The preliminary studies on PC3 human prostate cancer cells irradiated with the new X-ray microbeam facility

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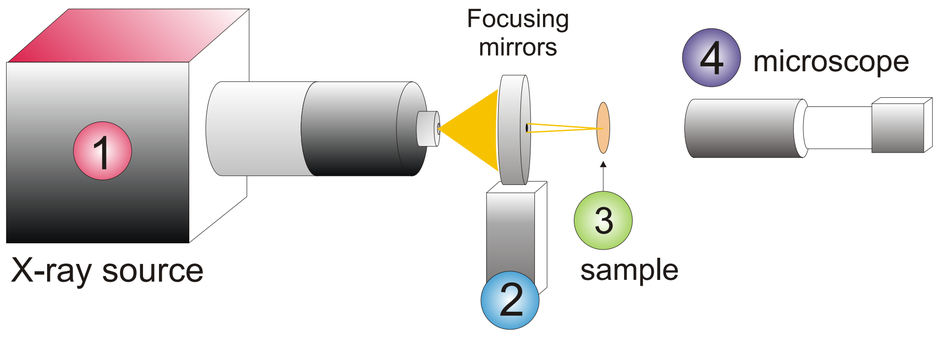
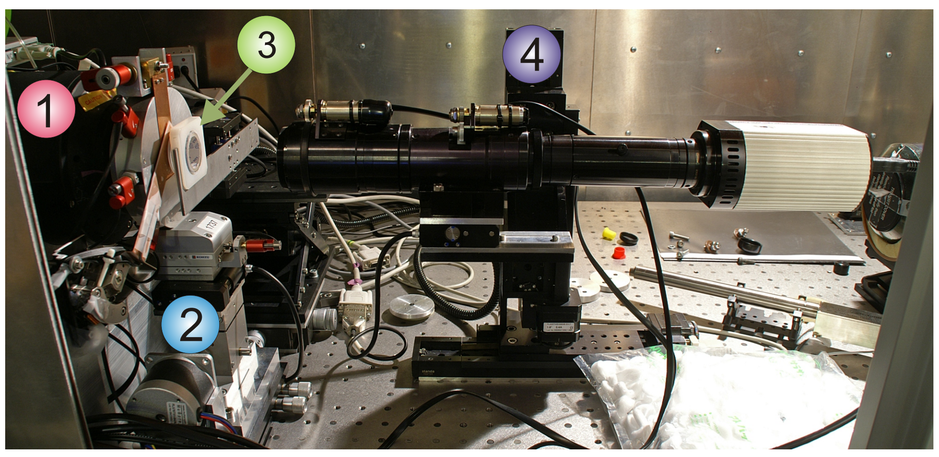
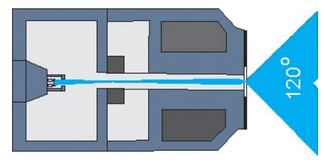
During medical treatments, patients are exposed to small doses of ionising radiation. While biological consequences of high exposition are well known [1], effects of small doses are still not-learned. The negative effects of low doses (i.e. cancer) are not unique, and could appear many years after irradiation. The only possibility to assess the risk (or benefit, according to the radiation hormesis concept [2]) is to analyse the response of biological systems to the ionising radiation at cellular level.

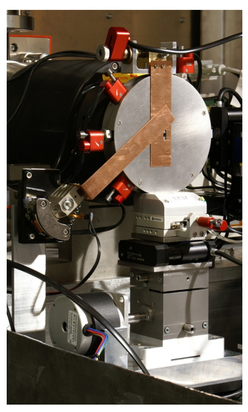




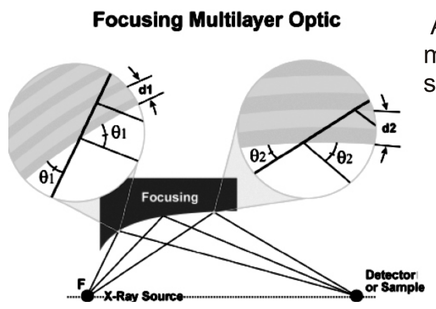
1 The facility is based on an open type X-ray source with microfocusing [3]. The accelerating voltage is in the range of **[20 - 160] kV**, the tube current of **[0 - 200] uA**, and the target current, depending on the voltage, varies from 0 to about **25 uA**. In this experiment a **Titanium** anode of K_α **4.5 keV** characteristic energy is used, and the source spot is about **3 um** in diameter.



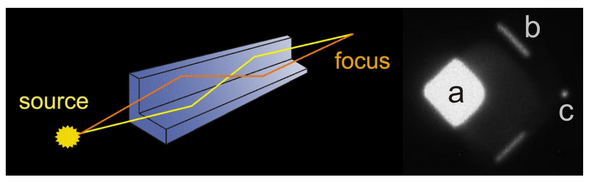
2 The radiation emitted from the source is focused on the sample with a use of the Rigaku multilayer mirrors in the **Kirkpatrick-Baez** arrangement. The Rigaku K-B mirrors are two ellipticaly curved multilayer elements perpendiculary fixed each to the other.

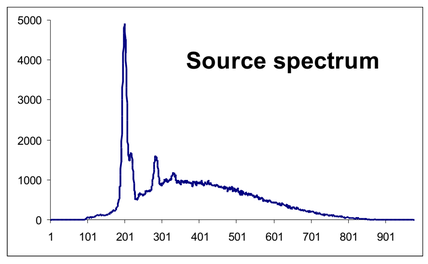


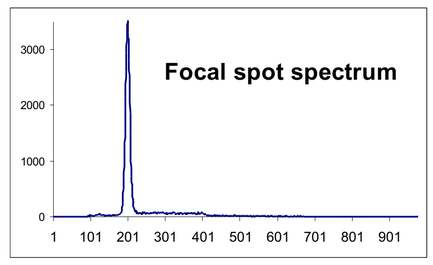
Due to the **Bragg interference law**, the radiation reflected from a multilayer is mono-chromatic, and the two multilayer elements of mirrors are **optimized** for **4.5 keV Ti K_α** line.

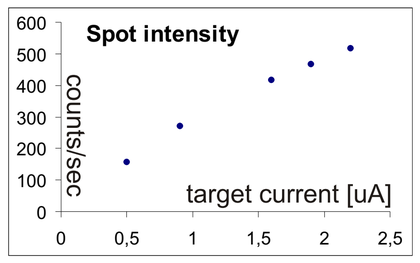


An x-ray could go through directly without touching the mirror (a), reflect from only one surface (b) or from both surfaces, which gives the the focal spot (c).

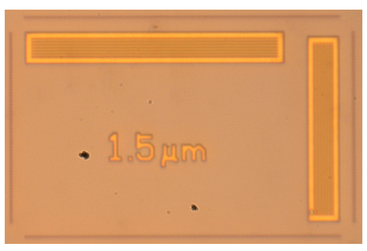


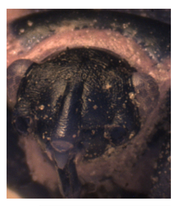


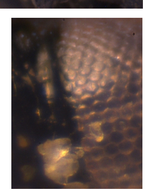




4 The optical microscope equipped with a camera and coaxial light source gives an image with the **resolution of 1.5 um.**







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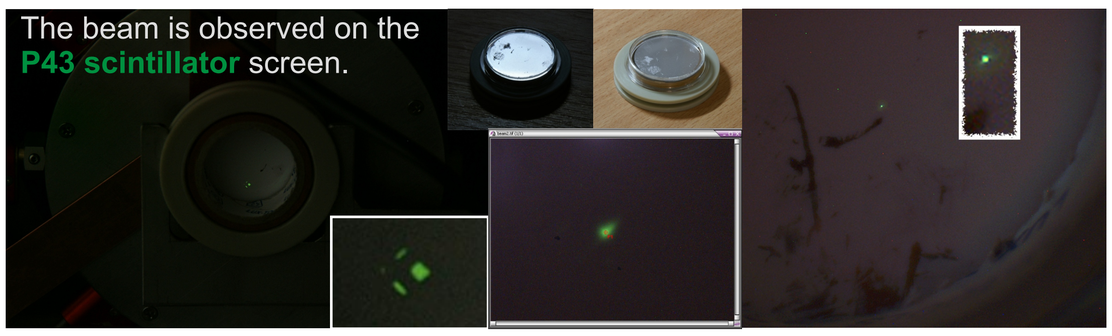
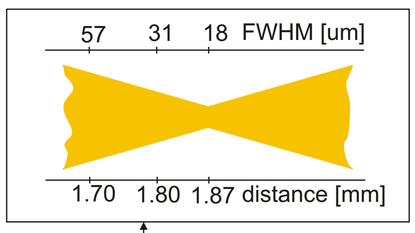
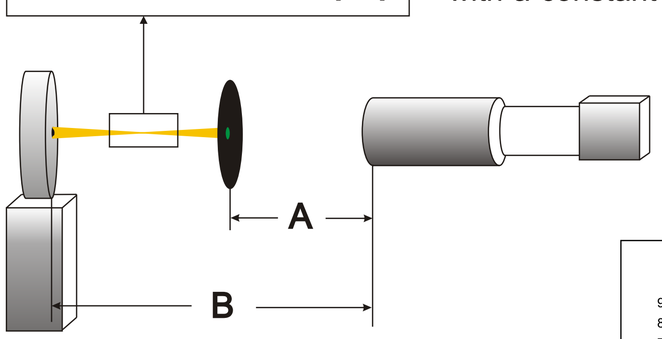
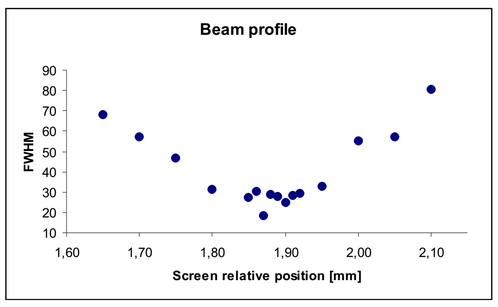
3 Cells are seeded and irradiated on **35 mm** diameter **Petri dishes** with 10 mm round holes in the central part of the bottom. The bottom is covered with the **1.5 µm** thick **Mylar foil**. A population of about **10^5 cells** in **4 µl medium** is seeded on the central part of the Mylar foil **16–18 hours** before the experiments [4].

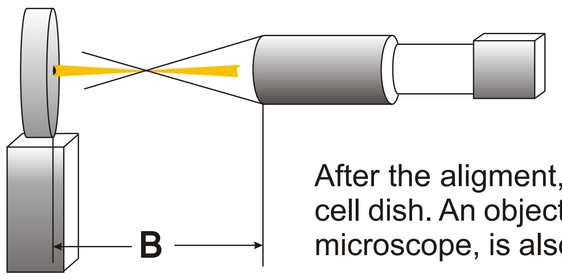




The distance **A** between the microscope and the scintillator is optimized for the highest zoom and magnification. It is done when the scintillator crystal grains are visible sharp. The scintillator and microscope are moving in the beam direction with a constant distance **A** from each other until the smallest beam spot is visible on the scintillator screen. Since then, the distance **B** remains fixed. The beam profile is also obtained with a use of this method.

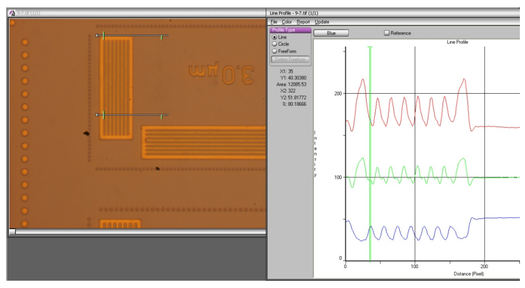


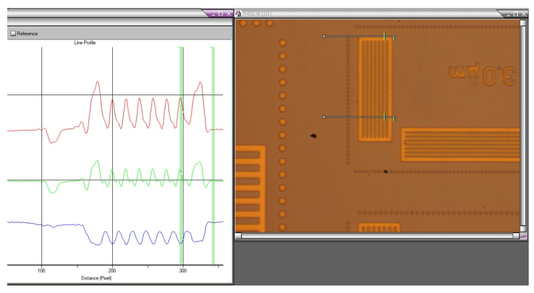




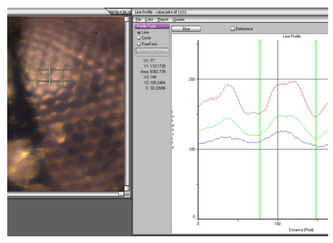
After the aligment, the scintillator could be replaced by the cell dish. An object, which is placed in the focal plane of the microscope, is also situated in the focal plane of the beam.

The microscope is used with maximal zoom and magnification. The **resolution pattern** enable to precisely establish the **micrometer per pixel** calibration ratio, as well as the resolution of sample positioning system.





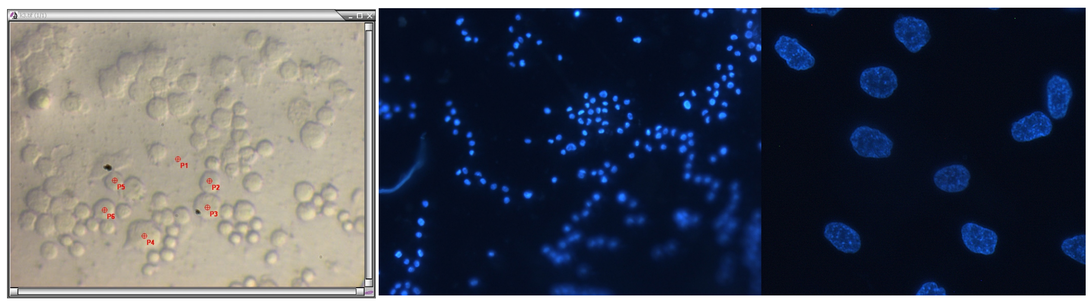
Positioner coordinates [mm]							
9,7	**9,694**	**9,688**	**9,682**	**9,676**	**9,67**	**9,664**	**9,658**
Position of maxima in the pixels readout							
46	63	83	103	121	**141**	**162**	181
66	83	103	122	**141**	**160**	181	199
84	102	121	**142**	**160**	179	200	219
104	121	**141**	**161**	179	199	220	238
123	**141**	**161**	180	198	218	238	257
142	**160**	180	199	218	237	258	277



The worms eye pixel ~22 um in diameter

In the pixels readout, the average distance between maxima is **19,275 ± 0.2 px**. In the resolution pattern the distance between centers of sticks is **6 um**. This gives **0.311 um/px** calibration ratio, and the positioning resolution is **60 nm.**

When a group of cells is chosen, the beam marker P1 is loaded, and the cells allotted to irradiation are also marked. Coordinates of marked cells are exported. Knowing the actual position of the sample positioners, coordinates of the P1 point in the image and the calibration coefficient, it is possible to calculate the positions where the stages have to move in order to target the marked cells. **The video on the computer presents the irradiation process.**



After irradiation, necrotic and apoptotic cells are being visualized under a fluorescence microscope. The focused monochromatic beam, delivered to 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.

ACKNOWLEDGEMENTS

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[2] K. Kant, S.K. Chakarvarti, "*Radiation hormesis: the validity of the linear no-threshold hypothesis*", Int. J. of Low Radiation, 3,1 (2006)
[3] S.Bożek, J.Bielecki, J.Baszak, H.Doruch, R.Hajduk, J.Lekki, Z.Stachura and W.M. Kwiatek, "*X-ray microprobe – A new facility for cell irradiations in Kraków*", Nuclear Instruments and Methods in Physics Research Section B, 267, (2009)
[4] W.M. Kwiatek , M. Podgórczyk, Cz. Paluszkiewicz, A. Balerna , A. Kisiel, "*Sulphur XANES analysis of cultured human prostate cancer cells*" , Acta Phys. Pol. A, 114, 463 (2008).