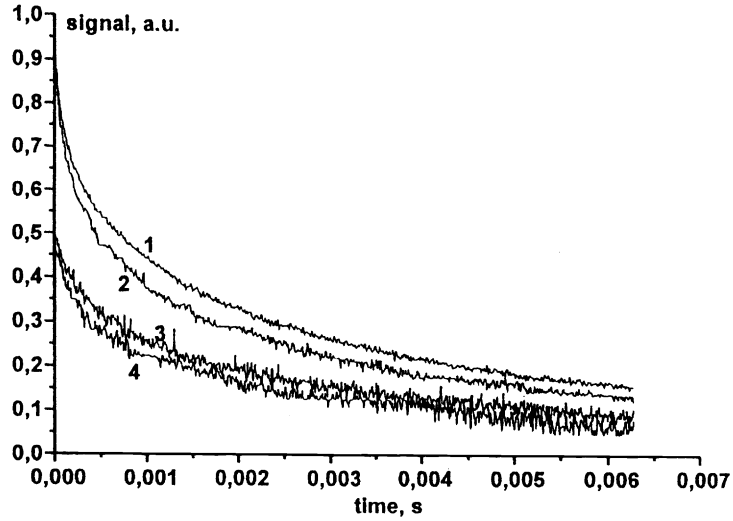
Taking into account that the characteristic time of decreasing of the IR thermo-radiation is much less than τ_R, it is possible to recalculate the recorded signal **S(t)** to the input of the preamplifier **Z(t).**

$$\mathbf{Z(t)=S(t)\ exp(t/\tau_R)} \qquad (2).$$

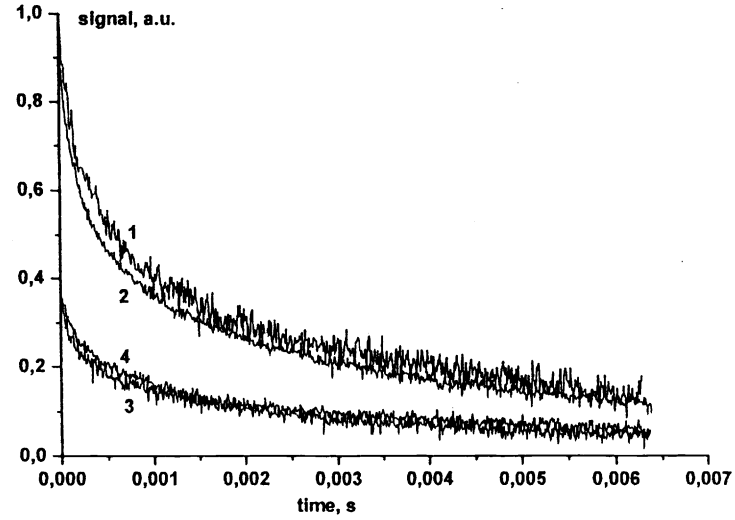
The last formula is true with an accuracy of a small factor of $\mathbf{1/(\tau_R\chi\alpha^2)}$. Here $\chi=\mathbf{1.4*10^{-3}\ cm^2/s}$ is the thermodiffusivity of the tissue[10]. In our calculations we have neglected by a factor $\mathbf{[1/(\tau_R\chi\alpha^2)]^b}$, for $\mathbf{b \geq 2}$.

The method of calculation of the absorption is based on the solution of the thermoconductivity problem.

$$(\partial T / \partial t) = \chi(\partial^2 T / \partial x^2) \qquad (3).$$



a



b

Fig .3. Radiometric signals from cartilage vs time, for the selected pairs of wavelength at different laser fluence:

(a): (1) λ=6.35 μm, E=0.08J/cm^2, (2) λ=5.92 μm, E=0.08J/cm^2, (3) λ=6.35 μm, E=0.04J/cm^2, (4) λ=5.92 μm, E=0.04J/cm^2.

(b): λ=3.22 μm, E=0.06J/cm^2, (2) λ=2.82 μm, E=0.07J/cm^2, (3) λ=2.82 μm, E=0.02J/cm^2, λ =3.28 μm, E=0.026J/cm^2.

All curves are normalized to the laser energy pulse density.

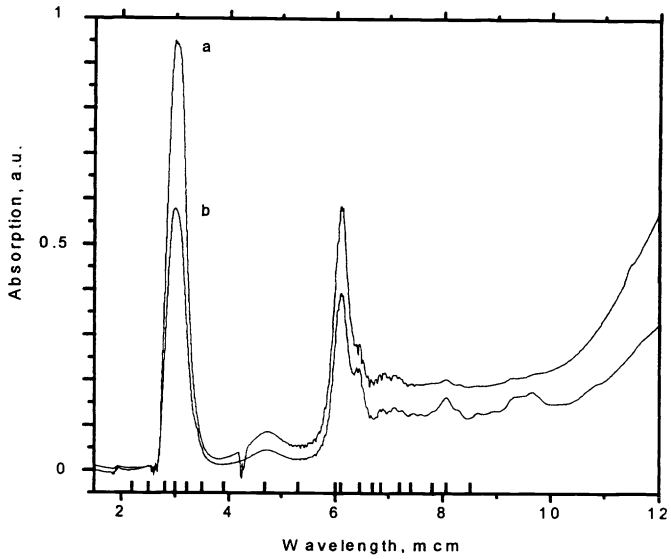


Fig.4. The IR absorption spectra of (**a**) cartilage and of (**b**) cornea. The bars at the bottom axes show the wavelengths for which our measurements have been carried out.

We consider the **1-D** approximation of the equation, because, for our experiments, the relation $\alpha^{-1} << \mathbf{r}_0$ is true. (Here $\mathbf{r}_0$ is the radius of the laser beam.)

In our experiments, we have measured the kinetics of IR response of the sample. This response is determined by the Plank's equilibrium thermal radiation of the black-body. The intensity of the black-body radiation $I(\lambda)$ can be written as:

$$I(\lambda) = 16\,\pi^2 h c^2 \lambda^{-5} / (e^{2\pi h c/\lambda kT} - 1) \qquad (4).$$

Our IR fluorimeter allowed us to measure the signal, that is proportional to the value of Z(t)[13]:

$$Z(t) = \int_0^{\infty} \mathbf{dx} \int_{5\mu}^{14\mu} \mathbf{d}\,\lambda * \alpha(\lambda) * \mathbf{I}\,[\lambda, \mathbf{T(x,t)}] * \mathbf{f}\,(\lambda) * \mathbf{exp}[-\alpha(\lambda)\mathbf{x}] \qquad (5).$$

Here **x** is the coordinate into the depth of the sample, measured from the irradiated surface; the limits of the integration in the eq.5 are determined with the limits of the sensitivity spectrum **f**(λ) of the IR detector used.

In the most studies using the PPTR, the absorption coefficient α in the eq.5 is assumed to be wavelength-independent and the limits of integration over the wavelength are extended from 0 to ∞. When integrating over the wavelength, the Plank's function (4) can be replaced by the Stefan's radiation law $\int_0^{\infty} d\lambda\, \mathbf{I}(\lambda) \sim \sigma \mathbf{T}^4$. The integral $\int_0^{\infty} d\lambda\, \alpha(\lambda) \sim \mathbf{N}$ is taken to be fixed and dependends only on the number of electrons **N.**

In the formula (5), the value $Z(t)$ contains the complicated nonlinear dependence on the spectrum α(λ) in the integrand. For relatively narrow limits of the integration in the eq.5, the approximation $\alpha = Const$ can be used, but, for our case, the limits of the integration are relatively broad. Sometimes, the approximation of the invariable α is used in view of the nescience of the

IR spectrum of the material[14]. We have measured the spectrum (Fig.4) and can use it for calculation according to the eq.5. The eq.5 has been also written in the paper[15] without discussion of its limitation concerning to the real function **f** (λ) and a solution of a thermoconductivity propblem with real boundary conditions. Here we will discuss the advantages and imitations of this approach and also the main assumptions used.

The differences between calculations performed with a simple approach using Stephan law of radiation and with the eq.5 taking into account the real spectrum of the tissue and spectral sensitivity of the detector, are, for our experimental conditions, from 20 to 30 percent. One of the assumption usually used is the neglecting in the eq.5 by the factor of «grayness» of the sample, $\varepsilon(\lambda)$. This cofactor can be written in the form:

$$\varepsilon(\lambda) = 4 n_\lambda / [(n_\lambda + 1)^2 + K_\lambda^2] \qquad (6),$$

$$K_\lambda = \alpha \lambda / 4 \pi \qquad (7).$$

Here n_λ is the reflection coefficient of the sample. It can be shown that the changes of ε with wavelengths are small. So, we measure the radiometric signal within a factor of ε which is unknown, but does not change, for all our experiments. Another assumption is connected with the calculation of he initial temperature profile **T(x, t=0)** determining from the measured signal. Here and further, time is reckoned from the end of laser pulse of 6 μs in duration.

The problem of calculation the initial temperature is a typical inverse problem, and the solution of this problem is not uniquely defined and can be obtained using the numerical methods[16]. There are several reasons required that the mathematical algorithm should be complicated:

1. The absorption spectra $\alpha(\lambda)$ is a complicated function. The integral equation (5) is a linear integral equation with respect to the function **I[λ,T(x,t)],** this integral equation cannot be solved ambiguously. From the theory of the ordinary methods in the linear integral equations, it is known that the desired function **I[T(x,t)]** is proportional to the inverse function $F^{-1}[\alpha(\lambda)]$ [17] which is not uniquely defined.
2. The function **S(t)** can be measured only with some errors caused by a noise (see Fig. 3). With regard of these errors, the solution of the inverse problem cannot be absolutely exactly determined. So, the uniquely determination of the desired function **I[T(x,t)]** is possible only with the use of some additional information or assumption. For this goal we use the set of test functions in the form:

$$T_{test}(x, t=0) = A \exp(-\alpha x) \qquad (8).$$

Here $A=E\alpha/C$; **E** is the laser energy density ; **C** is the heat capacity of the tissue. The simple eq. 8 can be used instead of more exact formula (see the book[18] p.4) describing the temperature field in the tissue heated with a laser pulse when the relation $(\chi\tau)^{-1/2} >> \alpha$ is valid. In our case (for $\chi = 1.4*10^{-3}$ cm^2/s, $\tau = 5$μs) this relation is true, for $\alpha << 1.2*10^4$ cm^{-1}. So, the test function (8) may give a significant error for high absorption coefficients. Since the real absorption coefficient depends on the temperature and may change during laser irradiation, the use of the approximation $\alpha = const$ in the eq.8 may also give some error.

In additional to the initial condition we have to formulate the correct boundary conditions for the thermo-conductivity problem. It is known that water evaporation from the irradiated surface effects very much on the temperature field[19]. The boundary conditions for the equation (3) can be written as:

$$(\partial T/\partial x)_{x=0} = (\nu QN / \sigma) \exp(-Q / RT_S); \quad (\partial T/\partial x)_{x=l} = - (\nu QN / \sigma) \exp(- Q / RT_S) \qquad (9).$$

Here **N** is the superficial concentration of water molecules (Assuming that water is 80 percent of cartilage and 70 percent of cornea we get $N \cong 8.2*10^{14}$ and $6.5*10^{14}$ molecule/cm^2 for these tissues correspondingly); **Q = 44 kJ/mole** is the evaporation heat of water; ***R*** is the universal gas constant, σ is a thermal conductivity, ν is oscillation frequency of the water molecules, $T_S = T(x=0,t)$ is the surface temperature, ***l*** is the thickness of a sample. The cooling of the surface due to the air blowing is assumed much less than that due to water evaporation.

The algorithm of the calculation includes the solution of the thermo-conductivity problem using the boundary conditions (9) and the initial condition (8) with various values of α, then the calculation of the time dependence of the signal **Z(t)** according to the eq.5. The comparizon of experimental and theoretical dependencies (Fig.5) allows us to obtain the proper value of the absorption coefficient. Neglect of water evaporation from the tissue surface may introduce an error up to 10 percent, for experimental conditions used.

It should be noted that water evaporation from the biological tissue during the preparation of the tissue samples leads to the desiccation of the superficial layer and this may change optical and thermophysical properties of this layer. This effect may results in some error of the calculations when assuming the homogeneity of the tissue properties. Also we have to take into account that the laser beam propagates inside the sample in the direction different from the right angle. The angle of propagation is approximately equal to φ=**arc sin** $[(1/\sqrt{2})\,(n^2+\alpha^2\lambda^2*0.025)^{-1/2}] \approx 0.57$ **rad.** This effect increases the value of absorption coefficient calculated on about 13%.

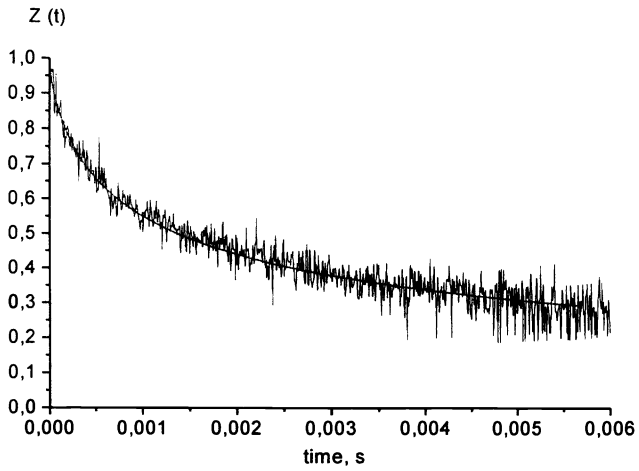


Fig. 5. The typical example of time dependence of the radiometric signals (experimental and calculated). The maximums of these curves are normalized to 1.

5. RESULTS OF CALCULATION AND DISCUSSION

The results of calculation of the absorption coefficient for cartilage are presented in the table 1.

Table 1. **Results of calculation of the absorption coefficients for cartilage.**

λ, μm	β, cm^{-1}	α_{320}, cm^{-1}	α_{320}/β	$\partial\alpha_{320}/\partial T$, cm^{-1}/K	α_{360}, cm^{-1}
2.81	4484	7430±616	1.66	-69±12	4950±250
2.82	4873	8041±150	1.65	-90±18	4400±100
2.87	6662	9669±520	1.45	-90±18	5400±600
3.00	9110	4787±228	0.52	-29±10	3750±150
3.15	6817	4962±210	0.72	-25±8	3450±450
3.22	4381	4301±164	0.98	-48±8	2550±150
5.92	1404	4014±125	2.85	-19±14	3400±100
6.00	2138	4729±116	2.21	-32±6	3450±50
6.09	2709	5146±50	1.90	-25±6	4350±150
6.20	2139	4076±134	1.90	-18±6	3425±75
6.29	1555	2863±76	2.04		
6.35	1398	2798±76	2.00		
6.45	1380	2843±163	2.06		

Here λ is the laser wavelength, β is the absorption coefficient measured with a FTIR spectrometer at the room temperature; α is the absorption coefficient calculated from the data of the PPTR measurements, for temperature of **320 K** (our experiments shown that α values do not depend on temperature, for $T < 320$ K). The accuracy of our measurements are 0.10 and 0.06, for water absorption bands 3 and 6.1 μm respectively. **$d\alpha/dT$** is the derivative of the absorption with respect to temperature; (our experiments shown that **$d\alpha/dT$** does not depend on temperature, for **$T < 370$ K**). α_{360} is the absorption coefficient at **360 K**, the averaged relative accuracy of measurements of the α_{360} is of 12%.

As is seen from the table 1, the wavelength corresponding to the maximal absorption in the **3 μm** band measured by the FTIR method (it falls on the wavelength λ = **2.97** μm) is distinct from that determined by the PPTR method (it falls near the wavelength λ=**2.87** μm). The positions of the absorption maximum, for the spectral band **6.1 μm,** determined by different methods better agree each to other. We think that the differences between values measured by PPTR and FTIR methods are due to the specific features of the superficial layer of the biological sample.

Table 2. **Integral absorption of the 3 and 6 μm absorption bands of cartilage for various maximal temperature increment.**

ΔT, K	η_3	η_6
10	1.00	1.00
20	1.00	1.00
40	1.00	1.00
50	0.91	0.96
60	0.82	0.92
70	0.73	0.88
80	0.64	0.84
90	0.55	0.80
100	0.46	0.76
120	0.28	0.68
140	0.10	0.60

The temperature dependence of the absorption spectrum of cartilage can be seen from the table 2 .

Here η_3 and η_6 are the relations of the integral absorption, for the 3 and 6 μm bands to the initial integral absorption at room temperature. The integral absorption (**I**), for the certain band can be evaluated as a sum $\mathbf{I} = \sum_i \alpha(\lambda_i)(\lambda_i - \lambda_{i+1})$.

It is seen from the table 2 that the amplitude of the absorption line decreases with temperature. The effect of self-bleaching of the absorption line of pure water with temperature has been shown in[1].

The results of the measurements of alterations in absorption for cornea are presented in the table 3.

Table 3. **The alterations in the absorption coefficient of cornea**

λ, μm	E, J/cm^2	α, cm^{-1}
3.22	0.071	2295 ± 114
3.22	0.103	3154 ± 250
3.00	0.030	11402 ± 359
3.00	0.058	5744 ± 150
3.00	0.096	3367 ± 298
2.81	0.045	8755 ± 95
2.81	0.063	4444 ± 81

The relative accuracy of these measurements is characterized by a value of about 9 percent. The increase of the absorption with temperature is observed for the wavelength 3.22 μm, but, for other wavelengths 3.00 and 2.81 μm, the α values decreased with temperature. There is a tendency to shift of the absorption line to the «red» side of the spectrum with laser energy density. When temperature increases the amplitude of absorption usually decreases and the width of the band tends to widen.

It is found, for all experimental results obtained using the PPTR technique, that the absorption coefficient is almost constant for temperature region from room temperature to $T^* \approx 50 - 60^\circ C$ and decreases with nearly constant rate, for $T > T^*$. One can assume that T^* is a characteristic temperature for which the molecular structure of the tissue changes, and this leads to the energy of the inter-molecular interaction decreases. One assumes[1] that the reason of the temperature dependence of pure water is the de-aggregation of water molecules. The phenomena of 'bound-to free' transition of water in cartilaginous tissue, taking place at ~70°C and resulting to de-aggregation of water and proteoglycan sub-systems have been studied elsewhere[18,19]
The temperature induced shift of water absorption bands for the tissues with high concentration of water can relate to the alterations in dipole-dipole interactions of water molecules. So, we can think that the effect of temperature on the absorption spectra of cartilage and cornea, is due to the temperature alterations in water –water, water-collagen and water proteoglycan interaction.

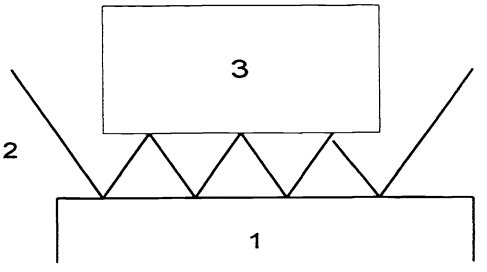


Fig.6. The block diagram of the ATR FTIR spectrograph.
1 is the measured sample; 2 is a crystal with a higher index of refraction than that for the tissue examined (ZnSe with $n_2 = 2.5$, for $\lambda = 2.5$ μ m); 3 is the optical IR mirror, the broken line shows the direction of IR light propagation.

The values of α obtained using the FTIR spectrometer differ from these obtained with the PPTR technique, for the minimal temperature increment studied. This difference may be due to differences in the water content in the tissue samples examined with different techniques and also probably due to some peculiarities of the FTIR method used. We have measured the FTIR spectra using the method of multiplied attenuated total reflection (ATR)[21]. For this method (Fig. 6), the superficial layer of the sample examined with a typical depth of α^{-1} determines the value of the effective absorption recorded by the FTIR spectrograph.

If the absorption of the measured sample is not so small, the relation of the reflected and of the incident light beam can be written as:

$$(I_R / I_I) = 1 - \gamma(\alpha\lambda) \qquad (10).$$

So the output signal I_{out} is

$$(I_{out}/I_I) = [\,1 - \gamma(\alpha\lambda)]^{k} \qquad (11).$$

Here γ is a constant in the order of 1, **k=20-50** is the number of reflections[21]. The FTIR spectrograph measures the absorption α using the eq.(11). assuming the *mirror* reflection of light by a sample. But actually the surface of a tissue sample is not perfect and can scatters light. According to[19], the coefficient of total superficial scattering and reflection could be of 12-15%. The effect of the small-angle light scattering manifests as a supplementary cofactor θ in the eq.11. There is an inequality for this cofactor $\theta \leq 1$. The characteristic value of $1-\theta$ is 0.04 - 0.07. We can write

$$(I_{out}/I_I) = [1 - \gamma(\alpha\lambda)]^{k}\,\theta^{k} \approx [1 - \gamma(\alpha\lambda) - (1-\theta)]^{k} \qquad (12).$$

It is seen from above formula that surface scattering may increase the value of the absorption coefficient determined by the FTIR spectrometer. This effect will be more pronounced for the wavelengths with high absorption. When one calculate the absolute value of the absorption coefficient from the data measured in arbitrary units (see the Fig. 4), one correlates the data, for λ=**2.93** μ**m** with the absorption value known for liquid water[4], taking into account the high water content in the biological tissue. So, the absorption coefficient may be overestimated, for other wavelengths, where α values are lower. Since we used FTIR spectra for the calculation the temperature dependent absorption coefficient from the PPTR measurements, non-accuracy FTIR measurements may be also a reason of additional error of the α values obtained with PPTR. From the other hand, the PPTR technique and the computation algorithm used give more correct results, for not very high absorption when the approximation (8) is still valid. For very high absorption coefficient, the computation algorithm is to be further improved.

6. CONCLUSION

We have shown that the IR coefficient of absorption of the cartilage and of the cornea may change materially under a pulse of the IR laser radiation. The tuned radiation of the FEL have been used as the source for the PPTR measurements. We have modified the computation algorithm taking into account the real FTIR spectra of the tissue, the effect of water evaporation from the tissue, and specific characteristics of the IR detector used. The alterations in absorption have been measured, for two water absorption bands near 2.9 and 6.1 μm. It is shown that the absorption of tissue usually decreases with laser energy density. The IR absorption spectra of cartilage and cornea have been measured by the FTIR spectrometer. The limitation and possible errors of two techniques used have been compared and discussed.

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