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Experimental stand for studying the impact of laser-accelerated protons on biological objects

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Abstract. **An original experimental stand is presented, aimed at studying the impact of high-energy protons, produced by the laser-plasma interaction at a petawatt power level, on biological objects. In the course of pilot experiments with the energy of laser-accelerated protons up to 25 MeV, the possibility is demonstrated of transferring doses up to 10 Gy to the object of study in a single shot with the magnetic separation of protons from parasitic X-ray radiation and fast electrons. The technique of irradiating the cell culture *HeLa Kyoto* and measuring the fraction of survived cells is developed. The ways of optimising the parameters of proton beams and the suitable methods of their separation with respect to energy and transporting to the studied living objects are discussed. The construction of the stand is intended for the improvement of laser technologies for hadron therapy of malignant neoplasms.**

Keywords: *laser-plasma interaction, high-energy protons, irradiation of cell cultures.*

1. Introduction

Modern laser sources [1–5] and their growing commercial availability offer wide possibilities for the development of a variety of applications of ultraintense radiation, one of which is the laser-induced acceleration of particles [6, 7]. Compact laser-plasma sources of accelerated particles are relevant for many applications, firstly as a cheaper and convenient alternative to classical accelerators. Besides, laser sources of particles possess an additional set of unique parameters, e.g., the point-like localisation and short pulse duration, which make them irreplaceable in some applications [8]. In medicine, the sources of accelerated ions are mainly considered as a means of the hadron therapy of cancer diseases, the essence of which consists in irradiating cancer cells with fast ions in order to destroy their mitotic ability.

Fast ion therapy allows increasing both the biological efficiency and the irradiation conformity, i.e., it provides the possibility to transfer the dose exactly to the tumour with a minimal loading on surrounding normal tissues. As a result, the survival indicators are increased and the patient's life quality is improved [9]. The selectivity of the ion beam impact is due to the Bragg absorption peak [10] localised at the depth depending on the energy of the proton entering the living tissue. In the world several dozens of centres for ion cancer therapy exists [11]; however, the main obstacle for the popularisation of this treatment is the high cost of the service caused by the use of cumbersome and expensice-to-maintain classical accelerators. The compact and relatively cheap laser-plasma accelerators can be an alternative to them in the future [12, 13].

However, it is worth noting that the presently existing technologies of laser-plasma acceleration do not provide sufficiently monoenergetic protons with maximal energy, and, therefore, they do not provide the required locality and action on deeply located tissues. The authors of Ref. [14] report the generation of laser-accelerated protons with a record-breaking energy of 67.5 MeV and a spectrum without any monoenergetic singularities. Such an energy corresponds to the Bragg peak at a depth of nearly 3 cm [15], which, in principle, allows the irradiation of the human eye retina, but is obviously insufficient for the treatment of deeply located inoperable tumours, e.g., those localised in the brain. In this relation, the studies aimed at the improvement of the laser sources and the regimes of laser-plasma interaction for increasing the maximal energy and the number of accelerated protons, as well as the development of the methods of separation with respect to energy, transportation and focusing of the laser-accelerated beams become particularly important. However, even with the existing laser sources in the studied acceleration regimes one can develop the basic approaches to the laser proton therapy and perform the experiments with cell cultures [16, 17].

Here we present the apparatus consisting of a vacuum laser accelerator of protons based on the PEARL petawatt laser system, magnetic energy separator and original unit for irradiating living cultures, including a vacuum – air interface and a multiwell plate for placing the living cell culture. We present the estimates of the apparatus capabilities from the point of view of attainable depths and locality of interaction and consider the ways of improving the particle acceleration regime and additional focusing of the proton pulse. We also describe the technique of affecting the cell culture *HeLa Kyoto* with laser-accelerated protons.

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2. Laser acceleration of protons

The experimental part of the studies was carried out using the PEARL laser-plasma stand (Institute of Applied Physics of RAS, Nizhnii Novgorod), based on the laser system of the same name [18]. In Ref. [19] our group demonstrated protons with the energies up to 43.3.MeV, accelerated in the TNSA regime [20] by focusing the p-polarised pulse (7.5 J, 60 fs) onto the aluminium target 0.8 μm thick, placed at the angle 45° to the optical axis.

For the pilot experiments with the biomodule, we used thicker targets (10 μm) that provide higher stability of the angular distribution of protons and smaller sensitivity of the acceleration regime to such parameters of the laser radiation as the duration and contrast. The typical pattern of dose distribution, received by the radiochromic films (RCFs), placed at the distance about 4 cm from the region of laser-plasma interaction in the course of focusing onto the foil 10 μm thick is presented in Fig. 1a, and the corresponding energy spectrum of protons is shown in Fig. 1b. The protons reach the studied object through the holes in the centre of the plates (see, Section 3), the dark area around the hole is not related to the dose on the plates.

In the experiments the proton beams with an exponentially decreasing energy spectrum were obtained, which is in good agreement with the results of the known theoretical and experimental studies [21–23].

The character of the effect of accelerated protons on the medium is mainly dependent on the medium density, so that the doses received by the plastic RCFs are close to the doses to be received by living tissues under similar conditions. It is seen that in a single shot the biotissues can receive the dose of tens and hundreds of Gy, which significantly exceeds the doses required for therapeutic purposes [24]. However, this dose is a result of the impact of protons with different energies, and, therefore, the action is not local. In the present paper, we will not dwell on the possible optimisation of the angular and energy spectra of the proton beam at the expense of changing the regime of the laser-plasma interaction. Instead, we restrict ourselves to the study of a potential possibility to use the TNSA regime in the laboratory experiments on the impact of beams of accelerated protons on biological objects.

For medical applications, it is essential to have monoenergetic protons that provide the locality of impact; therefore, it is necessary to carry out the separation with respect to energy. The simplest way is to use the magnetic separation, based on transmitting the protons through the region with the constant magnetic field, which was implemented in the present work. A negative factor of using such separation is the essential reduction of the dose, received by the object of study, which was also taken into account.

3. Experimental stand

For affecting the biological objects the laser acceleration stand was equipped with a biomodule for placing the living objects and the equipment for maintaining the vital activity of living cells.

The schematic of the experimental stand for irradiation of biological objects by a beam of accelerated protons is presented in Fig. 2. The laser pulse (the centre wavelength 910 nm, the energy 10 J, the pulse duration about 60 fs) arrived at the vacuum target chamber, where by means of an off-axis parabolic $f/4$ mirror it was focused on the target surface (aluminium foil 10 μm thick) making an angle of 45° with the direction of incident radiation. To measure the energy spectra of the protons we used the radiochromic film diagnostics [23, 25] mounted in the stack placed at a distance of 35 mm behind the target normally to its surface. In the stack, a hole was made with a diameter 3.5 mm, through which the generated beam of protons (after passing through the permanent magnet) was sent onto the substrate with the cell culture. To estimate the parameters of the proton beam passed through the hole we used the dose received by the film in the area adjacent to the hole; to simplify the calculations the angular distribution in the hole was assumed uniform. This is possible since the characteristic angular size of the proton beam in our experiments with the energy below 20 MeV is usually much larger than the size of the hole in the radiochromic plates (see Fig. 1a).

To accelerate the protons at the expense of laser-plasma interaction the experiment should be carried out under the pressure no higher than 10^{-3} Torr, which is incompatible with the survival of the cell culture. Therefore, the proton beam was injected into the special unit suited for providing the cell

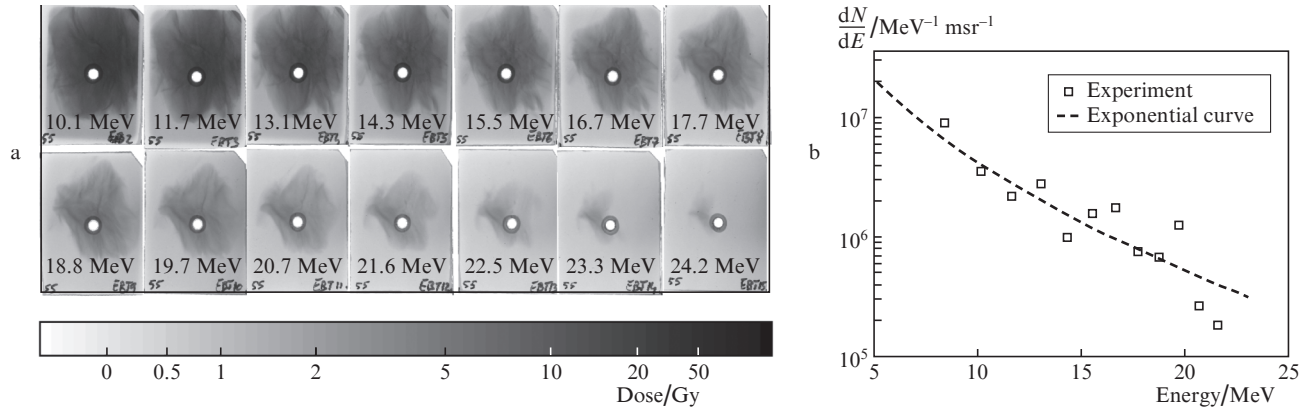

Figure 1. Patterns of dose distributions obtained using radiochromic films (a) as a result of irradiation by TNSA protons and the corresponding energy spectrum (b) for the laser pulse energy 5 J and the target 10 μm thick. The holes in the centre of the films are for passing the protons to the studied object, and the dark ring surrounding them is not related to the dose. The zero level is chosen by the background level at the plates, corresponding to high energy of protons.

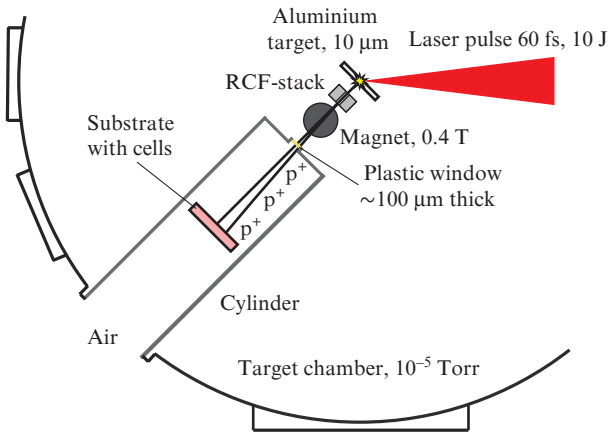

Figure 2. Schematic of the experiment on the impact of laser protons on biological objects.

culture survival and operating under the atmospheric pressure. The proton beam was ejected from the target chamber through the window 15 mm in diameter, closed with the plastic film ~100 μm thick. The unavoidable slight reduction of the proton energy (by 0.2 MeV for the protons with the energy 20 MeV) due to passing through the window was taken into account. The window was installed in the metallic cylinder 0.5 m long, mounted on one of the flanges of the target chamber. This allowed the installation of the substrate with cell culture at the distance not smaller than 0.3 m from the proton source.

The separation with respect to energy of protons was implemented by means of a system of permanent magnets (6 cm, 0.4 T) with the perspective of increasing the magnetic induction to 2.5 T; the system also allowed the differentiation of the proton impact and the impact of X-ray radiation. To control the doses received by the cell culture the RCFs were placed at the front and back side of the substrate.

In the pilot experiments with this geometry, the doses in the region of the multiwell plate attained tens of Gy with slightly expressed separation with respect to energy of protons.

4. Cell culture treatment technique

In further experiments with living cells, we plan to use the cell culture *HeLa Kyoto*, the cervical cancer [26], as the object of study. The culture is chosen because of the simplicity of keeping this type of cells under the laboratory conditions.

We plan to cultivate the cells in the DMEM medium, containing glutamine, 10% of serum and antibiotics, in the atmosphere with 5% CO at the temperature 37 °C. The number of cells per well is nearly 3000. A day after seeding the cell culture is irradiated with the proton beam. Immediately before the shot the nutritive medium is removed from the wells and the substrate is placed into the biomodule vertically, since the proton beam outgoing from the target lies in the horizontal plane. After irradiation, the wells are refilled with the fresh nutritive medium and the multiwell plate is placed into the CO_2 incubator for 24 hours. All manipulations are to be executed in accordance with the aseptic rules. On the next day after the shot, the MTT test is performed to evaluate the number of cells survived in the course of experiment [27]. The wells not subjected to irradiation are used as control ones.

The described procedure is very similar to that widely used in medical studies; however, it requires keeping the appropriate level of cleanness and disinfection in the physical laboratory, as well as installing the equipment for the cell culture treatment.

5. Calculation of the dose received by the cells

The information about the energy spectrum of the accelerated protons, obtained from the interpretation of RCF darkening around the hole, was the basis for evaluating the dose, absorbed by the studied cells. Assuming the angular distribution of protons passed through the hole to be uniform, the trajectories of protons and the dose accepted by the cells were calculated. The losses due to passing through the film closing the window and to the nonuniformity of the magnetic field were taken into account.

Figure 3 presents the calculated doses for the cell monolayer in the biomodule and the experimental proton spectrum (the maximal energy 43.3 MeV [19]), obtained using the PEARL laser. It is well seen that in the module the conditions are implemented that allow the transfer of essential doses to the studied cells by means of laser-accelerated protons, and that the use of the magnetic field allows the separation of the proton impact from that of accompanying electrons and X-ray radiation.

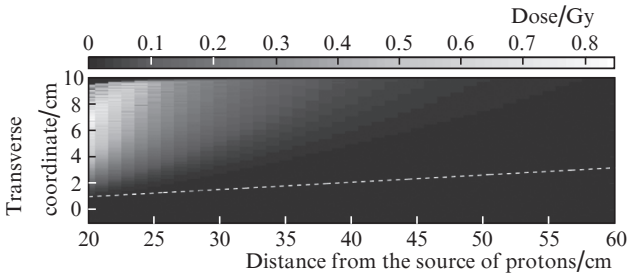

Figure 3. Dose distribution received per shot by a single cell or cell monolayer, placed in the experimental module, for the typical proton spectrum obtained using the magnet with the induction of 2.5 T. The dashed line limits the 'line-of-sight' zone for X-ray radiation.

The possibility to affect deeply lying tissues is demonstrated in Fig. 4 that shows the distribution of doses, received by a thick biological tissue placed at a distance of 30 and 60 cm for the magnetic system with the induction 2.5 and 1.8 T, respectively. One can see that in the topology with one magnet, used in our experiments, the increase in the distance leads to the improvement of depth separation keeping the depth of impact constant; however, it becomes possible at the expense of the dose reduction. At the same time in order to separate X-ray radiation from the particles it is necessary to vary the magnetic field, namely, it should be increased when approaching the source.

6. Discussion of results and possible ways of improving the system

The doses measured directly at the biological culture location, as well as the calculations based on the RCF spectrometer data, have shown that the setup allows the transfer of a dose, sufficient for damaging the studied cells in a single shot. On

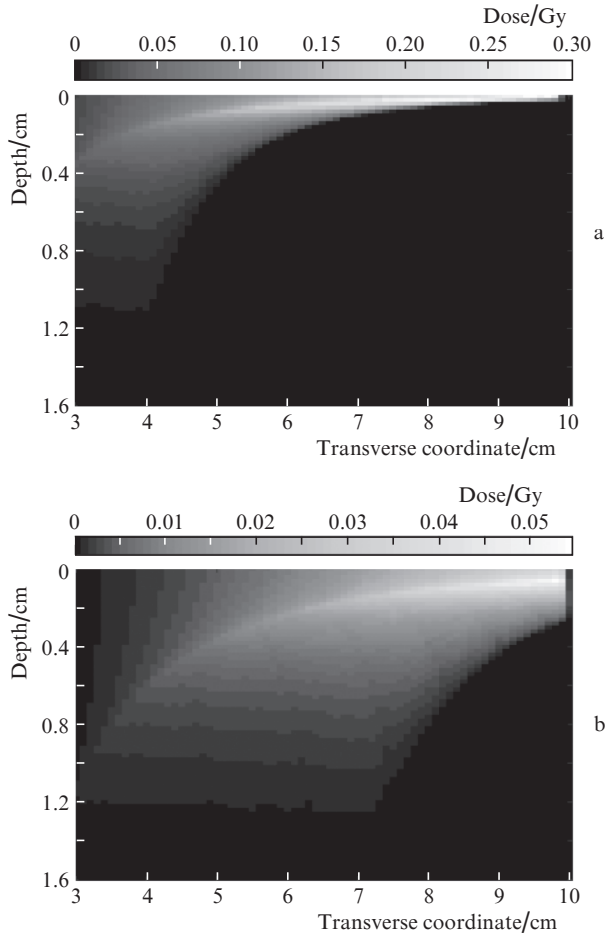

Figure 4. Distribution of doses received by the biotissue layer as a function of the depth and the transverse coordinate in the biomodule for the distance between the source and the object 30 cm and the magnetic field induction 2.5 T (a), and for the distance 60 cm and the magnetic induction 1.8 T (b).

the other hand, from the calculations it follows that the separation with respect to energy (and therefore the impact locality provision) using a single magnet has low efficiency, since it leads to the essential reduction of the dose. For example, as seen from Fig. 4b, for the therapy of a biological tissue at the depth of 1 cm using the present source of protons the accumulation of the impact during a few hundred shots is necessary.

At the same time, the used topology allows efficient separation of protons from X-ray radiation, which makes it possible to perform experiments in monolayers or slices.

Note that the presented work is not a pioneering one. For example, we can mention Ref. [16], where an analogous setup utilising laser-accelerated protons, based on a repetitively pulsed laser system with a pulse energy about 2 J is described. The difference from our case is that the used laser system has a higher power, which potentially allows the accumulation of a necessary dose during a smaller number (a few units) of shots.

In the future, the system can be obviously upgraded by using the magnetic optics for additional focusing of the proton beam, keeping the same separation efficiency. For example, one can use a selector of 'chicane' type [28] or a more complex system of permanent magnets [29].

The focusing fields can be also produced by the laser-plasma interaction. In our opinion, the most promising in the sense of focusing and separation of the proton beam with respect to energy in the TNSA regime are the toroidal magnetic fields arising in the interaction of a nanosecond laser pulse with a solid-state target [30], as well as the radial electric fields initiated by the irradiation of a hollow microcylinder [31]. Such schemes offer the possibility of raising the dose additionally at the expense of the compactness of the energy separator that allows one to place the studied object nearer to the strongly diverging proton beam in the TNSA regime. The the compactness results in the essential complication of the experiment setting.

At the same time, at the PEARL setup a complex of works is in progress aimed at increasing the peak intensity in the interaction region at the expense of increasing the pulse energetics [32, 33], optimisation of its focusing and duration [34]. We expect that the optimisation of laser parameters can essentially increase the maximal energies and the charge of the laser-accelerated proton beam [35].

7. Conclusions

The experimental biological stand is constructed consisting of a laser source of protons based on a PEARL laser system and a biomodule for placing the tested biological cultures with a film 'vacuum–air' interface. The simplest energy separator with a single permanent magnet allowed the separation of the proton impact from the parasitic impact of X-ray radiation and fast electrons. The pilot experiments demonstrated that the studied cultures can receive critical doses of 1–2 Gy per shot, which is enough for conducting experiments with living cells. The technique of performing the experiment with the cell culture *HeLa Kyoto* is developed and approved. It is shown that the topology of the experiment with a single magnet is low efficient, since the achievement of the required separation is accompanied by the essential reduction of the impact dose, which leads to the necessity to accumulate the effect during tens and hundreds of shots. The ways of upgrading the biological module and the energy separator by means of special magnetic optics and optimising the regimes of impact are outlined with the aim of increasing the dose and locality of the impact.

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