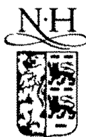

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Two approaches for irradiating cells individually: a charged-particle microbeam and a soft X-ray microprobe

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Abstract

We are developing two independent, but complementary microbeams for irradiating cells individually in vitro. Firstly, a charged-particle microbeam that uses a fine-bore glass capillary, combined with a transmission detector to precisely irradiate cells with exact numbers of energetic charge-particles and secondly, a soft X-ray microprobe that produces a very fine beam of carbon-K (278 eV) ultrasoft X-rays, focused to a spot size < 100 nm. X-ray focusing is achieved using zone-plate diffraction lenses developed initially for X-ray microscopy applications.

1. Introduction

The groundwork for the use of microbeams in radiation biology took place in the 1950's, by Zirkle and colleagues [1]. In recent years, there has been a resurgence of interest world-wide in the use of microbeams in this field [2,3]. Microbeams provide a unique opportunity to control precisely, the dose to individual cells in vitro and the localisation of dose within the cell. This makes it possible to study a number of important radiobiological processes in ways that cannot be achieved by other methods. For example, at the levels of dose relevant to environmental exposure to naturally occurring radioactive radon gas, virtually no cell receives more than one charged-particle traversal in its lifetime. Using a charged-particle microbeam, it is possible to deliver exactly one particle to a cell and is therefore ideally suited to developing an in vitro experimental model for reproducing the levels of exposure that occur in vivo. Microbeam methods are also useful in addressing conflicting observations related to cellular spatial sensitivity. For example, some studies [4] suggest that it is DNA close to the cell nuclear membrane that is most easily damaged by ionizing radiation, while other studies indicate different patterns of sensitivity [5]. Finally, there have been several reports that radiation effects may be transmitted from irradiated cells to neighbouring unirradiated cells [6]. A microbeam facility can be used to selectively irradiate individual cells which can be subsequently revisited to ascertain what changes have occurred to that cell, and to its unirradiated neighbours.

At the Gray Laboratory, we are developing two independent, but complementary microbeams. This

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report describes the development and current status of these facilities. The charged-particle microbeam uses a fine-bore glass capillary (1 μm bore) to collimate energetic protons, or $^{3}He^{2+}$ ions (produced using a 4 MV Van de Graaff accelerator) to irradiate cells in vitro individually with precise numbers of particles. This facility is now operational. We are also developing a facility that is capable of irradiating individual cells using a very fine beam of carbon-K (278 eV) ultrasoft X-rays, focused to a spot size < 100 nm using diffractive X-ray optical techniques. The charged-particle microbeam will be used for studies specifically related to the biological effects of charged-particles, while the superior spatial properties of the soft X-ray microprobe make this the source of choice for studies related to sub-cellular spatial sensitivity.

2. The charged-particle microbeam

The development of the physical aspects of our charged-particle microbeam fall broadly into four categories: the radiation source, particle collimation, particle detection and cell alignment. These are discussed below.

2.1. Radiation source

The microbeam utilises a 4 MV Van de Graaff accelerator sited at the Gray Laboratory, which can accelerate singly- or doubly-charged particles, generated using a radio-frequency ion source. A 90° analysing magnet is used to select accelerated particles of the desired mass and energy. A second 90° bending magnet is used to direct particles vertically upward into the irradiation area, passing through two sets of micro-adjustable slits (arranged orthogonally) and a mechanical shutter. The final section of beamline is braced by a substantial optical table at bench height, which also supports the sample irradiation stage, and a cell imaging system.

2.2. Particle collimation

For the charged-particle microbeam to realise fully its potential in a radiobiological application, it should be possible to irradiate a preselected target with an accuracy approaching 1 μm. This can be achieved either by focusing or by collimation. Although focusing is ultimately capable of the producing the finest beams, there are advantages to using collimation when the target is wet cells (i.e. collimator assemblies can be compact and relatively straightforward to "aim"). Also, one of the main benefits of using a focused system (namely, the fine spot size) can be compromised by the requirement for vacuum windows and detectors between the source and the cell (which introduce scattering).

Our collimators are constructed from fused silica capillary tubing, supplied to our specification by S.G.E. (UK) Ltd. An important feature of this capillary is that it is thick walled (the outside diameter is 225 μm) compared to the bore diameter (available down to 1 μm), and is therefore thick enough to confine the particles produced by our accelerator. A gimballed mount at the end of the accelerator beamline supports a 1 mm length of capillary, such that a very high aspect-ratio collimator is used (i.e. length:diameter up to 1000:1). This is covered by 3 μm thick Mylar film which serves as a vacuum window.

2.3. Particle detection

An essential feature of the microbeam is the ability to detect, and therefore control, the number of

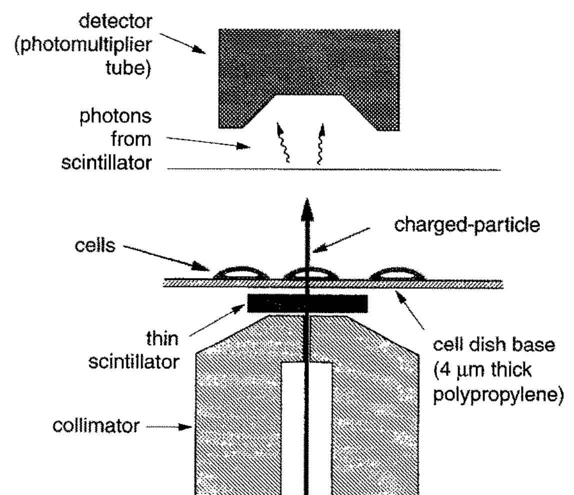

Fig. 1. The method of collimation and detection used with the charged-particle microbeam and the arrangement of the sample (not to scale).

particles that traverse the target. The configuration of our target (wet cells in solution, attached to a thin membrane through which the cells are irradiated) precludes the use of detectors beyond the cell. Instead, our microbeam uses a transmission-type detector based on a 18 μm thick plastic scintillator (type NE102A, Nuclear Enterprises Ltd, Edinburgh) mounted directly to the end of the collimator. A photomultiplier (PM) tube is used to detect the fluorescent pulse from the passage of each particle. The PM tube is a miniature (16 mm diameter by 13 mm long), single-anode device (Hamamatsu, type R5600U-06) mounted immediately above the dish containing the cells. The arrangement of the collimator and detector relative to the sample is shown in Fig. 1. The thickness of the scintillator used is sufficient to distinguish the signal (due to the passage of a charged-particle) from spurious noise within the detector. A drawback of this technique is that the irradiation stage must be in total darkness. During irradiation, the counts from the detector are used to control a fast electrostatic shutter to terminate exposures.

2.4. Cell alignment and irradiation

An important requirement of the cell alignment and irradiation system is the ability to rapidly identify and position sub-cellular targets for microbeam irradiation. Speed is essential because many assays of biological radiation effect require several hundreds, or even thousands of cells to be micro-irradiated individually. The nuclei of V79 mammalian cells (which we use) are typically 10–15 μm in diameter and are identified using a DNA-binding fluorescent stain (Hoechst 33258). The cells are viewed using an epi-fluorescent microscope.

The arrangement for aligning, irradiating and viewing the cells is shown in Fig. 2. A charge-coupled device (CCD) micro-imaging camera forms part of a highly-automated cell-finding and position-logging system. The dish of cells is supported on a motorised 3-axis micropositioning stage which operates under computer-control. A 5 mm square region of the cell dish is automatically scanned in a few minutes and the coordinates of all cells within the region are logged (typically 200–300 cells). Note that for this procedure, the PM tube is replaced by a

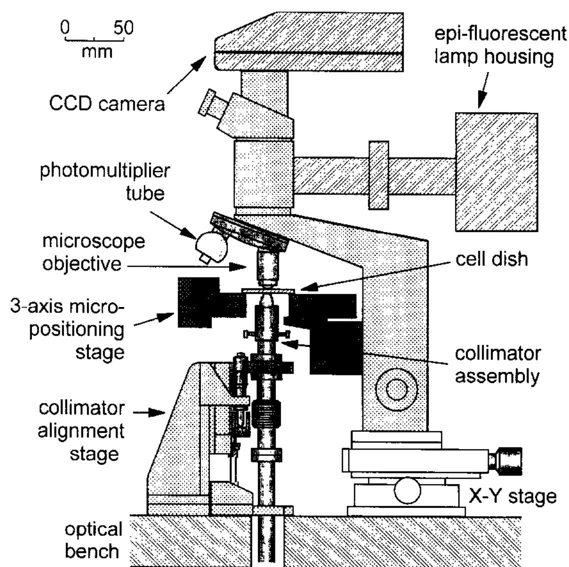

Fig. 2. The collimation, detection, imaging and micropositioning arrangement for irradiating cells individually in vitro using a charged-particle microbeam.

× 10 microscope objective (both are mounted on the microscope turret). The stored co-ordinates are then used during the irradiation phase of the experiment to automatically position each cell, in turn, over the collimator and deliver a pre-set number of particles. To maximise the targeting accuracy, it is important that the collimator exit is as close as possible to the cell. Therefore, prior each exposure, the collimator/detector assembly is moved upward about 1 mm until it just touches the base of the cell dish (made from 4 μm thick polypropylene, and to which the cells are attached). After the exposure, it is lowered to permit the next cell to be positioned. By automating the irradiation procedure, this sequence of operations is performed quickly. Typically it takes ~ 5 min to irradiate 250 cells (i.e. 1.2 s per cell).

2.5. Microbeam performance

The collimator can be assessed in terms of the spatial and energy resolution it provides. Using initially 3.5 MeV protons, the peak energy at the sample position is 3.15 MeV, with a full-width at half-maximum energy spread of 47 keV. Greater than 96% of the particles are within the full-energy peak. The targeting accuracy of the collimator has

been evaluated by exposing CR-39 "track-etch" plastic to the particle beam at the sample position. The passage of a particle is visualised as a micron-sized pit on the surface of the plastic, revealed by etching the plastic. Using this technique, we find that ~ 95% of 3.5 MeV protons are within ±5 μm of the target position and ~ 90% are within ±2 μm. Likewise, by aiming single particles at an array of imaginary targets on the track-etch plastic, the detector/shutter performance can be evaluated. Using the arrangement described in Section 2.3, the detector counting efficiency is better than 99%, with < 1% "false positive" counts (at a count-rate of 10 counts per second).

3. The soft X-ray microbeam

To complement our studies using the charged-particle microbeam, we are developing a facility that is capable of irradiating individual cells using a very fine beam of carbon-K (278 eV) ultrasoft X-rays, focused to a spot size < 100 nm. The X-ray focusing is achieved using diffraction lenses (zone-plates) developed initially for X-ray microscopy applications [7]. These are circular diffraction gratings (~ 100 μm diameter) with radially increasing line density, such that the diffraction angle also increases and X-rays may thus be brought to an axial focus. The development of zone plates has advanced significantly over the past ten years, and it is now possible to make zone plates than can focus X-rays to spots with diameters of 30 nm or less at energies of a few hundred electron volts.

The principle of targeting cells individually using focused X-rays is shown schematically in Fig. 3. The X-rays are generated using a beam of 20 kV electrons, magnetically focused to a micron-sized spot onto a carbon target. The produces 278 eV carbon-K X-rays and a significant quantity of unwanted bremsstrahlung radiation up the 20 keV (which is not properly focused by the zone-plate). Cells are irradiated using first-order diffracted X-rays. Zero-order (i.e. undiffracted) and higher-order focused X-rays are also produced, but these are blocked by an arrangement of masks (see Fig. 3). The bremsstrahlung radiation (which is more penetrating) is not removed by the masks however. Instead, the

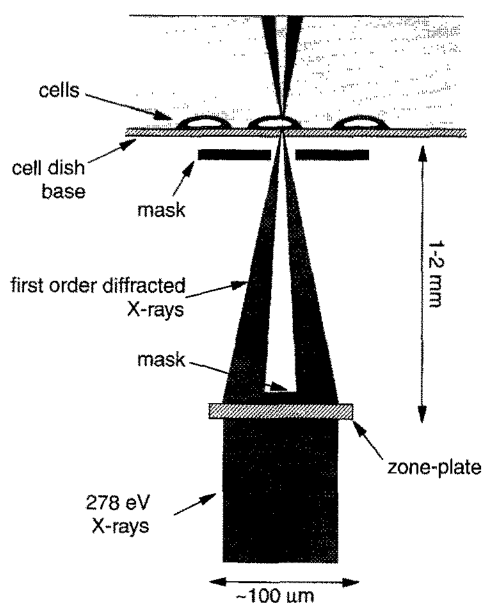

Fig. 3. A configuration for irradiating irradiating cells individually in vitro using a zone-plate diffractive lens to focus 278 eV carbon-K X-rays to a ~ 100 nm spot.

X-rays are reflected by a grazing-incidence (3° incidence angle) mirror sited midway between the source and the zone-plate. This efficiently reflects the characteristic radiation (75% efficiency) but not the higher energy bremsstrahlung component.

The alignment of cells with the focus will utilise imaging and micropositioning methods similar to those developed for the charged-particle microbeam. The position of the focus will be established by precisely scanning a knife-edge through the beam near the focus while measuring transmitted X-rays using a thin-window proportional counter. The scanning procedure can be achieved by using the sample micropositioning stage otherwise used to support the cell-dish. The proportional counter is used to characterise the energy spectrum and dose-rate at the sample-position. Pilot studies have indicated that using a well-specified zone-plate (typically, 10% efficiency in the first-order focus) it will be possible to deliver several tens, or hundreds of focused 278 eV X-rays per second, which is sufficient for a wide range of experiments.

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