Vol. **109** (2006) *ACTA PHYSICA POLONICA A* No. 3

Proceedings of the XL Zakopane School of Physics, Zakopane 2005

The GSI Heavy Ion Microbeam: A Tool for the Investigation of Cellular Response to High LET Radiations

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Since the mid 1990's, an increasing number of charged particle microbeams have been designed to deliver a preset number of ions to individual living cells with the micron resolution. These tools provide a powerful technique to investigate the cellular response to low doses of radiations. During the last years, the single ion hit facility in operation on the GSI microbeam since 1987 has been upgraded for the irradiation of individual living cells *in vitro*. This setup presents two main peculiarities compared to the microbeams used up to now for cell irradiation. First, the beam's micrometric size is obtained by magnetic focusing and not by a simple collimation. This allows obtaining a smaller beam spot, a better defined linear energy transfer, and a high irradiation throughput. Then, the GSI microbeam is able to focus ions from carbon to uranium with energies between 1.4 MeV/u to 11.4 MeV/u. The range of accessible linear energy transfer is thus considerably extended compared to light ions microbeam in operation today. The design of the GSI microbeam is described, including the beam control, the online cell localisation, the cell dish designed specifically for microbeam irradiation, and the cell irradiation procedures. Experimental tests performed to check the global aiming accuracy as well as the first cellular irradiations are presented.

PACS numbers: 41.75.Ak, 87.50.–a

1. Introduction

Charged particle microbeams, which allow delivering a precise number of ions to individual cells with a micrometer lateral resolution, are now recognised as powerful tools for the investigation of the cellular response to low doses of radiation [1]. Indeed, the high selectivity offered by this irradiation technique is of

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primary importance to investigate novel radiation responses, such as non-targeted effects, that do not fit with the conventional radiation response model based on direct DNA damage [2, 3].

Up to now, these studies have been performed using micrometric ion beams formed by small apertures of micrometer size [4, 5]. During the last years, several groups have reported the development of focused microbeams for cellular irradiation [6–8]. In these setups, the beam is focused down to a few micrometers by means of magnetic lenses. This technique presents several advantages: it reduces considerably the proportion of scattered ions, it offers the possibility of a better defined linear energy transfer (LET), a potentially smaller beam spot can be obtained and a higher aiming accuracy is possible. Additionally, such a microbeam can be deflected rapidly from cell to cell instead of mechanically aligning every individual cell with the stationary beam. Therefore, focused ion microbeams have also the potential of higher throughput. Up to date however, focusing microprobes have demonstrated their superiority only in vacuum, where cells cannot be kept alive. The solution adopted at GSI was to take advantage of the focusing properties of the existing single ion hit facility in operation since 1987 [9]. During the last years, the GSI microbeam has been upgraded to deliver a preset number of heavy ions, in air, on individual cells kept in their nutrient medium. For this purpose, a specific irradiation stage had to be developed to allow the extraction of an exact number of particles in air, the precise localisation of the cells and a precise alignment of the beam with the target. Compared to other micro-irradiation facilities which work mainly with light ions, the GSI microbeam offers new possibilities: ions from carbon to uranium with energies between 1.4 MeV/u and 11.4 MeV/u can be focused and so the range of accessible LET can be considerably extended.

2. Experimental setup

2.1. The microbeam line

The GSI heavy ion microbeam has been described elsewhere [10]. Briefly, the microbeam is situated at the end of the GSI linear accelerator (UNILAC). The ions entering the microbeam line through object slits are focused down to a focal spot of about 500 nm in diameter by means of magnetic quadrupole lenses. Deflecting magnets, situated in front of the focusing lenses, are used to move the beam spot in the focal plane. To ensure the irradiation with a preset number of particles, a fast electrostatic beam switch, situated in front of the object slits, is controlled by the hit detection system.

Since the living cells cannot be placed in vacuum, the beam is extracted at atmospheric pressure through a vacuum window. In our system, a 200 nm thick Si_3N_4 window is used because it presents both the properties to stand the pressure difference between the vacuum of the beam line and the atmospheric pressure and to be thin enough not to degrade too much the lateral resolution of the microbeam.

2.2. Particle counting

To ensure that an exact number of ions can be delivered on the target, a detection system presenting an efficiency close to 100% is required. Considering that the cells are kept in their nutrient medium during irradiation and that the heavy ions have a very limited range in matter, the hit detector has to be placed before the cell chamber. To avoid the degradation of the beam properties by the introduction of an excessive amount of matter before the target, the detection technique is based on the multiplication of the secondary electrons ejected from the vacuum window by a channeltron. In order to improve the amount of secondary electrons generated by every passing ion, the Si_3N_4 window has been covered with a 100 nm thick CsI layer.

Tests of this detection system have shown a detection efficiency of 99.5% in the case of carbon ions.

2.3. The cell chamber

Considering a horizontal microbeam line, a specific chamber that allows keeping the cells attached to a vertical membrane, in their nutrient medium is needed.

The cell chamber developed at GSI is based on a square shaped body made of stainless steel (type 1.4301) that fits exactly into the high resolution $x{-}y$ stage used to position the target with a sufficient accuracy. This chamber presents an 8 mm diameter round aperture in the centre. On the beam side, this aperture is closed by a 4 μm thick polypropylene foil on which the cells attach. The foil is glued and stretched onto the chamber body by means of candle wax. This material presents the interesting advantage to be non-toxic for the cells and easy to remove when the chambers have to be reused. The back side of the aperture is closed with a 200 μm thick cover glass pressed onto the steel body with a silicone-rubber O-ring to reduce the loss of liquid. An additional volume of liquid was provided above the round chamber to compensate for the loss of liquid, and a small air-leak has been added to equalise the pressure between inside and outside.

All materials used have been tested for biocompatibility by checking cell growth compared to standard culture conditions.

2.4. Cell localisation and sample positioning

During the irradiation procedure, the cells are visualised through the layer of medium using an epi-fluorescence microscope coupled to a high resolution CCD camera (12 bit PixelFly). According to the biological experiment, sub-cellular compartments are stained using fluorescent dyes and then can be easily distinguished from the background. The illumination is provided by a 50 W mercury arc lamp equipped with a heat-blocking filter. Additional filters can be inserted under remote control, which let pass only UV-light for cell imaging or visible light for focusing the microscope without damaging the cells. To minimise the UV dose delivered to the cells during the imaging process, a fast mechanical light shutter allows illuminating the cells only for the time necessary for the CCD camera to

collect a fluorescence image. The images taken from the CCD camera are transmitted to a personal computer where every cell is recognized using automatic image processing routines (Image-Pro Plus, Media Cybernetics®).

To bring the cells within the deflection range of the microbeam, a high precision $x-y$ stage has been developed. This stage is driven by DC-motors coupled to 0.25 mm pitch micrometer screws. The position is measured by an optical sensor (0.1 μm accuracy) and a given position can be set reproducibly within a few tenths of a micrometer.

3. Position calibration

To achieve the alignment of the beam focus with a target of interest, a calibration transforming its coordinates in the microscope image into deflection voltages is required. For this purpose, a thin scintillator, which can be placed on the sample holder, is used to visualise the microbeam spot directly with the microscope. By using an exposure time of 10 seconds, the beam spot can be easily localised in the microscope image frame. It is then possible to take a set of 3 beam spot positions and their corresponding deflection voltages in order to find the deflection voltage for any other coordinate by interpolation.

4. Experimental validation of the system

Two methods were used to check that the micro-irradiation facility is able to target individual cells with a sufficient accuracy.

The first method is based on the irradiation of pre-etched ion tracks randomly positioned on a CR39 nuclear track detector. The result is shown in Fig. 1. The

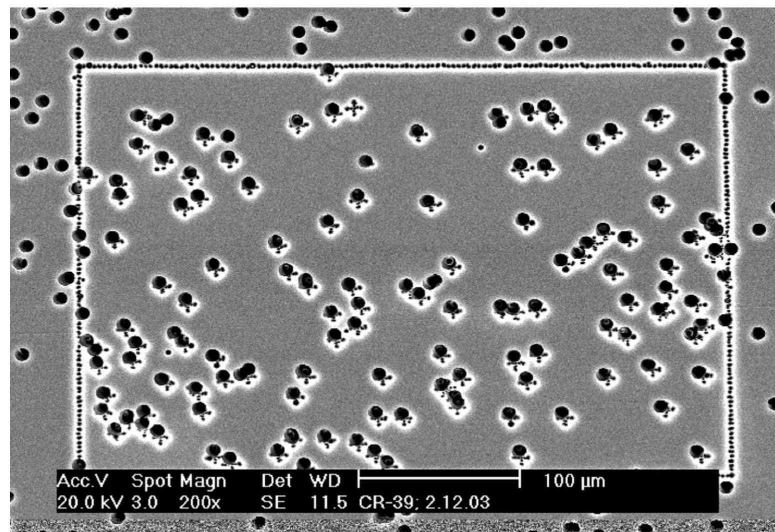

Fig. 1. CR39 sample with pre-etched tracks (large holes) irradiated with a cross pattern of single C ions. Ideally, the crosses should be centred on the pre-etched tracks. The frame written by ions indicates the field of view of the microscope.

slight shift between the expected positions and the actual irradiated positions is due to a slight bending of the CR39 foil leading to a distortion of the optical path. Nevertheless, this demonstrates that every target inside the field of view can be recognised and that the deflexion system allows reaching any position in the field without significant distortion.

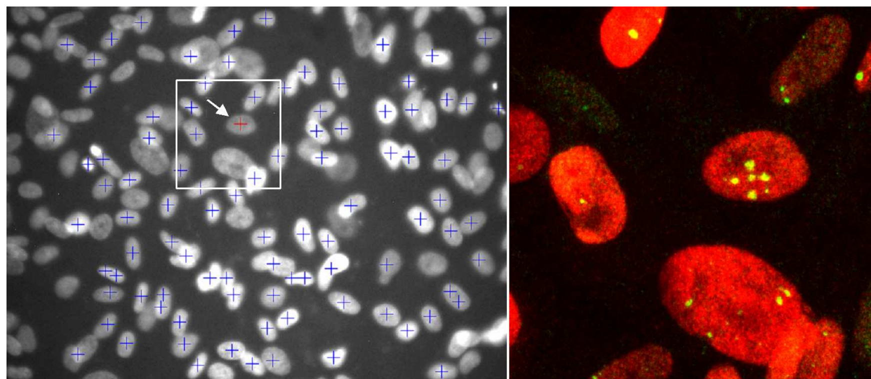

Fig. 2. Left: stained cell nuclei automatically recognised by computer are marked by crosses. Only the cell designated by the arrow is actually irradiated. Right: cell irradiated with a cross pattern after fixation and immuno-staining for 53BP (bright spots) and DNA counterstaining (cell nucleus). 5 ions/position, distance between the spots: 3 μm; 4.8 MeV/u carbon ions.

Another method consists in visualising the hits directly in the cell nucleus by means of an immuno-staining technique which reveals various proteins associated with DNA damage [11]. The example shown in Fig. 2 clearly demonstrates the ability of the GSI microbeam to automatically recognise, localise, and irradiate living cells in their nutrient medium with a counted number of particles. Using this technique it was possible to estimate the global accuracy of the irradiation process by measuring the mean distance between the cell nucleus centre and the centre of the cross pattern appearing in the nucleus after irradiation. This was found to be 1.3 ± 0.7 μm.

Acknowledgments

This work was partially supported by the EU grant CELLION MRTN-CT-2003-503923.

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