Construction of a X-Ray Cabinet for Live Cell Experiments*

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Introduction

Visualization of repair processes in living cells is of increasing importance to deduce molecular mechanisms in the cellular response to radiation. Observation of the dynamics of DNA repair factors directly at the beamline at ion induced damage sites provided insight into the regulation of the DNA damage response [1]. X-rays are frequently used as a reference in radiation biology. To compare our results of high LET to sparsely ionizing radiation, we constructed a x-ray cabinet for real time kinetic measurements of cellular radiation responses. Special emphasis was put on radiation safety aspects which were planed and conducted according to legal requirements by GSI radiation protection department.

Description

The new cabinet consists of an Isovolt 160M1/10-55 x-ray tube (GE Sensing + Inspection Technologies). The tube is operated by a voltage up to 35kV at a maximal current of 80 mA. Contribution of soft x-rays is minimized by an additional filtering of 0.5mm Al. At the target position of the microscope a dose rate of around 36 Gy/min can be achieved, which is sufficient for dynamic measurements which normally are performed at a dose below 2 Gy. The shielding of the housing was calculated in the radiation protection department according to these radiation parameters and safety requirements and manufactured in house. The front door of the cabinet is equipped with two independent interlock connectors with immediately power off the x-ray tube if opened accidentally. A blinking lamp on the wall signals operation. The cabinet was approved by the GSI radiation protection department, the TÜV and the radiation protection authority. For cell observation we use the same equipment as for beamline microscopy, which allows a direct comparison of results. The setup was described elsewhere [1]. Briefly it consists of an Olympus IX71 microscope (60x Planapo water NA1.2) with motorized stage and a piezo focussing system. Excitation light is provided using a fast switchable monochromator (Poly V, Till-Photonics). Image detection is done using a sensitive back illuminated EMCCD camera Andor ixon 888. The open cabinet equipped with the microscope and x-ray tube is shown in Fig. 1.

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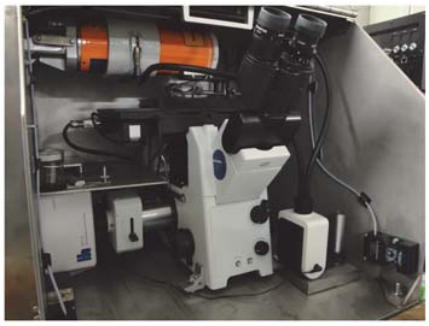

Figure 1: Open Cabinet

First experiments

First experiments were performed measuring the real time recruitment of GFP-tagged 53BP1 in human osteosarcoma cells (U2OS) after irradiation with 2 Gy. Figure 2 (left) shows the formation of radiation-induced foci at the time of 10min postirradiation. In Fig. 2 (right), the relative increase of the fluorescence at sites of double-strand breaks (DSBs) due to the accumulation of 53BP1 is shown.

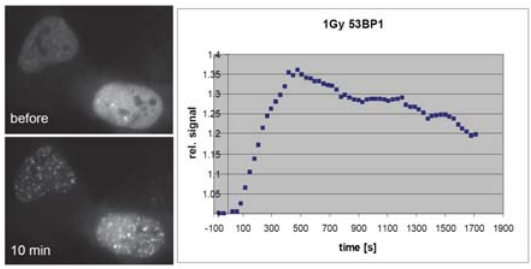

Figure 2: 53BP1 recruitment and corresponding kinetics

Outlook

In addition to conventional broadfiled x-irradiation, the setup will be equipped with micro-collimators made from stacks of GaAs with micro-channels (Microleman) in front of the cell samples to allow for a partial irradiation of cells. This setup tends to mimic the spatial dose distribution of charged particles and facilitates the analysis allowing a direct comparison of irradiated and non-irradiated areas.

References

[1] F. Tobias *et al.*, PLoS One. (2013);8(2):e57953

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