Master of Science Thesis

Irradiating Single Cells with Single Ions: A Feasibility Study

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Abstract

**Background:** To gain more knowledge of the mechanism behind the appearance of radiation induced cancer, the cellular response at low doses has to be studied. A Single Ion Hit Facility (SIHF) at the Lund Nuclear Microprobe Laboratory will be used for this purpose. The facility allows single MeV ions to hit single living cells.

**Purpose:** The goals of this work were to i) design a suitable cell dish where the cells are attached during irradiation and investigate how the cells tolerate being ii) exposed to air, iii) placed in a vertically position and if it is possible to iv) determine the lineal energy and the specific energy both theoretically and experimentally.

**Results:** It was shown that the specially designed cell dish, made of 200 nm thick Si$_3$N$_4$ glued on acrylic plastic fulfilled its purpose to fit in the irradiation chamber without any reconstructions of the sample holder had to be done, and it could easily be improved i.e. include more Si$_3$N$_4$ irradiation windows. This thesis shows that there is a linear relationship between time of air exposure and survival and consequently the time of exposure of air has to be considered when studying the cell response after irradiation. The lineal energy and the specific energy in cells were determined theoretically to be 13.9$\pm$2.7 keV$\mu$m$^{-1}$ and 82$\pm$16mGy respectively. From irradiation two energy spectra were obtained, with and without cells. No significant difference were found between the spectra and therefore, no lineal energy or specific energy could be calculated.

**Conclusion:** To minimize cell damage other than from irradiation, the cells cannot be handled in open air. It was shown that cells positioned vertically move, which cannot be allowed when aiming at sub micrometer structures.

A cell dish with five 200 nm thick Si$_3$N$_4$ windows, was constructed. V79 hamster cells were cultured on the dish before irradiation, from which two energy spectra were obtained, with and without cells. These two spectra were analyzed and the result could neither prove or discard the theoretical results due to no statistical significance.
1 Introduction

1.1 Background

When biological material is traversed by charged particles there is a possibility that they will directly interact with the critical targets, e.g. the DNA chain, in the cell. The target will be ionized or excited which leads to biological damages e.g. DNA strand breaks. Another possibility is the indirect action i.e. the charged particles will interact with other parts of the cells, e.g. water molecules, and produce free radical. The free radicals are highly reactive and can diffuse a short distance to critical targets [1, 2]. To describe the stochastic effects of radiation induced damages a linear-quadratic model is used. The model is linear up to about 1 Gy and at higher doses it is quadratic [3]. Data on ionizing radiation induced cancer in humans is of a low statistical value because the number of persons are small and doses are relative large [4]. To estimate the effects with low dose irradiation, data from high dose irradiation is back-extrapolated. Recent experiments indicate that these back-extrapolations do not provide an accurate description of the effect from low dose irradiation [5, 6]. One reason why the linear-quadratic model does not fit to actual measured results at low doses may be due to the so called bystander effect [7]. Previous research found that doses up to 1 Gy induces a higher cell death than estimated from the linear-quadratic model [3, 8]. To investigate the actual relationship between low dose irradiation and response, studies has been done using broad beams to irradiate cell colonies. This method is of low accuracy because of insufficient information about the number of irradiated cells, the number of particles traversed a particular cell and spontaneous cell death in control cell cultures [6]. However, when using a micro beam for irradiating cells, it will be possible to gain more knowledge of the radiobiological effects, due to the high hit accuracy and precision. Using the micro beam it is feasible to hit single cells with single ions e.g. protons [5]. The ions can even be aimed at very precise locations within the cells for example the nucleus. The use of a micro beam also facilitates studies in the
1. INTRODUCTION

The effects of non-hit cells [3, 6, 7, 9, 10, 11], i.e. the bystander effect.

1.2 Microbeam

The accelerator, at the Lund Nuclear Microprobe, The Division of Nuclear Physics, Lund Institute of Technology, used in this work is a single ended 3 MV van de Graaff accelerator that can generate hydrogen ions using a radio frequency ion source, described in more detail by Nilsson et al. [2]. Two pairs of slits are placed along the horizontal beam line to collimate the beam. Close to the irradiation chamber three magnetic quadrupoles and a magnetic steering system are placed (figure 1.1). The beam is focused by the quadrupoles and can be focused down to sub micro-meter size [12]. The ions are extracted at the end of the beam pipe through a 200 nm thick vacuum window, made of Si₃N₄. After passing the window they travel through a thin (100 µm) air gap and then hit the irradiation window, where the cells are attached. In this experiment it is required to hit each cell with just one single proton. To do this, the beam has to be blanked immediately after a cell hit. To be able to blank the beam intensity has to be below 1000 protons per second [2]. This is done with an electrostatic shutter that consists of two plates and an electric deflection field in between. The blanking system is activated by a particle detector (PIN) [13], placed close behind the cell sample [12].

![Figure 1.1: The microbeam facility](image-url)
1. INTRODUCTION

1.3 Aims

To understand the underlying mechanism by which charged particles interact with living matter could help us to better estimate the risk of ionizing radiation and to develop more efficient treatment by radiotherapy [2, 5, 10, 14]. This thesis is concerned with preparations for the study of the response on singular cells hit by single ions.

A horizontal microbeam facility similar to the one in Lund, has been developed by Reinert et al. [11]. For aiming at single cells they had to construct a specially made mini Petri dish, that fit into the irradiation chamber. Due to the horizontal beam line, the Petri dish had to be in a vertical position, which can affect the position of the cells. Targeting with a precision of sub micro meter level, it is of great importance that the location of the cells is precisely fixed. Because the vertical position the cell medium had to be removed from the Petri dish and the cells are consequently exposed to the air. Following their approach for solving these difficulties, this thesis aims (step 1-3 below) at reach further to the completion of the microbeam facility in Lund.

For studying the cellular response after irradiation, the energy deposition in the cells has to be determined (step no. 4).

1. Design a suitable cell dish.

2. Study how cells manage to be exposed to the atmosphere in the accelerator hall.

3. Investigate any possible movement of the cells when they are positioned vertically.

4. Determine the energy deposition in the cells after targeting them with single protons.
2 Materials and Methods

2.1 Cell culturing

Lung fibroblasts (V79) of the Chinese hamster were used in this work. V79 cells were cultured in a Petri dish, at 37 degrees Celsius in an atmosphere of 95% air and 5% CO$_2$ [5]. The atmosphere is chosen for simulating in vivo conditions. The cells were grown in a nutrient solution of DMEM (Dulbecco’s modification of Eagle’s Medium), fetal calf serum, penicillin, streptomycin, and glutamine [15].

2.2 Designing cell dish

The cell dish, made of acrylic plastic, had to fit into the existing equipment i.e. the sample holder inside the irradiation chamber. The dish was sawed out in the dimension of 80.0×30.0×3.2 mm$^3$. Five holes were drilled, 1.5 mm radius in the dish. Previous work regarding thickness and material of the irradiation window, where the cells are attached, suggest 200 nm thick silicon nitride, Si$_3$N$_4$ to be used for this purpose [2, 11]. The frame of the irradiation window was 5×5 mm$^2$ and 200 µm thick and the window was 1.5×1.5 mm$^2$. Around each hole a hollow was drilled, 200µm deep and 3.54 mm in radius. In these hollows the irradiation windows were positioned and attached with bee wax. The wax were put into a pipet tip which was twined in by a thin isolated copper wire (figure 2.1). The wire was connected to a adjustable power supply for heating the wire and thereby melting the wax. The melted wax was dispensed out around the Si$_3$N$_4$ frame, enough to fill the empty space between the frame and the dish.
2. MATERIALS AND METHODS

Figure 2.1: The thin isolated copper wire twined around the tip, which was filled with bee wax.

2.3 Exposure of the cells to the atmosphere in the accelerator hall

To prevent scattering and straggling of the protons, as much as possible of the nutrient solution has to be removed from the cell dish during irradiation. When removing the medium, the cells are exposed to the air and the cells will dry out, which could lead to unwanted damage or cell death. To exclude other possible damage factors than irradiation, it has to be established how the cells are affected by exposure to the air. Cells were plated in a 96 multi well dish (figure 2.2) with an equal number of cells in each well. The cell dish was placed in the incubator 20 hours. The solution was removed and the cell dish was brought to the accelerator hall. The 96 wells were divided into 6 groups, with 16 wells in each. The top of the multi well dish was removed in such a way that the first group was exposed to the air in 25 minutes and the last group in 0 minutes. The cell dish was brought back to the preparation room and 5 µl cell medium was added to each well and the cells were incubated for 24 hours. After that, the medium was sucked out and the cells were washed with PBS. The PBS solution was removed and Trypsine was added. The cells were again incubated for 10 minutes. The dish was carefully knocked to make the cells loose from the dish. The cells from each group (1-6) were sucked out and counted in a Bürker chamber.
2. MATERIALS AND METHODS

Figure 2.2: A 96 multiwell dish, where cells are exposed to air in different time intervals (0, 5, 10, 15, 20, and 25 minutes).

2.4 Positioning the cells vertically

Cells were grown in a Petri dish marked with a reference line (few µm thick) in the surface. The line was used as a fix position marker from which a possible movement of the cells could be detected. A picture was taken of the cells and the reference line. The Petri dish was later placed in a vertical position in the incubator. After 20 minutes a new picture was taken. The images were compared, by measuring four distances (L₁-L₄) between the marker and two cells using the imaging software ImagePro 4.0 [16]. The distances were measured four times in order to determine the accuracy of the measurements. This procedure was repeated with three different cell cultures.

2.5 Calculation of the energy deposition

To relate the cellular response to dose, the lineal energy, \( y \), and specific energy, \( z \), was calculated.

The lineal energy \( y \) is defined according to ICRU 33 [17] as the quotient of
2. MATERIALS AND METHODS

\( \epsilon \) by \( \bar{l} \):

\[
y = \frac{\epsilon}{\bar{l}} \left[ Jm^{-1} \right],
\]

were \( \epsilon \) is the energy imparted to the matter in a volume by one energy deposition event and \( \bar{l} \) is the mean chord length of the volume. The specific energy \( z \) [17] is the quotient of \( \epsilon \) by \( V \) and \( \rho \):

\[
z = \frac{\epsilon}{\rho V} \left[ Jkg^{-1}, Gy \right],
\]

were \( \epsilon \) is the energy imparted by ionizing radiation to matter in a volume \( V \) with density \( \rho \).

This was done both theoretically by stopping power calculations and experimentally by analyzing two energy spectra, before and after transmission through the cells.

The stopping power values and the energies were calculated by a software program, SRIM (The Stopping and Range of Ions in Matter) [18], described by Nilsson [2]. The two irradiations were done, with a continuous proton beam scanned over the irradiation window, the first scan with cells attached to the window and the second without cells. Two energy spectra were thus obtained, from which the energy deposited in the cells could be determined. For the calibration of the energy spectra the energy of the incoming protons and the thickness of each layer, i.e. vacuum window, air gap, irradiation window, and cell layer, the protons traverse has to be known. Then it is possible to simulate the same system in SRIM and the energy loss can be calculated in each layer. Then a calibration equation is adapted to match both situations i.e. the energy spectra and the simulated system in SRIM.
3 Results

3.1 Designing the cell dish

The final design of the cell dish is shown in figure 3.1, where two Si$_3$N$_4$ irradiation windows are glued with wax. Figure 3.2 shows in close up the melted wax dispensed out around one of the frames of the irradiation windows.

Figure 3.1: The acrylic cell dish placed in the sample holder inside the irradiation chamber. Two Si$_3$N$_4$ windows are glued with wax to the holder. The beam pipe, where the protons are extracted, is shown to the left (Please note that this setting is not optimized i.e. the distance between beam pipe and irradiation window is not minimized).

Figure 3.2: The melted wax is dispensed out around the Si$_3$N$_4$ frame.
3. RESULTS

3.2 Exposure the cells to the atmosphere in the accelerator hall

The cells in each well were counted in the 96 multiwell plate and a cell survival curve as a function of time was obtained. The number of surviving cells were found to decrease with time of exposure to air (figure 3.3) and there seems to be a linear relationship between time of exposure and cell survival.

![Cell survival as a function of time](image)

**Figure 3.3:** Cells exposed to the air in the accelerator hall. The graph shows a linear relationship between time of exposure and cell survival.

3.3 Positioning the cells vertically

A mean value of the four measured distances (L₁-L₄) for three different cell cultures (figure 3.4) were calculated. The measurement uncertainty was determined by Student’s t-test and the result shows that in 42% (5 out of 12) of the measurements there was a significant (p=0.01) movement of the cells after 20 minutes in a vertical position (Table 3.1).
3. RESULTS

Figure 3.4: Three different cell cultures, where the distances ($L_1$-$L_4$) were measured, for determine any possible movement when the cells are positioned vertically. A: Cells and marker before vertical position. B: After 20 minutes in a vertical position.

Table 3.1: The result from the measurements of distances ($L_1$-$L_4$) in three different cell cultures.

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Length no.</th>
<th>Mean Before [µm]</th>
<th>Mean After [µm]</th>
<th>Significance of movement (p = 0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$L_1$</td>
<td>118.15</td>
<td>126.01</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>$L_2$</td>
<td>108.63</td>
<td>115.12</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>$L_3$</td>
<td>91.44</td>
<td>90.43</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_4$</td>
<td>97.83</td>
<td>89.56</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>$L_1$</td>
<td>345.65</td>
<td>343.59</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_2$</td>
<td>317.43</td>
<td>317.58</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_3$</td>
<td>359.32</td>
<td>358.11</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_4$</td>
<td>385.79</td>
<td>391.16</td>
<td>yes</td>
</tr>
<tr>
<td>3</td>
<td>$L_1$</td>
<td>166.68</td>
<td>171.25</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>$L_2$</td>
<td>141.05</td>
<td>143.88</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_3$</td>
<td>327.78</td>
<td>327.40</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>$L_4$</td>
<td>357.89</td>
<td>357.45</td>
<td>no</td>
</tr>
</tbody>
</table>
3. RESULTS

3.4 Calculations of the energy deposition

3.4.1 Theoretical calculation of lineal energy and specific energy

The initial energy (1) given, the energy loss after passing the vacuum window (2), the air gap (3), the irradiation window (4), and the cell thickness [19] (5) (figure 3.5) could be calculated, using the stopping power values obtained from SRIM.

\[
E = 2.56\text{MeV} \quad (1)
\]

\[
E(43.52\mu m, \text{Si}_3\text{N}_4) = 2.55\text{MeV} \quad (2)
\]

\[
R(2.56\text{MeV}, \text{Si}_3\text{N}_4) = 43.72\mu m
\]

\[
x = 200 \times 10^{-3}\mu m
\]

\[
(43.72 - 200 \times 10^{-3})\mu m = 43.52\mu m
\]

\[
R(2.55\text{MeV}, \text{air}) = 103.08\text{mm}
\]

\[
x = 20.0 \pm 2\text{mm}
\]

\[
(103.08 - 20\text{mm} = 83.08\text{mm}
\]

Figure 3.5: The setting, where (1)/(2) is the proton energy before/after the vacuum window, (3) the proton energy after the air gap, (4) the proton energy after the irradiation window, and (5) is the proton energy after passing the cell layer.
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\[ E(83.08 \text{mm}, \text{air}) = 2.24 \text{MeV} \]  
(3)

\[
\begin{align*}
E &= 2.24 \text{MeV} \\
R(2.24 \text{MeV}, Si_3N_4) &= 34.48 \mu m \\
x &= 200 \times 10^{-3} \mu m \\
(34.48 - 200 \times 10^{-3}) \mu m &= 34.28 \mu m
\end{align*}
\]
irradiation window

\[ E(34.28 \mu m, Si_3N_4) = 2.23 \text{MeV} \]  
(4)

\[
\begin{align*}
E &= 2.23 \text{MeV} \\
R(2.23 \text{MeV}, H_2O) &= 92.11 \mu m \\
x &= 7.2 \pm 1.2 \mu m \\
(92.11 - 7.2) \mu m &= 85.11 \mu m
\end{align*}
\]
cell layer

\[ E(85.11 \mu m, H_2O) = 2.13 \text{MeV} \]  
(5)

The difference between the energy of the protons after passing the irradiation window (4) and energy of the protons traversed the cell layer (5) gives the energy imparted \( \epsilon \) in the cells.

\[ \epsilon = (2.23 - 2.13) \text{MeV} = 100 \text{keV} \]

The energy imparted \( \epsilon \) divided by the mean chord length \( l \), estimated to be equal to the cell thickness, gives the lineal energy, \( y \).

\[ y = \frac{100 \text{keV}}{7.2 \mu m} = 13.9 \text{keV} \mu m^{-1} \]

With the uncertainty, \( dy \):

\[ dy = y \sqrt{\left( \frac{da}{a} \right)^2 + \left( \frac{dc}{c} \right)^2} \]

were \( da \), is the estimated uncertainty (10%) of the measured air gap distance, \( a \), the distance of the air gap, \( dc \) the uncertainty in cell thickness, and \( c \) is the
3. RESULTS

cell thickness. The thickness of the Si$_3$N$_4$ windows is assumed to be known exactly.

\[
\frac{dy}{\mu m} = 13.9keV \mu m^{-1} \sqrt{\left(\frac{1.2}{7.2}\right)^2 + \left(\frac{2.0}{20.0}\right)^2} = 2.7keV \mu m^{-1}
\]

The energy imparted, $\epsilon$, divided by the volume $V$ and $\rho$ gives the specific energy, $z$. Were the $V$ of the cell is estimated to a sphere with the diameter of the cell thickness 7.2± 1.2 $\mu$m and the density $\rho$ equal to water, 1 g/cm$^3$

\[
z = \frac{100keV}{\frac{4}{3} \pi \left(\frac{7.2\mu m}{2}\right)^3 \times 1g/cm^3} = 82mGy
\]

With the uncertainty, $dz$:

\[
dz = z \sqrt{\left(\frac{da}{a}\right)^2 + \left(\frac{dc}{c}\right)^2},
\]

were $da$, is the estimated uncertainty (10%) of the measured air gap distance, $a$, the distance of the air gap, $dc$ the uncertainty in cell thickness, and $c$ is the cell thickness.

\[
dz = 82mGy \sqrt{\left(\frac{1.2}{7.2}\right)^2 + \left(\frac{2.0}{20.0}\right)^2} = 16mGy
\]

3.4.2 Experimental determination of lineal energy and specific energy

The energy calibration of the spectra (figure 3.6 and 3.7) gives,

\[
E(chn) = 21 \times chn - 763keV,
\]

where $chn$ is the channel number. The energy peak was at $chn=125$, FWHM = 11, with cells and $chn=126$, FWHM = 7, without cells.
3. RESULTS

It is assumed that the shape of the energy spectra is Gaussian, therefore the standard deviation, \( \sigma \), can be calculated through the relation

\[
FWHM = 2.25 \times \sigma
\]

\( \sigma \) of energy spectra with cells (FWHM=11) is:

\[
\sigma = \frac{11}{2.35} = 4.68
\]

\( \sigma \) of energy spectra without cells (FWHM=7) is:

\[
\sigma = \frac{7}{2.35} = 2.98
\]

The channel number of the peak for spectra with cells and without cells (CI=95\%) is 125±9.17 and 126±5.84 respectively. Since the confidence intervals overlap the difference between the two peak values is not statistically significant.
3. RESULTS

Figure 3.6: Energy spectra from irradiation with cells. The x-axis shows the calibrated energy after the channel number.

Figure 3.7: Energy spectra from irradiation without cells. The x-axis shows the calibrated energy after the channel number.
4 Discussion

4.1 Cell dish

The specially designed cell dish fulfilled its purpose i.e. the energy loss in the irradiation window was negligible (10keV) and the acrylic plastic fitted into the existing equipment. The advantage of the cell dish is the simple construction and that it is very easy to improve, e.g. by making the acrylic thinner for minimizing the air gap between irradiation window and detector and to include more irradiation windows.

4.2 Exposure of the cells to air

After the nutrient cell medium is removed, a cover glass is immediately placed over the holes in the cell dish so the cells do not have contact to the air while bringing them to the accelerator hall. The critical time of air exposure is when the cover glass is removed and the cell dish is placed in the irradiation chamber. It is assumed that once the dish is placed in the sample holder and the irradiation begins the cells have no contact to the air, because the detector is placed close behind the dish. This result shows that the time of handling the cells outside their natural environment has to be taken into account when evaluating the cellular response after irradiation. The time of placing the cell dish into the irradiation chamber has to be measured to make a correction for cell damage due to the air exposure.

When sucking out the nutrient cell medium, not all will be removed. A thin layer of the solution will remain, in which the pH-value will be changed during air exposure. This effect has not been investigated here, but it will most probably affect the cells in a negative matter.
4.3 Irradiation

For technical reasons, such as break downs of one of the focusing magnets and difficulties in aligning the beam, no irradiation when steering single protons to the single cells could be done. However, a method of finding and irradiating single cells has been developed. Cells will be plated on the Si$_3$N$_4$ windows and before irradiation an image of the cells is taken. The image is analyzed and the coordinates of the cells are found (figure 4.1) by a program specially made for this purpose by Ph.D. student Natalia Arteaga, at the Department of Nuclear Physics, Lund Institute of Technology. The program, made using IDL, searches the cells and provides the coordinates of the center of the cells. Figure 4.1 shows how the program finds the coordinates (red dots) of the cells. The coordinates are later sent to the computer that controls the magnets steering the protons to hit the selected location within the cells.

Figure 4.1: A: image of the cells attached to the irradiation window. B: The image is analyzed by the cell finding program, where the red dots represent the coordinates.

4.4 Hit verification

A simple test of the cell finding program was done where a pattern of dots was drawn in an image software. The pattern were analyzed by the program and
the coordinated were sent to the steering computer and a C-39 track film was irradiated. The C-39 film was later developed and studied in a microscope (figure 4.2). As seen in the picture the arrows are blurred, which is due to a poorly aligned and optimized beam.

At the moment it is not possible to ensure where within the cells the protons hit or even if the protons hit the cells at all. One possible solution to this problem is to place an ultra thin silicon detector just before the irradiation window and the cells as discussed by Nilsson [2]. Reinert et al. [11] have solved this by using a digital camera inside the irradiation chamber. Images from the camera provide the relative coordinates of the cells with respect to the corners of the beam exit window. The coordinates of the exit window’s corners are know from a beam scan over the entire exit window. Once the coordinates of the corners are known, these can be used as reference markers, and the cells position can be extracted.

![Figure 4.2: A: image of the cells attached to the irradiation window. B: The image is analyzed by the program where the red dots represent the coordinates.](image)

### 4.5 Verification of cellular response

To verify the cellular response after single ion targeting two methods are proposed. The first and the easier method of verifying the response is Colony
4. DISCUSSION

Forming Assay (CFA) where the irradiated cells’ ability to form colonies is compared to the non-irradiated cells’. After irradiation the cells are plated on Petri dishes and incubated. A control cell group is incubated at the same time. After three days, when the cells have formed colonies they are taken out of the incubator and the colonies are counted both irradiated and non-irradiated. If the cells have taken damage from irradiation a decreased level of colony forming is expected.

Prise et al. [20] suggest a method of scoring micronuclei. Micronuclei are formed when fragments are lost during cell division. Micronuclei are good pointers of biological response in cellular systems. There is a 1:1 correlation between the amount of micronuclei and cell lethality [20]. For measuring micronuclei acridine orange staining will be used. Micronuclei will be clearly visible beside binuclei within the orange background of the cytoplasm. This procedure provides a method to follow each irradiated cell, in contrast to CFA, where only the irradiated cells as a group can be studied.

To reach further a completion of the study of cellular response of low dose irradiation using the single ion hit facility, it is important to introduce these two methods in the research.

4.6 Cell movement

The investigation of cell movement in vertical position for 20 minutes proved that in 5 of 12 measurements there was a significant difference in position (largest movement was 7.86 µm). A movement of this magnitude could not be accepted for this kind of research, were targets of sub µm size are concerned. This result differs from Reinert [11] study, were it was shown that the cells did not move when positioned vertically. Reinert only positioned the cells put vertically for 15 minutes and it is possible that there is a time dependence of cell movement, which has to be examined closer.

4.7 Calculations of the energy deposition

The theoretical calculation of the lineal energy and the specific energy assumes that a single proton is traversing one single cell, which is the required
situation in this research. Due to the accelerator related problems, as mentioned above, it was not possible to steer the protons to the cells coordinates. The proton beam was scanned over the entire irradiation window, which means that the energy spectra obtained from the irradiation with cells is an average between traversed cells and no cells. It is therefor not possible to compare the experimental results with the theoretical. However, one could expect a difference of energy deposition between the irradiation with cells and without cells. The result from the irradiation showed a small numeric difference in the channel number of the two peaks (125±9.17, 126±5.84), but the confidence intervals overlapped. Due to the lack of statistical significance it was not adequate to calculate any energy deposition in the cells from the experiment. The experimental result neither prove or discard the theory. A possible reason for the small difference could be that the calibration of the energy was done quite roughly i.e. the distances between the exit window and irradiation window, and irradiation window to the detector was measured with low accuracy.
5 Conclusion and future outlooks

A cells dish made of five 200 nm thick silicon nitride windows was glued to a acrylic disc. The dish could easily be improved by making it thinner to minimize the air gap between the cells and the detector. This may be important when the microbeam is ready for more high precision targeting and the hit accuracy will be investigated in detail. Investigations of how the V79 hamster cells manage to be exposed to the air in the accelerator hall, where it was found that the cells tolerate air exposure of approximately 10 minutes (80% survival). It is suggested that the time when the cells are out in open air is important to keep as short as possible. The time of handling the cells outside their nutrient solution could be shortened by having an incubator near the accelerator and suck out the cell medium just before the irradiation. The effect of the cells’ position when positioning them vertically for 20 minutes was studied and in 42% of the measurements a cell movement were found. If this is time dependent has to be further investigated. The lineal energy and the specific energy were determined theoretically. How this is related to the cellular response could be investigated by using CFA and micronuclei scoring, as described by Prise et al. [20].
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