Cells irradiation complementary lines at the IFJ PAN

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Introduction

Nowadays, ionising radiation has found many applications in medical diagnostics and therapy. During these medical treatments patients are exposed to small doses of radiation. While biological consequences of high radiation doses are well known, effects of small doses are still not-learned. Some researchers are convinced, that any dose of radiation could be harmful to the body, while others believe that small doses of radiation are profitable and healthy (Fig.1). Unfortunately, it is not easy to determine the influence of small doses, because their negative consequences could appear after many years and a simple extrapolation of the risk from high doses region may be not justified. These consequences are unique (e.g. cancer disease) and could be induced by many others factors, as well as the positive effects of radiation if they exist. The only way to solve this problem is to analyse the response of biological systems to the ionising radiation at the cellular level. Such a research is possible with the use of microbeams, where the beam spot size is in the range of micrometers or hundreds of nanometers and enable to irradiate of singule cell by the exactly determined dose of radiation (Fig 2). Furthermore, only microbeams enable to study of bystander effect, where the radiation response is given also by numerous cells located in the neighbourhood of the irradiated one (fig.3).

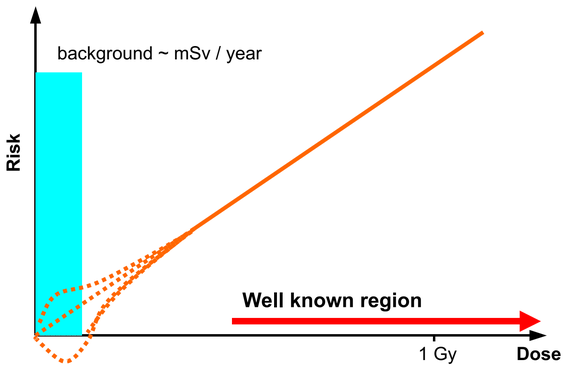


Fig. 1. Risk of negative biological effects vs exposition dose. The continous line represents the high doses linear relation obtained from clinical data (patients from Hiroshima). The dashed curves are hypoteical extrapolations of known data. The lowest curve illustrates the hormesis phenomena.

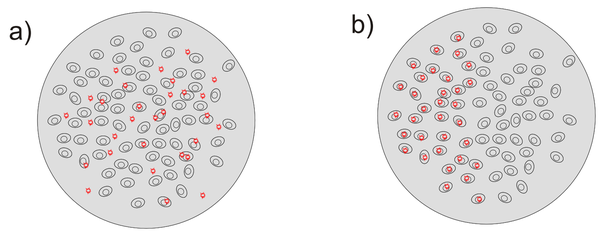


Fig. 2. Irradiation of a cell sample
a) with classical radiation source b) with microbeam

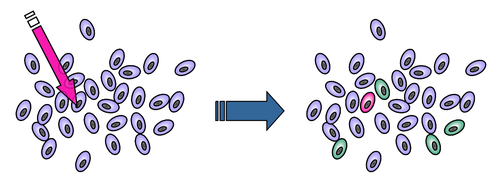


Fig. 3. Bystander effect

	Protons	X-rays
Ionisation density	high	low (photoelectrons)
Mean detph of penetration in cells	100 μm total range for 3 MeV protons	half of intensity at 100 μm for 4.5 keV x-rays

Biological consequences of irradiation depend on the energy and the type of particles. The table presents differences between photon and proton irradiation effects. At the Institute of Nuclear Physics proton cell irradiation facility is being used for over 3 years at the particle (proton and alpha) microprobe. The second irradiation line, situated at the X-ray microprobe, is now at the final stage of construction These two experimental lines will enable complementary cell irradiation studies.

Proton microprobe

Fig. 4. Proton microprobe

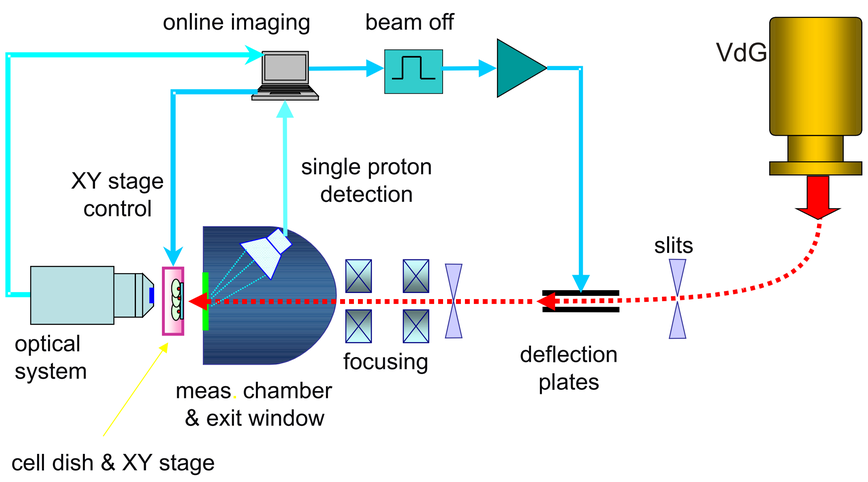


Fig. 5. Van de Graaff accelerator

Fig. 6. Ion beam lines

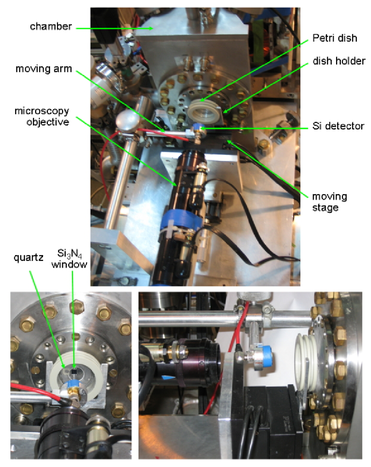


Fig. 7. Irradiation facility

Fig. 7a Fig. 7b

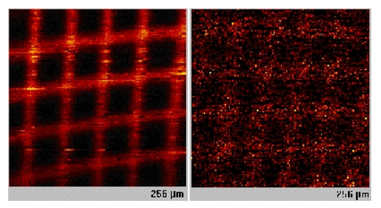
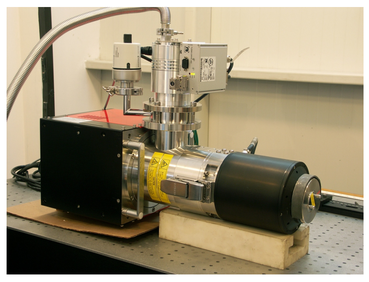


Fig. 8. Fluorescent image of the copper resolution grid imaged by the external beam
a) 200 um distance from the exit window
b) 1 mm distance

VdG accelerating voltage up to 2.5 MV
Accelerated ions: H, He
focusing - down to the size of ~3 μm
beam current 100 pA and more

X-ray microprobe

Fig. 9. Hamamatsu X-ray Source



Source parameters
Voltage range: 20 kV - 160 kV
Target current range: 0 ~ 30 μA
Spot size: 2 μm
Target material: Titanium (4.5 keV)

Fig. 10. X-ray microprobe

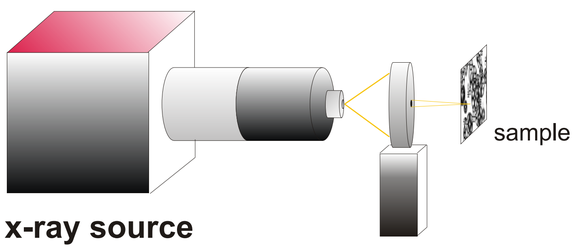


Fig. 13 a

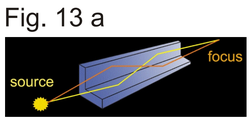


Fig. 13 b) Beam image
Focal spot ~ 8 um

Fig. 13. a) Rigaku multilayer mirrors
a) principle of work
b) x-ray image of the beam

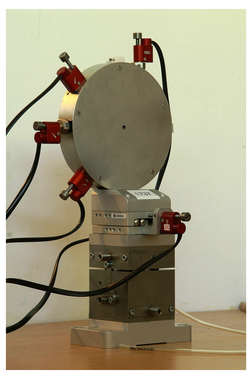


Fig. 14. Rigaku multilayer mirrors

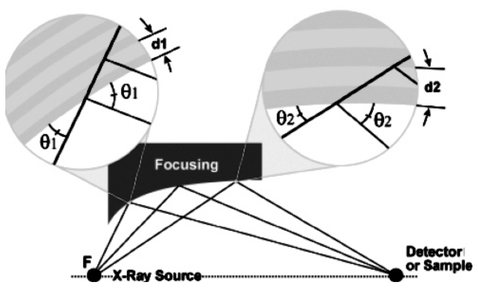


Fig. 15. Multilayer focusing

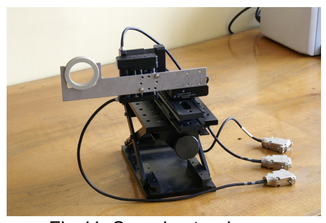


Fig.11. Sample stand

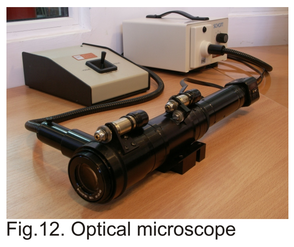


Fig.12. Optical microscope

Quantitative Phase Microscopy (QPM)

Cells targeting procedure requires to make them visible. Thin biological layers are almost transparent for visible light used in the optical microscope. Quantitative Phase Microscopy method generates phase contrast images from conventional amplitude images collected at different sample-to-objective distances. Phase map is calculated from an in-focus and a pair of equidistant de-focused (positive and negative) bright field images.

Fig. 16

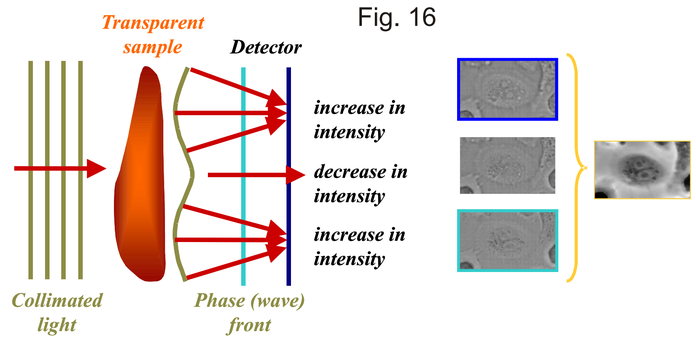
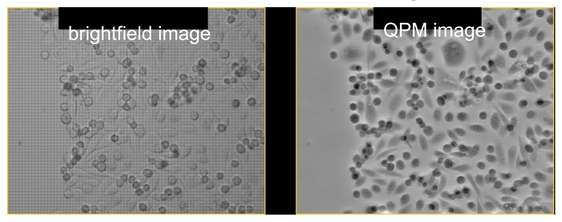


Fig. 17



Cells recognition software

The irradiation process begins automatically due to cells recognition and positioning software created at IFJ PAN. First, the microscope image of the sample (Fig. 18) is being binarized (Fig. 19). Next, with the use of median filter a noise is removed from the binarized image (Fig. 20). The third step is called opening (Fig 22). The opening of a binary image I by a structuring element B can be expressed as the union of all translates of B that fit inside I . It effectively removes any image components which cannot completely `hold' the structuring element.

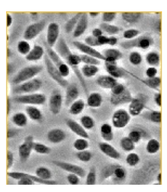


Fig. 18

binarization

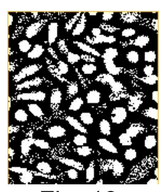


Fig. 19

noise reduction

Fig. 20

opening

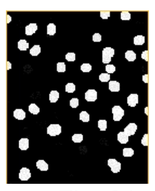


Fig. 21

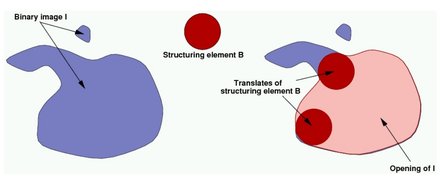


Fig. 22

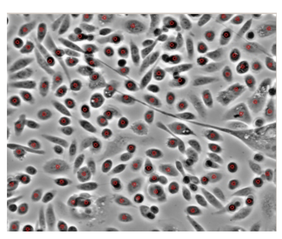


Fig. 23. TARGETS LOCATED