MICROBEAMWORKSHOP2013

BORDEAUX, FRANCE
OCTOBER 3RD & 4TH 2013

MICROBEAM PROBES OF CELLULAR RADIATION RESPONSE

ABSTRACT BOOKLET

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Editorial

Welcome in Bordeaux for the 11th edition of the Workshop on microbeam probes of cellular radiation response. This workshop, held regularly since 1993, brings together groups interested in developing and applying micro-irradiation techniques using ionizing radiation to study cell and tissue damage.

Besides the usual topics covered by the workshop, we introduce a new topic: radiobiology of laser accelerated particle. Although not strictly microbeams, this emerging field is developing quickly and raises new questions on radiation-induced damage at very high dose rates. Microbeams can be very useful tools to address these questions and we hope that gathering scientists from the different communities can lead to interesting exchanges of scientific ideas.

The organization of this meeting and its orientation towards laser accelerated particles takes advantage of the strong scientific activity around lasers in the Bordeaux region. We would like to specifically thank the continuous support from interdisciplinary research program IOPRA (Interface Optique-Physique-Radiothérapie en Aquitaine) launched by the region Aquitaine and gathering groups working at the interface of laser physics, radiation physics, modelling and radiation therapy.

We wish to all of you fruitful scientific discussions and a very pleasant stay in Bordeaux.

Philippe Barberet,
Chair of the workshop

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SCIENTIFIC PROGRAM

Thursday, October 3rd, 2013

8.00	Registration	
8.45	**Welcome**	
Session I	**Instrumentation of ion & x-ray microbeams**	
9.00	RECENT PROGRESS OF THE INSTITUTE OF MODERN PHYSICS HIGH ENERGY INTERDISCIPLINARY MICROBEAM *G. Du, Institute of Modern Physics, Chinese Academy of Sciences*	O1
9.20	RECENT PROGRESS IN DEVELOPMENT OF HEAVY-ION MICROBEAM SYSTEMS OF JAEA-TAKASAKI *T. Funayama, Microbeam Radiation Biology Group, JAEA*	O2
9:40	SUPER RESOLUTION MICROSCOPY AT THE COLUMBIA MICROBEAM *A.D. Harken, RARAF, Colombia University*	O3
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11.20	HIGH ENERGY ION ACCELERATION AND NEUTRON PRODUCTION USING RELATIVISTIC TRANSPARENCY IN SOLIDS *S. Busold, TU Darmstadt*	O6
11.40	PRODUCTION OF NARROW BANDWIDTH LASER-ACCELERATED PROTON BEAMS BY PASSIVE ENERGY SELECTION *R. Riquier, CEA DAM, Ecole Polytechnique*	O7
12.00	HOW TO CHARACTERIZE 10^{12} ENERGETIC PARTICLES IN A SUB-NANOSECOND BUNCH? *M. Tarisien, CENBG, University of Bordeaux*	O8
12.20	HIGH PEAK POWER LASER DEVELOPMENT FOR ION ACCELERATION *Amplitude Technologies*	
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14.00	**Poster walk & free discussion**	

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16.00	DNA REPAIR DYNAMICS FOLLOWING ION IRRADIATION AT ULTRAHIGH DOSE RATES FROM LASER-DRIVEN ACCELERATORS *F. Hanton, Queen's University Belfast*	O10
16.20	THE APPLICATION OF PARTICLE BEAMS FROM A LASER PLASMA WAKEFIELD ACCELERATOR TO CANCER THERAPY *A. Sorensen, SIPBS, University of Strathclyde*	O11
16.40	DOES THE RBE CHANGE FOR PROTONS WHEN USING LASER ACCELERATORS FOR TUMOR THERAPY? *G. Dollinger, Universität der Bundeswehr, München*	O12
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(Chair: Karen Kirkby)

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8.50	MONTE CARLO MODELING OF RADIATION EFFECTS FROM FOCUSED LOW LET PROTONS AND SINGLE CARBON IONS *W. Friedland, Helmholtz Zentrum München*	O14
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9.30	NANOPARTICLE DOSE ENHANCEMENT USING X-RAYS AND PROTONS *J.C.G Jeynes, Surrey Ion Beam Center*	O16
9:50	NANODOSIMETRIC STUDY OF MICROBEAM RADIATION THERAPY *A.L. McNamara, Institute of Medical Physics, University of Sydney*	O17
10.10	RESPONSE OF HUMAN NORMAL FIBROBLASTS TO PROTON IRRADIATION *A. Michaelidesová, Nuclear Physics Institute, ASCR, Prague*	O18
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S. Girst, Universität der Bundeswehr, München

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G. Gonon, Institut de Radioprotection et de Sûreté Nucléaire, France

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M. Merchant, Surrey Ion Beam Center

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A. Testa, Technical Unit of Radiation Biology and Human Health, ENEA

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S. Bourret, CENBG, University of Bordeaux

17.10 Concluding Remarks and Closing

20.00 Gala Dinner

Saturday, October 5th, 2013

9.00 **Visit of the AIFIRA facility**
12.00

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O1. RECENT PROGRESS OF THE INSTITUTE OF MODERN PHYSICS HIGH ENERGY INTERDISCIPLINARY MICROBEAM

Du G., Guo J., Wu R., Guo N., Liu W.

Institute of Modern Physics, Chinese Academy of Sciences

To study the radiation effect of cosmic heavy ions of low fluxes in electronics and living samples, a focusing heavy ion microbeam facility, for ions with energies of several MeV/u up to 100MeV/u, was constructed in the Institute of Modern Physics (IMP) of CAS. This facility has a vertical design and an endstation for both in-vacuum analysis and in-air irradiation, where the beam irradiated from the top down. GeV heavy ions have been successfully focused to 3×5 μm^2 spot at this interdisciplinary microbeam facility in 2011 [1].

To perform targeted cell irradiation and spatial analysis, a compact data acquisition and beam control system was developed at the microbeam facility recently. The system is composed of a PXI chassis, a PXIe-8108 controller, a PXI-6115 multifunction DAQ card from National Instruments, and in-house software called Cellular Exposure and Nuclear Application (CENA) was developed to integrate these modules together. The experimental system is currently capable of energy spectrum measurement, single ion hit/ion counting, raster scanning, arbitrary pattern irradiation and spectrum mapping [2].

Two 20A-20V operational power supplies (BOP20-20ML Kepco Inc, USA) were used for the scanning magnets, which can provide scanning current with a typical ripple of 0.01% amplitude. Experiments using 25 MeV/u Kr^{26+} beam (magnetic rigidity ~2.4 Tm) has demonstrated that it was able to raster-irradiate sample within 1 mm^2 square area. The beam switch could deflect away the beam in about 1 microsecond when the desired number of ions was detected [2]. Beam irradiation test at the IMP microbeam has also shown that vacuum window using Mylar foil of 8 μm coated with aluminium could be irradiated to break by focused carbon beam (7 MeV/u), while Kapton films of 7.5 and 12 μm coated with aluminium were radiated resistant and could survive several beamtimes.

Presently the high energy ions were detected using the PMT and silicon detector installed on the objective seat, where the ions are detected after traverse of the target. To detect ions in front of the target, a Channeltron detector (waiting for beam test) is installed in the vacuum nozzle to detect single ions using secondary electrons produced at the gold coated Kapton foil.

In addition, the vertical part of the microbeam facility has been reconstructed with frames for fine beamline alignment. A cell lab has been constructed close to the microbeam end-station for sample preparation and cell culture and is in routine operation now. Targeted cell irradiated program (with manual cell selection) has been integrated into the CENA software, and is waiting for beam test.

Acknowledgements. The authors thank the accelerator colleagues during facility construction and beam commissioning. This work was financially supported by the Hundreds Talent Program of CAS (Y150310BRO) and National Natural Science Foundation of China (Grant No. 31200630).

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G. Du *et al.*, Nucl Instrum Meth B, 306:29-34 (eb2013).

O2. RECENT PROGRESS IN DEVELOPMENT OF HEAVY-ION MICROBEAM SYSTEMS OF JAEA-TAKASAKI

Funayama T., Yokota Y., Suzuki M., Ikeda H., Sakashita T., Kobayashi Y.

Microbeam Radiation Biology Group, Medical and Biotechnological Application Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency

Heavy-ion irradiation has been employed in a wide range of biological applications, including heavy-ion radiotherapy and radiation breeding, because of its high and unique biological effectiveness. However, the elucidation of mechanisms underlying biological response of heavy-ion radiation is necessary to advance these useful applications.

Target irradiation of individual cells using microbeam is a useful means to investigate the mechanism of heavy-ion radiation action. Therefore, we have developed the heavy-ion microbeam systems in JAEA-Takasaki, and utilized for analyzing biological effects of heavy-ion.

In our facility, there are two microbeam systems for biological study: the collimating heavy-ion microbeam system, and the focusing heavy-ion microbeam system. Both systems are installed individually on the vertical beam lines of the AVF - cyclotron.

The collimating microbeam system, which was developed and installed at mid 1990's and utilized for various biological studies, generates heavy-ion microbeams using micro collimator [1-2]. The system is designed to irradiate living cells individually and precisely with a control of the number of ions to be delivered, and the experimental system is designed to detect hit positions directly and immediately after irradiation and enable to observe biological effects of ion hit after the irradiation. The system contains multiple microscope systems and the variable size of the microbeam spot from 10 to 250 μm in diameter, which can be utilized for target irradiation of various ranges of living materials from cultured cells to small individuals like silkworm and *C. elegans*.

Using the system, we have carried out a lot of biological studies concerning the effect of heavy-ion radiation including radiation induced bystander effect [3-4]. To explore the behaviour of the whole cell population after bystander irradiation, an integration of live-cell imaging technology into the cell targeting-and-observation system of the collimating microbeam system is under development.

On the other hand, the focusing microbeam system was developed to irradiate heavy-ions to the target cells with beams finer than the collimating system. In the system, the beam transported from the AVF-cyclotron was collimated by the micro slits then the beam was focused using a quadruplet-quadrupole lens system to generate finer beam spot. The size of the spot is minimum 1 μm in diameter in vacuum.

To carry out target irradiation of living cells, the beam spot was extracted into the air from the vacuum window, and its position was detected under microscopy using a plastic scintillator. The cell target system was installed below the vacuum window, and consists of 7 automatic stages to align the microscope position and to target the sample. The sample was placed just beneath the beam exit and the cell images were observed using a cooled CCD camera. To evaluate the function of the cell targeting system, HeLa cells stained with CellTracker Orange was inoculated on a CR-39 film, covered with a Kapton film, sealed with a petrolatum, then place on the sample stage. The cells were moved to the beam spot position detected by a scintillator, then the cells were irradiated with focusing heavy-ion microbeam. The number of ions irradiated on each cell was counted with a solid state detector and controlled by a fast beam shutter. Using the system, we established a method to irradiate finer heavy-ion microbeam on individual HeLa cells. For rapid and accurate delivery of heavy-ions to the individual cells, a method for targeting cells with scanned beam is under development.

The outline of these developments will be presented in the talk.

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O3. SUPER RESOLUTION MICROSCOPY AT THE COLUMBIA MICROBEAM

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As microbeam technology advances, the quest for the irradiation of ever smaller targets of interest increases. We have proposed the construction of a 'super' microbeam that will have a 70 nm diameter beam at our sample location. The targeting of samples at that resolution becomes difficult as the Abbe diffraction limit makes traditional microscopy below 200 nm unattainable. We propose to construct a super resolution microscope with 70 nm resolution to be used in conjunction with the super microbeam to irradiate sub-100 nm targets in the cells.

The technique we have chosen is Stimulated Emission Depletion (STED) microscopy.[1] This technique uses a pair of lasers, one which is diffraction limited as a traditional scanning laser microscope and a second, co-linear beam that has a helical phase shift creating a depletion donut around the excitation spot. Figure one show how the depletion donut depletes the excited state of the fluorophore in the donut area limiting emission to a sub-diffraction space in the center of the donut. The STED technique can be used to produce real-time images on live samples with minimal image analysis compared to other techniques which require long acquisition times and computationally intensive image construction.

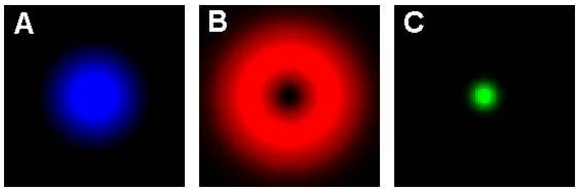

Figure 1: A) Excitation laser spot, B) depletion 'donut' which is centered on the excitation spot, and C) the effective fluorescence detected by the imaging system.

We will leverage off our established multi-photon microscope as our excitation beam and the previously developed laser introduction optics to the irradiation end station, as shown in Figure 2 [2]. The co-linearized beams will be scanned across the sample using precision galvanometric mirrors and the images are constructed by correlating the fluorescent intensity with each location measured using the existing photo multiplier tubes. We will be using a 592 nm continuous wave (CW) laser for the depletion laser which will allow the tunable capabilities of the multi-photon microscope to image both green and yellow fluorescent materials.

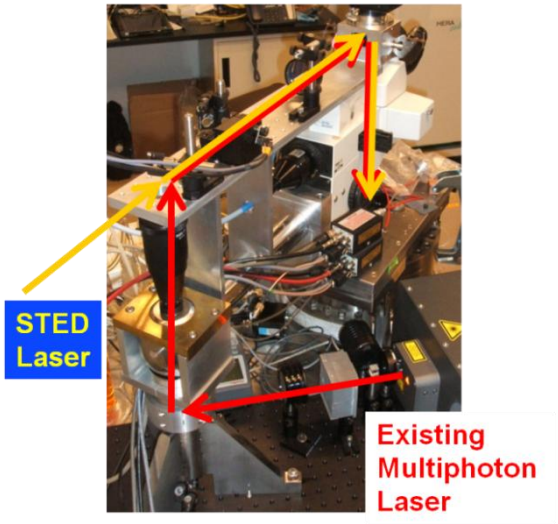

Figure 2: Combined laser path layout at the microbeam end station later bench. The scanning mirrors are just to the upper right of where the lasers converge.

The expansion of imaging into the sub-100 nm regime is a large step forward in the targeting and observational possibilities on the RARAF microbeam.

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O4. DEVELOPMENT PROGRESS OF THE HIGH ENERGY X-RAY MICROBEAM

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Radiobiological microbeams are technologies able to deliver precise radiation doses to individual cells (or parts of them) and follow their fate. By using microbeams, significant contributions have been made in understanding the risks associated with single charged particle traversals [1], the differential radio-sensitivity of sub-cellular elements [2], [3] and in better characterizing new biological mechanisms such as the bystander effects and adaptive responses [4], [5]. With the rapid molecular and technological developments [6], new probes, assays and cell monitoring options cellular are become available which will allow further exploitation of the microbeam technology.

X-ray microbeams offer unique prospects due to the specific characteristics of the interaction between X-rays of defined energies and biological samples, the higher resolution and limited scattering achievable. X-rays of energy higher than a few keV are classified as sparsely ionizing radiations (low Linear Energy Transfer, LET) producing less clustered lesions inside biological samples. The high penetration of hard X-rays (1/e ~900 µm for 8 keV Cu K_α X-rays) combined to the lack of scattering as the photons penetrate the samples, will also allow single cell irradiation experiments in 3D biological structures. Moving from single cells to more complex biological systems is of critical importance to validate and extrapolate current understanding of radiation action and phenomena to more relevant biological models and ultimately *in vivo*. At Queen's University Belfast, we are exploiting technological developments of the X-ray microscopy community [7], to develop the second generation of X-ray microbeams specifically aimed to increase the energy of the radiation probe up to 10s of keVs.

A commercially available electron bombardment X-ray source (UltraBright from Oxford Instruments) was chosen as X-ray source and optimized for our purposes. The UltraBright source is rated up to 80 W (60 kV, 2 mA max working parameters) with a dispenser type cathode whose electrons are focused by active electrically isolated grids into an elongated spot on a solid target. The X-ray emitting spot is viewed at 33 degrees angle through a Be vacuum window resulting in a round X-ray source of ~40 µm diameter. The current target is made of solid Cu but alternative materials (molybdenum, silver and tungsten) are possible including alloys which will offer different characteristic K_α lines for a quick X-ray energy selection. The X-ray output has been characterised and compared to prediction simulations (GEANT4) to finalise the design of filters and X-ray optics.

Whilst optics to focus soft X-ray beams (<5 keV) have been extensively explored and are based on diffraction principles (i.e. zone plates) to achieve nanometre resolution, relatively few options are available for hard X-rays. The new X-ray microbeam will be using reflective capillary systems to take advantage of the total reflection that occurs at shallow incident angles to channel the X-ray beam and focus or condense it down to micron and sub-micron size spots. Monolithic polycapillaries (Unisantis Europe GmbH) made by tens/hundreds of individual single piece micro-capillaries have been tested and characterized with the UltraBright X-ray source. Using such devices, 8 keV X-ray beam can be focused into a ~12 µm spot with an overall gain exceeding 1000.

For fine spatial resolution, we will be employing glass monocapillaries, i.e. single hollow tapered capillaries. Using the same principle of total reflection from the inner walls of a single glass channel, hard X-rays can be concentrated into a spot of micron and sub-micron size [8]. Two set of monocapillary lenses are currently being tested: 1) parabolic capillaries which are expected to produce ~2-5 µm spot with a gain of 50 and 1.5 degree divergence; 2) conical stretched glass pipettes with aperture < 100 nm and several cm length [9].

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SESSION II. LASER-ACCELERATED PARTICLE BEAMS

O5. RECENT PROGRESSES IN LASER ION ACCELERATION AND APPLICATIONS

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In the last few years, intense research has been conducted on the topic of laser-accelerated ion sources and their applications. Laser accelerated ion beams have exceptional properties, i.e. high brightness and high spectral cut-off, high directionality and laminarity, as well as short burst duration. In particular, for proton energies >10 MeV, the transverse (resp. longitudinal) emittance is at least 100-fold (resp. 104-fold) better than conventional accelerators beams. Beam optimization of laser-accelerated protons is now a crucial point for the development of applications in various areas. Several directions need to be pursued, namely (i) optimization of the high-energy end of the spectrum e.g. for dense plasma radiography and medical applications, (ii) optimization of the low-energy end of the spectrum e.g. for isochoric heating of matter, (iii) enhancement of laser-to-protons conversion efficiency and reduction of divergence e.g. for medical applications or ion irradiation. Recent experimental results and simulations on these topics will be presented. New ways to ensure tunable control of the beam divergence and energy selection, and to increase the maximum ion energy as well as the laser-to-ions conversion efficiency have been developed. I will first review the source characteristics and paths for optimization. I will show that maximum ion energy and the conversion efficiency can be enhanced using low-density plasmas or special targets, and discuss applications taking advantage of the unique characteristics of those beams. I will then describe some present-day applications as plasma radiography for density or electromagnetic field retrieval, isochoric heating of matter to eVs, and evoke some others, which are more prospective like fast ignition of fusion targets or proton therapy of deep-seated tumors.

O6. HIGH ENERGY ION ACCELERATION AND NEUTRON PRODUCTION USING RELATIVISTIC TRANSPARENCY IN SOLIDS

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The quest for high energy ions and secondary radiation for applications like cancer treatment has been going on for some years. Recently using high contrast short pulse lasers and the concept of relativistic transparency a breakthrough has been achieved with respect to ion energy and the production of neutrons. Using the 200 TW TRIDENT laser at LANL we have achieved proton energies well exceeding 100 MeV and intense pulses of neutrons in experiments this year. The prospects for medical applications and for upcoming experiments using the high contrast short pulse laser PHELIX and novel targets will be discussed.

In a recent campaign at LANL we have achieved proton energies exceeding 150 MeV and deuteron energies exceeding 180 MeV. We also have accelerated heavier ions to the point where the use in medical applications become promising. Furthermore we have been successful in converting those ion beams into a beam of neutrons that might be valuable to application in medicine, material science and security applications [1]. With more than 10^{11} neutrons in a single shot and energies up to 200 MeV these neutrons have been used for the first time to image a secondary object, using just 60 J of laser energy.

With the upcoming high contrast version of PHELIX, operational since a few weeks, we intend to continue this endeavor using shorter pulse length and up to four times the energy of TRIDENT. Simulations indicate proton energies of about 230 MeV, using cryogenic targets.

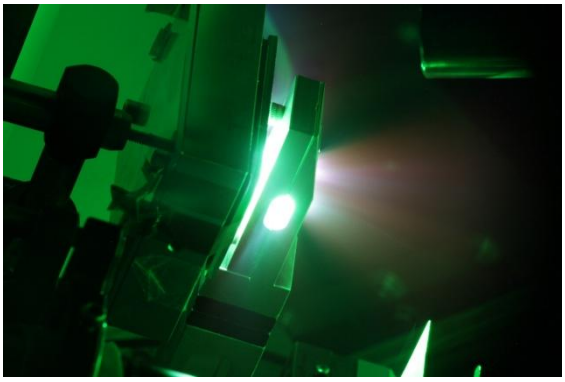

Figure 1: Experiment on a laser driven neutron source

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O7. PRODUCTION OF NARROW BANDWIDTH LASER-ACCELERATED PROTON BEAMS BY PASSIVE ENERGY SELECTION

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For tenth of years, radiotherapy has proven to be a very powerful and effective way to irradiate cells. Yet, for now this method uses mostly hard X-ray beams which deposit their energy in a decreasing exponential manner. Although more recently, hadrontherapy has also proven to be of high interest, mainly due the more precise energy deposition it allows, since protons deposit their energy mostly at the end of their range. Because this peak of deposition is very narrow in depth (a few microns in water). It allows one to chose very precisely the area to irradiate, using the correct interval of energy. The use of this technique is however still limited, mainly due to the high cost of such facilities using conventional ion accelerators. Laser accelerated protons and ions have been proposed has a potential way to reduce the cost of such facilities since they could permit a reduction of the gantry. However, one major impediment is that these beams have broadband energy spectrum. We present here a way to select a chosen energy interval from this spectrum : we used magnetic fields to bend the protons' trajectory and two shields to make a slit, in order to select at will the minimum and maximum of the energy. Experimental measurements as well as scaling to higher proton energies using future facilities will be presented.

O8. HOW TO CHARACTERIZE 10^{12} ENERGETIC PARTICLES IN A SUB-NANOSECOND BUNCH?

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The development of Ultra High Intensity (UHI) lasers this past decade has made possible to accelerate bunches of particles over a very short duration. These particles can be electrons with energies up to 1GeV, photons produced by electron deceleration (Bremsstrahlung), or ions (mostly protons) of several tens of MeV. These bunches contain around 10^{12} particles with continuous energy distributions and last tens of picoseconds [1]. To meet the challenge of characterizing these bunches of particles (energetic and spatial distributions), we use in parallel different kinds of passive detectors.

Radio Chromic Films (RCF) [2] are commonly used to visualize the spatial profile of a bunch of particles via the polymerization of the zone where the ionising particles passed through. Energy distribution of impinging particles can be extracted from the optical density of the irradiated zone, but the response of the film is not very reliable.

Otherwise, photostimulable phosphor plates have been used for 30 years for ionizing particle imaging. We have launched a program to measure and simulate via GEANT4 the response functions of the Image Plates (IP) for various particles ranging in energy from few keV to 30 MeV. We are now able to use the response functions of the IP to quantitatively characterize bunches of electrons, photons, protons and even ^{4}He particles up to tens of MeV [3,4].

Finally we have developed a technique based on nuclear activation [5] that complements those traditionally used by the plasma physics community. A sixteen β+ radioactivity counting station system (Fig.1) has been built in order to determine the number of nuclear reactions induced in activation samples set on the particle path. This number of reactions convoluted with the stopping power of a stack of activation samples, makes possible to determine the impinging particle energy distribution.

Our original approach is to combine the advantages of these 3 techniques in order to characterize the particle bunches in view of using them in nuclear physics experiments under extreme conditions.

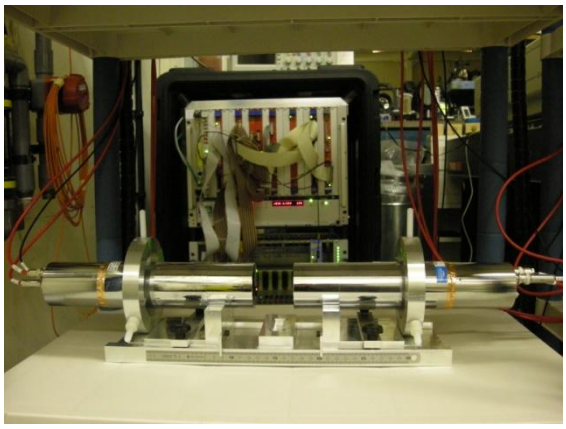

Figure 1: One of the sixteen β+ counting stations system [5].

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SESSION III. RADIOBIOLOGY WITH LASER ACCELERATED PARTICLE BEAMS

O9. REVIEW ON RADIOBIOLOGICAL STUDIES USING LASER DRIVEN PARTICLE BEAMS: FROM IN VITRO EXPERIMENTS TO HUMAN TUMOR IRRADIATIONS ON MICE

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Particle acceleration by high intensity laser promises more compact radiation sources but results in different beam properties like ultra-short and very intense particle pulses, low pulse repetition rate, inherent pulse-to-pulse fluctuation, large beam divergence and broad energy distribution compared with conventional particle beams [1]. Therefore, any medical application requires not only a high power laser system and appropriate laser target to produce the particle beam but also technical solutions for dose delivery including beam transport, radiation detection, irradiation field formation and dosimetry. Moreover, the radiobiological consequences of laser based and therewith ultra-short radiation pulses with high pulse dose have to be known.

During the last years, laser based irradiation technology was developed and established allowing the irradiation of in vitro cell samples and small animals. Radiobiological studies performed so far will be reviewed and the technical approaches used will be discussed with special focus on the development towards clinical particle radiotherapy.

Experiments started with X-ray pulses with energies ranging from keV to MeV produced by laser systems of Terawatt (TW) power [2-5]. Mammalian cells were irradiated with these laser-produced bremsstrahlung and characteristic X-rays. Cell survival and DNA DSB induction were studied as a function of absorbed dose and compared to those of conventional photon sources.

In a second step, systematic in vitro studies with laser driven electron beams have been carried out [6-9]. Using laser systems with ~10 TW power, electrons with energies of up to few 10 MeV have been produced. Again, dose effect relationships have been determined for different cell lines and biological endpoints.

Rapidly, similar in vitro studies with laser accelerated protons have been started by several groups [10-15]. An increased laser intensity of up to about 150 TW was applied to accelerate protons to energies of up to 20 MeV.

Recently, first in vivo experiments have been established [16]. Tumor irradiation was realized for murine sarcoma KHT and human squamous cell carcinoma FaDu on mouse ear. Doses up to 14 Gy were applied and irradiation induced tumor growth delay was investigated. One prerequisite for these experiments was the ability to deliver a prescribed dose to each tumor. Furthermore, the successful irradiation of more than hundred mice over a period of several weeks marks the achieved stability and reproducibility of all implemented setup components and methods.

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O10. DNA REPAIR DYNAMICS FOLLOWING ION IRRADIATION AT ULTRAHIGH DOSE RATES FROM LASER-DRIVEN ACCELERATORS

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Hadron therapy is currently a fast growing cancer strategy with huge potential benefits for patients and industries. Unfortunately, the cost and size of these current hadron therapy facilities are beyond the scope of a typical hospital and limit both the widespread use of proton treatments as well as the employment of heavier ion beams, which are potentially even more effective [1]. The idea of future facilities based on laser driven ion accelerators has been proposed as a way of reducing complexity and cost of these infrastructures [2]. Significant effort is ongoing to demonstrate the ion beam parameters required to make this a realistic proposition. In the meanwhile, several groups have started preliminary work on the methodology and viability of using laser driven ion sources for cell irradiation experiments, a necessary step in view of any future therapeutic use**.** The main aim of these investigations so far has been to establish a procedure for cell handling, irradiation and dosimetry compatible with a laser-plasma interaction environment [3-5]. One of the peculiarities of laser-driven ion beams is their ultrashort duration, as ions are emitted in bursts of picosecond duration at the source and their therapeutic use may result in dose rates many orders of magnitude higher than normally used. The biological effects of ions at these ultra-high dose rates are virtually unknown, and need to be carefully assessed prior to any medical use. We will report outcome from radiobiological experiments performed using the GEMINI and TARANIS laser system to carry out totally new investigations of the dynamics of cellular response to ion irradiation and damage at the unprecedented dose rates

Preliminary work was carried out at Queen's University employing the TARANIS multi-terawatt chirped-pulse amplified (CPA) laser system. A Ti:Sapphire – Nd:Glass laser capable of producing intensities of 10^{19} W/cm^2 translates to particle number densities of ~ 10^{11}/MeV/Sr for protons at the lower energy range (~ 3MeV). These parameters allowed required doses to be obtained in a single shot basis, this coupled with the experimental lay-out chosen allowed a dose rate of 10^9 Gy/s to be obtained [7].

A crucial element in the experimental arrangement was the proton beam irradiation system which had to be capable of separating charged particles of different energies, while maintaining a high flux on the cell plane. This was achieved through the implementation of a magnet of magnetic field strength~ 0.9T. The dispersed beam was then transported to the cell dish, placed outside the experimental chamber, through a Mylar window of 50 µm thickness, with transverse dimensions of a few cm. The cells were placed vertically on a specially designed dish during the exposure and then quickly removed for processing, as required.

The developed and tested experimental technique was then implemented at the GEMINI laser facility in the Rutherford Appleton Laboratory. This offered different parameters to those attainable with TARANIS, with cell irradiation being possible with both proton and carbon ions. The GEMINI laser can provide laser intensities ~ $5\text{x}10^{20}$ W/cm^2 providing particle densities of 10^{11}-10^{12}/MeV/Sr for protons and 10^9/MeV/Sr for Carbons. This allows delivery of clinically relevant doses in a single shot with a dose rate in excess of 10^9 Gy/s.

Both experiments carried out at TARANIS and GEMINI investigated DNA damage formation and associated repair kinetics in normal human fibroblasts cells (AG01522) exposed to laser-driven ions using the γ-H2AX immunofluorescence technique.

The biological outcome of radiation exposure is determined by both the amount and complexity of DNA damage [8]. While the dose absorbed by cells is a good indicator of the amount of damage induced, the complexity of the damage is linked to the clustering of DNA lesions, which result from direct ionizations from ions and electrons as well as reactions with radical species. Using pulsed laser-driven ion sources, the spatio-temporal distribution of the ionization pattern

is also pushed to limits which have not been investigated so far. Several effects related to the ultrashort nature of the deposition have been suggested to critically impact on the cellular responses: possible alteration of the radical production (oxygen depletion effect) [9]; spatio-temporal overlap of independent tracks resulting in collective effects [10]; lack of interaction between direct and indirect DNA lesions [11]. This is a regime where very little is known, and the only way to assess these predictions is via experimental testing. Work carried out so far has been limited to protons and in most cases Gy-level doses have been obtained by accumulating multiple irradiations with an effective dose rate of Gy-s, as in "conventional" irradiations, [3, 4] or using single shot irradiations with ion beams limited to < 5 MeV [7]. Experiments performed at GEMINI extended these studies to unexplored ranges with the aim of unveiling any of the effects described above via systematic investigations. Data is currently being analyzed and compared to data obtained with KeV X-rays and conventional proton beams.

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O11. THE APPLICATION OF PARTICLE BEAMS FROM A LASER PLASMA WAKEFIELD ACCELERATOR TO CANCER THERAPY

[2] Sorensen A., [1] Subiel A., [1,2] Farrell P., [1,2] Esposito M., [1] Reboredo Gil D., [1] Cipiccia S., [1] Welsh G., [1] Issac R., [3] DesRosiers C., [3] Moskvin V., [3] Mendonca M., [4] Maryanski M., [5] Seitz B., [6] Evans P., [7] Partridge M., [2] Boyd M., [1] Jaroszynski D.

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X-rays and electrons with energies of a few MeV are the most common form of ionizing radiation used for cancer radiotherapy. We have shown using Monte Carlo simulations that very high energy electrons (VHEE) (150 - 250 MeV) could be an attractive modality for radiotherapy as VHEE penetrate and deposit their largest dose much deeper into tissue than X-ray photons. However, due to the present lack of readily available VHEE accelerators, mainly theoretical studies have been undertaken so far. The rapid development in ultra-compact laser-plasma Wakefield accelerators (LWFAs) is now providing an alternative VHEE accelerator for radiotherapy. The "Advanced Laser-Plasma High-Energy Accelerators towards X-rays" (ALPHA-X) program at the University of Strathclyde has developed laser plasma accelerators for the production of reproducible ultra-short duration, high quality, high energy electrons. The relative biological effectiveness of these particles on tumour and normal cells is currently unknown. To interrogate the biological effectiveness of LWFA VHEE on cells it is crucial to determine the precise radiation dose delivered to the cells. We have used a range of dosimeters (ionisation chamber, image plates, bangel and Gafchromic film) with well know behaviour using Linac produced electrons beams with energies of 18 – 20 MeV to characterise absolute dose deposition and distribution of the ALPHA-X electron beam with energies around 140 - 160MeV. This has been followed by cell irradiation experiments to determine the toxicity and dose response relationship between LWFAs VHEEs (140 -160 MeV) and tumour cell kill in two lung cancer cell lines, using a custom designed and sequentially modified cell irradiation setup. This has been compared with X-ray photons delivered by an Xrad-225 cell irradiation cabinet. Furthermore, we have interrogated the magnitude and dynamics of DNA double strand breaks following irradiation with LWFA VHEE and compared this to that of X-ray photons using γ-H2AX as a molecular marker for DNA double strand breaks. One of the properties of LWFA electron beams is the emission of bremsstrahlung gamma irradiation as the beam propagates through water. The relative energy of these components varies with depth through a water medium and therefore the nature of the radiation experienced by the biological target at increasing depths changes with distance from the point of penetration. To understand the effect of radiation types at different depths on tumour cell kill, we determined the clonogenic cell survival in lung cancer cell lines, irradiated at different positions and depths from the radiation source, in a water phantom along the beam line. The potential of these findings in relation to current clinical radio therapy will be discussed.

O12. DOES THE RBE CHANGE FOR PROTONS WHEN USING LASER ACCELERATORS FOR TUMOR THERAPY?

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There is ongoing discussion whether the RBE of protons changes when tissue is irradiated by protons from femtosecond laser accelerators as they are proposed for possible cheaper proton therapy facilities. I will discuss the physical arguments and also the experimental evidence that there is no significant difference between nanosecond pulsed and continuous (millisecond to second) proton irradiation.

Although the proton pulses will be in the picosecond range or even shorter directly behind the laser accelerator the beam pulse will widen to the 100 ps to nanosecond time scale due to the beam transport that takes some meters even in ideal cases including energy filtering through magnets and slit systems. Thus, beam delivery in the 100 ps to nanosecond timescale has to be considered with respect to tumor therapy applications resulting in ultra-high dose rates in the 10^9 Gy/sec.

The general physical arguments why the RBE will not change are the following: a maximum dose of < 2 Gy is delivered in a single pulse of protons in realistic treatment plans meaning an average proton fluence of less than about 10^9 protons/cm². Since there will be an overlap of several pulses at a certain position to form the total dose due to beam scanning or by forming a spread out Bragg peak the individual dose and thus the proton fluence of a single proton pulse will be even less. The average proton-proton distance calculated from that fluence will be less than 0.3 µm. Thus, the microdose distribution of one proton is not much altered by the doses deposited by the neighboring protons impinging within the same pulse. It is not expected that ionisation densities and e.g. double strand break induction will change due to synergetic interaction of at least two protons from the same pulse considering the large distances and also the nanosecond pulse duration. Even if considering chemical interactions that can also lead to gene attacks or interaction of double strand breaks from two separated protons there are no considerable synergetic effects at those fluences [1].

The theoretical considerations that no RBE changes occur are proven already by a number of experiments. Ultra high dose rate experiments have already been performed by pulsed x-ray and pulsed electron experiments in earlier days. In order to simulate the pulsed proton irradiation from laser experiments we modelled nanosecond pulses with sufficient proton densities to deliver 2 Gy doses in a single proton pulse at the microprobe SNAKE and showed for several endpoints in cell culture and artificial tissue experiments [2, 3] that there is no significant change in RBE between pulsed and continuous proton irradiation. We performed even growth delay experiments of subcutaneous tumors in mice after pulsed and continuous proton irradiation [4] that did not show any changes in RBE on the 10% level (see paper T.E. Schmid et al).

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SESSION IV.RBE & THERAPY RELATED STUDIES

O13. ENHANCED RBE OF SUB-MICROMETER FOCUSED LOW LET PROTONS

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Due to their physical and radiobiological properties, in particular their increased relative biological effectiveness (RBE), high linear energy transfer (LET) radiation qualities are of special interest for tumour therapy. The aim of the present investigation is to quantify the influence of spatial dose distribution on the RBE of heavy ions. By focusing low LET 20 MeV protons (LET in water of 2.65 keV/µm) the spatial dose distribution of protons can be modified towards that of heavy ions where the dose is concentrated around the ion tracks [1]. By changing the spot size and the number of focused ions one has opened a wide field to study RBE effects in dependence of dose distributions on sub-micrometer scale.

In this work the influence of spatial dose distribution is studied on the endpoint of induction of dicentric chromosomes. Human-hamster hybrid (AL) cells were irradiated with focused 20 MeV protons in a quadratic matrix pattern with certain point distances and certain numbers of protons per matrix point. Three different matrices were used: 5.4×5.4 µm2, 7.6×7.6 µm2 and 10.6×10.6 µm2 with protons per point of 117, 232 and 451. All three irradiation modes deposit a mean dose of 1.7 Gy. For comparison cells are also irradiated with randomly distributed protons or 55 MeV carbon ions in a 5.4×5.4 µm2 matrix pattern and one ion per point, applying the same dose of 1.7 Gy.

Fig. 1 shows the pooled results from two independent experiments. The RBE for induction of dicentric chromosomes after irradiation with randomly distributed protons is measured to 1.33 ± 0.19 and increases with higher numbers of protons per point up to 2.60 ± 0.27 for 451 protons applied at each point of a 10.6×10.6 µm2 matrix. The RBE of carbon ions (3.21 ± 0.27) is about 25% higher than the RBE of 451 focused protons.

The enhanced RBE of focused protons is attributed to their inhomogeneous dose distribution, similar to heavy ions, where dose and hereby DNA double strand breaks (DSB) are concentrated around the matrix points. Thus the probability for joining wrong DSB ends, a prerequisite for induction of dicentric chromosomes, increases due to the decreasing mean distances to neighbouring DSB. On the other hand the even higher RBE of carbon ions is not reached because the dose distribution of focused protons is similar but not identical to that of heavy ions. In the centre of an ion track much higher doses occur and nonlinear effects, like clustering of single strand breaks enhancing the number of induced DSB or induction of dense clusters of DSB lead to an additional DNA damage for heavy ions.

These findings demonstrate the influence of spatial dose distribution to RBE and show the potential of using focused low LET protons as a model system for further understanding RBE of heavy ions.

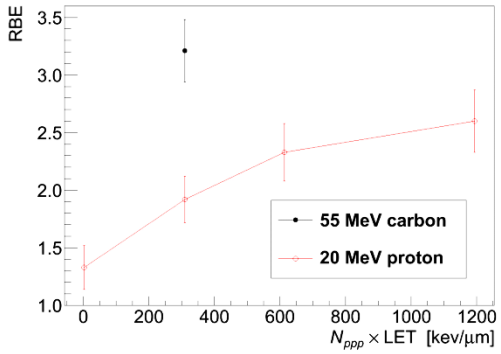

Figure 1: RBE of 55 MeV carbon ions and 20 MeV protons versus the number of particles per point, N_{ppp}, times the LET. RBE after randomly distributed 20 MeV proton irradiation is plotted at N_{ppp} = 1. Each data point is the pooled result of two independent experiments.

Supported by the DFG-Cluster of Excellence 'Munich-Centre for Advanced Photonics' and by Bmbf-project 02NUK031A "LET-Verbund".

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O14. MONTE CARLO MODELLING OF RADIATION EFFECTS FROM FOCUSED LOW LET PROTONS AND SINGLE CARBON IONS

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PARTRAC is a state-of-the-art tool for stochastic Monte Carlo-based simulations of radiation track structures, damage induction in cellular DNA and the processing of DNA double-strand breaks (DSB) via non-homologous end-joining [1]. Dedicated modules describe interactions of ionizing particles with the traversed medium, the production and reactions of reactive species, and score DNA damage by overlapping the track structures with multi-scale chromatin models. The DNA repair module describes stochastically the hierarchical action of repair enzymes processing the DNA ends on the basis of their calculated spatial distribution and DSB complexity in terms of nearby strand breaks and base lesions. A recent model extension allows the calculation of various types of chromosomal aberrations [2].

PARTRAC calculations have been thoroughly benchmarked against diverse experimental data. In particular, for both low- and high-LET radiation the simulations correctly reproduce the measured size distributions of radiation-induced DNA fragments in the whole detectable size range. Notably, the simulations enable assessing also DSB associated with undetectable, very short DNA fragments produced especially by high-LET radiation, and indicate that typical DNA fragmentation studies do significantly underestimate DSB yields of high-LET radiation [3].

The DNA repair module has been successfully adapted to describe the LET-dependent repair kinetics in cells irradiated with ^{60}Co γ-rays and diverse ion beams. The chromosome aberration module has provided correct predictions for the dose dependence of the relative yields of dicentrics after gamma- and alpha-particle irradiation, although the absolute yields were overestimated by factors of 5 and 2, respectively [2].

An excellent data set for advanced benchmarking of PARTRAC simulations has been provided by recent experiments comparing the effects of irradiation with single carbon ions and the corresponding number of low-LET protons focused to sub-micrometer bunches so as to closely mimic the carbon tracks [4]. Focused protons have been shown to possess enhanced RBE for micronucleus induction and dicentrics compared to randomly distributed protons, yet considerably below the effects of single carbon ions with the same energy deposit.

To represent these experiments, the PARTRAC modules have been extended to simulate matrices of proton bunches and single ions corresponding to the given setup [4] in terms of track structures, radiation damage to DNA and its repair by the cells. The results on DSB yields, DNA fragmentation and the production of dicentrics will be presented at the workshop.

Acknowledgements. This work has partially been supported by the KVSF- Project 'LET-Verbund' of the Federal Ministry of Education and Research.

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O15. REDUCED SIDE EFFECTS USING PROTON MICROCHANNEL IRRADIATION IN A HUMAN SKIN MODEL

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We propose a novel strategy to reduce the known side effects of radiotherapy by using proton microchannel irradiation. The goal is to minimize the risk of normal tissue damage by microchannel irradiation, while preserving local tumor control through a homogeneous irradiation of the tumor that is achieved because of beam widening with increasing track length by small angle scattering of beam particles. Only a small fraction of the cells in the skin obtains a high dose, even much higher than within the tumor, leading to high cell death rates along the microchannels, while the rest receives only little dose. DNA damage induced in the hit cells will thus not be transported into later cell generations because the cells do not divide any longer, reducing secondary tumor induction. In order to prove the hypothesis of reduced side effects in normal tissue through microchannel proton irradiation, we report on a comparative study of microchannel and broad beam irradiation of artificial skin tissue.

20 MeV protons were administered to human skin models (EpidermFT^{TM}) in 10 to 180 µm wide irradiation channels on a quadratic raster with distances of 500 to 1800 µm between each channel (center-to-center) applying an average dose of 2 Gy (cf. fig. 1). For comparison, other samples were irradiated homogeneously by protons at the same average dose (HF).

Normal tissue viability was significantly less reduced after proton microchannel irradiation compared to homogenous irradiation. Levels of inflammatory markers, such as cytokines and chemokines, were significantly lower in the supernatant of the human skin tissue after microchannel irradiation than after homogeneous irradiation. Furthermore, genetic damage as determined by the measurement of micronuclei in keratinocytes was also significantly reduced after microchannel irradiation compared to homogeneous irradiation (cf. fig. 2).

Our data show that proton microchannel irradiation maintains cell viability while significantly reducing inflammatory responses and genetic damage compared to homogenous irradiation, and thus might improve normal tissue protection after irradiation therapy. Developing future proton therapy centers which have the techniques to provide proton beams with beam widths $\sigma < 0.3$ mm in beam scanning mode would allow to profit from reduced side effects, especially in the skin region, while keeping tumor control on the same level as conventional proton therapy.

Supported by the DFG-Cluster of Excellence 'Munich-Centre for Advanced Photonics'.

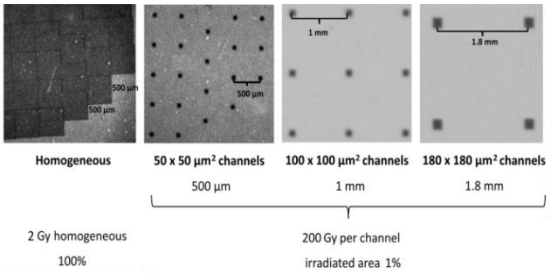

Figure 1: Irradiation fields of 20 MeV protons with a mean dose of 2 Gy visualized by Gafchromic films

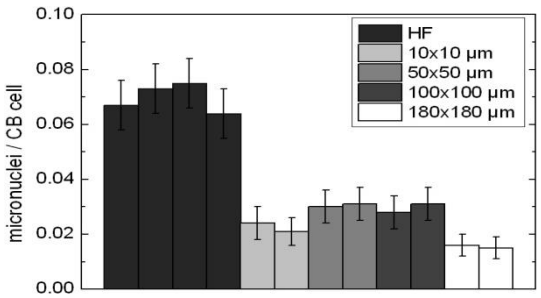

Figure 2: Micronuclei induction after 20 MeV protons with a mean dose of 2 Gy

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O16. NANOPARTICLE DOSE ENHANCEMENT USING X-RAYS AND PROTONS

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Nanoparticles (NPs) are a promising candidate for dose enhancement of therapeutic X-ray cancer treatments [1]. This is because they tend to agglomerate in tumours, are non-toxic and have a high cross-section for absorbing X-rays of certain energies. It is proposed that the overall dose given to a tumour could be reduced if the localised damage to a tumour is enhanced with NPs, thus sparing normal tissue affected by entrance and exit doses of X-rays. NPs of all types of materials have been studied including gold nanoparticles (GNPs) and NPs doped with rare earths [2].

In our work, we use ion beam analysis to analyse individual RT112 cancer bladder cells incubated with GNPs. We measure the mass of gold in each cell, together with the mass of each cell in pg of carbon. By using a cell penetrating peptide (TAT), the mass of gold imported into cells increases by at least five times with both 30 nm and 50 nm diameter GNPs.

By exposing these cells containing GNP to doses of X-rays (1-5 Gray) we show that cell death increases by at least 4 times, giving significant dose enhancement. We analyse the dynamics of this dose enhancement using the Monte Carlo code Geant 4 to simulate secondary electron production from X-ray gold nanoparticle interaction in a virtual cell. We find that irradiations with 225 kVp X-rays increases secondary electron production by about 300 % with GNPs compared to a control.

We are also interested in potential enhancement of dose using ions rather than X-rays. However, simulations show that ions yield a very modest increase in secondary electron production of up to about 30 % with 3 MeV protons and much less with low energy carbon ions. These simulations are tested in two ways using the Surrey vertical microbeam. Firstly, cells containing GNPs are irradiated using 3 MeV protons and as predicted, there is no enhancement using clonogenic survival. Secondly, we use a 2 μm proton beam to irradiate nanoparticles surrounding the nucleus of cells. We measure reactive oxygen species production by the beam using a dye (HD2CFDA) and compare the signal between cells incubated with NPs and cells that have no NPs in them.

In conclusion, dose enhancement using nanoparticles for cancer therapy seems feasible using X-rays, but much less so using low energy particles.

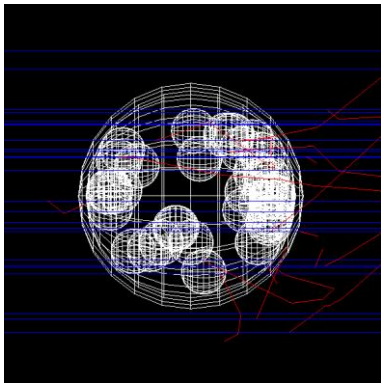

Figure 1: A Geant 4 Monte Carlo simulation of secondary electrons (red) emitted from gold nanoparticles (small spheres) inside a cell vesicle (large sphere) after 3 MeV proton irradiation (blue lines).

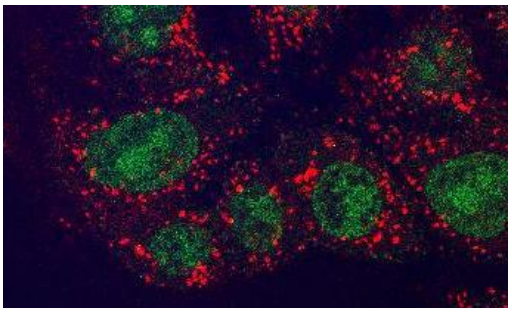

Figure 2: Nanoparticles contained in vesicles (red dots) are mainly in the cytoplasm of cells (the nucleus is shown in green).

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O17. NANODOSIMETRIC STUDY OF MICROBEAM RADIATION THERAPY

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Microbeam radiation therapy (MRT) is a new experimental oncological modality, intended for the treatment of inoperable brain tumours, particularly in difficult cases where conventional radiation therapy can cause irreversible damage. MRT consists of an array of highly collimated, quasi-parallel x-ray microbeams aimed at the tumour tissue, delivering high dose within the beam path and low doses in regions between the beams. For reasons still not fully understood, healthy tissue exposed to the microbeam array is able to regenerate while tumour volumes are significantly reduced[1]. Low energy Monte Carlo radiative transport simulations provide new insight into understanding the underlying mechanisms of MRT. In particular, predicting the ionisation cluster distribution, which is a significant cause of lethal damage to cells, would provide insight into the biological responses. Geant4-DNA was used to model an x-ray microbeam of width 20 µm in liquid water. Secondary electrons, predominately responsible for ionisation clustering, were tracked to predict damage to cells within and adjacent to the beams.

An x-ray microbeam of width 20 x 20 μm^2 incident on a liquid water cube of dimension 40 x 40 x 40 μm^3 was simulated using the Monte Carlo simulation toolkit Geant4 (version 9.6.p01). X-rays were modeled using the Low Energy Electromagnetic Physics models, including the following processes: Compton scatter, Rayleigh scatter, pair production and the photoelectric effect. Secondary electrons were modeled with the Geant4-DNA physics models[2,3] including the following processes: elastic scattering, electronic excitation, ionisation, vibrational excitation and attachment. Monoenergetic microbeams with 30 and 100 keV energies were investigated and in each case 10^6 incident photons were simulated.

Figure 1 shows the 3D electron track structure for 30 keV (top panel) and 100 keV (bottom panel) x-ray microbeams of width 20 µm centered at 0 on the yz plane, respectively.

Large numbers of ionisation and excitation processes, mostly responsible for biological damage, occurred in each case. However the distribution of the secondary electrons responsible for these processes differed with incident photon energy. Electrons in the 100 keV case were mostly confined to the beam (peak) region while in the 30 case, large numbers of electrons were found in both the peak and valley regions.

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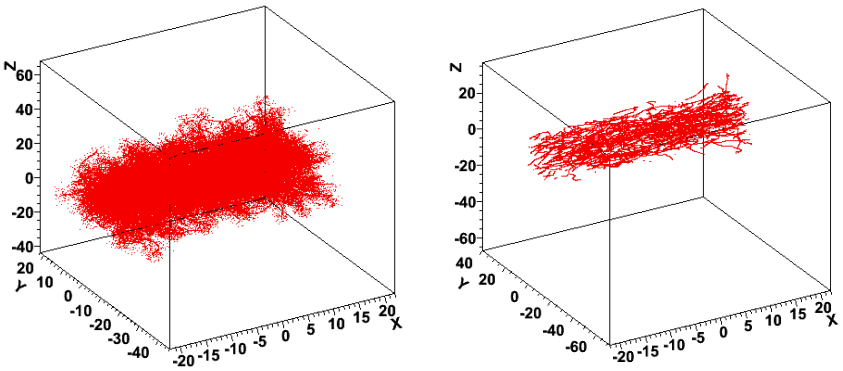

Figure 1: Electron track structure of a 30 keV (left) and 100 keV (right) x-ray microbeam.

O18. RESPONSE OF HUMAN NORMAL FIBROBLASTS TO PROTON IRRADIATION

[1,2,4] Michaelidesová A., [1] Vachelová J., [1] Havránek V., [1] Štursa J., [1] Zach V., [3] Falk M., [3] Falková I., [3] Ježková L., [4] Vondráček V., [1] Davídková M.

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Worldwide implementation of proton therapy to clinical practice requires detailed knowledge of biological response of normal and tumor tissue to proton irradiation. A new program of radiobiology studies started in the Nuclear Physics Institute in Řež near Prague (NPI). For proton irradiation, tandetron accelerator and two cyclotrons are used in order to cover proton energies from 2 MeV to about 226 MeV.

The accelerator Tandetron 4130 MC, one of the basic experimental facility of the NPI ASCR, is a source of accelerated ions of most of elements from H to Au with energies from 0.4-20 MeV and intensities up to tens of μA. The main laboratory accessories are devices for material characterization by standard nuclear analytical techniques (RBS, RBS-channeling, ERDA, ERDA-TOF, PIXE, PIGE, and Ion-Microprobe with 1 μm lateral resolution) and for high-energy implantation.

Isochronous cyclotron U-120M is also located in the NPI. Current parameters of accelerated and extracted beams are: p+/ H−: 5.4–38 MeV, D+/ D−: 11–20.5 MeV, 3He+2: 16.2–55 MeV, 4He+2: 22–40 MeV.

For proton energies up to 226 MeV, samples are irradiated at IBA cyclotron at the new Proton Therapy Center (PTC) in Prague. The mode of active pencil beam scanning is used in presented studies.

The mentioned proton beams have been used for irradiation of normal neonatal skin fibroblasts. The cells were grown on a 2.5 μm mylar foil stretched on a plastic ring (Chemplex Industries, USA). Special sample holders for the cell irradiation were constructed for the irradiation at tandetron and cyclotron accelerators. In starting experiments, confluent cell monolayers were irradiated at tandetron accelerator by 2 MeV protons by various fluences, U-120M cyclotron by 30 MeV protons and PTC center by protons of energies in the range from 100 to 226 MeV. The first results on cell survival, apoptosis, oxidative stress, DSB induction and repair obtained using proton microprobe and cyclotron proton beams will be presented and discussed.

The work is supported by the Grant Agency of CR (Center of Excellence P108/12/G108), the Ministry of Education, Youth and Sports of CR (COST LD12039, LD12008, OPVK CZ.1.07/2.3.00/30.0030).

SESSION V. LOW IRRADIATION DOSE STUDIES & BYSTANDER EFFECTS

O19. ESTABLISHMENT OF EXPERIMENTAL SYSTEMS FOR ANALYZING BYSTANDER EFFECTS INDUCED BY HEAVY-ION IRRADIATION BETWEEN NORMAL AND CANCER CELLS

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Radiation-induced bystander effects cause radiation influence on not only irradiated cells, but nearby non-irradiated cells [1]. Many of past researches on bystander effects had been focused on the influence between the cells of the same type like normal vs. normal cells [2] or cancer vs. cancer cells [3], induced by low linear-energy-transfer (LET) radiation such as X-rays and gamma rays. Therefore, no one can judge clearly whether the heavy-ion induced bystander effects between the normal cells and the cancer cells, which will be expected to be arisen in the process of heavy-ion radiotherapy, is harmful or beneficial. Thus, we investigated the heavy-ion induced bystander effects between different cell types, and the results were compared with those between the same cell types to understand the characteristic phenomenon of bystander effects between different cell types.

In this study, we used human lung normal fibroblast cell line WI-38, and human lung cancer cell line H1299/wt*p53* which is genetically modified to produce wild-type p53 proteins. To detect only the medium-mediated bystander effects, we adopted cell co-culture systems to isolate irradiated cells and bystander cells. The irradiated cells were exposed to the carbon-ion broad beams (LET = 108 keV/μm, WI-38: 0.13 Gy, H1299/wt*p53*: 0.5 Gy) of the cyclotron of JAEA-Takasaki, then the cells were co-cultured with non-irradiated cells under non-contact situation. After 6 and 24 hours of co-culture, the non-irradiated cells were harvested and used for colony formation assay to calculate survival rates.

The co-culture experiments were performed by changing the combination of cell types. When we co-cultured irradiated WI-38 with non-irradiated WI-38, it was found that the survival rates of non-irradiated WI-38 decreased after 6 and 24 hours of co-culture. On the other hands, when we co-cultured irradiated H1299/wt*p53* with non-irradiated WI-38 and vice versa, the rate of survival of non-irradiated cells showed a tendency to increase. The result indicated that the clonogenic abilities of the non-irradiated cells were increased, when the cells irradiated with carbon ions transmitted bystander signals via medium to the different type of the cells. From the result, it is suggested that there is a large difference in heavy-ion induced bystander responses via medium between the same type cells and different type cells.

In the co-culture experiment using the broad beam irradiation, we have been able to detect only the medium-mediated bystander response induced by signals of intercellular communication released from irradiated cells to the medium. However, since there is also known a bystander signalling pathway that was mediated by a gap junctional intercellular communications (GJIC), we adopted the target cell irradiation technique using the heavy-ion microbeam system of JAEA-Takasaki to clarify the molecular mechanisms concerned with the bystander effects induced both via medium and GJIC. The microbeam is an effective tool for inducing the bystander effects to the cell population [4] and it enables to elucidate mechanisms that are difficult to make clear with our previous study using broad beam irradiation. Although there are many reports of bystander effects between the same type cells using microbeam, there are few reports of bystander effects between different type cells using microbeam systems. Thus, we start the establishment of experimental systems for irradiating a part of confluent cells of mixed culture in the same dish using microbeam. The details of the method and the findings will be reported in the talk.

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O20. MICROBEAM X-RAY CELL IRRADIATION SYSTEM AT CRIEPI AND MECHANISMS OF X-RAY-INDUCED BYSTANDER RESPONSE

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2. *The Wakasa Wan Energy Research Center, Fukui, Japan*
3. *University of Fukui, Fukui, Japan*
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A radiation-induced bystander response, which is generally defined as a cellular response that is induced in non-irradiated cells that received bystander signals from directly irradiated cells within an irradiated cell population. Due to its nature, elucidation of the bystander response is important in evaluating the risk of low dose radiation. To investigate the bystander response induced by low-LET photons, a Microbeam X-ray Cell Irradiation System was developed at CRIEPI. Using this system, we are studying the mechanisms of the bystander response.

Our system [1] is characterized by (1) tabletop size, (2) an X-ray focusing system using Fresnel zone plate (FZP), and (3) an on-line confocal laser microscope. This system was initially designed for an aluminum K-shell (Al_K) X-ray (1.49 keV). Additionally, we are now modifying this system to generate C_K X-ray (0.278 keV). The electron gun with lanthanum hexaboride cathode is operated at voltages up to 30 kV, depending on the target. The FZPs were designed for Al_K and C_K X-rays and manufactured by the NTT Advanced Technology Corporation (NTT-AT, Japan). The order selecting aperture is used to pass only the first-order diffracted X-ray by blocking unwanted zero-th and higher-order X-rays. The minimal beam size of Al_K X-ray was about 2 µm in diameter. The positions of the cell nuclei and/or cytoplasm were determined with a fluorescence imaging. The position of the targets and the exposure period were controlled by the irradiation software. Confocal laser scanning microscope is combined with irradiation system to enable high resolution imaging analysis. Using this system, we are testing a time-lapse imaging of the cells inducing DNA damage responses or apoptosis.

When 5 cell nuclei of normal human fibroblast WI-38 cells bearing wild-type *p53* were irradiated in the population, the mode of bystander cell killing showed a biphasic behavior [1]. Additionally, the bystander cell killing was effectively suppressed by pretreatment with a scavenger of nitric oxide (NO) or an inhibitor of iNOS[1)]. Since the secretion of NO depends on the *p53* status, next the role of *p53* was assessed using two types of human non-small-cell cancer H1299 cells, one expressing wild-type *p53*, the other, mutated *p53* [2]. Surviving fraction of bystander cell of wild-type *p53* also showed a biphasic relationship to the irradiating dose such that the fraction was steeply reduced up to 0.45 Gy, recovered toward to 2.0 Gy, and then kept in the level of control up to 5.0 Gy. On the contrary, in the *p53* mutated cells, the surviving fraction was steeply reduced up to 1.0 Gy, and the reduced level was kept up to 5.0 Gy. These results suggest that the X-ray-induced bystander cell killing depends on *p53* status of the targeted and/or bystander cells.

Figure 1: Microbeam X-ray Cell Irradiation System at CRIEPI.

Acknowledgments. This work was supported in part by MEXT KAKENHI (21681006).

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O21. ANALYSIS OF BYSTANDER EFFECTS INDUCED BY HEAVY IONS AND GAMMA-RAYS

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Radiation-induced bystander effects manifest cell killing, chromosomal aberration, mutation and other effects in the cells that are not irradiated but are close to irradiated cells via intercellular and intracellular signalling pathways. It is necessary to make clear the mechanism of bystander effects in order to estimate the potential risks of low dose radiation accurately. In the present study, we thus investigate the influence of irradiated dose and of the fraction of irradiated cells on bystander cell-killing effects using heavy ion microbeam, carbon-ion broad beam and gamma-rays to shed light on the mechanism of bystander effects.

Human lung normal fibroblast cell line WI-38 was cultured with MEM medium supplemented with fetal bovine serum and antibiotics. Using the heavy-ion microbeam cell-targeting system of JAEA-Takasaki [1], about 0.001% of the confluent-cultured cell population in glass-based dishes were irradiated with 0.5 Gy of carbon ions (LET = 103 keV/μm) and 1.9 Gy of neon ions (380 keV/μm). Next, confluent-cultured cells in porous membrane-based inserts were irradiated with 0.125 Gy to 2 Gy of carbon-ion broad beam (108 keV/μm) and gamma-rays (0.2 keV/μm), and the inserts were then placed on corresponding companion plates, in which non-irradiated cells were cultured in the same way as irradiated cells. The fraction of irradiated cells was 33%. Following co-culture between irradiated cells and non-irradiated cells for 6-24 h, non-irradiated bystander cells were trypsinized, counted, and then diluted to a suitable cell density with the culture medium. Diluted cell suspension was transferred into a culture dish and cultured for 14 days to count colonies formed. Colonies comprising 50 or more cells were regarded as survivors. In addition, the concentration of nitrite in the co-culture medium was measured using the method of Saltzman with some modifications [2] to elucidate the mechanism of bystander effects mediated via nitric oxide (NO) radicals.

We investigated the survival of bystander cells following irradiation of carbon- and neon-ion microbeams, carbon-ion broad beam and gamma-rays to elucidate the influence of the fraction of irradiated cells on bystander effects. The survival of bystander cells did not change at 6 h but decreased at 24 h following microbeam irradiation. On the other hand, following irradiation of carbon-ion broad beam at 0.125 Gy and of gamma-rays at 0.5 Gy, survival of bystander cells decreased both at 6 h and 24 h. From these results, it was estimated that radiation-induced bystander effects were dependent on the fraction of irradiated cells as well as time following irradiation.

Next, we investigated the survival of bystander cells at 24 h following irradiation of different doses of carbon-ion broad beam and gamma-rays. Survival of bystander cells co-cultured with the cells irradiated by carbon-ion broad beam and gamma-rays decreased with increasing dose and its reduction was saturated at 0.5 Gy or higher doses. The dose-response curves of bystander cell-killing effects were similar between carbon-ion broad beam and gamma-rays. These results indicated that bystander cell-killing effects were partly dependent on dose but independent of radiation quality.

In the end, the concentrations of induced nitrite in the co-culture medium were measured at 24 h following irradiation of different doses of carbon-ion broad beam and gamma-rays. They increased with increasing dose and saturated at 0.25 Gy or higher doses of carbon-ion broad beam and gamma-rays. The saturation levels of induced nitrite tended to be higher in carbon ions than in gamma-rays. Taking the results of survival and induced nitrite together, we propose the possibility that NO radicals released from irradiated cells contribute to induce bystander cell-killing effects but their effective concentration has an upper threshold.

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SESSION VI. DNA REPAIR

O22. SUBDIFFUSION SUPPORTS JOINING OF CORRECT ENDS DURING REPAIR OF DNA DOUBLE-STRAND BREAKS

[1] Girst S., [1] Hable V., [2] Drexler G.A., [1] Greubel C., [1] Siebenwirth C., [1] Haum M., [2] Friedl A.A., [1] Dollinger G.

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The mobility of damaged chromatin regions in the nucleus may affect the probability of mis-repair. In this work, live-cell observation and distance tracking of GFP-tagged DNA damage response protein MDC1 was used to study the random-walk behaviour of chromatin domains containing radiation-induced DNA double-strand breaks (DSB). The DSB were induced by microbeam irradiation at ion microprobe SNAKE of the Munich tandem accelerator utilising 20 MeV protons (LET 2.3 keV/µm) to induce mainly isolated DSB from this low LET irradiation and 43 MeV carbon ions (LET = 370 keV/µm) to induce tracks of DSB with high DSB density.

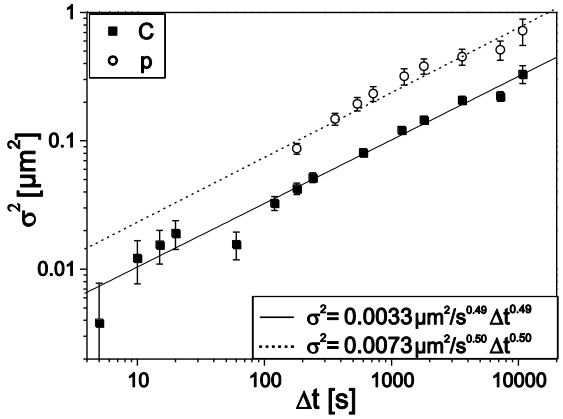

Figure 1: Double-logarithmic plot of the squared standard deviations σ^2 (± SEM) of the distance changes $\Delta l(\Delta t)$ between neighboring MDC1 foci in the nuclei of cells irradiated with carbon ions (filled squares, 657 foci pairs from 58 cells) and protons (open circles, 32 p^+ per irradiation point, 99 foci pairs from 12 cells), each 30-60 time frames analyzed per sample. The data are fitted with the power-law function (eq. (2)).

Our measurements indicate a subdiffusion-type random walk process with similar time dependence for isolated and clustered DSBs that were induced by the 20 MeV proton or 43 MeV carbon ion micro-irradiation (see figure 1). The subdiffusion is characterised to a square root behaviour of the mean squared deviation from the mean from the time differences Δt. It is also manifested in the distance distribution functions that are cusp like instead of Gaussian distributions.

As compared to normal diffusion, subdiffusion enhances the probability that both ends of a DSB meet, thus promoting high efficiency DNA repair. It also limits their probability of long-range movements and thus lowers the probability of mis-rejoining and chromosome aberrations.

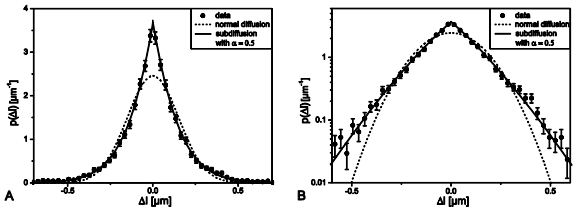

Figure 2: Distribution functions of foci distances for time differences of 60s showing a non-Gaussian, cusp like behaviour that is characteristic for sub-diffusion processes.

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S. Girst *et al*., submitted to *Nature scientific reports*

O23. FROM ENERGY DEPOSITION OF IONIZING RADIATION TO CELL DAMAGE SIGNALLING: BENCHMARKING SIMULATIONS BY MEASURED YIELDS OF INITIAL DNA DAMAGE AFTER ION BEAM IRRADIATION

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Ionizing radiation is an essential tool in the therapeutic arsenal of oncology. Recently, radiation therapy has become more complex with the emergence of new technologies derived from particle accelerators, able to perform conformal irradiations with different radiation qualities of high and low linear energy transfer (LET). This technological evolution has brought new benefits to patients but also new risks. Investigating and quantifying the existence of a causal link between the early effects and the late effects in tissues is a key issue for understanding the effects of ionizing radiation on the body. This study, at the micrometric scale, of the relationship between the topology of energy deposition and the initial biological events (mainly signs of cell damage) is the first step in this process.

The microbeam cell irradiation system is a powerful facility permitting us to hit specific cell nuclei with a pre-determined number of particles with a given pattern of irradiation. Microbeam technology allows studying the putative biological consequences that may happen along a particle track. The absorption of energy from radiation in biologic material may lead to clusters of ionizations and excitations of the target molecule that can generate DNA double strand breaks depending on radiation quality (type and energy of the particle).

The aim of this study is to evaluate the probability of interaction between the particle and the DNA as a function of radiation quality using the analysis of nuclear foci formation such as γ-H2AX and 53BP1 observed by immunofluorescence. The cell nuclei were irradiated with a certain pattern and number of ionizing particles of different types and energies using the microbeam facility at the Physikalisch-Technische Bundesanstalt (PTB).

Cultures of primary cells such as HUVEC cells (Human Umbilical Vein Endothelial) or fibroblasts (Normal Human Dermal Fibroblasts) from Lonza were exposed to 8 MeV or 20 MeV α particles with a respective LET of 160 keV/µm or 37 keV/µm or, also to 10 MeV protons (LET ~5 keV/µm). The karyotype and the genomic stability of cells were evaluated to ensure an equivalent DNA content in all cells of a population. Each cell nucleus was targeted at its center by a pattern of 5 particles placed at the corners of a square of 5 µm x 5 µm and one particle in the middle. Ten minutes after irradiations, the cells were fixed and the immunofluorescence protocol was followed.

The foci analysis is currently based on a platform of high-throughput microscopy including a powerful and robust infrastructure necessary for a massive image analysis (Scan-R software, Olympus). It enables the efficient measurement of numerous topological parameters on foci such as area, shape (i.e. circularity factor, elongation factor, etc.), relative positions in the cell nuclei, density, distance from neighbors, etc. With high-speed microscopy, statistical evaluation of these measurements can be undertaken in a large population of cell nuclei which provides a way to estimate a probability of foci formation related to a particle traversal. These biological measurements will be confronted with results obtained by Monte Carlo simulations, modeling the ionizing particle interactions on a virtual phantom of the cell nucleus with the same mean geometry and DNA density as the HUVEC and Fibroblast cells used. This should allow studying the relationship between the topology of energy deposition and early cell damaging.

For a better comparison between simulations and foci observations, some biological uncertainties and beam parameters have to be taken into account. Background foci due to cells in the division phase of the cell cycle and also due to the methodology of nuclei detection for microbeam irradiation using Hoechst staining have been considered. The protocol of cell seeding, localization of cell

nuclei and data filtering performed after image analysis were optimized and allowed us to reduce the impact of foci background on the final measurements. In addition, microbeam parameters, such as the beam size, detection thresholds and noise events in the particle counter, may also play a key role on the foci distribution observed. They can affect the initial pattern of irradiation leading to the deformation of the square pattern of irradiation with some hit out of the nucleus and some too close to each other resulting in the formation of one big focus instead of two. A script using Matlab has been developed to simulate how the beam size, but also the detector performance and the nucleus dimension, can disturb the initial pattern of irradiation and to calculate the probabilities of occurrence of different situations.

Knowing the different factors that drive the experimental uncertainties, we will be able to take them into account for the interpretation of the results. This allows us to obtain an accurate estimation of the probability of interaction, leading to foci formation, between particles of different LET and DNA.

This work is carried out within EMRP (European Metrology Research Programme) Joint Research Project SIB06 BioQuaRT (Biologically weighted Quantities in RadioTherapy). The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

O24. EFFECT OF A HISTONE DEACETYLASE INHIBITOR (SAHA) ON THE DIAMETER OF γ-H2AX FOCI AFTER MICROBEAM IRRADIATION

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Suberoylanilide hyroxamic acid (SAHA) is a Histone Deacetylase (HDAC) inhibitor and is known to modulate chromatin structure by hyperacetylation of histones. SAHA can increase radio-sensitivity for X-rays and Carbon ions for some cell lines [1,2], and is thought that SAHA interferes with DNA double strand break (DSB) repair. This experiment investigates the quality of γ-H2AX foci caused by proton irradiation of normal human skin fibroblast AG01522 cells treated with SAHA with a focused MeV ion beam.

A beam of 3.8 MeV H^+ ions, focused to a 3 µm beam diameter using the Surrey Ion Beam Centre Vertical Beamline (VNB) [3], was used to individually target single AG01522 cells plated in a 1 mm diameter droplet on a polypropylene cell dish. Samples were prepared with SAHA concentrations of 0.5 µM and 1 µM. 100 cells per sample were individually targeted and irradiated with precisely counted ions, of either 50 or 100 protons. The cell dishes were then processed with γ-H2AX and each irradiated cell revisited using a Nikon fluorescent microscope for foci observation.

Observation of the diameter of γ-H2AX foci created in the presence of SAHA indicates a difference in the damage to DNA from proton irradiation with an LET of 12 keV/µm. This difference may be due both to the relaxing effect to chromatin structure and disruption of DSB repair pathways caused by SAHA.

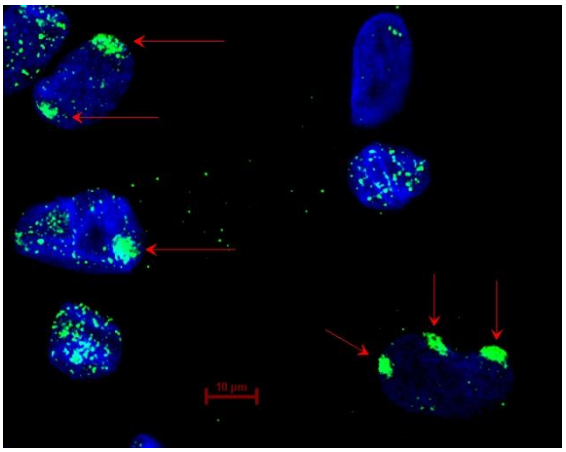

Figure 1: γ-H2AX foci in AG01522 fibroblast cells after 1 Gy irradiation with a 3.8 MeV proton microbeam.

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O25. STUDY OF RADIATION-INDUCED CHROMOSOME DAMAGE AT MICROBEAM FACILITIES: DEVELOPMENT OF A NOVEL IN SITU PROTOCOL DESIGNED WITHIN THE "BIOQUART " PROJECT

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The biological effects of single charged particles cannot be simulated by *in vitro* conventional broad-beam exposures, due to the random Poisson distribution of particle tracks traversing the target cells. Charged-particle microbeam facilities were designed to target the nuclei or cytoplasm of single cells with a predefined number of particles and to analyze the induced damage on a cell-by-cell basis. In such a way, the radiation-induced cell damage can be directly correlated to type and energy of the radiation and to the number of ions per cell. Within the "*BioQuaRT"* (Biologically weighted Quantities in RadioTherapy) Project, we developed an *in situ* protocol for the analysis of the unrepaired chromosome damage induced by charged particles irradiations at the PTB microbeam facility. The development of a special *in situ* assay was required in this microbeam irradiation system, because only a very limited number of cells (about 3000 cells/dish) could be seeded on the thin base made from a BioFoil (25 µm thick) of the specific irradiation dishes at PTB. This method was developed on Chinese Hamster Ovary (CHO) cells, among the most commonly used cell lines in *in vitro* radiobiology experiments. This present protocol has the great advantage of allowing the simultaneous scoring of chromosome aberrations and micronuclei on the same irradiated dish. Although this method was developed for single-ion microbeam studies, it could be extended to other radiobiological applications requiring the use of *in situ* cytogenetic assays in case of restricted experimental conditions.

This work was carried out within EMRP Joint Research Project SIB06 "BioQuaRT". The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

O26. TARGETED MICRO-IRRADIATION OF *C. ELEGANS* EMBRYO

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The capabilities of targeting single cells using a charged-particle microbeam significantly contribute to the knowledge of radio-induced biological effects at the cellular level. However, while useful, *in vitro* experiments have limitations and do not mimic a realistic biological response. Recent researches have shown that i*n vivo* studies using living organisms are needed to elucidate more complex mechanisms involved in the radiation-induced response [1, 2].

Recently, a charged particle microbeam has been designed at the CENBG (Centre d'Etudes Nucléaires de Bordeaux-Gradignan). This facility allows performing *in vitro* targeted micro-irradiation on living cellular models with a high accuracy. The aim of our work was to adapt experimental procedures to irradiate *in vivo* multicellular models. For this purpose, early *Caenorhabditis elegans* embryos were selectively irradiated with 3 MeV protons in a dose dependent manner. The induction of DNA damage and their consequences along the embryonic cell division were evaluated using time-lapse microscopy.

C. elegans strains and culturing: WS1433 (opIs34[hus-1::GFP]) and GZ264 (isIs17 [pie-1::GFP::pcn-1 + unc-119(+)] were obtained from the Caenorhabditis Genetics Center. The MG152 strain (xsIs[hisH2B::GFP + rol-6(su1006)] was given by Denis Dupuy (Gene regulation and evolution, IECB, INSERM U869, France). Animals were maintained at 25°C as previously describe [3].

C. elegans micro-irradiation. A horizontal charged-particle microbeam facility has been designed at CENBG on the AIFIRA platform (Applications Interdisciplinaires des Faisceaux d'Ions en Région Aquitaine). Using this facility, two-cell stage embryos were selectively targeted in the AB cell nucleus and exposed to 3MeV protons. The induction of radiation-induced DNA damage along the embryonic cell division was observed using time-lapse microscopy. The defined number of protons delivered within the embryo was detected downstream of the sample using a high efficiency particles detector. The deposited dose has been evaluated with the Geant4 toolkit.

We have developed an experimental procedure to selectively irradiate a multicellular model using a microbeam facility. For this purpose, the AB cell nucleus of a two-cell stage WS1433 *C. elegans* embryo, expressing the radiation-induced DNA repair protein HUS-1::GFP, was selectively irradiated in an absolute dose control manner. The accumulation of HUS-1–GFP protein at the DNA damage sites was observed few minutes after irradiation using time lapse microscopy and revealed persistent radiation-induced DNA damage along the cell division. The chromosomal integrity was also considered after targeted irradiation along the cell division. Two cell-stage MG152, expressing H2B::GFP fusion protein, and GZ264, expressing PCN-1::GFP fusion protein, were selectively irradiated and the dynamics of chromosomes segregation was observed in real-time. The induction of chromosome alterations along the embryonic cell division was revealed by the visualization of inter-chromosome bridges.

These data demonstrate our ability to specifically irradiate a single cell nucleus in a living multicellular organism in an absolute dose control manner. We were also able to target a cell nucleus in a specific cell division stage and to visualize and track a DNA repair protein *in vivo*. Finally, we have demonstrated our ability to follow the fate of radiation-induced DNA damage in real-time and in function of the incident dose. This experimental approach will be suitable to evaluate the relation between dos of ionizing radiation and radiation-induced biological effects on *C. elegans* from individual to populations.

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SESSION VII. POSTERS

P1. DEVELOPMENT OF A HIGH RESOLUTION SINGLE CELL IRRADIATION FACILITY (SCIF)

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There are about 20 SCIF systems worldwide using either ions of X-rays for irradiation of single cells and tissues with high resolution microbeams. The topic has its own series of conferences: the latest one will be an 11th International Workshop on Microbeam Probes of Cellular Radiation Response. However, the proposed system in Australia will be the world first which will offer sub-micron resolution for X-rays and with energies from 2 to 25 keV. A schematic overview of the planned X-ray microscopy facility at the Australian Synchrotron is given in Figure 1.

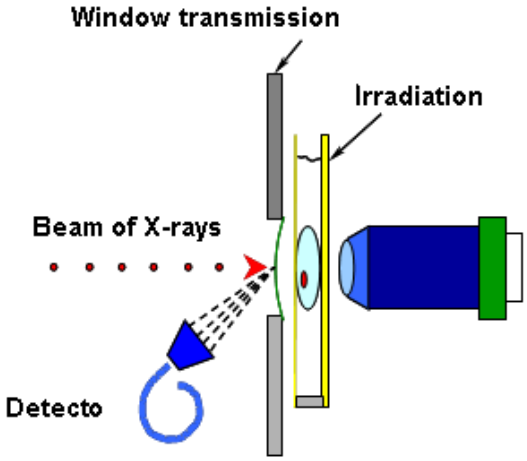

Figure 1: Schematic view of the Single Cell Irradiation Facility at the Australian Synchrotron. Beam of X-rays will be focused to 100 nm with zone-plate focusing system. Irradiation dish will enable to keep cells alive during the experiment. And the Microscope will enable to observe cells before and after irradiation with the beam. The dose will be monitored by the special detector or ionization chamber.

The single-cell irradiation project requires a close collaboration within mixed teams of specialists in biology, radiation biophysics, oncology, nanotechnology, synchrotron radiation, and solid state physics. We shall investigate biological effects of X-ray irradiation as a function of (i) energy, (ii) track location within the cell, (iii) number of X-ray tracks, (iv) cell species (V79, mouse fibroblasts, commercially available cultures of normal and pathological human cells), (v) cell state (cell cycle, functional status etc.). Using standard bio-medical assays and new solutions for the nanobiosensors we shall study the cellular response to radiation (survival or final destruction by necrosis or apoptosis), signalling pathways during processes of apoptosis (e.g. the bystander effect) or pathways of repair processes of the radiation induced damage. We shall also investigate mutagenic effects in surviving clonogenic cells. This facility will provide additional information for Microbeam Rdiation Therapy (MRT) which has been developed at the Australian Synchrotron.

System	Energy (keV)	Flux (ph/s×μm^2)	Dose rate (Gy/s)	Comments
Synchrotron based X-ray sources				
Photon Factory	5.35	10^2	0.3	Beamline BL27B
Australian Synchrotron	7	~$3x10^9$	~$3x10^7$	Energies 2-25 keV
Laboratory based X-ray sources				
Central Research Institute of Electric Power Industry, Tokyo, Japan	1.49	~$6x10^1$	1.0	4 μm^2
Laboratory based system at the Institute of Nuclear Physics, Krakow, Poland	4.50	~$1x10^3$	0.7	5 μm^2

Table 1: Comparison of SCIFs for X-ray microbeam

P2. THERMAL SIMULATIONS OF SQUID BASED MICRO-CALORIMETERS USED UNDER MICROBEAM IRRADIATION

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The increase in the number of proton and ion therapy centres worldwide (43 in operation and 24 proposed centres) necessitates a better understanding of the biological effect of such modalities [1]. Microdosimetry, the study of the initial energy deposition patterns at the length scale of the cell nucleus, is one of the quantitative methods used for comparing different types of radiation in Radiotherapy that can be used to predict relative biological effectiveness [2]. The present novel study is part of feasibility performance and development of a SQUID based micro-calorimeter that unlike other microdosimetry approaches provides a direct measurement of energy deposition at the micrometre scale.

The DC Superconducting QUantum Interference device (SQUID), Figure 1, measures the change in magnetic flux passing through the loop, equation (1).

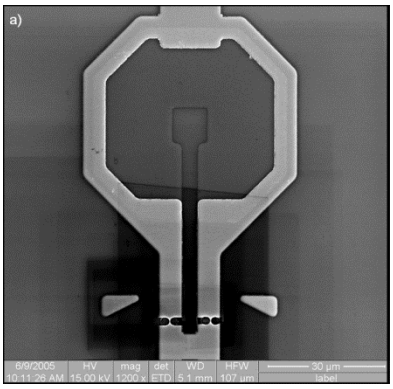

Figure 1: Inductive Superconductive Transition Edge Detector (ISTED) which consists of a DC SQUID and a centrally located superconducting absorber [3].

Energy is deposited from the passage of protons through the absorber within the SQUID loop. This causes a rise in temperature and the breaking of Cooper pair electrons into normal electrons. As a result the superconducting penetration depth of the thin film superconducting absorber changes, changing its effective area [4]. This in turn leads to a change in the output signal from the SQUID. Temperature rises of less than 1μK are readily detectable and when combined with the low specific heat of the absorber at cryogenic temperatures this leads to extremely high energy sensitivity [5]. A SQUID microcalorimeter of this type can have a sensitivity 1,000× that of semiconductors or 10,000× that of gas filled ionisation chambers [6].

$$\lambda\ T\ = \frac{\lambda(0)}{[1-(\frac{T}{T_c})^4]^{1/2}} \quad (1)$$

In practice the energy deposited in the absorber is required to be measured which needs to be related to energy deposition in the tissue equivalent absorber. The Comsol Multiphysics software was employed to determine the latter relation through solving of the heat equation for real case situations using the finite element method. As a preliminary investigation, an average 3.8MeV energy proton Percentage Depth Dose curve was modelled to interact with the Graphite and the resultant heat dissipation was recorded. The average temperature rise in each layer was than measured as an indication of the energy deposited within the layer.

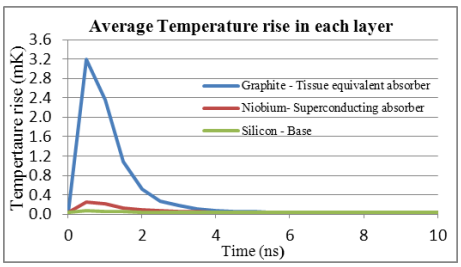

Figure 2: The average temperature rise over the entire volume of each layer is illustrated.

Simulations were performed for different thermal and interaction conditions. It is concluded that the temperature measurements in the tissue equivalent absorber can be directly related to that of the superconducting absorber. The temperature rise in the model is much greater than the threshold for the detector to produce a measureable signal. We are in the process of measuring the detector response experimentally to compare the findings.

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P3. DIGITAL IMAGE PROCESSING TECHNIQUES AT SURREY VERTICAL BEAMLINE FOR AUTOMATED SINGLE CELL IRRADIATION

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The Surrey Vertical Nanobeam (VNB) is able to target and irradiate single living cells or cells' internal structures with size less than 10 nm with single ions. The facility is designed to produce a range of ions from protons to calcium with energies from 0.5 to 12 MeV. A digital end-station microscopy facility is mounted on top of the beamline to provide the imaging means. Currently, the microscopy facility provides automated targeting and irradiation of single cells in fluorescence microscopy (Merchant et al., 2012). However, bright-field illumination microscopy relies on the advantage that cells can be imaged without any staining. Thus, it assists in avoiding the use of toxic dyes allowing the cells to physiologically evolve in the cell dish. Additionally, the VNB presents the capability of irradiating a maximum of 100,000 cells per hour, a performance that can be reached only with automated digital image processing techniques.

In this abstract, we briefly present some results of application of digital image processing techniques in bright-field illumination microscopy. The goal of this on-going project is to develop an automatic procedure in cells recognition, targeting and irradiation in order to provide a bulk single-cell irradiation, exploiting, simultaneously, the VNB capabilities for this type of specimen illumination.

The microscope is designed to provide either Bright-Field or Fluorescence microscopy images. The end-station facility can operate either in a 'region-scan' mode where a region of a cell dish is scanned acquiring images of sequenced sub-regions or in 'time-lapse' mode where a small region is selected and imaged within defined time intervals. Both modes can be used in bright-field or fluorescence microscopy. The images are saved in ICS (Image Cytometry Standard) format which allows a 'metadata' file to accompany every image with acquisition-related parameters.

However, bright-field acquired images depict not only cells but also other structures like debris, areas of uneven illumination or even artefacts due to the dish bottom material internal structure (i.e. polypropylene). On the other hand, cells appearance change as they progress through the cell cycle phases or if they are in an unhealthy condition (i.e. apoptosis).

In order to perform image processing and analysis, MATLAB® (The MathWorks, Inc., Natick, MA) has been used due to its capability of integrating various built-in toolboxes (e.g. Image Processing Toolbox™) with prototyping language. The goal of the applied image processing techniques is to produce a binary mask for each cell. Various techniques have been applied in order to build a cells recognition algorithm. Wavelets have been incorporated to denoise the acquired images, a technique that has substantially assisted in the following steps of cells recognition (Kingsbury, 1999). A combined top-hat transformation and edge detection technique has been applied in order to eliminate the background. An adaptive (from image to image and pixel to pixel) threshold has been used in order to determine undesired frequencies. The convex hull determines the cells' boundaries while morphological operators finalise the binary mask. Shape analysis determines the type of cells that are present in the image. Geometrical, statistical and textural features are extracted for further analysis. Moreover, the whole procedure has been automated for extracting cells from a bulk of images. A file of cells' centroids is extracted and can be directly fed to the irradiation system in order to automatically irradiate them.

Acknowledgements. The authors gratefully acknowledge the financial support of EC FP7 Marie Curie ITN and, particularly, the project ENTERIVISION as well as Ms Miriam Barry for providing numerous images for analysis.

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P4. MIRCOM: A NEW MICRO-IRRADIATION BEAMLINE ON THE IRSN AMANDE FACILITY

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The AMANDE facility, developed and operated by the Institute for radiological protection and nuclear safety (IRSN) in Cadarache, is used to produce reference monoenergetic neutron fields. This facility is based on a 2 MV Tandetron™ accelerator manufactured by HVEE [1]. In order to study the biological effects of ionizing radiations at the cellular scale, IRSN, in collaboration with the Centre d'Études Nucléaires de Bordeaux - Gradignan (CENBG), has decided to install on AMANDE an ion microbeam designed to perform targeted micro-irradiation of biological samples in vitro. In addition to the beamline itself, modifications on the Tandetron™ accelerator will be carried out to provide additional ion species, and an extension of the existing building will be realised to host the microbeam along with a fully equipped biology lab.

The modifications on the accelerator part will be carried out by HVEE. In addition to the existing multi-cusp ion source, able to provide protons and deuterons, two new sources will be installed. A second multi-cusp ion source will be mounted next to the existing one to provide He ion beam generation, with an energy up to 6 MeV. For the production of heavier ions, a new injector, with a multi-target sputter ion source, will be installed. It will allow the productions of B, C, N and O ion beams, with energies up to 8 MeV.

The new beamline will be hosted in an extension of the existing AMANDE building. This extension has been designed to comply with the constraints of a microbeam, such as mechanical movements (vibrations) and temperature stability. The microbeam room will be coupled with a fully equipped biology lab where cell culture, sample preparation and analysis will take place.

The micro-irradiation beamline itself is the fruit of a collaboration between IRSN and CENBG. It is based on an existing horizontal micro-irradiation beamline, developed and operated by CENBG on the AIFIRA (Applications Interdisciplinaires des Faisceaux d'Ions en Région Aquitaine) platform. The IRSN beamline is currently being built at CENBG. Its spatial resolution is expected to be around the micrometre under vacuum using a quadruplet of magnetic quadrupoles. Electrostatic scanning plates are inserted between the last quadrupole and the target to provide a precise and fast positioning of the beam spot on target. For cell irradiation, the beam is extracted in air through a 150 nm thick Si3N4 window. The culture dish is placed vertically in front of the extraction window, and a precise sample positioning stage has been developed to move it in the target plane. The cells are visualized and targeted online using an epifluorescence microscope (AxioObserver™ Z1, Carl Zeiss, Germany) positioned horizontally at the end of the beam line. This microscope is fully motorized and remote controlled. One of the main features of the beamline is the ability to perform online time-lapse microscopy. This feature allows the study of the dynamics of radiation response a few seconds after irradiation. All the calibration, irradiation and time-lapse microscopy processes are controlled using custom made software.

The performances of the beamline for alpha particles and protons are expected to be similar to those of the CENBG beamline: a beam spot size in air around 2 µm, and a targeting accuracy of ± 2 µm.

Three different particle detectors will be used on this beamline: a low pressure gas detector for alpha particles, a scintillation detector for protons, and a secondary electrons detector for heavy ions.

The modifications on the AMANDE accelerator will be carried out from April to November 2013, followed by the construction of the extension building until the end of 2014. The installation and commissioning of the beamline will take place next. The first irradiation of biological samples is expected in 2015.

Acknowledgments. This work is part of F. Vianna's PhD thesis, supervised by Ph. Moretto, Ph. Barberet & V. Gressier. The CENBG micro-irradiation beamline has been developed by Ph. Barberet & H. Seznec. The authors thank the technical staff of CENBG for their help in the technical and design developments.

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P5. PROPOSED 100 NANOMETER DIAMETER PROBE AT RARAF

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We began operating our microbeam at RARAF in 1994 using a 5 µm diameter aperture system. We have steadily reduced the beam size by successive systems comprising electrostatic quadrupole lenses first as single multiplets and then then compound multiplets. See Bigelow, *et. al.* [1] for a review. The present system utilizes a pair of identical triplet lenses in series in a 3.8 m long vertical beam line. In normal operation we use a resolution of 0.6 to 1 µm diameter. We are limited now to a minimum beam spot of 0.4 µm by spherical and parasitic aberration inherent in quadrupole focusing systems.

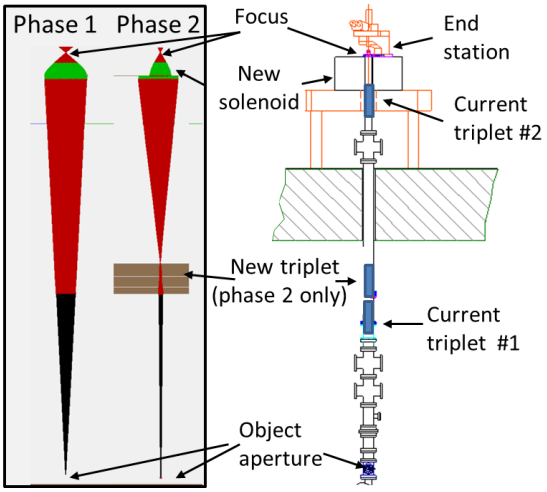

FIG. 1: LAYOUT OF THE SUPER MICROBEAM. THE INSET SHOWS CALCULATED BEAM PROFILES FOR PHASE 1 AND 2.

Our present resolution is entirely adequate for the experiments that are routinely performed at RARAF, namely irradiating cell nuclei other sub cellular regions of cells and for bystander studies. New developments in marking proteins with fluorescent tags is opening up an exciting class of experimental sub cellular targets which are smaller than our present capabilities. We decided that we should continue to keep our facility on the cutting edge by designing the next generation of microbeams. We want a design that will produce a beam smaller than 100 nm. We propose to construct a compound system comprising an electrostatic quadruplet followed by a solenoid.

Calculations involve two stages. First, using an assumed coil cross sections and current density, calculate the resulting field profiles using the program Superfish. Second the field strengths are used as input for the ray-tracing program, SIMION-8. Ray traces using various object sizes and limiting apertures are performed to determine the minimum beam spot dimensions. These calculations are performed iteratively to find the optimum design.

We will show examples of the calculation and the expected beam diameter vs. the object aperture diameter where the angle limiter is chosen to provide the same count rate that we obtain our present double quadrupole triplet system. We conclude that the proposed system can provide a beam with a 75 nm diameter and good count rate.

This work is supported by The National Institute of Biomedical Imaging and Bioengineering under Grant NIBIB 5 P41 EB0002033-17

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P6. THE DOSIMETRIC IMPACT OF LUNG TUMOUR MOTION IN STEREOTACTIC ABLATIVE RADIOTHERAPY

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Stereotactic Ablative Radiotherapy (SABR) is gradually becoming a very widely used technique in the treatment of early stage Non-Small Cell Lung Cancer. Its superiority over surgery is driving it in becoming the first treatment option [1,2]. However, the concern regarding the presentation of unfavourable interplay effects in the delivery of SABR still remains [2,3]. The combination of internal organ motion and the use of advanced radiotherapy techniques such as Intensity-Modulated Radiotherapy (IMRT) and Volumetric-Modulated Arc Therapy (VMAT), which are both involving multi-leaf collimator modulation of the dose, results in discrepancies between the calculated and the delivered dose deposition (4,5). This projects aims to experimentally compare and contrast the dosimetric differences of Conformal, IMRT and VMAT techniques on different breathing patterns and different tumour sizes.

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P7. REDUCTION OF MUTATION IN BYSTANDER CELLS CAUSED BY NITRIC OXIDE-MEDIATED BYSTANDER CELL DEATH

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Radiation-induced bystander responses had a great impact on radiobiology, because they may have important implications for the estimation of risks to human health associated with exposure to low-dose radiations. The microbeam cell irradiation system, which enables observation of cellular responses of individual irradiated and non-irradiated cells with an equal efficiency, is a powerful tool for the elucidation of mechanisms underlying the biological responses to low-dose radiations, including bystander responses. Using a synchrotron X-ray microbeam irradiation system developed at the Photon Factory, High Energy Accelerator Research Organization, KEK, our group demonstrated that bystander cell death was biphasically enhanced in a dose-dependent manner (1). Moreover, we found a dose-dependent biphasic reduction of mutation in bystander cells (2). Here, we analyzed the relationship between bystander cell death and mutation in bystander cells, as the next step.

V79 cells were seeded on custom-designed dishes with a polypropylene (3-μm-thick) base (1.0×10^5/dish) and incubated overnight. We irradiated 5 target cell nuclei with 10×10 μm^2 of 5.35 keV X-ray beams. After 3-h incubation, the cells were harvested by trypsinization, and the fraction of bystander cells that survived was measured using the conventional colony-formation assay. We also measured the mutation frequency using an *HPRT* mutation assay. Recently, we showed that nitric oxide (NO) is a principal mediator of bystander cell death (1). Therefore, we investigated the role of NO in bystander cell death and mutations. During the colony assays for the measurement of surviving fractions and of mutation frequencies, the cells were also incubated with a medium containing carboxy-PTIO, a specific scavenger of NO, instead of normal fresh medium.

The surviving fraction of bystander cells decreased to 0.87 ± 0.015 when nuclei were irradiated with 1 Gy; however, at higher doses, the surviving fraction was stable at approximately 0.94 (3). As shown in Figure 1, the mutation frequency at the null radiation dose was 2.6×10^{-5} (background level), and the frequency at a dose of approximately 1 Gy decreased to 5.3×10^{-6}. At high doses, the mutation frequency returned to the background level (3). A similar biphasic dose-response effect was observed during bystander cell death. Furthermore, we found that incubation with carboxy-PTIO suppressed not only the biphasic increase in bystander cell death but also biphasic reduction in mutation frequency of bystander cells (Figure 1) (3).

These results clearly show that NO plays an important role not only in the induction of death of bystander cells but also in the suppression of spontaneous mutagenesis in bystander cells. This study showed that radiation-induced bystander responses could affect processes that protect cells against naturally occurring alterations such as mutations.

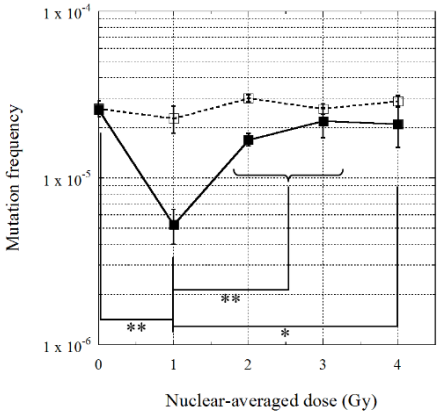

*Figure 1: HPRT mutation frequencies in bystander cells. Cells were incubated with (□) or without (■) carboxy-PTIO. * and ** represent P < 0.05 and P < 0.01, respectively.*

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P8. HYDROGEN SULFIDE SUPPRESSES RADIATION-INDUCED BYSTANDER EFFECT ON HYPOXIC HEPATOMA CELLS

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Radiotherapy is becoming an important therapeutic method for hepatocellular carcinoma. Growing evidence has shown that cells in response to radiation may release transmissible factors capable of induction of cellular responses in unexposed cells, which is refereed as radiation-induced bystander effect (RIBE). RIBE may have two-side effects on radiotherapy by enhancing tumor cell killing efficiency but increasing the secondary cancer risk once genetic damage occurred in the bystander normal cells. Solid tumor always contains a hypoxic area due to chronic insufficiency in blood supply. The radioresistance of hypoxic cells may result in unfavorable prognosis effects on tumor therapy. It has been suggested that, since radiation-induced DNA damage can not be fixed by oxidization and then is easy to be repaired under hypoxia condition, the hypoxic tumor cells have a higher ability to survive from harmful stresses. Endogenous hydrogen sulfide (H_2S) is a newly-found gasotransmitter that plays bio-regulation roles similar to NO and carbon monoxide in the body, especially in the cardiovascular and nervous system. H_2S can be endogenously synthesized in various mammalian tissues by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Endogenous H_2S might be a new anti-oxidation and protects organisms against hypoxic damage. However, it is unclear whether endogenous H_2S can contribute to RIBE under hypoxic condition and if so what is the mechanism. The roles of H_2S in hypoxia-induced radioresistance and RIBE were investigated in the present work.

Results showed that, when HepG2 cells were maintained in hypoxia circumstances for 4h, the cellular radioresistance was extensively increased so that the oxygen enhancement ratio of the survival fraction approached 2.68. Under this hypoxia condition, when the cells were treated with DL-propargylglycine (PPG) and aminooxyacetic acid (AOAA), a specific inhibitor of H_2S synthase of CSE and CBS respectively, radiation responses including cell killing, micronucleus (MN) formation, and caspase-3 activity were significantly enhanced. However, treatment of cells with low concentrations of NaHS (≤100μM) protected cells from these radiation damages. Western bolting assay showed that CSE and CBS were over-expressed in the irradiated hypoxic cells in a dose dependent manner. Moreover, when the hypoxic HepG2 cells were treated with NaHS together with glibenclamide, a specific inhibitor of K^+_{ATP} channels, the role of exogenous H_2S in radioprotection was partly eliminated.

More importantly, it was found that MN were induced in the non-irradiated cells after treatment with conditioned medium (CM) harvested from irradiated cells under hypoxia condition. This bystander effect was diminished when the irradiated cells were pretreated with NaHS, but on the contrary, it was increased when the irradiated cells were pretreated with an inhibitor of the synthase of H_2S. Interestingly, the expressions of CSE and CBS were reduced in the CM-treated bystander cells. Moreover, the activity of caspase-3 increased in the hypoxic bystander cells and the ratio of Bcl-2/Bax decreased along with the CM treatment time, which could be regulated by both NaHS and the inhibitor of endogenous H_2S.

This study also demonstrated that H_2S contributed to hypoxia-induced radioresistance probably via the opening of K^+_{ATP} channels. Moreover, under hypoxia condition the irradiated hepatoma cells could induce bystander responses by depressing the generation of endogenous H_2S and stimulating apoptosis in the bystander cells. Based on the current data and our previous investigations, an outline of H_2S-regulated signalling pathways in RIBE was shown in Fig.1. These findings suggest that the endogenous H_2S synthase could be a potential radiotherapeutic target for a hypoxic tumor.

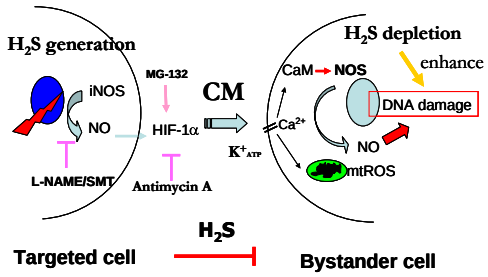

Figure 1: A schematic of H_2S-regulated signal pathways in RIBE

P9. THE NON-TARGETED EFFECTS OF LOW-DOSES OF IONISING RADIATION AND POTENTIAL RISK OF CANCER

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Proton therapy is a promising treatment modality for cancer which has distinct advantages over conventional radiotherapy. This includes its ability to deliver a very high dose of radiation to deeply or critically located tumours and thus minimizing radiation dose to the surrounding normal tissues [1]. Many proton therapy facilities are currently operating or under construction world-wide. Moreover, treatment of cancer with ion beam is considered as a rapidly developing field of research [3]. However, concerns still exist because of the rare but severe potential effects of the minimal low doses of radiation, which might be received by non-targeted normal cells or tissues adjacent a targeted tumour volume during proton therapy treatment [4]. Radiation-induced non-targeted effects, such as genomic instability (GI) have been proposed as a very early stage in radiation carcinogenesis. Many studies have identified genomic instability in a variety of cell lines immediately post-irradiation. In these studies, however cells involved were mostly tumour driven cell lines [2]. The novelty of this project is to investigate the potential induction of genomic instability in the progeny of irradiated normal human skin fibroblasts using low doses of radiations *in-vitro*. Our data shown that at a single X-ray dose of 0.2 Gy, the induction of γH2A.X foci and giant cells was increased in the progeny of irradiated cells compared to unirradiated controls or cells irradiated to higher doses (> 1 Gy). These results indicate a probability of genomic instability induction (which relates to cancer) at low doses of X-ray irradiation.

The normal human skin fibroblasts (AG01522D) purchased from Genetic Cell Repositories at Coriell institute for Medical Research (IMR) (Camden, USA) and maintained according to the recommended protocols. Cells prepared for irradiation in the Vertical Nanobeam Laboratory at University of Surrey. X-ray irradiations performed at Royal Surrey County Hospital using a Gulmay kilovoltage therapeutic unit. The unit was operated at 250 kVp, 12 mA and a dose rate of 0.6 Gy/min. Irradiations delivered to the targeted cells as a single dose of 0.2, 0.4, 0.6, 1, 2, 3, 4 and 5 Gy. After irradiations, all treated cells were incubated and allowed to proliferate (2-3 weeks) until the time at which they were fixed and stained for analysis. Then, clonogenic survival, γH2A.X staining and giant cell assays were used to identify and evaluate the effect of radiation in the progeny of irradiated cells. The images of γH2A.X foci and giant cells formations were captured using time-lapse fluorescence microscope. The surviving curve was constructed by OriginLab 8.6 software using Linear Quadratic (LQ) and Induced Repair (IndRep) models.

Cells irradiated with a dose of 0.2 Gy of X-rays were shown high levels of giant cells and γH2A.X foci formations in their progeny compared to controls and cells irradiated with higher doses of 2-5 Gy. This co-indicated with the hyper-radiosensitivity (HRS) region observed in the survival curve of AG01522D cells (data not shown). As expected, the cloning efficiency (CE) was decreased in a dose-dependent manner, which coupled with the downward binding of the survival curve, indicating a delayed cell death (associated with GI). These results suggest that low doses of X-rays have a hyper-radiosensitive effect on genomic instability and cancer induction in the progeny of irradiated AG01522 cells.

The X-ray results support the emerging link between the non-targeted effects of low doses of radiation and the potential induction of hyper-radiosensitivity and genomic instability in the progeny of irradiated cells. While they still remain to be confirmed, these effects could occur when using low doses of heavy charge particles, such as protons and they may have a significant contribution to cancer induction or progression.

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P10. EVALUATIONS OF GENOTOXIC EFFECTS AFTER 2.45 GHZ RADIOFREQUENCY RADIATION (RFR) EXPOSURE IN MALE MOUSE GERM CELL LINE

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Whether radiofrequency radiation (RFR) could cause DNA damage remained highly debated. In the present in vitro study, we used 2.45 GHz radiofrequency radiation at an average special absorption rate (SAR) of 4 W/kg to expose cultured male mouse germ cell line GC-2. CCK-8 assay was used to detect cell viability. Alkaline comet assay, immunofluorescence of γ-H2AX nuclear foci were used to identify DNA damage. Flow cytometry was used to confirm cell cycle arrest. Annexin V/PI double staining was used to detect apoptosis. The results demonstrated that the exposures of 2.45 GHz RFR at the SAR of 4 W/kg for 24 h in GC-2 caused cell cycle delay with a concomitant increase of histone H2AX phosphorylation, which was linked to the increase of DNA double-strand breaks, but using comet assay, we did not identify that RFR can induce DNA damage. We presumed that γ-H2AX detection is more sensitive than comet assay for detecting DNA damage. These results indicated that RFR induced DNA damage which was highly associated with cancer and reproductive disorder.

P11. IMPACT OF 1800 MHZ RADIOFREQUENCY ELECTROMAGNETIC RADIATION ON DNA BASE IN A MOUSE SPERMATOCYTE-DERIVED CELL LINE

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Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can produce genotoxic effects in male germ cells remains controversial. In the present study, we conducted a 24 h intermittent exposure (5 min on /10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1, 2 or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

Keywords: Radiofrequency electromagnetic radiation; DNA base damage; Reactive Oxygen Species; Comet assay; Germ cell line.

P12. POST-IRRADIATION BLEACHING OF GFP TRIGGERED BY SHORT PULSE PROTON IRRADIATION

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We present current experiments at our micro-beam cell irradiation at the pelletron accelerator laboratory at RIKEN. In contrast to other microbeamlines, we use tapered glass capillaries with a thin window at its outlet for focusing protons or helium ions with up to 3 MeV/u down to diameters below 1 µm [1-3]. Furthermore, the ions reach the cells from above at an angle of 45 degrees, such enabling the usage of standard cell dishes without the need of special cell preparation.

Presently, we are researching the effects of high dose irradiation of living cells with subcellular resolution. We recently have discovered a post irradiation bleaching of GFP stained nuclei of HeLa cells, being triggered by a short high dose proton irradiation of the nucleus (see Fig. 1). This effect is different from usual photobleaching, since it only occurs in live cells, and does not depend on the presence of blue excitation light.

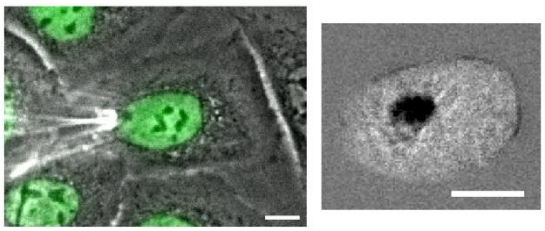

Figure 1: Left: HeLa cells together with the tip of the tapered capillary (window size: 3 µm°. Shown is the merged phase contrast and fluorescence image. Right: Difference of the fluorescence images before and 60 s after irradiation of the nucleus. The bleached area is visible as a dark spot. Scale bars are 10 µm.

The Hela cells we use have been genetically altered such that they express GPF fused to histone H2B. They were seeded in 35 mm Petri dishes two days before irradiation. During the experiment, nuclei have been irradiated during 1 s with 1 MeV protons, with doses ranging from 5000 to 10^5 protons. Directly after irradiation, the bleached area starts expanding while the total fluorescence signal in the irradiated area decreases exponentially with a half time of typically 30 s. Figure 2 shows an example of this bleaching taking place after irradiation of 25000 protons.

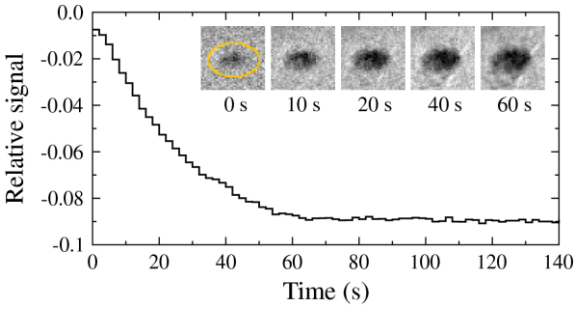

Figure 2: Development of the GFP bleaching following irradiation. The pictures (width 10 µm) show the bleaching at different times after irradiation. The graph shows the relative mean signal of the area within the orange oval (first picture) in dependence of time after irradiation.

Currently, we are further investigating this effect which indicates the presence of physiological responses in mammalian cells induced by proton irradiation.

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P13. PROTON THERAPY: BETTER FOR THE WHOLE BODY?

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Proton therapy gives a conformal dose to a tumour unlike in traditional X-ray radiotherapy. However, there is inevitably some dosage received by normal tissue, despite the Bragg peak being deposited within the tumour. With proton therapy coming to England the benefits are becoming well understood. However, we still need to understand the effects upon the whole body after proton therapy.

It has been shown that after standard X-ray radiotherapy DNA double strand breaks (DSB) are 14 times higher per lymphocyte 30 minutes after 2Gy irradiation and, after 24 hours the DSB count was still 2 and a half times higher than in control blood (Fleckenstein et al., 2011). Studies investigating the effects of X-rays in irradiated tissues have shown an average of 9-14 foci of γ-H2AX per Gy (Mandina et al., 2011; Redon, 2009), with some of these studies showing that after 8 hours 20% of the foci remain (Redon, 2009). Therefore, clinicians are also interested in the whole body effects of proton therapy after treatment.

Here we examine the DSB response using γ-H2AX. To do this, human lymphocytes were irradiated with the Surrey Vertical Beam using doses ranging from 0-5 Gy at varying energies between 1-3 MeV. The repair kinetics for double strand breaks was measured over 24 hours using the γ-H2AX assay. This allows the analysis of how protons affect the lymphocytes' ability to repair. Furthermore, this study was compared to X-rays to give a full understanding of how these radiotherapy types differ in repair kinetics and how permanent the effects of radiation are to the whole body.

This research could provide a useful source of information for clinicians while designing treatment plans.

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Redon, D. B. S. (2009). c-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin. *Advances in Space Research*, (43), 1171–1178.

P14. EFFECTS OF CARBON-ION MICROBEAM IRRADIATION ON LOCOMOTION AND PHARYNGEAL PUMPING IN C. ELEGANS

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The nematode *Caenorhabditis elegans* (*C. elegans*) is a good *in vivo* model system to examine radiobiological effects at behavioral level. We recently reported that locomotion in the nematode *C. elegans* using body-wall muscles was significantly reduced after broad-beam irradiation of the whole body [1]. Furthermore, to investigate the radiation effects on different types of movement using different muscles, we subsequently focused on pumping motion (chewing and swallowing) which is a rapid periodic motion involving the use of the pharyngeal muscles, and found that the proportion of animals in which the pumping motion stopped had increased after broad-beam irradiation [2]. In the present study, we examined whether or not the effects similar to those observed in the broad-beam irradiated animals on both the locomotion and pharyngeal pumping motion could be induced by region-specific microbeam irradiation.

Young adult wild-type *C. elegans* were used in the experiments. To investigate the effects of region-specific microbeam irradiation, we used energetic carbon ions ($^{12}C^{5+}$, 18.3 MeV/u, LET = 119 keV/μm) generated at the HZ1 port of TIARA at JAEA. *C. elegans* was enclosed in a polydimethylsiloxane microfluidic device [3] with buffer to inhibit locomotion during irradiation. The 'head' region including the pharynx, 'middle' region around the intestine, and 'tail' region were targeted independently; these regions were irradiated with 12,000 carbon ions corresponding to 500 Gy at a 20 mϕ micro-aperture region. In the case of locomotion assay, *C. elegans* was placed on an agar dish without food immediately after irradiation. The locomotion was video-recorded and 'body bends', which is defined as the number of bends in the anterior body region at 20-sec intervals, was counted. In the case of pharyngeal pumping assay, *C. elegans* was placed on an agar dish with a bacterial lawn (food) immediately after irradiation. 60 continuous shots of the pharyngeal pumping motion, each of 1 sec duration, were obtained using a high-speed camera and the frequency of the pumping strokes per 1 sec duration was counted.

Microbeam irradiation in each region (to head, middle, and tail regions) slightly decreased locomotion, and there were no significant differences in the degrees of the decrease between the irradiation regions (data not shown). This suggests that irradiation to very limited region is enough to decrease the locomotion in C. elegans. This may relate to the fact that the neural circuit for motor control of whole-body movement exists from the head to the tail. On the other hand, the proportion of animals in which the pharyngeal pumping motion had stopped increased in only the head-irradiated animals (Figure 1). This suggests that the stop of the pharyngeal pumping motion after whole-body irradiation [2] reflects radiation effects on the head (including the pharynx). From these results we found that effects of the region-specific microbeam irradiation differ depending on types of movements. Further studies involving the effects of microbeam irradiation on movements in C. elegans are in progress.

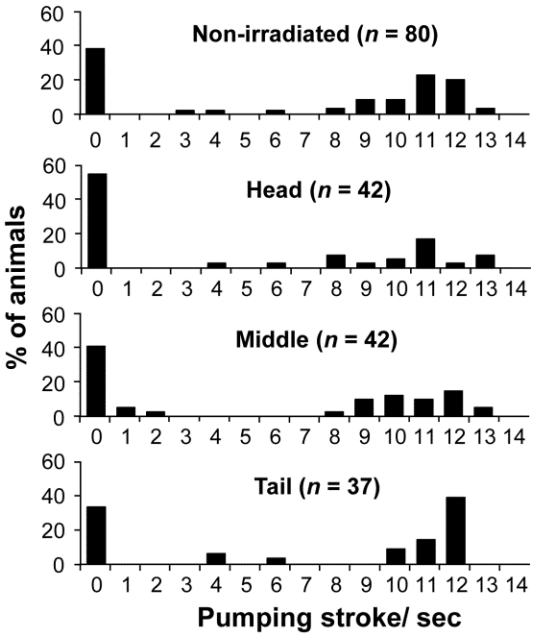

Figure 1: Pumping motion in the microbeam-irradiated C. elegans within 1 h after irradiation.

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P15. THE METHODOLOGY FOR DETERMINING SPATIAL FUNCTION OF RELATED GENES IN THE BYSTANDER SIGNALLING FROM SOMATIC CELLS TO GERM CELLS OF CAENORHABDITIS ELEGANS

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Although radiation-induced bystander effects (RIBE) have been well demonstrated in some animal models *in vivo* [1-5], there is little evidence regarding the RIBE between somatic cells and germ cells. In our previous study, the irradiation of posterior pharynx bulbs of *Caenorhabditis elegans* (*C.elegans*) with proton micro-beam led to an enhanced level of germ cell apoptosis in the bystander gonads, clearly demonstrating the bystander signalling from somatic cells to germ cells. It was also shown that DNA damage-induced germ cell death machinery and MAPK signalling pathways were involved in the induction of germ cell apoptosis by micro-beam irradiation [6]. However, it is still unclear how these related signalling pathways took part in the processes. In order to address this issue, a new worm-microbeam experimental system is being tested, in which two reciprocal tissue-specific RNAi mutants *rrf-1(lg)* and *ppw-1(lg)* are employed. Some genes in the DNA damage-induced germ cell death machinery, insulin-like signalling pathways and MAPK signalling pathways are knocked down separately in somatic cells and germ cells, and the spatial function of related signalling pathways is determined by scoring the level of germ cell apoptosis.

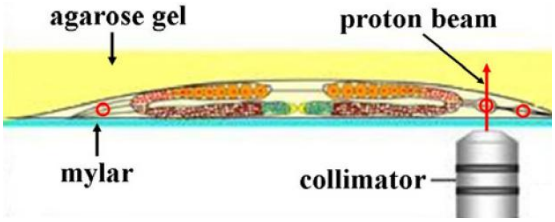

Figure 1: Schematics of microbeam-localized irradiation of C. elegans

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P16. MONTE-CARLO MODELLING, MICROBEAM IRRADIATION AND TIME-LAPSE IMAGING ON LIVING CELLS

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The study of radiation-induced DNA damage signalling and repair protein recruitment is one of the key features of charged particles microbeams [1]. By using these devices, it is possible to irradiate sub-nuclear regions with a defined dosimetry and, by using online time-lapse imaging, to visualize and quantify the expression and/or re-localization of fluorescence labelled proteins as a function of dose. In order to relate the observed response to the quantity of damage inflicted to DNA, it is necessary to assess the local dose delivered to the irradiated area. Indeed the concept of dose usually calculated in the whole nucleus is of limited use when only a sub-nuclear area is irradiated

We have recently developed a new end-station for our light ion microbeam presenting an improved beam resolution and equipped with time-lapse imaging online. This new setup has been presented at the last microbeam workshop (New York, USA, March 2012), along with its performances: a beam size on target of about 2 µm, for 3 MeV protons and alpha particles, and a targeting accuracy of 2.0 ± 0.7 µm. In parallel to these developments, we have developed a Monte-Carlo simulation code (based on Geant4) to calculate the energy deposition at the sub-micrometer scale in cellular phantoms obtained from confocal microscopy [2]. This code simulates the energy deposition in realistic nuclei geometries and thus the dose delivered to the whole nucleus as well as the local dose delivered to the irradiated area.

By using this methodology we have characterized the recruitment time of the XRCC1 protein as function of the local dose for irradiations with 3 MeV protons and alpha particles. The simulation data allow to correlate the local dose deposition with the irradiated sub-nuclear volume and the data obtained on the kinetics of the relocalization of XRCC1-GFP after irradiation. We can observe that the re-localization kinetics of XRCC1-GFP is clearly related to the delivered dose.

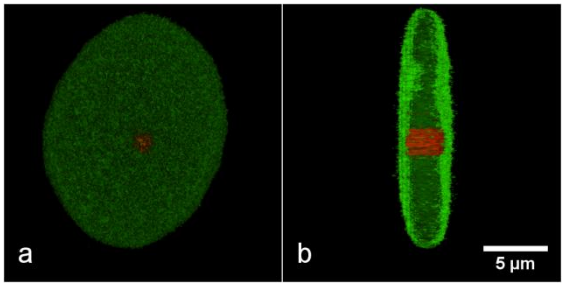

Figure1: Simulation of local energy deposition in a cellular phantom obtained from confocal microscopy. a) Front view of the cell nucleus b) Side view. The nucleus volume appears in green (H2B-GFP) and the proton tracks, modelled in Geant4, are shown in red. The simulation was performed with 1000 protons. Here the dose to the nucleus is 15.4 Gy and the local dose is 318 Gy.

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P17. MICROBEAM IRRADIATION TO STUDY HYPERSENSITIVE RESPONSE OF A549 NSCLC CELLS AFTER LOW-DOSE IRRADIATION WITH LOW-LET PROTONS

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Over the past decade, low dose effects of ionising radiation have been highlighted and studied, example include low dose hyper-radiosensitivity (HRS), bystander effect, and adaptive responses. Although these effects are largely reported after X-ray irradiation, it is of great interest to also study them after charged particle irradiation. Such studies can inform a range of separate but interlinked disciplines - radiotherapy, radio-protection and space radiobiology where low doses of high-LET radiation are encountered. In this context, we recently proved that the HRS is found after low dose irradiation of A549 lung cancer cells with X-rays and low-dose-rate beta particles [1]. Recent results indicate that is also observed after irradiation with charged particles [2, 3]. For these, a broad beam was used, leading to an inherent dose error due to the Poisson distribution of the beam. In this case, the probability of a cell being traversed by an ion is related to its surface and the number of incident ions (i.e. the dose) leading to a distribution of dose amongst the cell population. In this context, microbeam facilities are advantageous as every cell can be irradiated with a precise number of ions allowing delivery of exactly the same dose to each cell.

In this work the Wolfson vertical beam line of the University of Surrey is used to irradiate A549 lung cancer cells with a microbeam of 3.8 MeV protons [4] corresponding to a LET of 12 keV/µm. Clonogenic assays and phospho-histone H3 staining are undertaken to measure the cell surviving fraction and the mitotic ratio for doses ranging from 0.01 to 0.5 Gy. The results are finally compared to the results obtained after broad beam irradiation.

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