# Cell irradiation with laser-driven proton and carbon ions

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## Introduction

The viability of using protons and heavier ions as an alternative in radiotherapy has received increasing interest due to their superior dose-depth profile. Presently, several clinics exist worldwide to treat cancer patients with protons or carbon ions from synchrotrons, cyclotrons and linac accelerators, providing excellent clinical results. However, to date only a limited number of patients have been treated and this is due to the huge cost involved in building/running of current hadrontherapy clinics [1].

Laser-driven ion sources have been proposed as a way of overcoming this cost-limiting factor [2-8]. Since advancements in laser technologies have led to the realization that protons exceeding 50 MeV can be achieved experimentally, this has led to a concentrated effort to demonstrate ion beam parameters required for therapeutic application. In parallel, several groups have begun preliminary work on the methodology and viability of using laser-driven ion sources. This has partly focused on establishing procedures for cell handling, irradiation and dosimetry compatible with a laser-plasma environment.

The most significant difference between laser-driven ion and conventional ion sources is that laser-driven ion sources emit ultra-short ion pulses of picosecond duration in contrast to quasi-continuous beams from conventional accelerators. When irradiating cell media, this leads to an ultra-high dose deposition rate exceeding what currently is used in therapy by up to 9 orders of magnitude [9]. Interestingly, these deposition time scales are within the same time frame as most chemical processes (10ps to 50 us) occurring post-irradiation. It has been suggested that this may have an adverse effect due to local oxygen depletion [10] or lack of interaction between direct and indirect mechanisms [11]. Alternatively, Fourkal *et al*. suggested collective effects instead could arise from spatio-temporal overlap of independent tracks [12]. Essentially, the effects of using these ultra-high dose rates are virtually unknown and need to be carefully assessed prior to any therapeutic application.

Preliminary investigations into the biological effectiveness of laser-driven ions were carried out successfully at QUB's TARANIS laser, employing 2-10 MeV protons in single-shot studies with dose rates $>10^9 Gys^{-1}$ irradiating V79 Chinese hamster cells [9]. The experimental set-up used at QUB was utilized recently in an experiment carried out at the Astra-Gemini laser facility, which we report here. The main aim of the investigations carried out here was to establish a procedure for irradiating cells at the facility and to demonstrate ultra-high dose rate irradiation of human cells with proton and carbon ions, the two species of most current therapeutic relevance.

## Experimental Arrangement

A single beam of the Astra-Gemini of central wavelength $\lambda_L$=0.8 µm, energy on target $E_L$ ~ 6J and a duration $\tau_L$=45 fs was reflected off a double plasma mirror to provide a reduction of ASE contrast below $10^{-10}$ of peak intensity. An $90^o$ off-axis parabolic mirror (f/2) was used to focus the laser pulse of linear polarisation at normal incidence onto 25 nm amorphous carbon targets, which was chosen so that volumetric acceleration of carbon ions could be achieved, leading to intensity on target of $3x10^{20}$ W/cm$^2$. The ions were spatially selected with a large rectangular aperture slit of (900x400 µm$^2$) which was located behind the target. A dipole magnet (100 mm long, with maximum strength of 0.9 T) placed behind the aperture, was used to disperse the ions according to their energies prior to cell irradiation. The proton and carbon ions exited the vacuum chamber through a 50 µm Kapton window and irradiated the human fibroblast cells (AG01522) in air. The cell dish containing a thin monolayer of cells was positioned in a vertical position and due to the prior energy dispersion, a large region of cells in the cell dish were irradiated in a single shot with the full spectrum of protons and carbon ions.

The dosimetry was carried out by placing normal and customized (overlaminate and adhesive layers preceding active layer were not present) EBT2 Gafrchromic film directly behind the cell dish. The customized Gafrchromic film was placed on top of the normal film in the region exposed to carbon ions, in order to achieve an accurate dose measurement. The ion energies irradiating the cells were chosen in such a way to avoid Bragg Peak deposition in the cell layer. This allowed obtaining a measurement of the transmitted dose and inferring dose deposited on cells.

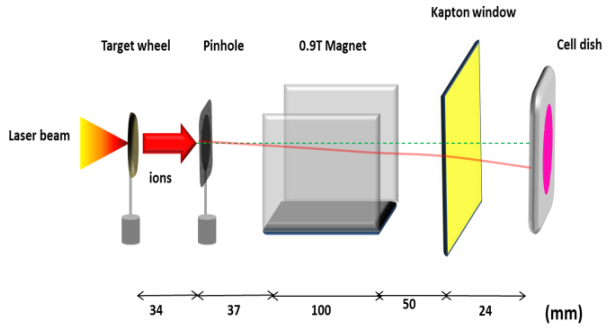


**Fig. 1: Schematic of the experimental layout**

Full characterization of the ion beam was carried out with conventional techniques (Thomson parabola spectrometer, RCF/CR39 stacks) prior to the cell irradiation campaign to ensure optimal conditions. A typical carbon-ion spectrum obtained by this procedure is shown in Fig 2.

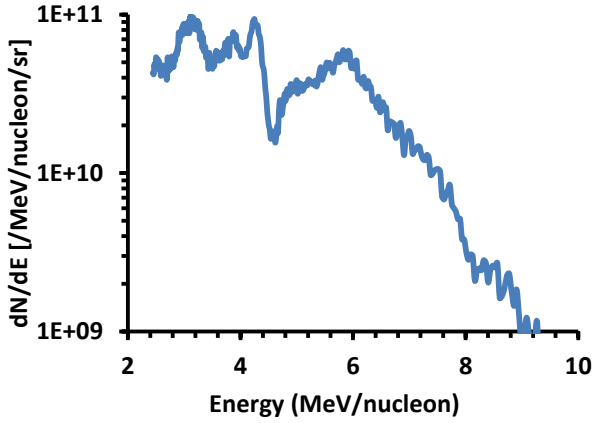


**Fig. 2: Typical carbon-ion (C6+) spectra from 25 nm amorphous carbon target measured using image plate with thomson parabola set-up, prior to cell campaign.**

## Cell Irradiation

The simultaneous proton and carbon ion irradiation was performed on AGO1522 cells (obtained from the Coriell cell repository). The AG01522 cells were irradiated in a single shot with doses of ~ 1-2 Gy for protons and ~ 1-3 Gy for carbon ions with dose rates ~ $10^{10}$ $Gys^{-1}$ and ~ $10^{9}$ $Gys^{-1}$ respectively. The AG01522 cells were cultivated in filtered alpha-MEM medium (Lonza, UK) supplemented with 20 % Fetal Bovine serum (FBS) and 1 % penicillin/streptomycin (GIBCO, Invitrogen, UK). The cells were kept in a tissue culture incubator with a 95% air, 5% $C0_2$ and 37° atmosphere. All work prior and proceeding irradiation was carried out either in a laminar flow cabinet or biosafety hood to avoid any contamination.
The cells were seeded 24 hours before irradiation on 3 µm mylar in the bottom of a customized cell dish. Prior to irradiation the cell dish was sealed with another layer of 3 µm sterile mylar film, after which culture medium was pumped into the cell dish to fill it completely. To prevent cell damage/death through drying out, cell culture medium remained in the cell dish until moments before irradiation. The cell culture medium was then drained by an automated pump and filled back directly after irradiation. The irradiated dishes were left in the incubator for a determined time after which they were fixed with chilled solution of methanol and acetone (1:1). Fixation of cells allowed us to capture the state of the cell at that moment of time and to measure directly the double strand break (DSB) formation and repair over a 24 hour period.

## Biological Analysis

DSB formation was detected by means of fluorescent antibodies against the active form of protein 53BP1. 53BP1 localises at DSB sites and promotes non-homologous end-joining [13]. 53BP1 provided visual and quantitative data that was recorded by imaging (Zeiss Axio Imager Z2 microscope with Isis and Metasystem Isis software) the cells in a selected horizontal line taken across the irradiated area. Fig. 3 shows the 53BP1 foci formed within cells at different positions along the horizontal line corresponding to irradiation from proton and carbon ion spectra. Imaging through this method allowed for the optical density to be matched with the corresponding average foci/ per cell for that area, as seen in Fig. 4, and allowed us to further target regions of specific energy/ion in order to consider the biological effectiveness of particles with low linear energy transfer (LET) compared to high LET particles. For statistical robustness of data a minimum of 50 cells were counted per energy point selected.

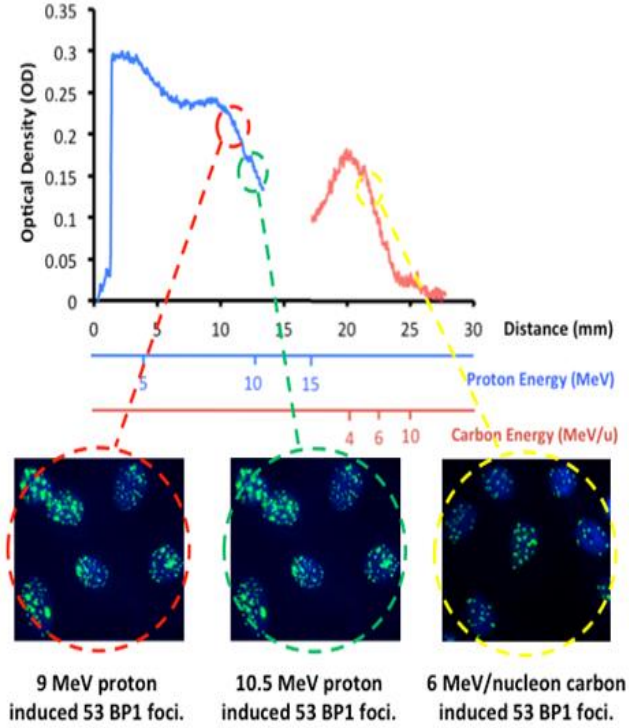


**Fig.3: Typical optical density profile obtained from radiochromic film placed directly behind the cell dish. Corresponding snapshots of typical nuclear foci observed in the regions of 9, 10.5 MeV protons and 6 MeV/nucleon carbon ions is shown.**

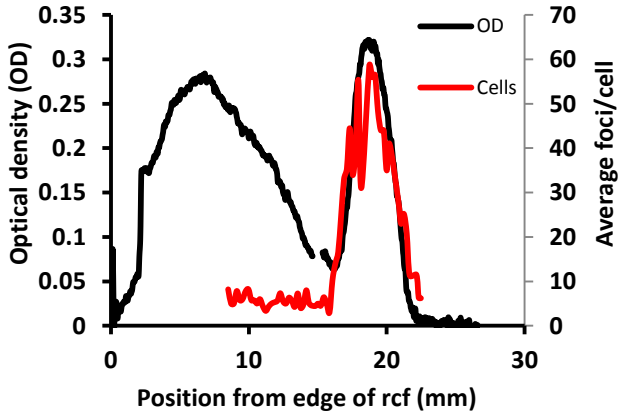


**Fig. 4: The relation between optical density and average foci per cell. The selected data represents the repair of foci irradiated by proton after 24 hours compared to residual foci from carbon-ion irradiation. This highlights the effects of high LET radiation compared to that of low LET.**

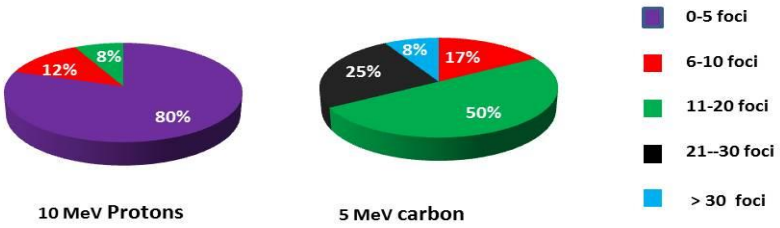


**Fig. 5: Pie charts represent the foci damage remaining at 24 hours post-irradiation.**

The data obtained has highlighted that the quality of damage caused by carbon ions is significantly different from protons. Fig. 4 shows the number of 53BP1 foci remaining 24 hours post-irradiation and highlights the complexity in DSBs formed from carbon ion irradiation. If we consider this further, considering a specific energy, 5 MeV carbon ions with LET ~ 276KeV/µm shows significant residual foci remaining at 24 hours with 83 % cells containing >10 foci. In contrast, lower LET radiation i.e.10 MeV protons (LET ~ 4.6 keV/µm) which show the majority of cells having less than 5 foci, as represented in Fig 5.

## Conclusion

The implemented set-up allowed us to achieve irradiation by laser-driven proton and carbon-ions successfully on AG01522 cells. This is the first reported experiment to be carried out using laser-driven carbon ions to date.
Proton and carbon-ion irradiation with a range of energies were used to obtain temporal kinetics for 53BP1 foci over a 24 hour period. We are able to show the effects of high LET carbon-ion irradiation with high number residual foci remaining after a 24 hour period compared to protons (low LET), which almost fully repaired. The residual foci for carbon are the result of more complex double strand breaks dominated by the direct effect, unlike that of proton data. Preliminary results are promising and pave the way for future investigations utilising laser-driven carbon ions with ultra-high dose rates in in-vitro studies.

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