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**Developments in dosimetry for cancer treatment
with proton therapy**

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A mis padres, Alicia y Ariel ♥

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GLOSSARY

ASTRO American Society for Radiation Oncology. 22

BA Break aberration. 40

BIANCA Blophysical ANalysis of Cell death and chromosome Aberrations. iv, 19, 47, 50, 58–60, 68, 73, 74

BNCT Boron Neutron Capture Therapy. 43, 48, 86

BP Bragg peak. 21, 30, 32, 53, 54, 62

CAR Carabe et al RBE model. 67, 80–83

CCR Complex Chromosome Rearrangements. 88, 89, 154

CDRA Compound Dual Radiation Action. 40, 41, 88, 89, 153

CeArP Argentine Proton Therapy Center. 5–7, 9–11, 13–15, 23, 24

CHE Chen and Ahmad RBE model. 67, 80–83

CIBIO Department of Cellular, Computational and Integrative Biology, University of Trento, Italy. 93

CNEA National Atomic Energy Commission. 23

COMPhID Continuous Microdosimetric Photon Isoeffective Dose Model. 6, 10, 11, 14, 19, 20, 44, 45, 85, 87, 91, 92, 99, 105, 111, 112, 117, 123, 126, 130, 132, 133, 135–139, 172, 173

CSDA Continuous Slowing Down Approximation. 28

DSB Double-strand break. 37, 40, 41, 89

EM Electromagnetic. 27

- FBS** Fetal bovine serum. 93
- GE** General Electric Company. 24
- GTV** Gross tumor volume. 66, 77–79
- H460** Non-small cell lung cancer cell line. 6, 10, 14, 19, 85, 92, 93
- HNSCC** Head-and-neck squamous cell carcinoma. iv, v, 6, 10, 14, 47, 50, 51, 58–60, 62, 63, 66–68, 73–77, 80, 81, 83
- HSF** Human skin fibroblasts. iv, 6, 10, 14, 47, 50, 58–61, 63, 73–75
- IBA** Ion Beam Applications Company. 23
- ICRU** International Commission on Radiation Units and Measurements. 51
- IsoE** Isoeffective. 5, 6, 9, 10, 13, 14, 49–51, 62, 66, 68–77, 79, 80, 82, 111, 112, 133–135, 137, 139
- JON** Jones RBE model. 67, 80–82
- LAIDEP** Proton Therapy Research and Development Laboratory. 23, 24
- LEM** Local Effect Model. 37
- LET** Linear Energy Transfer. iv, 5, 6, 9, 10, 13, 14, 18–21, 33, 37, 41, 42, 44, 47–49, 55–60, 68–70, 72–74, 76, 80, 82, 86–89, 104, 124, 125
- LNL** Laboratori Nazionali di Legnaro. 5, 9, 13
- LQ** Linear–quadratic. iii, 6, 10, 14, 18–21, 34, 36–38, 48, 67, 85, 92, 99, 111, 154
- MC** Monte Carlo. 50, 51, 95, 103, 104, 139, 169
- MCN** McNamara RBE model. 67, 80, 82
- MCS** Multiple Coulomb Scattering. 29, 32
- MFO** Multi-Field Optimization. 33, 64, 65, 77, 79
- MK** Microdosimetric-Kinetic. 41
- MKM** Microdosimetric Kinetic Model. 37
- MLQ** Modified linear–quadratic model. 37, 44, 47–49
- NSCLC** Non-small cell lung cancer. 6, 10, 14, 19, 85, 92
- ODR** Orthogonal Distance Regression. 99, 110

- p.d.f.** Probability density function. 41, 42, 153
- PB** Pencil beam. 48
- PBP** Pristine Bragg peak. iv, 51, 54, 55, 62, 70
- PBS** Pencil Beam Scanning. 23
- PE** Plate efficiency. 98, 117, 120
- PhID** Photon Isoeffective Dose. iv, 6, 10, 14, 43–45, 47, 48, 50, 51, 67, 87, 88
- PHITS** Particle and Heavy Ion Transport code System. 51–53, 57, 95, 96, 102–104, 112, 169, 170
- PLR** Peeler RBE model. 67, 80–82
- r.v.** Random variable. 153
- RBE** Relative biological effectiveness. iii, 5, 6, 9, 10, 13, 14, 17, 18, 20, 21, 35–38, 41–44, 51, 58, 63, 66, 67, 70–83, 111, 112, 133–139
- ROI** Region of interest. 115
- RORU** Rorvik et al RBE model. 67, 80–82
- SF** Survival fraction. 98
- SFO** Single-field optimization. 33
- SLD** Sublethal Damage. 37
- SOBP** Spread-Out Bragg Peak. iv, 6, 10, 14, 21, 32, 49–51, 53–58, 62, 67, 74, 75, 77, 80, 81, 85, 92, 93, 96, 98, 103–105, 108, 112, 126, 127, 135–138
- SSB** Single-strand break. 40, 41
- SSPT** Spot-scanning proton therapy. 32
- SV** Sensitive volume. 105, 106
- TCP** Tumor control probability. iv, 63, 66, 77, 79
- TDRA** Theory of dual radiation action. 48
- TEPC** Tissue-Equivalent Proportional Counter. 40, 85, 92, 104–106, 171
- TIFPA** Trento Institute for Fundamental Physics and Applications. 5, 9, 13
- TIL** Tilly et al RBE model. 67, 80–82

TPS Treatment planning systems. 29

UBA University of Buenos Aires. 23

UHDR Ultra-high dose rates. 42

V79 Chinese hamster fibroblasts. iv, 6, 10, 14, 47, 50, 58–60, 63, 73–75, 83

WE Water-equivalent. 95, 106, 107

WED Wedenberg RBE model. 67, 80–82

WIL Wilkens and Oelfke RBE model. 80–83

ABSTRACT

Proton therapy is an advanced radiotherapy modality that exploits the distinct physical properties of protons to achieve highly localized dose delivery. Due to their finite range in tissue and the presence of a pronounced Bragg peak, this technique allows for increased dose concentration within the tumor while sparing surrounding healthy tissues.

In clinical practice, doses in proton therapy are commonly prescribed in terms of photon-equivalent dose, accounting for the biological effectiveness of proton radiation through the use of the Relative Biological Effectiveness (RBE). In this context, the photon isoeffective (IsoE) dose refers to the photon dose that would produce the same biological effect as a given proton dose, i.e., an equivalent (isoeffective) biological outcome. This approach allows proton treatments to be directly compared with the extensive clinical experience available for photon therapy, facilitating treatment evaluation, outcome prediction, and clinical decision-making. Typically, a constant RBE value of 1.1 is adopted, following international recommendations. However, growing experimental and theoretical evidence indicates that RBE is not uniform and varies with several factors, with higher values often observed toward the distal region of the proton beam. These include the level of biological damage, dose and dose per fraction, specific cell or tissue type, and radiation quality, commonly expressed in terms of linear energy transfer (LET) or lineal energy (y).

This thesis is developed within the framework of the construction and commissioning of the first proton therapy center in Argentina and Latin America, the Argentine Proton Therapy Center (CeArP), representing a key step toward the regional implementation of advanced proton therapy. It is also part of an international collaboration with Italy, involving the Dept. of Physics "Alessandro Volta" of the University of Pavia, the Trento Institute for Fundamental Physics and Applications (TIFPA, INFN), and the Laboratori Nazionali di Legnaro (LNL, INFN).

Within this context, two cell-specific models are presented for the calculation of photon IsoE doses in proton therapy, aimed at improving the biological accuracy of dose prescription and supporting their potential integration into clinical treatment planning, without introducing an alternative fixed or variable RBE

formalism, but rather by directly computing photon-equivalent doses from radiobiological response. Both approaches link radiation quality—described through macrodosimetric (LET) or microdosimetric (y)—to the radiobiological parameters α and β of the linear–quadratic (LQ) cell survival model.

The first part of the thesis focuses on the macrodosimetric model, called the Photon Isoeffective Dose (PhID) model, which accounts for mixed charged-particle fields, LET-dependent radiobiological parameters, sublethal damage repair, and synergistic effects among field components. PhID is constructed for head-and-neck squamous cell carcinoma (HNSCC), human skin fibroblasts (HSF), and Chinese hamster fibroblasts (V79), using ion-specific, LET-dependent α and β parameters derived from the BIANCA biophysical model. PhID predicts depth- and absorbed dose-dependent IsoE doses that can significantly deviate from RBE-weighted doses, particularly in high-LET regions near the distal edge of the Bragg peak. Furthermore, it reproduces the increase of RBE with increasing LET and decreasing dose per fraction, showing strong dependence on the cell line and reference radiation. Alpha particles are identified as dominant high-LET contributors beyond protons. Compared with phenomenological RBE models, the model yields intermediate RBE ranges consistent with its cell-specific formulation and provides a mechanistic interpretation of the observed trends. Due to its formulation in terms of macroscopic quantities, the model is suitable for direct implementation in treatment planning systems.

The second part focuses on the microdosimetric model, referred to as the Continuous Microdosimetric Photon Isoeffective Dose (COMPhID). Within this framework, α and β are described as functions of the stochastic variable y through the first three moments of the linear energy distribution. The model is characterized by five parameters determined from combined microdosimetric and radiobiological experiments performed for the human non-small cell lung cancer (NSCLC) H460 cell line, for which the radiobiological parameters were generated in this work, at the Trento Proton Therapy Center, a facility with similar characteristics to the CeArP.

The COMPhID model accurately reproduces the main radiobiological trends across different irradiation conditions, showing a clear depth-dependent behavior of the IsoE dose and RBE along the spread-out Bragg peak (SOBP), with increasing values toward the distal region, consistent with the evolution of radiation quality as described by the linear energy distributions. RBE values ranged from approximately 0.7 to 1.5 (up to about 35% deviation from the conventional fixed value of 1.1). The model remains robust when applied to independent experimental and Monte Carlo microdosimetric inputs, capturing the expected trends beyond the calibration set. Overall, COMPhID provided a flexible and predictive framework for linking microdosimetric information with biological response, highlighting the importance of radiation quality variations in proton therapy.

The models presented in this thesis highlight the importance of adopting a cell-specific formulation, enabling a more accurate description of the underlying radiobiological response and contributing to a more biologically consistent dosi-

metric approach in proton therapy, bridging clinical implementations and more fundamental descriptions of radiation effects, with potential impact on the implementation of advanced treatment strategies in emerging centers such as CeArP.

ABSTRACT (SPANISH)

La protonterapia es una modalidad avanzada de radioterapia que explota las propiedades físicas distintivas de los protones para lograr una administración de dosis altamente localizada. Debido a su alcance finito en el tejido y a la presencia de un pronunciado pico de Bragg, esta técnica permite aumentar la concentración de dosis en el tumor mientras se preservan los tejidos sanos circundantes.

En la práctica clínica, las dosis en protonterapia se prescriben comúnmente en términos de dosis equivalente a fotones, teniendo en cuenta la efectividad biológica de la radiación de protones mediante el uso de la Efectividad Biológica Relativa (RBE). En este contexto, la dosis isoefectiva a fotones (IsoE) se refiere a la dosis de fotones que produciría el mismo efecto biológico que una dosis dada de protones, es decir, un resultado biológico equivalente (isoefectivo). Este enfoque permite comparar directamente los tratamientos con protones con la amplia experiencia clínica disponible para la terapia con fotones, facilitando la evaluación del tratamiento, la predicción de la respuesta al tratamiento y la toma de decisiones clínicas. Típicamente, se adopta un valor constante de RBE de 1.1, siguiendo recomendaciones internacionales. Sin embargo, una creciente evidencia experimental y teórica indica que la RBE no es uniforme y varía con varios factores, observándose a menudo valores más altos hacia la región distal del haz de protones. Entre estos factores se incluyen el nivel de daño biológico, la dosis y la dosis por fracción, el tipo específico de célula o tejido, y la calidad de la radiación, comúnmente expresada en términos de transferencia lineal de energía (LET) o energía lineal (y).

Esta tesis se desarrolla en el marco de la construcción y puesta en marcha del primer centro de protonterapia en Argentina y América Latina, el Centro Argentino de Protonterapia (CeArP), representando un paso clave hacia la implementación regional de esta modalidad avanzada. También forma parte de una colaboración internacional con Italia, que involucra al Departamento de Física de la Universidad de Pavia, el Trento Institute for Fundamental Physics and Applications (TIFPA, INFN) y el Laboratorio Nacional de Legnaro (LNL, INFN).

En este contexto, se presentan dos modelos, dependientes de la línea celular, para el cálculo de la dosis IsoE a fotones en protonterapia, con el objetivo de mejorar la precisión biológica de la prescripción de dosis y permitir su posible in-

tegración en la planificación clínica de tratamientos, sin introducir un formalismo alternativo de RBE fijo o variable, sino calculando directamente las dosis equivalentes a fotones a partir de la respuesta radiobiológica. Ambos enfoques vinculan la calidad de la radiación—descrita a través de magnitudes macrodosimétricas (LET) o microdosimétricas (y)—con los parámetros radiobiológicos α y β del modelo de supervivencia celular lineal–cuadrático (LQ).

La primera parte de la tesis se centra en el modelo macrodosimétrico, denominado Dosis Isoefectiva a Fotones (PhID, por sus siglas en inglés), que tiene en cuenta el campo mixto de partículas cargadas, parámetros radiobiológicos dependientes del LET, reparación de daño subletal y efectos sinérgicos entre los componentes del campo. PhID se construyó para la línea celular de carcinoma de células escamosas de cabeza y cuello (HNSCC), fibroblastos de piel humana (HSF) y fibroblastos de hámster chino (V79), utilizando parámetros α y β dependientes del LET y específicos de cada ion, derivados del modelo biofísico BIANCA. PhID predice dosis IsoE dependientes de la profundidad y de la dosis absorbida que pueden desviarse significativamente de las dosis ponderadas por el RBE, particularmente en regiones de alto LET cercanas al extremo distal del pico de Bragg. Además, reproduce el aumento del RBE con el incremento del LET y la disminución de la dosis por fracción, mostrando una fuerte dependencia con la línea celular y la radiación de referencia. El campo de partículas alfa se identifica como el contribuyente dominante de alto LET además del campo de protones. En comparación con modelos fenomenológicos de RBE, el modelo permite obtener rangos intermedios de RBE consistentes con su formulación específica por célula y proporciona una interpretación mecanística de las tendencias observadas. Debido a que se basa en magnitudes macroscópicas, el modelo es adecuado para su aplicación directa en sistemas de planificación de tratamientos.

La segunda parte de la tesis se centra en el modelo microdosimétrico, denominado Modelo Continuo Microdosimétrico de Dosis Isoefectiva a Fotones (COM-PhID). En este marco, α y β se describen como funciones de la variable estocástica y a través de los tres primeros momentos de la distribución de energía lineal. El modelo se caracteriza por cinco parámetros que se determinan a partir de experimentos microdosimétricos y radiobiológicos combinados realizados para la línea celular de cáncer de pulmón humano de células no pequeñas (NSCLC) H460, para la cual los parámetros radiobiológicos fueron generados en este trabajo, en el Centro de Protonterapia de Trento, una instalación con características similares al CeArP.

El modelo COMPhID reproduce con precisión las principales tendencias radiobiológicas bajo diferentes condiciones de irradiación, mostrando un claro comportamiento dependiente de la profundidad de la dosis IsoE y de los valores de RBE a lo largo del spread-out Bragg peak (SOBP), con un aumento hacia la región distal, en concordancia con la evolución de la calidad de la radiación descrita por las distribuciones de energía lineal. Los valores de RBE varían aproximadamente entre 0.7 y 1.5 (hasta alrededor de un 35% de desviación respecto del valor fijo convencional de 1.1). El modelo se mantiene robusto cuando se aplica a entradas

microdosimétricas independientes, tanto experimentales como provenientes de simulaciones Monte Carlo, capturando las tendencias esperadas más allá del conjunto de calibración. En conjunto, COMPhID proporciona un marco flexible y predictivo para vincular la información microdosimétrica con la respuesta biológica, destacando la importancia de las variaciones en la calidad de la radiación en protonterapia.

Finalmente, el modelo demostró ser robusto cuando se aplicó a entradas microdosimétricas independientes, incluyendo tanto espectros experimentales como aquellos obtenidos mediante simulaciones Monte Carlo, capturando exitosamente las tendencias esperadas incluso cuando los datos de entrada no formaban parte del conjunto de calibración. En conjunto, COMPhID proporciona un marco flexible y predictivo para vincular la información microdosimétrica con la respuesta biológica, destacando la importancia de las variaciones en la calidad de la radiación en protonterapia.

Los modelos presentados en esta tesis resaltan la importancia de adoptar una formulación específica por línea celular, permitiendo una descripción más precisa de la respuesta radiobiológica subyacente y contribuyendo a un enfoque dosimétrico más consistente desde el punto de vista biológico en protonterapia, tendiendo un puente entre las aplicaciones clínicas y las descripciones más fundamentales de los efectos de la radiación, con potencial impacto en la implementación de estrategias de tratamiento avanzadas en centros emergentes como el CeArP.

ABSTRACT (ITALIAN)

La protonterapia è una modalità avanzata di radioterapia che sfrutta le proprietà fisiche dei protoni per ottenere una distribuzione di dose altamente localizzata. Grazie al range finito dei protoni nei tessuti e alla presenza di un pronunciato picco di Bragg, questa tecnica consente di ottenere una distribuzione di dose altamente concentrata all'interno del tumore preservando i tessuti sani circostanti.

Nella pratica clinica, le dosi in protonterapia sono comunemente prescritte in termini di dose equivalente ai fotoni, tenendo conto dell'efficacia biologica dei protoni usando l'Efficacia Biologica Relativa (RBE). In questo contesto, la dose isoeffettiva ai fotoni (IsoE) si riferisce alla dose di fotoni che produrrebbe lo stesso effetto biologico di una data dose di protoni, ovvero un esito biologico equivalente (isoeffettivo). Questo approccio permette di confrontare direttamente i trattamenti con protoni con l'ampia esperienza clinica disponibile per la terapia con fotoni, facilitando la valutazione del trattamento, la previsione dell'esito e il processo decisionale clinico. Tipicamente, si adotta un valore costante di RBE pari a 1.1, seguendo le raccomandazioni internazionali. Tuttavia, crescenti evidenze sperimentali e teoriche indicano che RBE non sia uniforme e vari in funzione di diversi fattori, con valori spesso più elevati nella regione distale del fascio di protoni. Tra questi fattori vi sono il livello di danno biologico, la dose e la dose per frazione, il tipo specifico di cellula o tessuto e la qualità della radiazione, comunemente espressa in termini di trasferimento lineare di energia (LET) o energia lineare (y).

Questa tesi si sviluppa nel contesto della costruzione e messa in funzione del primo centro di protonterapia in Argentina e America Latina, il Centro Argentino di Protonterapia (CeArP), rappresentando un passo fondamentale verso l'implementazione regionale di questa tecnologia avanzata. Essa fa inoltre parte di una collaborazione internazionale con l'Italia, che coinvolge il Dipartimento di Fisica "Alessandro Volta" dell'Università di Pavia, il Trento Institute for Fundamental Physics and Applications (TIFPA, INFN) e i Laboratori Nazionali di Legnaro (LNL, INFN).

In questo contesto, si presentano due modelli specifici per linea cellulare per il calcolo delle dosi IsoE ai fotoni in protonterapia, con l'obiettivo di migliorare l'accuratezza biologica della prescrizione della dose e supportarne una possibile

integrazione nella pianificazione clinica dei trattamenti, senza introdurre un formalismo RBE alternativo, fisso o variabile, ma calcolando direttamente le dosi equivalenti ai fotoni a partire dalla risposta radiobiologica. Entrambi gli approcci collegano la qualità della radiazione, descritta tramite grandezze macrodosimetriche (LET) o microdosimetriche (y), ai parametri radiobiologici α e β del modello lineare-quadratico di sopravvivenza cellulare (LQ).

La prima parte della tesi si concentra sul modello macrodosimetrico, denominato Photon Isoeffective Dose (PhID), che tiene conto di campi misti di particelle cariche, parametri radiobiologici dipendenti dal LET, riparazione del danno subletale ed effetti sinergici tra i componenti del campo. PhID è costruito per carcinoma squamoso della testa e del collo (HNSCC), fibroblasti della pelle umana (HSF) e fibroblasti di criceto cinese (V79), utilizzando parametri α e β dipendenti dal LET e specifici per tipo di ione, derivati dal modello biofisico BIANCA. PhID predice dosi IsoE dipendenti dalla profondità e dalla dose, che possono discostarsi significativamente dalle dosi pesate con RBE, in particolare nelle regioni ad alto LET vicino alla parte distale del picco di Bragg. Inoltre, il modello riproduce l'aumento della RBE con l'incremento del LET e la diminuzione della dose per frazione, mostrando una forte dipendenza dalla linea cellulare e dalla radiazione di riferimento. Le particelle alfa sono identificate come contributori dominanti ad alto LET oltre ai protoni. Rispetto ai modelli fenomenologici di RBE, il modello fornisce intervalli intermedi di RBE coerenti con la sua formulazione specifica per cellula e offre un'interpretazione meccanicistica dei comportamenti osservati in funzione della dose. Grazie alla sua formulazione in termini di grandezze macroscopiche, il modello è adatto per essere implementato direttamente nei sistemi di pianificazione del trattamento.

La seconda parte si concentra sul modello microdosimetrico, denominato Continuous Microdosimetric Photon Isoeffective Dose (COMPhID). In questo contesto, α e β sono descritti come funzioni della variabile stocastica y attraverso i primi tre momenti della distribuzione di energia lineare. Il modello è caratterizzato da cinque parametri determinati a partire da esperimenti combinati microdosimetrici e radiobiologici effettuati sulla linea cellulare di carcinoma polmonare umano non a piccole cellule (NSCLC) H460, i cui parametri radiobiologici sono stati generati presso il Centro di Protonterapia di Trento, una struttura con caratteristiche simili al CeArP.

Il modello COMPhID riproduce accuratamente le principali tendenze radiobiologiche in diverse condizioni di irraggiamento, mostrando un chiaro comportamento dipendente dalla profondità della dose IsoE e dei valori di RBE lungo lo spread-out Bragg peak (SOBP), con un aumento verso la regione distale, in accordo con l'evoluzione della qualità della radiazione descritta dalle distribuzioni di energia lineare. I valori di RBE variano approssimativamente tra 0.7 e 1.5 (fino a circa il 35% di deviazione rispetto al valore convenzionale fisso di 1.1). Il modello si dimostra robusto anche quando applicato a input microdosimetrici indipendenti, sia sperimentali che da simulazioni Monte Carlo, riproducendo gli andamenti attesi anche oltre il set di calibrazione. Nel complesso, COMPhID fornisce un frame-

work flessibile e predittivo per collegare le informazioni microdosimetriche con la risposta biologica, evidenziando l'importanza delle variazioni della qualità della radiazione nella protonterapia.

I modelli presentati in questa tesi evidenziano l'importanza di adottare una formulazione specifica per ogni linea cellulare, consentendo una descrizione più accurata della risposta radiobiologica e contribuendo a un approccio dosimetrico più coerente dal punto di vista biologico. Questo approccio colma il divario tra le implementazioni cliniche e le descrizioni radiobiologiche degli effetti della radiazione, con un potenziale impatto sull'implementazione di strategie di trattamento avanzate in centri emergenti come CeArP.

INTRODUCTION

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 [1]. Treatment strategies typically include surgery, systemic therapies (such as chemotherapy, hormonal treatments, and targeted biological therapies), and/or radiotherapy.

Radiotherapy is a treatment that uses ionizing radiation to eradicate cancer cells or inhibit their proliferation by inducing DNA damage. This damage results from the interaction of radiation with the atoms and electrons that constitute biological tissue. Radiotherapy can be broadly classified into two main categories: internal radiotherapy and external beam radiotherapy. The latter includes conventional photon therapy, as well as particle therapy modalities such as electron therapy, heavy ions therapy, and proton therapy.

This work focuses on proton therapy, which involves the treatment of tumors using external beams of high-energy protons, typically in the range of 70–230 MeV. In contrast to conventional photon therapy, proton therapy is characterized by the finite range of proton beams. Photon beams exhibit a dose deposition pattern with an initial build-up region followed by an approximately exponential decrease with depth in tissue. As a result, achieving a higher dose in the tumor relative to surrounding healthy tissues generally requires the use of multiple beam directions and complex irradiation geometries.

In contrast, protons deposit energy in tissue in a depth-dependent manner governed by their energy loss through electromagnetic interactions with orbital electrons. As protons slow down, their energy deposition per unit path length increases, culminating in a pronounced maximum known as the Bragg peak. This distinct physical characteristic enables the delivery of highly conformal dose distributions, maximizing the dose within the target while minimizing exposure to surrounding healthy tissues and organs at risk.

Despite these differences in the underlying interaction physics between protons and photons, proton therapy is commonly applied in clinical practice in a similar manner, using a simplified biological scaling approach. Specifically, treatment doses are prescribed assuming a constant Relative Biological Effectiveness (RBE) factor of 1.1, such that the absorbed proton dose multiplied by this fac-

tor yields the photon-equivalent (isoeffective) dose. This assumption implies that protons are uniformly 10% more biologically effective than photons, regardless of irradiation conditions.

The RBE is defined as the ratio between the dose of a reference radiation and the dose of a test radiation required to produce the same biological effect. Although a fixed value of 1.1 is widely used in clinical proton therapy, numerous experimental studies have demonstrated that RBE is not constant and can vary significantly depending on irradiation conditions [2–12]. In particular, increased RBE values are typically observed at the distal edge of proton beams, where the linear energy transfer (LET) is higher. Indeed, RBE depends on a combination of physical and biological factors, including radiation quality (commonly expressed in terms of LET), particle type, dose and dose per fraction, dose rate, cell or tissue type, biological endpoint, and oxygenation conditions, among others [13].

The concept of RBE is particularly valuable when the biological endpoint is assessed through qualitative or graded clinical scales rather than direct numerical measurements. In such cases, RBE remains a quantitative metric, as it provides the ratio of doses required to produce the same biological effect, regardless of how that effect is initially characterized (e.g., lens opacity, skin erythema, or functional performance) [14]. However, a key limitation of experimentally determined RBE values is their limited predictive and extrapolative capability, especially when irradiation conditions differ from those under which the data were obtained. This limitation underscores the need for radiation biophysics models capable of providing predictive power beyond specific experimental setups.

Over the years, extensive data on the biological effectiveness of charged particle beams have been collected, and various RBE models have been proposed. These models are generally categorized as either phenomenological/empirical or mechanistic, although the distinction among them is not always strict. Most existing models are phenomenological, based on *in vitro* proton irradiation data from different cell lines and rely on the linear-quadratic (LQ) model [15] with cell inactivation as the biological endpoint [8, 11, 16–23]. In such models, RBE calculations depend on the proton dose, the $(\alpha/\beta)_x$ ratio of the reference radiation x , and the dose-averaged LET (LET_D) as a measure of radiation quality. However, while LET_D is theoretically capable of encapsulating the mixed-field nature of the beam, most existing RBE models typically rely on this single averaged physical quantity. In clinical practice, LET_D calculations often prioritize primary and secondary protons, potentially neglecting the biological impact of higher-LET fragments generated through non-elastic nuclear interactions. Furthermore, many current formalisms do not explicitly account for the individual biological contributions of each specific particle species within the mixed field, nor do they typically consider possible time-dependent synergistic effects between radiation components.

Moreover, the construction of these models is typically based on databases of *in vitro* experiments involving one (Chinese hamster fibroblasts, V79) or several cell lines. The dependence on the cell line is given by the LQ parameters of the reference radiation, and its use is often generalized to other types of cell lines,

despite having been obtained with limited or unrelated biological data. This suggests that a more detailed characterization of the field components could provide a more robust framework for predicting biological response in complex irradiation scenarios.

Within this framework, this thesis presents two cell-specific models for the calculation of photon isoeffective doses in proton therapy: a macrodosimetric approach, referred to as the Photon Isoeffective Dose (PhID) model, and a microdosimetric approach, referred to as the Continuous Microdosimetric Photon Isoeffective Dose (COMPhID) model. Both approaches are based on establishing a link between radiation quality—described through physical quantities such as LET or lineal energy (y)—and biological parameters, such as the α and β coefficients of the LQ cell survival model.

PhID is formulated on the basis of cell survival data and explicitly incorporates the main charged primary and secondary particles generated by the incident proton beam. It integrates LET-dependent radiobiological parameters and accounts for sublethal damage production and repair, as well as potential synergistic effects between different radiation field components. The radiation field considered in this framework includes contributions from protons (primary and secondary, without distinction), deuterons, tritium, alpha particles, ^3He ions, and electrons [24].

The model was developed for three representative cell lines, head-and-neck squamous cell carcinoma (HNSCC), human skin fibroblasts (HSF), and Chinese hamster fibroblasts (V79), using radiobiological parameters derived from the biophysical model BIANCA [25], which provides the LQ parameters α and β as functions of LET over a broad range for the charged particles considered.

With respect to the COMPhID model, this is formulated by describing radiation quality through the stochastic distribution of lineal energy, thereby preserving information on the microscopic patterns of energy deposition that are more directly related to the underlying mechanisms of biological damage. The model explicitly depends on the first three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, derived from microdosimetric spectra, while its parameters are linked to the radiobiological response through the LQ formalism via the coefficients $\langle\alpha\rangle$ and $\langle\beta\rangle$.

The determination of the parameters of the model requires a combined experimental approach in which microdosimetric measurements are coupled with radiobiological data. In this work, such experiments were carried out at the Trento Proton Therapy Center (Italy) under equivalent irradiation conditions for both the cell cultures and the microdosimetric detector, enabling a direct correlation between radiation quality and biological response. The COMPhID model was developed for the human NSCLC H460 cell line, selected due to the high clinical relevance of non-small cell lung cancer, which accounts for the majority of lung cancer cases, the leading cause of cancer-related mortality worldwide.

Based on the structure described above, this thesis is organized into three main chapters. The first chapter presents the theoretical background, introducing the

fundamental concepts of proton therapy, key physical quantities such as absorbed dose and LET, as well as essential aspects of radiobiology, the concept of photon isoeffective dose, and microdosimetry. These elements provide the foundation for the developments presented in the subsequent chapters.

Chapters 2 and 3 correspond to the macroscopic and microdosimetric approaches, respectively. For clarity and consistency, each chapter is structured into three main sections: a theoretical/mathematical formulation of the model, a description of the materials and methods employed, and the presentation of results followed by specific conclusions.

In particular, Chapter 2, devoted to the PhID model, presents in Section 2.3.2 a conceptual study of the formalism, where the impact of including all secondary charged particle components is evaluated and a sensitivity analysis of the model is performed, investigating its dependence on both the selected cell lines and the reference radiation. Furthermore, Section 2.3.3 introduces a tumor control probability (TCP) model, based on published data from recurrent HNSCC patients [26,27], which is employed to assess the clinical impact of calculating the photon isoeffective dose using the proposed model. Finally, Section 2.3.4 presents a comparison between the model response and phenomenological RBE models, where RBE ranges are evaluated by varying the physical dose per fraction for three representative values of LET_D . In all case studies, the resulting photon isoeffective dose is systematically compared with the dose obtained using the conventional fixed RBE value of 1.1.

Chapter 3, devoted to the COMPhID model, presents in Section 3.3.2 the analysis of cell survival curves obtained from radiobiological experiments, from which the α and β parameters of the LQ model are derived. Section 3.3.3 introduces the microdosimetric spectra obtained from the corresponding experiments, including the determination of the moments $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$. Based on these results, Section 3.3.4 presents the calculation and determination of the COMPhID model parameters. Section 3.3.5 then presents the calculation of photon isoeffective dose and RBE using the COMPhID model, together with a comparison with experimental data. Finally, Section 3.3.6 provides representative examples of model applications.

The thesis concludes with a general discussion that encompasses both models, analyzing their differences, complementarity, and respective potential, and finally outlines future research directions arising from this work.

CHAPTER 1

GENERAL BACKGROUND

This chapter provides the general framework and the theoretical foundations that underpin the present thesis. It begins with an overview of proton therapy and its clinical indications, highlighting the establishment of the Argentine Proton Therapy Center and its significance for Latin America.

The fundamental physics of proton interactions with matter is then presented, including stopping processes, multiple Coulomb scattering, nuclear reactions, and the characterization of the Bragg peak (BP) and its clinical extension through the Spread-out Bragg peak (SOBP).

Subsequently, the chapter introduces key concepts in radiation physics and radiobiology, such as absorbed dose, Linear Energy Transfer (LET), the Linear Quadratic (LQ) model, and the notion of Relative Biological Effectiveness (RBE), together with its empirical and mechanistic formulations and its expression within the LQ survival framework.

These aspects are complemented by a section on microdosimetry, which covers its fundamental principles and experimental approaches, as well as the theory of Compound Dual Radiation Action, thereby bridging the physical and biological scales of radiation effects.

Finally, the chapter addresses the concept of isoeffectiveness, focusing on the Photon Isoeffective Dose formalism for mixed radiation fields, its application to proton therapy, and its microdosimetric reformulation. Collectively, these sections establish a comprehensive theoretical basis for understanding the physical and biological principles that support the subsequent analysis and discussion of results.

1.1 Proton therapy

Proton therapy is an external beam radiotherapy modality that uses high-energy proton beams for the treatment of tumor tissues. Compared to conventional ra-

diotherapy techniques, proton therapy can provide improved dose distributions in selected clinical scenarios, potentially reducing the irradiation of healthy tissues and the risk of radiation-induced side effects, including secondary malignancies.

According to the American Society for Radiation Oncology (ASTRO) [28], disease sites that frequently benefit from the use of proton beam therapy include:

- Pediatric and adolescent/young adult tumors (curative treatments, with an emphasis on minimizing long-term toxicity)
- Patients with genetic syndromes requiring strict dose reduction
- Medically inoperable cases requiring dose escalation
- Re-irradiation scenarios
- Primary malignant or benign bone tumors
- Central nervous system tumors (e.g., skull base tumors such as chordomas and chondrosarcomas, ocular tumors, spinal, and craniospinal irradiation cases)
- Head and neck tumors (e.g., nasopharyngeal, nasal cavity, paranasal sinus tumors, advanced or unresectable cases)
- Thoracic tumors (e.g., esophageal cancer, mediastinal tumors, lymphomas, mesothelioma, locally advanced lung cancer)
- Abdominal tumors (e.g., hepatocellular carcinoma, intrahepatic biliary cancers, retroperitoneal sarcomas)
- Pelvic tumors (e.g., advanced unresectable pelvic tumors, cases requiring maximal organ sparing such as single or transplanted kidney)
- Genitourinary cancers, including prostate cancer cases requiring extended nodal irradiation (e.g., pelvic and/or para-aortic lymph nodes) beyond conventional treatment volumes.
- Breast cancer cases requiring complex irradiation, including bilateral disease, involvement of regional lymph nodes (particularly the internal mammary chain), unfavorable anatomical conditions, or situations where conventional techniques would result in excessive dose to organs at risk such as the heart.

The increasing number of clinical indications for proton therapy has been accompanied by a significant worldwide expansion of proton therapy facilities. Currently, 117 proton therapy centers are in clinical operation, 31 are under construction, and 34 are in the planning stage [29]. Figure 1.1 illustrates the global distribution of these centers, with facilities in operation shown in light blue and those under construction in red.

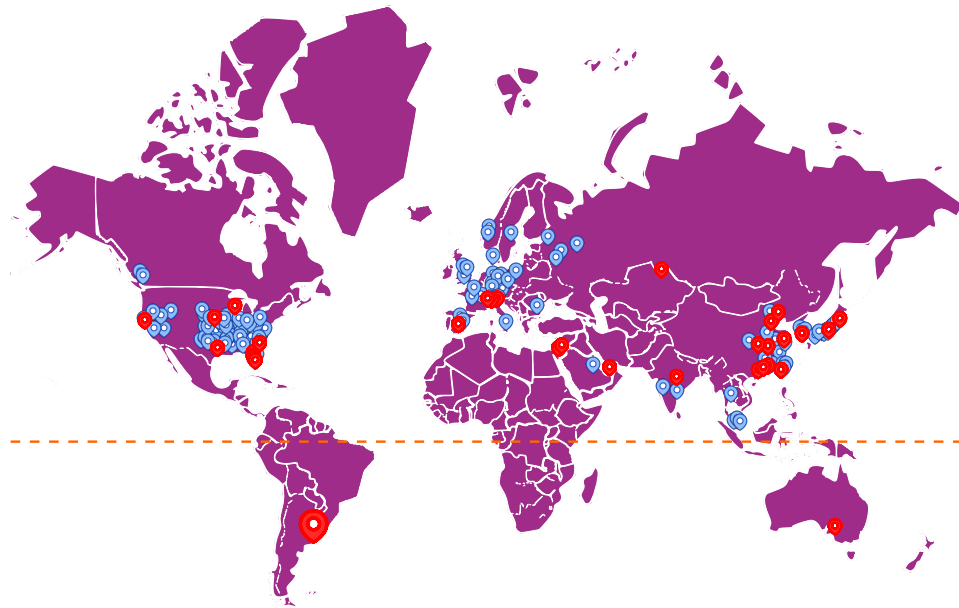


Figure 1.1: Global distribution of proton therapy centers. Facilities in clinical operation are shown in light blue, while centers under construction are indicated in red.

Among the operational centers, the highest concentration is found in the United States (49), Japan (19), and China (6), followed by Germany and Russia (5). Regarding centers under construction, China (8), the United States (6), and Italy (2) lead the expansion. Notably, two facilities under construction are located in the southern hemisphere, in Argentina and Australia. In particular, the Argentine Proton Therapy Center represents the first proton therapy facility in Latin America. The following section provides a more detailed description of this center, as this thesis is developed within the framework of its construction and commissioning.

1.1.1 The Argentine Proton Therapy Center

The National Atomic Energy Commission (CNEA) leads the development of the Argentine Proton Therapy Center (CeArP), in collaboration with INVAP and in partnership with the University of Buenos Aires (UBA). The construction phase of the facility has been completed, and it is currently undergoing commissioning. Once operational, CeArP will become the first proton therapy center in Latin America. It is located in the Autonomous City of Buenos Aires, in the Agronomía district, where it will form a high-level oncological hub together with the Instituto de Oncología Ángel H. Roffo (UBA) and the Centro Diagnóstico Nuclear (CNEA).

The center is equipped with a C230 cyclotron, part of the Proteus Plus® system provided by Ion Beam Applications (IBA). It will include two clinical treatment rooms equipped with 360 degree gantries with Pencil Beam Scanning (PBS) nozzles, and a third room dedicated to research within the Proton Therapy Research and Development Laboratory (LAIDEP), which will support activities in medical



Figure 1.2: *Rendered view of the Argentine Proton Therapy Center (CeArP), located in Buenos Aires, Argentina.*

physics, radiobiology, preclinical studies, instrumentation, computational dosimetry, and proton beam technologies.

LAIDEP will be equipped with a fixed clinical-grade proton beam line based on PBS technology, a dedicated control and monitoring room, and several specialized laboratories.

In addition to proton therapy, the center will include a conventional radiotherapy unit equipped with a Versa HD[®] linear accelerator (Elekta) and a CyberKnife[®] system for radiosurgery. Imaging capabilities will include a GE Revolution Dual Energy CT[®] scanner and a GE Signa Artist[®] MRI system for treatment planning and patient follow up.

This comprehensive technological infrastructure significantly enhances the clinical capabilities of the center and directly reinforces its regional standing. In particular, it will enable a larger number of patients from neighboring countries and the whole Latin America to access proton therapy and advanced radiotherapy treatments, without the need to travel to other continents.

The color map shown in Fig. 1.3 illustrates the distribution of cancer incidence, expressed as crude rates per 100 000 inhabitants for both sexes, across Latin America in 2022, based on data reported by the World Health Organization (WHO)¹.

In Argentina, the incidence reaches approximately 290 cases per 100 000 inhabitants. Among the different cancer types, the most prevalent are breast (71 per 100 000), prostate (45), colorectum (24), lung (20), and cervix uteri (17).

Comparable or higher incidence rates are observed in neighboring countries, such as Uruguay (481 per 100 000), Chile (311 per 100 000), and Brazil (291 per 100 000), further highlighting the regional need for advanced radiotherapy modalities such as proton therapy.

¹<https://gco.iarc.who.int/today/en>

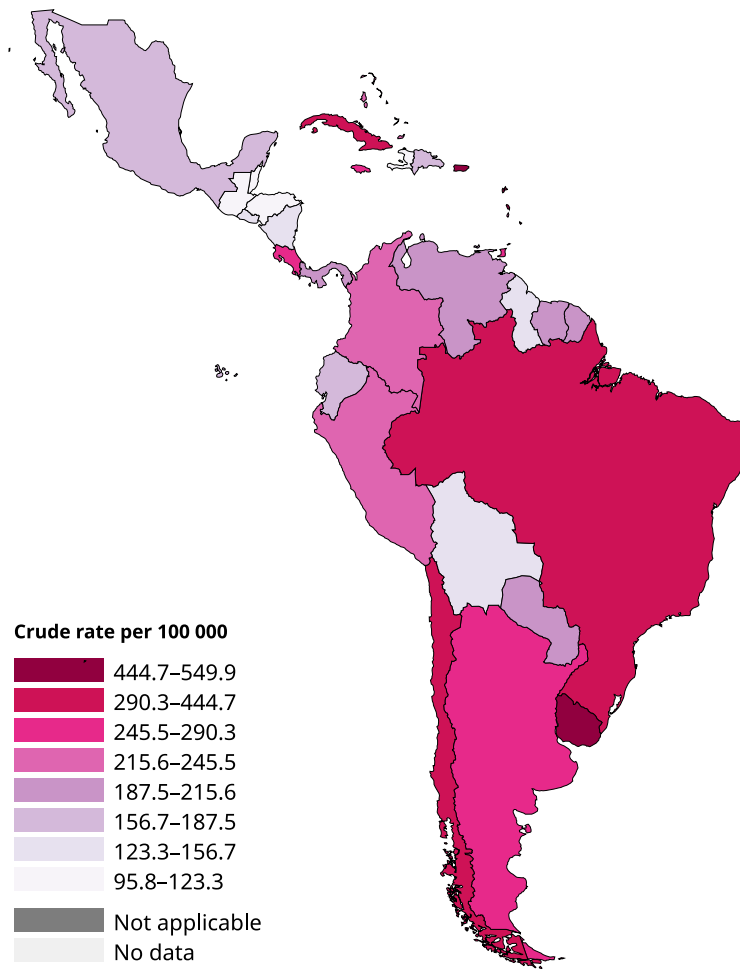


Figure 1.3: Distribution of cancer incidence in Latin America in 2022, expressed as crude rates per 100 000 inhabitants for both sexes.

Additional information about the center is available at <https://www.argentina.gob.ar/cnea/centro-argentino-de-protonterapia>.

1.1.2 Physics of interaction

This section describes the predominant types of interactions between protons and the atoms constituting the tissue. Figure 1.4 illustrates several mechanisms by which a proton interacts with an atom or nucleus: (a) Inelastic Coulomb interactions with atomic electrons, (b) Repulsive elastic Coulomb scattering with the nucleus, and (c) capture of the primary proton (p) and creation of secondary particles through inelastic nuclear interaction (p': secondary proton, e⁻: electron, n: neutron, γ: gamma rays) [30].

Protons lose kinetic energy mainly through inelastic interactions with atomic electrons, while traveling approximately along straight paths. Occasional elastic Coulomb interactions with atomic nuclei can deflect their trajectories. Less frequent nuclear interactions have a more significant impact, leading to the ab-

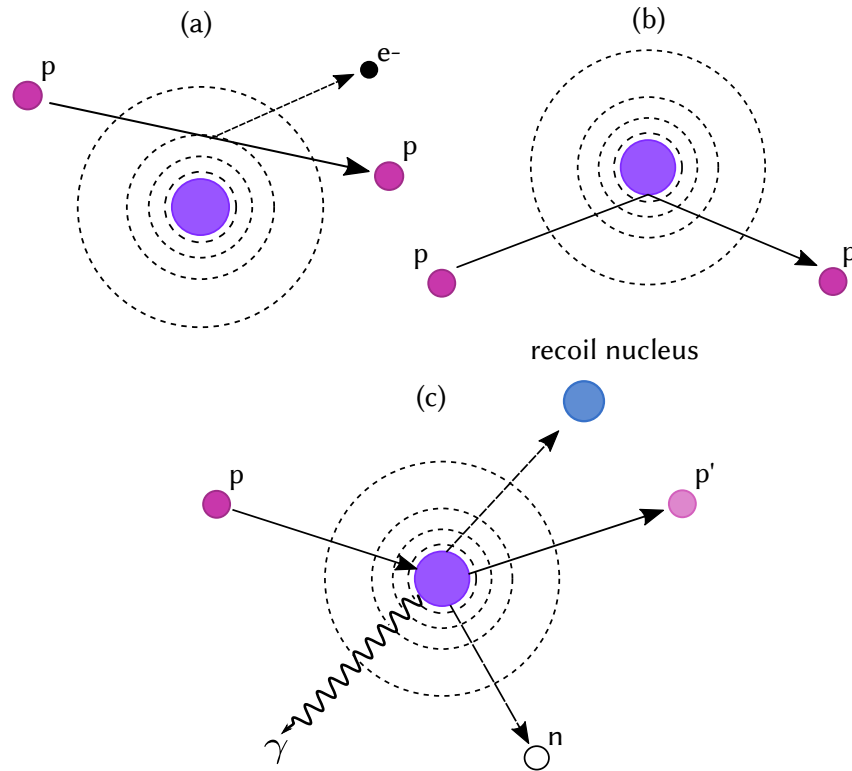


Figure 1.4: Schematic illustration of the mechanisms of interaction between protons and matter. Image inspired on [30].

sorption of the primary proton and the emission of secondary particles such as protons, neutrons, and heavier fragments (^2H , ^3H , ^3He , ^4He). Table 1.1 summarizes these interaction mechanisms and their dosimetric implications.

Interaction	Target	Result of the interaction	Effect on the projectile	Dosimetric manifestations
Inelastic Coulomb scattering	Atomic e^-	Primary p^+ , ionization of e^-	Continuous energy loss	Energy loss, determines the range in tissue
Elastic Coulomb scattering	Atomic nucleus	Primary p^+ , recoil nucleus	Change on the trajectory	Determines the gradient of the lateral penumbra
Inelastic Nuclear interactions	Atomic nucleus	Secondary p^+ and heavy ions, neutrons and γ rays	Elimination of primary p^+ of the beam	Primary fluence, production of free neutrons

Table 1.1: Summary of the different types of interaction with matter, targets, influence on projectiles, and selected dosimetric manifestations. Table extracted from [30].

Taking into account inelastic nuclear interactions, attention should be paid to the following effects:

- Often, the recoil nucleus, a product of the interaction, can be radioactive like ^{11}C , ^{13}N and ^{15}O among other species.
- Primary and secondary protons, together with secondary neutrons, contribute to material activation.

In summary, protons interact with matter in three different ways. They are slowed down by multiple collisions with atomic electrons and are deflected by a variety of collisions with atomic nuclei. Finally, they can collide head-on with a nucleus, setting secondary particles in motion as a result of a nuclear reaction. These processes are called *stopping scattering* and *nuclear interactions*. The processes of scattering and stopping result from the electromagnetic (EM) interaction between the charge of the proton and that of the electrons or the nucleus, depending on which interaction occurs. The following sections will discuss each type of interaction in detail.

1.1.3 Stopping

Protons are slowed down in matter mainly through multiple collisions with atomic electrons. While the appropriate description of the collision process must be formulated within the framework of quantum mechanics, it can be said, from a *classical* point of view, that the proton loses more energy the longer it interacts with the orbital electrons. Therefore, the rate of energy loss increases as the proton slows down, giving rise to the Bragg ionization peak near the end of the range, the distinctive feature of proton radiotherapy [31]. This section will cover the concepts of *Stopping power*, *Range* and *Straggling*.

Stopping power The mean rate of ion energy loss, or linear stopping power, is defined as the differential dE/dx , where E is the mean energy and x is the position variable along the beam axis. It is often more convenient to express the rate of energy loss in a manner that is independent of mass density; mass stopping power is defined as:

$$\frac{S}{\rho} = -\frac{dE}{\rho dx}, \quad (1.1)$$

where ρ is the mass density of the absorbing material. It should be noted that the stopping power is defined for a beam, not for an individual particle.

There are several formulas and theories that can be used to describe the rate of energy loss:

- Formula based on the *Bragg-Kleeman rule*, which derives from alpha particles.
- More complete physical theory developed by Bohr (1915), which is based on the calculation of the momentum of a stationary, unbound electron and the impact parameter.

- More accurate formula, attributed to Bethe (1930) and Bloch (1933), which takes into account quantum-mechanical and relativistic effects:

$$\frac{S}{\rho} = -\frac{dE}{\rho dx} = 4\pi N_A r_e^2 m_e c^2 \frac{Z}{A} \frac{z^2}{\beta^2} \left[\ln \frac{2m_e c^2 \gamma^2 \beta^2}{I} - \beta^2 - \frac{\delta}{2} - \frac{C}{Z} \right], \quad (1.2)$$

where N_A is Avogadro's number, r_e is the classical radius of the electron, m_e is the mass of the electron, z is the charge of the projectile, Z is the atomic number of the absorbing material, A is the atomic weight of the absorbing material, c is the speed of light, $\beta = \frac{v}{c}$ where v is the velocity of the projectile, $\gamma = (1 - \beta^2)^{-1/2}$ and I is the average excitation potential of the absorbing material. In addition, δ corresponds to the density correction derived from the shielding of remote electrons by nearby electrons, resulting in a reduction in energy loss at higher energies, and C is the layer structure correction element where the particle velocity is close to the velocity of the atomic electrons. The two correction terms in the Bethe-Bloch equation involve relativistic theory and quantum mechanics and need to be considered when the proton energies used in these calculations are either very high or very low.

From the Bethe-Bloch equation 1.2 [31], it emerges that the rate of energy loss of protons in the human body depends strongly on the density of the material (which can vary by approximately three orders of magnitude from air in the lung to cortical bone), and the ion velocity, which can cause the linear stopping power in water to vary by a factor of approximately 60 for proton energies between 1 and 250 MeV [30].

Range The *range* of a proton beam is defined as the depth in the medium at which approximately half of the incident protons have come to rest. As the beam penetrates the material, the number of primary protons gradually decreases due to nuclear interactions that remove particles from the beam. Near the end of the range, a sharp reduction in proton fluence is observed, corresponding to protons that have progressively lost their kinetic energy through electromagnetic interactions and are ultimately stopped in the medium. This distal fall-off does not occur at a single well-defined depth, but instead exhibits a smooth, quasi-sigmoidal shape. This behavior arises from *range straggling*, a consequence of the stochastic nature of energy loss processes, which causes individual protons to stop at slightly different depths.

As can be inferred from the Bethe-Bloch equation (see Eq. 1.2), the relationship between the logarithm of the range and the logarithm of the energy is approximately linear. Therefore, the range R_{CSDA} of a proton can be calculated using the Bragg-Kleemann rule, assuming the Continuous Slowing Down Approximation (CSDA) [32]:

$$R_{CSDA}(E) = aE^p, \quad (1.3)$$

where a is a material-dependent constant, E is the initial energy of the proton beam in MeV, R_{CSDA} is the CSDA range in cm, and the exponent p takes into

account the dependence on the energy or velocity of the proton. For the energy range of 100 to 200 MeV in water, the value of a is equal to 0.00244 and that of p is equal to 1.75.

Since energy loss is a stochastic process, both in terms of number and energy transfer in each individual collision, it has a probabilistic nature. Therefore, protons, even if their initial energy is exactly the same, will not stop at exactly the same depth. This is called **range straggling**, or *energy straggling* if the focus is on fluctuations in energy loss rather than range.

1.1.4 Multiple Coulomb Scattering

In addition to energy loss, protons undergo scattering primarily due to multiple interactions with atomic nuclei. Individual deflections are small, but their cumulative effect leads to an angular spread of the beam, known as *Multiple Coulomb Scattering* (MCS). As a result, the spatial distribution of scattered protons is approximately Gaussian (see Fig. 1.5).

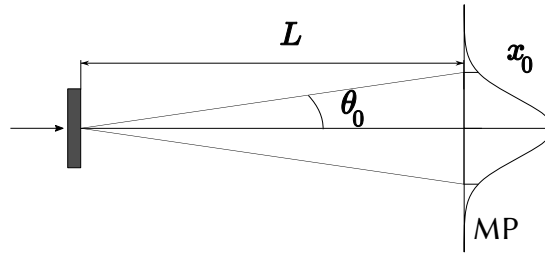


Figure 1.5: Multiple Coulomb scattering in a thin block of matter. MP: measurement plane. Image taken from [31].

The angular distribution of MCS is approximately Gaussian as it results from the accumulation of many small random deflections, being the theoretical approximation in accordance with the Central Limit Theorem [31]. However, the full distribution exhibits a Gaussian core with a low-probability tail. For most radiotherapy applications, the Gaussian component, which contains about 98% of the protons, is sufficient.

Several analytical models based on Coulomb scattering theory have been developed to describe MCS, including the well-known formulation by Molière with subsequent refinements [33–35].

An accurate description of MCS is essential for dose calculations in treatment planning systems (TPS) and Monte Carlo simulations. In particular, Monte Carlo methods rely on MCS theory within the condensed history approach, where multiple interactions are grouped to improve computational efficiency.

1.1.5 Nuclear interactions: primary and secondary particle field

In addition to the interaction mechanisms described in sections 1.1.3 and 1.1.4, protons can interact with the nucleus through inelastic nuclear reactions in which the nucleus is irreversibly transformed. The main effect of nuclear reactions is a small decrease in the absorbed dose due to the elimination of primary protons, which is largely compensated for by the release of secondary protons and other ions. Important aspects of nuclear reactions will be discussed in this section.

In proton therapy, *primary particles* are protons from the beam that slow down and stop in matter at any given depth after undergoing electromagnetic interactions. This group constitutes the **primary particle field**.

Particles from inelastic or non-elastic nuclear reactions are called *secondary particles* and constitute the *secondary particle field*. Those secondary particles, such as protons constituting the nucleus or neutrons, which emerge from inelastic collisions, tend to have much lower energies and much larger scattering angles than primary protons.

Secondary particle field The field of secondary particles, which come from inelastic reactions at therapy energies, is composed of secondary protons (p'), deuterons (^2H), tritons (^3H), ^3He , alpha particles (^4He), residual nuclei, neutrons (n) and gamma rays (γ).

Table 1.3 summarizes the lowest-threshold nuclear reactions between primary protons and ^{16}O , ^{12}C , and ^{14}N —the main constituents of soft tissue (Table 1.2)—that give place to secondary particles. From this Table, it can be seen that for each target, the reactions that produce alpha particles are, apart from p' , those with the lowest threshold energy. For this reason, a higher production of these particles is to be expected at greater depths in soft tissue compared to other fields of secondary charged particles.

Atomic number	Fraction by weight
1	0.101172
6	0.111000
7	0.026000
8	0.761828

Table 1.2: Atomic composition of soft tissue (ICRU) [36].

1.1.6 Bragg peak and Spread-out Bragg peak

Bragg Peak In proton therapy Bragg peak (BP) refers to the entire dose-depth distribution measured in a given material using a proton beam wide enough to achieve lateral, quasi-energetic equilibrium. The physical processes governing the location and/or height of the peak are mainly proton stopping power (Sec. 1.1.3)

Target	Reaction products	Threshold [MeV]
^{16}O	$^{17}\text{F} + \gamma$	0
^{16}O	$^{16}\text{O} + \text{p}'$	0
^{16}O	$^{15}\text{O} + \text{d}$	14.3
^{16}O	$^{14}\text{O} + \text{t}$	21.7
^{16}O	$^{14}\text{N} + {}^3\text{He}$	16.2
^{16}O	$^{13}\text{N} + \alpha$	5.5
^{16}O	$^{15}\text{O} + \text{n} + \text{p}'$	16.7
^{12}C	$^{13}\text{N} + \gamma$	0
^{12}C	$^{12}\text{C} + \text{p}'$	0
^{12}C	$^{11}\text{C} + \text{d}$	17.9
^{12}C	$^{10}\text{C} + \text{t}$	25.3
^{12}C	$^{10}\text{B} + {}^3\text{He}$	21.3
^{12}C	$\text{p} + 3\alpha$	7.9
^{12}C	${}^9\text{B} + \alpha$	8.2
^{12}C	$^{12}\text{N} + \text{n}$	19.6
^{14}N	$^{15}\text{O} + \gamma$	0
^{14}N	$^{14}\text{N} + \text{p}'$	0
^{14}N	$^{13}\text{N} + \text{d}$	8.9
^{14}N	$^{12}\text{N} + \text{t}$	23.7
^{14}N	$^{12}\text{C} + {}^3\text{He}$	5.1
^{14}N	$^{11}\text{C} + \alpha$	3.1
^{14}N	$^{14}\text{O} + \text{n}$	6.4

Table 1.3: Lowest-threshold nuclear reactions between primary protons and the main elemental constituents of soft tissue [36] (^{16}O , ^{12}C , and ^{14}N), responsible for the production of secondary particles [37].

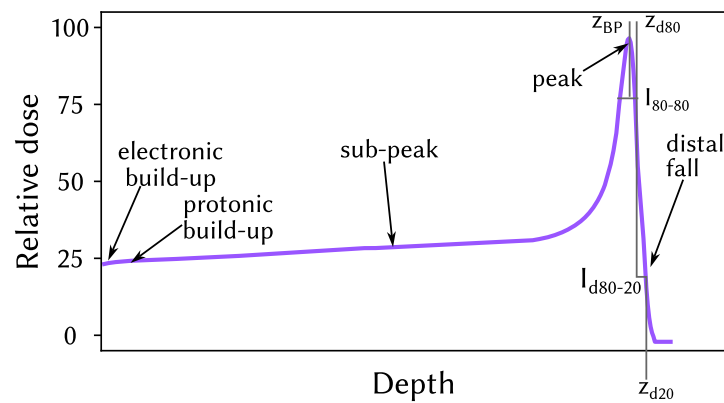


Figure 1.6: Relative dose as a function of depth in water. The build-up is not visible in this graph. Modified image, taken from [30].

and energy straggling; nuclear reactions (Sec. 1.1.5) a much lesser extent and, for very small fields, MCS (Sec. 1.1.4).

Figure 1.6 shows a proton BP along with labels identifying different regions. In order of increasing depth, these are: the build-up region, proton build-up, sub-peak, peak, and distal fall. The figure also shows characteristic depths (e.g., the z_{BP} depth at which the peak occurs) and several characteristic lengths (such as the distal fall length from 80% to 20% and the peak width from 80% to 80% proximal). For more details on each of these regions, see [30].

Spread-Out Bragg Peak A spread-out Bragg peak (SOBP) is the superposition of several Bragg peaks of different energies and assigned weights in order to achieve a uniform dose distribution in the tumor region, maximizing the dose in these regions and minimizing it in normal tissue. A weight, in practice, is essentially the beam delivering time of the corresponding peak (at constant current).

By definition, the SOBP extends from the depth corresponding to 90% of the proximal dose to 90% of the dose at the distal fall-off (see Fig. 1.7); this region is commonly referred to as the *modulation width*.

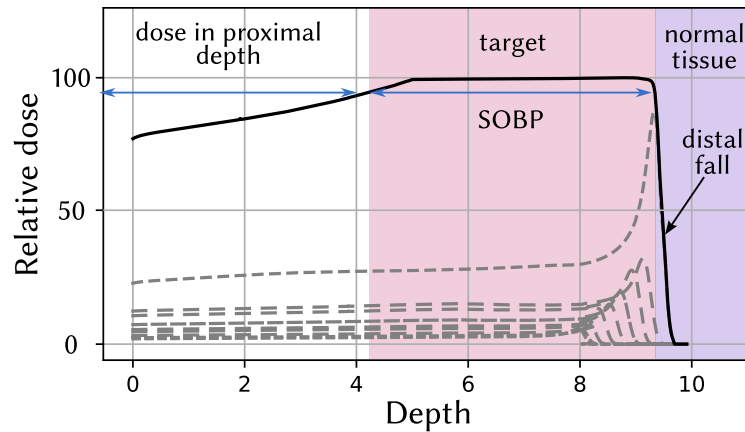


Figure 1.7: SOBP dose-depth distribution (solid line) and Bragg peak components (dashed lines). Modified image, taken from [31].

In clinical proton therapy, uniform dose delivery is commonly achieved using either *passive scattering* or *pencil beam scanning*, the latter also referred to as *spot-scanning proton therapy (SSPT)*. Passive scattering relies on beam-modifying devices placed in the treatment nozzle to laterally spread the proton beam, together with a rotating range modulator wheel, a compensator, apertures, and/or a ridge filter, in order to shape the dose distribution within the target volume.

In contrast, pencil beam scanning employs a narrow proton beam that is magnetically steered to cover the lateral extent of the target, while variations in beam energy are used to achieve the required depth coverage. In this technique, the dose is delivered as a collection of discrete spots, each corresponding to a proton Bragg peak at a specific position and energy. The dose contribution of each spot is determined by its assigned weight.

In SSPT treatment planning, the prescribed target dose is obtained by select-

ing appropriate spot positions and energies and by optimizing their corresponding weights. This optimization can be performed using either *single-field optimization* (SFO), in which each proton field is optimized independently, or *multifield optimization* (MFO), where spots from all fields are optimized simultaneously. For more details on these techniques, see [31, 38–41].

1.2 Fundamental concepts in radiation physics and radiobiology

1.2.1 Absorbed dose

The absorbed dose, D , at a given point in a radiation field is defined as the energy deposited per unit mass of the irradiated medium [42]:

$$D = \frac{dE}{dm}, \quad (1.4)$$

and is expressed in SI units as joules per kilogram (J/kg). The unit of absorbed dose is the Gray (Gy), where 1 Gy = 1 J/kg.

As a typical example, a standard proton therapy treatment (e.g., Head and Neck cancer) may involve the delivery of a total dose of approximately 70 Gy, administered in around 35 fractions of 2 Gy per session.

1.2.2 Linear Energy Transfer

The linear energy transfer (LET) is a fundamental nonstochastic physical quantity widely used to characterize radiation quality in relation to its biological effectiveness. It represents the average energy deposited by a charged particle per unit path length as it traverses matter, typically expressed in units of keV μm^{-1} [42].

In physical terms, LET reflects the density of ionization events along the particle track: higher LET values correspond to a higher concentration of energy deposition over short distances, which is generally associated with increased biological damage.

Two practical definitions of LET are commonly used: the track-averaged LET (LET_T) and the dose-averaged LET (LET_D). The first one represents the fluence-weighted mean LET and is closely related to the original physical definition. The latter accounts for both LET and deposited dose, making it more suitable for describing biological effects [43].

A practical way to compute these quantities from the LET distribution is given by:

$$\text{LET}_T = \frac{\sum_i \phi_i \cdot \text{LET}_i}{\sum_i \phi_i}, \quad \text{LET}_D = \frac{\sum_i \phi_i \cdot \text{LET}_i^2}{\sum_i \phi_i \cdot \text{LET}_i}, \quad (1.5)$$

where ϕ_i denotes the fluence of charged particles with a given LET_i value [44].

The fluence, ϕ , is defined as the number of particles crossing a differential area element dA perpendicular to the direction of motion in a given time interval, and can be expressed as:

$$\phi = \frac{dN}{dA}, \quad (1.6)$$

with units of particles per unit area (e.g., protons cm^{-2}).

1.2.3 Linear Quadratic Model

The linear–quadratic (LQ) model is widely used in both experimental and clinical radiobiology to describe cellular response to ionizing radiation, showing good agreement with observations in both *in vitro* and *in vivo* conditions [45]. In this framework, cell survival (S) is expressed as:

$$S = \exp(-\alpha D - \beta D^2), \quad (1.7)$$

where D is the absorbed dose, α has units of Gy^{-1} , while β has units of Gy^{-2} . Figure 1.8 illustrates a typical survival curve, which is a plot of surviving fraction SF against the absorbed dose D .

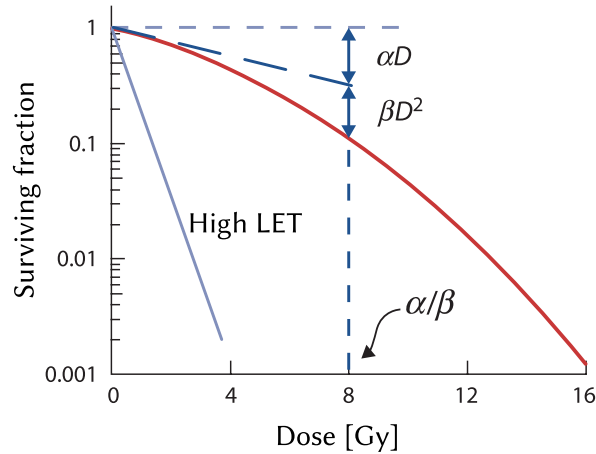


Figure 1.8: Example of a survival curve. Modified image, taken from [45].

The linear term (αD) represents damage arising from single radiation tracks that are sufficient to induce lethal lesions in the DNA. The quadratic term (βD^2) accounts for damage produced by the interaction of two independent radiation tracks, leading to combined sublethal lesions that become lethal when occurring in close proximity.

Clonogenic assay and calculation of surviving fraction One of the standard methods to quantify cellular radiosensitivity is the clonogenic assay, which is a technique in cell biology that evaluates the ability of a single cell to proliferate and form a colony, quantifying the reproductive cell surviving fraction after exposure to ionizing radiation or other cytotoxic agents *in vitro*.

In this assay, cells are first prepared as a single-cell suspension and divided into several groups. One group is kept as a non-irradiated control, while the remaining groups are exposed to different irradiation conditions. All samples are then seeded in culture dishes or multi-well plates under identical growth conditions.

The number of cells seeded in each dish depends on both the delivered dose and the intrinsic radiosensitivity of the cell line. In general, higher doses lead to increased cell killing; therefore, a larger number of cells must be initially plated to ensure that a statistically significant number of colonies can be counted after irradiation.

Following irradiation, the cells are incubated for a period that depends on the growth rate of the specific cell line, typically ranging from a few days up to one or two weeks. During this time, surviving cells proliferate and form colonies, which are subsequently stained and counted.

The first quantity evaluated is the plating efficiency (PE), determined from the control (non-irradiated) samples. It is defined as the ratio between the mean number of colonies formed and the mean number of seeded cells:

$$PE = \frac{\langle N_{col} \rangle}{\langle N_{seed} \rangle}, \quad (1.8)$$

where N_{seed} is the mean number of cells seeded per well and $\langle N_{col} \rangle$ is the mean number of colonies, calculated as

$$\langle N_{col} \rangle = \frac{1}{N_{wells}} \sum_{i=1}^{N_{wells}} N_{col,i}, \quad (1.9)$$

with N_{wells} being the total number of wells and $N_{col,i}$ the number of colonies counted in the i -th well.

Once the plating efficiency is determined, the surviving fraction (SF) for irradiated samples is calculated on a well-by-well basis as

$$SF_i = \frac{\frac{N_{col,i}}{N_{seed,i}}}{PE}, \quad (1.10)$$

where $N_{seed,i}$ is the number of cells initially seeded in the i -th well.

1.2.4 Relative Biological Effectiveness (RBE)

The biological effectiveness of particle beams used for therapeutic purposes is commonly characterized by the Relative Biological Effectiveness (RBE). RBE is defined as the ratio between the dose of a reference radiation and that of a test radiation required to produce the same biological effect, for instance, a given surviving fraction in an irradiated cell culture:

$$RBE = \frac{\text{Dose of reference radiation (for level of effect } s)}{\text{Dose of test radiation (for level of effect } s)} \quad (1.11)$$

RBE is an experimentally well-defined quantity, both conceptually and in its derivation, designed to isolate differences in biological effectiveness between radiation types while minimizing the influence of assay-specific factors. It provides a useful measure for comparing the effectiveness of different radiations, even for endpoints that are not easily quantifiable.

However, experimental RBE has limited predictive capability, particularly when applied to irradiation conditions that differ from those under which it was determined (e.g., changes in dose rate or fractionation). This limitation highlights the need for empirical and mechanistic models in radiation biophysics to extend RBE predictions beyond experimental conditions.

Differences between the biological effectiveness of different radiation types depend on multiple factors and variables: the biophysical characteristics of the different radiation fields, the spatial distribution (i.e., the ionization density) of energy deposits at the microscopic level, the level and type of the described effect (targeted or not), the dose and irradiation time-dependence concerning the interplay between production and repair of the sublethal damage in tissues and tumors, the oxygenation level of cells, etc.

In clinical practice, the RBE in proton therapy is commonly assumed to be equal to 1.1, using as reference radiation photons from ^{60}Co or clinical-quality X-rays (6–10 MV). However, extensive experimental evidence indicates that RBE is not constant but varies with depth in tissue, increasing toward the Bragg peak and particularly in the distal fall-off region as the particle energy decreases.

This depth dependence is a key aspect of ion beam radiobiology and should be considered in treatment planning, especially when targets are located near critical structures. Due to range uncertainties and the enhanced biological effectiveness at low energies, normal tissues beyond the Bragg peak may be exposed to radiation with increased damage potential.

At present, commercial treatment planning systems do not incorporate variable, voxel-wise RBE models that account for its underlying dependencies. As a result, conservative strategies in dose prescription, beam arrangement, margins, and robustness are typically adopted, limiting the full exploitation of the physical and radiobiological advantages of charged particle beams. This limitation has been widely recognized by the international community, and addressing it is considered essential for improving dose optimization in ion beam therapy.

1.2.4.1 Empirical and Mechanistic RBE models

Over the years, extensive data on the biological effectiveness of charged particle beams have been collected, and various descriptive models of RBE have been developed. These models are generally classified as either empirical or mechanistic, though with some degree of overlap between approaches. All of them seek to characterize the dependencies of the RBE factor, with varying levels of detail.

The most widely used empirical models parameterize RBE in terms of the LQ model, which relates cell survival to absorbed dose as described in Sec. 1.2.3. These models incorporate dependencies on radiation quality—through additional

parameterization using an average quantity, the dose weighted LET, embedded in the α and β parameters of the LQ model—as well as on damage repair as a function of time, dependent on dose rate [8, 18, 19, 21]. Models based on experimental microdosimetry aim to relate the first and second moments of the energy deposition distribution in sensitive sites of approximately one micron or smaller to the behavior of RBE, adopting certain hypotheses to explain the supralinear dependence on dose [46, 47]. However, none of these models differentiate between primary and secondary particles, thereby precluding consideration of possible time dependent synergistic effects.

The best known mechanistic models are founded on the hypothesis that the macroscopic effect arises from the deposition of local energy. In the Local Effect Model (LEM), the same *local* dose is assumed to produce the same biological effect, regardless of radiation quality [48]. Other models are based on the theory of dual radiation action [49], incorporating a conversion kinetics, as the Microdosimetric Kinetic Model (MKM) which incorporates a kinetic description to translate the damage into DSB or lethal chromosomal aberrations [50, 51].

1.2.4.2 Expression of the RBE based on the LQ survival model

In its simplest form, the analytical expression for the relative biological effectiveness of a test radiation T with respect to a reference radiation X , at a given survival level s , is defined as

$$RBE_s = \frac{D_X}{D_T} \Big|_s.$$

By equating the LQ survival expressions (Eq. 1.7) for both radiations and expressing the result in terms of D_X , a quadratic equation is obtained. Its positive solution, divided by D_T , yields the usual expression:

$$RBE_s = \frac{D_X}{D_T} \Big|_s = \frac{1}{2D_T} \left[\sqrt{\left(\frac{\alpha}{\beta}\right)_X^2 + \frac{4}{\beta_X} (\alpha_T D_T + \beta_T D_T^2)} - \left(\frac{\alpha}{\beta}\right)_X \right]. \quad (1.12)$$

A more comprehensive, albeit still simplified, expression, referred to as the Modified LQ model (MLQ), accounts for the creation, repair and pairwise combination of repairable double-strand breaks (DSB), also referred to as sublethal damage (SLD), for both the reference and test radiations. The quadratic terms include the Lea-Catcheside time factor [52] which depends on the characteristic repair times and dose rate.

However, it is worth noting that, for constant dose rates, the irradiation time varies for each experimental point. Consequently, the Lea-Catcheside factor for SLD production and repair is different at each point, also depending on the delivered dose, which prevents obtaining a closed-form expression for the RBE. Neglecting this aspect may influence the estimated alpha and beta parameters, given the assumptions underlying their derivation.

Another aspect arises when considering radiations that generate secondary particles coexisting with the primary ones, thus constituting a mixed radiation field. Such fields, defined by their individual parameters and dose contributions, are described not only by separate linear and quadratic terms but also by mixed terms accounting for SLD creation. These mixed terms are also subject to repair but, if not repaired, may ultimately lead to mixed lethal combinations between pairs of sublesions produced by each component.

A detailed description of radiation-induced DNA damage, including the different types of lesions and the cellular response mechanisms, is beyond the scope of this work and can be found in Chapter 2 (“Irradiation-induced damage and the DNA damage response”) of [45].

Phenomenological RBE models considered in this study The PhID model introduced in this thesis provides dose estimates from which RBE values can also be derived. For comparison, results are benchmarked against phenomenological RBE models (see Sec. 2.2.6) which estimate RBE values that can be translated into corresponding dose predictions: Wilkens and Oelfke (WIL) [8], Tilly *et al* (TIL) [11], Chen and Ahmad (CHE) [17], Carabe *et al* (CAR) [18], Wedenberg (WED) [19], Jones (JON) [20], McNamara (MCN) [21], Rørvik *et al* (RORU) [22] and Peeler (PLR) [23]. These models are based on parameterizations of the LQ model, where the calculation of the RBE depends on the proton dose D_p , the $(\alpha/\beta)_x$ ratio of the reference radiation, and on the model-specific terms RBE_{min} and RBE_{max} . This relationship, analogous to the expression 1.12, can be written as:

$$RBE = \frac{1}{2D_p} \left(\sqrt{\left(\frac{\alpha}{\beta}\right)_x^2 + 4D_p \left(\frac{\alpha}{\beta}\right)_x RBE_{max} + 4D_p^2 RBE_{min}^2} - \left(\frac{\alpha}{\beta}\right)_x \right). \quad (1.13)$$

The considered LQ-based RBE models can be expressed in the common form given in Eq. 1.13, following the reformulation of phenomenological RBE models presented in [16]. Within this framework, the models differ only in the definition of the RBE_{max} and RBE_{min} functions. The specific functional forms of these terms for each model are provided in Appendix A.

1.3 Microdosimetry

1.3.1 Fundamentals of microdosimetry

The effects of radiation at the cellular level are governed by the local patterns of energy deposition within the cell and its substructures. In this context, microdosimetry is the branch of radiation biophysics that studies the spatial, temporal, and spectral characteristics of the stochastic process of energy deposition in microscopic volumes. By accounting for the random nature of energy losses and the

track structure of charged particles, it provides a physical description that can be directly linked to the biological effects of radiation.

Microdosimetry, in its modern sense, was established through the pioneering work of Harald H. Rossi (1917–2000), who recognized the fundamental distinction between macroscopic absorbed dose and energy deposition within microscopic structures. He identified that the relevant quantities at the microscopic scale are inherently stochastic variables. Building on this insight, Rossi and his collaborators developed advanced techniques to measure the random fluctuations of energy deposition, constructed a novel conceptual and mathematical framework, and applied these new principles and methods to the study of radiobiology [53].

Regional microdosimetry, also known as the microdosimetry of Rossi counters, examines the deposition of energy within a site of specified dimensions without accounting for the microscopic distribution of individual energy transfers—that is, the ionization pattern generated along the trajectory of a charged particle. This formulation is particularly significant because it introduces quantities that, at least in principle, can be experimentally determined and correlated with biological effects.

Building on this framework, the following sections introduce the fundamentals of both regional and experimental microdosimetry. In particular, experimental microdosimetry enables the characterization of complex radiation fields through the measurement of stochastic energy deposition in microscopic volumes, expressed in terms of the quantities defined within regional microdosimetry, such as the lineal energy and its associated distributions.

1.3.1.1 Regional microdosimetry

In regional microdosimetry, two fundamental quantities are introduced as the microscopic, stochastic analogs of dose and LET: the specific energy z and the lineal energy y . Both are random variables that can be experimentally determined under appropriate conditions. The specific energy is defined as the ratio of the energy imparted ϵ by one or more events to the mass of the site m , i.e. $z = \epsilon/m$. The lineal energy corresponds to the energy imparted in a single event divided by the mean chord length $\langle l \rangle$, namely $y = \epsilon/\langle l \rangle$. Under an isotropic and uniform field of random straight lines, the mean chord length of a convex body is given by Cauchy's formula $\langle l \rangle = 4V/S$, where V denotes the volume and S the surface area of the body.

The fundamental mathematical construct in regional microdosimetry is the single-event probability density function, $f(y)$, expressed in terms of the stochastic variable lineal energy y . From this distribution, the moments of the lineal energy can be defined in a general form as

$$y^{(n)} = \int_0^\infty y^n f(y) dy. \quad (1.14)$$

In particular, the first moment, $y^{(1)}$, corresponds to the frequency-mean lineal energy, commonly denoted as y_F . The dose-mean lineal energy, y_D , is defined as

the ratio of the second to the first moment, and can therefore be expressed as

$$y_D = \frac{y^{(2)}}{y^{(1)}} = \frac{1}{y_F} \int_0^\infty y^2 f(y) dy. \quad (1.15)$$

Then, considering y_F , the following function:

$$d(y) = \frac{yf(y)}{y_F}, \quad (1.16)$$

is referred to as the dose-weighted probability density function.

1.3.1.2 Experimental microdosimetry

Experimental microdosimetry enables the characterization of radiation quality in mixed fields by means of ionization measurements, which are then converted into energy imparted spectra and subsequently into the lineal energy distributions defined earlier.

A widely used device for the characterization of complex radiation fields is the Tissue-Equivalent Proportional Counter (TEPC). Its operation relies on a relationship that, under appropriate conditions, allows the energy deposited in a gas-filled cavity of macroscopic dimensions (g) to be related to that deposited in a microscopic site of biological tissue (t):

$$\overline{\Delta \varepsilon} = \Delta x_g \left(\frac{S}{\rho} \right)_g \rho_g = \Delta x_t \left(\frac{S}{\rho} \right)_t \rho_t,$$

where Δx is the distance traveled by a particle, S/ρ the mass-stopping power and ρ the density, either in the gas cavity or in the tissue microscopic site. If the detector cavity is made of *tissue equivalent* gas and walls, the mass-stopping power cancels out and a direct relationship arises that permits obtaining microdosimetric spectra representative of the actual energy deposition in a microscopic site [53].

1.3.2 Theory of Compound Dual Radiation Action

The theory of Compound Dual Radiation Action (CDRA) [54] states that lesions arise by pairwise combination of sublethal lesions at two different spatial scales:

- at the nanometer scale of about 30 nm, related with the maximum distance over which a pair of single-strand breaks (SSB) can destabilize the double DNA chain and provoke a double-strand break (DSB);
- at the micrometer scale, where previously formed pairs of DSB can combine to form a two-break aberration (2-BA) at distances typical of the Brownian random displacement of both broken strands.

In this way, CDRA establishes a sequential process for the formation of lethal lesions, starting from the DNA scale where essentially single events are causing

the effect (both SSB are produced by the same event²), and then by the combination of DSBs that can be produced, either by a single event or by uncorrelated events, occurring at micrometer distances.

In both cases, the probability of pairwise combination of sublethal lesions is proportional to the square of the specific energy deposited in the corresponding volumes. Subsequently, these probabilities are averaged over the corresponding probability densities: the single event probability density function (p.d.f.) for nanometric volumes and the multi-event p.d.f. for micrometer volumes. The former gives place to an average number of lesions proportional to the dose, while the latter gives place to two terms: linear with dose and quadratic with dose. The resulting number of lesions depends on the dose through a linear–quadratic relationship. Figure 1.9 illustrates the mechanism.

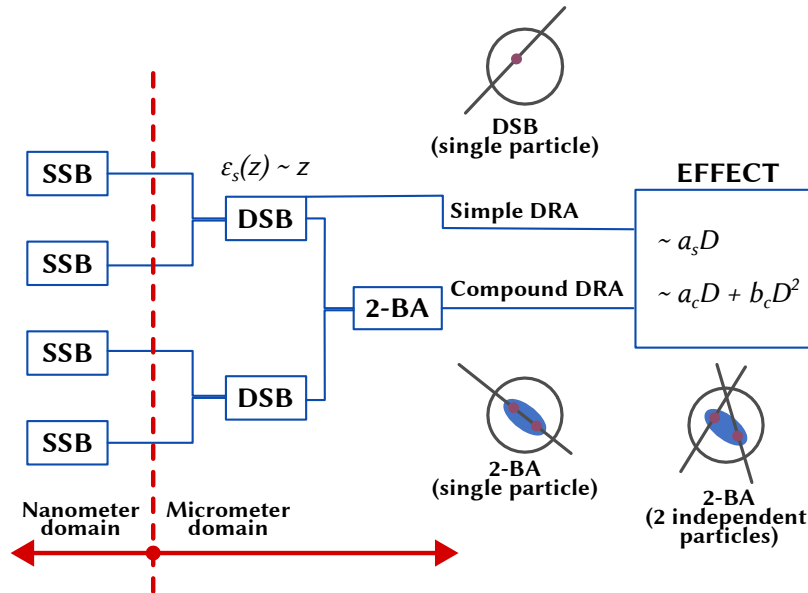


Figure 1.9: CDRA mechanism. The figure was adapted from [54].

One of the most widely used models derived from the CDRA theory and applied to particle therapy to obtain the RBE of protons is the Microdosimetric-Kinetic (MK) model [55]. In its initial version, the mean number of lethal lesions, $\langle w \rangle$, which are assumed to be Poisson distributed among the cell population, is written as follows:

$$\langle w \rangle = -\ln S = \left(\alpha_0 + \frac{0.204 y_D}{d^2} \beta \right) D + \beta D^2, \quad (1.17)$$

where d is the sensitive site diameter (or domain, in Hawkins's formalism), α_0 is the limiting value for α as lineal energy (or LET) approaches zero, and β is the

²For a spherical volume of 10 nm in diameter, a radiation with an average of 10 keV/μm yields a mean number of events per Gy of about 0.005, implying that even at a dose as high as 200 Gy, only one event is expected on average.

quadratic term coefficient, assumed to be constant and independent of LET. In an approximation where LET is almost constant, i.e., the so-called track-segment approximation (TSA), the moments of order n are the n th powers of the LET:

$$L^{(n)} = \int_0^\infty L'^n \delta(L' - L) dL' = L^n. \quad (1.18)$$

Since $y_D \sim L$, it means that the expression between brackets in Eq. 1.17 increases linearly with LET. However, it is found that the dependence of α on LET shows a departure from a straight line for LET values higher than about 50 – 100 keV/ μ m. Then Hawkins, following the approach of Rossi and Kellerer, introduced a multiplicative term to account for saturation effects [56]:

$$RBE_1 = \frac{1 - \exp\left(-\frac{0.14\alpha_R RBE_{1P}}{\sigma} y_D\right)}{\frac{0.14\alpha_R RBE_{1P}}{\sigma} y_D} RBE_{1P}, \quad (1.19)$$

with $RBE_{1P} = \frac{\alpha}{\alpha_R} = \frac{\left(\alpha_0 + \frac{0.204 y_D}{d^2} \beta\right)}{\alpha_R}$, where σ is the inactivation cross-section, i.e., the cross-sectional area associated with the intersection of the ion trajectory as it passes through the chromatin and inactivates the cell with probability one. The subscript P indicates that the relationship holds for a Poisson distribution of lethal lesions. RBE_1 , in turn, is the RBE for a progressive departure from the Poisson distribution of lethal lesions as LET increases.

Even with this correction, the expression for α still depends at most on the second-order moment of the single-event p.d.f..

1.4 The Isoeffectiveness Concept

The concept of Isoeffectiveness (IsoE), historically the basis for defining the RBE, can be extended beyond a purely empirical comparison of different radiation types at a given level of effect. Through the application of appropriate biophysical models, it enables a more comprehensive description of radiation action by linking two radiations through the observed effect and its magnitude, whether quantitative or qualitative.

Within this framework, isoeffectiveness can be formulated in terms of different descriptors of radiation response, including RBE as well as other relevant end-points such as tumor control probability (TCP) and normal tissue complication probability (NTCP), which provide quantitative measures of the biological effect (see Fig. 1.10). Moreover, there is no inherent restriction on the choice of the reference. For example, the reference radiation or conditions may depend on the specific context of interest, such as comparing proton therapy delivered at conventional dose rates with FLASH proton therapy at ultra-high dose rates (UHDR), where the reference corresponds to protons delivered at conventional dose rate and the test radiation to protons delivered at UHDR.

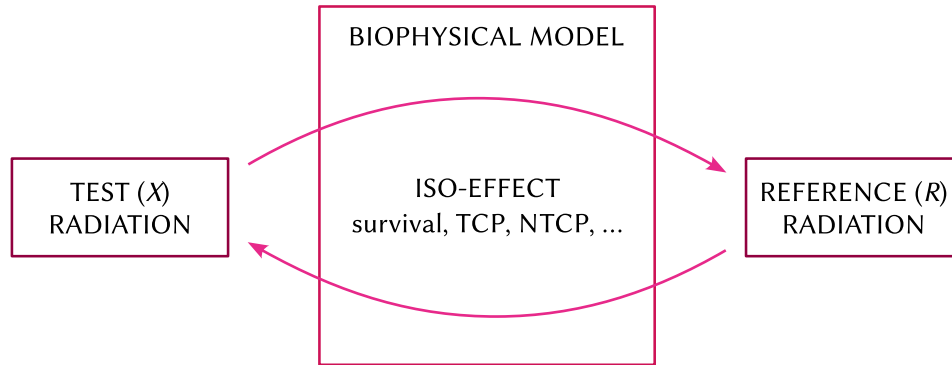


Figure 1.10: Conceptual scheme of isoeffectiveness. A biophysical model links a test radiation to a reference radiation by equating their biological effects. This equivalence can be established using different endpoints, including cell survival, TCP, and NTCP, allowing flexibility in the choice of the reference radiation.

Clearly, two major challenges arise within this framework: one experimental, related to the determination of the relevant biological effects and the acquisition of reliable biological, physical, or clinical data; and the other model-related, involving the development and validation of appropriate approaches, ranging from empirical to mechanistic descriptions at different spatial scales.

1.4.1 The Photon Isoeffective Dose formalism for mixed radiation fields

The Photon Isoeffective Dose (PhID) model [57] was originally developed within the framework of Boron Neutron Capture Therapy (BNCT) [58], a modality of hadron therapy characterized by complex dosimetry involving four interdependent radiation components with markedly different biological effectiveness. In BNCT, the total absorbed dose is typically decomposed into four contributions: gamma dose, thermal neutron dose, fast neutron dose, and the dose arising from the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction.

This decomposition is based on the experimental quantities used to characterize a BNCT neutron beam, namely photon KERMA rate, thermal neutron flux (to calculate both the ^{14}N and ^{10}B dose rates), and fast neutron KERMA rate [59].

Historically, fixed RBE weighting factors were commonly applied to each dose component in order to express all contributions in terms of a photon-equivalent dose. However, it was later demonstrated that the use of constant RBE values is not appropriate in this context, as it leads to RBE-weighted equivalent doses that systematically overestimate the photon dose required to reproduce the observed biological effect in tumors, while underestimating the corresponding dose in normal tissues [27, 57, 60].

In contrast, the PhID formalism provides a framework in which the combined biological effect of mixed radiation fields can be described without relying on fixed

RBE factors, allowing for a more consistent and accurate estimation of isoeffective doses. This approach has been successfully applied to improve the prediction of mucosal toxicity in patients with head and neck cancer [60], and more recently to predict grade 2 or higher somnolence syndrome in patients with brain cancer, both in the context of BNCT [61].

1.4.2 Extension of the PhID approach to Proton Therapy for discrete mixed radiation fields

The PhID formalism can be naturally extended to describe fields composed of an arbitrary number of radiation components, which may be defined according to selected physical or biological characteristics, such as particle type or average LET values. In this framework, a proton therapy field can be conceptually decomposed into a discrete number of components: primary protons, secondary protons, and other secondary charged particles. Each component is characterized by a quantity describing its effectiveness, e.g., the average LET, along with a particle, and cell specific set of LET-dependent parameters from a chosen model (e.g., MLQ), obtained either experimentally or through biophysical mechanistic descriptions of radiation action. This decomposition provides a practical and flexible way to apply the PhID approach to complex proton fields, enabling a more accurate estimation of isoeffective doses for both target volumes and normal tissues.

The PhID formalism can be naturally extended to describe fields composed of an arbitrary number of radiation components, which may be defined freely or distinguished by specific physical or biological characteristics, such as particle type or average LET values. In this framework, a proton therapy field can be conceptually decomposed into a discrete number of components: primary protons, secondary protons, and other secondary charged particles. Each component is characterized by a quantity describing its effectiveness, e.g., the average LET, along with a particle- and cell-specific set of LET-dependent parameters from a chosen model (e.g., MLQ), obtained either experimentally or through biophysical mechanistic descriptions of radiation action. This decomposition provides a practical and flexible way to apply the PhID approach to complex proton fields, enabling a more accurate estimation of isoeffective doses for both target volumes and normal tissues.

1.4.3 Microdosimetric reformulation of the Photon Isoeffective Dose model for Proton Therapy

The derivation of the continuous Photon Isoeffective Dose formalism proposed in this thesis, referred to as the COMPhID model, is grounded in the framework of microdosimetry (see Sec. 1.3). In this approach, radiation quality is described by stochastic quantities, such as lineal energy –the stochastic analogue of LET. Even for a monoenergetic, single-species particle field, the energy deposition in microscopic structures is a random variable and, consequently, so is the lineal

energy, defined as the energy deposited in a single event divided by the mean chord length of the site (these microdosimetric quantities and their interpretation are introduced in detail in the following section).

Unlike the discrete PhID formalism, which decomposes the field into a finite number of particle components, the COMPhID model represents a reformulation in which the description of radiation quality is expressed in terms of lineal energy distributions. This shift from a particle-based to an energy-deposition-based framework allows a direct connection with the stochastic nature of microscopic energy deposition, while retaining a physically meaningful link to the biological effect. However, in this reformulation, the explicit dependence of the biological effect on the particle type is no longer retained, as different radiation components contributing similar lineal energy values become indistinguishable within the microdosimetric description. The continuous description is carried out at microscopic scales, consistent with the observation that the biological effectiveness of radiation is expressed through the amount of energy deposited at the micrometer scale, essentially within the sensitive structure of the cell nucleus, where DNA damage leads to cell inactivation.

In this context, a radiation field can be conceived as a distribution of components whose number can be made arbitrarily large, distributed over differential intervals of lineal energy. Such a distribution is described by the microdosimetric spectrum, i.e., the dose-weighted probability density as a function of lineal energy. The statistical weight of each differential component is given by the height of the corresponding differential interval of that probability density or, if the information originates from experimental measurement, by the height of the spectrum at a given lineal energy within the corresponding bin.

CHAPTER 2

THE PHID MODEL IN PROTON THERAPY

Chapter overview

As previously noted in Sec. 1.4.2, the original isoeffective dose approach is directly applicable to proton radiation fields within the context of proton therapy. These fields can be modeled as a discrete set of components, defined by the primary protons and the main secondary charged particles produced along their trajectory through tissue. This mixed radiation field includes contributions of protons (primary and secondary, without distinction), deuterons, tritons, alpha particles, ^3He ions, and electrons set in motion by photon interactions (see Table 1.3) [24].

The discrete version of the PhID approach developed in this chapter will consider, through the MLQ model, the main contributions to the absorbed dose distinguished by particle type and parameterized by their average LET, and specific cell lines for calculating photon isoeffective doses in proton therapy.

The model is formulated on the basis of cell survival data and includes the main charged components of proton fields, incorporating LET-dependent parameters as well as sublethal damage production, repair, and interactions between field components. It is constructed for three cell lines - head-and-neck squamous cell carcinoma (HNSCC), human skin fibroblasts (HSF), and Chinese hamster fibroblasts (V79)- using radiobiological parameters derived from the BIANCA biophysical model (Sec. 2.2.2).

The performance of the model is first assessed for a representative pristine Bragg peak configuration and subsequently for spread-out Bragg peak (SOBP) fields, with particular attention to the influence of cell line selection, reference radiation, and field composition. The results are compared with the conventional fixed RBE approach. These analyses are presented in Sec. 2.3.2, Sec. 2.3.2.1, and Sec. 2.3.2.2.

In addition, the model is evaluated in clinically relevant scenarios using Tumor Control Probability frameworks (Sec. 2.3.3) and is benchmarked against phenomenological RBE models (Sec. 2.3.4). Finally, the dependence of the predicted RBE on dose and LET is analyzed across representative conditions.

2.1 The photon isoeffective dose model in proton therapy

2.1.1 The Photon Isoeffective Dose Formalism

As described earlier, the Photon Isoeffective Dose (PhID) model is a formalism that, given the dose due to the proton radiation field, allows calculating the photon equivalent dose that yields the same biological effect, e.g. the same level of cell survival. Originally developed in the context of Boron Neutron Capture Therapy (BNCT) [57], PhID was adapted to proton therapy taking into account the mixed charge radiation field resulting from non-elastic nuclear interactions of the proton beam with the target medium [62]. This field comprises six main contributions: primary and secondary protons (without distinction), deuterons, tritons, alpha particles, ^3He ions, and electrons generated by secondary photons produced in non-elastic nuclear interactions (see Table 1.3). The energy deposition from charged particles (protons and fragments) is simulated under the Continuous Slowing Down Approximation (CSDA), where the kinetic energy transferred to secondary electrons (delta rays) not originating from photons is inherently accounted for in the collisional stopping power.

Although PhID is not uniquely associated with a single survival model and alternative formulations may be used to characterize radiobiological isoeffects, in this work the biological effect underlying the definition of photon isoeffective dose is quantified in terms of cell survival. The corresponding dose–response is assumed to be adequately represented by the linear–quadratic (LQ) model, extended to account for dose rate–dependent sublesion production and repair, i.e., the Modified LQ (MLQ) model.

When synergistic effects are considered, the dose–response is further described by incorporating the additional mixed terms from the theory of dual radiation action (TDRA) [49, 54, 63], modulated by the generalized Lea-Catcheside time factor G [52, 64]. Moreover, through the radiobiological parameters α and β , specific to both the particle type and the cell line, the model exhibits a dependence on LET.

Considering that multiple pencil beams (PB) are employed in proton therapy to deliver dose to the target volume, and that several particle types are taken into account in this work, radiobiological parameters, as well as other quantities (e.g., LET, dose), are hereafter denoted using subscripts. Latin indices (e.g., i, j) identify the particle type, while Greek indices (e.g., ν, μ) label the pencil beams contributing to the energy deposited in a given voxel.

2.1. The photon isoeffective dose model in proton therapy

By equating the natural logarithm of cell survival for the reference radiation to that of proton-induced radiation, the Photon Isoeffective Dose model at a given spatial point $\bar{r}$ can be expressed as follows:

$$\begin{aligned} \alpha_x D_x + G_x(\theta') \beta_x D_x^2 = & \sum_{v=1}^N \sum_{i=1}^6 f_{iv} \alpha_{iv}(L_{iv}) D_T \\ & + \sum_{v,\mu=1}^N \sum_{i,j=1}^6 G_{ij}(\theta) \sqrt{\beta_{iv}(L_{iv}) \beta_{j\mu}(L_{j\mu})} f_{iv} f_{j\mu} D_T^2, \end{aligned} \quad (2.1)$$

where D_x , from now on called the *isoeffective dose* (IsoE), is the dose of the reference radiation x ; α_x and β_x are the radiobiological parameters of the MLQ model, and $G_x(\theta')$ the generalized Lea-Catcheside time factor for the reference radiation, where θ' denotes the irradiation time for the reference radiation. The right-hand side corresponds to the mixed charged radiation field generated by the interaction of the primary proton beam with the target, consisting of six components i (or j): primary and secondary protons, deuterons, tritons, alpha particles, ^3He ions, and electrons. The radiobiological parameters α_{iv} and β_{iv} , which depend on the average LET for particle i and beam spot v (hereafter denoted as L_{iv}), represent the parameters associated with particle type i in beam spot v (or μ), where $v, \mu = 1, \dots, N$. Here, N denotes the total number of proton beams of energies $E_{0,v}$, used to form the SOBP or, more generally, in treatments involving multiple beam directions. The absorbed total dose of the field is denoted by D_T , and the relative contribution of each component iv (or $j\mu$) is given by $f_{iv} = D_{iv}/D_T$. The quadratic term accounts for the synergistic effects between components iv and $j\mu$, where $G_{ij}(\theta)$ is the Lea-Catcheside factor that describes the first-order production and repair of sublethal lesions caused by radiation i (or j) that reduces the probability of interaction with sublethal lesions produced by radiation j (or i) during irradiation, with θ denoting the particle field irradiation time.

If the survival experiments are performed with irradiation times θ (or θ') that are much shorter than the characteristic repair time, t_0 , of the cell line, the repair process during irradiation can be neglected. Since irradiation times in survival experiments, as well as in conventional radiotherapy and proton therapy, are on the scale of minutes, whereas the repair times of the three cell lines considered are on the order of hours [9, 65–67], the calculation yields $G_x(\theta') \approx 1$ and $G_{ij}(\theta) \approx 1$ (see Appendix B). Thus, equation (2.1) can be solved analytically and reduces to:

$$\begin{aligned} \alpha_x D_x + \beta_x D_x^2 = & \sum_{v=1}^N \sum_{i=1}^6 f_{iv} \alpha_{iv}(L_{iv}) D_T \\ & + \sum_{v,\mu=1}^N \sum_{i,j=1}^6 \sqrt{\beta_{iv}(L_{iv}) \beta_{j\mu}(L_{j\mu})} f_{iv} f_{j\mu} D_T^2 \end{aligned} \quad (2.2)$$

Since the main goal of this work is to compute the IsoE dose, equation (2.2) is

solved to obtain D_x . In the particular case of a single pencil beam ($N = 1$), this reduces to:

$$D_x = \frac{-\alpha_x + \sqrt{\alpha_x^2 + 4 \cdot \beta_x \cdot \left(\sum_{i=1}^6 f_i \alpha_i D_T + \sum_{i,j=1}^6 \sqrt{\beta_i \beta_j} f_i f_j D_T^2 \right)}}{2 \cdot \beta_x}. \quad (2.3)$$

Based on this equation, the IsoE dose, at a given spatial point $\bar{r}$, can be determined if D_T , the relative contribution f_i of each component in the mixed radiation field, and the corresponding $\alpha_i(L_i)$ and $\beta_i(L_i)$ parameters are known, along with the radiobiological parameters α_x and β_x of the specific cell line, for the reference radiation x .

In this work, the IsoE dose will be computed for two irradiation scenarios: a single proton pencil beam ($N = 1$) and a SOBP generated with 26 proton pencil beams ($N = 26$). The irradiation of a soft-tissue phantom with a proton beam of energy $E_{0,v}$ will be simulated by Monte Carlo (MC; see Sec. 2.2.1). For each voxel, the simulations will provide the total absorbed dose D_T , the dose due to each component D_{iv} and particle fluence Φ_{iv} corresponding to each of the six considered charged particle types. From these quantities, the relative dose fractions f_{iv} and the corresponding average L_{iv} values will be derived (Sec. 2.2.1.4). Through a biophysical model, the radiobiological parameters $\alpha_{iv}(L_{iv})$ and $\beta_{iv}(L_{iv})$ will be derived for each particle field (Sec. 2.2.2). The following sections describe in detail how each of these quantities is obtained.

2.2 Materials & Methods

This section begins with a description of the Monte Carlo simulations performed to obtain the total absorbed dose, D_T , the dose due to each component D_{iv} , and the particle fluence, Φ_{iv} , as well as the derived quantities f_{iv} and L_{iv} for each voxel of the irradiated phantom (Sec. 2.2.1).

Section 2.2.2 then describes the approach used to obtain the radiobiological parameters $\alpha_{iv}(L_{iv})$ and $\beta_{iv}(L_{iv})$ for the three cell lines considered—HNSCC, V79, and HSF— from the BIANCA model. In addition, the radiobiological parameters of the reference radiation α_x and β_x employed for each cell line are presented in Section 2.2.3.

Once all quantities required to compute the IsoE dose according to Eq. 2.2 have been determined— $\alpha_{iv}(L_{iv})$, $\beta_{iv}(L_{iv})$, D_T , f_{iv} , and α_x and β_x —Section 2.2.4 presents the conceptual analysis of the PhID model. This analysis investigates the model response when considering the full mixed radiation field compared with a proton-only description (Sec. 2.2.4.1), and examines the sensitivity of the PhID model to the selected cell line and reference radiation (Sec. 2.2.4.2).

In addition, Sec. 2.2.5 presents the methodology used to assess the clinical impact of calculating photon IsoE doses with the PhID model, in comparison with the conventional fixed-RBE (1.1) approach. This evaluation was performed in a

clinical-like scenario using a tumor control probability model for non-uniform, single-fraction photon doses in HNSCC, and includes an analysis of the volumetric RBE distribution.

Finally, Sec. 2.2.6 describes the methodology adopted to compare the response of the PhID model within a SOBP with phenomenological RBE models.

2.2.1 Monte Carlo simulations

As mentioned, the IsoE dose was computed for a single proton beam ($N = 1$) and a SOBP with 26 proton beams ($N = 26$). The following section describes the procedure carried out through MC simulations to obtain D_T , D_{iv} , f_{iv} , and L_{iv} in every voxel of an irradiated phantom.

2.2.1.1 Pristine Bragg peaks (PBPs)

PHITS Monte Carlo code [68] (version 3.341) was employed in this study to simulate the irradiation of an ICRU soft tissue phantom (see Table 1.2) using a monoenergetic, monodirectional proton beam with an initial energy E_{0v} and 5-mm diameter disk of uniform intensity. In the present work, a homogeneous medium with a density of 1 g/cm^3 was used as a simplified scenario to illustrate the main features of the PhID model and its sensitivity to radiobiological parameters. While clinical practice involves tissues with varying densities (e.g., bone $\approx 1.2 \text{ g/cm}^3$ or lungs $\approx 0.6 \text{ g/cm}^3$), the formalism of this model allows for a natural extension to heterogeneous media. Since the PhID model relies on accurate physical quantities (dose and LET distributions) obtained from particle transport simulations, it can be applied on a voxel-by-voxel basis regardless of the material composition, provided the corresponding α and β parameters are known.

A total of 26 different incident energies were considered, ranging from 80 MeV to 119 MeV, which resulted in the generation of $v = 1, \dots, 26$ pristine Bragg peaks. For each energy E_{0v} , simulations were performed in parallel for all six charged particle fields (^1H , ^2H , ^3H , ^3He , ^4He , e^-). Calculations were carried out on a high-performance computing cluster. By simulating $3 \cdot 10^9$ primary protons per run, a maximum relative Type A uncertainty below 5% was achieved in the central axis of the beam for all particle fields and calculated quantities (see Figure 2.1). This value represents a conservative estimate across all radiation components; for the proton dose specifically, the uncertainty remains below 1%, leading to a propagated uncertainty in the IsoE dose of less than 0.1% at clinically relevant dose levels.

The initial proton beam energies E_{0v} were determined following the procedure described in Section 2.2.1.2, based on PHITS MC simulations of monoenergetic proton irradiations in an ICRU soft tissue phantom.

Electron transport calculations were performed using the built-in EGS5 code in PHITS, following the recommended settings from [69]. As mentioned, the transport included primary protons and secondary charged particles (^2H , ^3H , ^3He , and

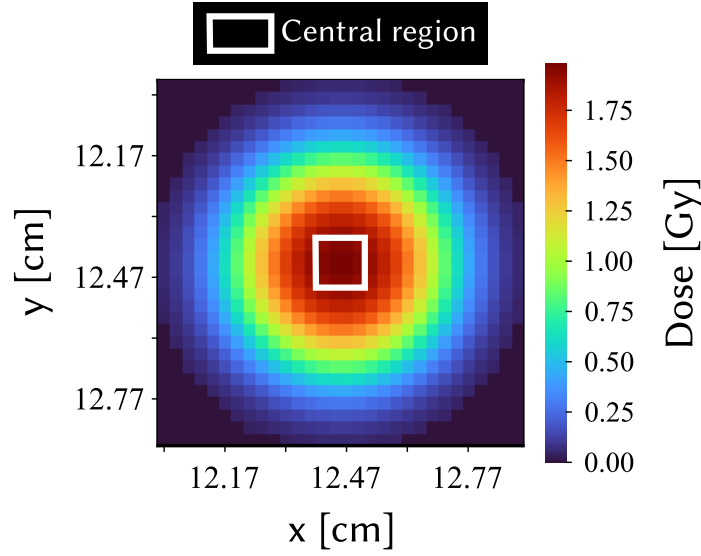


Figure 2.1: Axial section of a 110 MeV proton beam, where the central region selected for calculations is outlined (in white).

^4He), with an energy cut-off of 1 keV (default value in PHITS), while electrons were transported with a corresponding energy cut-off of 100 keV.

The stopping power of protons and other charged particles was calculated using the SPAR model as implemented in PHITS [70], whereas nuclear interactions and the production of secondary particles were simulated using the NMTC model within the same framework. Energy straggling was modeled using the Landau-Vavilov distribution, and Coulomb diffusion (angular straggling) was included according to Lynch's formula.

For each combination of beam energy ν (or μ), and particle type i (or j), two quantities recorded within scoring voxels of dimensions $0.3 \times 0.3 \times 0.2 \text{ mm}^3$ were obtained: (i) the absorbed dose D_{iv} , scored using the PHITS tally [T-deposit], and (ii) the particle fluence Φ_{iv} , determined with the tally [T-track]. The latter quantity was calculated to determine the L_{iv} value in each voxel, as will be detailed in Section 2.2.1.4.

Given D_{iv} , the total absorbed dose D_T in each voxel was calculated as:

$$D_T = \sum_{\nu=1}^N \sum_{i=1}^6 D_{iv}, \quad (2.4)$$

and the corresponding fraction as:

$$f_{iv} = \frac{D_{iv}}{D_T}. \quad (2.5)$$

2.2.1.2 Maximum dose position as a function of proton beam energy

In order to determine the initial energy $E_{0\nu}$ of a given proton pencil beam ν , associated with the position of its maximum dose, Z_{BP} (see Sec. 1.1.6 and Fig. 1.6),

the Z_{BP} values obtained from PHITS as a function of the incident proton energy were fitted using Eq. 1.3, as shown in Fig. 2.2.

Instead of $R_{CSDA}(E)$, the quantity $Z_{BP}(E_{0,v})$ was considered, yielding fitted values of $a = (0.0199 \pm 0.001) \frac{mm}{MeV}$ and $p = 1.782 \pm 0.004$, comparable to those reported in section 1.1.3.

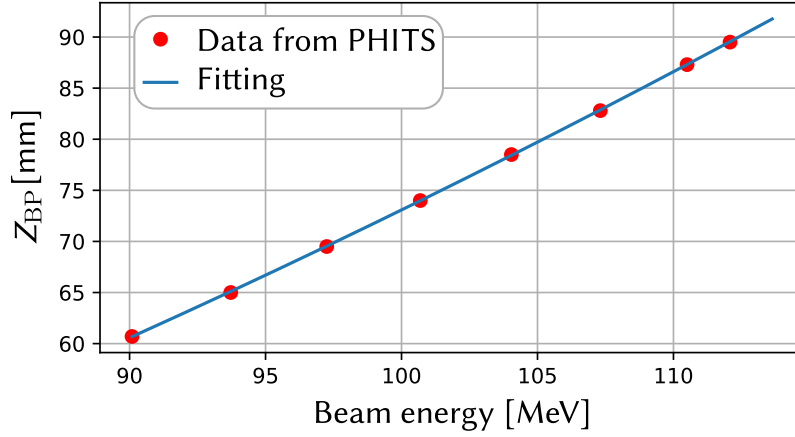


Figure 2.2: Maximum dose position, Z_{BP} , as a function of the initial energy of the proton beam.

This fitting provides the basis for determining the initial energies of the individual proton beams forming the SOBP, which constitute one of the input parameters of the PHITS Monte Carlo code.

2.2.1.3 Spread-out Bragg peak (SOBP)

Analysis of the minimum separation required between consecutive Bragg peaks

In order to obtain a quasi-uniform dose distribution, a requirement was imposed that the relative percentage difference between the maximum and minimum dose points within the SOBP region be less than 5%. One parameter influencing this percentage is the spacing between adjacent BPs. Assuming that a BP, neglecting the sub-peak region (see Sec. 1.1.6), exhibits an approximately Gaussian distribution, it is possible to estimate the separation $X_2 - X_1$, which corresponds to the separation between the central values X of each Gaussian, as illustrated in Fig. 2.3.

Considering that the Gaussians follow the form $f(x) = A \cdot e^{-\frac{(x-X)^2}{2\sigma^2}}$, where A denotes the amplitude, and defining $p = d \cdot A$ (with p representing the distance from the Gaussian maximum to the point p , parameterized by the percentage variability between peaks and valleys d), the following approximation is obtained:

$$X_2 - X_1 = 2 \cdot \sqrt{-2 \cdot \sigma^2 \cdot \ln \left(\frac{1-d}{2} \right)} \approx Z_{BP_2} - Z_{BP_1}, \quad (2.6)$$

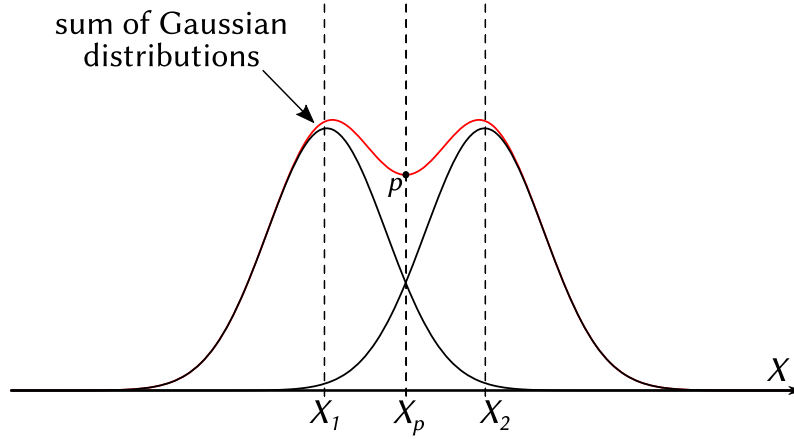


Figure 2.3: Representative diagram of the sum of two consecutive Gaussians.

where Z_{BP_1} and Z_{BP_2} are the positions of maximum dose of the consecutive BPs.

Using Eq. 2.6 and assuming a ripple value of $d = 5\%$, the separation $X_2 - X_1$ can be calculated once the value of σ of the distribution is known. Based on this, Gaussian fits were performed for several Bragg peaks in order to obtain an average value of σ , which allowed the range difference between consecutive peaks to be determined, yielding a maximum allowable separation of 2.5 mm to satisfy the established criterion.

In the literature, more conservative values for the separation between consecutive Bragg peaks are often adopted, such as $Z_{BP_2} - Z_{BP_1} = 0.8$ mm [71]. In the present work, a separation of 2.0 mm was employed for the construction of the SOBP, which remains well within the limit established by the above analysis, while reducing the associated computational cost.

Construction of the SOBP

Considering a separation of 2.0 mm between consecutive BPs and an approximate distance of 7 cm between the position Z_{BP_1} of the first BP and the position Z_{BP_N} of the highest-energy BP, with $Z_{BP_N} \approx 10$ cm, a total of 26 BPs were determined to be required to generate a SOBP with the desired range and modulation width.

Using the function $Z_{BP}(E_{0_v})$ defined in Sec. 2.2.1.2, the initial energies of the individual proton beams were then calculated by taking the corresponding Bragg peak positions Z_{BP_v} as input. Accordingly, the initial proton beam energies used to generate the SOBP were: 80.8, 82.6, 84.4, 86.1, 87.8, 89.5, 91.2, 92.8, 94.4, 96.0, 97.6, 99.2, 100.7, 102.2, 103.7, 105.2, 106.7, 108.1, 109.6, 110.9, 112.4, 113.8, 115.2, 116.5, 117.9, and 119.2 MeV.

Considering these 26 Bragg peaks, the SOBP was constructed by summing the individual PBP dose distributions, each associated with a specific weight factor w_v . These weights were initialized with different values and subsequently optimized by minimizing an objective function defined as the relative difference between the maximum dose, $D_{\max}(\text{SOBP})$, and the minimum dose, $D_{\min}(\text{SOBP})$, within

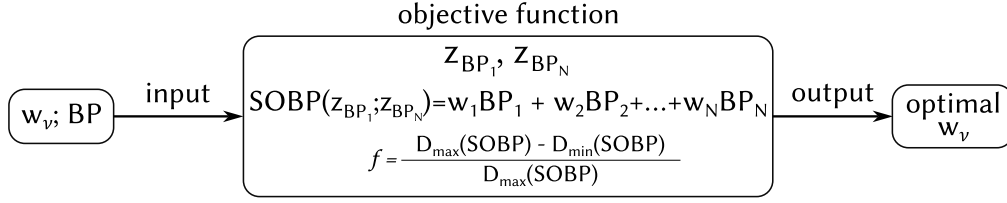


Figure 2.4: Flowchart corresponding to the optimization code for obtaining the optimal weights of the Bragg peaks.

a specified region of the SOBP (see Figure 2.4), employing the Sequential Least Squares Programming method in Python (version 3.8.5).

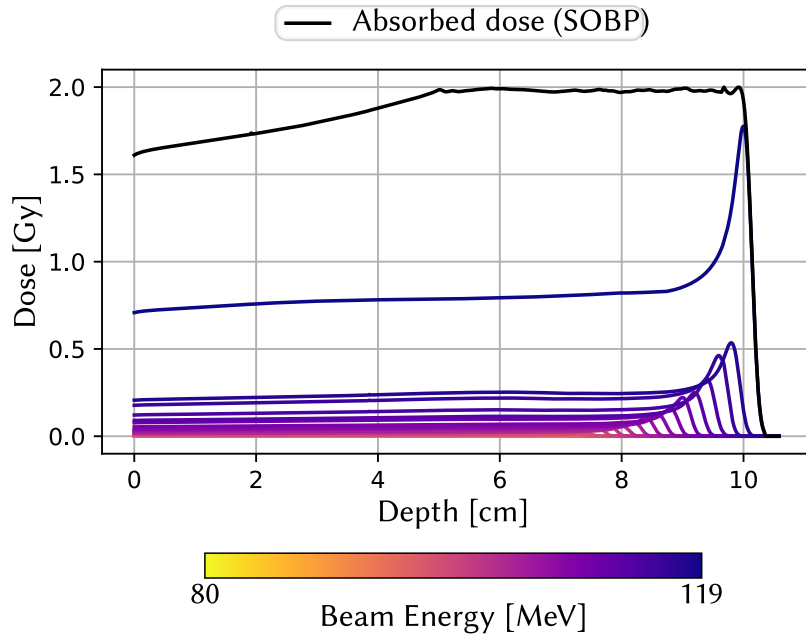


Figure 2.5: Dose profile of the spread-out Bragg peak (black curve) together with the individual PBP that compose the SOBP.

Figure 2.5 shows the resulting SOBP, with a range of 10 cm and a modulation width of 6.7 cm (see Sec. 1.1.6), obtained by combining the 26 PBP using the optimal weighting factors. This optimization ensured a dose uniformity of 98% within the SOBP region.

This absorbed dose is subsequently used throughout this work for the calculation of the photon isoeffective dose, as will be presented in the following sections.

2.2.1.4 LET and LET_D calculation

Calculation of the LET

The L_{iv} , corresponding to the track-averaged LET (L_F), was calculated according to Eq. (2.7), using the dose D_{iv} and particle fluence Φ_{iv} evaluated in each voxel. This LET derivation assumes an isotropic or parallel particle field over a sphere of

radius r . The average energy deposited per particle, $\langle \epsilon \rangle$, is obtained by multiplying the dose D_{iv} by the volume V (assuming a density of 1 g/cm^3) and dividing by the number of particles n . The corresponding LET is then computed by normalizing $\langle \epsilon \rangle$ by the mean chord length $\langle I \rangle$ of the spherical volume. For a convex body, Cauchy's formula gives $\langle I \rangle = 4V/S$, where S denotes the surface area of the sphere:

$$L_{iv} = \frac{\langle \epsilon \rangle}{\langle I \rangle} = \frac{D_{iv} \cdot V/n}{4V/S} = \frac{D_{iv}}{n/\pi \cdot r^2} = \frac{D_{iv}}{\langle \Phi_{iv} \rangle}. \quad (2.7)$$

This approach provides a practical way to obtain the average L_{iv} in each voxel of the phantom, consistently derived from the two basic tallies provided by PHITS ([T-deposit] and [T-track]), in line with the formalisms presented in standard dosimetry texts [72] and the ICRU Report 78 [73].

To verify the validity of this approach, the resulting average LET obtained from Eq. 2.7 was compared with the L_F values calculated from the $L \cdot f(L)$ spectra obtained using the [T-Let] tally at different scoring positions defined in the Monte Carlo simulations in two cases: (i) along a 119 MeV proton beam (Fig. 2.6), and (ii) within the SOBP. The 119 MeV proton beam was chosen because, as shown in Fig. 2.5, it corresponds to the beam with the highest weighting factor contributing to the SOBP.

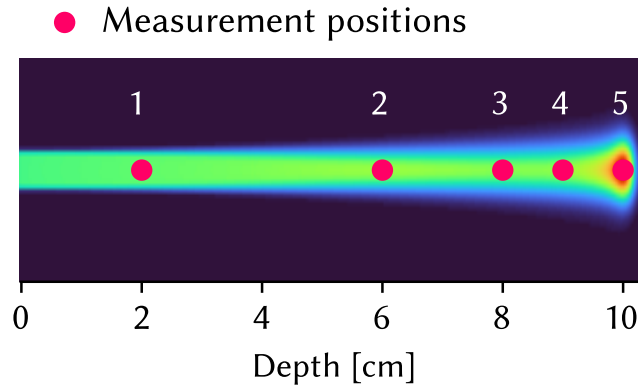


Figure 2.6: LET spectrum scoring positions along a 119 MeV proton beam.

In both cases the $L \cdot f(L)$ spectra were obtained within spherical regions located along the central axis of the proton beam. The volume of these regions was chosen to match that of the voxel ($0.3 \times 0.3 \times 0.2 \text{ mm}^3$) used for the calculation of the absorbed dose D and the particle fluence Φ , thus ensuring a consistent comparison. Figure 2.7 shows an example of an $L \cdot f(L)$ spectrum, in this case obtained at position 5 of the 119 MeV proton beam.

Once the spectra were obtained, they were integrated in order to calculate the L_F values at each position, as follows:

$$L_F = \int L \cdot f(L) dL. \quad (2.8)$$

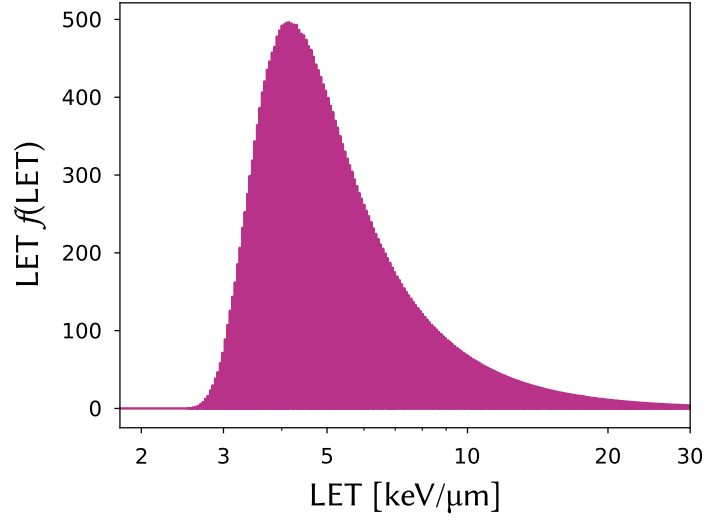


Figure 2.7: $L \cdot f(L)$ spectrum obtained at position 5 of the 119 MeV proton beam, as shown in Fig. 2.6.

This value was then compared with that obtained using the approximation given by Eq. 2.7, which, for practical purposes, will be referred to in this section as L_{approx} . Table 2.1 summarizes the values of L_F and L_{approx} , obtained at selected positions along the 119 MeV proton beam and within the SOBP.

Position/ Depth	119 MeV			SOBP		
	L_{approx} [keV/ μ m]	L_F [keV/ μ m]	Rel. Diff.* [%]	L_{approx} [keV/ μ m]	L_F [keV/ μ m]	Rel. Diff.* [%]
1 / 2 cm	0.74	0.73	1.5	0.75	0.76	1.2
2 / 6 cm	0.98	0.97	1.0	1.03	1.05	1.5
3 / 8 cm	1.31	1.30	0.6	1.49	1.52	2.0
4 / 9 cm	1.74	1.83	5.1	2.21	2.19	0.8
5 / 10 cm	5.53	5.57	0.8	5.78	6.08	5.2

Table 2.1: Comparison between L_{approx} (Eq. 2.7) and L_F derived from the $L \cdot f(L)$ spectra at different positions along the 119 MeV proton beam and the SOBP. (*) Absolute relative percentage difference with respect to L_F , defined as $\frac{|L_F - L_{approx}|}{L_F}$.

The results show that the largest discrepancy between L_F and L_{approx} , of the order of 5%, occurs near the end of the proton range in both cases. This level of disagreement is considered acceptable, particularly in view of the substantial reduction in computational time achieved by using Eq. (2.7) to estimate the LET.

A more accurate determination of LET based on the explicit calculation of $L \cdot f(L)$ spectra would require obtaining such spectra in every voxel of the phantom, which is not practical when using PHITS. Moreover, achieving statistical convergence of the LET spectra (for all the particle fields) would demand simulations

with at least 10^{10} primary histories per run, rendering this approach computationally unfeasible for routine calculations.

Calculation of the LET_D

As anticipated, the response of the model within a SOBP was compared with phenomenological RBE models (Sec. 2.2.6). Since these models rely on the dose-averaged LET, L_D , this quantity was calculated from the LET (Eq. (2.7)), following the approach described in [44]:

$$L_{D_{iv}} = \frac{\sum_{v=1}^N \sum_{i=1}^6 \langle \Phi_{iv} \rangle \cdot L_{iv}^2}{\sum_{v=1}^N \sum_{i=1}^6 \langle \Phi_{iv} \rangle \cdot L_{iv}}, \quad (2.9)$$

which can be equivalently expressed as:

$$L_{D_{iv}} = \sum_{v=1}^N \sum_{i=1}^6 L_{iv} \cdot f_{iv}. \quad (2.10)$$

2.2.2 Radiobiological parameters as a function of the LET: BIANCA model

Radiobiological parameters α and β as a function of LET were considered for three distinct cell lines: a human tumor cell line (head-and-neck squamous cell carcinoma, HNSCC), a non-cancerous human cell line (human skin fibroblast, HSF), and a normal tissue animal cell line (Chinese hamster fibroblast, V79). These LET-dependent parameters were obtained from the Biophysical ANALysis of Cell death and chromosome Aberrations (BIANCA) model [74,75]. In brief, BIANCA is a biophysical model that describes radiation-induced chromosome aberrations and cell death based on the induction and processing of DNA critical lesions. Within the PhID framework, these parameters are not merely numerical coefficients but reflect the intrinsic radiobiological properties of each cellular system. Specifically, the α and β values incorporate characteristics such as DNA repair capability, chromatin organization (DNA packaging), and cell-cycle properties. Consequently, the model accounts for the fact that the same radiation field can produce markedly different biological responses depending on the specific cell type being irradiated, reflecting the complex interplay between radiation quality and biological factors.

To implement these parameters into our calculations, the α parameter for each cell line and particle type was obtained by fitting a given functional form—summarized in Table 2.2—with the `curve_fit` function from the SciPy library in Python. Since the β parameter does not exhibit a well-defined functional dependence on LET, its value was estimated through local interpolation between β values at neighboring LET points. It should be noted that the availability of experimental datasets differs among the cell lines considered. In particular, for the V79 cell line, experimental data are available separately for ^3He and ^4He , whereas for the HNSCC and HSF lines, the available experimental data are typically grouped by atomic number ($Z = 1$ and $Z = 2$ ions).

The following section reports the set of radiobiological parameters α and β provided by the BIANCA model for each cell line. Additionally, the parameters are presented as a function of LET, along with the corresponding fits of the α parameter, as shown in Figure 2.8.

2.2.2.1 Head-and-neck squamous cell carcinoma line (HNSCC)

The α parameter as a function of LET was fitted using two separate datasets: one for ^1H and another for ^4He . Due to the lack of experimental data for hydrogen and helium isotopes, a practical approximation was adopted [76], whereby data for ^1H were used for deuterons (^2H) and tritons (^3H), and data for ^4He were used for ^3He .

For $Z=1$, the alpha parameter was fitted using a linear function of LET. For $Z=2$, a linear function was applied for $\text{LET} \leq 20 \text{ keV}/\mu\text{m}$, whereas for $\text{LET} > 20 \text{ keV}/\mu\text{m}$ a Lorentzian function was used with the natural logarithm of LET.

2.2.2.2 Chinese hamster fibroblast cell line (V79)

The α parameter as a function of LET was fitted using three datasets: one for atomic number $Z=1$ (^1H , ^2H & ^3H), another for $Z=2$ (^3He); and a third one for $Z=2$ (^4He) [74].

For $Z=1$ (^1H , ^2H & ^3H) and $Z=2$ (^3He), the alpha parameter was fitted using a quadratic function of LET. For $Z=2$ (^4He), the α parameter was fitted using the same Lorentzian function dependent on $\ln(L)$ as introduced above.

2.2.2.3 Human skin fibroblast (HSF)

The α parameter as a function of LET was fitted using two datasets: one for $Z=1$ (^1H , ^2H & ^3H) and another for $Z=2$ (^3He & ^4He). In both cases, the α parameter was modeled using a Lorentzian function dependent on $\ln(L)$. Further information on the α and β parameters database for HSF cells is available in [77–79].

Figure 2.8 illustrates the dependence of the α and β parameters on the LET for each cell line and ion type, along with the corresponding fit applied to the α parameter. The resulting fit coefficients for all cell lines and ion species are reported in Table 2.2.

2.2.3 Radiobiological parameters for the reference photon radiation

The reference radiation used for each cell line is as follows:

- HNSCC: 10 MV photons delivered by a CD Clinac irradiator, ^{60}Co gamma rays, and 250 kVp X-rays [80,81].
- V79: 250 kVp X-rays, ^{60}Co gamma rays, and 6 MV photons [12,82].

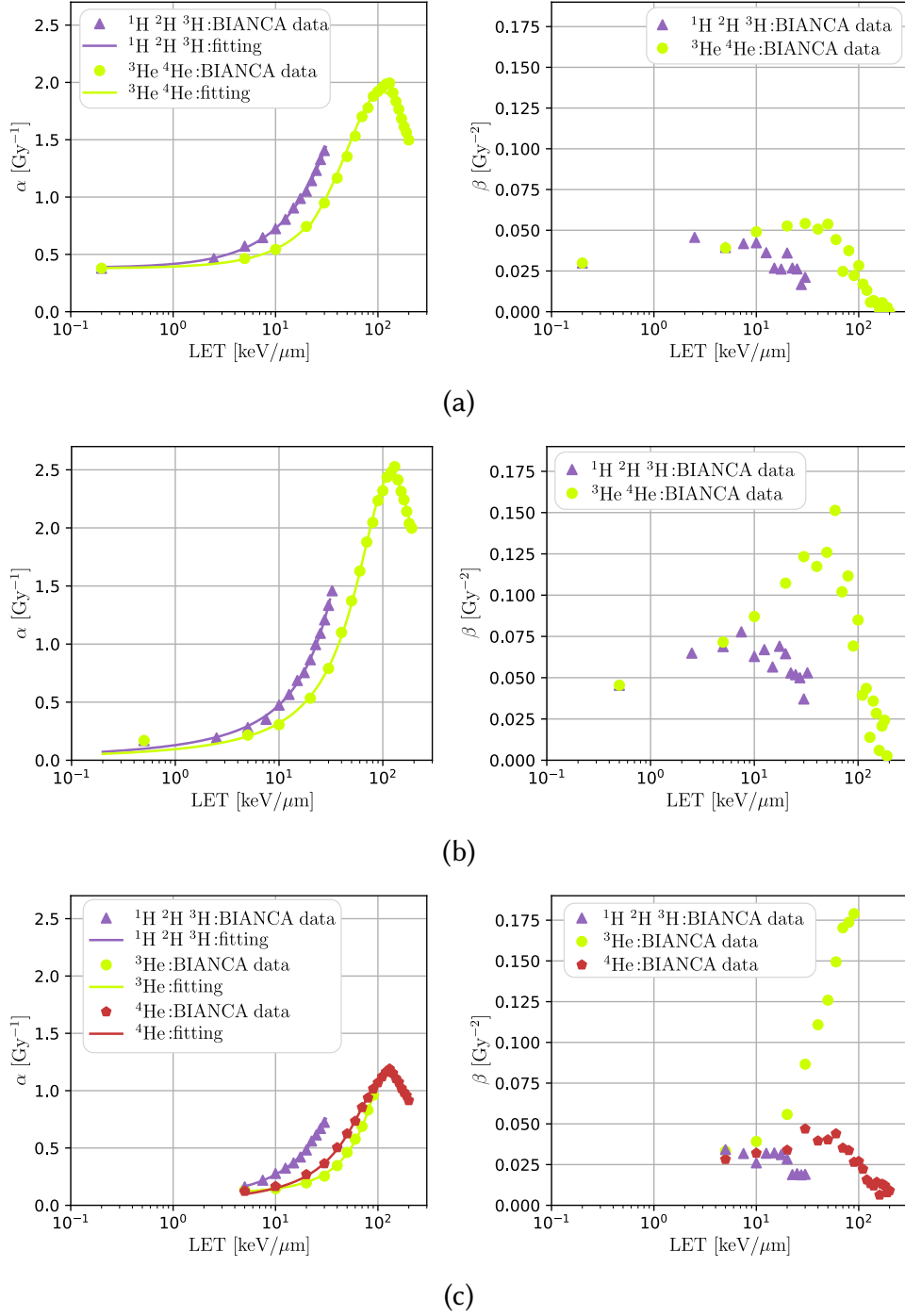


Figure 2.8: Dependence of the radiobiological parameters α and β on LET, obtained from BIANCA model and the corresponding fits for α , for (a) HNSCC (human tumor), (b) HSF (human normal), and (c) V79 (non-human normal) cell lines.

2.2. Materials & Methods

	BIANCA data	Fitting function	Fitted coefficients	R^2
HNSCC	Z=1	$\alpha(L) = a + b \cdot L$	$a = (3.85 \pm 0.05) \cdot 10^{-1}$ $b = (3.40 \pm 0.02) \cdot 10^{-2}$	0.99
	Z=2 $L \leq 20$	$\alpha(L) = a + b \cdot L$	$a = 0.37 \pm 0.01$ $b = (1.84 \pm 0.06) \cdot 10^{-2}$	0.99
	Z=2 $L > 20$	$\alpha(L) = \frac{a}{(\ln(L)-b)^2+c^2}$	$a = 3.24 \pm 0.35$ $b = 4.69 \pm 0.03$ $c = 1.30 \pm 0.07$	0.97
V79	Z=1	$\alpha(L) = a + b \cdot L + c \cdot L^2$	$a = (6.72 \pm 1.28) \cdot 10^{-2}$ $b = (1.92 \pm 0.16) \cdot 10^{-2}$ $c = (9.72 \pm 4.61) \cdot 10^{-5}$	0.99
	Z=2 (^3He)	$\alpha(L) = a + b \cdot L + c \cdot L^2$	$a = (1.02 \pm 0.12) \cdot 10^{-1}$ $b = (3.69 \pm 0.60) \cdot 10^{-3}$ $c = (6.65 \pm 0.62) \cdot 10^{-5}$	0.99
	Z=2 (^4He)	$\alpha(L) = \frac{a}{(\ln(L)-b)^2+c^2}$	$a = 1.09 \pm 0.04$ $b = 4.82 \pm 0.01$ $c = 0.98 \pm 0.02$	0.99
HSF	Z=1	$\alpha(L) = \frac{a}{(\ln(L)-b)^2+c^2}$	$a = 2.84 \pm 0.50$ $b = 4.60 \pm 0.28$ $c = -0.83 \pm 0.18$	0.99
	Z=2	$\alpha(L) = \frac{a}{(\ln(L)-b)^2+c^2}$	$a = 2.26 \pm 0.08$ $b = 4.78 \pm 0.01$ $c = 0.96 \pm 0.02$	0.99

Table 2.2: Summary of the fitted datasets for the HNSCC, HSF, and V79 cell lines, including the fitting functions employed and the corresponding fitted coefficients. Dimensional units are omitted for clarity.

- HSF: ^{60}Co gamma rays [83].

The corresponding values of the α_x and β_x parameters for each case are listed in Table 2.3.

Cell line	Reference radiation	α_x [Gy^{-1}]	β_x [Gy^{-2}]	$\left(\frac{\alpha}{\beta}\right)_x$ [Gy]
HNSCC	10 MV	0.379	0.030	12.6
	^{60}Co	0.570	0.031	18.4
	250 kVp	0.630	0.035	18.0
HSF	^{60}Co	0.170	0.045	3.8
V79	250 kVp	0.121	0.037	3.3
	^{60}Co	0.112	0.030	3.7
	6 MV	0.209	0.016	13.1

Table 2.3: Radiobiological parameters α_x and β_x corresponding to the reference radiation for each cell line.

2.2.4 Conceptual analysis of the photon isoeffective dose model

2.2.4.1 Consideration of the total charged particle field

The response of the Photon Isoeffective Dose model was studied using a PBP of 100 MeV, representing an intermediate energy within the considered range 80–119 MeV, with a maximum absorbed dose of 2 Gy. The analysis was performed considering the HNSCC line, whose radiobiological parameters are shown in Figure 2.8(a) and listed in Tables 2.2 and 2.3. As reference radiation, 10 MV photons were considered.

To assess the impact of including the full charged particle field on the isoeffective dose, calculations were performed according to Eq. (2.3) under two scenarios: (i) including all six charged particle fields, and (ii) considering only the proton field (primary and secondary without distinction). The impact was quantified by calculating the percentage relative difference between the IsoE dose obtained with only the proton field (IsoE_{PF}) and that obtained with all particle fields (IsoE_{AF}), defined as:

$$\text{RD}_{\text{IsoE}(\text{AF vs PF})} = \frac{|\text{IsoE}_{\text{AF}} - \text{IsoE}_{\text{PF}}|}{\text{IsoE}_{\text{AF}}}.$$

Furthermore, in order to study the dependence of IsoE on dose and on the L_D , the IsoE dose was calculated as a function of absorbed dose for four positions along the BP (see Fig. 2.15):

1. z_1 : at the beam entrance or plateau.
2. z_2 : at the sub-peak.
3. z_3 : at the maximum dose position.
4. z_4 : at the distal fall-off where the dose is equal to 80% of the maximum dose.

For each position, the IsoE was calculated considering all particle fields (IsoE_{AF}) and only the proton field (IsoE_{PF}). The relative difference between these two cases was then evaluated. In addition, the relative difference between each IsoE and the dose weighted with a fixed $\text{RBE} = 1.1$ was calculated as follows:

$$\text{RD}_{\text{RBE}=1.1 \text{ vs IsoE}} = \frac{|\text{dose}_{\text{RBE}=1.1} - \text{IsoE}|}{\text{dose}_{\text{RBE}=1.1}}.$$

2.2.4.2 Photon isoeffective dose sensitivity to cell line radiobiological parameters

The sensitivity of the photon isoeffective dose was investigated under two scenarios considering the SOBP described in Section 2.2.1.3:

- **Case 1 – Varying cell lines:** Three cell lines (HNSCC, HSF, and V79) were analyzed using the same reference radiation (^{60}Co gamma rays).
- **Case 2 – Varying reference radiation:** The HNSCC cell line was analyzed under three different reference radiations: 10 MV photons, ^{60}Co gamma rays, and 250 kVp X-rays.

In both cases, the RBE at 10% survival ($\text{RBE}_{10} = (D_x/D_T)|_{10\%}$) was calculated as a function of depth.

2.2.5 Tumor control probability model for HNSCC: proof-of-concept assessment

A tumor control probability (TCP) model for non-uniform, single-fraction photon doses was employed to assess the clinical impact of the PhID model compared to the fixed RBE of 1.1 approach [27]. This model was constructed from published clinical data of 96 patients with recurrent HNSCC treated with hypofractionated stereotactic body radiotherapy [26]. Briefly, the TCP for a single-fraction uniform photon dose is described by a Poisson-based distribution: $\text{TCP} = \exp(-c_1 V c_2 S)$, where V is the tumor volume (in cm^3), S is the cell survival fraction, and c_1, c_2 are parameters modulating the volume effect on local control. To account for the non-uniform dose distributions, the model was extended using the equivalent subvolume formalism [84], such that the total TCP for a tumor T of volume V is given by:

$$\text{TCP} = \exp \left[-c_1 V c_2 \left(\frac{1}{V} \int_T S(D(x))^{1/c_2} dx \right)^{c_2} \right], \quad (2.11)$$

where $S(D(x))$ is the cell survival fraction at point x for a dose D . The model parameters ($c_1 = 2.4$, $c_2 = 0.12$, and $\alpha = 0.022 \text{ Gy}^{-1}$) were obtained by fitting the clinical data after converting the original fractionated regimes to single-fraction equivalent doses using the linear-quadratic formalism with $\alpha/\beta = 10 \text{ Gy}$. While a simplified phantom was used for this proof-of-concept, it provides a controlled environment where observed differences are strictly attributable to the biophysical models, avoiding the uncertainties of specific patient anatomies. In this context, the TCP is employed as a comparative radiobiological figure of merit rather than a predictive clinical tool, providing a controlled environment where observed differences are strictly attributable to the biophysical models. In addition, the volumetric distribution of RBE within the irradiated region was analyzed and compared with those observed in the previous sections.

2.2.5.1 Multi-Field Optimization in a soft-tissue phantom:

The TCP was assessed in a clinical-like scenario involving the irradiation of a soft-tissue parallelepiped phantom of approximately 40 cm^3 using two opposed proton fields composed of 10 energies each (ranging between 106 and 119 MeV). Within

this volume, a spot-scanning configuration was created by subdividing the target into 20 isoenergy layers. Laterally, the spot pattern alternated between 6×6 and 5×5 square grids, with the 5×5 grids geometrically centered between adjacent 6×6 grids so that each spot lies midway between four spots of the neighboring layer, forming a staggered configuration commonly referred to as an orange-crate pattern. This configuration generates a three-dimensional voxelized structure that ensures sufficient lateral overlap across layers. In total, 610 spots were included. However, because of the symmetry of the irradiation arrangement, several spots shared the same weight, resulting in a total of 60 independent spot weights that required optimization.

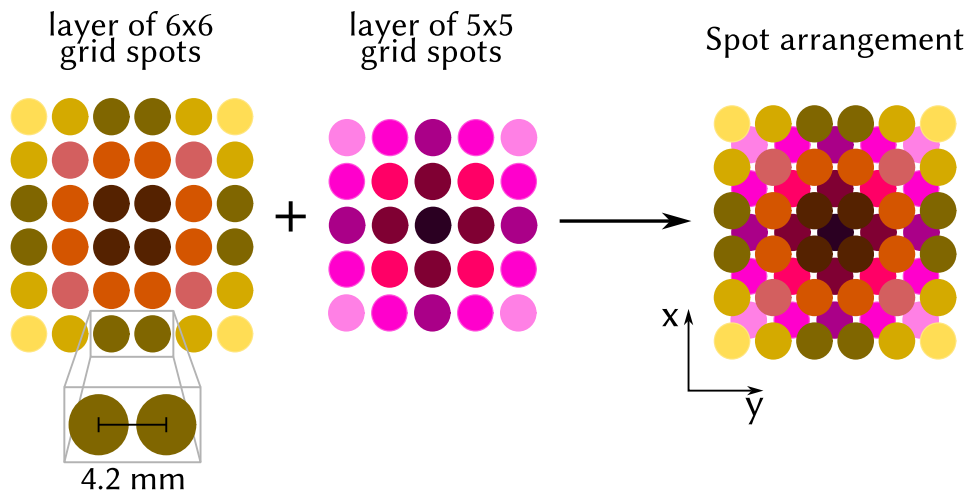


Figure 2.9: Schematic representation of the axial-plane spot arrangement used for phantom irradiation.

Figure 2.9 schematically illustrates the axial-plane spot arrangement used for phantom irradiation. Spots sharing the same color correspond to identical weighting factors, reflecting the symmetry of the arrangement. Owing to this symmetry, only six independent spot weights need to be optimized per energy layer. As also shown in the figure, the center-to-center distance between adjacent spots is 4.2 mm, a value determined through an analysis analogous to that described in Sec. 2.2.1.3.

After defining this spatial layout, a Multi-Field Optimization (MFO) approach was applied to simultaneously adjust all spot weights. The optimization was carried out in MATLAB® [85] using the *fmincon* routine, minimizing an objective function defined as the difference between the maximum and minimum dose within a selected central region of the phantom-corresponding to a sub-volume of approximately 8 cm³. Figure 2.10 shows the dose distribution resulting from the MFO in axial and sagittal slices of the phantom, together with the corresponding dose profiles.

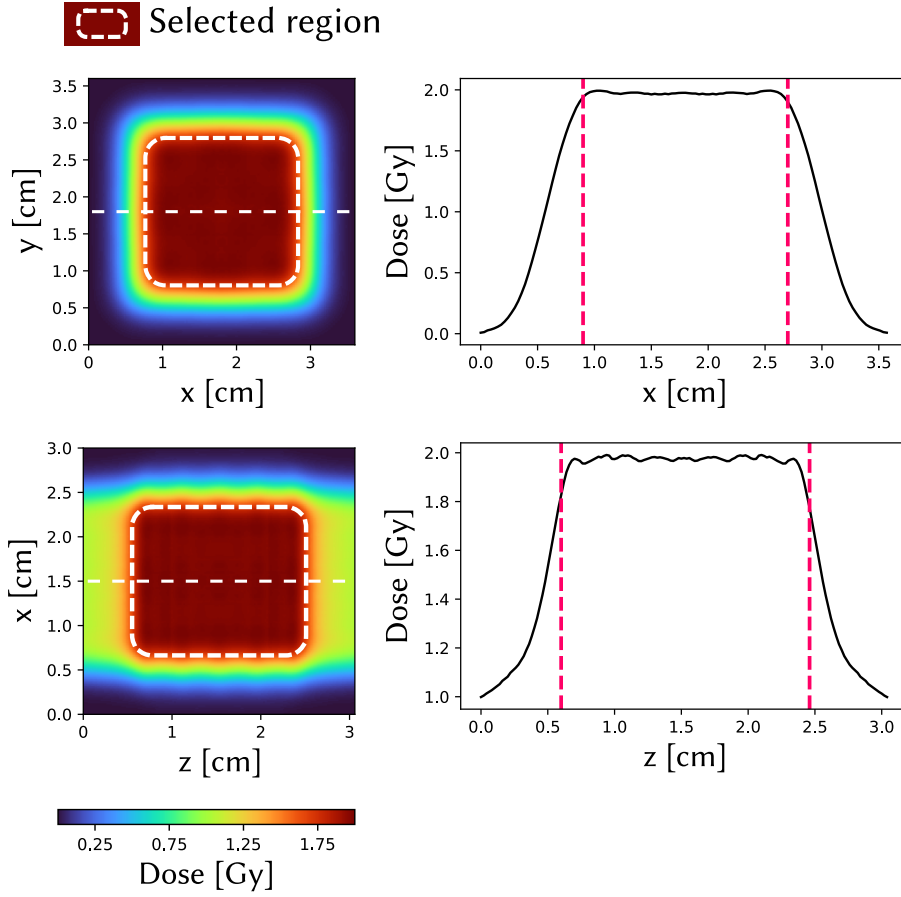


Figure 2.10: Axial and sagittal sections showing the dose distribution obtained from the MFO. The dashed lines indicate the region where a 90% dose uniformity is achieved, corresponding to a volume of 8 cm^3 . This criterion, less restrictive than the $\pm 5\%$ uniformity condition adopted earlier in this work, is used here to reflect more clinically realistic conditions. The associated dose profiles are shown on the right.

2.2.5.2 Photon isoeffective dose calculation

From the MFO result, the IsoE_{AF} dose was computed in each phantom voxel using Eq. 2.2 considering the mixed charge particle field and accounting for the number of spots contributing to that voxel. The value of N corresponds to the number of spots whose dose contribution f_{iv} is non-zero at that location, and therefore varies across the phantom, ranging from approximately 320 in high-overlap regions to about 10 in peripheral areas (see figure 2.11).

It should be noted that this maximum number of elements, $N = 320$, increases when secondary particles are included, as it must be multiplied by a factor of 6, corresponding to the total number of particle fields considered.

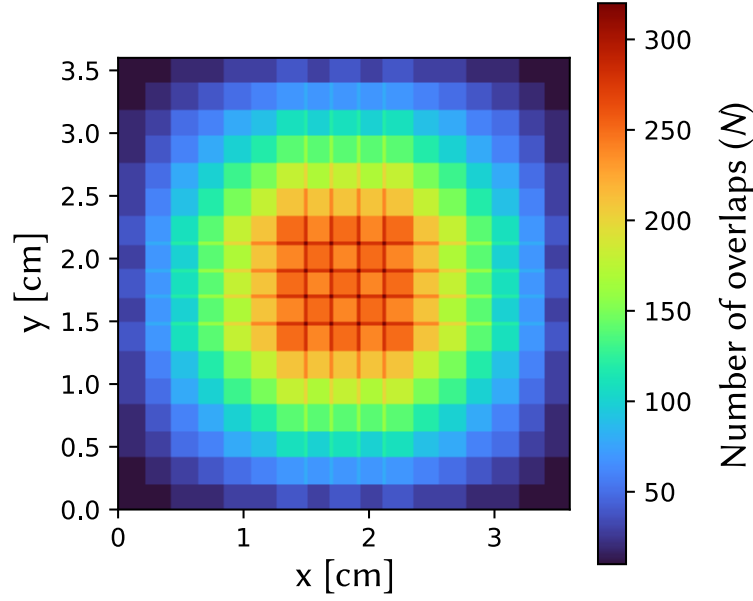


Figure 2.11: Axial slice of the phantom showing the spatial overlap of spots contributing to the irradiation field.

2.2.5.3 TCP calculation for HNSCC

For the TCP calculation, the treatment was assumed to correspond to a standard fractionation scheme of a total absorbed dose of 70 Gy delivered in 35 fractions of 2 Gy each. The fractionated doses were first converted to single-fraction equivalent doses, D_{SF} , using the linear-quadratic formalism (eq. 2.12) with an α/β ratio of 10 Gy, a value commonly adopted for HNSCC [27, 86, 87]. This equivalent dose was calculated as follows:

$$D_{SF} = \frac{1}{2} \cdot \left(-\frac{\alpha}{\beta} + \sqrt{\left(\frac{\alpha}{\beta}\right)^2 + 4 \cdot G \cdot \left(\frac{\alpha}{\beta}\right) \cdot D \cdot \left(1 + \frac{d}{\alpha/\beta}\right)} \right), \quad (2.12)$$

where $D = 70$ Gy, d is the maximum dose of the calculated isoeffective dose distribution, and $G \approx 1$.

The gross tumor volume (GTV) was defined to coincide with the previously described 8 cm^3 subvolume of the phantom (see Fig 2.10). Once the GTV was defined, TCPs were calculated for both dose distributions under comparison: the IsoE-based dose distribution and the proton absorbed dose weighted by a fixed RBE of 1.1. The TCP values were computed according to the model given by Eq. 2.11, which describes the probability of tumor control for a non-uniform dose distributions within the tumor T [27].

While a simplified phantom was used for this proof-of-concept, it provides a controlled environment where observed differences are strictly this context, the TCP is employed as a comparative radiobiological figure of merit rather than a predictive clinical tool.

2.2.6 Model comparison

The PhID model was compared against phenomenological RBE models: those proposed by Wilkens and Oelfke (WIL) [8], Tilly *et al.* (TIL) [11], Chen and Ahmad (CHE) [17], Carabe *et al.* (CAR) [18], Wedenberg (WED) [19], Jones (JON) [20], McNamara (MCN) [21], Rorvik *et al.* (RORU) [22] and Peeler (PLR) [23]. These models are based on parameterizations of the LQ model [16], where the calculation of the RBE depends on the proton dose D_p , the $(\alpha/\beta)_x$ ratio of the reference radiation, and on the model-specific terms RBE_{min} and RBE_{max} (see Eq. 1.13).

All the considered models, through the RBE_{min} and RBE_{max} terms, depend on the L_D . These terms and their dependencies are presented in detail in Section 1.2.4.2 and Appendix A.

It should be noted that while these phenomenological models were originally parameterized as a function of LET_d , the PhID model is intrinsically based on the LET of each radiation component in the voxel, following the BIANCA model formalism. To ensure a consistent comparison, each model was implemented using its original physical quantities: LET_d for the literature models and voxel-by-voxel LET for the PhID model.

Although the Photon Isoeffective Dose model inherently accounts for the total dose from the mixed charged-particle field, D_T , the other models do not explicitly account for the individual components of the mixed charged-particle field. Consequently, to ensure a fair and consistent comparison, we restricted the isoeffective dose calculation to the proton dose, D_p . The comparison was carried out for the HNSCC cell line, using 10 MV photons as the reference radiation.

In order to investigate the dependence of the models on the absorbed dose, RBE curves as a function of dose were calculated for three distinct L_D values, corresponding to the following positions (Fig. 2.15):

- the entrance of the SOBP,
- the depth of maximum dose at the most distal Bragg peak composing the SOBP,
- the position of maximum LET_D .

2.3 Results

2.3.1 Characterization of the mixed charged particle field

From the Monte Carlo simulations described in Section 2.2.1, the doses D_{iv} , dose fractions f_{iv} , and L_{iv} were obtained in each voxel of the irradiated phantom for each charged particle, i , of the mixed field (1H , 2H , 3H , 3He , 4He and e^-) and for each of the 26 considered energies E_{0v} .

Using the dose contributions D_{iv} and the total dose D_T (Eq. 2.4), the corresponding values of f_{iv} were then calculated in each voxel according to Eq. 2.5.

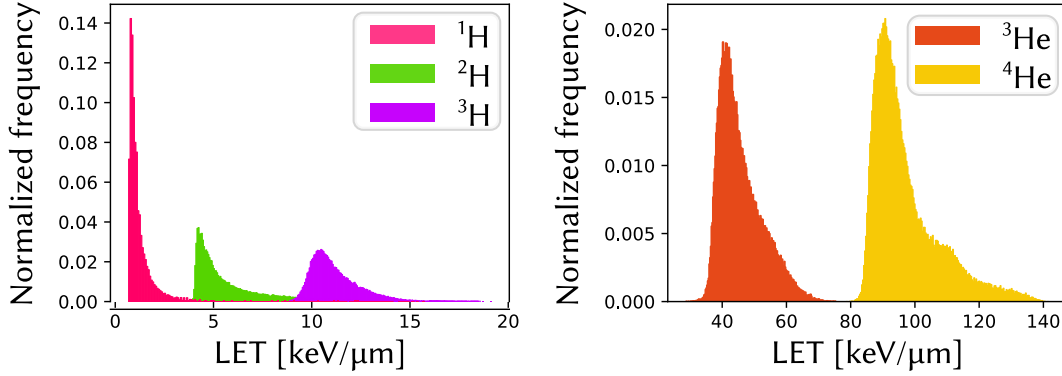


Figure 2.12: LET distributions for the different charged-particle fields considered, including contributions from all simulated energies. Electron contributions are not shown.

Figure 2.12 presents the LET distributions corresponding to the different charged-particle fields, with the exception of electrons, which are not shown. The reported distributions include LET values from all considered energies.

In this work, LET is not considered for electrons, as it is not well defined due to their highly scattered transport and energy-loss fluctuations. Accordingly, electrons are treated like the reference radiation, and the corresponding α and β parameters are assumed equal to those of the reference field.

The proton LET spans from approximately 1 keV/ μm up to about 15 keV/ μm . For the remaining particle species, the LET ranges extend from 4 to 15 keV/ μm for ^2H , from 8 to 20 keV/ μm for ^3H , from 30 to 135 keV/ μm for ^3He , and from 80 to 140 keV/ μm for ^4He .

Figures 2.13 and 2.14 show the α parameter as a function of LET for the HN-SCC cell line, derived for each particle type from fits to the BIANCA model data (see Table 2.2). While Figure 2.12 only reports the LET range and frequency for each particle type i , it does not reflect the actual impact of different LET values on the IsoE dose calculation. This impact can be captured by the dose contributions associated with $\alpha(L)$, which are shown in Figures 2.13 and 2.14 through the corresponding f_{iv} factors for all considered energies.

As can be observed, in all cases, the α parameters obtained from fits to the BIANCA model data lie within the range covered by the original model outputs.

As shown in Fig. 2.13, top left panel, the relative dose contribution factor f_v initially increases with LET, reaches a maximum, and then decreases for protons. This behavior is expected, since higher doses delivered by individual Bragg peaks are intrinsically associated with higher LET values, leading to larger f_v contributions at increasing LET.

For the secondary particle fields, an increase in LET is instead accompanied by a gradual reduction in f_{iv} . This trend is particularly pronounced for ^2H , where f_{iv} decreases by approximately one order of magnitude as LET increases from about 4 keV/ μm to 13 keV/ μm . In addition, these secondary particle fields exhibit a higher density of contributions at lower LET values within their respective LET

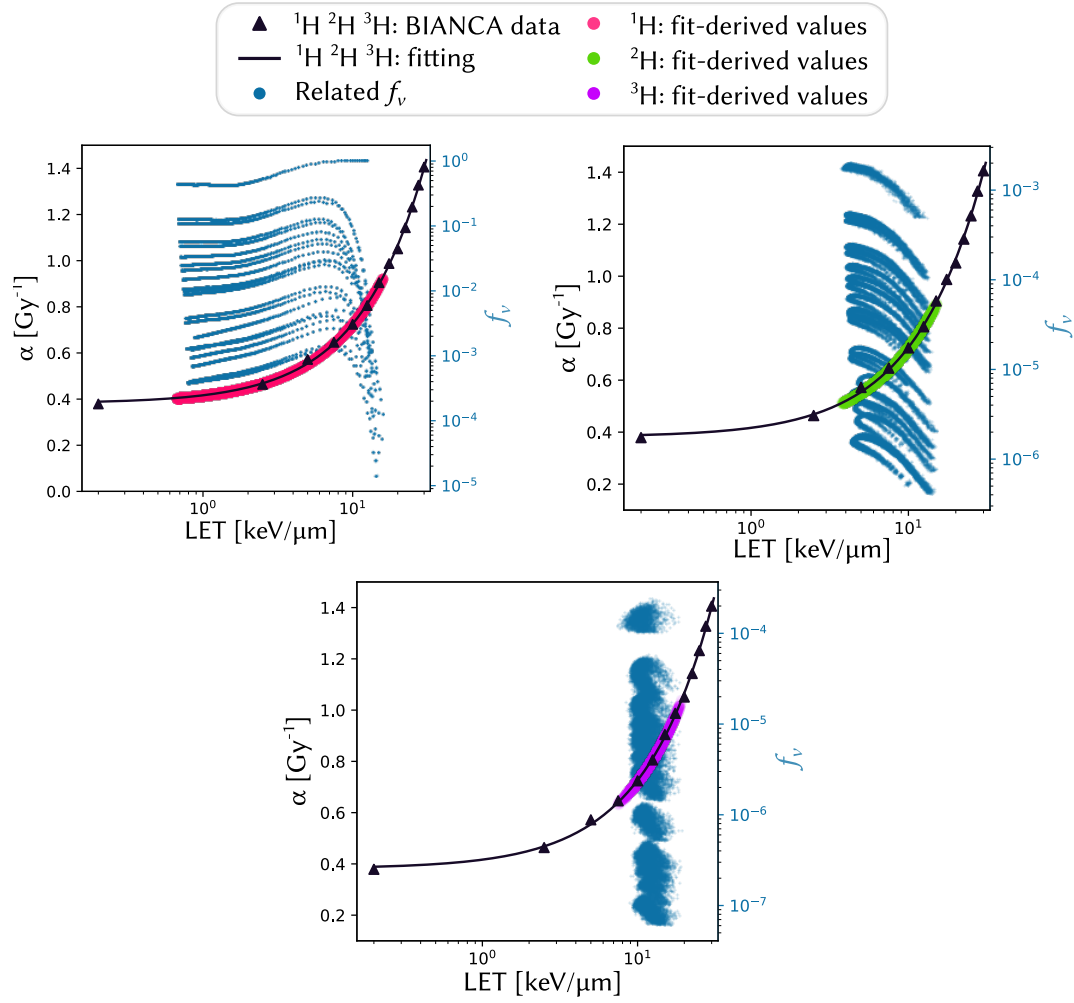


Figure 2.13: Dependence of the α parameter on LET for ^1H , ^2H , and ^3H , including the corresponding dose contribution factors f_v for all energies considered.

ranges. This behavior can be clearly observed when zooming into the distributions, as shown in Fig. 2.14.

2.3.2 Conceptual analysis of the photon isoeffective dose model

2.3.2.1 Impact of considering the total charged particle field

Figure 2.15(a) shows the depth-dose profiles for the isoeffective dose (IsoE) calculated by considering either all particle fields (IsoE_{AF}) or only the proton field (IsoE_{PF}), together with the absorbed dose and the dose weighted by a fixed RBE = 1.1. Both IsoE dose curves deviate progressively from the absorbed dose with increasing depth. In the proximal region (z_1), smaller deviations are observed, with the IsoE_{AF} and IsoE_{PF} doses exceeding the absorbed proton dose by 11% and 9%, respectively. At the maximum dose position (z_3), both IsoE doses reach ap-

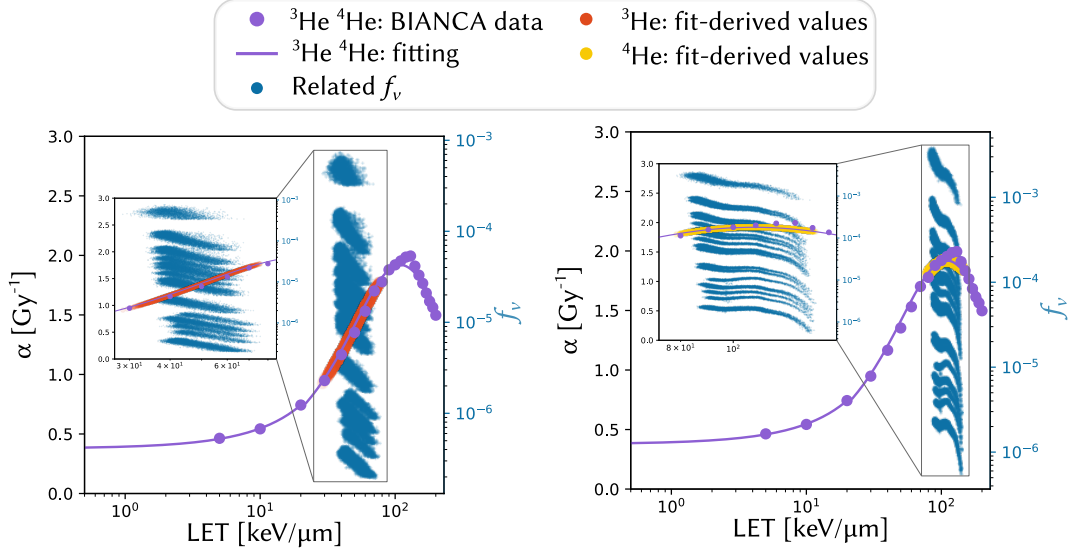


Figure 2.14: Dependence of the α parameter on LET for ^3He and ^4He , including the corresponding dose contribution factors f_v for all energies considered.

proximately 3 Gy — about 50% higher than the corresponding absorbed dose of 2 Gy and 35% higher than the RBE-weighted dose. In the distal region (z_4) both IsoE doses show an even larger deviation, being about 50% higher than the RBE-weighted dose.

These differences with respect to the clinically adopted $\text{RBE} = 1.1$ dose are noteworthy, as they highlight potential regions of dosimetric inaccuracy, particularly in the distal fall edge of the Bragg peak where tumor–normal tissue conflicts may arise.

When directly comparing the IsoE_{PF} to the IsoE_{AF} , the average relative difference is about 2.5% in the first centimeter of depth (Fig. 2.15(b)), gradually decreasing to negligible values at greater depths.

To better illustrate these effects at specific locations within the PBP, Figure 2.15(c) presents the IsoE_{AF} , IsoE_{PF} , and $\text{RBE} = 1.1$ doses as a function of the absorbed dose at four selected positions (z_1 , z_2 , z_3 , and z_4).

The figure also presents the relative difference between IsoE_{PF} and IsoE_{AF} ($\text{RD}_{\text{IsoE (AF vs PF)}}$), as well as the relative differences of each IsoE dose with respect to the $\text{RBE} = 1.1$ dose ($\text{RD}_{\text{RBE}=1.1 \text{ vs IsoE AF}}$ and $\text{RD}_{\text{RBE}=1.1 \text{ vs IsoE PF}}$).

At z_1 , both IsoE curves are close to the RBE-weighted dose, with differences below 2% at 2 Gy and around 2–3% at 30 Gy. However, at z_2 , z_3 , and z_4 , both IsoE doses exceed the $\text{RBE} = 1.1$ dose for all absorbed dose values, with discrepancies increasing progressively with depth. At 2 Gy, the relative differences ($\text{RD}_{\text{RBE}=1.1 \text{ vs IsoE}}$) reach 14%, 33%, and 46% at z_2 , z_3 , and z_4 , respectively, while at 30 Gy the deviations decrease to 11–16%.

These findings confirm that radiation quality — expressed by the dose-averaged LET, which for the total proton field increases from approximately 1 to 9 keV/ μm between z_1 and z_4 — plays a crucial role in determining the agreement (or lack

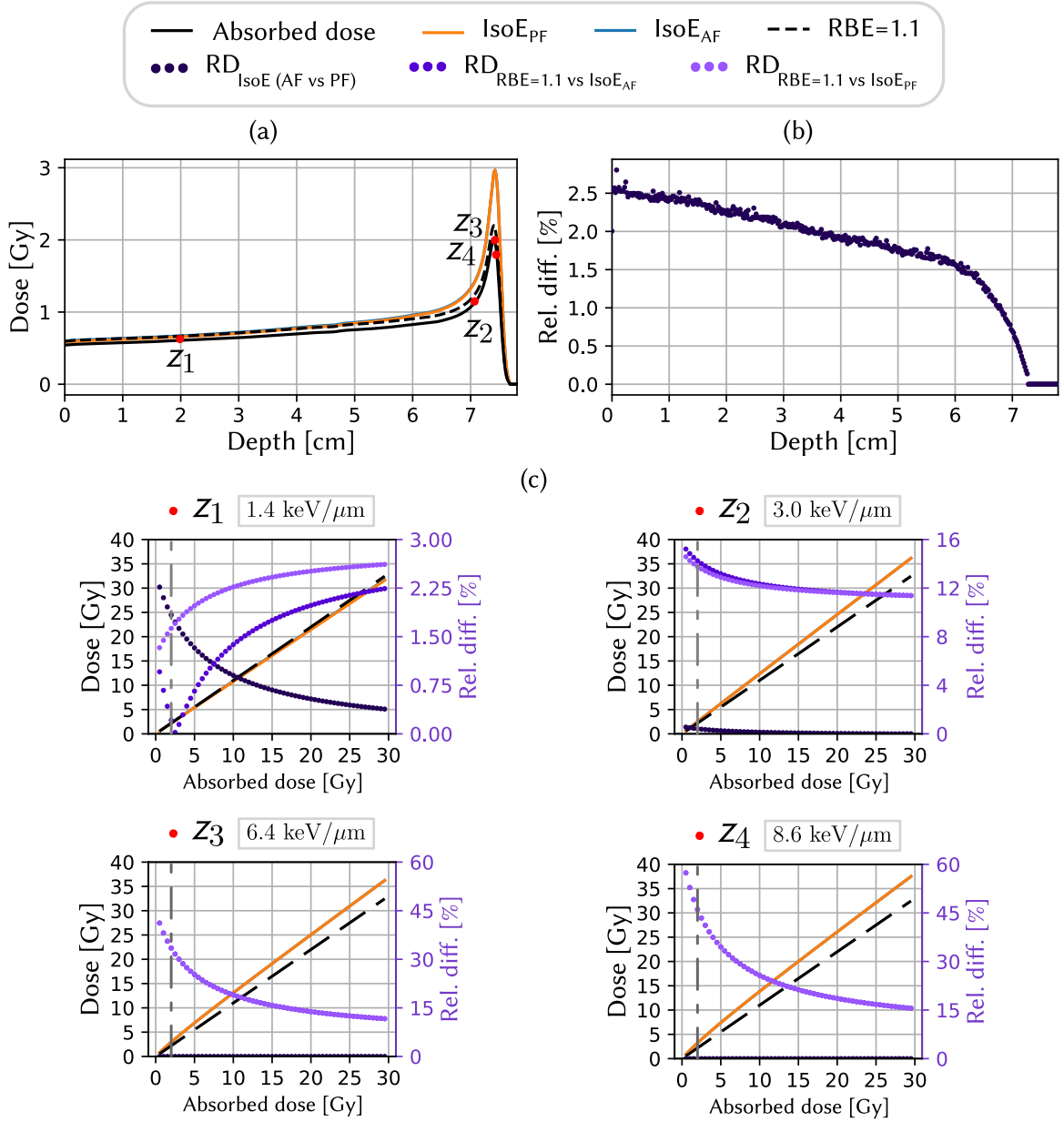


Figure 2.15: Isoeffective dose for a 100 MeV proton beam. (a) Depth-dose curves for the absorbed dose (solid black), $\text{RBE} = 1.1$ dose (dashed black), and isoeffective doses IsoE_{AF} (light blue) and IsoE_{PF} (orange). (b) Relative difference between IsoE_{AF} and IsoE_{PF} versus depth. (c) Dose curves versus the absorbed dose for positions z_1 , z_2 , z_3 , and z_4 , selected at different L_D values, showing the $\text{RBE} = 1.1$ dose (dotted black), IsoE_{PF} (orange), and IsoE_{AF} (light blue; not visible due to overlap with the orange curve). The secondary y-axis displays the relative differences between each IsoE and the $\text{RBE} = 1.1$ dose, as well as between the two IsoE doses. The vertical gray dashed-dotted line indicates the 2 Gy absorbed dose.

thereof) between different isoeffective dose models and the fixed RBE approach.

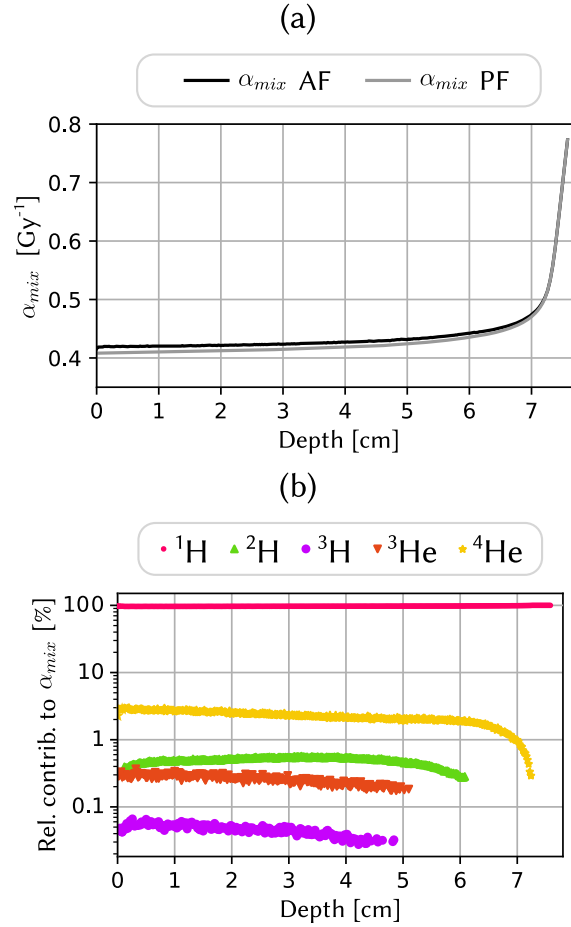


Figure 2.16: (a) Depth profiles of the α_{mix} parameter for a 100 MeV proton beam, calculated either considering all particle fields (AF) or only the proton field (PF). (b) Relative contribution of each charged particle field to the α_{mix} parameter as a function of depth.

The increase in the IsoE doses shown in Figure 2.15(a) is associated with a rise in LET of the mixed charge particle field, which in turn leads to an increase in the α parameter resulting in a higher IsoE dose. As shown in Fig. 2.16(a), the depth-dependent values of α_{mix} (defined as $\alpha_{mix} = \sum_{i=1}^s f_i \alpha_i$ with $s = 6$ for AF) are higher in the proximal region, but converge to proton-only values (α_{mix} with $s = 1$ for PF) at greater depth. This behavior is consistent with the LET ranges of the secondary charged particles described in Sec 2.3.1. These values highlight the greater contribution of high-LET components near the beam entrance, which diminishes with increasing depth, explaining why differences between IsoE_{AF} and IsoE_{PF} become negligible at larger depths.

The proximal region corresponds to where most secondary charged particles are produced as a result of nuclear reactions between primary protons and light nuclei such as ^{16}O , ^{12}C , and ^{14}N [88]. These reactions require a minimum thresh-

old energy to occur, which varies depending on the type of emitted particle (see Table 1.3). As protons slow down with increasing depth, fewer of them exceed these thresholds, resulting in reduced production of secondary particles. Consequently, the relative difference between the two IsoE doses decreases with depth as the proton field becomes dominant. This is illustrated by the relative contribution of each charged-particle field to α_{mix} as a function of depth (Fig. 2.16(b)). With increasing depth, the contribution of charged particles other than protons decreases, with alpha particles being the second largest contributors, at about 3%.

This analysis clearly shows that the isoeffective dose depends on both the absorbed dose and the depth in the irradiated tissue. The depth dependence reflects that, at each position, the mixed radiation field has a different quality, which in turn affects the LET and the radiobiological parameters calculated using the BIANCA model. In this specific case, the differences between IsoE doses calculated considering all six charged-particle fields or only the proton field are negligible. Moreover, the isoeffective doses would not differ significantly from those calculated considering only protons and alpha particles, rather than all six particle fields. This suggests that, for simplified modeling beyond the pure proton field, including alpha particles provides the most meaningful improvement in accuracy.

The contribution of the alpha-particle field has been investigated in previous studies [24, 89], where its relevance arises from its higher LET compared to protons (see Fig. 2.12), which leads to increased biological damage and, consequently, a higher associated RBE. This contribution enhances the effective RBE of proton beams, particularly in the entrance region, an effect also observed in other works [90, 91]. Moreover, even at very low fluences, alpha particles can induce disproportionately high biological effects. According to studies investigating the mutagenic potential of alpha particles in normal mammalian cells [92], when only about 10% of the cells in a culture were directly traversed by a single alpha particle, the resulting mutation yield was nearly identical to that observed when all cells were irradiated. This bystander response, mediated by intercellular communication, highlights that even a small dose contribution from alpha particles may significantly influence the overall biological outcome. These findings also suggest that regions of normal tissue exposed to very low doses of high-LET particles could, over time, be susceptible to the development of radiation-induced secondary tumors. This issue is particularly relevant for young patients, such as those in pediatric proton therapy, where although the fraction of secondary particles is small from a dosimetric standpoint, their carcinogenic potential cannot be neglected.

2.3.2.2 Photon isoeffective dose sensitivity to cell line radiobiological parameters

Case 1 - Varying cell lines: Figure 2.17(a) shows the depth profiles of the absorbed dose (solid black), the dose weighted with $RBE = 1.1$ (dashed black), and the IsoE doses corresponding to the HSF, V79, and HNSCC cell lines, calculated using ^{60}Co gamma rays as reference radiation. The observed dependence of the

isoeffective dose on the cell line reflects the intrinsic differences in cellular radiosensitivity discussed in Sec. 2.2.2. As previously mentioned, these variations arise from the cell-specific α and β parameters, which encapsulate biological factors such as DNA repair efficiency and chromatin organization. The results presented here highlight the well-known interplay between radiation quality (LET) and the intrinsic biological characteristics of the irradiated tissue, where the cellular milieu can be as decisive as the radiation type in determining the final RBE.

The figure also presents the L_D depth profile. As observed, the IsoE dose depends on the specific cell line and, for all the ones considered, increases with depth, reaching its maximum at the most distal Bragg peak edge of the SOBP. This rise highlights a loss of uniformity in the isoeffective doses, in contrast to the RBE-weighted dose, which remains uniform within the target region. This increase in the IsoE dose, as described in section 2.3.2, is explained by the gradual rise in L_D . This rise leads to a corresponding increase in the α values with LET, according to the data from BIANCA, while the β values remain relatively constant in that range. L_D reaches its maximum value of 12.2 keV/ μ m when the absorbed dose decreases to 1% of its peak. The three isoeffective doses attain their maximum at an L_D value of 6 keV/ μ m.

Taking IsoE_{HSF} as an example, the fact that the isoeffective dose can be either lower or higher than the total absorbed dose of the mixed radiation field can be explained by comparing the α_x and β_x parameters of ^{60}Co gamma rays (see Table 2.3) with the radiobiological parameters reported in Fig. 2.8(b). Two specific examples illustrate this point:

- at a depth of 4 cm, where $L_D = 1.1$ keV/ μ m, the parameters α_{mix} and β_{mix} , defined as $\beta_{mix} = \sum_{i,j=1}^s \sqrt{\beta_i \beta_j} f_i f_j$, $s = 6$ for AF, are approximately 0.129 Gy $^{-1}$ and 0.048 Gy $^{-2}$, respectively. Compared with the reference radiation (^{60}Co : $\alpha_x=0.170$ Gy $^{-1}$ & $\beta_x=0.045$ Gy $^{-2}$), it can be concluded that, for the same level of effect, the photon dose required is lower than the total absorbed dose of the mixed field, which explains why IsoE_{HSF} is lower than the absorbed dose at this depth.
- at a depth of 9.5 cm, where $L_D = 3.6$ keV/ μ m, the α_{mix} and β_{mix} values are approximately 0.239 Gy $^{-1}$ and 0.066 Gy $^{-2}$, respectively. Following the same reasoning as above, and compared with the reference radiation, IsoE_{HSF} dose is greater than the total absorbed dose for the same level of effect.

The same reasoning can be applied to predict or explain the behavior of isoeffective doses calculated in different scenarios, i.e., for different cell lines and reference radiations.

Figure 2.17(b) shows the RBE₁₀ as a function of depth for each cell line. As observed, in certain regions the RBE₁₀ values fall below the clinically adopted constant value of 1.1, reaching minimum values of 0.8, 1.0, and 0.7 for HSF, V79, and HNSCC, respectively, and maximum values of 1.6, 1.5, and 1.3. In all three cases, the maximum RBE₁₀ occurs in the distal fall-off region, coinciding with the

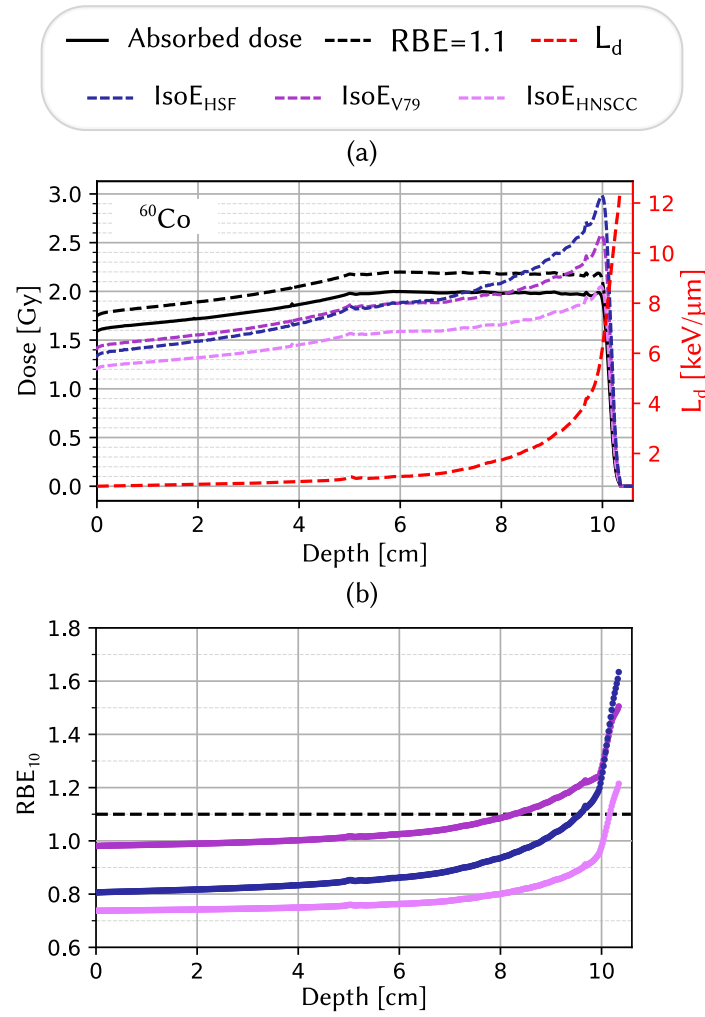


Figure 2.17: Dependence of the isoeffective dose on the cell line, considering ^{60}Co gamma rays as the reference radiation. (a) Depth–dose profiles of the absorbed dose (solid black), the dose calculated with a fixed RBE = 1.1 (dashed black), and the IsoE dose for the HNSCC, HSF, and V79 cell lines. The depth– L_D profile is also shown (red dashed). (b) RBE₁₀ profiles as a function of depth.

maximum L_D , about 3 mm beyond the SOBP. RBE₁₀ values below 1 indicate that the radiation field due to protons are less effective than ^{60}Co gamma rays. Thus, to achieve the same biological effect (10% survival), a higher proton dose is needed compared to ^{60}Co gamma rays. Consequently, adopting a fixed RBE of 1.1 may either underestimate or overestimate the photon-equivalent dose, depending on the radiation quality of the mixed field and the cell line considered.

Case 2 – Varying reference radiation: Fig. 2.18(a) shows the depth profiles of the absorbed dose (solid black), the dose weighted with RBE = 1.1 (dashed black), and the IsoE doses corresponding to the HNSCC cell lines, calculated considering 10 MV photons, ^{60}Co gamma rays, and 250 kVp X-rays as reference radi-

ations.

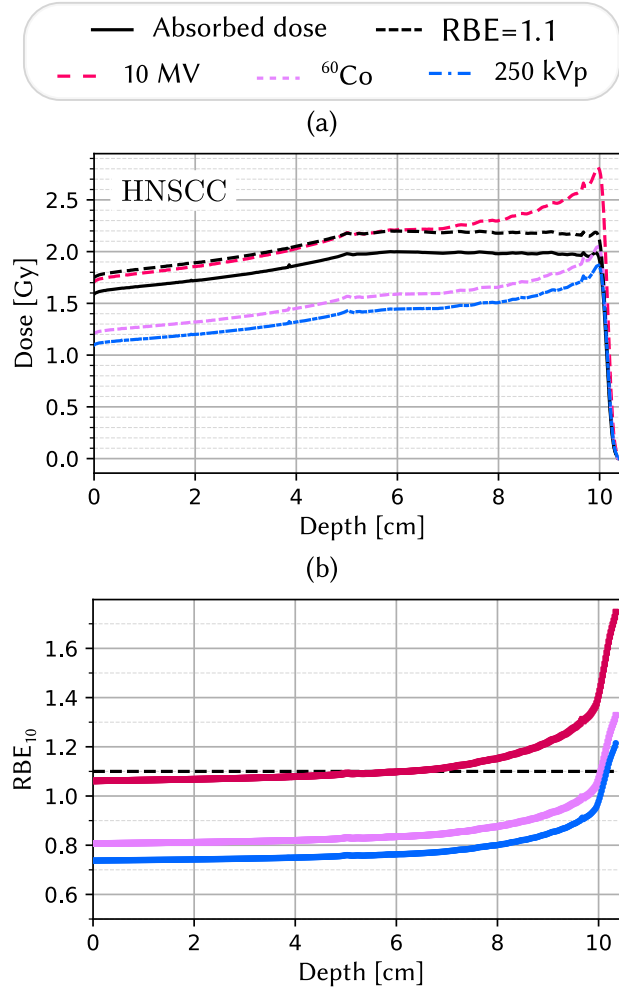


Figure 2.18: Dependence of the isoeffective dose on the reference radiation. (a) Depth-dose profiles of the absorbed dose (solid black), the dose calculated with a fixed $\text{RBE} = 1.1$ (dashed black), and the IsoE doses for the HNSCC cell lines calculated considering different reference radiations (10 MV photons, ^{60}Co gamma rays, and 250 kVp X-rays). (b) RBE_{10} profiles as a function of depth.

A strong dependence of the isoeffective dose on the reference radiation is observed for the HNSCC cell line. Reference photons can be grouped according to their mean energy [93]: megavoltage beams (10 MV, 6 MV, and ^{60}Co , $\text{LET} \approx 0.2 \text{ keV}/\mu\text{m}$) and kilovoltage X-rays ($\text{LET} \approx 1\text{--}1.5 \text{ keV}/\mu\text{m}$) [94]. As expected, in HNSCC cells, kilovoltage X-rays are more effective than megavoltage photons, requiring lower IsoE doses to achieve the same biological effect. Notably, a significant difference is also observed between the 10 MV and ^{60}Co results. While these two beams are physically similar in terms of $\text{LET} (\approx 0.2 \text{ keV}/\mu\text{m})$, the divergence in the calculated IsoE doses stems from the different experimental datasets used to characterize the reference biological parameters (α_x, β_x). This result highlights the

sensitivity of the PhID model to the baseline experimental data and reflects the well-known inter-laboratory variability in survival assays, where factors beyond LET, such as dose rate or specific culture conditions, can influence the reference radiobiological parameters.

The impact of these different reference baselines is evident in Fig. 2.18(b), where the depth-dependent RBE_{10} profile for 10 MV photons is higher than that those for both ^{60}Co and for 250 kVp X-rays. Here, D_x - the dose required to achieve the selected effect level of 10% cell survival for each reference radiation - remains constant with depth, whereas the corresponding absorbed dose delivered by the mixed charged-particle radiation field, D_T , varies with depth. Consequently, as the dose D_T required to reach 10% survival decreases with depth, the RBE_{10} increases, resulting in calculated RBE_{10} ranges for HNSCC of 1.1–1.7 for 10 MV photons, 0.7–1.3 for ^{60}Co and 0.7–1.2 for 250 kVp X-rays.

Cases 1 and 2 highlight the dependence of the photon IsoE dose on the cell line (Fig. 2.17), the chosen reference radiation (Fig. 2.18), and on L_D , as discussed in section 2.3.2. In all the exposed cases, the position of the maximum L_D coincides with that of the maximum RBE_{10} , although in this region, beyond the SOBP, the absorbed dose is around 1% or less of the maximum dose delivered within the region of interest. This maximum RBE_{10} value, which occurs beyond the SOBP, causes an extension of the effective range, as reported in other works [13,95].

2.3.3 Tumor control probability model for HNSCC: proof-of-concept assessment

Figure 2.19 shows the isoeffective dose, $IsoE_{AF}$, obtained from the MFO described in Sec. 2.2.5. The left panels display axial and sagittal slices of the RBE distribution associated with the $IsoE_{AF}$ dose at $z = 1.5\text{ cm}$ and $y = 1.8\text{ cm}$ respectively. The right panels present dose profiles along the x and z directions, comparing the absorbed dose, the $IsoE_{AF}$ dose, and the corresponding RBE profile.

As shown in the figure, the $IsoE_{AF}$ dose exhibits a gradual and slight increase from the center of the irradiated volume toward its lateral edges. Along the transverse direction (x or y , which show identical behavior due to symmetry), the corresponding RBE increases toward the periphery, reaching values close to 1.45, whereas along the beam direction (z) the RBE attains values of approximately 1.2.

Figure 2.20 shows axial and sagittal slices of the D_{SF} dose distribution, corresponding to the fractionated $IsoE_{AF}$ doses converted to single-fraction equivalent doses using the linear-quadratic-based transformation given in Eq. 2.12. In both slices, the GTV region is indicated by white dashed lines. The right panel presents the tumor control probability curve as a function of the single-fraction photon dose. From this curve, the TCP corresponding to the $IsoE_{AF}$ dose is $TCP_{IsoE} = 0.60$, whereas the TCP obtained using the proton absorbed dose weighted with a fixed RBE of 1.1 is $TCP_{RBE} = 0.55$. The absolute value of the relative difference with respect to TCP_{RBE} is therefore approximately 9%. While this differ-

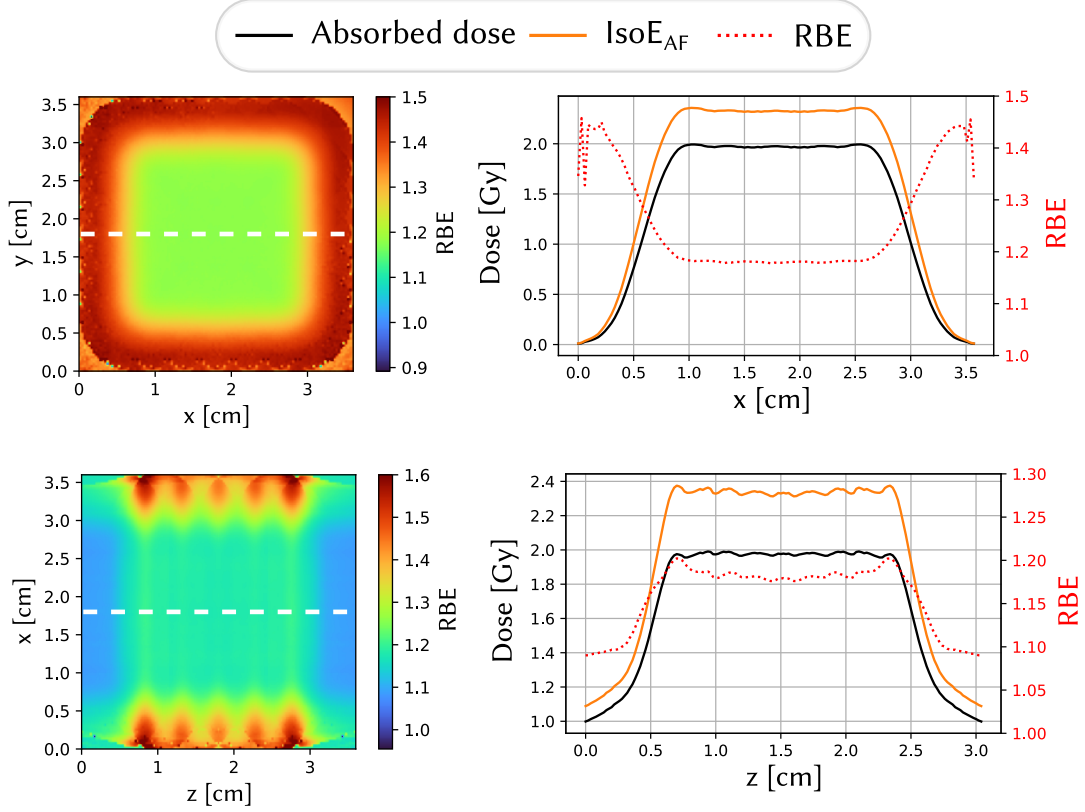


Figure 2.19: Axial and sagittal slices of the RBE distribution associated with the IsoE_{AF} dose (left) and corresponding dose profiles along the x and z directions comparing absorbed dose, IsoE_{AF} dose, and RBE (right).

ence is numerically robust within the framework of the model, being well above the propagated Type A uncertainty of the Monte Carlo calculations ($< 0.1\%$), it is important to note that the intrinsic uncertainties associated with the biological parameters (α, β) and the TCP model fit were not explicitly propagated in this study. Consequently, while the PhID model shows a clear sensitivity compared to the fixed RBE approach, its clinical significance should be interpreted with caution within the scope of this proof-of-concept assessment.

Regarding the RBE distribution, the RBE values derived from IsoE_{AF} within the GTV range from 1.1 to 1.2, with a mean value of 1.17 ± 0.01 (mean $\pm$ standard deviation). Across the entire irradiated volume, the RBE spans from 1.1 to 1.7 (see Fig. 2.21), with the highest values located at the periphery of the irradiated region, and a volume-averaged value of 1.22 ± 0.10 .

Furthermore, this volumetric analysis allows to characterize the full three-dimensional distribution of RBE. Under this configuration, approximately 70% of the RBE values lie within the 1.1–1.2 range, while the remaining 30% extend beyond this interval up to a maximum value of 1.7. Within this higher-RBE subset, values around 1.3 (60%) and 1.4 (35%) are the most representative, followed by values around 1.5 (4.2%), while only a negligible fraction corresponds to RBE values

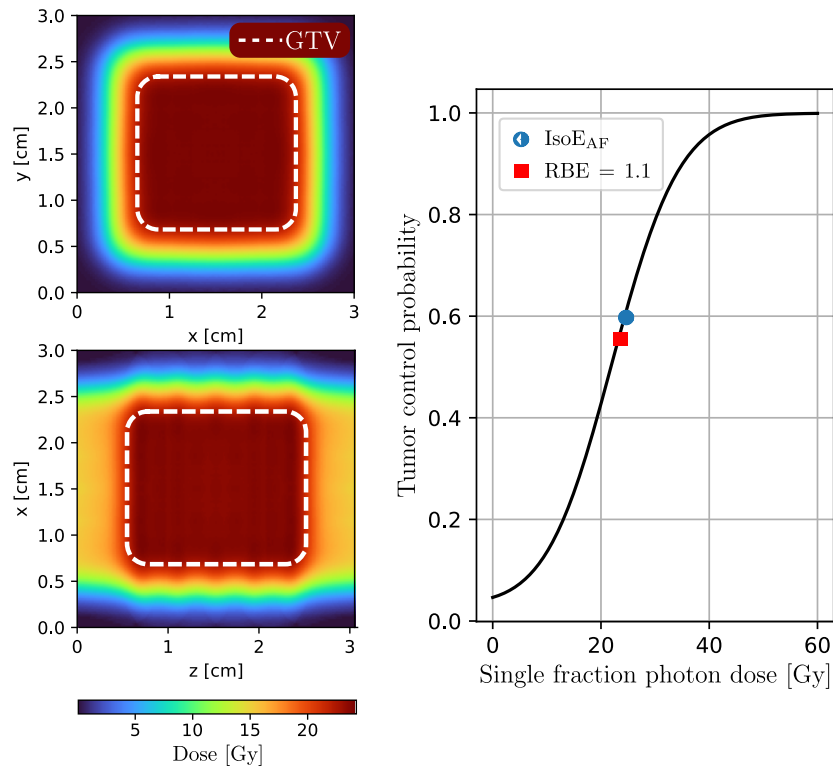


Figure 2.20: Left: Axial and sagittal sections showing the dose distribution obtained from the MFO. The GTV region, where the TCP was evaluated, is indicated by white dashed lines. Right: TCP curve as a function of the single-fraction photon dose, including the two TCP values calculated using the IsoE dose distribution and the RBE-weighted dose that were converted to single fraction values.

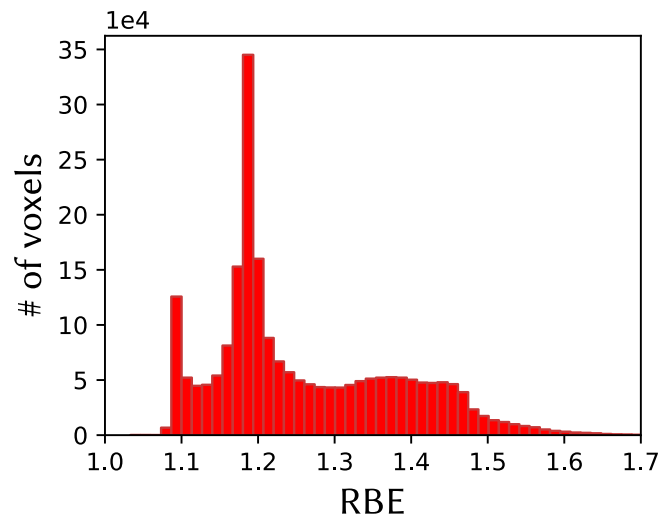


Figure 2.21: Histogram of RBE values, where the frequency represents the number of phantom voxels associated with each RBE.

close to 1.7.

It is important to note that while this proof-of-concept assessment was performed in a homogeneous soft-tissue phantom, the observed RBE variations and the resulting TCP differences would persist—and likely become more complex—in the presence of tissue heterogeneities. In a real clinical geometry, density variations (such as those from bony structures in HNSCC treatments) would alter the Bragg peak positioning and the local LET distribution. However, as the PhID model is inherently voxel-based, its integration into a CT-based Treatment Planning System (TPS) would allow for a precise biological characterization of the treatment, accounting for both the physical perturbations caused by tissue density and the specific radiobiological response of the involved organs at risk.

This analysis is clinically relevant, as the majority of the RBE values lie close to the fixed RBE of 1.1 commonly adopted in clinical practice. Nevertheless, the high RBE values observed at the periphery of the irradiated volume should not be overlooked, as these regions may correspond to normal tissue. In the present analysis, the IsoE dose was computed using radiobiological parameters representative of HNSCC across the entire volume, including the peripheral regions. While a more realistic assessment would require the use of normal-tissue-specific radiobiological parameters in those regions, an increased RBE toward the periphery is nonetheless expected.

2.3.4 Model comparison

Figure 2.22 shows the depth dose profiles (top panel) corresponding to the absorbed dose D_p (solid black), the dose weighted with $RBE = 1.1$ (black dashed), the IsoE dose (light blue dashed), and the doses calculated with different RBE models mentioned in section 2.2.6. The lower panel shows the RBE profiles in depth for all models considered, together with the RBE intervals obtained for each model.

The comparison shows that the IsoE dose model follows the same general trend as all phenomenological RBE models — an increase with depth — reflecting its dependence on LET, or L_D in the case of the other models. This increase becomes more pronounced near the distal fall edge, around 10 cm, corresponding to the position of the most distal Bragg peak of the SOBP.

In all models, except for the PLR model, the position of the maximum RBE coincides with that of the maximum L_D ($\sim 12 \text{ keV}/\mu\text{m}$). The JON and WIL models have the widest RBE ranges, extending from approximately 1.1 up to 3.4 and 3.1, respectively. These are followed by the proposed IsoE model and the RORU model, with RBE ranges from about 1.1 up to 2.1 and 1.6, respectively. The TIL, CHE, WED, and MCN models have narrower ranges, with minimum RBE values as low as ~ 0.9 and maximum values averaging ~ 1.3 . Finally, the CAR and PLR models predict RBE values below 1.0 across most of the depth range, except for the distal fall-off region in the CAR model, where the RBE reaches a maximum of 1.2. The PLR model shows the narrowest variation, with RBE values ranging only from 0.9 to 1.0.

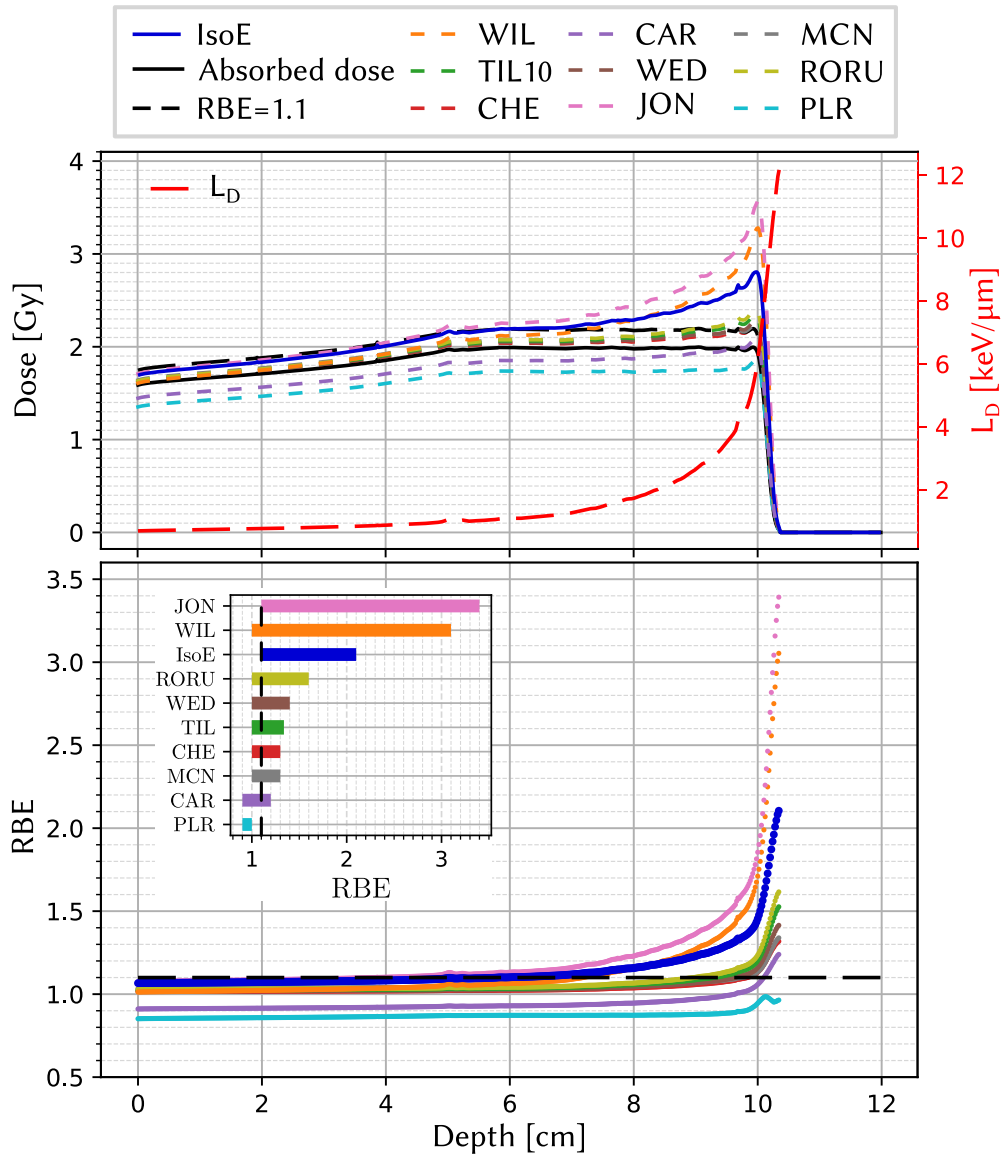


Figure 2.22: Depth–dose and RBE profiles of a SOBP in soft tissue for the HNSCC cell line, considering 10 MV photons ($(\alpha/\beta)_x = 12.6$ Gy) as the reference radiation. (Top panel) Depth–dose profiles (left axis) calculated with different phenomenological RBE models are shown as colored curves. The dashed red curve represents the L_D profile (right axis). (Bottom panel) Depth–RBE profiles derived from the doses shown in the top panel.

Figure 2.23 shows the RBE predictions from different models as a function of the dose per fraction, evaluated at the selected L_D values.

At a low L_D of $1.1 \text{ keV}/\mu\text{m}$, corresponding to the proximal region of the SOBP, most models (except JON) predict RBE values below 1.1 at 2 Gy, and in some cases even below to 1.0 (~ 0.9 for the CAR and PLR models). The WIL, TIL, CHE, WED, and RORU models exhibit a similar trend, showing a decrease in RBE with increas-

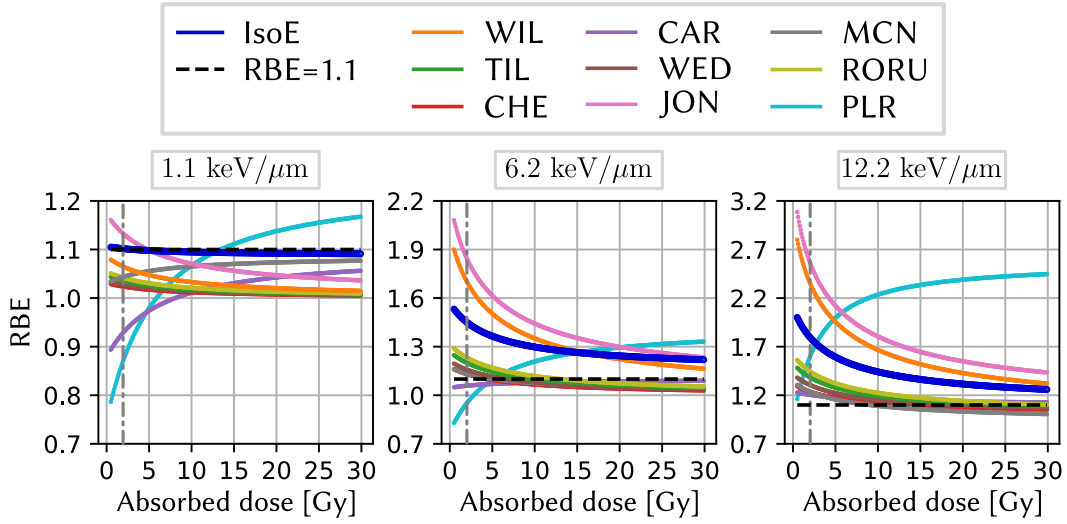


Figure 2.23: RBE predictions from different models as a function of dose at three selected L_D values. The vertical gray dashed-dotted line indicates the 2 Gy absorbed dose value.

ing dose, from ~ 1.1 down to ~ 1.0 . In contrast, the CAR, MCN, and PLR models display the opposite behavior, with RBE values rising with dose, reaching between 1.0 and 1.1 in CAR and MCN, and up to ~ 1.2 in PLR. Our model predicts a gradual decrease in RBE with increasing dose. While the values remain numerically within a close range around 1.1 for this specific cell line and LET, it is important to note that even small RBE variations (in the order of 5–10%) can lead to clinically significant differences in biological dose distribution. Such variations could potentially influence treatment planning decisions, particularly when organs at risk are located in high-LET regions or near the distal edge of the beam. In contrast, at higher L_D values, all models—except CAR and PLR—show a decrease in RBE with increasing absorbed dose, resulting in higher RBE values at lower doses.

At $L_D = 6 \text{ keV}/\mu\text{m}$, the TIL, CHE, WED, MCN, and RORU models predict RBE values in the range 1.1–1.2 at 2 Gy. At the same dose level, the IsoE model, together with JON and WIL, yield higher RBEs of 1.4, 1.8, and 1.7, respectively. In contrast, the CAR and PLR models predict lower RBEs of 1.0 and 0.9.

At the position of maximum LET, where the highest RBEs are observed, the IsoE, JON, and WIL models report values of 1.8, 2.6, and 2.3 at 2 Gy, respectively. The remaining models—CAR, CHE, MCN, WED, TIL, and RORU—fall within the range 1.2–1.4. In this region, the PLR model exhibits a distinct dose-dependent increase, reaching an RBE of 1.6 at 2 Gy and a maximum of 2.4 at 30 Gy.

As presented in Section 2.3.2.2, the range of RBE values depends purely on the radiobiological parameters α and β , which are specific of the cell line and the chosen reference radiation. For example, when the same cell line is considered but the reference radiation is changed from 10 MV photons to 250 kVp X-rays, the corresponding RBE range varies from 1.1–1.7 to 0.7–1.2, respectively, illustrating how the choice of reference radiation influences the resulting RBE values.

Furthermore, the differences observed among the RBE ranges predicted by different models primarily arise from how these models were constructed. The Photon Isoeffective Dose model developed in this work is cell-line-specific, having been built exclusively from data corresponding to the HNSCC cell line. In contrast, most RBE models were developed using experimental data from multiple cell lines, which introduces a broader biological variability [16]. An exception to this is found in the WIL, CHE and CAR models, which were derived solely from experiments with the V79 cell line, yet are frequently applied to other cell types. Such differences in model construction explain the variability observed in the predicted RBE ranges even when the same cell line and reference radiation are considered. Finally, the uncertainty associated with the Monte Carlo dose calculation was estimated to be on the order of 5%. However, an error propagation analysis indicates that the corresponding uncertainty in the isoeffective dose remains below 0.1% at clinically relevant dose levels (e.g., ~ 2 Gy), and only approaches 5% at very low doses (below ~ 0.03 Gy), such as near the distal edge of the Bragg peak. Therefore, the differences observed between RBE values from different models are primarily driven by the underlying model assumptions rather than numerical uncertainties.

The clinical relevance of these findings is twofold. First, as observed in the comparisons, even small RBE variations (in the order of 5–10%) can lead to significant differences in biological dose distribution. In a clinical environment, such variations can shift the balance between tumor control and normal tissue toxicity, particularly when organs at risk are located in high-LET regions or near the distal edge of the beam.

Second, the translation of the PhID model into current clinical workflows is feasible through existing advanced computing and scripting frameworks. Modern proton therapy TPS platforms already incorporate dose engines capable of providing voxel-level physical dose and LET distributions. While most commercial systems currently rely on a fixed RBE of 1.1, their research-oriented Application Programming Interfaces (APIs), such as those based on Python, allow for the external integration of cell-line-specific radiobiological parameters. This framework enables the calculation of photon-isoeffective dose distributions as a biological reporting layer that utilizes the detailed physical data already generated by the system. Consequently, the PhID model acts as a bridge toward more personalized and tissue-specific treatment planning without requiring major structural changes to existing TPS physics.

2.4 Conclusions

This study introduces a cell line-specific model for computing photon isoeffective doses in proton therapy, referred to as the PhID model, constructed for three cell lines: head-and-neck squamous cell carcinoma (HNSCC), human skin fibroblasts (HSF), and Chinese hamster fibroblasts (V79). The model is based on the modified LQ formalism and explicitly accounts for the mixed field of charged particles generated by the interaction of the incident proton beam with tissue, including

both primary and secondary components. Within this framework, alpha particles were identified as the second most significant contributors to the high-LET field after protons, indicating that their inclusion provides the largest gain in accuracy beyond a proton-only description. Moreover, the model incorporates sublethal damage production and repair processes, as well as synergistic effects among the different field components.

The PhID model depends on the dose delivered by the contributing particle fields, on LET-dependent radiobiological parameters α and β , and on the reference-radiation parameters α_x and β_x . Thus, the model predicts higher RBE values for lower doses per fraction and increasing LET, as well as for lower $(\alpha/\beta)_x$ ratios; for similar $(\alpha/\beta)_x$ values, the α_x parameter predominantly governs the resulting RBE magnitude.

It should be emphasized that, although the concepts of RBE and RBE_{10} were used throughout this work exclusively to interpret trends and enable comparisons, the PhID model is not intended to convert proton dose into a photon-equivalent dose based on fixed biological effectiveness values. Instead, the model does not rely on any predefined endpoint, as it incorporates information from the entire survival curves. In this respect, the PhID formalism is fully flexible with respect to the chosen dose–effect description and can be constructed from any experimental or modeled relationship between dose and biological effect, including cell survival, Tumor Control Probability (TCP), or Normal Tissue Complication Probability (NTCP), which is particularly relevant for the characterization of radiotoxic effects in dose-limiting normal tissues.

A key strength of the PhID model is its integration with the BIANCA biophysical model, which allows the determination of LET-dependent radiobiological parameters while explicitly accounting for both the cell line and the ion species involved. In this respect, the capability of BIANCA to provide radiobiological parameters for cell lines beyond those considered in this study opens the possibility of extending the PhID model to additional tumor and normal tissue types, while preserving its cell-specific formulation and contributing to a more biologically accurate dosimetric approach in proton therapy.

Finally, the PhID model is not limited to proton therapy and provides a flexible framework that can be extended to other particle-based radiotherapy modalities, such as carbon-ion therapy or alpha-particle therapy, where secondary particle fields and high-LET components may play a more prominent role in determining biological effectiveness.

CHAPTER 3

COMPHID MODEL

Chapter overview

This chapter introduces the COMPhID model, a formulation designed to describe radiation effectiveness within a continuous microdosimetric framework. In this approach, radiation quality is represented in terms of lineal energy, enabling the transition from a particle-based description of mixed radiation fields to an energy-deposition-based representation at the microscopic scale.

Within this framework, the model depends explicitly on the first three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, obtained from microdosimetric spectra. At the same time, its parameters are linked to the radiobiological response through the LQ formalism via the coefficients $\langle\alpha\rangle$ and $\langle\beta\rangle$. The determination of these parameters requires a combined experimental approach, in which microdosimetric measurements are coupled with radiobiological experiments.

These experiments were conducted at the Trento Proton Therapy Center (Italy) under equivalent irradiation conditions for both the cell cultures and the microdosimetric detector, allowing a direct link between radiation quality and biological response. The experimental methodology is described in Secs. 3.2.1 and 3.2.3.

Irradiations were performed at selected positions along the central axis of a passively modulated 148 MeV proton beam forming a SOBP. Cell survival curves for the human NSCLC H460 cell line were obtained in the dose range 0.5–5 Gy (Sec. 3.3.2), while microdosimetric spectra were measured using a mini-TEPC under equivalent conditions. From these spectra, the lineal energy moments were derived (Sec. 3.3.3).

The model parameters were obtained from the combined analysis of the microdosimetric and radiobiological data (Sec. 3.3.4). Once determined, the COMPhID model was applied to calculate the photon isoeffective dose using ^{137}Cs as reference radiation under two scenarios: (I) a fixed survival level of 10% and (II) a fixed

proton dose of 2 Gy. These calculations were performed considering four of the five experimental irradiation positions, and the results were compared with the experimental data to evaluate their consistency (Sec. 3.3.5).

Finally, the model was applied to three different cases: (i) using an experimentally measured microdosimetric spectrum at a given irradiation position, (ii) calculating the isoeffective dose along the SOBP, and (iii) analyzing an additional irradiation position for which the spectrum was obtained from Monte Carlo simulations. The results of these analyses are presented in Sec. 3.3.6.

3.1 The Continuous Microdosimetric Photon Isoeffective Dose (COMPhID) Model

3.1.1 Derivation of the COMPhID Model

As anticipated in Chapter 2, the isoeffective dose model, originally formulated within the framework of BNCT and subsequently applied in this thesis to describe the isoeffect of a radiation field composed of protons and their secondary particles, can be reformulated in a continuous framework. This reformulation stems from recognizing that at the microscopic scale where the effect manifests as DNA damage, the microdosimetric quantity lineal energy, enables the description of radiation quality in terms of a continuous variable.

The discrete partitioning of a mixed-radiation field reflects the different effectiveness of its components and relies on the association of a representative quality descriptor, such as the average LET, with each particle field. In this sense, the model depends on both the nature of the particle and its associated energy deposition characteristics.

In this context, characterizing the quality of the radiation field in terms of lineal energy—the stochastic analogue of LET—constitutes an appropriate approach for describing the radiation field within a continuous framework. Within this representation, radiation quality is expressed in terms of lineal energy, providing a unified description at the microscopic scale. As a result, the explicit dependence on particle type is not retained, as different radiation components may contribute to overlapping regions of lineal energy.

The discrete formulation provides a physically transparent description of the distinct particle components of the field, whereas the continuous approach offers a representation directly linked to the stochastic nature of energy deposition at the microscopic scale. Therefore, rather than constituting a strict extension of the discrete formulation, the continuous model represents a reformulation in which the description of radiation quality is shifted from a particle-based to an energy-deposition-based framework. While this approach enables a more direct connection with the stochastic nature of microscopic energy deposition, it also reflects a change in how radiation quality is parameterized, moving from particle identity to energy-deposition descriptors. This methodology situates the model at the spatial scale where radiation interactions within biological structures are

most directly related to biological effect, providing a complementary perspective to the discrete representation.

3.1.2 The COMPhID model

The Continuous Microdosimetric Photon Isoeffective Dose (COMPhID) model arises from a reformulation of the Photon Isoeffective Dose (PhID) model presented in Section 2.1, as introduced in the opening of this chapter, based on a conceptual shift from a particle-based description to a continuous microdosimetric representation of radiation quality.

This reformulation into the continuum is achieved by identifying, in the discrete PhID expression (Eq. 2.1), the corresponding quantities as functions of lineal energy y , which represents the stochastic analogue of the linear energy transfer (LET). Within this framework, radiation quality is not characterized by discrete particle components, each associated with a specific LET_i and corresponding radiobiological parameters α_i and β_i .

Instead, the model is expressed in terms of the microdosimetric lineal energy spectrum, where all particle species contributing to the mixed radiation field are naturally embedded within a single distribution $f(y)$. Consequently, the radiobiological parameters are formulated as functions of lineal energy, $\alpha(y)$ and $\beta(y)$, reflecting their dependence on the local radiation quality as described by y , rather than on discrete particle-specific quantities.

The expression in Eq. 1.16, corresponding to the dose-weighted probability density function, represents the continuous counterpart of the discrete distribution describing the relative contribution of each radiation component i to the total dose, with fractions f_i ($i = 1, \dots, n$).

In the standard representation of microdosimetric spectra, where $yd(y)$ is plotted as a function of y using logarithmic binning, the area under the curve between two lineal energy values y_1 and y_2 corresponds to the fraction of the total dose deposited within the interval $\Delta y = y_2 - y_1$ (see Fig. 3.1).

Based on this representation, the α and β coefficients can be reformulated in terms of the dose-weighted lineal energy distribution as:

$$\langle \alpha \rangle = \int_0^{\infty} \alpha(y) d(y) dy, \quad (3.1)$$

$$\langle \beta \rangle = G(\theta) \left(\int_0^{\infty} \sqrt{\beta(y)} d(y) dy \right)^2, \quad (3.2)$$

where $\alpha(y)$ and $\beta(y)$ are expressed as functions of the lineal energy.

It can be seen that the expression in the continuum retains the synergistic behavior between different components of the discrete expression, ($i \neq j$), by considering an alternative expression, completely equivalent, that can be derived as follows:

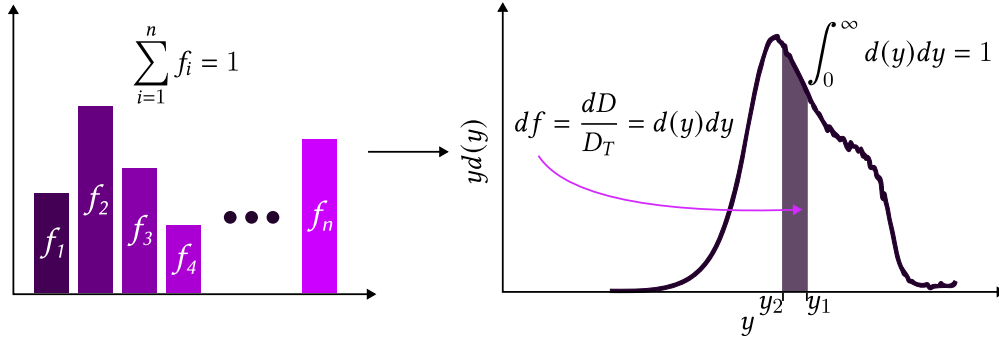


Figure 3.1: Conceptual scheme illustrating the reformulation from a discrete to a continuum description of the mixed radiation field: within the COM-PhID formalism, the differential areas under the dose-weighted lineal energy spectrum curve (right figure), defined over infinitesimal intervals of the lineal energy variable, are conceptually equivalent to the relative weights assigned to each discrete component (left figure), but irrespective of which particle of the mixed field contributed to that differential interval.

$$\begin{aligned} \left(\int_0^\infty \sqrt{\beta(y)} d(y) dy \right)^2 &= \int_0^\infty \sqrt{\beta(y)} d(y) dy \int_0^\infty \sqrt{\beta(y')} d(y') dy' \\ &= \iint_0^\infty \sqrt{\beta(y)} \sqrt{\beta(y')} d(y) d(y') dy dy'. \end{aligned} \quad (3.3)$$

Synergism is manifested whenever $y \neq y'$, i.e., practically always (the probability that two energy deposits are exactly equal in value is zero).

Finally, by combining Eqs. 3.1 and 3.2, the expression of the PhID model (Eq. 2.1) can be reformulated within the continuous microdosimetric framework as:

$$\alpha_x D_x + G_x(\theta') \beta_x D_x^2 = \langle \alpha \rangle D_T + \langle \beta \rangle D_T^2, \quad (3.4)$$

where the radiobiological parameters α and β characterizing the mixed radiation field are now formulated as dependent on lineal energy.

The specific functional forms proposed for $\alpha(y)$ and $\beta(y)$ are presented in the following section.

3.1.3 Proposed model for $\alpha(y)$ and $\beta(y)$

The starting point of the proposed model describing the dependence of α and β on lineal energy is the theory of Compound Dual Radiation Action (CDRA), introduced in Sec. 1.3.2.

In this work, an extension of the CDRA framework is proposed to account for the formation of Complex Chromosome Rearrangements (CCR), which are known to occur more frequently following irradiation with high-LET particles [96].

CCR are defined as chromosome aberrations involving three or more Double-Strand Breaks (DSB) distributed over two or more chromosomes. Motivated by the increased relevance of such complex damage at high LET, the CDRA formalism is extended in a natural way by considering the interaction of three DSB, which can combine to form a CCR. In this framework, the probability of forming a three-break CCR (3-B-CCR) is assumed to be proportional to the cube of the specific energy deposited within a micrometric volume, either from single or multiple events (see Fig. 3.2).

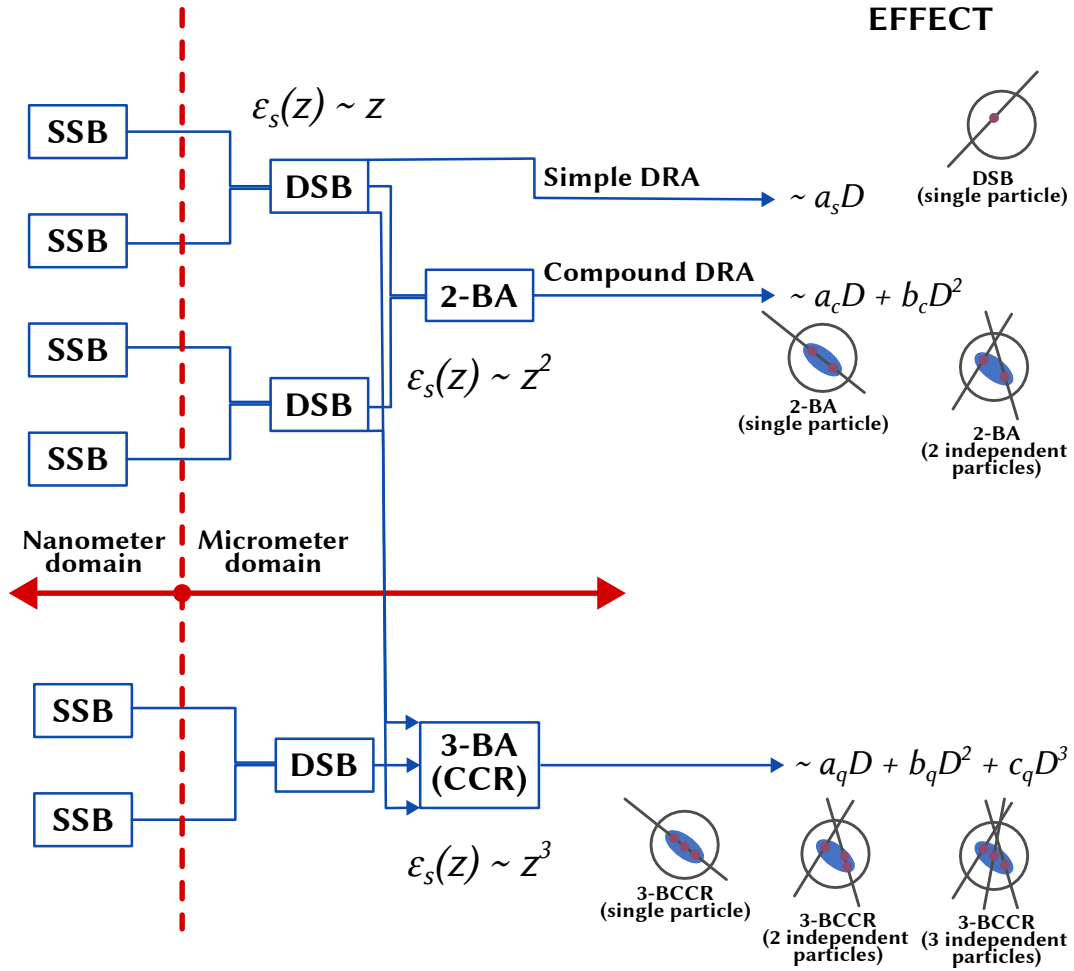


Figure 3.2: The inclusion of a third DSB that interacts with another two gives place to the simplest form of CCR.

Within the CDRA formalism, cell inactivation is described in terms of the formation of lethal lesions. Therefore, the proposed extension implies a corresponding modification of the effectiveness function, $\epsilon(Z)$, that relates the specific energy deposition, Z , to the average number of lethal lesions. In particular, the inclusion of higher-order interactions naturally leads to an additional cubic dependence on the specific energy.

A detailed derivation of this extended framework, including the formulation of

the average number of lethal lesions and its connection to the moments of the microdosimetric distribution, is provided in Appendix C. This derivation establishes the basis for the expressions proposed in the following section for $\alpha(y)$ and $\beta(y)$ as functions of the lineal energy.

3.1.3.1 Proposed expressions for α and β as a function of lineal energy

Proposed expression for the α term

Motivated by the dependence of Eq. C.12 on lineal energy, we propose the following form for the α parameter:

$$\alpha(y) = \alpha_0 + \alpha_1 y + \alpha_2 y^2. \quad (3.5)$$

The quantity $\alpha(y)$ is a function of the random variable y and, therefore, it is also a random variable. It can be seen that the first two terms, when integrated with the dose-weighted probability density function $d(y)$, are equivalent to the α term in Hawkins's expression, Eq. 1.17, with

$$\alpha_1 = \frac{0.204}{d^2} \beta = ca_2, \text{ and } a_2 = \beta. \quad (3.6)$$

Then, taking the expectation of Eq. 3.5 with respect to $d(y)$, we obtain

$$\langle \alpha \rangle = \int_0^\infty \alpha(y) d(y) dy = \int_0^\infty (\alpha_0 + \alpha_1 y + \alpha_2 y^2) d(y) dy, \quad (3.7)$$

$$\boxed{\langle \alpha \rangle = \alpha_0 + \alpha_1 \frac{y^{(2)}}{y^{(1)}} + \alpha_2 \frac{y^{(3)}}{y^{(1)}}}, \quad (3.8)$$

which results in an extension of Hawkins's expression with an additional term $\alpha_2 \frac{y^{(3)}}{y^{(1)}}$.

Proposed expression for the β term

Similarly to what was proposed for the alpha term, and again motivated by the dependence of the beta factor on the second moment of lineal energy relative to the first one (see expression C.7 in the Appendix), we now propose that β will depends linearly on the random variable y , i.e.,

$$\beta(y) = \beta_0 + \beta_1 y. \quad (3.9)$$

Note that the quantity integrated with respect to $d(y)$ is not $\beta(y)$ but $\sqrt{\beta(y)}$.

In the following, we show that a suitable approximation of 3.2 leads to a linear dependence of the generalized beta coefficient, $\langle \beta \rangle$, on y_D .

Let us define $g(y) = \sqrt{\beta(y)}$, which is a random variable. The integral of $g(y)$ with respect to $d(y)$ is the expected value of $g(y)$, i.e.,

$$E_d(g) = \int_0^\infty g(y)d(y) dy, \quad (3.10)$$

whose square is the expression 3.2 (ignoring the G factor). On the other hand, the expected value of the square of $g(y)$ with respect to $d(y)$ is:

$$\begin{aligned} E_d(g^2) &= \int_0^\infty g^2(y)d(y)dy = \int_0^\infty (\beta_0 + \beta_1 y) d(y)dy \\ &= \beta_0 + \beta_1 \frac{y^{(2)}}{y^{(1)}} \end{aligned} \quad (3.11)$$

Therefore, the variance of g with respect to $d(y)$ is:

$$V_d(g) = E_d(g^2) - E_d(g)^2. \quad (3.12)$$

Since the square of equation 3.10 is, beyond the G factor, the generalized beta coefficient $\langle\beta\rangle$, then we can write

$$\langle\beta\rangle = \beta_0 + \beta_1 \frac{y^{(2)}}{y^{(1)}} - V_d(g). \quad (3.13)$$

Up to this point, no approximation has been introduced. However, the practical applicability of Eq. 3.13 depends on the magnitude of the variance term $V_d(g)$. It is therefore necessary to quantify its contribution and assess whether it can be safely neglected under clinically relevant proton irradiation conditions.

The variance of $g(y)$, treated as a random variable, can be estimated by simulating a sensitive site of given size exposed to a particle field depositing energy within it. Assuming protons as the dominant particle component, and considering either monoenergetic beams at different energies or simulated proton beams in water, $V_d(g)$ can be evaluated by Monte Carlo sampling across different irradiation scenarios. Details of these calculations are provided in Appendix D.

The results show that $V_d(g)$ can be neglected, as its magnitude is comparable to the experimental uncertainties associated with the beta coefficients. Consequently, Eq. 3.13 can be applied without introducing a significant approximation. Then, expression 3.13 can be written as:

$$\boxed{\langle\beta\rangle \cong \beta_0 + \beta_1 \frac{y^{(2)}}{y^{(1)}}} \quad (3.14)$$

Considering expressions 3.8 and 3.14, the COMPhID model is characterized by five free parameters, namely α_0 , α_1 , α_2 , β_0 , and β_1 . The determination of these parameters requires the combined use of microdosimetric and radiobiological information.

The model explicitly depends on the first three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, which must be computed from microdosimetric spectra according to Eq. 1.14. On the other hand, the model parameters are

linked to the radiobiological response of the system through the LQ framework. In this context, the radiobiological parameters α and β , hereafter associated with the averages $\langle\alpha\rangle$ and $\langle\beta\rangle$, are obtained by performing dedicated radiobiological experiments and fitting the corresponding cellular survival curves.

In the following section, the procedure employed to determine the parameters of the model through combined microdosimetric-radiobiological experiments is detailed.

3.2 Combined radiobiological-microdosimetric experiments at the Trento Proton Therapy Center

Within the framework of the MAMBA¹ project, *WP5 - Proton Radiobiology (Task 5.7: Development of novel biophysical models)*, combined radiobiological and microdosimetric experiments were conducted at the Trento Proton Therapy Center (Italy) with the aim of determining the parameters α_0 , α_1 , α_2 , β_0 , and β_1 of the COMPhID model (see Eqs. 3.8 and 3.14).

These experiments are referred to as *combined* because they are specifically designed to link the biological response of irradiated cell cultures to radiation quality, quantified through microdosimetric spectra. To this end, both the cell monolayers and the sensitive volume of the microdosimetric detector were exposed to the same radiation quality under almost identical irradiation conditions.

The experiments were performed at selected points of interest along the central axis of an SOBP generated by the passive modulation of a 148 MeV proton beam.

According to the most recent global cancer statistics, lung cancer remains the leading cause of cancer-related incidence and mortality worldwide [97]. Among all diagnosed lung malignancies, approximately 85% correspond to non-small cell lung cancer (NSCLC) [98]. Given its high clinical prevalence and the increasing implementation of proton therapy in the management of thoracic tumors, NSCLC represents a particularly relevant disease model for radiobiological investigations [99].

In this context, cell survival curves were constructed for a dose range of 0.5 to 5 Gy, considering the human NSCLC H460 cell line available at the Department of Cellular, Computational and Integrative Biology (CIBIO).

Additionally, measurements of microdosimetric spectra were performed using a Mini-TEPC tissue-equivalent proportional counter, described later in 3.2.3.1, by replicating the setup used in the radiobiological experiment.

Finally, the parameters of the COMPhID model were derived from the combined analysis of microdosimetric spectra and radiobiological experiments.

¹<https://www.mamba-msca.eu/>

The following sections describe in detail the methodology adopted to carry out the radiobiological and microdosimetric experiments.

3.2.1 Radiobiological experiments

A series of radiobiological experiments was carried out involving the growth of the H460 cell line, followed by proton irradiation at the Trento Proton Therapy Center, Trento, Italy. Irradiations were performed at selected points of interest (P1–P5) along the central axis of an SOBP generated by the passive modulation of a 148 MeV proton beam (see Fig. 3.5). These biological experiments were conducted at CIBIO, University of Trento, Italy.

After irradiation, cell survival was quantified by means of clonogenic assays. The experimental survival data were then used to construct survival curves for each irradiation point, from which the corresponding parameters, α and β , of the linear-quadratic model were extracted.

In the following, each stage of the experimental procedure is described in greater detail.

3.2.1.1 Cell culture of the H460 cell line

The non-small cell lung cancer cell line H460 was obtained from the American Type Culture Collection (ATCC²). Cells were cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine (Life Technologies, Grand Island, NY), and maintained in T25 culture flasks at 37°C in a humidified atmosphere containing 5% CO₂ until reaching exponential growth at approximately 80% confluence.

The day before the irradiation, cells were harvested using a 0.25% trypsin/EDTA solution and subsequently counted to assess viability by trypan blue exclusion. The cell suspension was then carefully diluted and gently pipetted to ensure a homogeneous single-cell suspension and to avoid the presence of cell aggregates.

Subsequently, cells were seeded in 96-well cell culture plates at different densities, selected according to the planned delivered dose (see Table 3.1). A centered 5 × 5 well grid was used within each plate, ensuring 25 replicates for each irradiation condition as shown in Fig. 3.3. Each well was filled with a total volume of 100 µL of cell suspension, using an electronic micropipette to ensure high volumetric precision. Following seeding, the plates were placed in a humidified incubator at 37°C and 5% CO₂ and left overnight to allow for cell attachment prior to irradiation.

To mitigate edge effects associated with multi-well plate cultures, the wells surrounding those containing cells were filled with 100 µL of culture medium without cells. This strategy reduces evaporation-induced variations in medium volume, pH, and osmolarity, particularly at the plate periphery, and promotes uniform incubation conditions during the clonogenic assay [100].

²<https://www.atcc.org/products/htb-177>

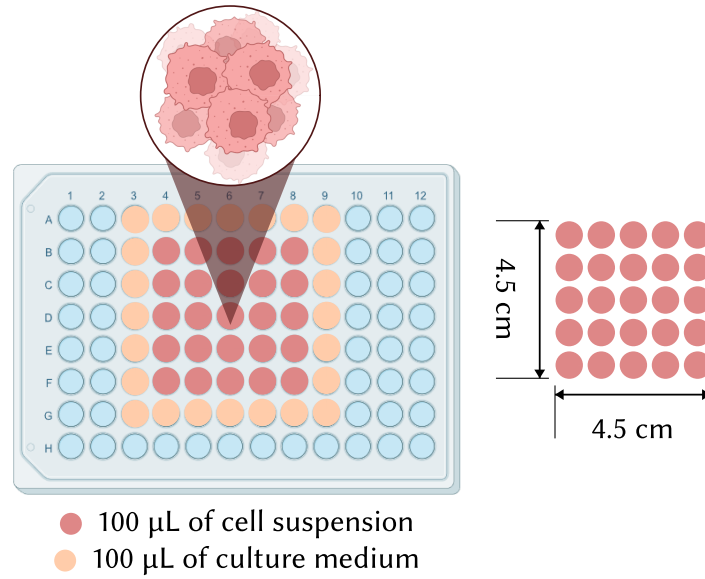


Figure 3.3: Layout of the 96-well plate showing the centered 5×5 seeding grid.

3.2.1.2 Irradiation of cell cultures at the Trento Proton Therapy Center

Three irradiation campaigns involving proton exposures of cell cultures were carried out at the Trento Proton Therapy Center (Agenzia Provinciale per i Servizi Sanitari, APSS), using the beam line dedicated to radiobiology research. All experimental activities conducted in the irradiation room were supervised by the Trento Institute for Fundamental Physics and Applications (TIFPA).

The radiobiology-dedicated beam line is based on a passive scattering delivery system (see Fig. 3.4), in which lateral beam modulation is achieved by means of a dual-ring scattering system, while energy modulation is performed using a custom-designed modulator. For the irradiation experiments, a 148 MeV proton beam was employed. Under this passive scattering configuration, the beam produces an irradiation field with an approximate diameter of 6 cm at the target position, which is sufficiently large to ensure dose delivery with a uniformity of about 98% [101]. All irradiations were performed at a constant nominal beam current, measured at the cyclotron exit, of approximately 40 nA and a dose rate of about 0.8 Gy/min.

The experimental setup adopted for the radiobiological experiments is illustrated in Fig. 3.4. As shown in the figure, the irradiation of the 96-well plates was performed from the bottom of the plate. This configuration was intentionally selected to ensure accurate definition of the proton range at the cell layer, leveraging the well-characterized material composition and thickness of the plate base. Irradiation from the top would have required the proton beam to traverse a non-uniform water column (the culture medium above the cells), complicating both homogeneous dose delivery and precise control of the proton range.

In addition, two Gafchromic EBT-XD films of $6.5 \times 6.5 \text{ cm}^2$ (Gafchromic, Ashland Advanced Materials) were affixed to the bottom surface of the plate. These

3.2. Combined radiobiological-microdosimetric experiments at the Trento Proton Therapy Center

Exp.1					
P1		P2		P4	
Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells
0	50	0	50	0	50
0.5	50	0.5	50	0.5	50
1	50	1	50	1	50
2	150	2	150	2	150
3.5	300	3.5	300	3.5	300
5	500	5	500	5	500
Exp.2					
P1		P2		P4	
Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells
0	50	0	50	0	50
0.5	50	0.5	50	0.5	50
1	50	1	50	1	50
2	150	2	150	2	150
3.5	300	3	300	3	300
4.5	500	4	500	4	500
Exp.3					
P1		P3		P5	
Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells	Dose [Gy]	Seeded cells
0	50	0	50	0	50
0.5	50	0.5	50	0.5	50
1	50	1	50	1	50
2	150	2	150	2	150
3	300	3	300	2.5	300
4.5	500	4	500	3	500

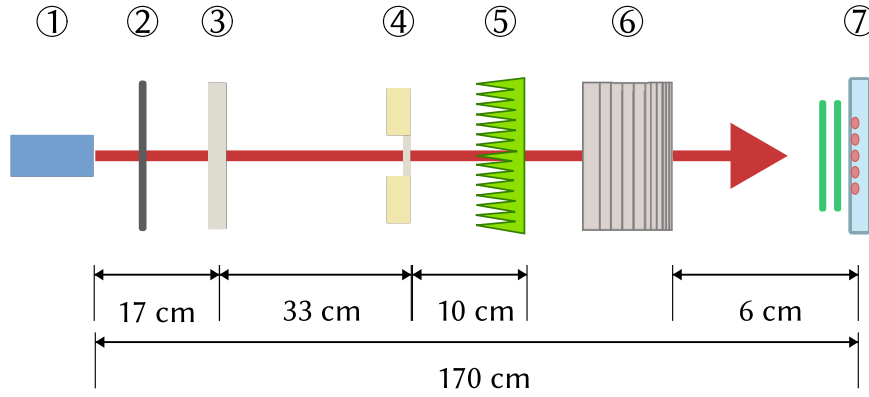
Table 3.1: Summary of the three experiments, including selected irradiation positions (P1–P5), planned doses, and the number of cells seeded per dose.

films served two purposes: to verify that the proton field reaching the cell monolayer matched that of the microdosimetric detector (see Sec. 3.2.3.1 for details), and to assess the surface dose distribution across the irradiated area and confirm that each well of the 96-well plate received the same dose.

Figure 3.5 presents the depth–dose and dose-averaged LET (LET_D) profiles along the central beam axis together with the selected measurement positions, P1–P5. These quantities were obtained from MC simulations performed with PHITS (see Sec. 3.2.2 for further details).

The different irradiation positions were obtained by combining layers of solid water of predefined thicknesses (RW3 (IBA³), $\rho = 1.032 \text{ g/cm}^3$) placed between the ridge filter and the irradiation position. Table 3.2 summarizes the thicknesses used to reach each position and the corresponding water-equivalent (WE) depth

³iba-dosimetry.com/product/solid-phantoms



- ① Beam pipe ④ Dual ring ⑦ Irradiation position
 ② First scattering foil ⑤ Ridge filter 96-well plate + 2
 ⑥ Solid water gafchromic films

Figure 3.4: Experimental set-up at the Trento Proton Therapy Center.

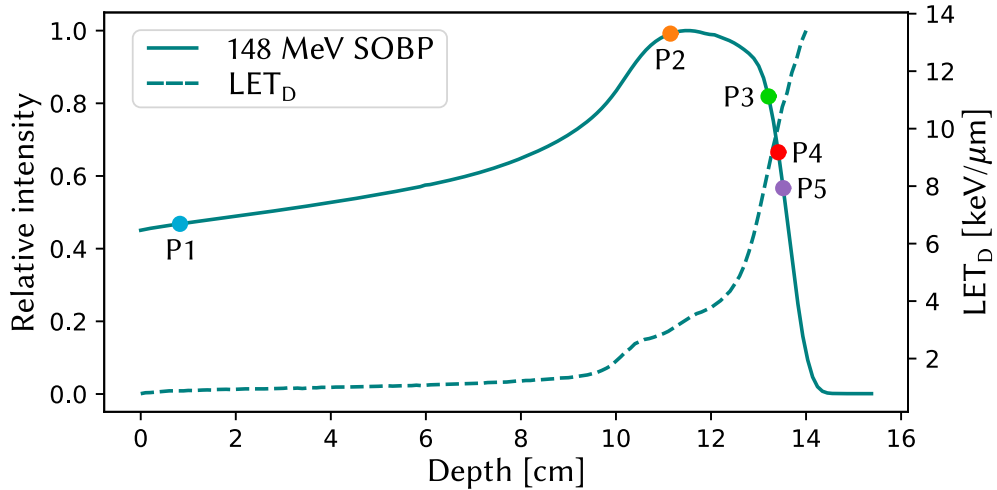


Figure 3.5: Relative depth-dose profiles and the depth profile of dose-averaged LET (LET_D) along the central axis of the SOBP, simulated with PHITS. The five selected irradiation positions are indicated.

calculated according to the procedure the presented in Sec. 3.2.3.1.

	P1	P2	P3	P4	P5
Solid water layer (RW3) [mm]	6	106	126	128	129
WE depth [mm]	8.22	111.42	132.06	134.13	135.16

Table 3.2: Summary of solid water thicknesses used to reach each irradiation position and the water-equivalent (WE) depth.

3.2. Combined radiobiological-microdosimetric experiments at the Trento Proton Therapy Center

In each irradiation campaign, a total of fifteen 96-well plates were irradiated (see Table 3.1). The cell cultures were maintained in an incubator available at the facility both before and after irradiation.

Control cell cultures were maintained under the same conditions as the irradiated samples, including transport from CIBIO to the proton therapy center facility, ensuring identical pressure, temperature, and humidity.

After irradiation, the samples were transported back to the CIBIO facility, where the 96-well plates were maintained in a tissue culture incubator for an additional 7 days undisturbed. After this time, a clonogenic assay was performed on all samples, as detailed in Sec. 3.2.1.3.

3.2.1.3 Clonogenic assay

Following the incubation period described above, a clonogenic assay, described in Sec. 1.2.3, was performed on all samples, including both irradiated and control cell cultures. Figure 3.6 shows a schematic representation of the clonogenic assay performed.

For this assay, cell colonies were stained using a 0.5% crystal violet solution in a 20/80 methanol/water mixture for approximately 20–25 minutes at room temperature. After staining, the solution was carefully removed, wells were gently rinsed several times with distilled water, and plates were left to air-dry overnight under a chemical hood.

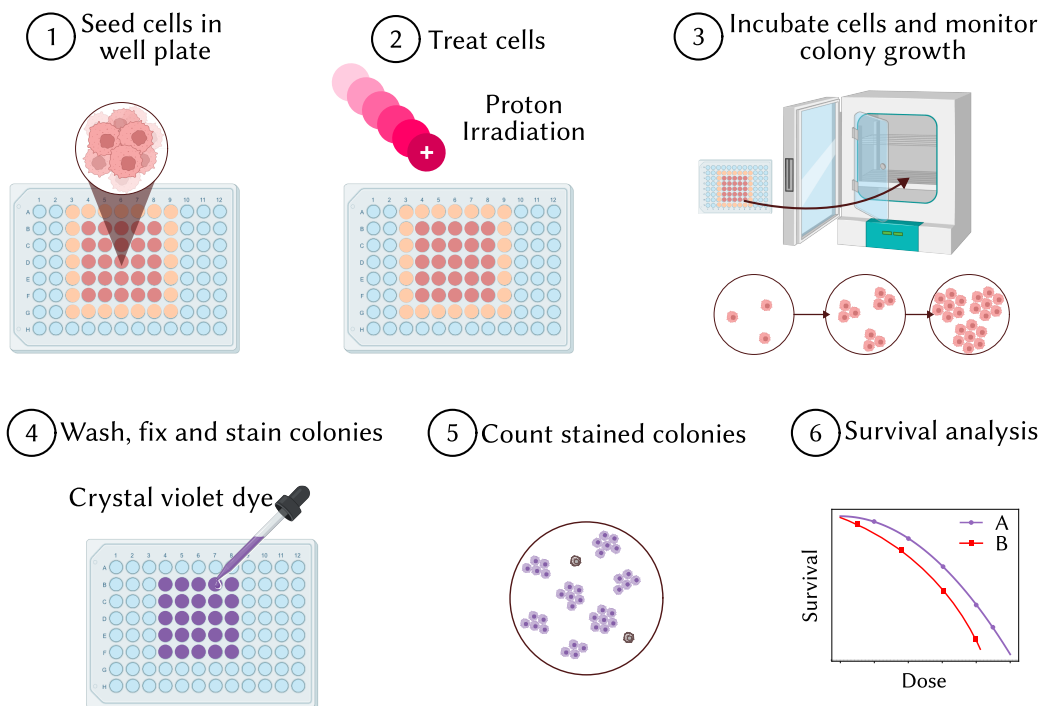


Figure 3.6: Experimental workflow of the clonogenic assay, adapted from a scheme developed by A. Faggian.

Colony counting was subsequently performed using the *Cell Counter* plugin⁴ available in ImageJ. This tool enabled rapid manual counting of colonies and allowed the results to be exported as CSV files, facilitating subsequent data processing and analysis in Python (version 3.8.5). To minimize observer bias, colony counting was performed independently by two researchers.

3.2.1.4 Construction of survival curves and derivation of radiobiological parameters α and β

The construction of the survival curves involved several sequential steps. First, the experimental data were processed through an outlier removal procedure. Subsequently, the plate efficiency (PE) was calculated for each irradiation point in each experimental campaign. The results from the three irradiation campaigns were then combined to build a single survival curve for each irradiation point. Finally, the radiobiological parameters were derived by fitting the resulting survival curves at each point.

In the following, each of these steps will be described in detail.

Outlier analysis Based on the colony counts obtained from the clonogenic assay, the resulting data were analyzed for each irradiation point and for each experimental campaign. The analysis included a data-cleaning step aimed at identifying and removing outliers. For a given irradiation point P_i and delivered dose D_{P_i} , data points were classified as outliers if they deviated by more than $\pm 2\sigma$ from the corresponding mean value, for that irradiation point and experiment.

Calculation of PE and cell survival After outlier removal, the plate efficiency was calculated for each irradiation point (P1–P5) and for each experiment (Exp. 1–3) according to Eq. 1.8.

Once the PE was determined, the cell surviving fraction, SF, was calculated for each irradiation point (P1–P5) and for each experimental campaign (Exp. 1–3, Table 3.1). The surviving fraction was evaluated on a well-by-well basis according to Eq. 1.10:

$$SF_i^{(P_d, \text{Exp}_k)} = \frac{\langle N_{\text{col}, i} \rangle}{\langle N_{\text{seed}, i} \rangle \langle \text{PE}_{P_d, \text{Exp}_k} \rangle}, \quad (3.15)$$

where $\langle N_{\text{col}, i} \rangle$ is the number of colonies counted in the i -th well, $\langle N_{\text{seed}, i} \rangle$ is the number of cells initially seeded in that well, and $\langle \text{PE}_{P_d, \text{Exp}_k} \rangle$ denotes the plate efficiency corresponding to irradiation point P_d in experimental campaign Exp_k .

Results from all experimental campaigns were then combined to construct a single survival curve for each irradiation point. This yielded one unified survival curve for each position along the SOBP, for a total of five curves.

⁴<https://imagej.net/ij/plugins/cell-counter.html>

Derivation of the radiobiological parameters α and β For each irradiation point, the cell survival data were fitted to the LQ model (see Sec. 1.2.3), from which the radiobiological parameters α and β were obtained. These parameters correspond to the right-hand side values of Eqs. 3.8 and 3.14 in the COMPhID model.

The fitting procedure was performed in Python using the Orthogonal Distance Regression (ODR) method implemented in the SciPy library [102]. This approach accounts for uncertainties in both the independent variable (dose) and the dependent variable (cell survival), ensuring a proper propagation of experimental and dosimetric uncertainties to the fitted parameters. In particular, the uncertainty associated with the dose values used to fit the survival curves was defined based on the dose uniformity analysis described in Sec. 3.2.1.5 and reflects the spatial non-uniformity of the delivered dose within the irradiated region.

3.2.1.5 Dosimetric characterization

The dosimetric characterization of the radiobiological and microdosimetric experiments was performed through two complementary approaches:

- *Measurement of the absorbed dose at each irradiation position:* The absolute absorbed dose was determined using the PPC05(IBA⁵) ionization chamber (in-air ionization chamber, active volume: 0.05 cm³ for absolute dose measurements in a water phantom or in free air), providing point-dose measurements at the selected irradiation depths.
- *Assessment of the transverse dose distribution of the proton beam:* The lateral dose uniformity was evaluated using pre-calibrated Gafchromic EBT-XD films with dimensions of 6.5 × 6.5 cm² (Gafchromic, Ashland Advanced Materials), allowing for a two-dimensional mapping of the dose in the irradiation plane.

The following sections describe each approach used for the dosimetric characterization in detail.

Measurement of the absorbed dose at each irradiation position As part of the dosimetric characterization, the absorbed dose was first determined using the PPC05 ionization chamber. For each experimental session, a dedicated calibration was performed prior to the irradiations. This calibration consisted of measuring the absorbed dose at the selected irradiation positions (P1–P5) along the central axis of the proton beam, thereby determining the number of protons required to deliver a dose of 1 Gy at each position.

Based on this calibration, the number of protons needed to deliver a planned dose at position P_d was calculated as

$$\# \text{ of protons} = D_{P_d} \cdot K_{P_d}^{\text{Gy}}. \quad (3.16)$$

⁵iba-dosimetry.com/product/ppc05-plane-parallel-chamber

Here, $K_{P_d}^{\text{Gy}}$ ($d = 1 \dots 5$) is the position-dependent calibration factor, representing the number of protons passing through the monitor chamber required to deliver 1 Gy at P_d for the planned dose D_{P_d} (see Table 3.1).

All measurements were corrected for ambient pressure and temperature, as the response of the ionization chamber depends on these environmental conditions.

Once the calibration was completed, the number of protons required to deliver the planned dose was calculated for each irradiation according to Eq. 3.16. This number was monitored online through the monitor chamber, while beam delivery was initiated and stopped using a beam shutter system to precisely define the irradiation duration.

Assessment of the transverse dose distribution of the proton beam In the second approach, $6.5 \times 6.5 \text{ cm}^2$ pre-calibrated Gafchromic EBT-XD films, specifically designed for best performance in the dose range from 0.4 to 40 Gy, were used in each irradiation to investigate the dose uniformity in the transverse plane of the proton beam. A proper analysis of the dose distribution using Gafchromic films requires a prior calibration at each irradiation position, since variations in radiation quality along the beam path lead to changes in the film response and, consequently, in the corresponding calibration curve.

In the present work, due to limitations in beam availability, the Gafchromic film calibration was performed only at a single irradiation position. As a result, the absolute dose values obtained by applying this calibration to films irradiated at other positions are not quantitatively valid. For this reason, Gafchromic films were employed exclusively to assess the spatial uniformity of the delivered dose across the transverse beam plane, rather than to determine the absolute absorbed dose.

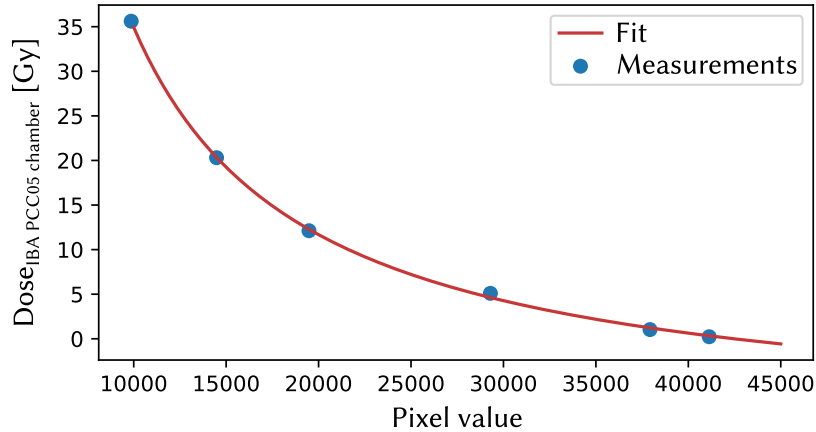


Figure 3.7: Calibration curve of the absorbed dose measured with the IBA PPC05 ionization chamber as a function of the pixel value associated with the Gafchromic film response to the delivered dose. The relative standard deviation at each fit point is $\sim 3\%$. Data provided by A. Fasolini.

This calibration was carried out by the TIFPA group. During the calibration

3.2. Combined radiobiological-microdosimetric experiments at the Trento Proton Therapy Center

procedure, absorbed dose measurements were obtained using the IBA PPC05 ionization chamber positioned downstream of the Gafchromic film, providing a reference dose for the calibration process.

After irradiation, the films were digitalized using an Epson Perfection V750 Pro flatbed scanner. Scans were acquired in transmission mode at a spatial resolution of 72 dpi. Image analysis was performed exclusively on the red channel of the 48-bit RGB images, which provides the highest sensitivity for Gafchromic EBT-XD films. The resulting TIF (Tagged Image File) images were processed using the ImageJ software [103] to extract the spatial distribution of pixel values over the XY plane, thereby obtaining a two-dimensional pixel value, pv , distribution for each film.

The resulting data were fitted using a function of the form

$$f(pv) = \frac{a}{pv + b} + c, \quad (3.17)$$

where the fitted parameters were $a = (4.20 \pm 0.24) \times 10^5$, $b = -653 \pm 430$, and $c = -10.1 \pm 0.6$. The set of experimental calibration points together with the corresponding fit are shown in Fig. 3.7.

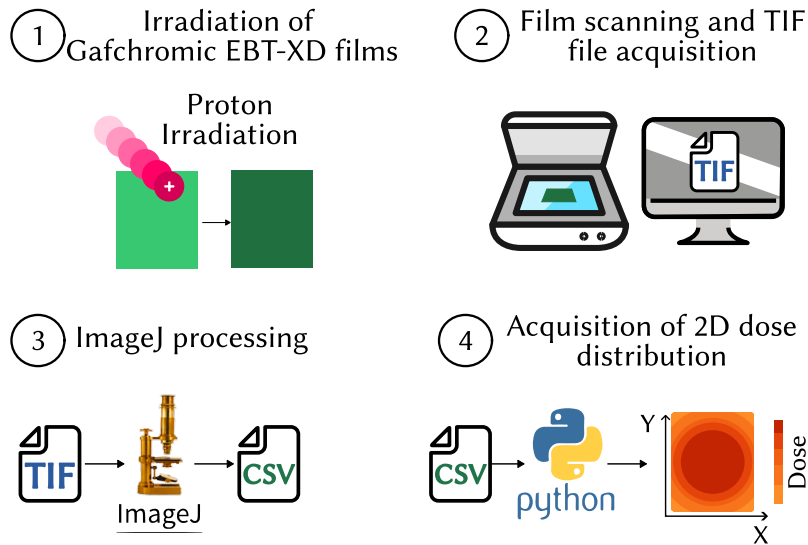


Figure 3.8: Schematic overview of the procedure to obtain the 2D dose distribution from Gafchromic film irradiations.

Following each irradiation experiment, the two-dimensional dose distribution of each film was obtained by processing the data in Python, using the same procedure as in the calibration, schematically illustrated in Fig. 3.8.

Once the two-dimensional dose distribution was obtained, the dose uniformity was investigated by analyzing representative dose profiles along the x and y axes, corresponding to the 5×5 seeding grid. The uniformity for each profile was calculated according to the following expression:

$$U[\%] = 100 \cdot \left(1 - \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}} \right), \quad (3.18)$$

where $I_{\min}$ and $I_{\max}$ denote the minimum and maximum pixel intensity values within the region of interest, respectively.

3.2.2 Monte Carlo simulations in PHITS

The geometry of the experimental setup and the beam characteristics were implemented in the Monte Carlo code PHITS (version 3.341), as illustrated in Figs. 3.4 and 3.12. This implementation was obtained by translating the geometry and beam configuration originally developed in the Geant4-based Monte Carlo tool TOPAS into the PHITS framework [104].

In TOPAS (based on Geant4, physics list G4EmStandardPhysics_option4), the electronic stopping power of protons is modeled using the Bethe-Bloch formalism for energies above 2 MeV, while the Bragg ionization model is employed at lower energies to accurately reproduce energy loss near the end of the proton range. In contrast, PHITS relies on the ATIMA code⁶, developed at GSI, which calculates the slowing down of protons and heavy ions in matter over a wide energy range, from 1 keV/u up to 450 GeV/u.

Comparative studies have shown that, in the energy range between approximately 1 MeV/u and 100 MeV/u, the stopping power calculated using the ATIMA code can be higher than that obtained with Geant4/TOPAS models [105]. To reproduce in PHITS the depth-dose profile previously calculated with TOPAS, the initial proton beam energy was therefore adjusted from a nominal value of 147.8 MeV (used in TOPAS) to 148.4 MeV in PHITS. In addition, the density of the ridge filter material was modified from 1.4 g/cm³ in TOPAS to 1.2 g/cm³ in the PHITS code.

Figure 3.9 compares the depth-dose profiles obtained with TOPAS and PHITS.

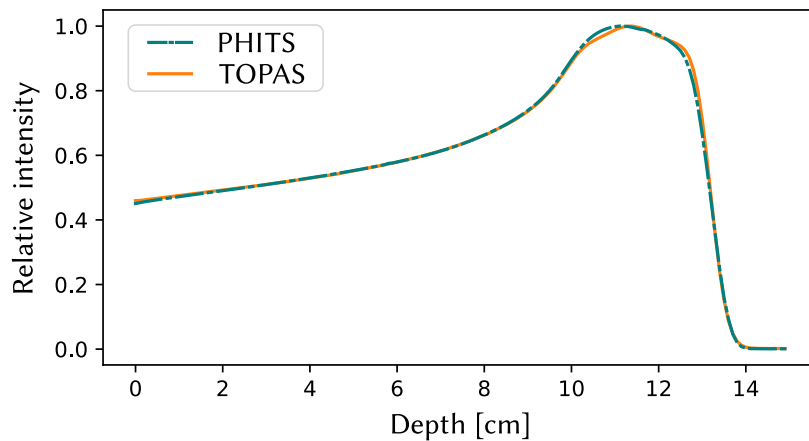


Figure 3.9: Comparative Depth-Dose Profiles between PHITS and TOPAS after adjusting the beam energy and ridge filter density in PHITS.

⁶<https://web-docs.gsi.de/~weick/atima/>

As can be observed, very small discrepancies in the shape of the SOBP are present between the simulations. These differences arise from the distinct ways in which the ridge filter geometry was implemented in the two MC codes.

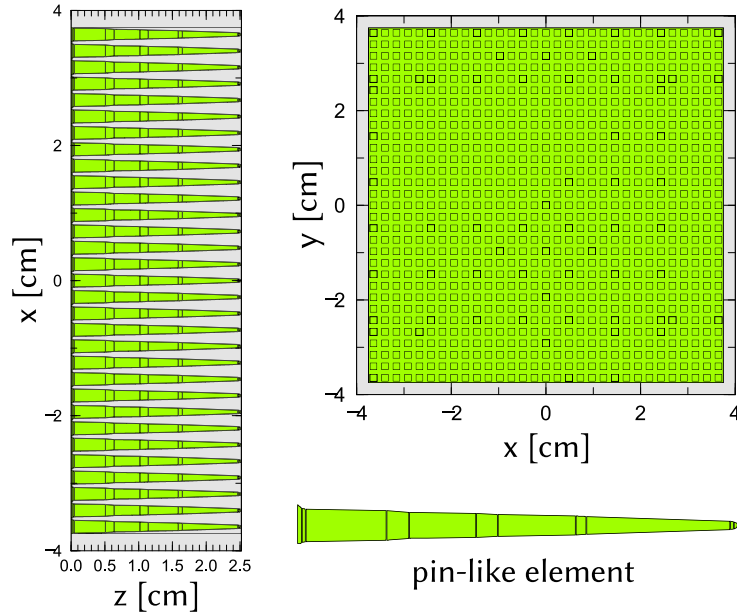


Figure 3.10: Geometry of the ridge filter simulated in PHITS. Left: lateral view showing the pin-like elements forming the structure. Top right: top view displaying the 61×61 pin arrangement. Bottom right: schematic representation of a single pin-like element, illustrating its trapezoidal composition.

The ridge filter consists of a 61×61 matrix of pin-like elements, where each individual pin is composed of ten trapezoidal segments (see Fig. 3.10). In TOPAS, the geometry is defined through C++ classes that are compiled together with the Geant4 kernel. Within this framework, a dedicated trapezoidal macro body is available, allowing each segment to be defined analytically and the complete pin structure to be constructed by the consecutive alignment of these macro bodies with high geometrical precision.

In contrast, PHITS does not currently provide a native trapezoidal macro body. As a consequence, each trapezoidal segment of the ridge filter pins must be approximated through the intersection of six planar surfaces. This alternative geometrical construction introduces small differences in the effective shape and material distribution of the ridge filter elements, which propagate into the simulated energy modulation and ultimately affect the resulting depth-dose profile in the SOBP region.

Once the geometry of the experimental setup and the beam characteristics had been implemented in PHITS, the following quantities were obtained through MC simulations:

- The absorbed dose and particle fluence, considering all radiation fields, were

scored using the tallies [T-deposit] and [T-track], respectively, in a solid water (RW3) phantom. Specifically, a $30 \times 30 \times 30 \text{ cm}^3$ phantom was simulated and discretized into cubic voxels with a 1 mm edge length. Both the phantom geometry and the scoring mesh were defined to be identical to those used in TOPAS, in order to enable a consistent comparison between the two MC tools. In both simulation frameworks, a total of 10^8 primary particles were simulated to ensure adequate statistical accuracy.

- From the tally outputs mentioned above, the dose-averaged linear energy transfer, LET (L_D), was calculated following the procedure described in Sec. 2.2.1.4.
- Microdosimetric spectra at the five selected positions along the SOBP (P1–P5) were calculated using the [T-Sed] tally available in PHITS. This tally enables the calculation of the probability density of energy deposition in microscopic sites using a mathematical formalism that evaluates energy deposition in the vicinity of charged-particle trajectories. The method is based on track-structure simulations and explicitly accounts for the production of δ -rays and Auger electrons [106, 107].

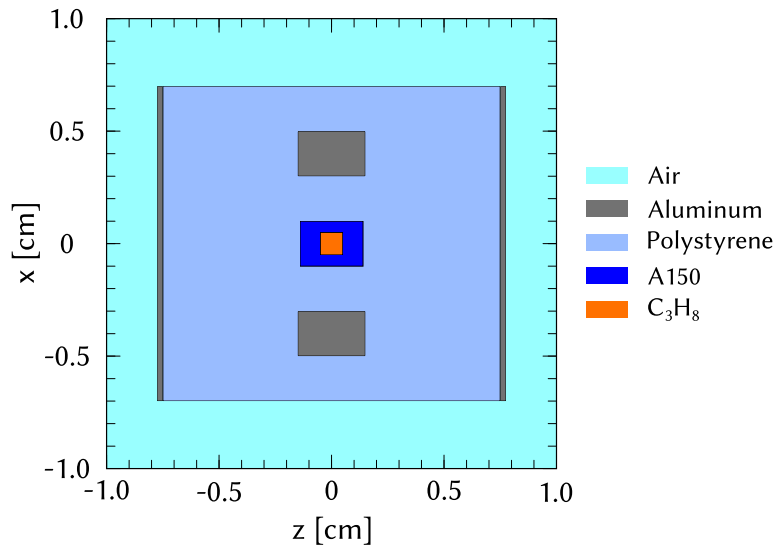


Figure 3.11: Axial view of the mini-TEPC geometry implemented in PHITS.

Using this approach, microdosimetric spectra accounting for all particle contributions were obtained in the form of lineal energy distributions, $yf(y)$. The scoring region was defined as the sensitive volume of the mini-TEPC detector implemented in PHITS (see Fig. 3.11). A biological site diameter of $1 \mu\text{m}$ was assumed for the calculation of the lineal energy distributions, consistent with the experimental sensitive site size of the mini-TEPC detector described in Sec. 3.2.3.1.

The calculations were performed employing the latest model developed by the PHITS group for the calculation of the probability density of energy

deposition in microscopic sites. This model was applied assuming water as the target material, as it was specifically developed and validated for this medium [108].

3.2.3 Microdosimetric experiments

In order to determine the first three moments of lineal energy, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, that constitute the microdosimetric input required by the COMPhID model (see Eqs. 3.8 and 3.14), a series of measurements of microdosimetric spectra were carried out following an experimental configuration similar to that adopted for the radiobiological experiments described in Sec. 3.2.1.2. The procedure followed to obtain the microdosimetric spectra is presented in the following sections.

3.2.3.1 Irradiation of a mini-TEPC at the Trento Proton Therapy Center

The microdosimetric measurement campaign was carried out in collaboration with Anna Bianchi and Valeria Conte (INFN, Laboratori Nazionali di Legnaro, LNL). Measurements were performed at the selected irradiation positions (P1–P5) along the SOBP, as in the radiobiological experiment (Sec. 3.2.1.2).

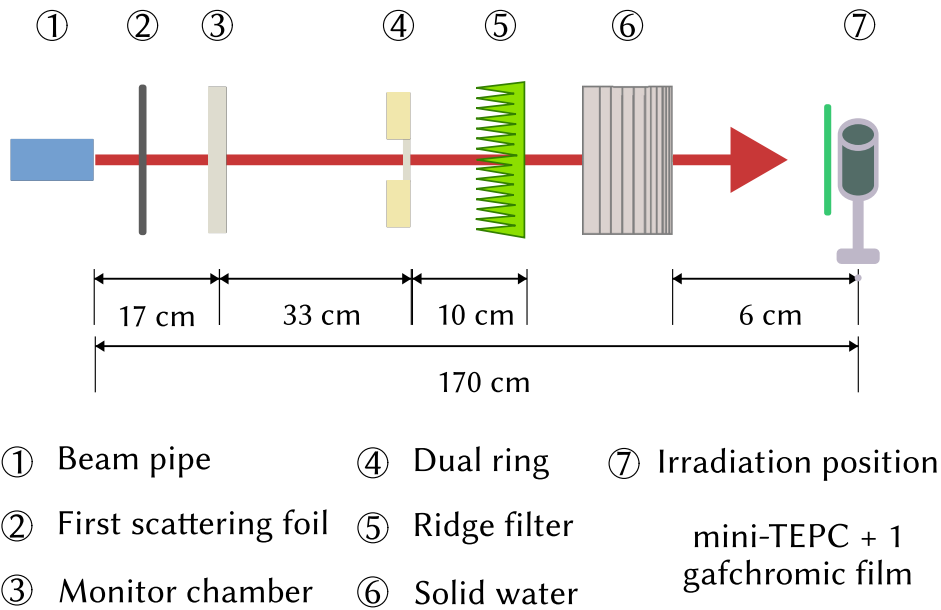


Figure 3.12: Experimental set-up corresponding to the microdosimetric experiment, where the thickness of solid water varies as specified in Table 3.4.

Figure 3.12 shows the experimental setup used for the microdosimetric measurements. These were performed using a mini-TEPC (see Sec. 1.3.1.2) positioned along the central axis of the proton beam, with its alignment ensured by means of a laser positioning system available at the facility. The detector, developed at the INFN Laboratori Nazionali di Legnaro by the group led by V. Conte, features a cylindrical sensitive volume (SV) with equal height and diameter of 1 mm. This

SV was filled with pure propane at a pressure of 408 mbar to simulate a biological site of about $1\ \mu\text{m}$ in diameter. The cathode wall is made of 0.9 mm-thick tissue-equivalent A150 conductive plastic and is surrounded by 6 mm of polystyrene, resulting in a water-equivalent wall thickness of 7.8 mm. A schematic representation of the mini-TEPC is shown in Fig. 3.13. More details about this microdosimetric detector can be found in (Bianchi et al., 2023) [109].

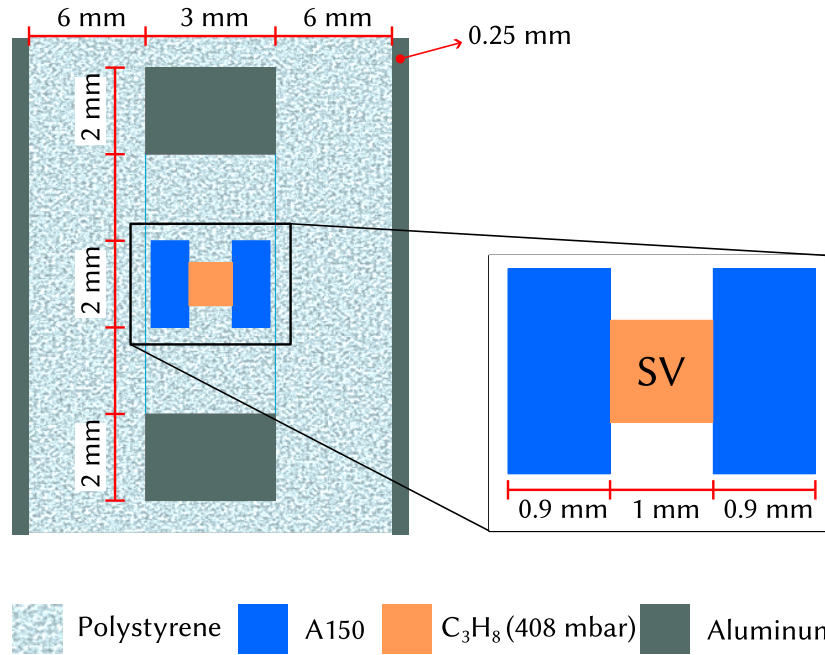


Figure 3.13: Schematic view of the mini-TEPC geometry and materials. The sensitive volume (SV) is presented in orange.

As shown in Fig. 3.12, a Gafchromic EBT-XD film was placed between the detector and the solid water slabs in order to study the dose distribution in the transverse plane, as discussed in Sec. 3.2.1.5.

On the other hand, the thicknesses of the RW3 solid water layers were calculated so that the measurement positions P1–P5 coincided with the irradiation positions used in the radiobiological experiment. To this end, the water-equivalent (WE) thickness was determined using the ratio of the stopping powers of the different materials relative to water, as provided by the NIST PSTAR database [110].

Figure 3.14 schematically illustrates all the materials traversed by the proton beam before reaching the target (the cell culture monolayer in the radiobiological experiment or the sensitive volume of the mini-TEPC in the microdosimetric measurements). Table 3.3 summarizes the different materials traversed by the proton beam downstream of the ridge filter to reach position P1 in both the radiobiological and microdosimetric experiments, together with the corresponding calculation of the WE thickness. Material densities were obtained from the NIST STAR database ⁷.

⁷physics.nist.gov/cgi-bin/Star/compos.pl

3.2. Combined radiobiological-microdosimetric experiments at the Trento Proton Therapy Center

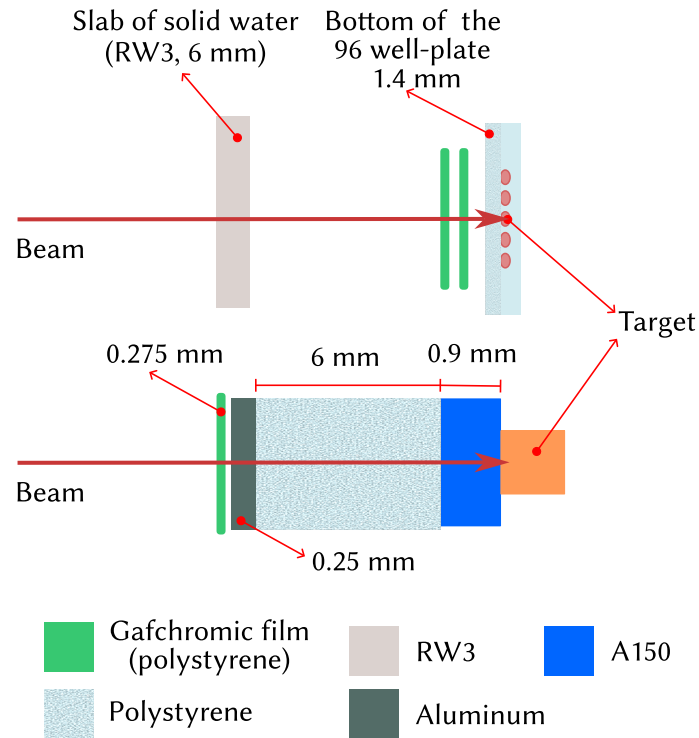


Figure 3.14: Schematic representation of the materials traversed by the proton beam downstream of the ridge filter until reaching the target, either the cell culture monolayer in the radiobiological experiment or the sensitive volume of the mini-TEPC in the microdosimetric experiment.

	Material	ρ [g/cm ³]	Thickness [mm]	WE _{thickness} [mm]	Total WE _{thickness}
Radiobiological experiment	RW3	1.032	6.00	6.19	8.22
	Gaf. films ×2	1.060	0.55	0.57	
	Polystyrene	1.060	1.40	1.46	
Microdosimetric experiment	Gaf. films ×1	1.060	0.275	0.29	8.08
	Aluminum	2.699	0.25	0.49	
	Polystyrene	1.060	6.00	6.27	
	A150	1.127	0.90	1.03	

Table 3.3: Summary of the materials crossed by the proton beam downstream of the ridge filter to reach position P1 in both the radiobiological and microdosimetric experimental setups, together with the calculated water-equivalent (WE) thickness.

As shown in Table 3.3, there is a difference of approximately 0.15 mm in water-equivalent (WE) thickness between the two experimental configurations. However, this mismatch does not produce a significant impact on the resulting micro-

dosimetric distributions and can therefore be considered negligible for the purposes of the present analysis.

Based on this analysis, the thickness of the solid water (RW3) layers required in the microdosimetric experiment to reproduce each of the five selected positions (P1–P5) was determined. These values are summarized in Table 3.4. In addition, this table reports the polarization voltage applied to the detector for each position, the beam current, and the number of recorded events at each irradiation point.

Positions	P1	P2	P3	P4	P5
Voltage [V]	750	720	720	720	720
Current [nA]	10	10	20	25	30
Thickness of RW3 [cm]	0	10	12	12.2	12.3
# events	1.00E+07	1.00E+06	1.00E+06	6.00E+06	6.00E+06

Table 3.4: *Experimental parameters of the microdosimetric measurements at the five selected positions along the SOBP (P1–P5), including the detector polarization voltage, beam current measured at the cyclotron exit, solid water (RW3) thickness, and number of recorded events at the mini-TEPC.*

As can be observed in Table 3.4, the number of events recorded by the detector at position P1 is higher than at the other positions (P2–P5). This is because higher statistics are required at this depth to properly resolve the contribution of secondary fragments, particularly alpha particles. This contribution is especially relevant in the entrance region, as discussed in Sec. 2.3.2.1. For the subsequent positions, from P2 to P5, the microdosimetric distributions are expected to progressively shift toward higher lineal energy values with increasing depth. This behavior reflects the increasing ionization density of protons as they slow down in matter, which progressively dominates the spectra and reduces the relative contribution of secondary particles.

It can also be observed that the beam current was increased from P1 to P2 in order to maintain an approximately constant counting rate of about 10^4 counts per second at the detector.

In addition, for all irradiation positions, lateral scans of the detector sensitive volume were performed with respect to the central beam axis, by shifting it by ± 2.4 cm in the transverse plane. Microdosimetric spectra were measured at these off-axis positions to verify the uniformity of the radiation field. This procedure ensured that all wells of the 96-well plate containing the cell cultures were exposed to the same radiation field and, consequently, to the same microdosimetric spectrum (see Fig. 3.3).

After completion of the microdosimetric measurement campaign, the lineal energy calibration of the mini-TEPC and the subsequent data processing were carried out by A. Bianchi. Further details on these procedures can be found in

[109, 111].

3.2.3.2 Statistical uncertainty estimation of microdosimetric spectra

Microdosimetric spectra are obtained by counting individual stochastic energy-deposition events in a sensitive volume. Since these events occur independently and randomly, the number of events recorded in each bin can be described by Poisson statistics [112]. Under this assumption, the statistical uncertainty of each bin of the microdosimetric spectrum was estimated directly from the number of recorded events.

Let y_i denote the center of bin i , and $f(y_i)$ the normalized probability density, such that

$$\sum_i f(y_i) \Delta y_i = 1, \quad (3.19)$$

where Δy_i is the bin width. The total number of recorded events, N_{tot} , is reported in Table 3.4 as # events.

The number of events associated with each bin is obtained from the normalized probability density as

$$N_i = N_{\text{tot}} f(y_i) \Delta y_i. \quad (3.20)$$

Assuming Poisson counting statistics, the standard deviation associated with the number of events in each bin is

$$\sigma_{N,i} = \sqrt{N_i}. \quad (3.21)$$

Therefore, the uncertainty associated with the spectral density is

$$\sigma_f(y_i) = \frac{\sigma_{N,i}}{N_{\text{tot}} \Delta y_i} = \frac{\sqrt{N_i}}{N_{\text{tot}} \Delta y_i}. \quad (3.22)$$

This uncertainty represents the statistical fluctuation associated with the number of events recorded in each individual bin of the microdosimetric spectrum.

3.2.3.3 Calculation of the first three lineal energy moments

Once the microdosimetric spectra at the five selected positions were obtained in the form of lineal energy distributions, $y f(y)$, the first three moments of the lineal energy were calculated according to Eq. C.10. The parameter q was set to 1, 2, and 3, yielding the moments $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, respectively.

$$y^{(q)} = \int_0^\infty y^q f(y) dy \quad (3.23)$$

From the calculated lineal energy moments, the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ (Eqs. 3.8 and 3.14) were derived.

The statistical uncertainties associated with these quantities were obtained by propagating the Poisson-based uncertainties of the spectral density described above. The bin-wise statistical errors of $f(y)$, previously estimated, were propagated to the calculated moments and then to the derived ratios using standard uncertainty propagation.

3.2.4 Determination of the parameters of the COMPhID model

The model parameters, α_0 , α_1 , α_2 , β_0 , and β_1 , were estimated using the ODR method which, as described in Sec. 3.2.1.4, enables the incorporation of uncertainties in both the dependent and independent variables. In the present case, the independent variables correspond to the moment ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$.

After estimating the model parameters, the goodness of fit was evaluated by computing the reduced chi-square statistic, which is defined as

$$\chi^2 = \sum_{i=1}^N \frac{(Y_i^{\text{obs}} - Y_i^{\text{pred}})^2}{\sigma_i^2}, \quad (3.24)$$

where Y_i^{obs} and Y_i^{pred} denote the experimental and model-predicted values, respectively, σ_i represents the experimental uncertainty, and N is the number of data points. In the present work, N corresponds to the number of irradiation positions.

In the parameter estimation procedure, four of the five irradiation positions were used to determine the model parameters, while position P4 was intentionally excluded from the fitting dataset. This position was subsequently used as an independent validation point, where the experimentally measured microdosimetric spectrum was employed to estimate the corresponding radiobiological parameters (α and β) and assess the predictive capability of the COMPhID model. Consequently, the parameter estimation was performed using $N = 4$ irradiation positions.

The reduced chi-square is given by

$$\chi_v^2 = \frac{\chi^2}{N - k}, \quad (3.25)$$

where k is the number of fitted parameters and $N - k$ corresponds to the number of degrees of freedom. For the α model (Eq. 3.8), $k = 3$, resulting in $\nu = 1$ degrees of freedom. For the β model (Eq. 3.14), $k = 2$, leading to $\nu = 2$ degrees of freedom.

Once the model parameters were determined, the ability of the model to reproduce the experimentally observed radiobiological parameters was evaluated. For this purpose, the model-predicted parameters $\langle \alpha_{\text{pred}} \rangle$ and $\langle \beta_{\text{pred}} \rangle$ were calculated according to Eqs. 3.8 and 3.14. These values were then compared with the experimental radiobiological parameters, α_{obs} and β_{obs} , obtained as described in Sec. 3.2.1.4.

In addition the performance of the model was quantified by evaluating the prediction accuracy, defined as the percentage agreement between the predicted and experimental parameters. For the α parameter, the precision was calculated as

$$\text{Precision} = \left| 1 - \frac{\langle \alpha_{\text{pred}} \rangle - \alpha_{\text{obs}}}{\alpha_{\text{obs}}} \right| \times 100, \quad (3.26)$$

and similarly, the same applies to the β parameter.

3.2.5 Calculation of the Photon Isoeffective Dose with the COMPhID model

The photon isoeffective (IsoE) dose was calculated using ^{137}Cs photons as the reference radiation, with radiobiological parameters $\alpha_x = 0.290 \text{ Gy}^{-1}$ and $\beta_x = 0.083 \text{ Gy}^{-2}$ [113].

For every parameter set obtained as described in Sec. 3.2.4, the corresponding position-dependent $\langle \alpha \rangle$ and $\langle \beta \rangle$ coefficients were reconstructed according to equations 3.8 and 3.14. The photon IsoE dose was then computed by solving the quadratic LQ survival equation 3.4 under two scenarios: (I) a fixed survival level of 10% (RBE_{10}), and (II) a fixed proton absorbed dose of 2 Gy.

In scenario (I), the reference photon dose D_x was obtained analytically from the LQ survival equation using the radiobiological parameters corresponding to the ^{137}Cs reference irradiation. In scenario (II), the quadratic equation derived from the LQ model was solved to determine the photon IsoE dose corresponding to a fixed proton dose of 2 Gy. In all cases, the physically meaningful real root was retained.

Once these doses were determined, the RBE for each scenario was calculated as $D_{\text{ref}}/D_{\text{protons}}$, where the reference dose corresponds to the photon dose D_x in scenario (I) and to the photon IsoE dose in scenario (II).

The uncertainties reported for these quantities were obtained through uncertainty propagation, taking into account the uncertainties associated with the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, as well as the uncertainties of the COMPhID model parameters, calculated as described in Section 3.2.4.

3.2.6 Application of the COMPhID model

To illustrate the application of the COMPhID model, the photon isoeffective dose and the corresponding RBE were calculated under the two scenarios (I) and (II) described in the previous section. These quantities were evaluated using both an experimentally measured microdosimetric spectrum and a spectrum obtained from Monte Carlo simulations, with the aim of exploring the applicability range of the COMPhID model and assessing the consistency of the results with the experimental trends observed. The following irradiation positions were considered:

- **Position P4**, using the experimentally measured microdosimetric spectrum and the corresponding ratios of the lineal energy moments, $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, calculated as described in Sec. 3.2.3.3.
- **Photon IsoE doses along the SOBP**, the photon IsoE dose was calculated using the COMPhID model at the five irradiation positions (P1–P5). The corresponding RBE values were also determined as a function of depth, using ^{137}Cs photons as the reference radiation. These calculations were performed based on the SOBP obtained from PHITS, shown in Fig. 3.9, assuming a maximum absorbed dose of 2 Gy along the SOBP. In this case, only scenario (II) was considered, but with the difference that the absorbed proton dose is fixed and varies from P1 to P5.
- **Position Pn**, corresponding to a new irradiation position located between P2 and P3, considering a solid water (RW3) thickness of 11 cm (see Table 3.4). For this position, the microdosimetric spectrum was obtained through Monte Carlo simulations performed with the PHITS code following the procedure described in Section 3.2.2. Once the spectrum was obtained, the first three lineal energy moments and their corresponding ratios were calculated as described in Section 3.2.3.3.

Using the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ for these irradiation positions, the same calculations described in Section 3.2.5 were performed. The results obtained under these conditions were then compared with those derived from the experimental data in order to assess their consistency.

3.3 Results

3.3.1 Dosimetric characterization

As mentioned in Sec. 3.2.1.5, the dosimetric characterization was carried out using two complementary approaches. The first approach consisted of measuring the absorbed dose at each irradiation position with the PPC05⁸ plane parallel chamber, while the second relied on the use of pre-calibrated Gafchromic films to investigate the transverse dose distribution in the irradiation plane. The results obtained with each approach are presented below.

Measurement of the absorbed dose at each irradiation position Tables 3.5–3.7 summarize the dose measurements performed for the selected positions (P1–P5) during the three different experiments (see Table 3.1). For each position, the tables report the planned dose D_{P_i} (planned), the calibration factor $K_{P_i}^{\text{Gy}}$, the number of protons calculated according to Eq. 3.16, the number of delivered protons controlled through the manual beam shutter, and the resulting delivered dose D_{P_i}

⁸iba-dosimetry.com/product/ppc05-plane-parallel-chamber

(delivered). The latter corresponds to the dose used for the construction of the cell survival curves.

D_{P_1} (planned)	$K_{P_1}^{Gy}$	# protons	# delivered protons	D_{P_1} (delivered)
0.5	2.62E+11	1.31E+11	1.33E+11	0.51
1	2.62E+11	2.62E+11	2.63E+11	1.00
2	2.62E+11	5.24E+11	5.26E+11	2.01
3.5	2.62E+11	9.17E+11	9.17E+11	3.50
5	2.62E+11	1.31E+12	1.31E+12	5.00
D_{P_2} (planned)	$K_{P_2}^{Gy}$	# protons	# delivered protons	D_{P_2} (delivered)
0.5	1.26E+11	6.30E+10	6.40E+10	0.51
1	1.26E+11	1.26E+11	1.27E+11	1.01
2	1.26E+11	2.52E+11	2.53E+11	2.01
3.5	1.26E+11	4.41E+11	4.43E+11	3.52
5	1.26E+11	6.30E+11	6.32E+11	5.02
D_{P_4} (planned)	$K_{P_4}^{Gy}$	# protons	# delivered protons	D_{P_4} (delivered)
0.5	1.63E+11	8.15E+10	8.37E+10	0.51
1	1.63E+11	1.63E+11	1.63E+11	0.99
2	1.63E+11	3.26E+11	3.27E+11	2.00
3.5	1.63E+11	5.71E+11	5.71E+11	3.50
5	1.63E+11	8.15E+11	8.13E+11	4.99

Table 3.5: Summary of the dose measurements performed in Exp. 1 at positions P1, P2, and P4.

Overall, the delivered doses showed excellent agreement with the planned values across all positions and experiments. The relative differences between planned and delivered doses were generally below 2%, confirming the stability of the beam delivery and the reliability of the calibration procedure. A conservative dose uncertainty of 5% was adopted, based on the analysis of the transverse dose distribution, as will be discussed in the following section.

Assessment of the transverse dose distribution of the proton beam After all irradiations (cell cultures, microdosimetric detector and Gafchromic EBT-XD films), the corresponding films were processed according to the procedure illustrated in Fig. 3.8 to obtain the two-dimensional dose distribution.

For the radiobiological experiment, Fig. 3.15 shows the positioning of the 96-well plate downstream of the solid water layers. In this image, the dashed yellow lines delineate the 5×5 seeding grid, which defines the irradiation target area. The right panel of the figure illustrates the fixation of the two Gafchromic films at the base of the plate, so that they were located between the 96-well plate and the solid water layers.

D_{P_1} (planned)	$K_{P_1}^{Gy}$	# protons	# delivered protons	D_{P_1} (delivered)
0.5	2.50E+11	1.25E+11	1.28E+11	0.51
1	2.50E+11	2.50E+11	2.49E+11	0.99
2	2.50E+11	5.00E+11	5.02E+11	2.01
3	2.50E+11	7.50E+11	7.52E+11	3.01
4.5	2.50E+11	1.13E+12	1.15E+12	4.60
D_{P_2} (planned)	$K_{P_2}^{Gy}$	# protons	# delivered protons	D_{P_2} (delivered)
0.5	1.21E+11	6.05E+10	6.35E+10	0.52
1	1.21E+11	1.21E+11	1.25E+11	1.03
2	1.21E+11	2.42E+11	2.42E+11	2.00
3	1.21E+11	3.63E+11	3.66E+11	3.02
4	1.21E+11	4.84E+11	4.84E+11	4.00
D_{P_4} (planned)	$K_{P_4}^{Gy}$	# protons	# delivered protons	D_{P_4} (delivered)
0.5	1.55E+11	7.75E+10	8.20E+10	0.53
1	1.55E+11	1.55E+11	1.52E+11	0.98
2	1.55E+11	3.10E+11	3.10E+11	2.00
3	1.55E+11	4.65E+11	4.65E+11	3.00
3.5	1.55E+11	5.43E+11	5.46E+11	3.52

Table 3.6: Summary of the dose measurements performed in Exp. 2 at positions P1, P2, and P4.

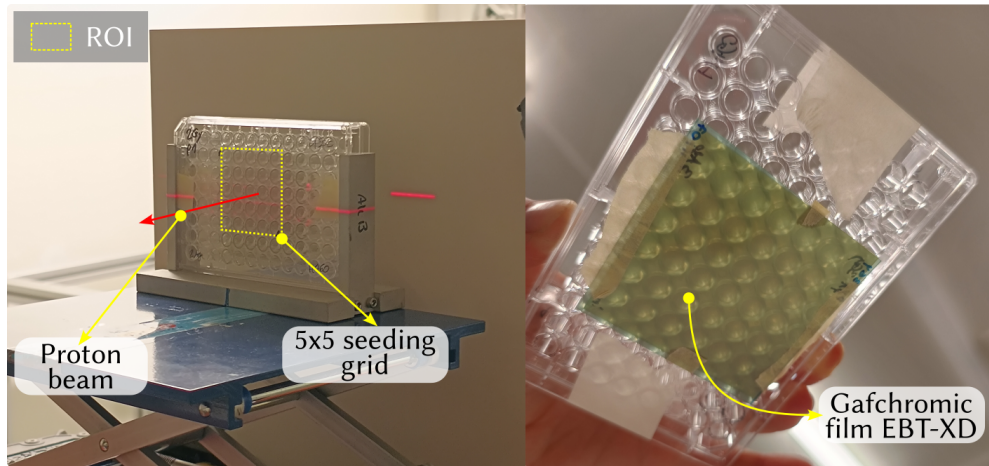


Figure 3.15: Positioning of the 96-well plate downstream of the solid water layers. The dashed yellow lines indicate the 5×5 seeding grid defining the irradiation area, while the right panel shows the placement of the two Gafchromic films fixed at the bottom of the plate.

Figure 3.16 shows a representative example of the analysis performed on all

D_{P_1} (planned)	$K_{P_1}^{Gy}$	# protons	# delivered protons	D_{P_1} (delivered)
0.5	2.57E+11	1.29E+11	1.25E+11	0.49
1	2.57E+11	2.57E+11	2.59E+11	1.01
2	2.57E+11	5.14E+11	5.15E+11	2.00
3	2.57E+11	7.71E+11	7.73E+11	3.01
4.5	2.57E+11	1.16E+12	1.13E+12	4.40
D_{P_3} (planned)	$K_{P_3}^{Gy}$	# protons	# delivered protons	D_{P_3} (delivered)
0.5	1.28E+11	6.40E+10	6.70E+10	0.52
1	1.28E+11	1.28E+11	1.28E+11	1.00
2	1.28E+11	2.56E+11	2.55E+11	1.99
3	1.28E+11	3.84E+11	3.84E+11	3.00
4	1.28E+11	5.12E+11	5.15E+11	4.02
D_{P_5} (planned)	$K_{P_5}^{Gy}$	# protons	# delivered protons	D_{P_5} (delivered)
0.5	2.06E+11	1.03E+11	1.03E+11	0.50
1	2.06E+11	2.06E+11	2.00E+11	0.97
2	2.06E+11	4.12E+11	4.13E+11	2.00
2.5	2.06E+11	5.15E+11	5.12E+11	2.49
3	2.06E+11	6.18E+11	6.16E+11	2.99

Table 3.7: Summary of the dose measurements performed in Exp. 3 at positions P1, P3, and P5.

Gafchromic films used in the three experiments summarized in Table 3.1. In this specific case, the analysis corresponds to Exp. 2, irradiation position P5, and a delivered dose of 2.5 Gy.

At the top of the figure, a dose distribution map obtained for the selected film is shown, together with the dose profiles at different positions along the y -axis, highlighted in yellow and selected for the uniformity analysis. These profiles lie within the region of interest (ROI), shown in Fig 3.15. On the right-hand side of the figure, the relative dose intensity is shown as a function of the x -axis, where the relative dose intensity profiles corresponding to the selected y positions are displayed. Analogously, the bottom part of the figure shows the same analysis performed along the x direction. Note in this figure that the peaks or maximum relative dose intensity values that can be observed in the periphery correspond to image artifacts, such as black backgrounds or annotations made on the film.

For each of these profiles, the dose uniformity was evaluated according to Eq. 3.18 within an ROI defined by x and y values covering the $4.5 \times 4.5 \text{ cm}^2$ region corresponding to the 5×5 grid of seeded cells.

After the analysis of all Gafchromic films, an average dose uniformity of $(94.8 \pm 2.0)\%$ was obtained, with maximum uniformity values reaching 98.6%, in agreement with the reported in [101], and minimum values of 90%. It is worth noting

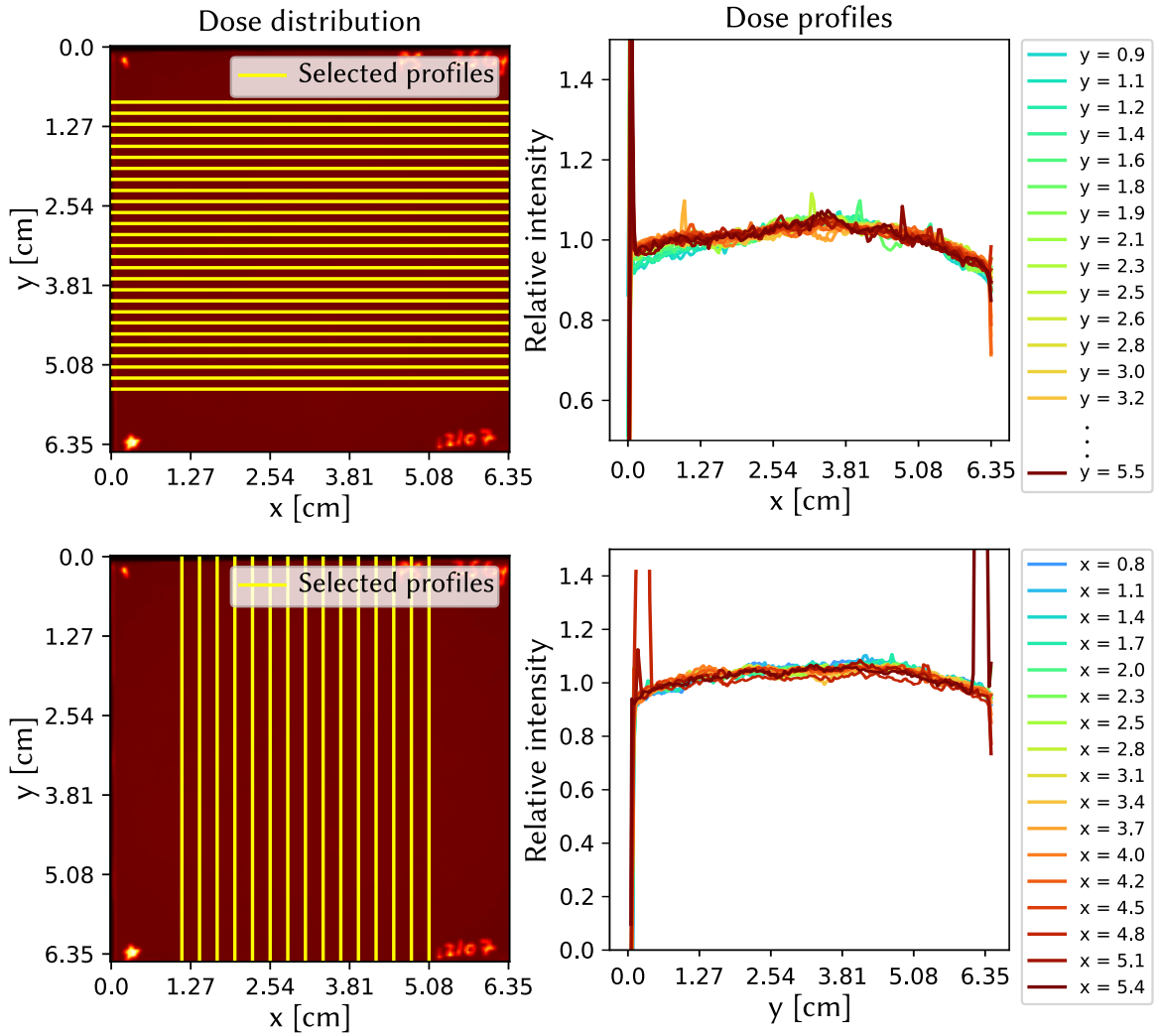


Figure 3.16: Representative Gafchromic film analysis (Exp. 2, P5, 2.5 Gy). Top panel: dose map with selected y -profiles within the ROI. Right panel: corresponding relative dose intensity along y . Bottom panel: analogous analysis along the x direction.

that the lowest uniformity values observed in some profiles correspond to delivered dose levels on the order of 0.5-1 Gy. These dose values are close to the lower limit of the optimal performance range of Gafchromic film, which extends from 0.4 to 40 Gy, as indicated in section 3.2.1.5, which could affect the correct response of the film and posterior analysis.

This study confirmed the dose uniformity within the irradiated region, as a dose uniformity of approximately 95% falls within acceptable limits. Consequently, each well within the 5×5 grid in which the cells were seeded was effectively exposed to the same radiation field. As can be observed, the choice of this grid was strategic: as expected, the dose uniformity degrades when the region of interest is expanded by one or two wells (1-2 cm) in both directions, in which case

dose uniformity values below 90% can be observed.

Taking this level of uniformity into account, a relative uncertainty of 5% was assigned to each of the reported dose values (D_{P_i} (delivered)) in Tables 3.5-3.7. This uncertainty was propagated and explicitly considered during the fitting of the experimental data, as anticipated in Sec. 3.2.1.4.

3.3.2 Radiobiological experiments

Figure 3.17 shows a photograph of the experimental setup used for the radiobiological experiment. The image illustrates the main components of the passive scattering system, as detailed in the schematic shown in Fig. 3.4, including the monitor chamber, the dual-ring system, the ridge filter, the stack of solid water layers, and the positioning of the 96-well plate. Based on the number of solid water layers employed, this photograph corresponds to the irradiation performed at position P5.

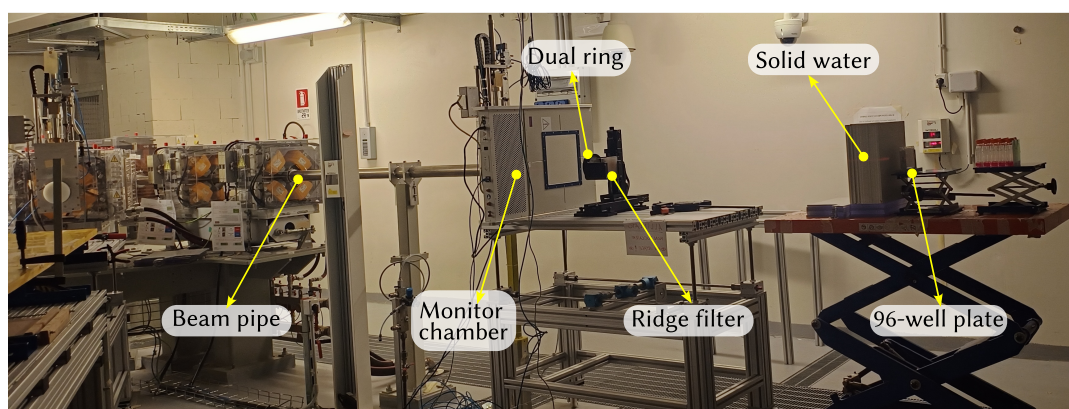


Figure 3.17: Photograph of the experimental setup used for the radiobiological experiment, showing the main elements of the passive scattering system and the positioning of the 96-well plate for irradiation at position P5.

In the following, the results obtained from the clonogenic assay are presented. These include counting of surviving colonies, subsequent outlier analysis, calculation of the plate efficiency (PE) and cell survival, construction of survival curves for each irradiation position (P1–P5), and finally the determination of the radiobiological parameters α and β required for the COMPhID model.

3.3.2.1 Clonogenic assay

Colony counting As described in Sec. 3.2.1.3, following the incubation period and the appropriate processing of the irradiated cell cultures according to the scheme shown in Fig. 3.6, manual counting of the stained colonies was performed. Figure 3.18 presents an example of this procedure, showing one representative irradiation position from each experiment (P1 in Exp. 1, P2 in Exp. 2, and P5 in

Exp. 3). For each position, three dose levels (low, medium, and high) were considered.

As can be observed in all three cases, increasing dose — and consequently the higher number of cells initially seeded to ensure measurable survival — makes colony counting more challenging due to the presence of dead cells that form a background layer across the well. Nevertheless, colonies can still be identified based on their higher staining intensity and the presence of a compact cellular “button” or cluster. Although this feature is not clearly appreciable in the images, it is readily distinguishable under microscopic observation.

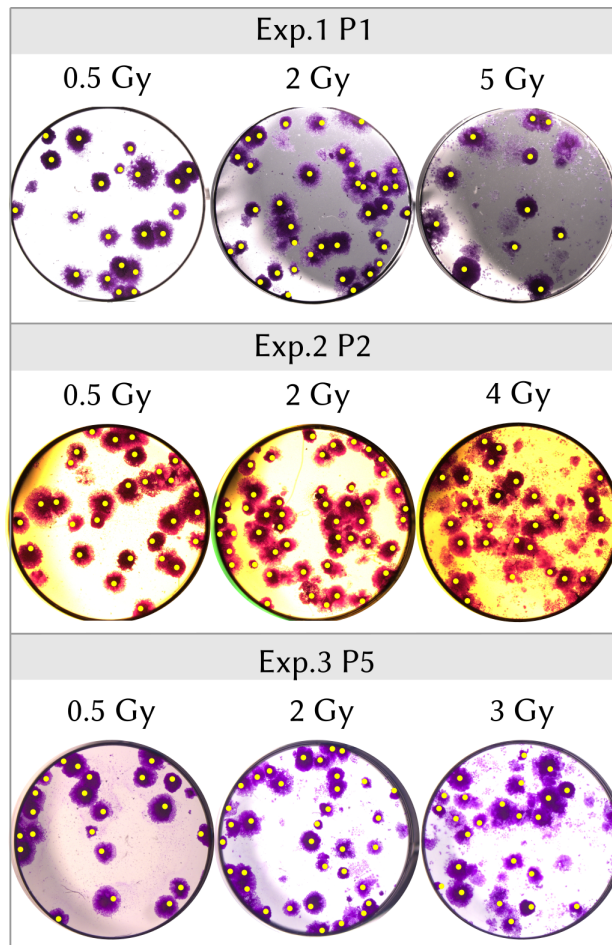


Figure 3.18: Representative examples of manual colony counting for one irradiation position from each experiment: P1 (Exp. 1), P2 (Exp. 2), and P5 (Exp. 3). For each position, three dose levels (low, medium, and high) are shown.

Figure 3.19 shows the final colony counts corresponding to irradiation position P1 of experiment 1 (Exp. 1) for each of the five dose levels considered. The colony counts are displayed on a representative mesh reproducing the layout of the 96-well plate grid used for cell seeding. In this mesh, each well is represented by a square color-coded according to a colormap, allowing rapid visual identification of the corresponding values.

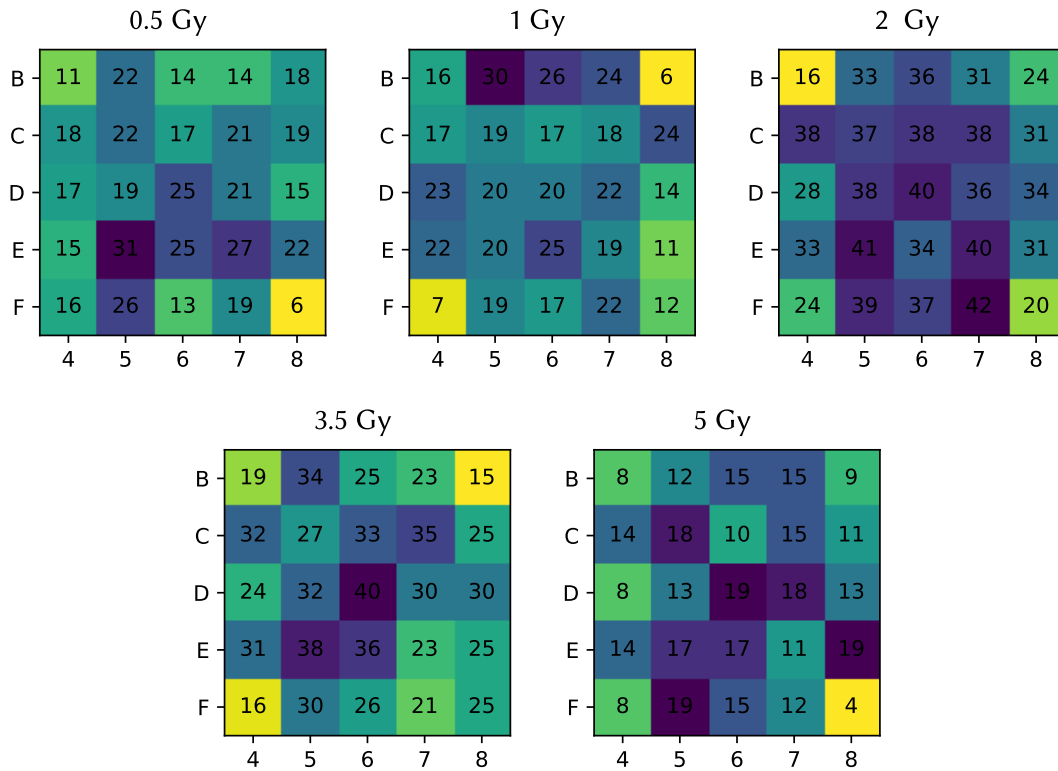


Figure 3.19: Final colony counts for irradiation position P1 of experiment 1 (Exp. 1) at the five evaluated dose levels. The counts are displayed on a representative mesh reproducing the 96-well plate layout used for cell seeding.

Analogous figures showing the colony counts for the control samples, as well as for all irradiation positions and dose levels listed in Table 3.1, are provided in the Appendix E.

Based on this final cell colony count, the outlier analysis was performed as described in section 3.2.1.4, the results of which are presented below.

Outlier analysis Figure 3.20 shows a representative 6×6 mesh reproducing the layout of the 96-well plate. The analysis was performed over all evaluated plates (a total of 45 plates, excluding control samples; see Table 3.1). For each plate, outliers were identified, and their corresponding spatial positions within the mesh were recorded. Subsequently, the number of detected outliers was counted for each well across the entire dataset. The resulting frequency per well was then mapped onto the representative mesh, where each position is color-coded according to the total number of outliers observed, as indicated by the color bar.

As shown in Fig. 3.20, no clear evidence of the “edge effect” described in Section 3.2.1.1 is observed. If such an effect were present, an increased number of outliers—particularly those lying at -2σ from the mean—would be expected at the borders of the grid. This behavior is not apparent in the present analysis.

A noticeable trend, however, is observed along the first row of the grid (with the exception of position B6). In positions B4 and B5, the detected outliers are

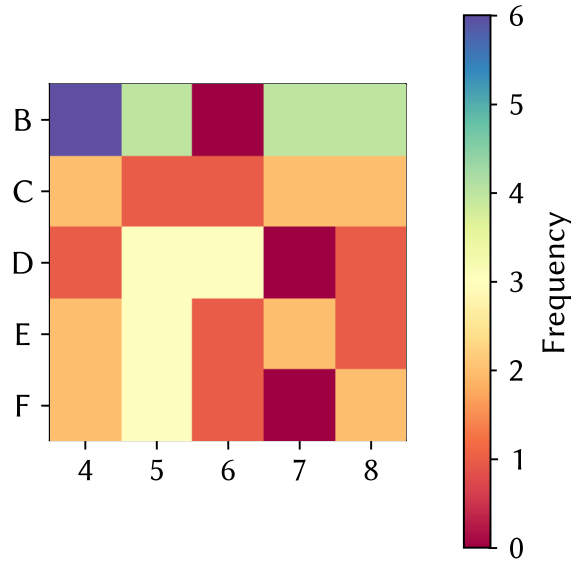


Figure 3.20: Frequency map of detected outliers across all analyzed plates. The 6×6 grid reproduces the 96-well plate layout, with each well color-coded according to the total number of outliers identified at that position.

predominantly located above $+2\sigma$ relative to the mean, whereas no clear tendency is observed in the remaining wells of that row.

Furthermore, from position B4 to F8, a gradual transition can be observed in the nature of the outliers, shifting from values above $+2\sigma$ to values below the mean. One possible explanation for this behavior may be progressive changes in the effective cell concentration during the seeding procedure. Although an electronic micropipette ensured high volumetric precision, cell suspensions are susceptible to sedimentation and aggregation. If the suspension was not continuously homogenized, the effective cell concentration may have decreased over time, leading to higher cell numbers in the initial wells and progressively lower numbers in subsequent ones.

Calculation of PE and cell survival Once the outlier analysis was completed, the wells identified as outliers were excluded from further analysis. For each experiment, the PE was then calculated according to Eq. 1.8. Table 3.8 summarizes the resulting PE values for all experiments.

	Exp.1	Exp.2	Exp.3
PE	0.420	0.639	0.668

Table 3.8: Plate efficiency (PE) values calculated for each experiment after outlier exclusion.

Based on the PE calculated for each experiment, the cell survival fraction was determined for each irradiation position using Eq. 1.10. For each irradiation position, all available survival data from the experiments in which that position was

included were pooled together. Thus, positions present in multiple experiments (e.g., P1 in Exp. 1, 2, and 3) combine the corresponding datasets. In contrast, positions included in fewer experiments (e.g., P4 in Exp. 1 and 2, or P2 in a single experiment) comprise only the respective available measurements, as detailed in Table 3.1.

Figures 3.21-3.25 present the resulting surviving fractions for positions P1 to P5, respectively. In all figures, the black scatter points correspond to the original data prior to outlier exclusion, while the colored points represent the filtered data after applying the outlier criterion.

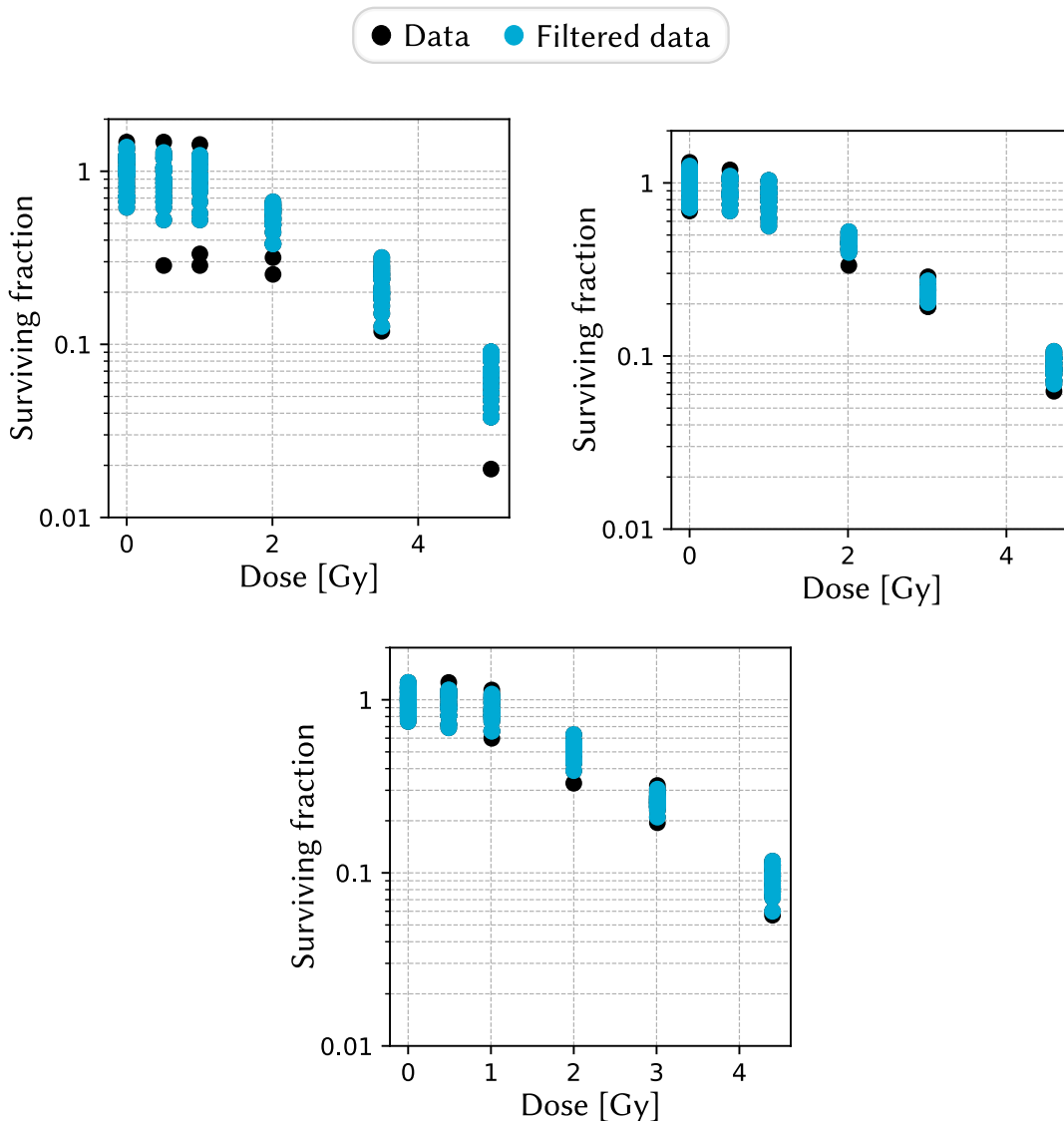


Figure 3.21: Cell surviving fraction at irradiation position P1 (see Table 3.1 for details). Black points represent the original data, while light blue points correspond to the dataset after outlier exclusion.

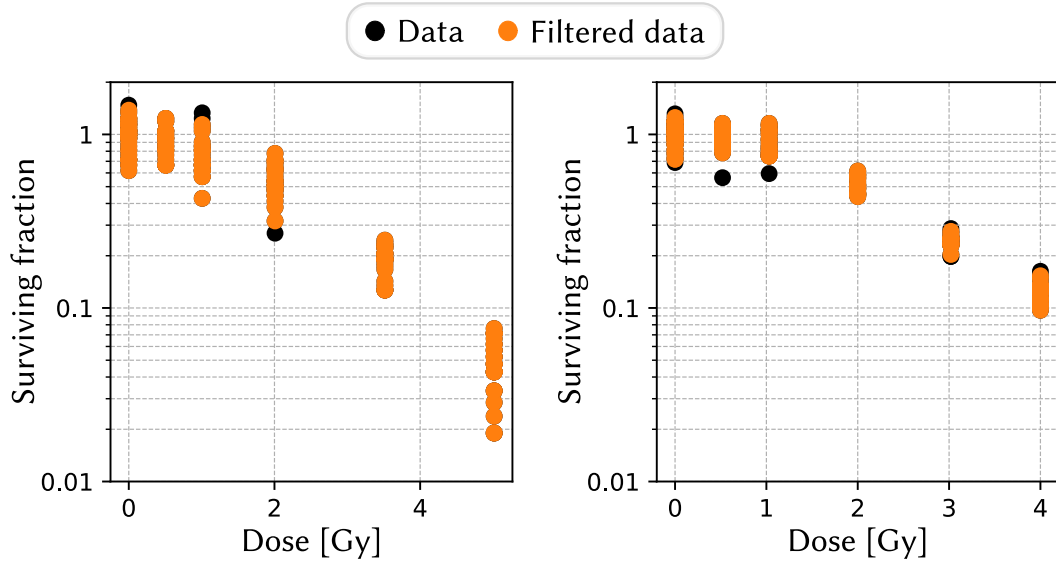


Figure 3.22: Cell surviving fraction at irradiation position P2 (see Table 3.1 for details). Black points represent the original data, while orange points correspond to the dataset after outlier exclusion.

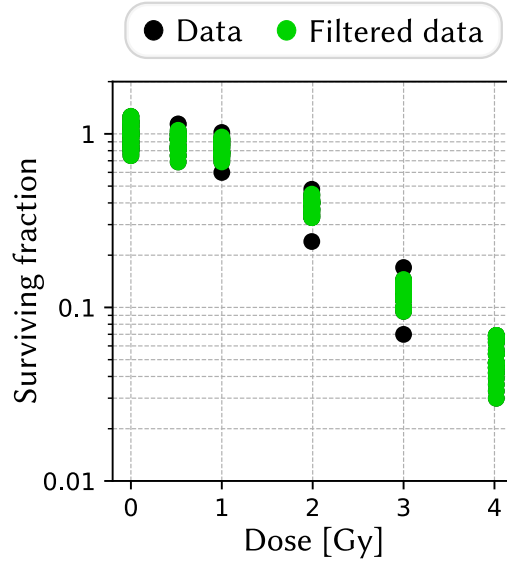


Figure 3.23: Cell surviving fraction at irradiation position P3 (see Table 3.1 for details). Black points represent the original data, while green points correspond to the dataset after outlier exclusion.

3.3.2.2 Derivation of the radiobiological parameters α and β

Finally, once the final cell survival data were obtained and the corresponding delivered doses D_{p_n} —summarized in Tables 3.5-3.7—were available, survival curve fitting was performed to determine the α and β parameters for each irradiation position, as described in Section 3.2.1.4. These parameters were subsequently used

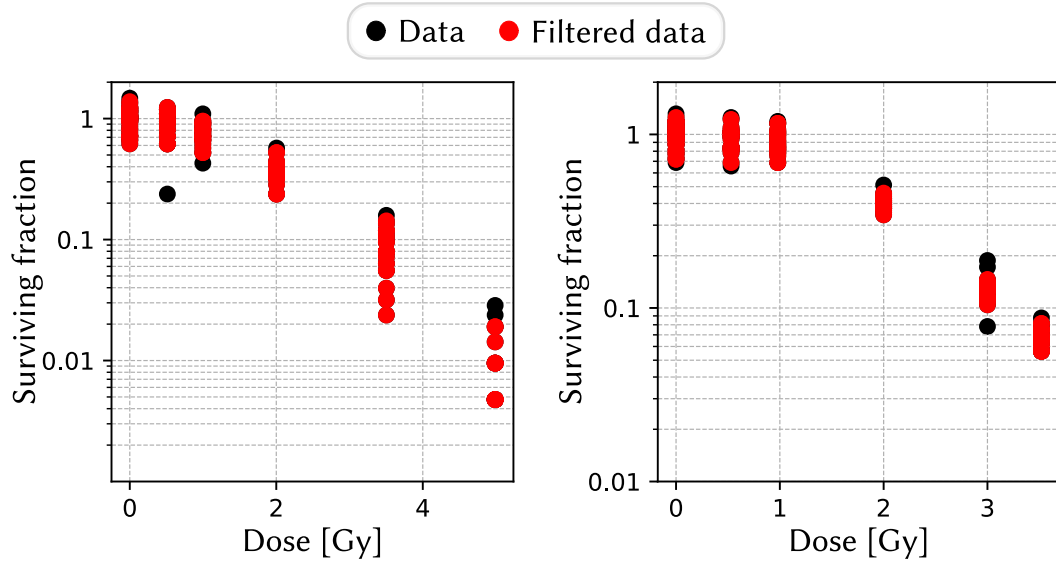


Figure 3.24: Cell surviving fraction at irradiation position P4 (see Table 3.1 for details). Black points represent the original data, while red points correspond to the dataset after outlier exclusion.

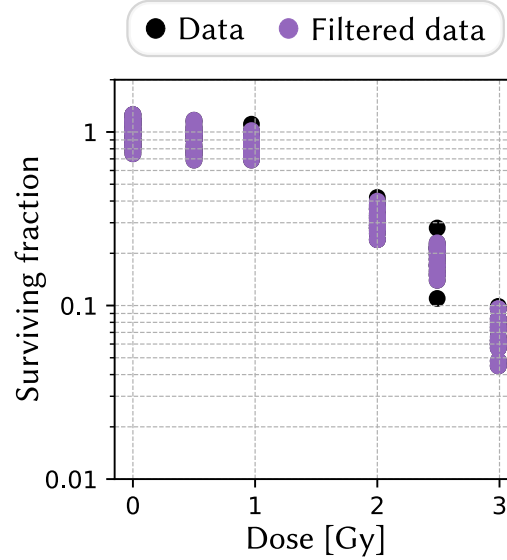


Figure 3.25: Cell surviving fraction at irradiation position P5 (see Table 3.1 for details). Black points represent the original data, while purple points correspond to the dataset after outlier exclusion.

in the relationships given in Eqs. 3.8 and 3.14 of the COMPhID model.

Figure 3.26 presents the fitted survival curves for irradiation positions P1–P5, while Table 3.9 summarizes the corresponding α and β values, their associated standard deviations, and the coefficient of determination (R^2) for all positions.

Finally, Fig. 3.27 displays the survival curves for all irradiation positions within a single plot. In addition, the reference survival curve corresponding to photon ir-

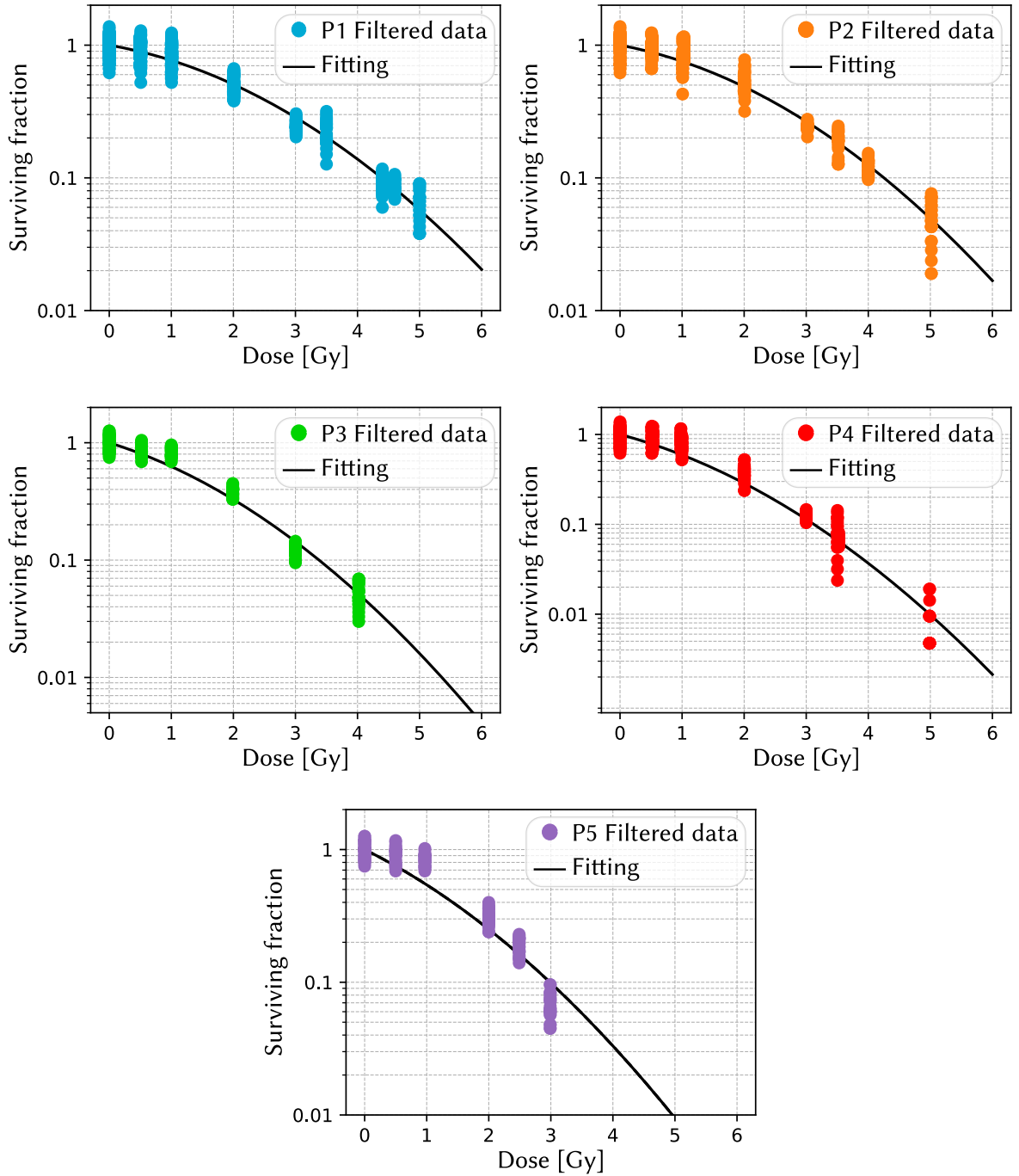


Figure 3.26: Experimental survival data for irradiation positions P1–P5. The fitted curves are shown in black.

radiation from ^{137}Cs is also shown for comparison. The radiobiological parameters reported for ^{137}Cs are $\alpha_x = 0.290 \text{ Gy}^{-1}$ and $\beta_x = 0.083 \text{ Gy}^{-2}$ [113].

A clear depth-dependent trend can be observed: as the irradiation position increases—corresponding to greater depth and therefore higher LET (see Fig. 3.5)—the survival curves become progressively steeper. In particular, P5 exhibits the steep-

Positions	$\alpha \pm \sigma$	$\beta \pm \sigma$	R^2
P1	0.186 ± 0.013	0.077 ± 0.003	0.96
P2	0.201 ± 0.017	0.080 ± 0.004	0.95
P3	0.377 ± 0.029	0.090 ± 0.008	0.97
P4	0.425 ± 0.128	0.100 ± 0.033	0.97
P5	0.532 ± 0.063	0.080 ± 0.024	0.90

Table 3.9: Fitted α and β parameters, their standard deviations, and the corresponding R^2 values for irradiation positions P1–P5.

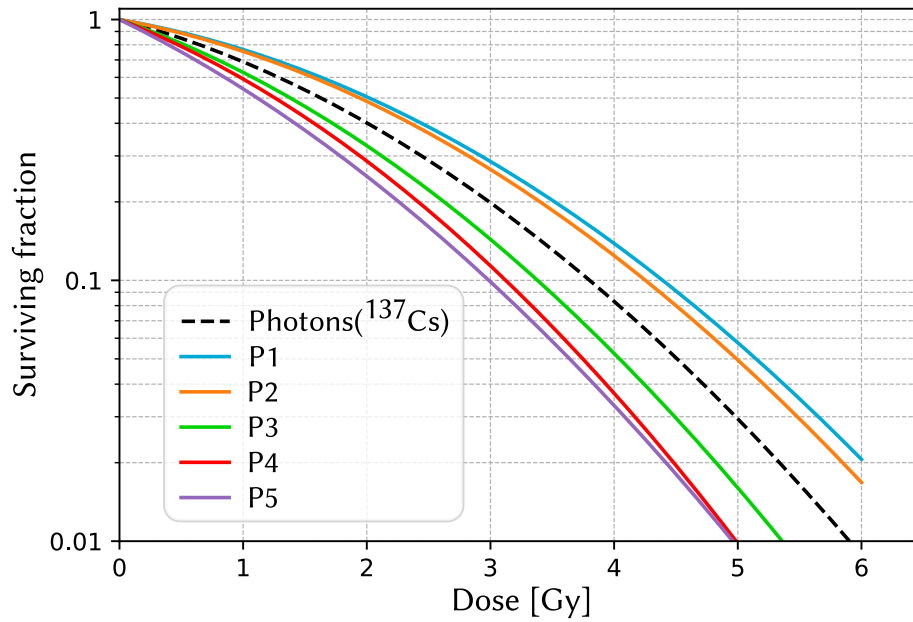


Figure 3.27: Survival curves for irradiation positions P1–P5. The ^{137}Cs photon reference dashed curve is included for comparison.

est survival curve (i.e., the most pronounced biological effect), whereas P1 shows the shallowest response, with positions P4, P3, and P2 lying in between.

Consistent with the expected radiobiological behavior, for a given survival level, a lower dose is required at higher depths (higher LET). The ordering of the curves therefore follows the sequence P5–P4–P3–P2–P1. At lower doses, partial overlap or crossing of the survival curves is observed, particularly between P1 and P2 and between P4 and P5.

Regarding the reference radiation, the ^{137}Cs survival curve lies below those of P1 and P2 over the entire investigated dose range, indicating a higher apparent biological effectiveness of photons under these conditions.

This implies that, relative to P1 and P2, photon irradiation produces a greater biological effect for a given dose. In comparison with P3 and P4, the effectiveness is lower across the entire dose range studied, whereas for P5 the relative effectiveness depends on the dose range considered. Overall, these findings suggest that, under certain dose conditions, the photon reference irradiation exhibits a biologi-

cal effectiveness comparable to or even exceeding that of some proton irradiation positions.

3.3.3 Microdosimetric experiments

In this section, the results of the microdosimetric experiment (see Sec. 3.2.3.1) are presented. The primary objective of this experiment was to determine the first three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, which constitute the microdosimetric input required by the COMPhID model (see Eqs. 3.8 and 3.14).

First, the measured microdosimetric spectra for each irradiation position are presented as the frequency- and dose-weighted lineal energy distributions, $yf(y)$ and $yd(y)$. Subsequently, the corresponding lineal energy moments, calculated from the microdosimetric data, and their ratios ($y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$) are presented.

3.3.3.1 Microdosimetric spectra for positions P1 to P5

Microdosimetric spectra measured with the mini-TEPC at the five different depths across the SOBP are shown in Fig. 3.28 as frequency lineal energy distributions $yf(y)$. A linear-log representation is adopted, with a logarithmic scale on the x-axis and a linear scale on the y-axis.

A clear progressive shift of the spectra toward higher lineal energy values can be observed when moving from position P1 to P5. This behavior reflects the increasing ionization density associated with protons as they slow down while penetrating deeper into the irradiated medium.

A similar behavior is observed in the corresponding $yd(y)$ distributions. As described in [109], the spectrum at position P1 — where no solid water was used (see Table 3.4) — exhibits distinct regions that can be clearly identified.

First, a pronounced maximum is observed in the region of approximately 0.5 – 0.6 keV/ μm . According to the PSTAR database (NIST) for protons in water, this value corresponds closely to the stopping power of 148 MeV protons, which is approximately 0.55 keV/ μm .

Second, a shoulder extending up to about 18–20 keV/ μm can be distinguished. This structure is associated with the so-called proton edge and originates from low-energy protons with higher stopping power traversing chord lengths close to the detector diameter. The upper limit of this feature corresponds to the maximum lineal energy delivered by the lowest-energy protons present at that depth when crossing the sensitive volume approximately along a diameter.

For proton energies of the order of 10^{-3} MeV, the stopping power in water is approximately 18 keV/ μm . Therefore, events beyond this value cannot be attributed to primary protons and are instead associated with secondary particles, such as secondary protons and target fragments generated through inelastic interactions between the primary protons and the nuclei of the irradiated medium [109, 114, 115].

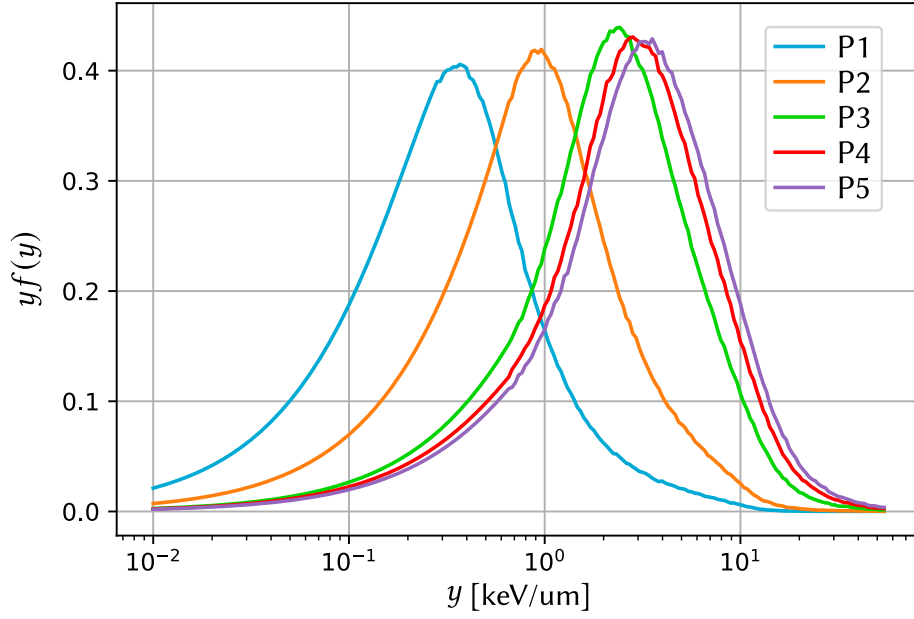


Figure 3.28: Microdosimetric spectra $yf(y)$ measured with the mini-TEPC at irradiation positions P1–P5 across the SOBP.

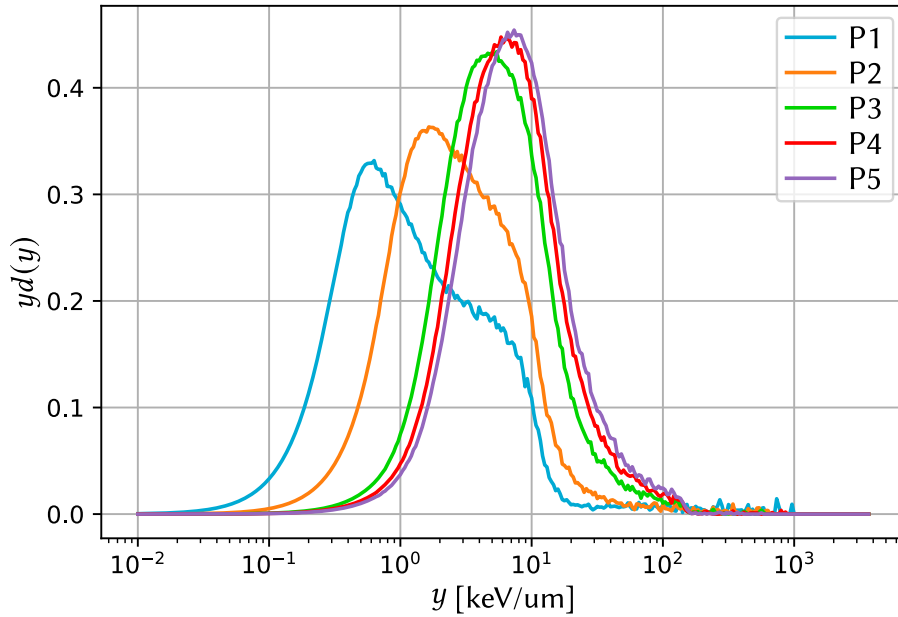


Figure 3.29: Dose-weighted lineal energy distributions $yd(y)$ at positions P1–P5 across the SOBP.

This analysis also explains why the number of recorded events at position P1 is higher than at the other irradiation positions, as previously reported in Table 3.4. As discussed in Sec. 3.2.3.1, as the spectra shift to the right with increasing depth, the main distribution associated with slowing-down protons progressively extends toward the same y region. As a result, the fragment contribution becomes

partially masked or embedded within the broadened high- y tail of the spectrum, making it less clearly distinguishable.

Regarding the spatial characterization of the radiation field, the lateral scans described in Sec. 3.2.3.1 showed no significant variations in the microdosimetric spectra when the sensitive volume was displaced by ± 2.4 cm with respect to the central beam axis. This confirms the lateral uniformity of the proton field at each irradiation position within the explored region. Accordingly, both central and peripheral wells of the 96-well plate can be considered as exposed to the same radiation field, allowing all 25 wells to be associated with a single representative microdosimetric spectrum.

3.3.3.2 Calculation of the first three lineal energy moments

Table 3.10 summarizes the values of the first three moments of the lineal energy distribution and associated uncertainties, calculated according Eq. C.10. Figure 3.30 presents these results graphically for each irradiation position.

Positions	$y^{(1)} \pm \sigma$ [keV/ μ m]	$y^{(2)} \pm \sigma$ [(keV/ μ m) ²]	$y^{(3)} \pm \sigma$ [(keV/ μ m) ³]
P1	0.57 ± 0.04	3.39 ± 0.23	$(8.88 \pm 1.76) \cdot 10^2$
P2	1.37 ± 0.10	8.06 ± 0.55	$(8.03 \pm 2.75) \cdot 10^2$
P3	3.17 ± 0.22	28.33 ± 0.56	$(13.25 \pm 2.57) \cdot 10^2$
P4	3.94 ± 0.28	42.78 ± 0.36	$(22.79 \pm 2.48) \cdot 10^2$
P5	4.45 ± 0.31	52.70 ± 0.19	$(19.59 \pm 0.57) \cdot 10^2$

Table 3.10: First three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, calculated for irradiation positions P1 to P5.

As can be observed, the calculated moments $y^{(1)}$ and $y^{(2)}$ increase progressively with depth from position P1 to P5. In contrast, the depth-dependent behavior of $y^{(3)}$ is less monotonic. The third-order moment exhibits a slight decrease from P1 to P2, followed by an increase from P2 to P4, and finally a slight reduction from P4 to P5.

Regarding the individual behavior of each moment, the relative uncertainty of the first moment of lineal energy, $y^{(1)}$, remains approximately constant at about 7% for all irradiation positions.

In contrast, for the second and third moments, $y^{(2)}$ and $y^{(3)}$, a clear decrease in relative uncertainty is observed from P1 to P5. For $y^{(2)}$, the relative uncertainty is approximately 7% at P1 and decreases progressively to about 0.4% at P5. An even more pronounced behavior is observed for the third moment: the relative uncertainty reaches values as high as $\sim 20\%$ and decreases to approximately 3% at P5.

The larger relative uncertainties observed for $y^{(2)}$ and particularly for $y^{(3)}$ at low depths can be explained by the mathematical definition of higher-order mo-

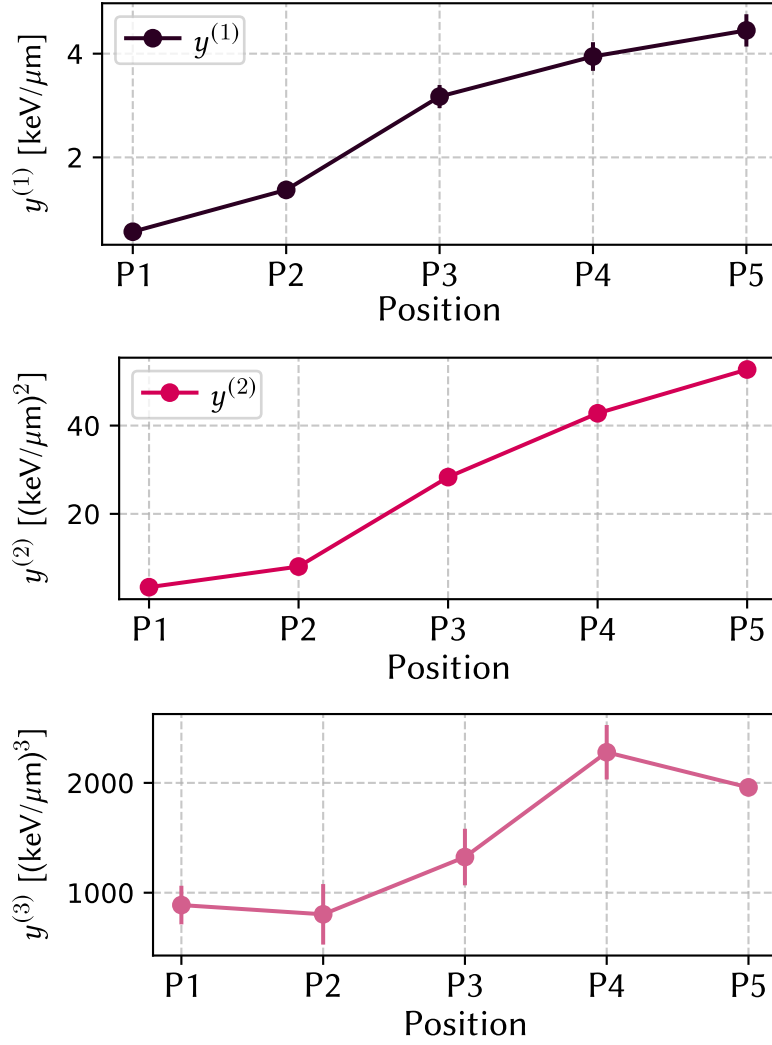


Figure 3.30: Graphical representation of the first three moments of the lineal energy distribution as a function of irradiation position. Error bars indicate one uncertainty.

ments. Since the q -th moment involves a weighting factor proportional to y^q , higher-order moments strongly amplify the high- y region of the spectrum. This behavior is clearly illustrated in Fig. 3.31, where the normalized differential contribution to the second ($q = 2$) and third ($q = 3$) moments of the lineal energy distribution is shown for positions P1 and P5. This quantity, $w_q(y)$, was calculated as follows:

$$w_q(y) = \frac{y^q f(y) dy}{\int_0^\infty y^q f(y) dy}.$$

In P1, the higher-order moments are predominantly determined by a narrow high- y region, indicating that only a limited portion of the spectrum contributes significantly to the integral. In contrast, in P5 the contribution is more broadly

distributed across y , resulting in a more balanced integration over the spectrum and improved statistical stability.

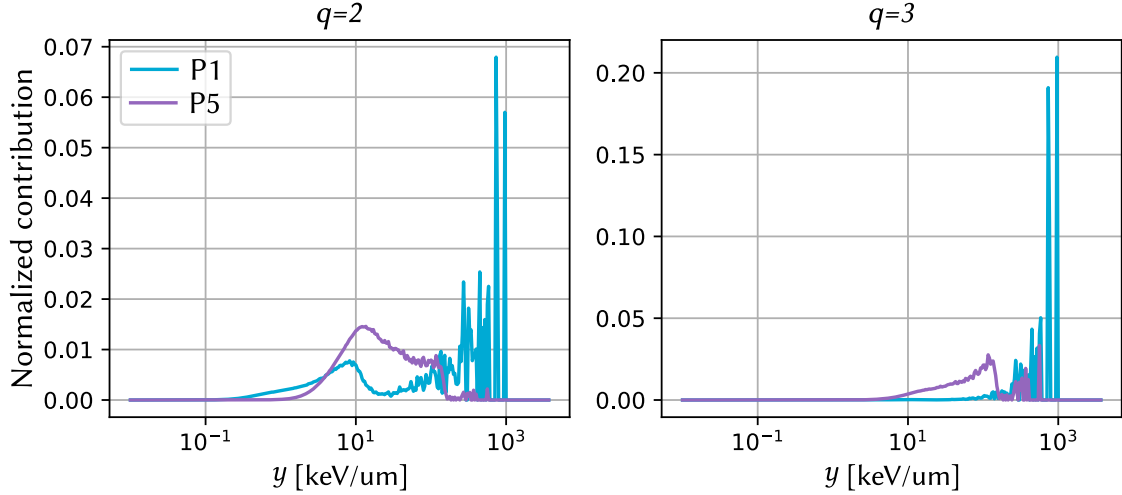


Figure 3.31: Normalized differential contribution to the second ($q = 2$) and third ($q = 3$) moments of the lineal energy distribution for irradiation positions P1 and P5. Each curve represents $y^q f(y) \Delta y$ normalized to the corresponding moment value, such that the total area under the curve is unity.

Under irradiation conditions where the high lineal energy tail is sparsely populated or statistically limited, the integral defining the moment becomes dominated by a small number of high- y events. This concentration of weight in few bins enhances statistical fluctuations and leads to the large relative uncertainties observed for $y^{(2)}$ and particularly for $y^{(3)}$ at shallow depths.

This behavior indicates that the higher-order moments are increasingly dominated by the statistical properties of the spectral tail, making them more sensitive to limited statistics.

Calculation of ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ Table 3.11 summarizes the values of the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, together with their associated uncertainties, obtained for each irradiation position. Figure 3.32 presents these ratios graphically as a function of irradiation depth (P1–P5).

As can be observed, the ratio $y^{(2)}/y^{(1)}$ increases with depth, whereas $y^{(3)}/y^{(1)}$ shows a progressive decrease from P1 to P5. In both cases, the associated uncertainty decreases with increasing depth, consistent with the behavior previously observed for the individual moments.

The values summarized in Table 3.11 are subsequently used for the determination of the COMPhID model parameters, according to Eqs. 3.8 and 3.14.

Positions	$\frac{y^{(2)}}{y^{(1)}} \pm \sigma$ [keV/ μm]	$\frac{y^{(3)}}{y^{(1)}} \pm \sigma$ [(keV/ μm) ²]
P1	5.99 ± 0.58	$(1.57 \pm 0.33) \cdot 10^3$
P2	5.88 ± 0.58	$(5.85 \pm 2.04) \cdot 10^2$
P3	8.93 ± 0.65	$(4.18 \pm 0.86) \cdot 10^2$
P4	10.84 ± 0.76	$(5.78 \pm 0.75) \cdot 10^2$
P5	11.84 ± 0.83	$(4.40 \pm 0.33) \cdot 10^2$

Table 3.11: Ratios of the second and third moments to the first moment of the lineal energy distribution, $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, for irradiation positions P1 to P5.

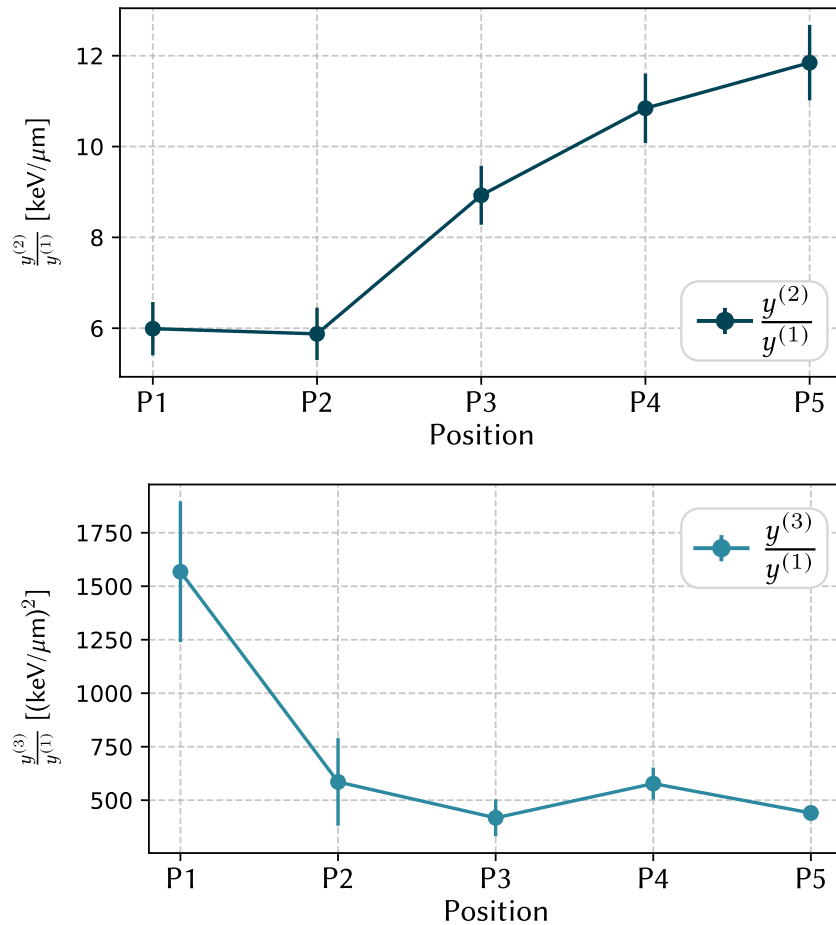


Figure 3.32: Ratios $\frac{y^{(2)}}{y^{(1)}}$ and $\frac{y^{(3)}}{y^{(1)}}$ as a function of irradiation position (P1–P5).

3.3.4 Determination of the COMPhID model parameters

Once the radiobiological parameters α and β (Table 3.9), together with the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ (Table 3.11), had been determined for each of the five irra-

diation positions (P1–P5), the COMPhID model parameters α_0 , α_1 , α_2 , β_0 , and β_1 were calculated using Eqs. 3.8 and 3.14. The estimation of these model parameters was carried out following the methodology described in Sec. 3.2.4.

Table 3.12 summarizes the estimated values and their 1σ standard uncertainties for the five parameters of the COMPhID model. The corresponding reduced χ^2 values (Eq. 3.25) are also reported for both the α and β parameters.

Parameter	Fit-estimates	$\chi_{red}^2\alpha$	$\chi_{red}^2\beta$
α_0	$(-10.96 \pm 0.92) \cdot 10^{-2}$	0.01	0.44
α_1	$(5.53 \pm 0.09) \cdot 10^{-2}$		
α_2	$(-2.26 \pm 0.36) \cdot 10^{-5}$		
β_0	$(6.19 \pm 1.01) \cdot 10^{-2}$		
β_1	$(2.78 \pm 1.58) \cdot 10^{-3}$		

Table 3.12: Estimated values for the five parameters of the COMPhID model. Reported values correspond to the ODR best-fit estimates and their 1σ standard uncertainties obtained from the fit covariance matrix. The reduced χ^2 values associated with the α and β fits are also reported.

As can be observed, the fit yields relatively small standard uncertainties for all α parameters, particularly for α_1 and α_2 , indicating good parameter identifiability. The fitted value of α_0 is found to be negative, at more than 8σ from zero. While in isolation this could be interpreted as implying a negative linear component for negligible values of $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, in the present formulation α_0 should be understood as part of a combined parameterization in which the effective value of $\langle\alpha\rangle$ is determined by the full set of terms. Within the range of experimentally relevant conditions, the resulting $\langle\alpha\rangle$ remains physically meaningful. Moreover, the reduced χ^2 values remain below unity ($\chi_{red}^2\alpha = 0.01$ and $\chi_{red}^2\beta = 0.44$), indicating that the model reproduces the data within the assigned experimental uncertainties.

Regarding the β model parameters, β_0 and β_1 , were both found to be positive, with relative uncertainties of approximately 16% and 60%, respectively.

To evaluate the reliability of the obtained model parameters, Fig. 3.33 presents a direct comparison between the experimentally observed radiobiological parameters, α_{obs} and β_{obs} (reported in Table 3.9), and those predicted by the model, α_{pred} and β_{pred} , calculated according to Eqs. 3.8 and 3.14. The corresponding numerical values are provided in Tables F.1 and F.2 in Appendix F, where the observed and predicted parameters are listed together with the prediction accuracy defined in Eq. 3.26.

As shown both graphically and in the corresponding tables, the predicted and observed α values exhibit a strong agreement, with precision values exceeding approximately 98% across all irradiation positions.

For the β parameters, a satisfactory level of agreement with the experimental data is observed, with precision values ranging from 82% to 98%.

Overall, the agreement with the experimental data supports the validity of

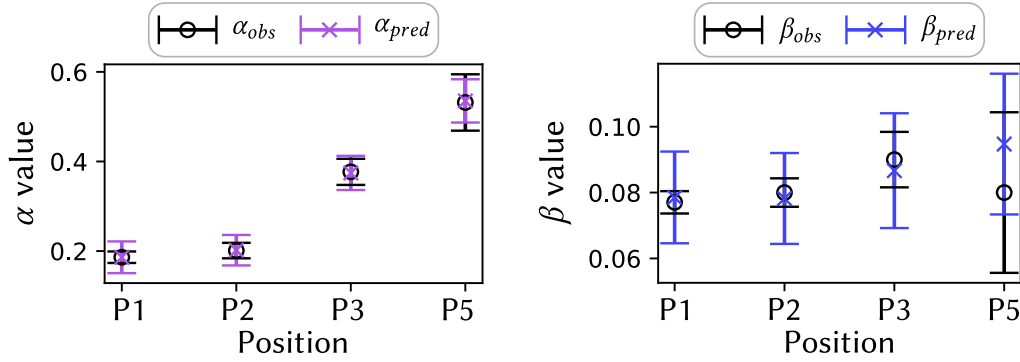


Figure 3.33: Comparison between experimentally observed and model-predicted α and β values at positions P1–P5. Error bars represent 1σ uncertainties.

the derived parameters, which are therefore adopted as the defining parameter set of the COMPhID model, as they provide an accurate representation of the observed radiobiological trends. Finally, the coefficients $\langle\alpha\rangle$ and $\langle\beta\rangle$ of the COMPhID model are the following:

$$\begin{aligned}\langle\alpha\rangle &= -10.96 \cdot 10^{-2} + 5.53 \cdot 10^{-2} \frac{y^{(2)}}{y^{(1)}} - 2.26 \cdot 10^{-5} \frac{y^{(3)}}{y^{(1)}}, \\ \langle\beta\rangle &= 6.19 \cdot 10^{-2} + 2.78 \cdot 10^{-3} \frac{y^{(2)}}{y^{(1)}}.\end{aligned}\tag{3.27}$$

In the following sections, these results will be used to evaluate the predictive performance of the COMPhID model, as well as the level of agreement between the experimentally determined radiobiological parameters and those predicted by the model.

3.3.5 Calculation of photon IsoE dose and RBE using the COMPhID model and comparison with experimental results

As described in Section 3.2.5, the photon IsoE dose was calculated using ^{137}Cs photons as the reference radiation. The calculations were performed for two scenarios at each irradiation position (P1–P3 and P5): (I) for a fixed survival level of 10%, and (II) for a fixed proton dose of 2 Gy.

The resulting photon isoeffective doses for both scenarios are summarized in Table 3.13, and obtained using the expressions in Eq. 3.27. The corresponding RBE values are also reported.

When compared with the experimental values, which can be graphically (and analytically) estimated from the survival curves shown in Fig. 3.27 for positions P1, P3–P5, the model predictions show a good overall agreement for both scenarios.

In scenario (I), where a fixed survival level of 10% is considered, the comparison between model predictions and experimentally inferred RBE_{10} values is summarized in Table 3.14. As observed, the relative differences remain within 1–4%

(I) for a survival level of 10%			
Position	IsoE [Gy]	Proton dose [Gy]	RBE ₁₀
P1	3.80	4.36 ± 1.33	0.87 ± 0.27
P2	3.80	4.29 ± 1.31	0.89 ± 0.27
P3	3.80	3.43 ± 1.27	1.11 ± 0.41
P5	3.80	2.86 ± 1.26	1.33 ± 0.58
(II) for a proton dose of 2 Gy			
Position	IsoE (COMPhID) [Gy]	Proton dose [Gy]	RBE
P1	1.62 ± 0.16	2.0	0.81 ± 0.08
P2	1.67 ± 0.15	2.0	0.84 ± 0.08
P3	2.28 ± 0.15	2.0	1.14 ± 0.08
P5	2.78 ± 0.17	2.0	1.39 ± 0.08

Table 3.13: IsoE dose and RBE values obtained using parameters from Table 3.12 for each irradiation position (P1–P5). IsoE values are shown for a fixed proton dose of 2 Gy, and RBE values are reported for both survival level 10% (RBE₁₀) and the 2 Gy scenario.

across all irradiation positions, confirming that the model accurately reproduces the experimentally inferred RBE₁₀ values.

Position	RBE ₁₀ (COMPhID)	RBE ₁₀ (exp)	Δ (%)
P1	0.87 ± 0.27	0.86	1
P2	0.89 ± 0.27	0.88	1
P3	1.11 ± 0.41	1.12	1
P5	1.33 ± 0.58	1.27	4

Table 3.14: Comparison between model-predicted and experimentally inferred RBE₁₀ values at the different irradiation positions. The relative difference Δ is calculated with respect to the experimental value.

In scenario (II), where the isoeffective dose is calculated for a fixed proton dose of 2 Gy, the IsoE dose increases with depth from P1 to P5, leading to a corresponding increase in RBE. The RBE values predicted by the model, shown in Table 3.13, are in good agreement with the experimentally inferred values, with relative differences within 1–3%.

Considering this level of agreement across the evaluated irradiation positions, the next section investigates an application of the model by calculating the IsoE dose at position P4 under scenarios (I) and (II), using the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ reported in Table 3.11 for this position. In addition, this analysis was also performed considering the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ calculated from a microdosimetric spectrum obtained between positions P2 and P3.

3.3.6 Application of the COMPhID model

To illustrate the application of the COMPhID model, the photon isoeffective dose and the corresponding RBE were calculated under the two scenarios (I) and (II) described in the previous section. The resulting values were then analyzed in terms of their consistency with, and ability to reproduce, the trends observed in the available experimental data. These quantities were evaluated for the following irradiation positions:

- Position P4, using the experimentally measured microdosimetric spectrum. The corresponding ratios of the lineal energy moments, $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, are reported in Table 3.11.
- Position P1–P5 along the SOBP. Once the radiobiological parameters $\langle\alpha\rangle$ and $\langle\beta\rangle$ were determined for the position P4 through the COMPhID model, the photon IsoE dose was calculated for the different irradiation positions P1–P5 along the SOBP, following the procedure described in Section 3.2.6.
- Position Pn, which corresponds to a new irradiation position located between P2 and P3, as described in Sec. 3.2.6. For this position, the microdosimetric spectrum was obtained through Monte Carlo simulations performed with PHITS. For further details, see Appendix G.2.

The results obtained for each of these positions are presented in the following.

Position P4 Table 3.15 summarizes the photon isoeffective dose and the corresponding RBE values calculated for scenarios (I) and (II) at irradiation position P4. Using the expressions given in Eq. 3.27, the radiobiological parameters obtained for this position were $\langle\alpha\rangle = (0.477 \pm 0.077) \text{ Gy}^{-1}$ and $\langle\beta\rangle = (0.092 \pm 0.019) \text{ Gy}^{-2}$.

These values show good agreement with the radiobiological parameters derived from the experimental survival curve for position P4 reported in Table 3.9, namely $\alpha = 0.425 \pm 0.128 \text{ Gy}^{-1}$ and $\beta = 0.100 \pm 0.033 \text{ Gy}^{-2}$, within the associated experimental uncertainties.

When compared with the radiobiological parameters obtained for the other irradiation positions (P1, P2, P3, and P5), reported in Tables F.1 and F.2, these values are found to be consistent with the overall trend along the SOBP. In particular, as shown in Table F.1, the value of α obtained for P4 lies between those corresponding to P3 and P5, i.e., $\alpha_{P3} < \alpha_{P4} < \alpha_{P5}$ ($0.377 < 0.477 < 0.532$), following the expected monotonic increase with depth.

A similar behavior is observed for the β parameter, with the value obtained for P4 remaining fully consistent with those reported for the neighboring irradiation positions in Table F.2, and lying between the values obtained for P3 and P5.

An analogous analysis can be performed using the proton doses and the corresponding RBE₁₀ values obtained for scenario (I), as well as the calculated photon IsoE doses and the associated RBE values for scenario (II).

(I) for a survival level of 10%			
Position	IsoE [Gy]	Proton dose [Gy]	RBE ₁₀
P4	3.80	3.04 ± 1.26	1.25 ± 0.52
(II) for a proton dose of 2 Gy			
Position	IsoE (COMPhID) [Gy]	Proton dose [Gy]	RBE
P4	2.61 ± 0.16	2.0	1.30 ± 0.08

Table 3.15: IsoE dose and RBE values obtained using parameters from Table 3.12 for position P4. IsoE values are shown for a fixed proton dose of 2 Gy, and RBE values are reported for both survival level 10% (RBE₁₀) and the 2 Gy scenario.

Focusing on the RBE results, the value obtained for P4 follows the same increasing trend observed across the irradiation positions. For scenario (I), the RBE₁₀ value obtained for P4 (1.25) lies between those calculated for P3 and P5, as reported in Table 3.13 ($1.11 < 1.25 < 1.33$), consistently with the monotonic increase of RBE₁₀ from P1 to P5 along the SOBP. A similar behavior is observed for scenario (II). The photon isoeffective dose calculated for P4 (2.61 Gy) falls between the values obtained for P3 and P5 ($2.28 < 2.61 < 2.77$ Gy), resulting in an RBE value of 1.30, which is fully consistent with the trend observed between these positions ($1.14 < 1.30 < 1.39$).

Overall, these results indicate that the COMPhID model is able to consistently reproduce the radiobiological behavior across the investigated irradiation positions when an experimental microdosimetric spectrum is available. In this context, the model can be used to estimate the radiobiological parameters α and β , as well as the photon isoeffective dose and derived quantities such as the RBE for a given proton dose or the commonly reported RBE₁₀. It should be noted that the model was derived from experiments performed with the H460 cell line and that the model is cell-specific; consequently, the calculated radiobiological parameters are strictly applicable to this cell line.

The applicability of the model is constrained to the range of lineal energies covered by the experimental microdosimetric spectra presented in Fig. 3.28. Within this range, the model provides a consistent and flexible framework, as it does not require reproducing the exact experimental setup employed in this work; rather, it can be applied whenever an experimental microdosimetric spectrum within the range defined by the spectra measured between positions P1 and P5 is available. The current assessment is therefore restricted to this experimentally validated domain. Since no additional measurements outside this range are presently available, the predictive capability of the model beyond these conditions remains to be established.

Photon IsoE doses along the SOBP Once the radiobiological parameters α and β predicted by the COMPhID model (reported in Tables F.1 and F.2), together with those obtained for position P4 in this section, were determined, the photon isoeffective dose was calculated for the irradiation positions P1–P5 along the SOBP.

Figure 3.34 shows the depth–dose profile of the SOBP, where the solid line represents the physical dose distribution. The filled markers indicate the irradiation positions P1–P5 along the SOBP. At these same positions, the photon isoeffective doses calculated using the COMPhID model are also shown using the same marker style. The symbols “+” denote the corresponding RBE values derived from the calculated IsoE doses, and the dashed line represents the dose weighted with a constant RBE of 1.1. The numerical values, together with the associated uncertainties of the IsoE doses and the derived RBE values, are reported in Table F.3 of the Appendix.

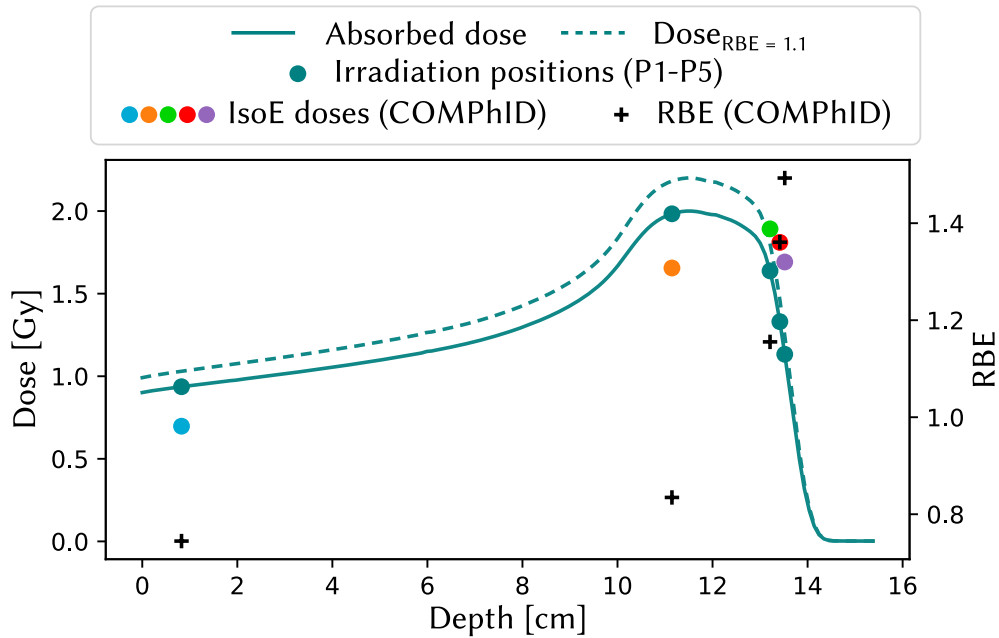


Figure 3.34: Depth–dose profile of the SOBP (solid line). Filled markers indicate the irradiation positions (P1–P5), while the corresponding photon IsoE doses calculated using the COMPhID model are shown with the same markers in different colors. The “+” symbols denote the derived RBE values, and the dashed line represents the dose weighted with a constant RBE of 1.1.

As can be observed from the figure, a clear depth-dependent behavior is obtained for both the photon IsoE dose and the corresponding RBE values along the SOBP. In particular, an increasing RBE trend is observed from P1 to P5, reflecting the progressive change in radiation quality with depth.

For positions P3–P5, corresponding to the distal region of the SOBP, the calculated RBE values show a monotonic increase from approximately 1.2 to 1.5. In this

region, the IsoE dose increases from P3 to P4 and slightly decreases at P5, while preserving the overall increasing RBE trend when normalized to the absorbed proton dose.

At shallower depths (P1 and P2), the photon IsoE doses are lower than the corresponding absorbed proton doses, resulting in RBE values below unity (approximately 0.7–0.8) and therefore below the clinically used reference value of RBE = 1.1.

Overall, these results highlight the capability of the COMPhID model to capture the depth-dependent variation of RBE along the SOBP, from values below the conventional RBE = 1.1 at shallow depths to significantly higher values at larger depths, reaching relative differences of up to approximately 35% with respect to the fixed value of 1.1.

Position Pn Based on the lineal energy ratios of the irradiation position Pn presented in Section G.2 of the Appendix, and considering the expressions given in Eq. 3.27, the following radiobiological parameters were obtained for Pn: $\langle\alpha\rangle = (0.179 \pm 0.076) \text{ Gy}^{-1}$ and $\langle\beta\rangle = (0.078 \pm 0.014) \text{ Gy}^{-2}$.

When compared with the values reported in Tables F.1 and F.2, it can be observed that the mean value of the α parameter is slightly lower than the value obtained for position P1 ($0.179 < 0.186 \text{ Gy}^{-1}$), while the β parameter lies within the range of the values reported for positions P1 and P2, remaining consistent within uncertainties.

Considering these results, the photon isoeffective dose for this position in scenario (II), assuming a fixed proton dose of 2 Gy, is expected to be lower than the value reported for P1 under the same scenario. This behavior is consistent with the lower α value and the similar β value compared with P1, which implies a reduced biological effectiveness. Consequently, a higher proton dose would be required to achieve a survival level of 10%. The corresponding results are reported in Table 3.16, where this behavior can be observed.

(I) for a survival level of 10%			
Position	IsoE [Gy]	Proton dose [Gy]	RBE ₁₀
Pn	3.80	4.48 ± 1.40	0.85 ± 0.26
(II) for a proton dose of 2 Gy			
Position	IsoE (COMPhID) [Gy]	Proton dose [Gy]	RBE
Pn	1.59 ± 0.29	2.0	0.79 ± 0.15

Table 3.16: IsoE dose and RBE values obtained using parameters from Table 3.12 for position Pn. IsoE values are shown for a fixed proton dose of 2 Gy, and RBE values are reported for both survival level 10% (RBE₁₀) and the 2 Gy scenario.

Table 3.16 shows that, in scenario (I) a proton dose of 4.48 Gy is required to

reach a survival level of 10%, resulting in a mean RBE_{10} value of 0.85. This compares well with the values reported for P1 (4.36 Gy and 0.87), demonstrating very good agreement within uncertainties.

For scenario (II), the calculated photon IsoE dose is 1.59 Gy. Considering a fixed proton dose of 2.0 Gy, this corresponds to an RBE value of 0.79. In contrast, for position P1 the corresponding values are 1.62 Gy and 0.81, respectively. Again, the agreement is very good and the results follows the behavior predicted from the radiobiological parameters α and β obtained for position Pn.

If the case of position P4 discussed previously is considered—where the obtained results were consistent with those reported for the neighboring irradiation positions P3 and P5—a similar behavior would be expected for position Pn. Since Pn is located between P2 and P3, the proton doses, photon isoeffective doses, and the corresponding RBE values would be expected to lie between those obtained for P2 and P3, following the trends previously reported in Table 3.13.

However, as mentioned at the beginning of this section, the spectrum for Pn was obtained from MC simulations. As discussed in Appendix C, the microdosimetric spectra derived from MC simulations show a systematic shift with respect to the experimental spectra, toward either lower or higher lineal energy values depending on the irradiation position. In the case of Pn, the spectrum is clearly shifted toward lower lineal energy values, which leads to slightly lower predicted radiobiological parameters and, consequently, a reduced biological effectiveness at this irradiation position.

Overall, these results further support the robustness of the COMPhID model given in Eq. 3.27, which provides consistent predictions when experimental microdosimetric spectra are used as input. In the case of microdosimetric spectra obtained from MC simulations, the model still captures the expected trends and provides meaningful estimates of the IsoE doses at a given irradiation position, particularly when the associated uncertainties in the predicted RBE values—such as those reported in Table 3.16—are taken into account.

It should be noted that, although the spectra obtained with the PHITS code do not fully reproduce all the features of the experimentally measured spectra, they nevertheless provide a reasonable approximation of the microdosimetric distributions and correctly describe the overall trends. Moreover, previous studies have shown that simulations performed with the TOPAS code yield microdosimetric spectra that are in good agreement with experimental measurements, reproducing both the peak positions and the overall shape of the distributions for the same proton beam conditions considered in the present study [116]. This indicates that the predictive capability of the approach can be further enhanced by improved modeling of the microdosimetric spectra, potentially allowing even more accurate predictions of the corresponding radiobiological quantities in future studies.

3.4 Conclusions

In this chapter, a continuous formulation of the isoeffective dose model based on microdosimetric quantities was developed. In this framework, radiation quality is described through the stochastic variable lineal energy, enabling a representation directly connected to microscopic energy deposition. The resulting Continuous Microdosimetric Photon Isoeffective Dose (COMPhID) model establishes a relationship between the first three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, and the radiobiological response within the linear-quadratic (LQ) formalism through the coefficients $\langle\alpha\rangle$ and $\langle\beta\rangle$, which now include additional supra-quadratic terms related to the creation of complex chromosome rearrangements, as a possible extension of CDRA theory.

The model is characterized by five free parameters, α_0 , α_1 , α_2 , β_0 , and β_1 , determined from combined microdosimetric and radiobiological experimental data for the H460 cell line. Within this calibration framework, the model provides an explicit link between measurable microdosimetric spectra and biological response, allowing the estimation of radiobiological parameters from physical quantities.

The reliability of the parameterization was assessed by comparing the predicted $\langle\alpha\rangle$ and $\langle\beta\rangle$ values with those derived experimentally. A high level of agreement was observed, with deviations below 2% for the α parameter and within 1–17% for β , indicating that the model is able to reproduce the main radiobiological trends across different irradiation conditions.

Based on this validation, the model was used to calculate photon isoeffective dose and corresponding RBE values under two conditions: (I) fixed survival fraction of 10%, and (II) fixed proton dose of 2 Gy. In both cases, the model predictions showed good agreement with experimental values, with relative differences within a few percent. The results also exhibit a depth-dependent increase in RBE along the SOBP, reflecting the progressive change in radiation quality as captured by the lineal energy distributions.

An application study was performed to evaluate the predictive capability of the model using microdosimetric spectra not included in the parameter fitting. Two types of input were considered: experimentally measured spectra and spectra obtained from Monte Carlo simulations using the PHITS code.

When an experimental microdosimetric spectrum was used, the predicted $\langle\alpha\rangle$ and $\langle\beta\rangle$ values showed good agreement with the corresponding experimentally derived parameters. In addition, the results were consistent with the trends observed along the SOBP, namely an increase of the α parameter with depth as the lineal energy increases, with a similar behavior observed for β . A consistent agreement was also found for the IsoE dose and the corresponding RBE values.

Alternatively, when microdosimetric spectra obtained from Monte Carlo simulations were considered, the model was still able to reproduce the expected trends and provide meaningful estimates of the IsoE dose and RBE. Although the spectra generated with PHITS do not fully reproduce all features of the experimental distributions, they provide a reasonable approximation and correctly describe the overall trends. These results highlight the potential of combining Monte Carlo

simulations with the COMPhID framework to investigate irradiation scenarios where experimental data may be limited. In this context, further improvements in the simulation of microdosimetric spectra would contribute to enhancing the applicability of the model.

It should be noted that the applicability of the model is restricted to the range of lineal energies covered by the experimental spectra used for its calibration. Within this domain, the model provides a flexible framework that requires only a microdosimetric spectrum as input, without the need for additional radiobiological experiments. Extending its validity beyond this range remains an open aspect for future work.

In addition to the analyses presented above, the photon IsoE dose along the 148 MeV SOBP was also investigated. This analysis showed a clear depth-dependent behavior of both the IsoE dose and the corresponding RBE values. An increasing RBE trend was observed from the proximal to the distal region, reflecting the progressive change in radiation quality with depth. RBE values ranged from approximately 0.7 to 1.5, corresponding to deviations of up to about 35% with respect to the conventional fixed value of 1.1.

Overall, the results presented in this chapter demonstrate that the COMPhID formulation provides a consistent and predictive framework for describing radiobiological effects based on microdosimetric information. By expressing radiation quality in terms of lineal energy, the model offers a coherent description at the microscopic scale and establishes a direct link between physical energy deposition patterns and biological response. Within its range of validity, the approach is able to reproduce experimental observations and extend predictions to new conditions, supporting its role as a robust formulation of the isoeffective dose model in a continuous microdosimetric framework.

GENERAL CONCLUSIONS

Proton therapy is currently undergoing a significant phase of global expansion, driven by its potential to improve treatment precision and reduce normal tissue toxicity. In this scenario, the accurate quantification of biological effectiveness remains one of the central challenges for its safe and optimized clinical implementation. This issue acquires particular relevance in Argentina and Latin America, where the ongoing development of the Argentine Proton Therapy Center (CeArP) represents a major milestone in the regional adoption of this technology. In this context, the present work contributes to bridging the gap between physical dose and biological response by advancing clinically oriented dosimetric frameworks. Beyond the specific models developed, this thesis provides new radiobiological data and methodological tools that are directly aligned with the needs of emerging proton therapy programs, supporting the transition from conventional RBE assumptions toward tumor-specific, radiobiology-based proton therapy strategies enabling a more accurate and tailored dosimetric description.

In clinical proton therapy, dose prescription is commonly based on a fixed RBE value of 1.1, implicitly assuming a uniform relationship between physical dose and biological effect across different irradiation conditions. However, extensive evidence indicates that RBE varies with radiation quality, dose, tissue type, and position along the beam, which may lead to inaccuracies in the estimation of the actual biological effect delivered to the patient. Within this context, the present work addresses the need for more accurate and physically grounded approaches to account for such variability. Specifically, this thesis develops two complementary, cell-specific approaches for the calculation of photon isoeffective doses in proton therapy, namely the macroscopic PhID model and the microdosimetric COMPhID model, avoiding the need for a constant RBE assumption. By linking radiation quality descriptors—expressed in terms of the linear energy transfer (LET) or the lineal energy (y)—to radiobiological response, these models allow for the estimation of position-dependent RBE values and provide a consistent framework to interpret proton therapy treatments in terms of photon-equivalent dose, thereby facilitating a more accurate and tissue-specific dosimetric assessment.

The macroscopic approach, represented by the PhID model and developed for

the HNSCC, HSF, and V79 cell lines, describes radiation fields in terms of averaged quantities, such as LET and particle-specific contributions. This formulation provides a simplified and operational framework that is directly applicable in clinical contexts, enabling an efficient translation of physical dose into biologically relevant quantities.

In contrast, the microdosimetric formulation, developed for the H460 cell line, expresses radiation quality through the stochastic distribution of lineal energy, preserving information on the microscopic patterns of energy deposition that are more closely related to the underlying mechanisms of biological damage. In this context, a key contribution of this work is the generation of the radiobiological dataset for the H460 cell line, which enables the parameterization of the model. Through this framework, it is possible to derive the α and β parameters for different radiation qualities, provided that the corresponding microdosimetric spectrum is known. While this approach offers a more detailed and mechanistically grounded physical description, it also requires the availability of microdosimetric spectra, which are not routinely accessible and may involve additional computational or experimental effort.

In this sense, both approaches can be regarded as complementary: the macroscopic formulation enables practical and clinically feasible implementation, whereas the microdosimetric model provides a powerful framework for investigating the relationship between radiation quality and biological effectiveness at a more fundamental level. This complementarity highlights the potential for future developments that integrate both perspectives, for example by using microdosimetric information to inform, calibrate, or refine macroscopic models, thereby providing a pathway from detailed physical descriptions to clinical applicability. Furthermore, the cell-specific nature of both models emphasizes the importance of personalized treatment planning in proton therapy, tailored to the biological characteristics of each tissue, ultimately contributing to more targeted treatments and improved patient outcomes.

In addition to the development of these models, this work led to the generation of a consistent and valuable set of radiobiological data, obtained under controlled and well-characterized irradiation conditions. This dataset constitutes a significant outcome in itself, as it provides a robust basis not only for further analyses and model validation, but also for informing and constraining biophysical models such as BIANCA, as well as microdosimetric approaches like the Microdosimetric Kinetic Model (MKM), for the specific cell lines investigated. Furthermore, it enables future extensions to other cell lines, irradiation conditions, and treatment scenarios.

Looking ahead, the approaches developed in this thesis open several directions for future work. In particular, validation against radiobiological and clinical datasets, including patient treatment plans and outcome modeling (e.g., TCP and NTCP), represents a natural next step to evaluate the robustness and predictive performance of the proposed frameworks. Furthermore, the integration of these models into treatment planning systems could facilitate their translation into clin-

ical practice. The radiobiological dataset generated in this work also provides a valuable basis for the development and validation of predictive radiobiological models, enabling a deeper exploration of the relationship between radiation quality and biological response in proton therapy. In this sense, this work not only addresses current limitations but also establishes a foundation for future developments aimed at strengthening the link between physics, radiobiology, and clinical practice in proton therapy.

LIST OF PUBLICATIONS

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- Valeriano, M.L., Santa Cruz, G., Ramos, R., Carante, M.P., Casali, A., Ballarini, F., Bortolussi, S. and González, S.J., 2024. THE PHOTON ISOEFFECTIVE DOSE MODEL IN PROTON THERAPY: AN APPLICATION WITH EMPHASIS ON HEAD AND NECK SQUAMOUS CELL CARCINOMA TREATMENT. Conference proceeding, International Journal of Particle Therapy, 12, p.100158, doi: [10.1016/j.ijpt.2024.100158](https://doi.org/10.1016/j.ijpt.2024.100158).
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- Dattoli Viegas A. M., Gossio S., Riback J., Valeriano L. M., Santa Cruz G., Casali A., Carante M., Ballarini F., Fatemi S., Postuma I., Vercesi V., Bortolussi S., Ramos R. L., González S. J., 2025. An extended photon isoeffective dose model accounting for the energy-dependent effectiveness of secondary charged particles in BNCT. Accepted for publication in the Medical Physics journal.

APPENDIX A

PHENOMENOLOGICAL RBE MODELS BASED ON THE LQ FORMALISM

The considered LQ-based RBE models can be expressed in the common form given in Eq. 1.13, following the reformulation of phenomenological RBE models presented in [16]. As introduced in Sec. 1.2.4.2, the models differ only in the definition of the RBE_{max} and RBE_{min} functions. In the following, the functional forms adopted by these terms are presented for each model.

Wilkins (WIL) model

$$RBE_{max}(LET_d) = 0.892 + 0.179(\text{keV}/\mu\text{m})^{-1}LET_d,$$
$$RBE_{min} = 1.$$

Tilly (TIL) model

$RBE_{max}(LET_d, (\alpha/\beta)_x) = 1 + \frac{0.309 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} LET_d$, for low $(\alpha/\beta)_x$ values around 2 Gy (TIL2),

$RBE_{max}(LET_d, (\alpha/\beta)_x) = 1 + \frac{0.550964 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} LET_d$, for high $(\alpha/\beta)_x$ values around 10 Gy (TIL10),

$$RBE_{min} = 1.$$

Chen (CHE) model

$$RBE_{max}(LET_d) = 1 + \frac{1 - e^{-0.0013(\text{keV}/\mu\text{m})^{-2}LET_d^2}}{0.045(\text{keV}/\mu\text{m})^{-1}LET_d},$$
$$RBE_{min} = 1.$$

Carabe (CAR) model

$$\text{RBE}_{\max}(\text{LET}_d, (\alpha/\beta)_x) = 0.843 + 0.154 \frac{2.686 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} \text{LET}_d,$$

$$\text{RBE}_{\min}(\text{LET}_d, (\alpha/\beta)_x) = 1.09 + 0.006 \frac{2.686 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} \text{LET}_d.$$

Wedenberg (WED) model

$$\text{RBE}_{\max}(\text{LET}_d, (\alpha/\beta)_x) = 1 + \frac{0.434 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} \text{LET}_d,$$

$$\text{RBE}_{\min} = 1.$$

Jones (JON) model

$$\text{RBE}_{\max}(\text{LET}_d, \alpha_x) = 1 + \frac{\text{LET}_d - 0.22 \text{ keV}/\mu\text{m}^{-1}}{30.28 \text{ keV}/\mu\text{m}^{-1}} \left(\frac{\alpha_u}{\alpha_x} - 1 \right),$$

$$\text{RBE}_{\min}(\text{LET}_d, \beta_x) = \sqrt{1 + \frac{\text{LET}_d - 0.22 \text{ keV}/\mu\text{m}^{-1}}{30.28 \text{ keV}/\mu\text{m}^{-1}} \left(\frac{\beta_u}{\beta_x} - 1 \right)},$$

where $\alpha_u = 2.696 \text{ Gy}^{-1} \times (1 - e^{-3.92 \text{ Gy}^{-1} \alpha_x})$ and $\beta_u = 0.06 \text{ Gy}^{-2} \times (1 - e^{-50 \text{ Gy}^{-2} \beta_x})$.

McNamara (MCN) model

$$\text{RBE}_{\max}(\text{LET}_d, (\alpha/\beta)_x) = 0.99064 + \frac{0.35605 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} \text{LET}_d,$$

$$\text{RBE}_{\min}(\text{LET}_d, (\alpha/\beta)_x) = 1.1012 - 0.0038703 \text{ Gy}^{-1/2}(\text{keV}/\mu\text{m})^{-1} \sqrt{(\alpha/\beta)_x} \text{LET}_d.$$

Rørvik (RORU) model

The unweighted model, based on the linear unweighted fit:

$$\text{RBE}_{\max}(\text{LET}_d, (\alpha/\beta)_x) = 1 + \frac{0.645 \text{ Gy}(\text{keV}/\mu\text{m})^{-1}}{(\alpha/\beta)_x} \text{LET}_d,$$

$$\text{RBE}_{\min} = 1.$$

Peeler (PLR) model

$$\text{RBE}_{\max}(\text{LET}_d, (\alpha/\beta)_x) = 0.75 + \frac{0.00143 \text{ Gy}(\text{keV}/\mu\text{m})^{-3}}{(\alpha/\beta)_x} \text{LET}_d^3,$$

$$\text{RBE}_{\min}(\text{LET}_d, (\alpha/\beta)_x) = 1.24 + 0.00074 \text{ Gy}^{-1}(\text{keV}/\mu\text{m})^{-3} (\alpha/\beta)_x \text{LET}_d^3.$$

APPENDIX B

LEA-CATCHESIDE G FACTOR

The Lea–Catcheside factor, $G_{ij}(\theta)$, introduced in Sec. 2.1.1, accounts for the temporal repair of sublethal damage produced during irradiation time θ . In particular, it describes the probability that two sublesions—induced by radiation components i and j —interact before being repaired. Within the modified linear–quadratic formalisms, this factor acts as a multiplicative term that modulates the quadratic dose contribution.

For a given irradiation time θ , the factor can be expressed as

$$G(\theta, t_0) = \frac{2t_0}{\theta} \left(1 - \frac{t_0}{\theta} \left(1 - e^{-\frac{\theta}{t_0}} \right) \right), \quad (\text{B.1})$$

where t_0 represents the characteristic repair time, which may correspond to fast or slow repair components.

When the formation of sublesions from different radiation components can be considered independent, the mixed-term factor $G_{ij}(\theta)$ can be decomposed into a weighted sum of individual contributions, namely

$$G_{ij}(\theta) = a_i G_i(\theta) + a_j G_j(\theta), \quad (\text{B.2})$$

where a_i and a_j denote the relative contributions of each component, with $a_i + a_j = 1$.

APPENDIX C

MEAN NUMBER OF LETHAL LESIONS IN CDRA AND ITS EXTENSION

The average number of lethal lesions is obtained by defining a new random variable (r.v.) that describes the number of lethal lesions as a function of the specific energy deposited in micrometric sites by one or more events. The following quantity,

$$\varepsilon(Z) = a_1 Z + a_2 Z^2 + a_3 Z^3, \quad (\text{C.1})$$

is the effectiveness function that, averaged over a population of sensitive sites, not necessarily identical, gives the average value of lethal lesions as a function of the mean specific energy for multiple events, $\bar{Z}$, where Z is the specific energy accumulated in a micrometer¹ site by zero, one or more events:

$$\langle \varepsilon \rangle(\bar{Z}) = \int_0^\infty \varepsilon(Z) f(Z, \bar{Z}) dZ = a_1 M_1 + a_2 M_2 + a_3 M_3. \quad (\text{C.2})$$

The quantities M_j are the j -order moments of the multievent p.d.f. There exists a recursive relationship between the multievent moments, M , and the single-event moments, m [117]:

$$m_{k+1} = \frac{M_{k+1}}{e} - \sum_{j=0}^{k-1} (kj) M_{k-j} m_{j+1}; \quad k = 0, 1, 2, \dots \quad (\text{C.3})$$

where e is the average number of events in the site at the dose $\bar{Z}$. Therefore:

¹In the theory of Compound Dual Radiation Action (CDRA) [54], uppercase zeta is used to denote specific energy deposited at micrometer sites, and lowercase zeta to denote the energy deposition at nanometer sites.

$$\begin{aligned}
 M_1 &= \bar{Z} = em_1, \\
 M_2 &= \bar{Z}^2 + \bar{Z} \frac{m_2}{m_1}, \\
 M_3 &= \bar{Z}^3 + 3\frac{m_2}{m_1}\bar{Z}^2 + \frac{m_3}{m_1}\bar{Z}.
 \end{aligned} \tag{C.4}$$

If we associate $\bar{Z}$ with absorbed dose, D , as is usually the case, then:

$$\langle \varepsilon \rangle(D) = a_1 M_1 + a_2 M_2 + a_3 M_3 = a_1 D + a_2 \left(D^2 + D \frac{m_2}{m_1} \right) + a_3 \left(D^3 + 3\frac{m_2}{m_1} D^2 + \frac{m_3}{m_1} D \right), \tag{C.5}$$

which can be rewritten as

$$\langle \varepsilon \rangle(D) = \langle \alpha \rangle D + \langle \beta \rangle D^2 + \langle \gamma \rangle D^3, \tag{C.6}$$

with

$$\begin{aligned}
 \langle \alpha \rangle &= a_1 + a_2 \frac{m_2}{m_1} + a_3 \frac{m_3}{m_1}, \\
 \langle \beta \rangle &= a_2 + 3a_3 \frac{m_2}{m_1} \text{ and } \langle \gamma \rangle = a_3.
 \end{aligned} \tag{C.7}$$

Assuming that the survival probability corresponds to the probability of zero lethal lesions and that these lesions follow a Poisson distribution, survival can be expressed as ²:

$$S(D) = e^{-\langle \varepsilon \rangle(D)}. \tag{C.8}$$

The α and β coefficients can be determined experimentally from cell survival data within the conventional LQ framework. In the present extension, however, the formal expansion of $\langle \varepsilon \rangle(D)$ naturally gives rise to a cubic term in D . Nevertheless, for consistency with the standard LQ description, we retain the expansion up to second order in D (although in general survival curves tend to depart from LQ behavior at high doses), recalling that the resulting α and β coefficients are not merely phenomenological parameters but incorporate information about the CCR.

Effectiveness function as a function of the moments of $f(y)$

A simple relationship exists between lineal energy and the specific energy per event, Z_1 , based on their respective definitions and on the Cauchy expression for the mean chord length of the sensitive site. Using this relationship, the coefficients of $\langle \varepsilon \rangle(D)$ can be written as functions of the moments of $f(y)$:

²It can be shown from microdosimetric considerations that lethal lesions are not strictly Poisson distributed. Nevertheless, experimental evidence indicates that the probability of cell survival exhibits an exponential dependence on the average number of lethal lesions. [118]

$$Z_1 = \frac{4}{\rho S} y, \quad (\text{C.9})$$

where ρ is the material density of the site, and S is its surface area. Let us define $c = \frac{4}{\rho S}$. Then,

$$\int_0^\infty y^q f(y) dy = \frac{1}{c^q} \int_0^\infty Z_1^q f_1(Z_1) dZ_1, \quad (\text{C.10})$$

or

$$m_q = c^q y^{(q)} \quad (\text{C.11})$$

where $y^{(q)}$ are the q -order moments of $f(y)$. Then, the coefficients α and β can be written as:

$$\langle \alpha \rangle = a_1 + c a_2 \frac{y^{(2)}}{y^{(1)}} + c^2 a_3 \frac{y^{(3)}}{y^{(1)}}, \quad (\text{C.12})$$

$$\langle \beta \rangle = a_2 + 3c a_3 \frac{y^{(2)}}{y^{(1)}}. \quad (\text{C.13})$$

The coefficient $\langle \gamma \rangle$ remains equal to a_3 and is independent of the moments of $f(y)$.

APPENDIX D

MONTE CARLO ESTIMATION OF THE VARIANCE OF g

D.1 Monte Carlo methodology

The Monte Carlo sampling procedure employed to evaluate the variance of g is outlined as follows (see Figure D.1):

- **1. Proton energy selection.** A proton with incident energy E , originating at $x = 0$, is sampled either at a fixed energy (monoenergetic) or from a track-by-track distribution obtained through a macroscopic PHITS calculation, corresponding to a monoenergetic 200 MeV proton beam slowed down to specific depths in water. The resulting track-by-track energy distribution at those depths includes protons of varying energies, reflecting energy straggling accumulated along their trajectories. Scoring depths are chosen around the Bragg Peak, both proximal and distal.
- **2. Range calculation and straggling.** Once the proton energy is selected, its projected range is determined using an Energy-Range Look-up Table (ER-LUT), under the Continuous Slowing-Down and Track Segment approximations. Additional energy straggling is introduced by sampling a Gaussian distribution according to Bohr's theory (see [30]). This yields the final distribution of proton energies traversing the site and their corresponding ranges.
- **3. Chord sampling.** A chord is randomly selected by sampling from the chord-length distribution of a sphere under isotropic randomness. The sphere diameter is set to $1\ \mu\text{m}$.
- **4. Event classification.** The distance from the source plane ($x = 0$) to the sphere surface is computed. The event type is then determined: if the resid-

D.2. Results

Single Energy E [MeV]	$\text{Var}(\sqrt{\beta_0 + \beta_1 y})$	Bragg Peak proton spectra E[MeV]	$\text{Var}(\sqrt{\beta_0 + \beta_1 y})$
5	$4.93 \cdot 10^{-4}$	7.5	$5.16 \cdot 10^{-4}$
10	$1.45 \cdot 10^{-4}$	8.7	$3.83 \cdot 10^{-4}$
20	$5.33 \cdot 10^{-5}$	10.4	$2.92 \cdot 10^{-4}$
30	$1.81 \cdot 10^{-5}$	12.7	$2.34 \cdot 10^{-4}$
40	$9.46 \cdot 10^{-6}$	15.8	$1.60 \cdot 10^{-4}$
50	$6.02 \cdot 10^{-6}$	19.5	$8.18 \cdot 10^{-5}$
60	$4.24 \cdot 10^{-6}$	23.5	$5.01 \cdot 10^{-5}$
70	$3.19 \cdot 10^{-6}$	27.5	$3.56 \cdot 10^{-5}$
80	$2.52 \cdot 10^{-6}$	31.1	$2.78 \cdot 10^{-5}$
90	$2.05 \cdot 10^{-6}$	34.4	$2.80 \cdot 10^{-5}$
100	$1.72 \cdot 10^{-6}$	37.5	$2.16 \cdot 10^{-5}$

Table D.1

The average experimental beta coefficient across all experiments (Table 3.9) is $\langle \beta \rangle = 0.085 \text{ Gy}^{-2}$. Considering this value, it can be observed that $\text{Var}(\sqrt{\beta_0 + \beta_1 y})$ is significantly smaller (see Table D.2).

Beam	Variance Average value (Gy^{-2})	$\langle \beta \rangle / \langle \text{Var}(g) \rangle$
Monoenergetic beams	$6.71 \cdot 10^{-5}$	$1.27 \cdot 10^3$
PHITS Bragg Peak	$1.66 \cdot 10^{-4}$	$0.51 \cdot 10^3$

Table D.2

This comparison shows that, even around the Bragg Peak and for a wide variety of energies and distributions, the variance of $\sqrt{\beta_0 + \beta_1 y}$ (which is of the order of the experimental uncertainties) can be neglected, and expression 3.14 can be applied without approximations.

APPENDIX E

CLONOGENIC ASSAY

This appendix presents the detailed colony counts obtained from the clonogenic assays, including both control samples and all irradiation positions and dose levels listed in Table 3.1. The results are displayed using a representative mesh reproducing the layout of the 96-well plates employed for cell seeding.

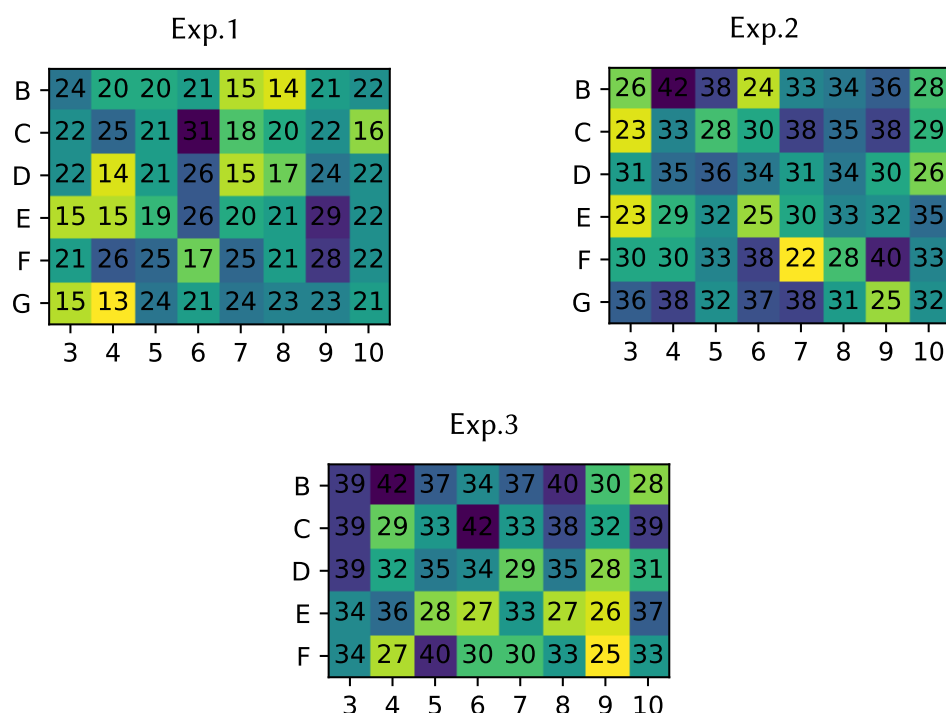


Figure E.1: Counting of colonies from control samples corresponding to experiments 1, 2, and 3.

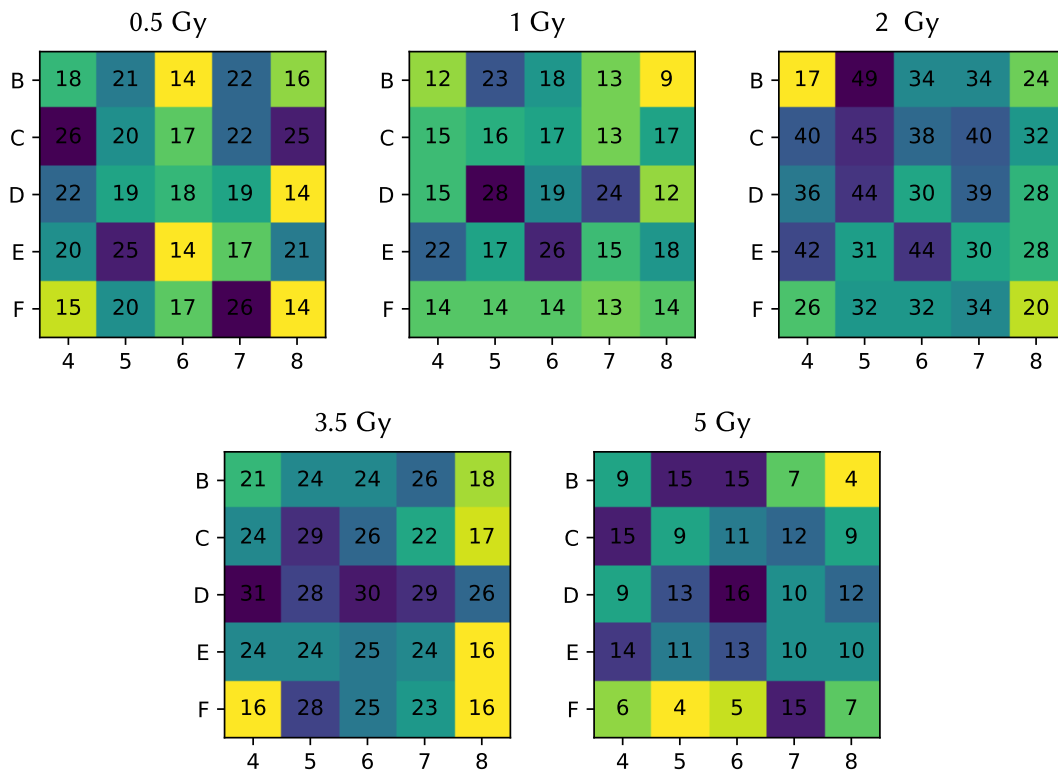


Figure E.2: Colony counts corresponding to Experiment 1 at irradiation position P2.

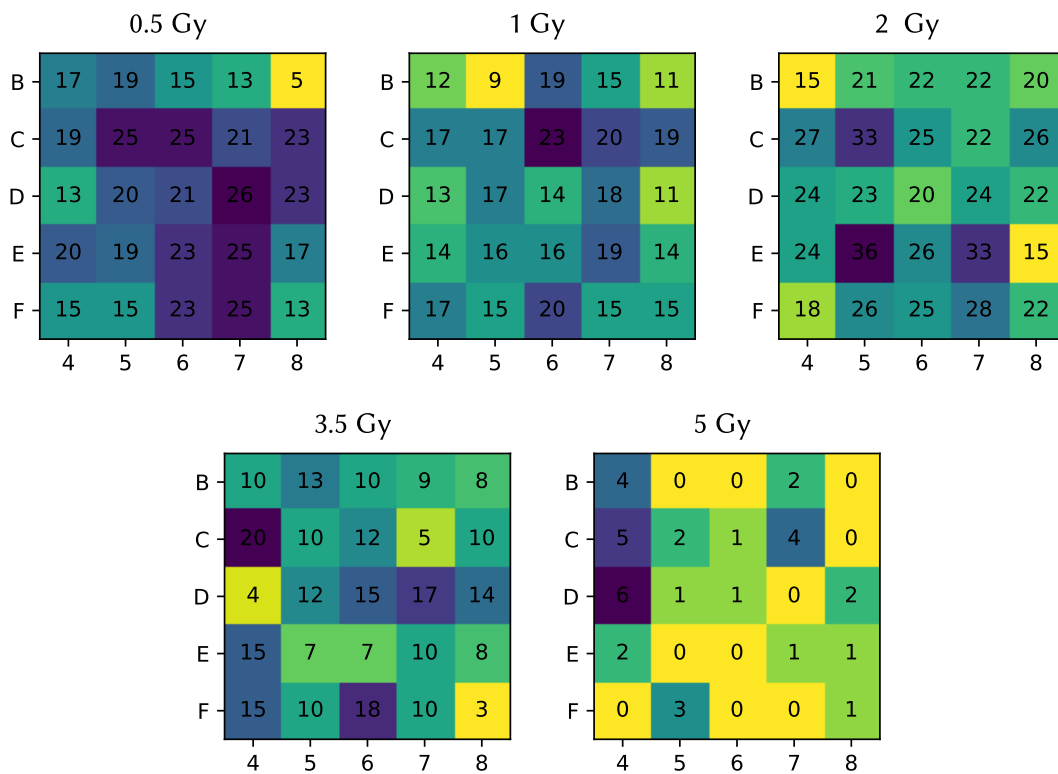


Figure E.3: Colony counts corresponding to Experiment 1 at irradiation position P4.

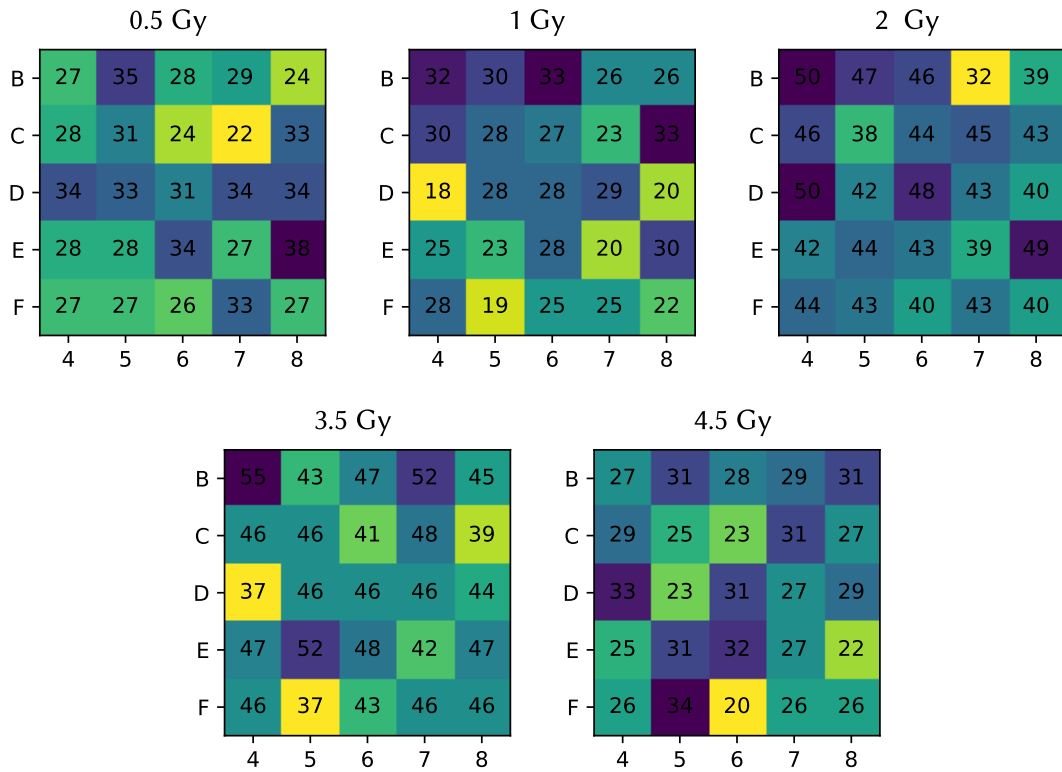


Figure E.4: Colony counts corresponding to Experiment 2 at irradiation position P1.

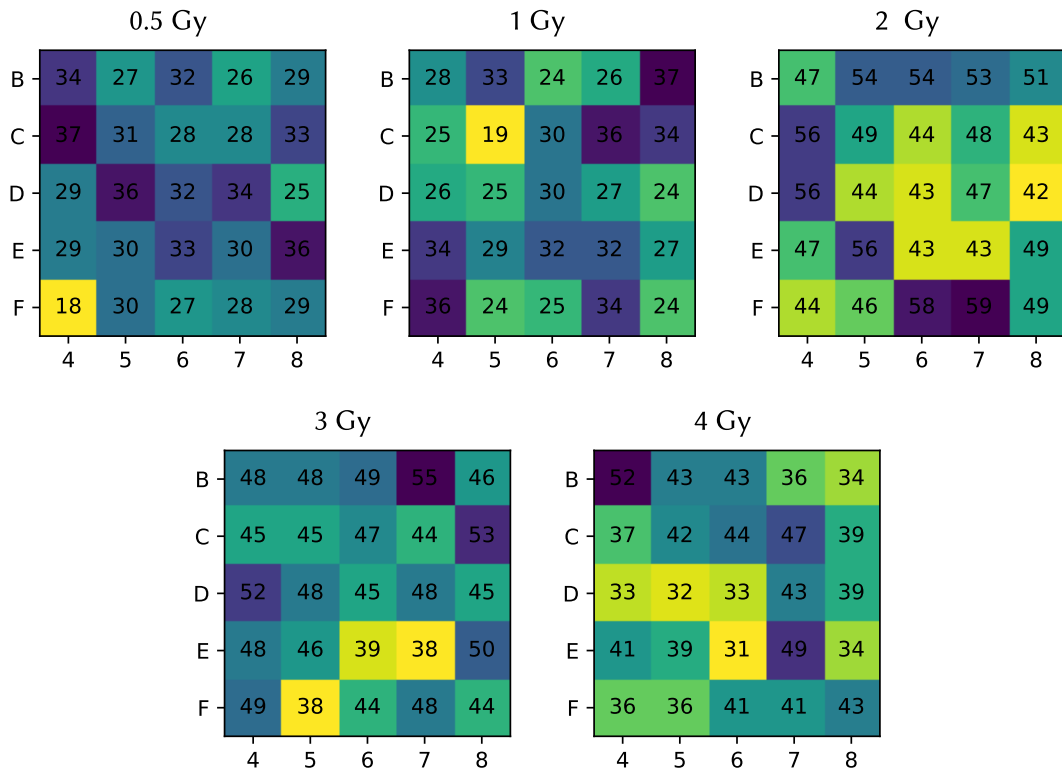


Figure E.5: Colony counts corresponding to Experiment 2 at irradiation position P2.

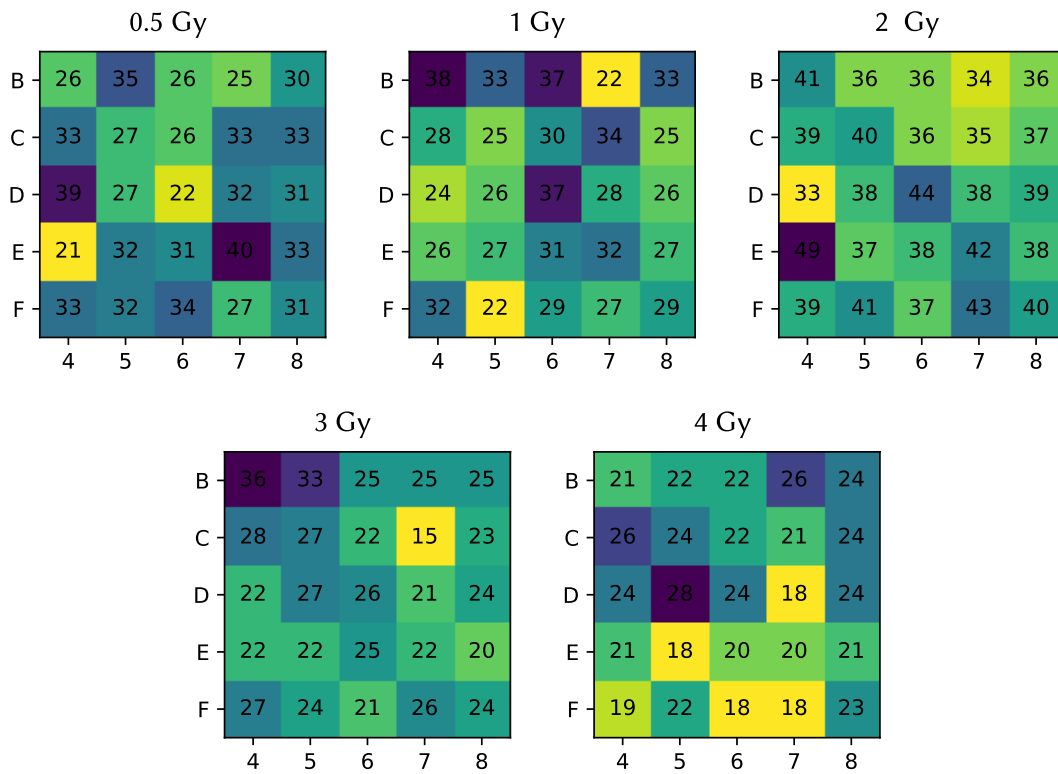


Figure E.6: Colony counts corresponding to Experiment 2 at irradiation position P4.

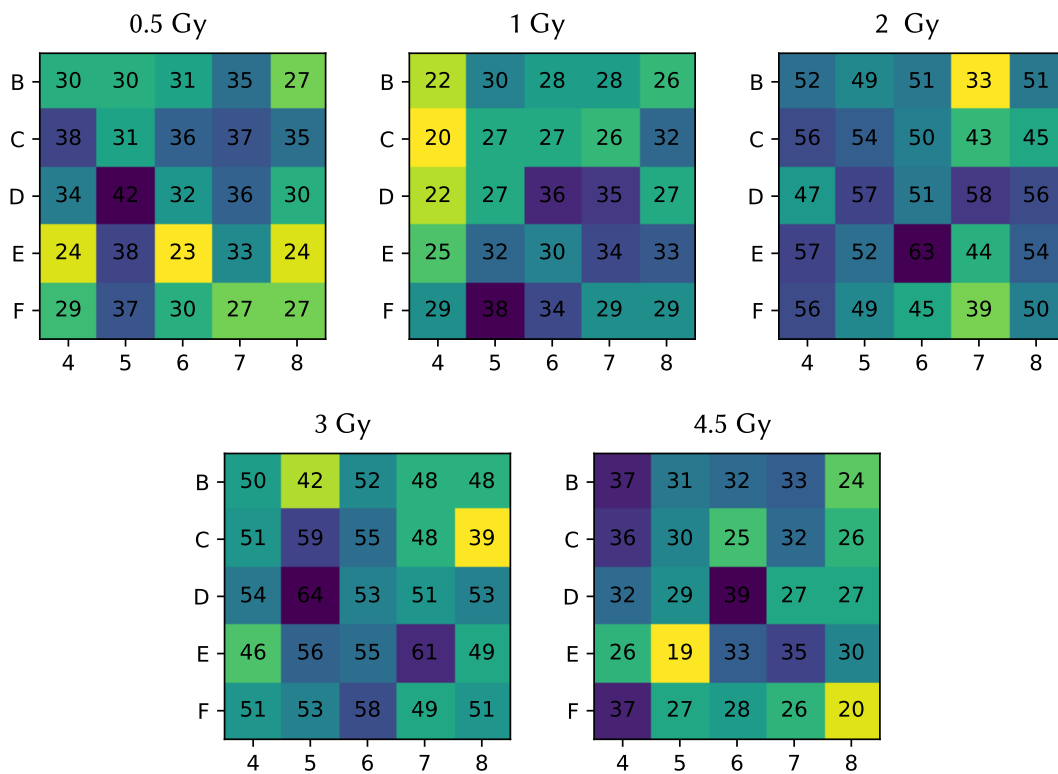


Figure E.7: Colony counts corresponding to Experiment 3 at irradiation position P1.

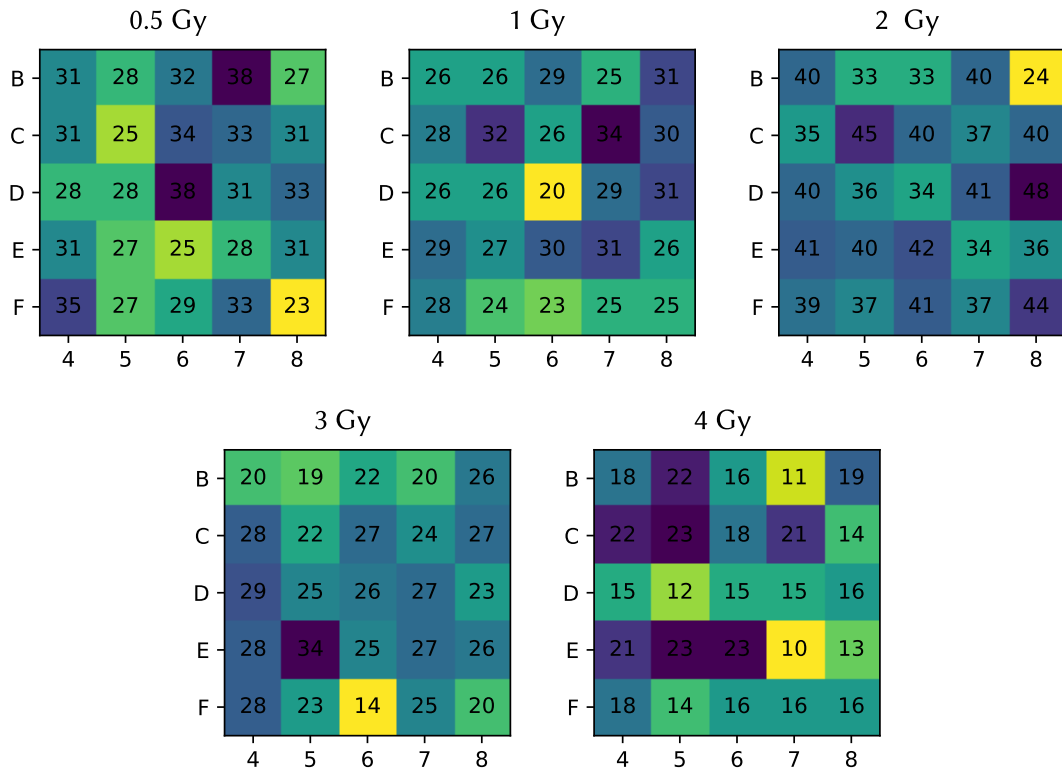


Figure E.8: Colony counts corresponding to Experiment 3 at irradiation position P3.

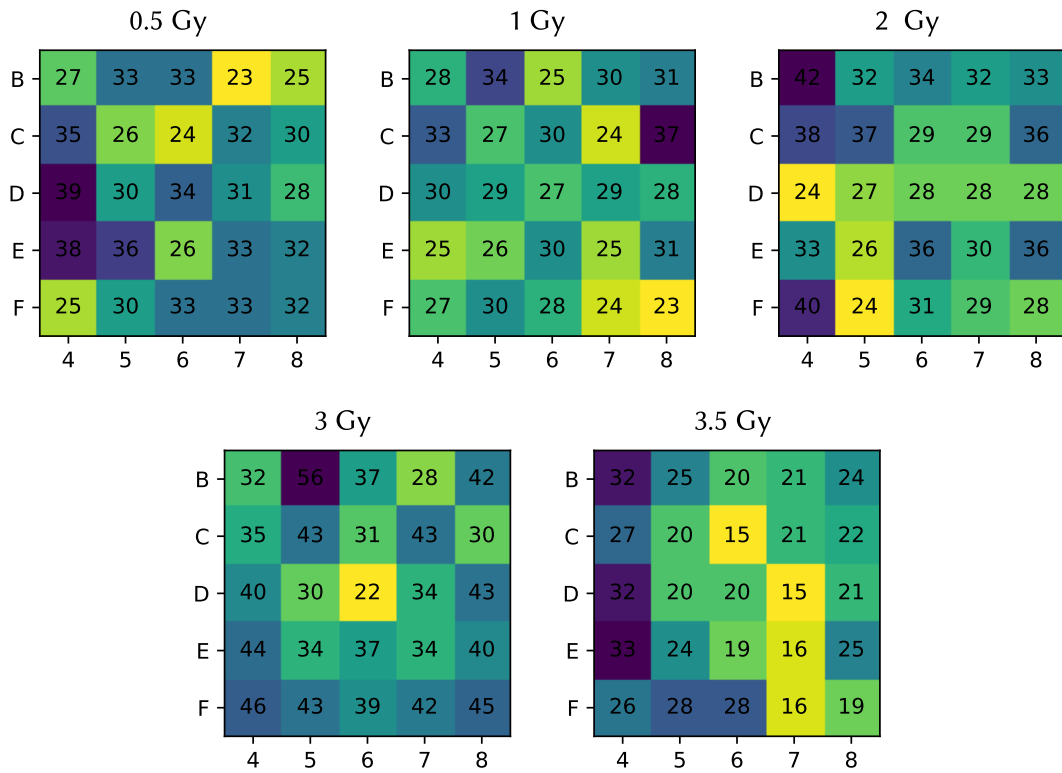


Figure E.9: Colony counts corresponding to Experiment 3 at irradiation position P5.

APPENDIX F

DETERMINATION OF THE COMPHID MODEL PARAMETERS

This appendix summarizes the numerical values corresponding to results presented in graphical form in the main text.

The data shown in Fig. 3.33 (Sec. 3.3.4) are reported in Tables F.1 and F.2.

Similarly, the numerical values associated with Fig. 3.34 (Sec. 3.3.6) are provided in Table F.3.

Position	α_{obs}	$\langle \alpha_{pred} \rangle$	Precision [%]
P1	0.186 ± 0.013	0.186 ± 0.035	99.9
P2	0.201 ± 0.017	0.202 ± 0.034	97.6
P3	0.377 ± 0.029	0.374 ± 0.038	99.4
P5	0.532 ± 0.063	0.535 ± 0.048	99.3

Table F.1: Observed and model-predicted α values at positions P1–P5, together with the corresponding point-wise precision. The precision was calculated according Eq. 3.26.

Position	β_{obs}	$\langle \beta_{pred} \rangle$	Precision [%]
P1	0.077 ± 0.003	0.078 ± 0.014	98.1
P2	0.080 ± 0.004	0.078 ± 0.014	97.7
P3	0.090 ± 0.008	0.087 ± 0.017	96.3
P5	0.080 ± 0.024	0.095 ± 0.021	81.6

Table F.2: Observed and model-predicted α values at positions P1–P5, together with the corresponding point-wise precision.

	Physical dose [Gy]	IsoE dose COMPhID [Gy]	RBE
P1	0.94	0.70 ± 0.08	0.74 ± 0.09
P2	1.98	1.66 ± 0.15	0.83 ± 0.08
P3	1.64	1.89 ± 0.13	1.15 ± 0.08
P4	1.33	1.81 ± 0.18	1.36 ± 0.14
P5	1.13	1.69 ± 0.11	1.49 ± 0.09

Table F.3: Photon IsoE doses calculated using the COMPhID model for irradiation positions P1–P5 and the corresponding RBE values derived for each position. All values are reported together with their associated uncertainties.

APPENDIX G

COMPARISON AGAINST MONTE CARLO SIMULATIONS

G.1 Comparison between experimental and simulated spectra

In this section, a comparison is presented between the microdosimetric spectra obtained from Monte Carlo simulations using PHITS and those measured experimentally. The comparison is shown in Fig. G.1, where the spectra are presented in terms of $yf(y)$. The spectra obtained from PHITS simulations are displayed as dashed lines, while the experimental spectra are shown as solid curves.

As can be seen in the figure, the microdosimetric spectra obtained computationally follow the same general trend as the experimental ones, i.e., a progressive shift of the spectra toward higher linear energy values from positions P1 to P5.

However, when comparing the experimental spectra with those obtained from MC simulations, a systematic shift between them can be observed, whose direction depends on the irradiation position. At positions P1 and P2, the PHITS spectra exhibit a slight shift toward higher linear energy values compared to the experimental spectra, which is more pronounced at position P1. In contrast, for positions P3, P4, and P5, the PHITS spectra are shifted toward lower linear energy values with respect to the experimental ones.

A quantitative assessment of the spectral shifts discussed above can be obtained by comparing the linear energy moments listed in Table G.1 with those derived from the experimental spectra (see Table 3.10). In particular, the first moment $y^{(1)}$, corresponding to the frequency-mean lineal energy y_F , provides a direct measure of the shift between simulated and experimental spectra.

At positions P1 and P2, the PHITS simulations yield slightly higher y_F values than the experimental measurements, consistent with the shift of the simulated spectra toward larger lineal energy values observed in Fig. G.1. Experimentally,

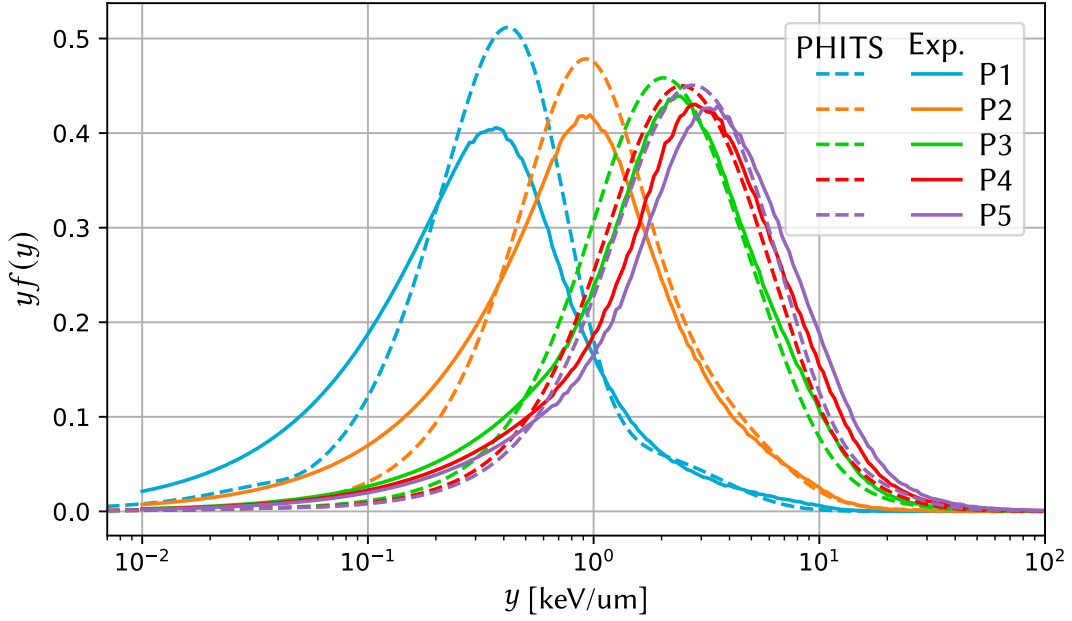


Figure G.1: Experimental and PHITS-simulated microdosimetric spectra $yf(y)$ at irradiation positions P1–P5. Solid lines correspond to experimental data and dashed lines to PHITS simulations.

Positions	$y^{(1)} \pm \sigma$ [keV/ μm]	$y^{(2)} \pm \sigma$ [(keV/ μm) ²]	$y^{(3)} \pm \sigma$ [(keV/ μm) ³]
P1	0.59	3.02 ± 0.14	$(3.97 \pm 0.36) \cdot 10^2$
P2	1.48	7.89 ± 0.08	$(2.70 \pm 0.09) \cdot 10^2$
P3	2.93	26.85 ± 0.50	$(1.89 \pm 0.13) \cdot 10^3$
P4	3.43	26.96 ± 0.11	$(5.19 \pm 0.08) \cdot 10^2$
P5	3.74	32.54 ± 0.15	$(7.07 \pm 0.10) \cdot 10^2$

Table G.1: First three moments of the lineal energy distribution, $y^{(1)}$, $y^{(2)}$, and $y^{(3)}$, calculated for irradiation positions P1 to P5 through MC simulations in PHITS. The uncertainty of $y^{(1)}$ is lower than 0.3%.

mean values of 0.57 and 1.37 were obtained at P1 and P2, respectively, whereas the MC simulations resulted in y_F values of 0.59 and 1.48. These correspond to relative differences of approximately 4% and 8% with respect to the experimental values.

In contrast, for irradiation positions P3, P4, and P5, the simulated y_F values are systematically lower than the corresponding experimental values. The experimental spectra yield mean values of 3.17, 3.94, and 4.45, whereas the MC simulations provide values of 2.93, 3.43, and 3.74, respectively. These correspond to relative differences of approximately 8% at P3, which increase to 13% and 16% at P4 and P5, respectively.

Regarding $y^{(2)}$, which corresponds to the dose-mean lineal energy y_D , the rel-

ative differences with respect to the experimental values are 11%, 2%, 5%, 37%, and 38% for irradiation positions P1 to P5, respectively. Larger discrepancies are observed for the third lineal energy moment $y^{(3)}$, for which the relative differences range between 43% and 77%.

The observed differences between simulated and experimental microdosimetric spectra and microdosimetric quantities, may arise from several factors related to both the Monte Carlo modelling and the experimental measurement. One possible source of discrepancy is the material assumed in the calculation of the microdosimetric distributions. As described in Sec. 3.2.2, the [T-sed] tally from PHITS used in the present work was applied considering water as the target material, since the model has been developed and validated for this medium [108]. In contrast, the sensitive volume of the mini-TEPC detector is filled with propane gas. Since the stopping power (or LET) in propane is lower than in water, the use of water-equivalent sites in the simulations may lead to a systematic shift of the spectra toward higher lineal energy values, as well as to differences in the contribution of secondary particles.

Additional discrepancies may also originate from statistical limitations associated with the number of events recorded by the detector, which particularly affect the estimation of higher-order lineal energy moments [109, 116]. These moments are known to be highly sensitive to the high- y tail of the spectra, where the number of events is typically limited. Experimental factors may also contribute to the observed differences, such as electronic noise in the detection system, uncertainties in the detector calibration, or the effective energy resolution of the microdosimetric detector.

Finally, differences may also arise from the physical models implemented in the Monte Carlo code to describe nuclear interactions. As discussed in Sec. 2.2.1, these models determine quantities such as stopping powers, secondary particle production, and energy deposition processes, all of which directly influence the resulting microdosimetric spectra.

Positions	$\frac{y^{(2)}}{y^{(1)}} \pm \sigma$ [keV/ μ m]	$\frac{y^{(3)}}{y^{(1)}} \pm \sigma$ [(keV/ μ m) ²]
P1	5.13 ± 0.23	$(6.73 \pm 0.60) \cdot 10^2$
P2	5.32 ± 0.06	$(1.83 \pm 0.06) \cdot 10^2$
P3	9.15 ± 0.17	$(6.44 \pm 0.46) \cdot 10^2$
P4	7.86 ± 0.03	$(1.51 \pm 0.02) \cdot 10^2$
P5	8.71 ± 0.04	$(1.89 \pm 0.02) \cdot 10^2$

Table G.2: Ratios of the second and third moments to the first moment of the lineal energy distribution, $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$, for irradiation positions P1 to P5 calculated through MC simulations in PHITS.

These differences in the microdosimetric spectra, as expected, affect the values of the calculated lineal energy moments and, consequently, the resulting ra-

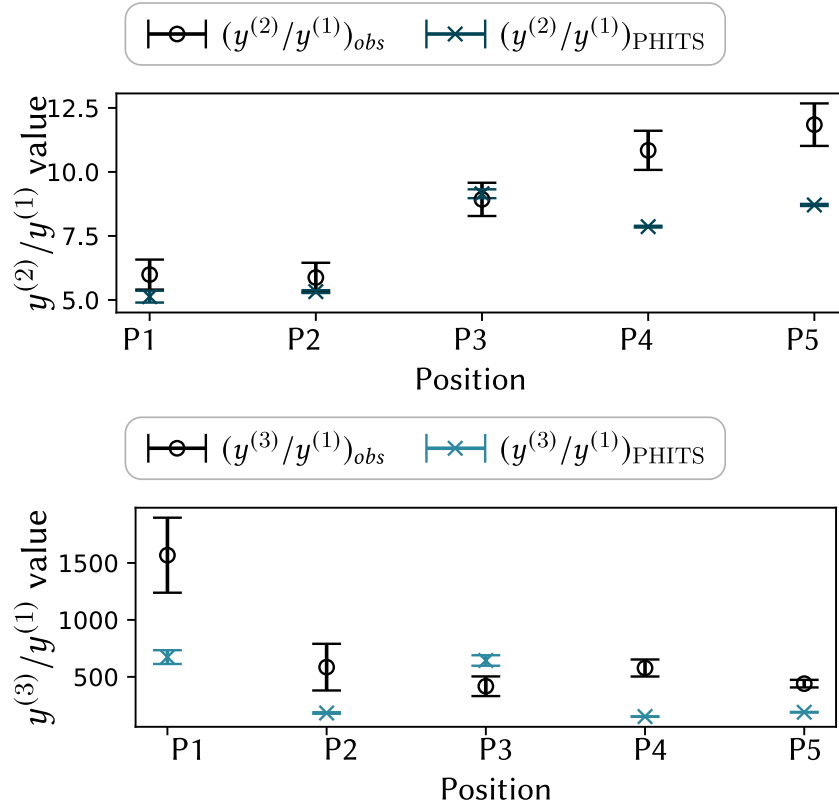


Figure G.2: Comparison of the ratios $(y^{(2)}/y^{(1)})_{obs}$ and $(y^{(3)}/y^{(1)})_{obs}$, obtained from the experimental microdosimetric spectra, with the corresponding values $(y^{(2)}/y^{(1)})_{PHITS}$ and $(y^{(3)}/y^{(1)})_{PHITS}$ derived from PHITS simulations for the irradiation positions P1–P5.

tios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$. Table G.2 summarizes the values of the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$ obtained from the PHITS simulations for each irradiation position. The comparison between the ratios derived from the simulations and those obtained experimentally (see Table 3.11) as a function of the irradiation position is illustrated in Fig. G.2.

As can be observed, the discrepancies between the experimental and MC-derived lineal energy moment ratios vary significantly from point to point along the SOBP positions. For the $y^{(2)}/y^{(1)}$ ratio, the relative differences with respect to the experimental values are 14, 9, 2, 27, and 26% for positions P1 to P5, respectively. Larger discrepancies are observed for the $y^{(3)}/y^{(1)}$ ratio, with relative differences of approximately 57, 69, 54, 74, and 57% for P1–P5.

These differences are not negligible, as they may propagate into the calculation of the isoeffective dose when using the COMPhID model. In particular, as shown in the following section, the model parameters were determined using the experimentally derived microdosimetric quantities; therefore, deviations between simulated and experimental lineal energy moments could lead to systematic differences in the predicted biological effectiveness when the model is applied to MC-generated spectra.

G.2 Determination of a microdosimetric spectrum between irradiation positions P2 and P3

In order to extend the application of the COMPhID model to an additional irradiation position, a corresponding microdosimetric spectrum was obtained through Monte Carlo simulations. Figure G.3 presents the set of microdosimetric spectra calculated for the irradiation positions P1–P5. An additional position, denoted as Pn and located between P2 and P3, is also included and represented by a brown dashed line.

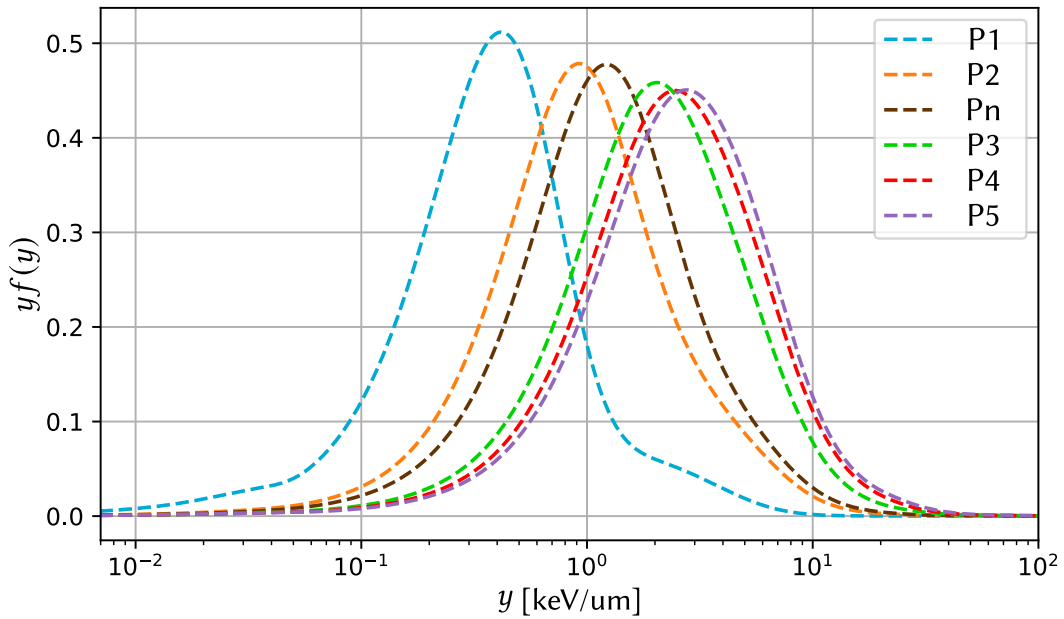


Figure G.3: Simulated microdosimetric spectra $yf(y)$ at irradiation positions P1–P5 and the new position Pn.

Considering this additional microdosimetric spectrum at position Pn, the first three lineal energy moments were calculated, together with the ratios $y^{(2)}/y^{(1)}$ and $y^{(3)}/y^{(1)}$. The obtained values are:

- $y^{(1)} = 1.80 \text{ keV}/\mu\text{m}$, with an uncertainty lower than 0.1%
- $y^{(2)} = 9.24 \pm 0.06 \text{ (keV}/\mu\text{m})^2$
- $y^{(3)} = (2.17 \pm 0.05) \cdot 10^2 \text{ (keV}/\mu\text{m})^3$
- $y^{(2)}/y^{(1)} = 5.14 \pm 0.03 \text{ keV}/\mu\text{m}$
- $y^{(3)}/y^{(1)} = (1.21 \pm 0.03) \cdot 10^2 \text{ (keV}/\mu\text{m})^2$

This set of values will then be used in Section 3.3.6 to estimate isoeffective photon doses and RBE values using the COMPhID model.

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