X-ray microbeam line for single cells irradiation

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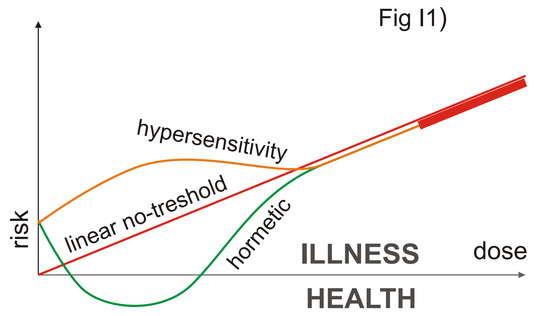
Introduction

Fig I1)



Influence of the ionising radiation on living organisms is well known in the area of high doses [1]. In the area of low doses health consequences are still not-learned. According to the linear extrapolation model, ionising radiation could be harmful in all range of doses, and the probablity of negative health effects reveal a linear dependence on the dose (fig I1). Other hypothesis, known as a radiation hormesis theory [2], propagates that the radiation is unharmful below a treshold dose, or even healthy in the area of small doses. However, influence of low doses of radiation cannot be determined through a "victim" examination, because possible negative consequences (i.e. cancer) are not unique, and moreover could also appear many years after the exposition. The only possibility to assess the risk (or benefit, according to the radiation hormesis theory) is to study the radiation effects at the cellular level, analysing the biological response of cells after irradiation one by one with an exact dose. This microdosimetry research could be realised with a use of a classic wide beam, however the accurate quantitative analysis are possible only with the application of microbeam facilities.

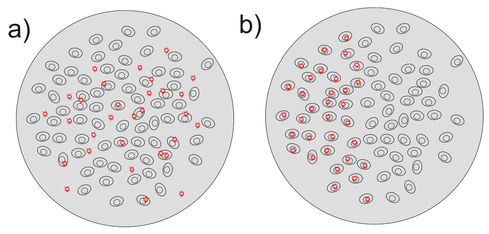
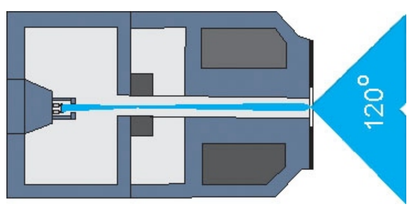
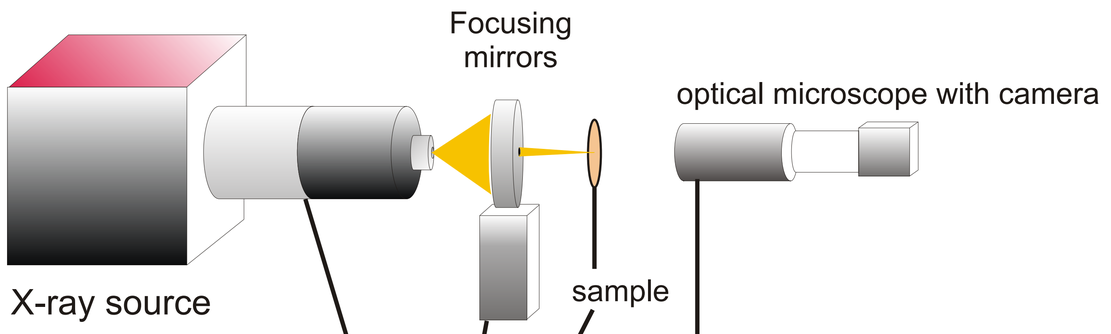


Fig I2) Cells irradiation with a classic source (a) and with the microbeam (b)

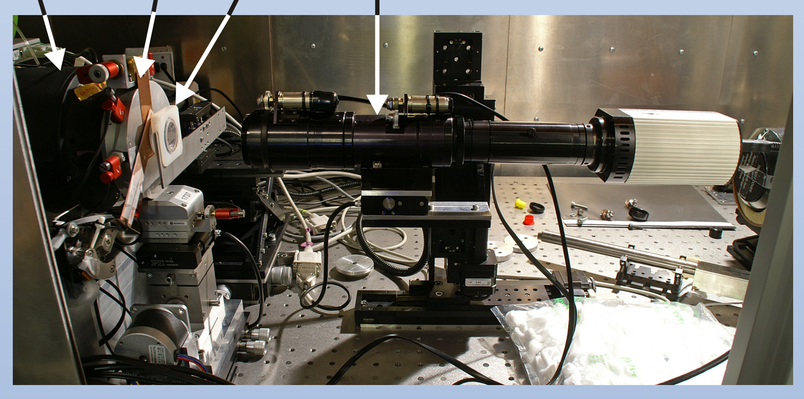
Open type X-ray source with microfocusing



Anode	Titanium	
Accelerating voltage:	20 - 160	kV
Tube current	0 - 200	uA
Target current up to	25	uA
Applied energy (K_α line)	4.5	keV
Source spot diameter	~ 3	um

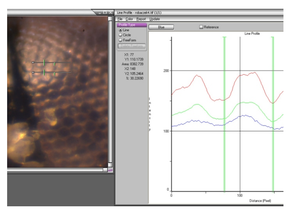


The source is fixed. The focusing mirrors, sample and microscope have 3D movement ability. The focusing distance is 30 mm.



Optical microscope

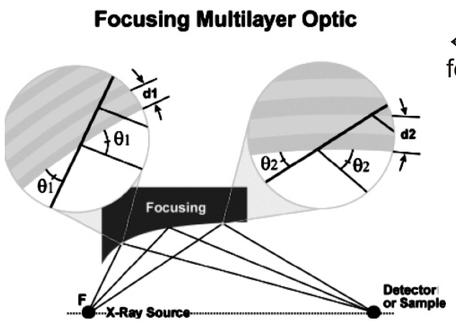
- resolution 900 lp / mm
- motorized zoom and focus
- coaxial light source
- digital camera with the Image Pro software is applied



A worms eye pixel ~22 um in diameter

Focusing mirrors

The principle of work is based on the Bragg constructive interference of the radiation reflected from a multilayer (fig. F1). Two ellipticaly curved multilayer elements are perpendicularly fixed each to the other (fig. F3 a).



← Fig. F1) Multilayer focusing principle [5]

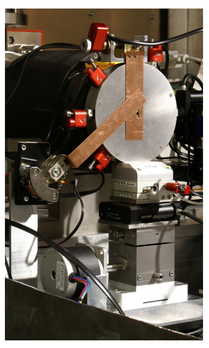


Fig. F2) → Rigaku multilayer mirrors with home made facilitations

An x-ray could go through directly without touching the mirror, which gives the direct beam (a) in the beam image (fig F3 b). Reflection from only one surface gives the single reflected beam (b). Reflection from both surfaces in cascade gives the focal spot (c). Only the focal spot remains during irradiation process, while other elements are apertured. The dose of radiation is controlled with a precise beam shutter (fig F2).

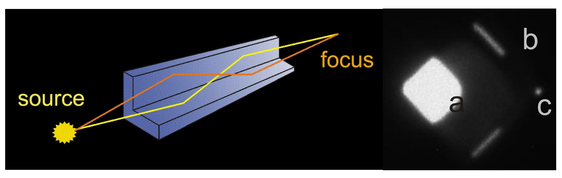
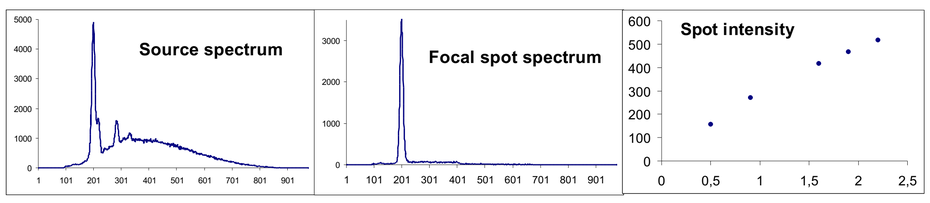


Fig. F3 a)

Fig. F3 b)

a - direct beam
b - single reflected beam
c - double reflected beam (the focal spot)

Due to the Bragg interference law, the radiation reflected from a multilayer is monochromatic, and the two multilayer elements of mirrors are optimized for 4.5 keV Ttanium K_α line. The spot diameter is 20 micrometers less.



Sample

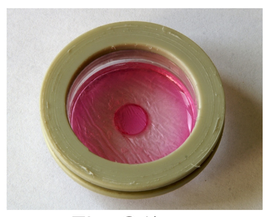


Fig. S1)

The experiments are being performed on cultured cell lines of PC3 human prostate cancer cells and CHO Chinese hamster ovary cells. Cells are seeded on 35 mm diameter Petri dishes with 10 mm round holes in the central part of the bottom (fig. S1). The bottom is covered with a 1.5 μm thick Mylar foil. A population of about 10^5 cells in 4 μl medium is seeded on the central part of the Mylar foil 16–18 hours before the experiments [4]. For the duration of an experiment the medium is removed from the cell dish in order to reduce the paralaxis effect, and the top of the dish is covered with the Mylar foil in order to isolate the cells from enviromental infections.

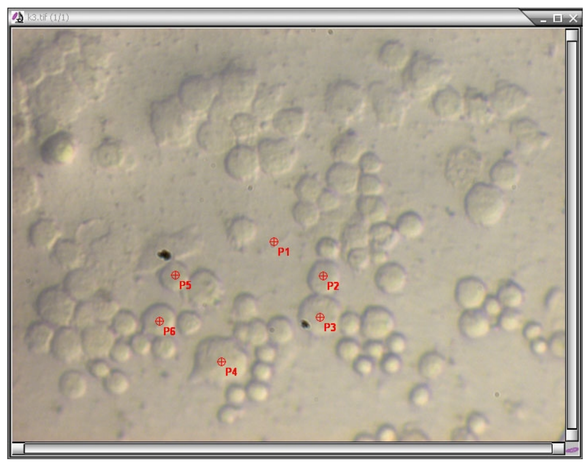


Fig. S2) Cells dedicated for irradiation are marked in the microscope image. Targeted cells are irradiated automatically one by one with an exact dose.

The video on the computer presents the irradiation process

After irradiation, cells are being visualized under a fluorescence microscope (fig. S3, S4). The focused monochromatic beam, delivered into a single cell in an exact period of time, enable precise assessment of the energy deposited in the target, and thus the analysis of a single cell damage as a function of the radiation dose.

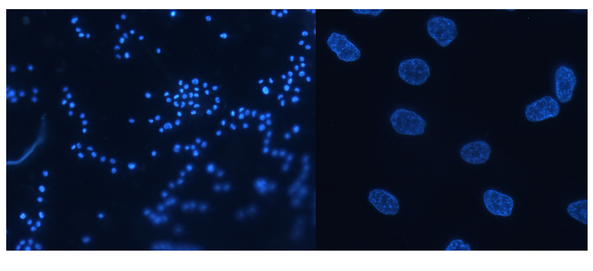


Fig. S3) All cells are visualized with the Hoechst 33342 dye. Hoechst is a bis-benzimidazole derivative compound, which permits through a cell membrane and binds to the minor groove of DNA. Under UV excitation this marker emits blue light. Cell damages caused by irradiation could lead to a micronucleus apperance after cell division. These separated parts of the cell nuclei observed with Hoechst could indicate cell radiation damages.

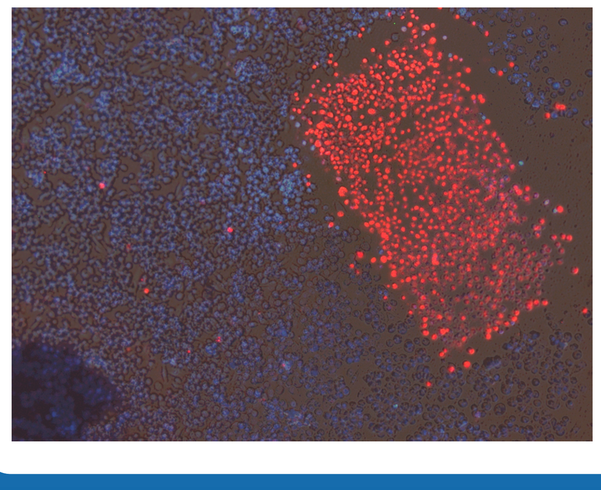


Fig. S4) After the Hoechst application, a propidium iodine marker is added. The propidium iodine also intercalates with the DNA, however this dye is being removed by the live cells, and remains only in necrotic and apoptotic ones. The Image presents a cell population partially irradiated with the proton microbeam at the IFJ PAN. Cells were visualized with Hoechst 33342 and the propidium iodine respectively. Cells situated in an irradiated rectangle area became PI-positive, which means that the applied dose of radiation was lethal for them.

For more information visit our website
http://www.microbeam.eu

Acknowledgements:

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