Ion-Microbeams and Their Role in Radiobiology Research in Europe

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I Motivation and history

A goal of research in radiobiology is to identify the radiation-sensitive target(s) in cells and characterise the mechanisms of damage and repair. To this end, a microbeam of ionising radiation (ions or x-rays) able to deliver a defined dose to individual cells or sub-units of cells is a useful tool. Low energy, micro-focus X-ray-generators are now commercially available, but only few of them are used in radiation biology experiments. Therefore I'm dealing in the following with ion microbeams only.

It was known long before the availability of microbeams, that ionising radiation results in cell death and hereditary defects (already before the role of DNA was clarified), and that the hereditary information was within the cell nucleus. So, it was reasonable to assume that only the irradiation of the cell nucleus was causing these effects. This assumption has first been proved in a ingenious experiment by Munro [1] using alpha-particles from a polonium-tipped micro-needle.

The first European experiment that, in principle, could detect the radiation sensitive targets in cells directly, was the "Biostack-Experiment" flown on Apollo 16 in 1972 [2]. In this experiment various cells (in a dried or dormant state) had been sandwiched between plastic nuclear track detectors and exposed to galactic cosmic rays. The path of the cosmic rays through the cells could later be traced within +/- 1µm by etching the plastic detectors. One of the strange results of this experiment was, that even particles that missed the cells by some micrometers seemed to cause some damage.

In 1987 a primitive, collimated ion-microbeam had been developed at GSI by Weisbrod [3], who used a single, etched track hole in mica as a collimator to create a microbeam of theoretically 1µm diameter to target single dried yeast cells. The position of the microbeam was found by observing the hole in the mica foil with a light microscope, then the yeast cells have been manually positioned behind the etched hole, and a the vacuum valve along the beam line was opened until one ion hit was detected by a surface barrier detector.

Another motivation for the use of an ion microbeam was related to studies of the health risk of environmental radon exposure. Initial studies were conducted by using broad beam irradiators. Due to the statistical nature of the broad beam every cell gets a fluctuating dose following a Poisson distribution. If the dose is reduced to around 1 particle hit per cell, the uncertainty of the dose is obvious. While one cell may get 3 hits another one will not be hit at all. Therefore, a possible solution was to irradiate every cell with exactly one or a defined number of ions and find the radiation risk using a mathematical model.

Connected to the problem of the extremely low dose effect was a report showing, that CHO cell cultures, irradiated with an α-source in a way that only few cells had actually been hit, showed sister chromatid exchanges also in a large fraction of non-hit cells [4]. This effect is now designated as a "bystander effect." Its importance stems from the fact that, depending on its magnitude, existing linear extrapolations of risk to low doses could underestimate the risk if they did not account for the effect.

Microbeams are obvious tools to investigate the bystander effect. In the same dish, hit and non-hit cells are exactly known. Using fluorophores bound to anti-bodies, and fluorescence microscopy one can visualise the hits as bright spots called foci. Cells can even be irradiated by artificial patterns to discriminate radiation induced foci from foci generated spontaneously. Understandably, the enigmatic bystander effect triggered an

avalanche of microbeam experiments. Numerous reports have been published presenting the results of investigations into the mechanisms underlying the bystander effect [5 *and references therein*]. However, some investigators report finding little or no evidence for a bystander effect [6, 7, 8], and the issue remains somewhat controversial.

II Technical choices for microbeams

a) Vertical versus horizontal microbeam

For a long time there had been only vertical microbeams in radiobiology, possibly because biologists have been used to looking into vertical light-microscopes and traditional, horizontal, open cell dishes could still be used. In fact, these open cell dishes are sometimes advantageous, because the cells or cell organelles to be targeted can then be observed at the highest possible magnification by water immersion microscope lenses. The use of water immersion lenses can also be beneficial to avoid targeting errors due to optical aberrations [9].

On the other hand, most accelerators are horizontal. Therefore, it seems not worthwhile to invest in costly bending magnets and adapt buildings for a vertical beam, when vertical cell dishes can also be used. Against some fears, cells in a vertical cell-dish keep attached, due to molecular forces, as long as they are alive.

Therefore, focused microbeams are now mostly horizontal.

b) Collimated microbeam

The easiest way to produce a microbeam is to restrict the wide beam coming from an accelerator by a small collimator. This facilitates tabletop experiments with the added advantage of using a light microscope to help in cell targeting. First one has to find the hole in the collimator and then one aligns the cell to be irradiated with that hole. (Fig.1)

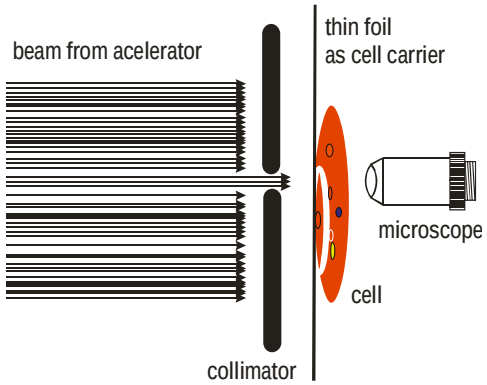

Fig. 1 *Targeted irradiation of cells by a collimated microbeam. A small aperture lets a small beam pass onto the cells attached to a thin carrier foil. To irradiate the cells with a counted number of ions, one needs also an hit detector and a fast beam switch not shown here.*

However this simplicity comes at a cost. There is always a small zone at the edges of a collimator, which particles can still pass with reduced energy and changed direction. Therefore, one cannot avoid that some particles hit a cell at an unknown position and with unknown LET.

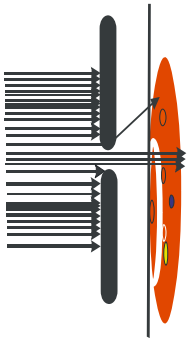

Fig.2 *Particle scattering at collimator edges.*

As the fraction of particles scattered at a collimator is inversely proportional to its aperture diameter, scattering becomes dominant for micron-size apertures [10]. Therefore, a great effort went into the development of collimators having the smallest possible scattering. Presently, the best low scattering collimators are 1 mm long glass capillaries having apertures of a few micrometers [11]. One can also minimise the microbeam halo of scattered particles by keeping the distance between collimator and

cell dish as small as possible. Still, even in that case, micro-apertures below 2 µm diameter do no longer produce smaller micro-beams, because the diameter of these beams is dominated by the halo of scattered particles [11].
Another disadvantage, relative to focused beams described later, is that the microbeam is stationary. Therefore, the cells in the cell dishes are moved into the beam mechanically, by translating the microscope stage. This severely limits throughput even when fast voice-coil motors are used to move the stage.

c) Focused microbeam
Focused microbeams do not only have much smaller beam spots, but they also have inherently much less scattered particles. (Fig.3)

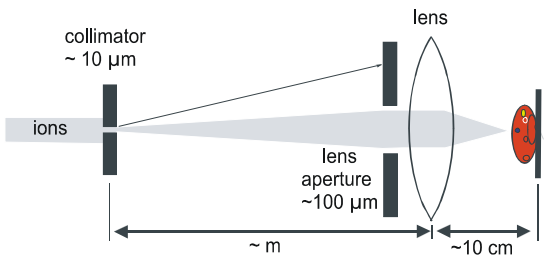

Fig.3 *Basic focused microbeam. It still needs some collimator to cut a small beamlet out of the accelerator beam. But to get the same micro-focus, this micro-aperture can be enlarged by the demagnification factor of the focusing lens.*

This larger micro-aperture is easier to produce. It also scatters less particles, as the fraction of scattered particles scales inversely proportional to the diameter of the micro-aperture [10]. Additionally, most of the scattered particles produced at this micro-aperture are stopped at the lens-aperture. Therefore, focused microbeams can have smaller beam spots and well defined energies (or LET). There exist now microbeams with beam spots down to 20 nm in vacuum [12]. And microbeams for radiobiology where the beam has to pass a vacuum window and some distance in air before it enters the cell dish, have achieved diameters of a few hundred nm and a targeting accuracy of about 700 nm [13].

Due to the small lens aperture and great focal length, the depth of focus is typically 1mm for a 1µm wide micro-beam. Therefore, the beam size is essentially a constant over the diameter of a cell.

Typically, focused microbeam installations are some meters long, have a micro-collimator of 10µm diameter and a lens aperture of 100µm diameter. Apart from blocking most of the particles scattered at the micro-collimator, the small lens-aperture is needed to limit spherical and chromatic aberrations of the lens. Therefore, microbeams are inherently low current beams, because most of the beam is stopped at these small apertures. But this is no problem for targeted irradiations, where beams with 1000 particles/s are sufficient.

With few exceptions, lenses are of the magnetic quadrupole type. As an exception of this rule, an electrostatic quadrupole triplet is used at RARAF, Columbia University [14], a superconducting solenoid at Bochum University [15], and there has been a microprobe with coaxial electrostatic lenses in Sidney in the nineteen-seventies [16]. The latter lenses are especially interesting, because they can be made converging and diverging. And as in light optics one can build achromatic systems from rotationally symmetric, converging and diverging lenses.
Among the magnetic quadrupole lenses any configuration from doublets, triplets and quadruplets are in use with no clear-cut advantage for one configuration.
Typical beam spots achieved with these microprobes are around 1µm.

Smaller beam spots can be achieved with microprobe systems having two demagnifying stages (Fig.4). That technique is standard in electron microscopes. It has first been applied for ion microbeams at the ETH Zürich [17].

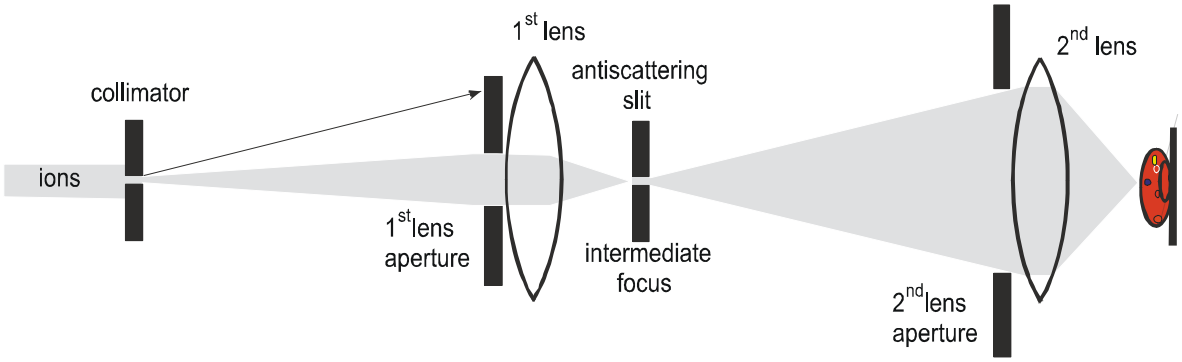

Fig.4 *Microprobe with two focusing lenses. Here the intermediate focus is demagnified once more by a second lens, and the total demagnification is the product of the demagnifications of the two single lenses. However, the beam divergence in the final focus is increasing too and one normally needs to decrease the 2nd lens aperture further to limit spherical aberrations.*

One can get smaller beam spots with two-stage systems or one can get the same beam spot with a much larger collimator aperture, which helps to reduce the fraction of scattered particles. A survey of two stage microprobe lenses can be found in [18].

Unfortunately, according to Liouville's theorem, the smaller beam spot of a two stage lens system means a larger beam divergence at the final focus or in other words a larger chromatic and spherical aberration. To mitigate the chromatic aberration, one needs an accelerator having a very low energy spread. To reduce the spherical aberration one has to close down the 2nd lens aperture with the consequence of a very low beam current. Happily, accelerators with an energy spread of $dE/E \sim 10^{-5}$ equipped with high brightness sources have been developed for these microbeam applications from High Voltage Engineering Europe [19].
Today, two stage systems do not only have demagnification factors of up to 1000, but scattered particles can also be removed very efficiently at their intermediate focus.
Therefore, microbeams with 2 demagnification stages together with dedicated accelerators have now the smallest beam spots and highest currents.

Another practical aspect of focused microbeams is that the micro-focus can be moved rapidly by electrostatic or magnetic deflectors, which speeds up targeted irradiations of cells considerably.

d) Finding cell- and beam-position

For the targeted irradiation of a cell one first needs to know its position and the position of the undeflected beam. From both positions one can calculate how to deflect the beam to hit the cell.
The position of the cell can only be determined by light microscopy to avoid damage before the irradiation. In principle the beam position could be found without this limitation. But to avoid calibration problems it is reasonable to find the beam position using the same microscope, digital camera and computer program.

This introduces a basic problem: Targeted irradiation of cells is limited in accuracy by the resolution limit of the light microscope. In addition, this accuracy is further limited by refraction effects, when the microscope is looking at the cells through thin windows and nutrient medium at even a very small angle. The same effects limit the accuracy when the beam is localised by looking at the light spot the beam is producing in a scintillator [9].

Besides these basic problems, there is the practical problem of irradiating many cells in a short time in order to get statistically valid results. Therefore, most facilities for targeted cell irradiation use computer programs for cell recognition and finding cell coordinates.

Staining and fluorescence microscopy are very convenient for the recognition process, because appropriate staining can highlight specific cellular structures so that these structures can often be recognised using a simple brightness threshold, while the recognition of unstained structures requires the complex and time consuming evaluation of structure information.

Commercial image recognition programs need only a few seconds (with a standard PC) to recognise some hundreds of stained cells illuminated by UV-light and imaged by fluorescence microscopy. Often the DNA-binding stains Hoechst 33258 or 33342 are used at concentrations between 5 nM [20] and 1000 nM [21] to recognise the cell nucleus. These stains are known to disrupt DNA replication during cell division [22]. Unfortunately, information about cell damage at these low concentrations is almost non-existent. The influence of Hoechst 33342 on the growth curves of CHO-K1 cells has been investigated by Heiß in his PhD-Thesis [23] at concentrations between 50nM and 1000nM, and no effect has been found relative to unstained cells up to 200nM.

For cell recognition, a lower stain concentration can be compensated to some extent by longer UV-exposure which can also damage the cells. Consequently, one needs more information about the optimum combination of stain-concentration and UV-illumination for the image quality needed for cell recognition.

Some researchers argue that UV-light and staining should generally be avoided. Others use GFP (green fluorescent protein)-transfected cells, because their fluorescence can be excited by visible (470 nm) light, which is thought to be harmless. But, to the best of my knowledge, still nobody has tested yet if fluorescence microscopy of GFP-transfected cells does not affect cellular physiology.

So some researchers try to recognise unstained cells using phase-contrast microscopy. This is still very time consuming and less reliable. It remains to be seen, if cells suffer less, when they need to be kept longer in a cell dish for recognition before the irradiation.

e) **Beam scanning**

In facilities with a collimated microbeam, cells have to be moved into the fixed beam position by a mechanical stage. One exception is CEA Saclay [CEA 2009, 2], where the beam is moved relative to the stationary cell dish by moving the collimator.

Much faster yet is to move the microbeam by electric or magnetic fields. Electrostatic beam scanners are usually believed to be fastest. But comparing the speed of the electrostatic beam scanner at PTB [24] (1 ms for full deflection at 4 kV) with the magnetic one at GSI (1 ms for full deflection at 5 A) one finds that they have a similar speed. Still one has to be aware of the hysteresis of magnetic beam scanners using iron or ferrite cores. Hysteresis means that one needs different currents to reach a particular point in the scanning field, depending on where the beam has been before. Happily, there exist ferrite materials with negligible hysteresis. We could, for example, prove for the beam scanner at GSI that, within the accuracy of the microscope image, the beam is always deflected to the same place independently of its starting point, when we use the same deflection-current.

Afraid of increasing spherical aberrations, some place their beam deflectors after the lens (seen in the beam direction). But then the working distance must increase and the focal spot as well. Therefore, at GSI the beam deflecting coils have been placed in front of the lens with the argument, that if the microprobe is used as an ion microscope, small objects will usually be looked at with high magnification, that means with a small beam scanning field and hence with small

spherical aberrations. If one looks at large objects within a large scanning field, a larger spherical aberration will not be noticed.

A more elaborate beam scanning system deflects the beam some way before the lens and reflects it again so that the beam crosses the lens axis at the first principal plane. That way, one can have a large scanning field with still small spherical aberrations. This method has been first applied by D. Heck [25] and it is now used in the new microbeam in Surrey [Surrey 2010] under the name "dogleg" deflection system.

f) Hit detection

To apply just one or a counted number of hits, one needs a hit detector which is nearly 100% efficient and leaves the microbeam intact.
There are essentially two solutions for these requirements. The hit detector can be placed in front or behind the cell dish, and none of these solutions (Figs. 5, 6 and 7) is without disadvantages.

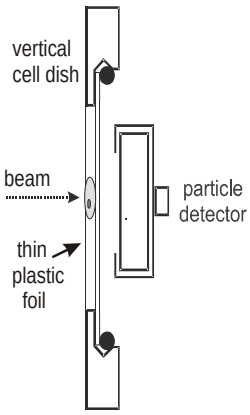

Fig. 5 *Surface barrier detector behind cell dish.*

Advantage: If the particles have enough energy to pass the cell dish, one is able to detect all particles (from protons to uranium) with 100% detection efficiency.
Disadvantages: Microbeams have rarely enough energy to pass through cells, nutrient medium and, in the case of vertical cell dishes, cover glass. The removal of medium can stress the cells and therefore interfere with the outcome of the experiment.

Moreover, between cell recognition and irradiation it is necessary to exchange the positions of the microscope and the surface barrier detector. That is not only time consuming, but it can also compromise the targeting accuracy if the microscope does not return to its former position within a fraction of a μm.

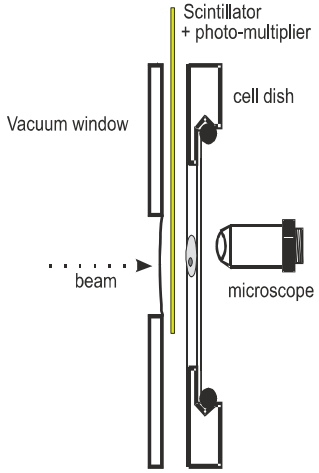

Fig.6 *Thin scintillator in front of the cell dish.*
Advantage: Lower energy particles can still pass detector and cells. But they are scattered by the scintillator and therefore the targeting accuracy suffers. A hit detector which avoids any unnecessary scattering uses secondary electrons emitted from the vacuum window (Fig. 7).

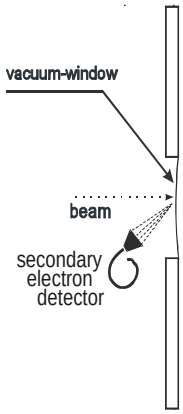

Fig. 7 *Hit detection by detecting the secondary electrons emitted from the vacuum window when an ion passes.*

Advantage: There is no scattering beyond the unavoidable scattering by the vacuum window and 100 nm thick 1 mm x 1 mm vacuum windows are commercially available which are stable at atmospheric pressure.
Disadvantage: Low secondary electron emission for low Z ions. Even with secondary electron emission enhancing CsJ coating of the vacuum window, only about 50% of the proton hits and 97% of the Alpha particle hits could be detected at GSI by that method.
Secondary electron detectors can spontaneously produce fake hit signals at a rate of about one "fake hit" per minute.

g) Beam switch

For a precise dose control, one needs a beam switch, which prevents more hits after the desired number of hits has been detected. Usually, the beam is switched off by applying a high voltage to an electrostatic deflector, which deflects the beam onto a beam stopper. (Fig.8)

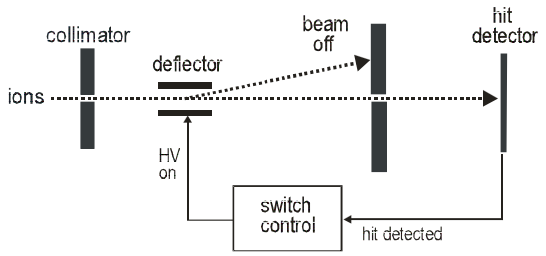

Fig.8 *Basic beam switch configuration*

If a high voltage is applied to the deflector plates, ions already on the way between deflector and hit detector continue to move on to the detector. Therefore, even a very fast reacting switch control circuit cannot prevent unwanted hits, if the beam switch is located too far from the hit detector. On the other hand, one needs some distance to deflect the beam sufficiently.
The probability for unwanted hits grows with this distance and the ion current. Let's, for example, assume an ion current of 1000 ions per second (a reasonable current for targeted irradiations) , 3m distance between switch and hit detector, and 5MeV/nucleon ions propagating at 10% of the speed of light. Then, on average, ions will appear at the hit detector every millisecond. For the distance between beam switch and hit detector the ions need 100ns. If a second ion follows within 100ns, the beam switch is not able to stop it. The probability for another ion appearing within 100ns is $100ns/1ms = 10^{-4}$.
If one additionally assumes that the high voltage needs about 1µs to deflect an ion far enough, the probability for an additional hit rises to 10^{-3}. Taking into account that a hit detector may be only 99% efficient, when thin transmission-type detectors (Fig.6 and 7) are used, one will get 1% additional hits. That is still an excellent value compared to the usual dose uncertainty of 5% for broad beam irradiations.

h) Cell dish

For ion beam irradiation, cell dishes usually have a thin membrane at the side where the beam enters. This membrane has to be as thin as possible to keep beam scattering low. It should also have a hydrophilic surface onto which cells attach easily, and it should be non-fluorescent when the cells are observed by fluorescence microscopy. Currently in use are silicon-nitride membranes [26], polypropylene and polyethylene terephtalate (mylar) foils. The plastic membranes are glued to the cell-dish-frame with candle wax [GSI 2006] or epoxy-glue [PTB 2004]. Candle wax is *usually* non-toxic (but one has to check every batch) and easy to remove in hot water, which is convenient for the reuse of expensive cell dishes. Epoxy-glue is toxic, and the cell medium must not come in touch with the glue. Therefore the PTB cell dish has a special design to prevent that.
Silicon-nitride membranes are available in thicknesses down to 100 nm. There is no fluorescence from silicon-nitride. Cells easily attach to it, but it is expensive and tricky to handle. Much cheaper are the plastic foils, but foils sold under the same brand name have often varying properties. In our experience with 2 different brands of polypropylene, the cells attached easily to one brand but not to the other. Similarly, we had been warned that mylar film would fluoresce, but our film did

not. Therefore, all plastic films have to be tested for their required properties.

Usually, the irradiated cells need to be revisited to follow their fate. Therefore, some cell dishes are provided with fiducial marks [26]. Others are designed in a way that they fit into the microscope stage reproducibly so that cells can be revisited with an accuracy of 20 µm without the use of fiducial marks [GSI 2006].

III Ion-microbeams for radiobiology in Europe

In Europe, focused ion microbeams came in use after the pioneering work of Cookson in Harwell [27] in the late nineteen-sixties. There, a microbeam had first been used for material analysis by nuclear reaction analysis and later by proton induced X-rays.

The biophysics community seemed to have developed their collimated microbeam facilities independently perhaps because the technique of focused microbeams looked too complicated and still insufficient for accurate targeted irradiations. The development of collimated microbeams culminated in the development of the facility in 1994 at the Gray Cancer Institute that was for a long time regarded as the "lighthouse" of radiobiology until its recent shutdown [20].
Shortly before, the irradiation with single ions had been introduced for focused ion microbeams at GSI [10] first for single ion micromechanics, and later for the investigation of radiation effects in microelectronics. Interestingly, the latter technique has a lot in common with radiobiology. There are microscopic radiation sensitive targets inside a microcircuit. Cell killing corresponds to "single event latchup", and DNA damage corresponds to "single event upset." But still the microbeams didn't need to leave the vacuum for that.

It is difficult to say when *focused* microbeams first entered the field of radiation biology. But a strong boost came with the European project CELLION starting officially in 2004 which united 10 institutions (exception: TU Munich and CEA Saclay) with the goal to develop or improve single hit facilities for radiation biology. Most of them are in the list of microbeam facilities below.

In order to avoid any personal bias, emphasising unimportant details while not mentioning important information, the references below contain publications selected by the facilities themselves.

At the end there is a table giving a short overview of some properties of these microbeams, also selected by the representatives of these facilities. Properties which seem unique are emphasised by a yellow background.

CENBG, Bordeaux

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Vertical Nanobeam

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	CENBG Bordeaux	CEA Saclay	GSI Darmstadt	Institute of Nucl. Physics, Crakow	Lund Nuclear Microprobe	Surrey Ion Beam Centre Vertical Nanobeam
Type of microbeam, collimated or focused	Focused , horizontal, being upgraded	collimated	Focused, horizontal	Horizontal Focused, X-ray microbeam also available	Focused	Focused, vertical, first facility specially designed for targeted irradiation of cells
If focused, one/two stage demagnifying s	One stage / Russian quadruplet		One stage	One stage (2 doublets separated by 50 cm)	Two stage	Single stage
Lens types	Magnetic quadrupoles (made in Bordeaux)		Magnetic quadrupole triplet made at GSI	MARC (Melbourne)	Oxford OM52	Magn. Quad. Triplet (Oxford Microbeams OM52)
Total demagnification	10		8 / 16	17	170 (calculated)	Dx ~ 80, Dy ~ 30
Ion species	H^+, He^+ (D^+ are available but not used for cell irradiation)	1H+, 3He+, 4He+	carbon up to uranium rarely p, He, Li	protons	Protons, deuterons, alpha particles	Protons through to Calcium.
Range of available energies	1 to 3.5 MeV	1-3 MeV	1.4 up to 11.4 MeV/nucleon	~1 MeV to 2.5 MeV, typically 2 MeV;	< 3 MeV	H^+ 4MeV, α 6MeV, O^{5+} 12 MeV 2MV Tandetron, with duoplasmatron source.
Beam spot size for targeted irradiation of cells	About 8 µm at the cell position	5 µm	0.5µm	~ 14 µm at 0.2 mm distance from vacuum window	2 µm	Planned: Sub-micron resolution 50 nm
Type of hit detector	Low pressure gas detector in front of cell dish	Silicon Surface barrier detector	Secondary electrons from vacuum window	Silicon surface barrier Ortec B-019-300-150	Post-cell, windowless Hamamatsu pin diode	PIN diode, scintillator & PM tube. Secondary electron detection system in development.
Fixed beam or moving beam	Fixed	mechanically moving (motorised) collimator	Moving beam	fixed	Scanning	Scanning
Recognition of fluorescent / non-fluorescent cells	Fluorescent cells	Fluorescent cells	Fluorescent cells	Unstained / non fluorescent	SeACell software, unstained cells	Stained cells and unstained cells in brightfield
Fluorescent labelling?	GFP tagged cell lines Hoechst vital dye	Hoechst 33342 (DNA intercalant)	Hoechst GFP	Propidium iodine, Hoechst 33342, Alexa fluor, Gamma-H2AX	Not used	Hoechst for nucleus
Maximum number of irradiated cells/h for fluorescent / unstained cells	About 2000	~ 3000/h	9000/h at 20% beam duty-cycle, filling, transport and mounting of cell dish ~ 10 min	Real experiments: < 500/irradiation typ. ~200 / 10-15min incl. target mount., cell selection + irradiation	- (not applicable)	300-10000, depending on endpoint assay (i.e clonogenic/micronuclei/gamma H2.AX)
Targeting accuracy	3µm	5 µm	700 nm	~30 µm	Better than 3 µm, no recent measurement	1 micron - nucleus/cytoplasm
Which radio-biological effects did you investigate with the microbeam (mainly)?	Radiation induced DNA damage	Bystander effect	Bystander, recruitment of repair proteins, live cell imaging	Cell survival, DSB formation and repair	Bystander effect	Low dose hypersensitivity with clonogenic assays/ RBE of different ions/ effects of protons with drugs e.g

						temozolamide / broadbeam vs focussed beam survival curves

	PTB Braunschweig	**SNAKE, Maier Leipnitzlaboratorium, Garching and Univ. der Bundeswehr München**	**INFN – Laboratori Nazionali di Legnaro (Padova)**	**Lipsion**
Type of microbeam, collimated or focused	Focused , 90° bent, beam from above	Horizontal, Focused, most sophisticated lens system	Horizontal, Collimated (Collimator: 100 um thick tantalum pinhole, 2 or 5 um diameter)	focused
If focused, one/two stage demagnifying s	Two stage	One stage, optionally two stages	--	two stage
Lens types	Magnetic quadrupole doublets	Superconducting magnetic quadrupole doublet, multipole (8-, 12-, 16-pole) correction of spherical aberrations	--	2 separated magnetic doublets
Total demagnification	8 / 20	Variable depending on application from 100 / 25 (one stage) to 200 / 200 (two stage)	--	130 x 130
Ion species	H^+, $^4He^+$	P, He, Li, Be, B, C, O, F, Si, Cl, I	$^1H^+$, $^2H^+$, $^3He^{+,++}$, $^4He^{+,++}$	p, 4He+
Range of available energies	2 - 20 MeV	p : 4 – 28 MeV He: 1.4 – 10.5 MeV/nucl; Li – O: 1 – 8 MeV/nucl Si, Cl: 1 – 4 MeV/nucl; .....I: 05 – 2 MeV/nucl => LET from 2 keV/ µm – 1000 keV/µm	0.8 – 12 MeV (in air)	0.9 – 2.4 MeV
Beam spot size for targeted irradiation of cells	2 – 3 µm (fwhm)	0.35 µm – 0.7 µm	10 um beam spot diameter on cell, after 100 um air gap;	350 nm without cells
Type of hit detector	BC 400 scintillator foil	Scintillator behind cell sample: 170 µm thick scintillator as cell substrate or 2 mm separated scintillator (for proton detection)	Silicon surface barrier detector (placed downstream of the cell sample)	Hamamatsu S1223-01 photodiode behind Petri dish
Fixed beam or moving beam	scanning	Scanning, range: ~ 0.5 x 0.5 mm²	Fixed beam	scanning
Recognition of fluorescent / non-fluorescent cells	Fluorescent or GFP	Phase contrast online and offline microscopes Epifluorescence online and offline microscope	Semi-automatic recognition system of unstained cells using phase contrast optical microscope.	Automatic, unstained
Fluorescent labelling?	Hoechst 33342, 33258	GFP, YFP, scratch label, . . .	--	No staining
Maximum number of irradiated cells/h for fluorescent / unstained cells	50 000 (stained)	Targeted irradiation not yet automated, Maximum scanning frequency, 1000 points / sec Particle rate: 1000 Hz – 100 000 Hz	Not available; the automatic cell recognition system is necessary to reduce time for cell recognition; the semi-automatic system is time consuming at the moment.	2000 cells/min plus 5 min Petri-dish handling
Targeting accuracy	1 – 2 µm	1.5 µm	5um (mainly affected by the beam spot size/particle spatial distribution in air at the cell position)	1.5 µm
Which radio-biological effects did you investigate with the microbeam (mainly)?	Bystander effects, foci formation with live cell imaging	Competition effect Kinetics of protein accumulation at radiation induced foci Kinetics of protein release from foci after DNA repair Dynamics investigation of irradiation induced foci Pulsed proton irradiation of cells, tissue and mice LET effects of foci formation	Micronuclei; DNA-damage; cell survival. Preliminary biological experiments performed; Radio-biological effect investigations not performed yet, due to the long time necessary for cell recognition (at the present).	Track-visualisation using DSB-markers

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