According to the World Cancer Report, cancer rates could further increase by 50% to 15 million new cases in the year 2020. In the year 2000, malignant tumours were responsible for 12% of the nearly 56 million deaths worldwide from all causes. In many countries, more than a quarter of deaths are attributable to cancer [1]. To rescue these patients, surgery (with long history) has been used in the majority of cases as the primary form of treatment and it leads to good therapeutic results in a range of early non-metastatic tumors. Additionally, cytotoxic chemotherapy also has been used for patients [2–4]. However, for the tumors of the head and neck, cervix, bladder, prostate and skin, surgery or chemotherapy is not sufficient as a treatment. Here, radiotherapy is an efficient tool to achieve a reasonable probability of tumor control.

Radiation has been used for treating cancer for the last 100 years [5]. In the early days, simple low-energy X-rays were found to be very effective for treating superficial skin cancers. Higher energy machines and cobalt gamma sources were developed [6] that could treat deeper seated cancers [7, 8]. However, these early machines often gave a higher dose to the skin than to the tumor. Thirty years ago, linear accelerators, which accelerate electrons to near the speed of light to generate even higher energy X-rays, were developed. These have allowed treatment with far higher doses while staying below the tolerance of surrounding normal tissues. This has contributed to the higher cure rates for many cancers that we see today. In 1970 the overall cure rate for cancer was 25%; today it is 60% [9].

Although treatments by X-rays are in common use, they have an intrinsic draw
Introduction

back namely the depth–dose distribution. The dose decreases with penetration depth, thus most of the dose is deposited in and just below the skin. Given in sufficient doses, X-ray radiation techniques will control many cancers. But, healthy tissue that is crossed by the X-rays may receive a similar dose and thus can be damaged. Consequently, a less-than-desired dose or a combination of many fields is frequently used to reduce damage to healthy tissues and avoid unwanted side effects.

In 1946, Robert Wilson published a study that suggests that energetic protons could be used to treat cancer because they are capable of delivering an increased dose of radiation to a tumor while simultaneously decreasing radiation exposure to surrounding healthy tissue [10]. In 1948, the first proton therapy experiments were conducted at the University of California at Berkeley. Tumor control was achieved in the chest and lungs of animals [5]. The University of California at Berkeley treated the first patient with protons in 1954. Patients were treated with proton therapy also at other research institutions, including Harvard University in Boston [5]. By the end of 2007 there were about 34 hospital-based proton therapy centers running or under construction in the world [11]. This single number justifies the statement that proton therapy increases. Proton therapy centers are also planned for the Netherlands.

As seen in the figure 1.1, compared to other kinds of ionizing radiation such as electrons or photons, atomic particles such as $^{12}$C-ions and protons have a different depth distribution of the deposited dose, peaking at an energy-dependent depth (the Bragg peak) at the end of the particle’s track and dropping to zero beyond this peak. It is the volume selectivity given by the existence of a well localized Bragg-peak region, where the deposited dose is maximum, that can be shifted by energy variation over the target volume that makes proton and heavy ion therapy such a promising technique in cancer treatment. With photons the dose decreases exponentially with increasing depth. In figure 1.1 next to protons, carbon ions are shown because of the recent focus on heavy ions such as carbon ions. The main reason for the transition to carbon ions is the increased Relative Biological Effectiveness (RBE) in the last few centimeters of the carbon range [11]. The first heavy ion treatment of tumors was pioneered in Berkeley at the Bevalac facility in 1975 [12, 13]. In Germany, the Heidelberg Ion Therapy Centre HIT is a carbon ion radiotherapy facility [14].

On the macroscopic scale, the beneficial effects of energetic particle beams in radiotherapy are established, but what happens on a molecular level. Ionizing radiation induces a variety of damages in cellular DNA, which is thought to be the critical target of biological effects of radiation, by both direct energy deposition into DNA (direct effect) and reactions with diffusing radicals (indirect effect) [15]. Direct ionization of the DNA produces cation radicals, many of which were studied by electron spin resonance techniques. It is believed that the most probable cation radical in DNA is guanine [16] either as a result of initial ionization of guanine or from radical trans-
Figure 1.1: Schematic of the comparison of the depth–dose distribution of photons, protons and carbon ions. The figure is courtesy of D. Schardt, GSI.

...fer to guanine from other ionization sites. The biological effects of ionizing radiation are thought to arise from the formation of double-strand break (DSB) and clustered DNA lesions, e.g. two or more lesions (base lesion, single-strand break (SSB), a basic site or DSB) formed within about 10 base pairs separation by a single radiation track. Most mechanistic studies to date [17] however have focused on the indirect effects using dilute, aqueous solutions containing DNA, indicating that the hydroxyl radical (\(\cdot\)OH) is the main water radical that induces single- (SSB) and double-strand breaks (DSB) in DNA whereas hydrated electrons, H-atoms and \(\cdot\)OH radicals induce DNA base lesions. Experimental [18] and theoretical [19] studies have indicated that in living cells or under highly scavenging conditions mimicking those for \(\cdot\)OH scav- enging in the cell, \(\sim40\%\) of the lesions induced in DNA by low linear energy transfer (LET) radiation can be ascribed to direct effects increasing to \(\sim70\%\) for high LET \(\alpha\)-particles. The experimentally obtained ratios between direct and indirect effects examined for various biological end points have been summarized by Becker and Sevilla [16]. Within the living cell a complex repair machinery operates in order to maintain the integrity of DNA. Most of the lesions can be repaired but if the damage is very severe, it can be the origin of a tumor. The connection between the initial lesions and the cellular end points is not fully understood [20].

To understand strand breaks at the molecular level, it is worth to study the frag-
mentation and energetics of individual building blocks under different kinds of irradiation and in the different regions of the Bragg curve. Up to now, the detailed understanding of the effect of radiation on a cell or DNA has been limited. In this thesis, we extend the experiments to a broad comparison between not only low and high Linear Energy Transfer (LET) radiation but also low and high energy for high LET irradiations. LET which is a measure of the energy transferred to material as an ionizing particle travels through is an important parameter. In the Bragg peak regime, collisions involving atomic particles are very complex. Until recently, it was commonly believed that the main differences between irradiation with atomic particles and with electrons/photons lied in the different track structures and in the higher density of ionization events along the track in the case of atomic particles. The track structure of heavy particles in cells or tissue including all interactions present in the media were simulated in great detail [21–25].

In an attempt to understand better the nature of DNA damage associated with ionizing radiation, we have examined SSB and DSB formation in plasmid DNA irradiated in dilute aqueous solutions containing plasmid. A plasmid is a DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA [26]. Plasmids are double stranded and in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms, in mitochondria or chloroplasts. Plasmids are a convenient model system to study DNA damage, since they are of a well defined size and are relatively easy to prepare in milligram quantities in sufficiently high purity for radiation chemistry. SSB detection is readily accomplished by gel electrophoresis. In this work, we use the pBR322 plasmid DNA which is commercially available. A model environment of a cell is also studied by investigation of ion irradiation of plasmid DNA in an environment containing scavenger material.

- In chapter 2, the experimental set-up is described, together with the main features of the experimental techniques used throughout this thesis or relevant for the results presented. The preparation of the samples and the sample holders for experiments of high and low energy ion irradiation are described in detail.

- In chapter 3, the theoretical concepts underlying processes studied in this thesis will be briefly described and the method used to calculate the stopping power will be summarized.

- Chapter 4 contains the experimental results about the influence of irradiation on plasmid DNA under two different regimes of carbon ion beams. We have investigated the SSB and DSB induction in plasmid DNA upon irradiation. SSB and DSB yields per plasmid per dose were found to be lower in the Spread Out Bragg Peak (SOBP) than in the plateau region. Moreover, we could also
determine the amount of non-scavengable plasmid damage at high scavenging capacities. Surprisingly not only DSB but also a sizable fraction of SSB are due to direct effects.

- In chapter 5, quantification of the "non-scavengable" part of plasmid DNA damage induced by $^{12}$C ions under spread-out Bragg peak conditions (high LET radiation) or by $^{137}$Cs $\gamma$-photons (low LET radiation) is achieved. Furthermore, DNA damage is quantified as a function of scavenger concentration up to levels where saturation of the damage is observed i.e., for high dose. The results are also compared to the existing data.

- In chapter 6, the first explorative results of low-energy heavy ion irradiation are presented. The trends in the data can be understood on basis of LET and vacancy production estimates calculated with the TRIM code.

- Chapter 7 summarizes the conclusions and outlook of this thesis.

Finally the summary of the thesis is given in Dutch.