Report No 1955/AP

Development of Kraków external microbeam – single ion hit facility

W. Polak, R.Hajduk, S. Lebed, J.Lekki, T. Horwacik, S. Maranda, T.Pieprzyca, C. Sarnecki, Z.Stachura, Z. Szklarz, O. Veselov, J.Styczeń

Abstract:

The main purpose of building the microbeam setup for the external (i.e out-of-vacuum) measurements is single cell with single ions irradiation. The single-ion hit facility is based on the existing Kraków microbeam setup characterized by the spatial resolutions of about 3 μm. Present work introduces the setup and the measurement chamber that was developed, constructed and assembled in IFJ PAN. The passage of single ions from vacuum to atmosphere (where the cell dish is located) is registered using the channeltron detector installed inside the chamber. Channeltron registers secondary electrons emitted from CsI layer covering the Si_3N_4 exit window. The system of very precise diaphragms reduces the beam intensity down to a fluence of about 10^3 protons/sec. The beam blanking, correlated with single proton passage is provided by the fast, electrostatic deflecting system. The precise 3D table, installed outside the chamber, allows positioning the cell dish at a 200 μm distance from the exit window and change the targeted cell with a sub-micrometer precision within the dish. The paper shows results of the preliminary investigations aimed towards optimization of the most important issues: resolution of external microbeam, proton registration efficiency, efficiency of deflecting system and accuracy of single-proton-hit system.

Henryk Niewodniczański Institute of Nuclear Physics
Polish Academy of Science
Kraków 2004

The aim of the project

Our understanding of the biological effects of radiation exposure to very low doses is still far from complete, despite of a tremendous progress in recent years. Any estimation of radiation hazards requires profound knowledge on interaction of ions with biological cells and tissue. In particular, there is a still unsolved debate about the influence of a very low dose radiation on living organisms, which was up to now only estimated by high dose data extrapolation. Results of the extrapolation based on irradiation cell by conventional broad beams are of low statistical value because of insufficient information about number of irradiated cells, number of particles traversed particular cell and spontaneous cell death in control cell cultures.

Therefore, some laboratories have introduced the use of collimated ion beams [Savant (2001), Watson (2000), Belyakov (2001), Belyakov (2002), Nagashawa (1999), Zhou (2001), Folkard part I,II (1997), Moretto (2001), Folkard (2001)], where cells, or even certain cell compartments, can be individually irradiated with an exact ion number. The precise information of hit and non-hit cells enables also studies of the radiation damage transfer from hit cell to neighboring cells, a so called "bystander effect". The genotoxic effect of charged particle radiation on living cells is a result of interactions among biological matter; primary particles traversing the cell, positive ions and secondary electrons produced along the track of charged particles and of chemical reactions with free radicals or other reactive oxygen species created by radiolysis of water in the cell. Those interactions can be tracked down to a level of a single chromosome or even of a single DNA strand. The development of focused microbeams allows achieving of a better linear energy transfer (LET) definition, greater range of LET using heavy ion beams, and higher aiming accuracy.

The main aim of this project is to investigate cell response after hitting it with the certain number of ions. Biological effects of ion irradiation will be studied as a function of energy and atomic number of the irradiating ions, number of the ions traversing the cell, the cell species and the cell state (alterations of the cell cycle and the functional status). Measurements connected with the bystander effect, especially the number of non-hit dead cells and the spatial range of the phenomena will be tested for various cell cultures, various locating of the ion traversal through the cell and the different cell status. The ultimate aim of the project is to understand the processes relevant to particle radiation-induced cancer as well as medical and industrial applications of the radiation which cause occupational hazards. Investigation over bystander effect phenomena may provide vital knowledge for radiation cancer therapy. This new branch of nuclear microprobe application grows very fast thanks to the great importance of the area under investigation and is supported by the European Commission through a Marie Curie Research Training Project CELLION, grouping ten scientific European institutions and coordinated by IFJ PAN.

The measurement setup

To enable the irradiations of living cells using single ions, the new measurement setup has been developed in Kraków Institute of Nuclear Physics. This new microbeam facility allows cell irradiations in atmosphere using protons with energy up to 2.5 MeV from the Van de Graaff accelerator [Lekki (2002)]

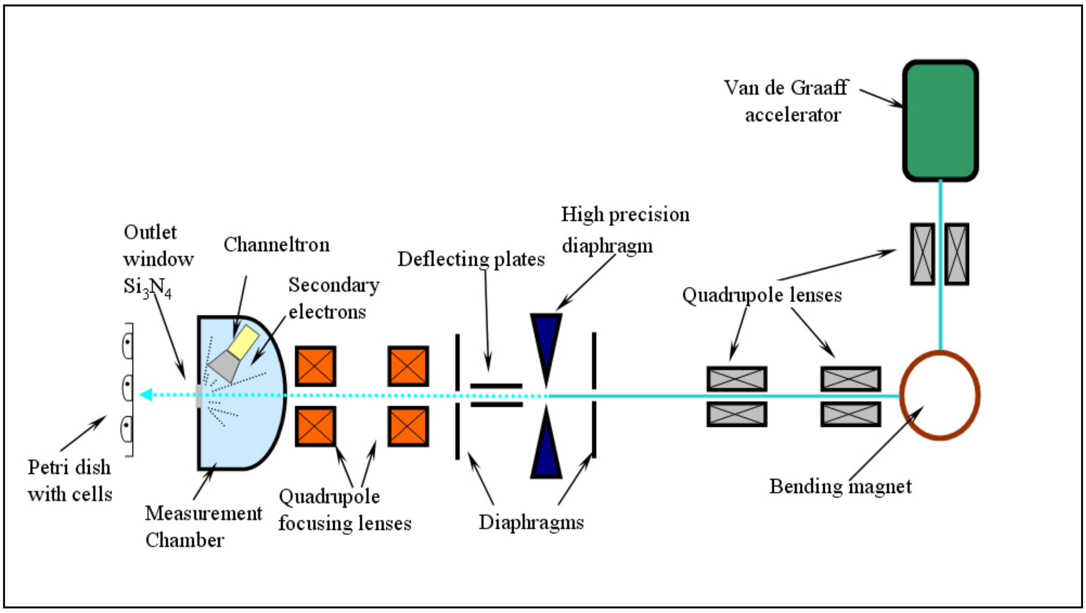


Fig. 1 Schematic view of microbeam single-hit-facility in Kraków

Proton beam from the accelerator, after passing the 90° analysing magnet, is pre-focused by 3 quadrupoles before entering the microprobe system (Fig 1). Further on, the precise diaphragms reduce the beam to the current of about 0.16fA, what corresponds to about 1000 protons/s. Two electrostatic deflecting plates (made by Technisches Büro S. Fisher) mounted after the slits allow rapid beam blanking (reaction time of single microseconds). Final focusing of the beam is provided by the two quadrupoles manufactured in the Micro Analytical Research Centre (MARC, Melbourne, Australia) [Lebed (2001), Lekki (2002)]. The best spatial resolution of about 3 x 3 μm^2 was achieved; however, 5 x 5 μm^2 resolution is a standard operational value. However, in experiments described in the present work, only resolution of about 12 x 12 μm^2 was applied. Design of the measurement chamber permits to focus proton microbeam close to the exit window. The window is made of a thin (200 nm) Si_3N_4 membrane, and separates the vacuum inside the chamber from the air where the biological sample is hold during investigations. Protons passing to the air are counted by detection of the secondary electrons emitted from the window foil by the channeltron [Sjuts Electronics] installed in the chamber. The computer controlled, precise 2D table (Physik Instrumente, Voice Coil type V-106 2S, resolution of 0.1μm) allows positioning the cells in the focus. The distance of the sample from exit window is controlled by a micrometer screw and, to minimize protons scattering, should be set to a value not exceeding 200 μm.

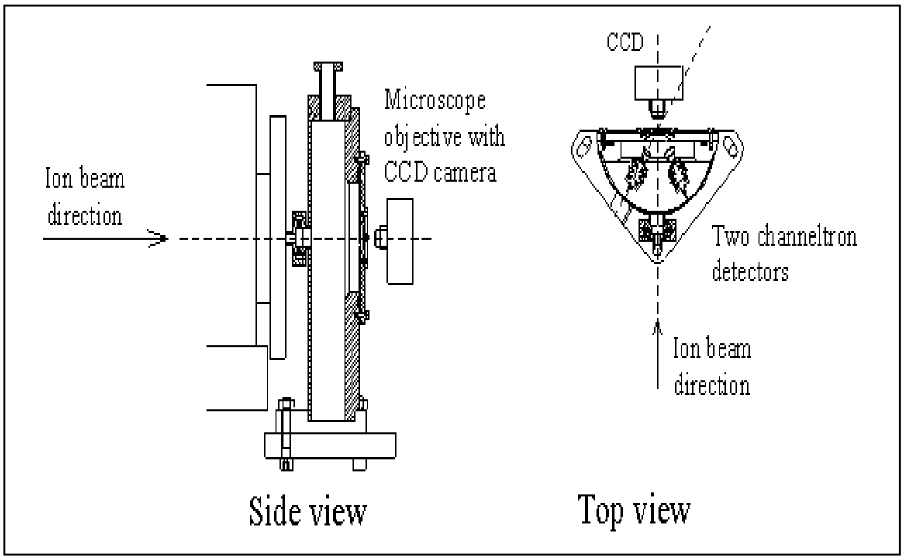


Fig. 2 Top and side view of the measurement chamber

The chamber (Fig. 2) has a possibility to rotate that enables precise focusing of the beam using additional quartz (Fig. 2a), located 1 cm aside from exit window. After focusing the beam and reducing its intensity, the chamber position is changed back to central axis to allow passing the ions through the outlet window (Fig.2a).

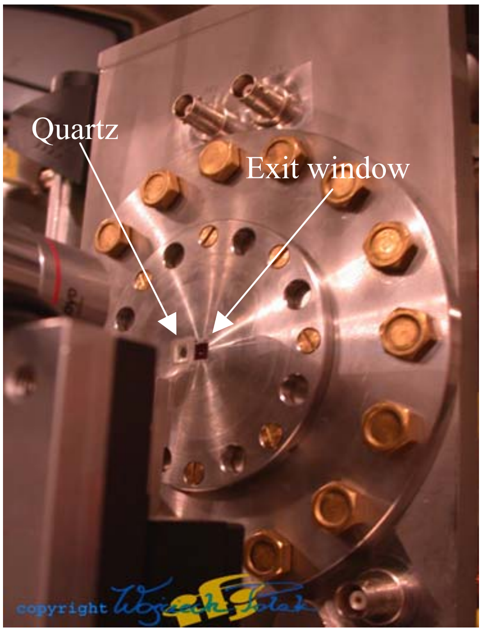


Fig. 2a Back side of the measurement chamber

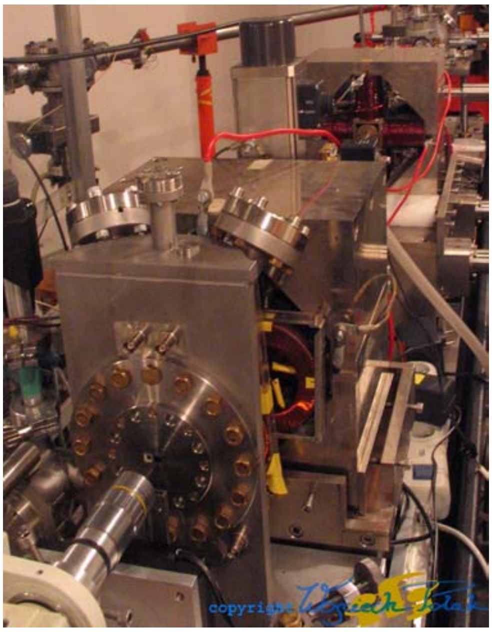


Fig. 2b Picture of the chamber and the microbeam facility

The first step – the external microbeam.

In order to irradiate the living biological material, the proton beam must leave the ion guide and experimental chamber and enter the atmosphere.

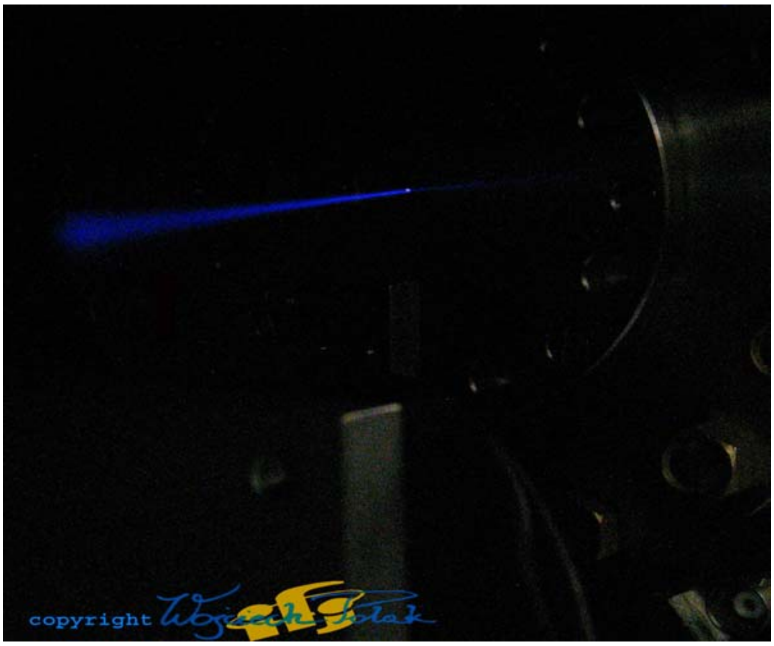


Fig. 3 Picture of ionized air due to proton beam entering the atmosphere

The photo in Fig. 3 proves that the proton beam actually enters the atmosphere. Protons passing through the window to the air are scattered in the Si_3N_4 window membrane and thus their direction diverges from central axis. This effect, and not the scattering in atmosphere, is the main cause of loosing the targeting accuracy of the irradiation, what was proven using SRIM (The Stopping and Range of Ions in Matter) [Ziegler (1998)] simulations. The further from the exit window the sample is situated the worse the targeting resolution can be achieved. The SRIM simulations show that at 200 μm distance and 200 nm thick exit window, the targeting resolution decreases to 4 μm from the ideal case. This result concerns the estimation of angular spread of the beam that was initially parallel. For a 1 mm distance, the resolution deteriorates by a factor of about 4 that makes a significant difference and is unacceptable for the planned application. These estimations were supported by direct measurements using scanning transmission ion microscopy (STIM). The calibration grid (400 mesh) located at several distances from the exit window was scanned using the external beam in air. The PIN diode positioned behind the grid was used as a proton detector. The achieved resolution at a distance of 200 μm was about 11 μm (Fig. 4a), which was in good agreement with calculations, taking into account that resolution in vacuum was of about 10 μm and the partial variances added in square values.

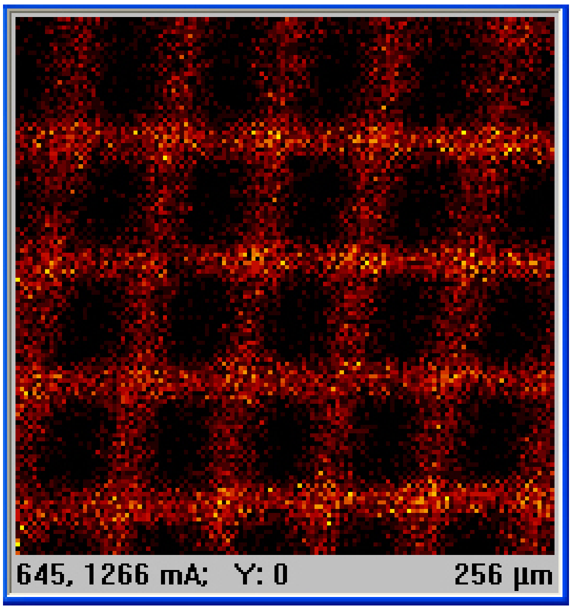


Fig. 4a Image of the calibration grid at 200µm distance from exit window;   resolution ≈11µm

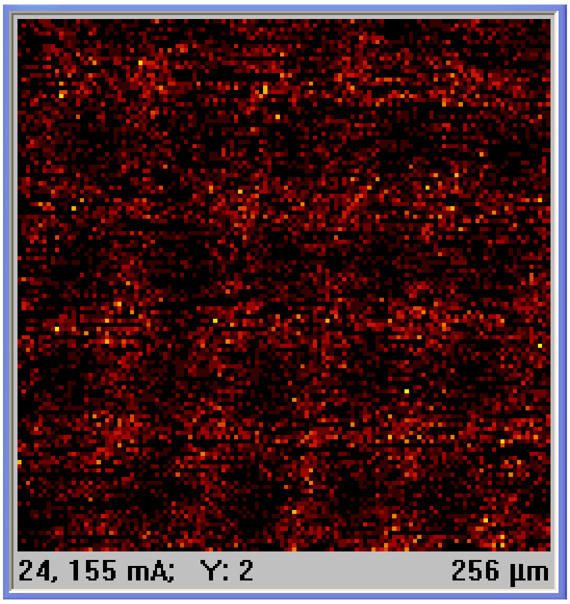
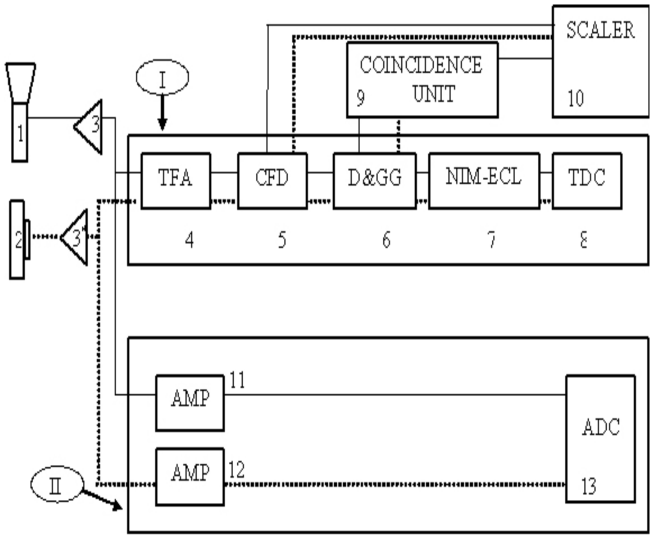


Fig. 4b Image of the same grid at 1mm distance from the exit window; resolution worse than 25µm

Proton registration efficiency

Protons which had passed through the Si_3N_4 membrane (commercially available at Silson company, UK) were registered by a semiconductor, ion implanted silicon detector characterized by the 100% efficiency for the particle registration. Those protons passing through the window ejected the secondary electrons from the membrane and the electrons were registered by a channeltron. The channeltron signals were collected in coincidence with the silicon detector signals in two ways:

- Time spectra were recorded using SILENA TDC with 1200ns base and 4096 channels and then were extracted and evaluated in the microbeam data acquisition program CMB [Lekki (2000)].
- Coincidences were registered by an electronic coincidence logic unit and counted in a scaler



I- Fast track (timing)
II- Slow track (energy)

1. Channeltron (electrons detector) – Sjuts Electronics
2. Si particle detector – Ortec
3. and 3^*. Ortec Preamplifiers
4. Timing Filter Amplifier - Ortec 836
5. Constant Fraction Discriminator – Caen mod.N415
6. Delay and Gate Generator- Ortec 8010
7. NIM – ECL – NIM translator- Caen mod.92
8. Time to Digital Converter- Silena 4418/T
9. Coincidence logic unit – Caen mod.N454
10. Scaler – Polon 401
11. Active Filter Amplifier – Ortec 572A
12. Active Filter Amplifier – Ortec 572
13. Amplitude to Digital Converter – Caen mod.C420

Fig. 5 Electronic system to register protons and electrons signal

Both of the above approaches have some advantages. Using the first way, the time spectra of protons, electrons and the coincidences of both signals can be easily extracted from event–by–event data files and displayed in a computer screen; therefore we have good control over experiment. The second way gives just the number of registered coincidences but is completely independent from computer system and a dead time created by the software of the acquisition system. Such a cross-checking of the results is very valuable, as it must be taken into account that the computer controlled data acquisition system has certain, not well defined reaction time (e.g. the Windows operating system is characterized by the rather high interrupt latency, the TDC unit is closed for pulses during time when computer reads data from it, etc.). Therefore some event losses are unavoidable. Efficiency of the proton detection using the secondary electrons registered in the channeltron was defined as the ratio of the coincident events to the total number of the particle detector signals.

Apart from the fast timing tracks, slow energetic signals were also collected by Caen ADC (Fig. 5). The electronic system enabled to register protons and electrons from both tracks (energetic and fast), additionally protons and electrons number were counted in the scaler. Therefore, the coincidences of slow energetic proton and electron signals could have been compared with coincidences between fast timing signals and simultaneously with hardware coincidences from logic unit. CMB program allowed extracting data, registered event-by-event, all from the spectrum. This provided information on coincidences of all four tracks (energetic and fast timing), and cut off the electronic noise.

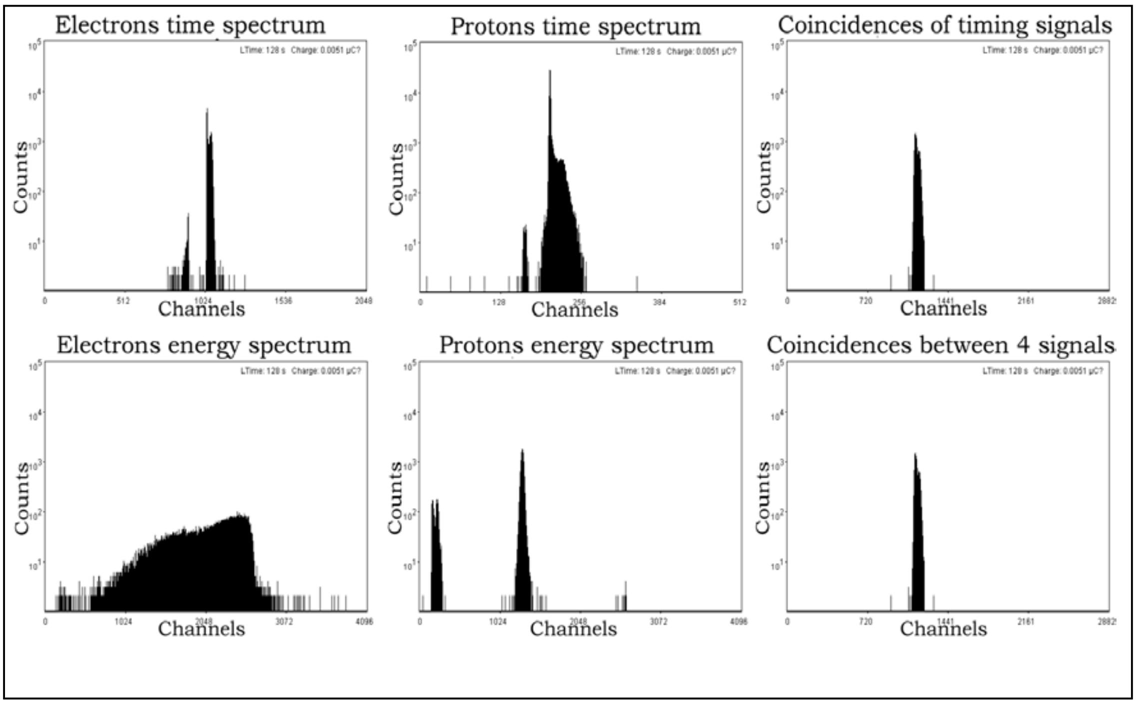


Fig. 6 Examples of received spectra from one measurement

Table 1.

Number of counts in proton energy spectrum	Number of counts in electron energy spectrum	Number of counts in proton time spectrum	Number of counts in electron time spectrum	Number of protons counted in scaler	Number of electrons counted in scaler	Number of coincidences between time protons and time electrons impulses	Number of coincidences between all 4 signals	Number of coincidences counted by Coincidence Logic Unit
73102	**56689**	**73234**	**56195**	**89964**	**58317**	**43940**	**43302**	**50190**

Table 1 gives an example of results from one of the measurements, spectra of which are presented in the Fig. 6.

The efficiency evaluated from the particular spectrum (shown in fig. 6) amounts to

- 57% considering fast timing tracks, and
- 56% considering counts in Coincidence Logic Unit

We observed that the number of the coincidences between all four signals was comparable to the number of coincidences between two timing signals from protons and electrons (discrepancy smaller than 0.02%).

From the previous experiments [Cholewa (2001), Fisher(2001), Polak (2003)] the Si_3N_4 membrane covered with the CsI layer proved to be more efficient than Si_3N_4 itself. The similar results were obtained in the present work. The Si_3N_4 membrane efficiency for proton registration was 10% only, while for Si_3N_4 covered with CsI the best result achieved was 61%. The 61% efficiency is not sufficient for the investigation purposes, where efficiency well above 90% is vital to hit single cell with single proton. Furthermore, we observe that the efficiency is deteriorated in time as shown in the graph below (Fig. 7). This means that the use of a new window is recommended each day. In the figure below each bar corresponds to a slightly different beam spot position on the window surface; therefore efficiency degradation can not be attributed to local destruction of the window surface due to the proton bombardment.

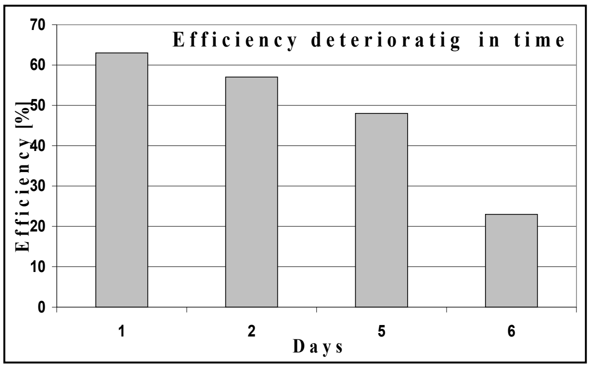


Fig. 7 Efficiency deteriorating in time

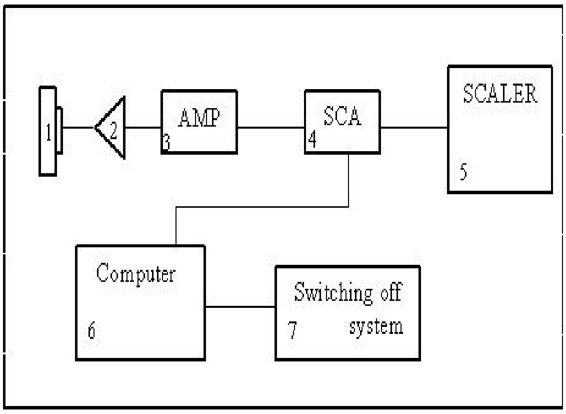
We will attempt to solve the problem of poor efficiency of proton registration in two different ways.

1. The further test with Si_3N_4 covered with the CsI layer will be performed in better geometrical conditions inside the standard vacuum chamber. Additionally, a mesh with positive voltage will be applied in front of the channeltron. The vacuum conditions also proved to be important to register secondary electrons [Cholewa (1998)]. The vacuum level during our measurements was about 2.3×10^{-5} Tr while following conclusions from abovementioned Cholewa's publication at least 5.0×10^{-6} Tr seems to be crucial. If those future improvements allow registering protons with efficiency better than 90%, this method will be used in the single-hit-system.
2. Protons will be registered by a detector (Si or PIN diode) behind the cell dish. This method, although giving about 100% efficiency, has some disadvantages:
   - During the time of the experiment medium must be taken out from the cell dish, otherwise protons will not reach the detector. The SRIM evaluations prove that 100% of protons will be transmitted to the detector considering: i) 200μm distance from the outlet window to the bottom of the cell dish, ii) 3μm mylar foil thickness, iii) 30μm of the cell thickness, iv) 5mm distance from the cell to the detector). This approach also decreases the possible experiment time to less than 10 minutes which guarantees survival of the cells.
   - Observation of the cell dish is more difficult because the detector must be positioned in front of the microscope objective for the time of measurement.

The single-event system

The fast blanking of the beam (Fig. 8) is very important to obtain just one proton hit per cell (providing 100% efficiency of proton registration was achieved).

Both the theoretical calculations and the experimental data proved that 440V applied to deflecting plates was enough to divert protons from entering the chamber. In time the blanking system was switched on, neither the protons nor electrons were registered by the corresponding detectors. The electronic system was rearranged to perform testing experiments for the beam blanking. Pulse from amplifier (electrons or protons) went to Single Channel Analyzer SCA (Polon 1201) where the applied thresholds (Fig. 9) cut off the electronic noise.



1. Silicon detector
2. Ortec Preamplifier
3. Active Filter Amplifier – Ortec 572
4. Single Channel Analizer (SCA) – Polon 1201
5. Scaler – Polon 401
6. Computer with Analog Output Board card which runs the blanking process and collects data from experiment
7. Switching off system – Technisches Büro S. Fisher

Fig. 8 Electronic system for measuring deflection efficiency

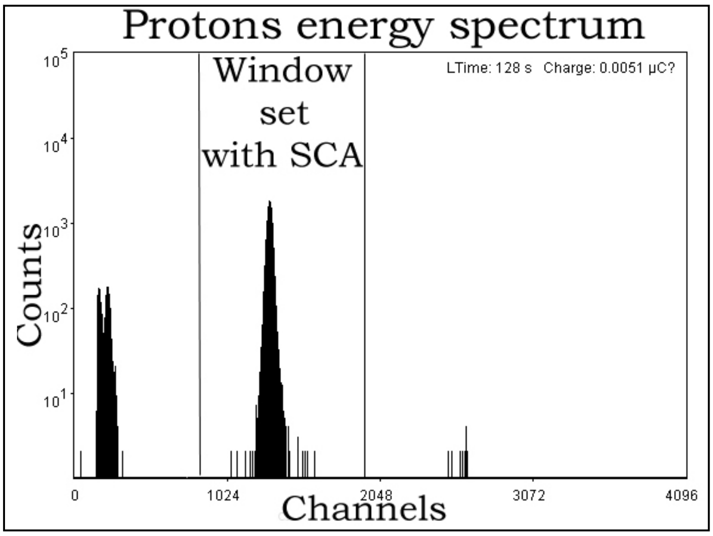


Fig. 9 Picture shows the window set with SCA on energy of protons to cut off electronic noise

The computer after obtaining the outgoing TTL signal, created in SCA, rapidly switched the beam off. After 500 ms the beam was switched on until the proton pulse was registered and the beam was switched off again. From calculations we found out that even for 50 µs resolution of the blanking system, the probability of registration two protons was about 0.1% for 0.1 fA beam current. Measured delay between the pulse from detector to the moment of application of 440 V onto deflecting plates was of about 13 µs.

The beam blanking efficiency was tested using two different methods:

Method 1.

Switching the beam off after registering pulse from the proton detector located just behind the exit window and, then, measuring the number of protons still reaching the detector after the beam blanking signal was sent. To assure proper statistics, those measurements were repeated many times and for various beam currents.

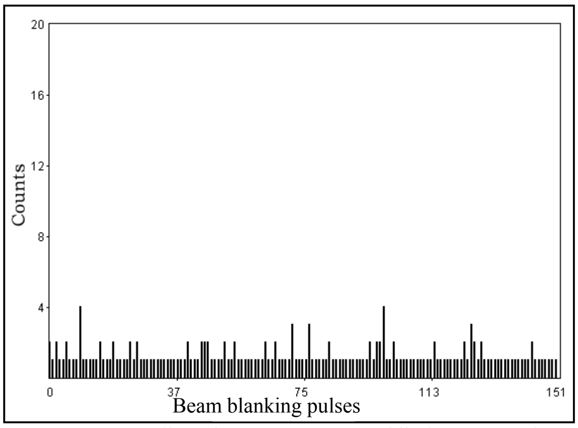


Fig. 11 Deflecting Protons efficiency using silicon detector signals
- beam intensity 800 protons/sec

In most occasions the deflecting system managed to switch the beam off fast enough to prevent more than one proton hitting the detector. However, there were cases that more than one count was accumulated before next blanking of the beam (Fig.10).

The experiment presented in Fig.11 shows that increasing the beam current causes malfunction of the system and more than one proton hit detector in many occasions. The graph shows mean values for the particular beam current for the whole measurement when real results (Fig.10) vary from one to few protons per one switch off event. For beam intensity smaller than 3000 protons/sec the blanking system has a constant performance of about 20% away from the ideal case (the ideal case is 1 one proton per one blanking event that is a value of 1 in Figure 11, while currently this value is about 1.2). The cases when more than one proton hit the detector are due to the data acquisition system performance that can be improved by switching the beam off using just electronic hardware (not by a computer system). The abovementioned beam intensity of 3000 protons/sec is more than sufficient for successful cell irradiation investigations.

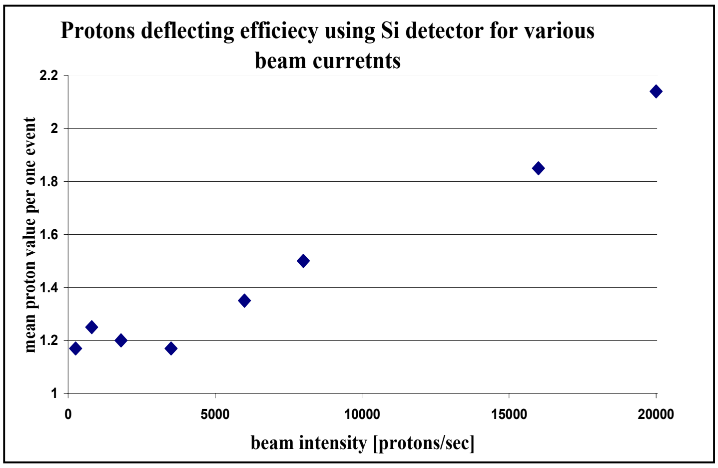


Fig. 11 Efficiency of deflecting proton using silicon detector for various beam intensities

Method 2.

Beam blanking after registering an event of proton passage through the exit window (i.e. secondary pulse from the channeltron) and measuring the number of protons reaching the detector afterwards. If a sufficient, close to 100% proton passage detection were achieved, this would be the principal method of carrying out the irradiation. However, due to the use of this particular exit window for five consecutive days, the measured efficiency of registering protons via registering secondary electrons was only of about 25%. Consequently, the expected value of successful beam blanking was close to this number, what was confirmed by the results shown in Figure 12 where, in average, 4.4 protons were registered per one blanking event. Unfortunately, the corresponding proton number distribution extended from one proton up to even 35 in extreme cases. That showed that at the moment the described system did not match experimental requirements whatsoever (Fig. 12)

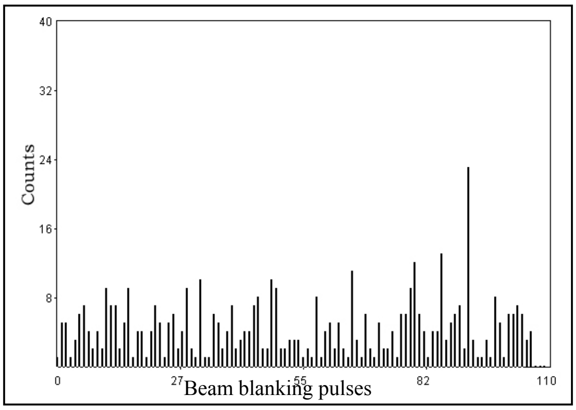


Fig. 11a Deflecting Protons efficiency using chaneltron signals
- beam intensity 250 protons/sec

Single hit resolution

The results of Fig. 12 may be illustrated using a different approach, used in another test experiment. To estimate the resolution of single-proton-hit system and the performance of positioning system, a solid state CR-39 detector (1.5 mm thick) was installed on a 3D moving table at a distance of about 1 mm from the exit window and then irradiated with protons. Unfortunately, the CR-39 detector was too thick to simultaneously register the protons with another particle detector located behind the CR-39. The beam was switched off using the secondary electrons signal from the channeltron. A pattern of 10 x 10 grid was created by changing the table position by 100 µm after each blanking event. After the irradiation process, the CR-39 detector was etched in a water solution of NaOH (6.25 mol/dm^3) at the temperature of 70°C for 6 hours. Next, after washing in ethanol, the sample was investigated using an optical microscope.

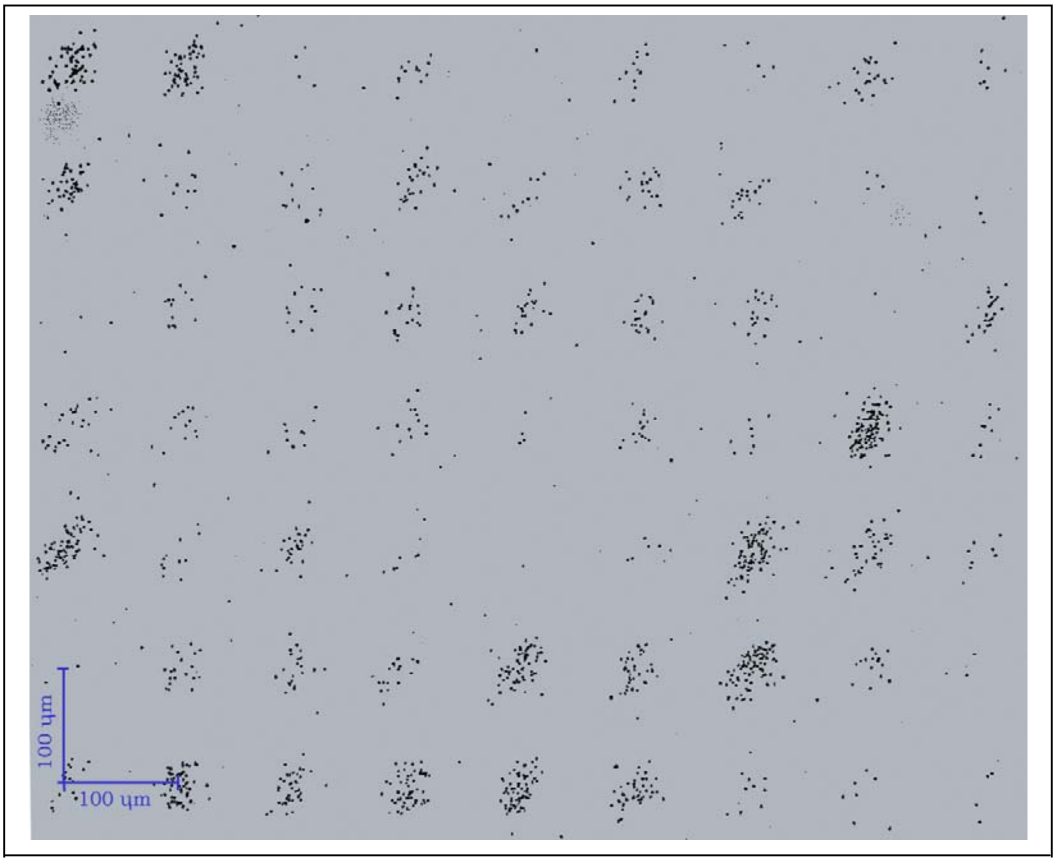


Fig. 13 Picture showing proton tracks obtained after etching CR-39 detector

As shown in Fig.13, every spot corresponding to a temporary beam position contains from one up to 50 proton track aggregations. The method allows displaying single proton tracks. The beam targeting resolution estimated from the picture is of about 40 µm which is not yet good enough to target the single cell. Poor spatial resolution of the beam is caused by a large (1mm) distance of the CR-39 detector from the exit window. Moreover, behaviour of one of the quadrupole lenses during the present experiment was not "normal" and the dumping of vibrations of the vacuum connection to the turbo-molecular pump was not perfect. To improve the accuracy, primarily, the good beam resolution (in vacuum) should be restored to 3 x 3 µm^2. The distance between sample and window must be reduced (cf. results shown in Fig. 4a), after installing emergency vacuum alarm (task currently in progress) preventing consequences following the possible exit window rupture. The anti-vibrating system must be applied because the quivering may alter the table movement and the precision of beam targeting during measurement.

Conclusions

The experiments described above show preliminary results of the performance of the first external microbeam in Poland. Although the system is not yet ready to irradiate single cells with single ions in a regular way, many useful and necessary tests can be carried out. The resolution of 40μm in the single hit mode is a good and promising start. The following experiments with modified Si_3N_4 windows in the vacuum chamber should give an answer which system of proton detection will be used in our facility. Electronic systems of detecting protons and electrons have been successfully tested as well as the deflecting system which allows switching into single-hit mode. The information gathered so far will be used in near future to improve the system performance and to make the first cell irradiation possible.

References:

Sawant SG, Randers-Pehrson G, Geard CR, Brenner DJ and Hall EJ: The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells in vitro can be initiated in the unirradiated neighbors of irradiated cells. - *Radiat Res*, (2001) 155:3, 397-401.

Watson GE, Lorimore SA, Macdonald DA and Wright EG: Chromosomal instability in unirradiated cells induced in vivo by a bystander effect of ionizing radiation. - *Cancer Res*, (2000) 60:20, 5608-11.

S.Lebed, Z.Stachura, M.Cholewa, G.J.F.Legge, J.Lekki, S.Maranda, A.Potempa, C.Sarnecki, Z.Szklarz, J.Styczeń, B.Sulkio–Cleff – „The new Cracow scanning nuclear microprobe", *Nucl. Instr. Meth. In Phys. Res. B* 181 (2001) 95–98.

Belyakov OV, Malcolmson AM, Folkard M, Prise KM and Michael BD: Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts. - *Br J Cancer*, (2001) 84:5, 674-679.

Nagasawa H and Little JB: Unexpected Sensitivity to the Induction of Mutations by Very Low Doses of Alpha-Particle Radiation: Evidence for a Bystander Effect. - *Radiat Res*, (1999) 152:5, 552-557.

Zhou H, Suzuki M, Randers-Pehrson G, Vannais D, Chen G, Trosko JE, Waldren CA and Hei TK: Radiation risk to low fluences of alpha particles may be greater than we thought. – *Proc Natl Acad Sci USA*, (2001) 98:25, 14410-14415.

M.Folkard, K.M.Prise, B.Vojnovic, S.Gilchrist, G.Schettino, O.V.Belyakov, A.Ozols, B.D.Michael, "The impact of microbeams in radiation biology", *Nucl. Instr. Meth. In Phys. Res. B* 181 (2001) 426–430

Folkard M, Vojnovic B, Prise KM, Bowey AG, Schettino G, Michael BD: A charged paticle microbeam: I. Development of an experimental system for targeting cells individually with counted particles - *Int. J. Radiat. Biol.* (1997) 72:375

Folkard M, Vojnovic B, Prise KM, Bowey AG, Schettino G, Michael BD: A charged paticle microbeam: II. Development of an experimental system for targeting cells individually with counted particles - *Int. J. Radiat. Biol.* (1997) 72:387

Moretto Ph. et al: Development of a single ion irradiation system at CENBG for applications in radiation biology – , *Nucl. Instr. Meth. In Phys. Res. B* 181 (2001) 104-109

W. Polak, J. Lekki, J. Gryboś, R. Hajduk, M. Cholewa, O. Kukharenko, Z. Stachura: Testing the Efficiency of the Si_3N_4 Membranes for Charged Particles Registration, *Nukleonika* **48** (2003) 25

Cholewa M, Kamiya T, Saint A, Prawer S, Legge G, Butler JE, Vestyck DJ
Diamond membranes: applications for single ion detection using secondary electron emission - *Diamond and Related Materials* 7 (1998) 510-512

Fischer BE, Cholewa M, Hitoshi Noguchi Some experiences on the way to biological ingle ion experiments: ", *Nucl. Instr. Meth. In Phys. Res.B* 181 (2001) 60-65

Cholewa M, Heiß M: Preparatory experiments for a single ion hit facility at GSI ", *Nucl. Instr. Meth. In Phys. Res.B* 210 (2003) 296–301

Belyakov OV, Folkard M, Mothersill C, Prise KM and Michael BD: Bystander-induced apoptosis and premature differentiation in primary urothelial explants after charged particle microbeam irradiation. - *Radiation Protection Dosimetry*,
(2002) 99:1-4, 249-251.

J. Lekki, R. Hajduk, S. Lebed, A. Potempa, T. Pieprzyca, Z. Stachura, M. Ziębliński, and J. Styczeń: CMB v. 1.1 Data Acqusition and Evaluation System of the Cracow Nuclear Microprobe" – *IFJ Report No 1856/AP* (2000)

J.F.Ziegler – SRIM version from 1998

Technisches Büro S.Fisher, Am Berg 9, D-64 372 Ober-Ramstadt, Germany,
tel. +49-6154-4818

J.Lekki, M.Cholewa1, E.Dutkiewicz, A.Gwiazdowska, R.Hajduk, K.Irzyńska, S.Lebed2, P.Mazur, W.Polak, A.Potempa, T.Pieprzyca, C.Sarnecki, Z.Szklarz, A.Veselov, R.Zając, Z.Stachura, J.Styczeń: SPH IFJ Project of Single Proton Irradiation of Biological Cells- *IFJ Report No 1915 (2002)*

http://www.sjuts.com

http://www.silson.com