FLUCOME 2009
10th International Conference on Fluid Control, Measurements, and Visualization
August 17–21, 2009, Moscow, Russia

SCHLIEREN VISUALIZATION OF LASER-INDUCED FLOW IN BIOLOGICAL TISSUES AND GELS WITH NANOABSORBERS

Alexander Omelchenko[1] and Emil Sobol[2]

ABSTRACT

Paper presents the results of schlieren visualization of flow induced by laser in hydrated biological tissues and gels with photo absorbing nanoparticals. This flow generated by stress in the tissue due to its interstitial water thermal expansion at laser heating. The heating of nanoparticles in the tissue used a near IR-laser, which have proper absorption spectra for endo- and exogenous nanoparticals in the tissue.

Object for the study was a hydraulic laser-tissue interaction within a small region in the tissue-fiber contact. In this region, we observed thermo-induced stress and flow generation as in CW, as in pulse modes of irradiation. Imaging of laser-irradiated zone of the tissue with a transparent probing light showed that stress from water thermal expansion causes the changes in optical density of tissue/gel near contact of fiber. These changes are essential for shadowgraph observing in direct Toepler's illumination and in off-axis schemes. Shadowgraph image processing showed high hydraulic pressure occurred in laser irradiated zone. Pressure distribution was calculated using Abel's method. These measurements are important for the numbers of medical technologies using the nanoabsorbers and laser radiation (hyperthermia, tissue engineering, etc.)

Keywords: Laser, biotissues, nanoparticles, schlieren, visualization, flow, thermo-elastic stress

INTRODUCTION

Laser hyperthermia, as a low invasive method of thermal treating of pathological tissues, is more spreading in medical practice: tumor hyperthermia (Steger *et al.* 1989; Muller *et al.* 1995), laser engineering of biotissues (Sobol *et al.* 2006). Laser procedure for cartilage reshaping is based on short-time hyperthermia of tissue. This procedure demands very precise control of cartilage temperature to avoid excessive over heating of pathological tissue, as well as it circumstances. Temperature control is necessary condition for cartilage reshaping, but not sufficient. Sometimes, it is prefer of pressure control in the tissue to obtain desirable effect of laser radiation.

---

[1]Corresponding author: Institute on Laser and Information Technologies, Russian Academy of Sciences, e-mail: omelch@laser.ru
[2] Institute on Laser and Information Technologies, Russian Academy of Sciences

If a near IR-laser is used as a source of laser heating, it is needed that biotissues have proper absorption spectra of endo- and exogenous nanoparticals, are included in these tissues. Main component for the most of hydrated biotissues absorbing in IR spectra (from 0.9 μm to 3 μm) is interstitial water, which is bounded in protioglycan aggregates. It forms integrity of matrix network, which defines all mechanical properties of tissue (strength, hydraulic conductivity, internal friction). Interstitial water, as a main absorbent of tissues, may be in “free” and “bound” states with endogenous nanostructures of proteoglycans (chondroitine and keratansulfate with the size of ~50-100 nm). According to structural organization of cartilaginous matrix (Pavlova *et al*. 1988), “free” water may be movable and pass through the nanopores and nanochannels in general substance of tissue (Maroudas and Schneiderman 1987; Omelchenko, Sobol *et al.* 2000), consisting of proteoglycan aggregates. Together with proteins and other glicosoaminoglycans they form a high viscosity gels in interstitial water (Hardingham *et al*. 1989). Another mucopolysacharides forms viscous gels as well. For example, it is mucin, as a main component of mucosa.

At the present, nanoabsorbers (absorbing nanoparticles of Ag, Au, γ-Fe_3O_4) have been applied to laser hyperthermia for enhancement of its efficacy. They locally change absorbance of tissue in the limit of size from 10 nm to 100 nm (Terentyuk and Tuchin *et al.* 2009, Nikiforova *et al.* 2008). As a result these absorbers induced thermal effect of laser heating of tissue. However, the mechanism of laser interaction with nanopartilces in the tissues stays unelucidated until the present. Therefore application of these unbiogenous particles should be regulated by special medical permission.

Laser heating causes the water thermal expansion, diffusion and hydraulic mass transfer from the light adsorption zone of tissue. Because of the low permeability of matrix and viscosity of general substance, water thermal expansion generates thermo-elastic stress in the tissue. There are hydrodynamic flow can be observed during laser-induced stress-relaxation: Omelchenko and Sobol (1999, 2008).

Aim of this work is the study of flow and laser-induced stress in the tissues and gels using its schlieren visualization, imaging and digital image processing.

MATERIAL AND METHODS

To study flow and laser-induced stress in semitransparent biological tissues (sclera, cartilage) and gels we used methods of schlieren visualisation, CCD imaging and image processing, which are developed and described in our previous work by Omelchenko and Sobol 2008. Samples were prepared from fresh tissues were selected from the animals sacrificed at the slaughterhouse. Then samples were placed in transparent slide filled with gel to prevent scattering of illumination light. Viscous and scattering gel was prepared from kit of components by GelTek®, Russia. Certified by FDA nanoparticles of γ-Fe_3O_4 from Austin, USA has been used in our experiments.

Erbium fiber laser with the wavelength of 1.56 μm, power up to 5 W (LS-2, IRE-Polus, Russia) working in CW and in repetitive pulse mode has been used to irradiate samples. A 600 μm (in diameter) fiber from laser delivery system was inserted in the slide to irradiate samples at the point of flow visualization. Laser delivery system was adopted with green pilot beam the wavelength of 532 nm.

To induce absorbance of tissue/gel investigated we used aqueous solution of 10-mg/ml concentrations of γ-Fe_3O_4 nanoparticles. The tissues were saturated in this solution during a 2 hour before experiment and gel were doped by the particles when its preparation (Nikiforova *et al.* 2008).

When samples irradiation, there was shadowgraph recording with the Sony DCR-TRV40E camcorder. Next off-line, it was carried out a frame-by-frame image processing of the record with the software has been developed by Omelchenko and Sobol 2008. Image processing included a color to gray scale image decoding, frame decomposition into raw/column to obtain brightness distribution on the selected direction of scan. These brightness distributions were used in calculations of radial distribution of optical density of the samples according of Abel's method (Burret, 1987)

RESULTS

At the effect of the delivered by 600 μm - fiber of 1.5 W power of CW laser radiation on the pure transparent gel we observed shadowgraph of the flow, which was occurred 40 s after beginning of irradiation (Fig.1).

Fig.1.Shadowgraph of the flow observed in zone of laser irradiation of gel. (Teopler's scheme. At the up of picture, it is seen the 600 μm fiber of laser delivery system).

Axial symmetric shadowgraphs were observed at the effect of the cylindrical gauss-beam of CW/pulse laser on gel at the direct illumination of the samples, visualized in Teopler's scheme and in off axis illumination, as well. So, the field of thermal stress and flow were seen in zone of laser irradiation of gel, evidently. This flow was developing in the motionless gel at the laser absorption depth at the 40^{th} s and next leading to bubbles formation at the 50^{th} s after beginning of irradiation. Before these moments it was observed steady growth of hydraulic pressure in zone of laser irradiation (Fig.2a). Meanwhile the pressure were reduced with the distance from beam axis (Fig.2a,b,c,d).

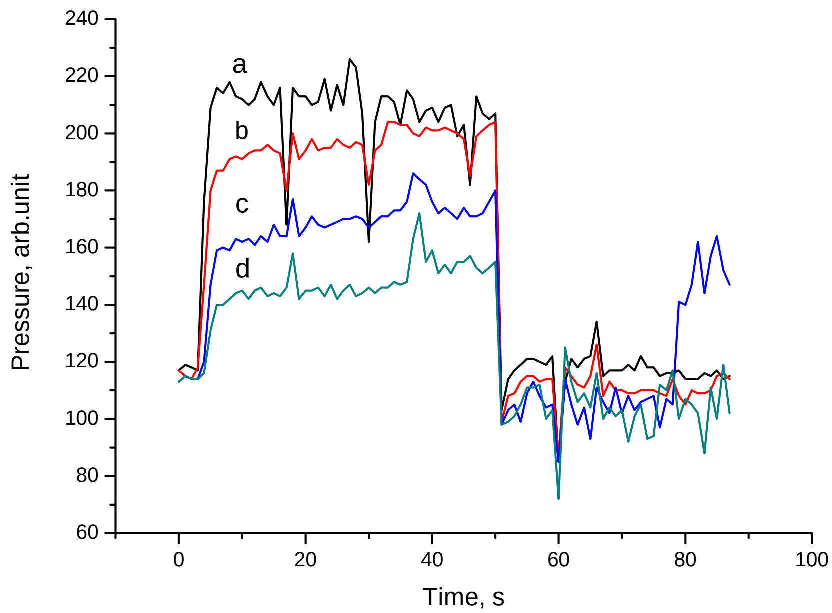


Fig.2. Dynamics of hydraulic pressure near zone of absorption of CW laser radiation with power of 1.5 W. Pressure at the different distance from beam axis: a-0.3 mm, b-0.4 mm, c-0.5 mm, d-0.6mm.

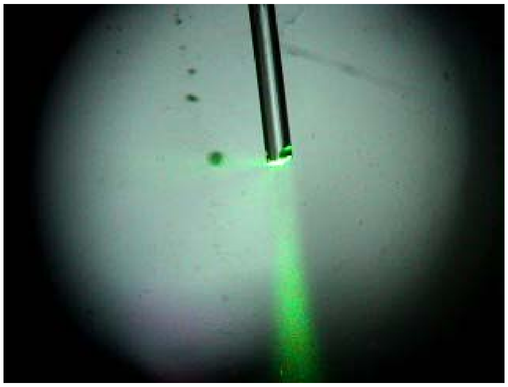
Note, to the moment of 50th s after beginning of laser irradiation of gel, fast flow has been developed in radial direction of beam axis. This flow caused cavitations and bubbles formation in zone of laser-gel interaction. It is seen from Fig.2 by the fast drop down of hydraulic pressure in this zone to the moment of 50th s.

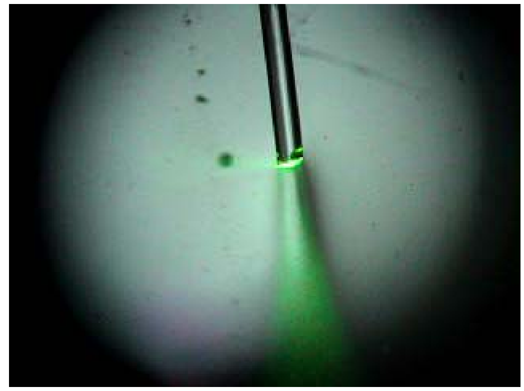
At the effect of repetitive pulse laser radiation (λ=1560 nm) on the low scattering gel in the place near its contact with fiber tip, it was observed the changes in angle of divergence of pilot laser radiation (λ=532 nm), which was distinctly seen in the images. Shadowgraphs of this effect are shown at the Fig.3.

Its need to note, that there was observed a temporary dependency of the divergence angle on the time of laser irradiation of gel (Table 1). The divergence angle was increased with irradiation time and was proportionally dependent on the power of laser radiation.

Table 1. Changes of angle of divergence of laser radiation dependent on irradiation time

Irradiation time, s	Angle Without nanoparticles	Angle With nanoparticles
0	9°	13°
10	19°	24°
20	20°	26°
30	21°	-
40	22°	-
50	46°	-





a **b**

Fig.3. Changes in angle of divergence of laser radiation near the fiber tip at the effect on pure gel of laser pulse of 1 s duration: a- at the beginning of the pulse, at the end

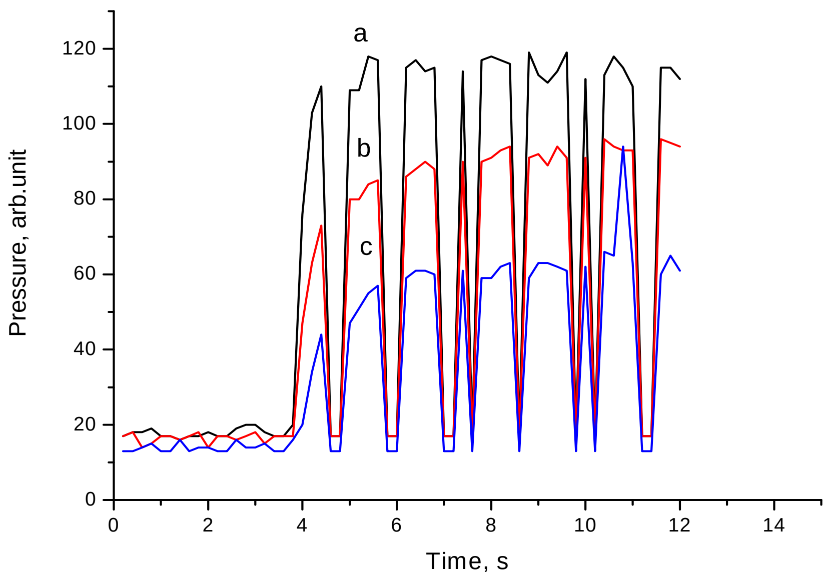


Fig. 4. Dynamics of hydraulic pressure near zone of absorption of repetitive pulse laser radiation with power 1.5 W, pulse duration of 0.6 s, repetition rate of 0.7 Hz in gel at the different distance from beam axis: a- 0.3 mm, b-0.4 mm, c-0.5mm

In the gels doped with nanoparticles, we observed shadowgraph with the reduced length. This shadowgraph was characterized by increased density of shadow with the growth of angle of divergence of probe light (Table 1).

As a result of imaging of cartilage (pure and with nanoparticles) laser-irradiated in Toepler's optical scheme, we obtained shadowgraph images. These images were processed with software and shadowgraph density distributions in the region of laser-tissue interaction have been calculated. Fig.5 presents cartilage images a)- before action of laser pulse, b)- in the act of laser.

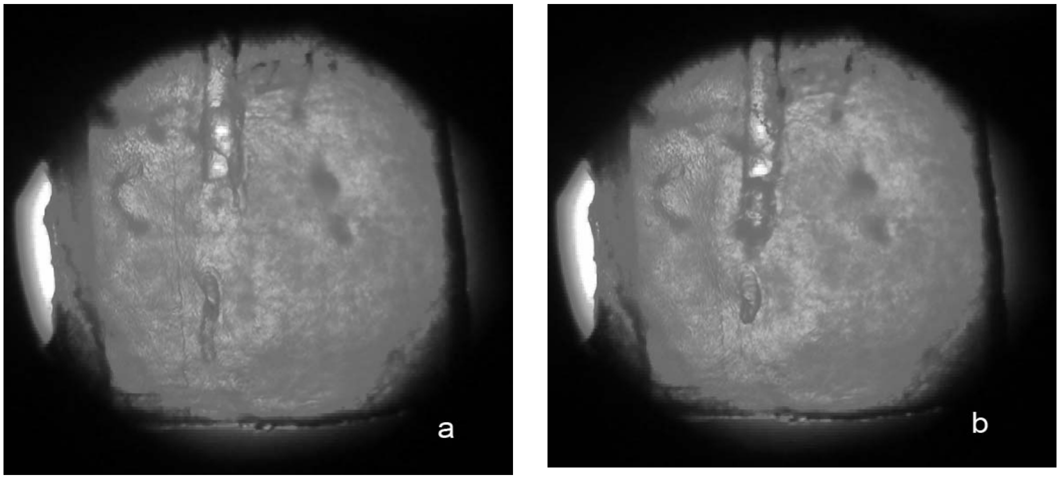


Fig. 5. Shadowgraph images of laser-irradiated cartilage (a- before action of laser pulse, b-in the act of laser)

Results of laser effect on cartilage with nanoparticles are presented at the Fig.6. This picture presents shadowgraph density distribution along line passed through the region of laser-tissue interaction.

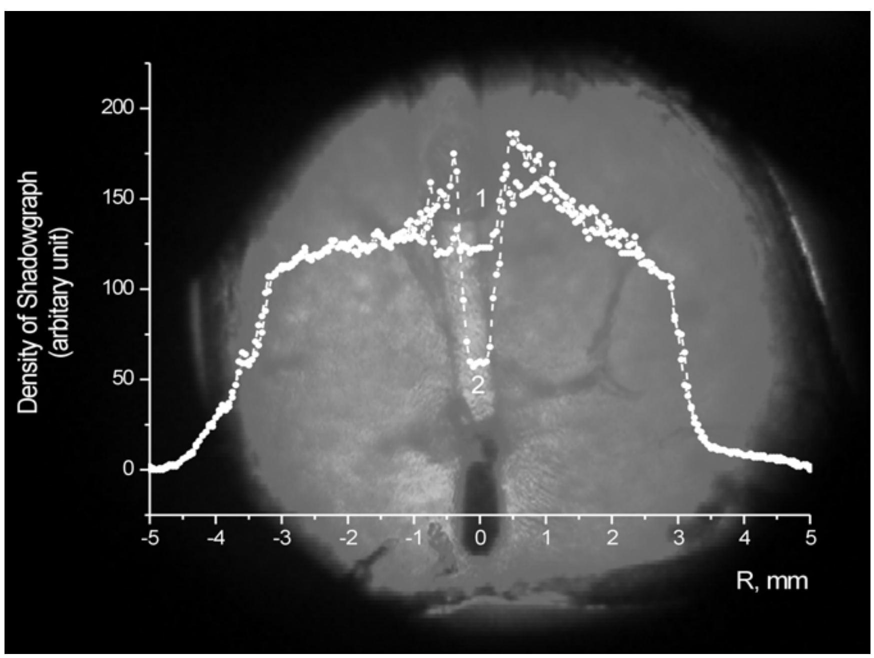


Fig. 6. Shadowgraph and density distribution in the laser-irradiated cartilage with nanoparticles (1- before action of laser pulse, 2-in the act of laser)

DISCUSSION

The presented results on schlieren visualization of flow in biological media showed that is noted a temporary dependency of optical density of shadowgraph observed at the absorption of a near-IR laser radiation in nanoabsorbers. Also flow visualization showed the dependencies of angle of divergence of light on power and duration of laser radiation, absorption and the mechanical property of transporting media. Similar effects are observed in viscoelastic media, when dynamic properties of biomolecular assemblies are studying by the methods of molecular hydrodynamics (Jumel, Harding *et al.*1989, 1992; Jumel, Harding and Sobol, Omelchenko *et al.*2002).

The laser-induced optical effects observed in semitransparent media near tip of fiber, we consider to relate with the growth of hydraulic pressure from water thermal expansion. The value of this pressure can be extremely high and, as it estimated by Omelchenko and Sobol (2008), reaches of ~10 MPa. This pressure leads to reducing of material density in zone of laser irradiation. As a result of gradient of material density it is reduced an optical density along beam axis near fiber tip. These effects are confirmed by increase of angle of divergence of probing light at the laser irradiation of gel. Increasing of the angle of divergence of light in gel doped with nanoparticles is the evidence of increase of thermal effect from absorption laser radiation. Also, thermal water expansion results in radial extension of optical density from axis to beam boundary.

On the strength of axial symmetry of the field of stress distribution it is established symmetrical distributions of refraction and absorption indexes with regard to beam axis in transparent media.

In result of the solution of inverse problem for absorption index profile (see Appendix II) by Abel's method (Burret, 1987; Vasilyev, 1963), it has been calculated values of hydraulic pressure for external region of absorption volume (Fig.2 and Fig 4). To obtain absolute value of the pressure for internal region it needs to use data on the changes of refraction angle at laser irradiation (Table 1). Change of this angle after 50^{th} s talks about sharp change of optical density near tip of fiber, and next dynamics is related with light scattering on irregularity of flow generated and bubbles formation. Observed pressure dropping down after 50^{th} s (Fig.2) is the evidence of stress relaxation in zone of laser heating. It can be expected that flow generation induce this stress-relaxation.

Schlieren visualization of cartilage laser-irradiated allows us to see stress distribution in the region of laser-tissue interaction (Fig.5). Application of nanoparticals in laser treating of cartilage improved methods of laser engineering of biotissues. It makes possible to treat cartilage and other tissues doped with nanoparticles in more effective and safe mode of laser irradiation.

All these results may be useful to design of new diagnostic instruments for laser hyperthermia and engineering of tissues.

REFERENCES

Steger, A.C., Lees, W.R., Walmsley K., Bown S. (1989) "Interstitial laser hyperthermia: a new approach to local destruction of tumor," *Br. Med. J.,* **299**(5), 362-365.

Muller, G., and Roggan, A. (1995) "Laser-induced interstitial thermotherapy," **PM25**, SPIE press, Bellingham.

Bagratashvili, V.N., Sobol, E.N., Shekhter, A.B., et al. (2006) "Lasernaya Engeneria kchrashey (Laser engineering of cartilage)", FizMatLit, Moscow.

Maroudas, A. and Schneiderman, R., (1987) "Free" and "exchangeable" or "trapped" and "non-exchangeable' water in cartilage. J. Orthopaed. Research (5), 133-138.

Hardingham, T.E., Hughes, C., Mow, V.C. and Lai, W.M. (1989) "Flow properties of proteoglycan solutions", 246-255 in: Dynamic properties of biomolecular assemblies. Ed. Harding, S.E., and Rowe, A.J. - Royal Society of Chemistry, Nottingham, UK.

Harding, S.E. (1989) *ib id.*

Harding, S.E.(1992), Laser light scattering in biochemistry. Royal Society of Chemistry, Cambridge, UK

Jumel, K., Harding, S.E., Sobol, E., Omelchenko, A.I. *et al.* (2002) "Aspects of the structural integrity of chondroitine sulphate after laser radiation" Carbohydrate Polymers, **48**, 241-245.

Sobol, E., Omelchenko, A., Mertig, M. and Pompe, W. (2000) "Scanning force microscopy of the fine structure of cartilage irradiated with CO2-laser" Laser in Medical Science, **15**(1), 15-23.

Pavlova, V.N., Kopyeva, T.T., Slutsky, L.I. and Pavlov, G.G. (1988), Khryash, Medizina, Moscow.

Omelchenko, A., Sobol, E., *et al.* (1999), "Acoustic control of laser shaping of cartilage." Proc. of SPIE, **3732**, 312-318.

Omelchenko, A. and Sobol, E. (2008), "Imaging of laser-induced thermo-elastic stress in biotissues with shadowgraph," Proc. of SPIE, **7000**, 700015-(1-8).

Barrett, H.H. (1984) "The Radon transform and its applications" In: Progress in Optics /Ed. E.Wolf., Amsterdam: Elsevier, **21,** 217-286.

Vasiliev, L.A . (1963), Tenevye metody. Nauka, Moscow.

Nikiforova, T.E., Omelchenko, A.I. and Sobol, E.N. (2008) " Magnetolasernoe upravlenie raspredeleniem ferromagnitnykh nanochastitz v hydratirovanykh biotkanyakh i gelyakh" Perspectivnye Materialy (rus.) Advanced Materials, spec.issue **6**(I), 450-453.

Terentyuk, G., S., Maslyakova, G.N., Suleymanova, L.V., Khlebtsov, N.G.. and Khlebtsov, B.N. (2009) "Laser-induced tissue hyperthermia mediated by gold nanoparticles: toward cancer phototherapy" J. Biomed. Optics. **14**(2), 021016(1-9).

Kiezel, V.A. (1973), Reflection of light. Nauka, Moscow.

Timoshenko, S.P. and Goodier, J.N.(1970), Theory of Elasticity. 3 ed, McGraw-Hill, N.Y.

APPENDIX I. NOTATION

The following symbols were used:

D-shadow density,

r– Radial coordinates,

α - absorption coefficient,

n - Refraction index,

a- beam diameter,

c –concentration of absorber,

χ- Coefficient of extinction,

l – Optical length,

θ_0 - angle of divergence,

I_0-intensity of illumination,

I_τ-intensity of transmitted light,

R-coefficient of reflection,

P_h-hydraulic pressure,

α_t-coefficient of water thermal expansion,

β_h-water compressibility,

T- Temperature.

APPENDIX II. CALCULATION OF PRESSURE IN LASER IRRADIATED MEDIA

The case of gauss-beam and axial symmetry of shadowgraph:

Let us assume, that from silica fiber with refraction index n_c=1.5 a cylinder light beam emitted into media with refraction index n_m=1.3 as it is shown at the Fig.5.

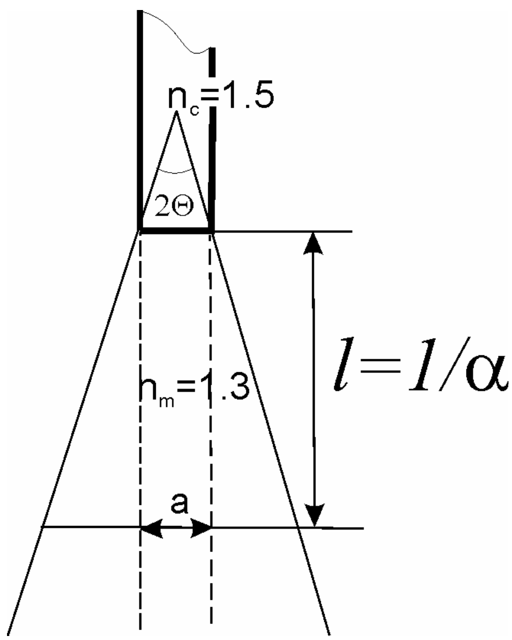


Fig.5

As a result of laser interaction with media there will be grade of refraction index Δn, as it is shown at the Fig. 6.

Changes in angle of light divergence are determined by Snell's low:

$$Sin\ \theta_1 / Sin\ \theta_0 = n_c / n_m \qquad (1)$$

Changes in angle of divergence at the fiber tip, as it is shown at the Fig.3, can be calculated according to (1):

$$Sin\ \theta_1 / Sin\ \theta_0\ (1+ \Delta\theta/ \theta_1) \approx n_1/ n_0(1+\Delta n/n_1) \qquad (2)$$

Value of the angle of internal total reflection depends on grade of refraction index Δn:

$$Sin\ \theta_m = n_0/(n_0-\Delta n) \approx 1/2; \quad \theta_m = 30°; \qquad (3)$$

Value of sight parameter of the maximal density of the shadows is defined by formula:

$$r_m = a/2\sqrt{(1-Sin^2\theta_m)} \approx 0.8a/2; \qquad (4)$$

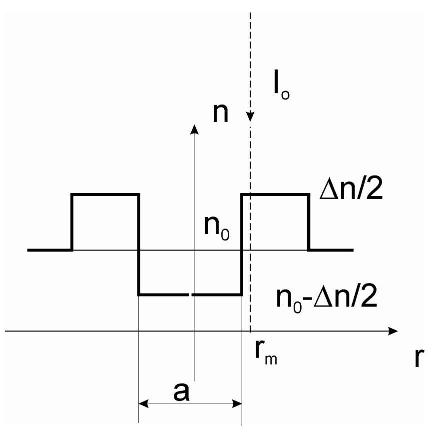


Fig.6

Definition of shadowgraph density: $D(r) = I_\tau/I_0$, where I_τ, I_0 – intensity of illumination and transmitted light can be find in the case of low absorbing media (αl<<1):

$$I_\tau = (1-R(r))I_0\ (1-e^{-\alpha l}) = I_0\ (1-R(r))\ c(r)\ \chi\ l \qquad (5)$$

, where R(r) - coefficient of light reflection from the layer with the grade of refraction index Δn is defined by Frennel's formulas: (Kiezel, 1973).

$$D(r) = (1-R(r)) \int c(r)\ \chi l\ dl \qquad (6)$$

Radial distribution of absorber's concentration is determined by Abel's method and pressure distribution can be found from the solution of thermo elastic problem (Timoshenko and Goodier 1970):

$$P(r) = \alpha_t/\beta_h\ T. \qquad (7)$$

Next, assuming for the temperature of media is proportional to absorbers concentration, ones can find hydraulic pressure: $P_h \approx \alpha_t/\beta_h\ c(T)$ (8)