Appendix 2

Summary of Professional Accomplishments

The particle track structure and its effect on DNA damage and repair in human cells exposed to ionising radiation

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Contents

1 Name

Beata Brzozowska

2 Diplomas, degrees conferred in specific areas of science, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation

Master's degree, dissertation: *Hadron production in the region of limited fragmentation*, Department of Particles and Fundamental Interactions, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, 2005

PhD degree, dissertation: *Scaled momentum spectra in deep inelastic scattering at HERA*, Department of Particles and Fundamental Interactions, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, 2010

3 Employment in research institutes or faculties/departments

Employment: adjunct at the Biomedical Physics Division, Faculty of Physics, University of Warsaw (01/09/2011-present)

Postdoctoral fellowship: Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University (01/04/2015–31/08/2016)

4 Description of the achievements

4.1 Title of the scientific achievement

The title of the scientific achievement, which is the basis for the habilitation procedure, is ***The particle track structure and its effect on DNA damage and repair in human cells exposed to ionising radiation***. The achievement refers to the study of the mechanisms of DNA damage and repair in cells exposed to ionising radiation. The effect of ionising radiation on the cellular material was measured in *in vitro* using radiobiological tests and studied in *in silico* using Monte Carlo (MC) methods.

4.2 List of works constituting the basis of the habilitation procedure

The list of publications, which constitutes a consistent cycle, which is the basis for the habilitation procedure, is included in the table below. My individual contributions are described in the list of publications below and in Appendix 3 (List of scientific achievements).

Authors (year)	**Title**	**Journal**
(**BB1**) M. Eriksson, P. Hååg, **B. Brzozowska**, M. Lipka, H. Lisowska, R. Lewensohn, A. Wojcik, K. Viktorsson, L. Lundholm (2017)	Analysis of chromatin opening in heterochromatic non-small cell lung cancer tumour initiating cells in relation to DNA damaging anti-tumour treatment	International Journal of Radiation Oncology * Biology * Physics 100(1):174-187 (IF=8.013) MNiSW 140 number of citations: 5

Individual contribution: — conception and analysis of data obtained using a clonogenic survival assay; — proposal of an innovative method for examining the size of colonies as a measure of the DNA damage and its complexity in comparative studies of the effects of radiation with different ionisation densities; — participation in the discussion of the results and editing the final version of the manuscript.		
(**BB2**) A. Sollazzo, **B. Brzozowska**, L. Cheng, L. Lundholm, S. Haghdoost, H. Scherthan, A. Wojcik (2017)	Alpha Particles and X Rays Interact in Inducing DNA Damage in U2OS Cells	Radiation Research 188(4): 400-411 (IF=3.372) MNiSW 70 number of citations: 10
Individual contribution: — development of software tools for analysing the size of repair foci; — preparation of tools for automatic focus counting based on the taken microscopic images; — development and application of the envelope of additivity method to study the interaction of two radiation types based on DNA repair foci which was an essential element of the study; — participation in the discussion of the results and editing the final version of the manuscript.		
(**BB3**) A. Sollazzo, **B. Brzozowska**, L. Cheng, L. Lundholm, H. Scherthan, A. Wojcik (2018)	Live Dynamics of 53BP1 Foci Following Simultaneous Induction of Clustered and Dispersed DNA Damage in U2OS Cells	International Journal of Molecular Sciences 19(2): 519 (IF=6.208) MNiSW 100 number of citations: 24
Individual contribution: — the conception and implementation of the analysis of the repair focus mobility, which constitutes a significant part of the quantitative results presented in the work; — participation in the discussion of the results and editing the final version of the manuscript.		
(**BB4**) L. Cheng, **B. Brzozowska**, A. Sollazzo, L. Lundholm, H. Lisowska, S. Haghdoost, A. Wojcik (2018)	Simultaneous induction of dispersed and clustered DNA lesions compromises DNA damage response in human peripheral blood lymphocytes	PLOS ONE 13(10):e0204068 (IF=3.752) MNiSW 100 number of citations: 13
Individual contribution: — development of a method to study radiation interaction synergy using the comet assay (envelopes of additivity); — proposing a new method of comparing radiobiological data based on Weibull distribution analysis; — participation in the discussion of the results and the writing of the manuscript.		
(**BB5**) **B. Brzozowska**, M. Gałecki, A. Tartas, J. Ginter, U. Kaźmierczak, L. Lundholm (2019)	Freeware tool for analysing numbers of cell colonies	Radiation and Environmental Biophysics 58(1): 109-117 (IF=2.017) MNiSW 70 number of citations: 16
Individual contribution: — design and implementation of software for cell colony analysis (image analysis, parameter optimisation, application development); — implementation of an algorithm for measuring an additional data feature, colony size, which can be used to study the biological effectiveness of ionising radiation; — analysis of the data obtained as a result of the clonogenic survival assay using the number and size of the colonies formed; — discussion of results and writing of the manuscript.		

(**BB6**) M. Gałecki, A. Tartas, A. Szymanek, E. Sims, L. Lundholm, A. Sollazzo, L. Cheng, Y. Fujishima, M. Yoshida, J. Żygierewicz, A. Wojcik, **B. Brzozowska**[1] (2019)	Precision of scoring radiation-induced chromosomal aberrations and micronuclei by unexperienced scorers	International Journal of Radiation Biology 95(9): 1251-1258 (IF=3.352) MNiSW 70 number of citations: 2
Individual contribution: — conceptualisation of the project; — scoring chromosomal aberrations and micronuclei; — conception and implementation of statistical analysis; — discussion of results and writing of the manuscript.		
(**BB7**) L. Cheng, **B. Brzozowska**[2], H. Lisowska, A. Wojcik, L. Lundholm (2019)	Impact of ATM and DNA-PK Inhibition on Gene Expression of and Individual Response Human Lymphocytes to Mixed Beams of Alpha Particles and X-Rays	Cancers 11(12): 2013 (IF=6.575) MNiSW 140 number of citations: 6
Individual contribution: — development of a synergy test method for gene data (envelopes of additivity); — performing an additivity envelope analysis for selected biological effects; — participation in the discussion and editing of the final version of the manuscript.		
(**BB8**) **B. Brzozowska**, A. Tartas, A. Wojcik (2020)	Monte Carlo modeling of DNA lesions and chromosomal aberrations induced by mixed beams of alpha particles and X-rays	Frontiers in Physics, Medical Physics and Imaging (IF=3.718) MNiSW 20 number of citations: 3
Individual contribution: — conceptualisation of the project (setting a research hypothesis and performing calculations confirming the synergistic effect of X and alpha radiation in the induction of chromosome aberrations); — development of the methodology and conditions for the simulations; — running simulations (PARTRAC codes); — discussion of results and writing of the manuscript.		
(**BB9**) M. Pietrzak, M. Mietelska, A. Bancer, A. Ruciński, **B. Brzozowska** (2021)	Geant4-DNA modeling of nanodosimetric quantities in the Jet Counter for alpha particles	Physics in Medicine and Biology 66(22): 2021 (IF=4.174) MNiSW 100
Individual contribution: — setting a research hypothesis and defining tasks; — determination of simulation parameters, selection of quantities included in the analysis (physics options, nanodosimeter performance, sensitive volume size, scaling procedure); — supervision over the implementation of the project; — discussion of results and writing of the manuscript.		
(**BB10**) A. Tartas, M. Filipek, M. Pietrzak, A. Wojcik, **B. Brzozowska** (2023)	Modeling of dose and linear energy transfer homogeneity in cell nuclei exposed to alpha particles under various setup conditions	International Journal of Radiation Biology doi: 10.1080/09553002.2023.2161659 (IF=3.352) MNiSW 70

[1]In this publication, a two-part surname appears: Brzozowska-Wardecka
[2]In this publication, a two-part surname appears: Brzozowska-Wardecka

Individual contribution:
— concept of the research topic, defined on the basis of experience gained in radiobiological experiments, in particular regarding the characteristics of radiation sources;
— methodology of computer simulations and definition of tasks;
— supervision over the implementation of the project;
— discussion of results and writing of the manuscript.

The content of these articles can be found in Appendix 5, and the statements of the authors of joint publications (except (**BB3**), (**BB4**), (**BB7**) and (**BB8**), where the *Author contribution* paragraph is included) are attached in Appendix 6.

4.3 Discussion of the scientific achievement

The purpose of the research described in the series of publications (**BB1**–**BB10**) mentioned above was to examine how the structure of the particle track, understood as the spatial distribution of ionisations, affects the response of irradiated cells and what is the effect of the simultaneous irradiation of cells with particles of different ionisation densities. An example of densely ionising particles are alpha particles, which interact with the atoms of the medium locally along their tracks. Photon radiation is characterised by a low ionisation density (they ionise the medium uniformly in a large volume).

Will ionising radiation with different ionisation density lead to the strengthening of the effect of simultaneous action (synergy)? Is the mechanism responsible for this the induction of complex DNA damage? The answers to these questions bring us closer to understanding the mechanism of DNA damage and repair and are of great importance in radiation therapy of cancer patients, where ionising radiation with different ionisation densities (e.g. photon and proton radiotherapy) is used to kill cancer cells.

Another motivation of my research was the investigation of characteristics of the radiation interaction with cellular material in the nanoscale, and parameters other than those used so far in radiobiology and radiotherapy (dose, linear energy transfer) to define the probability of complex DNA damage.

The research described in this series of publications contributed to the creation of new descriptors of the biological effectiveness of ionising radiation, taking into account local energy deposits. The studies are distinguished by a holistic approach that covers all known types of processes that affect the final biological effect. The evaluation of the biological effect is crucial both in radiotherapy (including the optimisation of patient irradiation techniques) and in the evaluation of contamination in radiation accidents, where the classification of injured persons is the basis for effective and quick assistance. Research on the interaction of ionising radiation with human cells, discussed in the following, was carried out using both experimental and theoretical approaches.

4.3.1 Introduction

Historical view

Tracks of the interaction of ionising particles with the matter have long been used in nuclear physics. The analysis of particle paths in an emulsion exposed to cosmic rays enabled Marian Danysz and Jerzy Pniewski to discover the first hypernucleus (Danysz and Pniewski, 1953). The characteristic track registered in the photographic emulsion indicated the disintegration of an unstable nuclear fragment, where a recently discovered lambda particle appeared instead of a neutron. The discovery that lambda hyperons can exist as components of the atomic nucleus was of great importance for research in high-energy physics, and in particular for the research group that Marian Danysz created at the Institute of Experimental Physics at Hoża Street in Warsaw to analyse the interactions of high-energy particles.

Similar mechanisms of interaction are used not only in basic research. Particles with lower energies have already found their application in medical diagnostics and therapy, e.g. thanks to the discovery of X-rays by Wilhelm Conrad Röntgen in 1895 (Röntgen, 1895). Maria Skłodowska-Curie used this radiation in the mobile X-ray machines she developed for planar imaging of injuries to the skeletal

system of soldiers during World War I. The injuries of personnel handling these diagnostic X-ray machines were among the first indications that ionising radiation causes damage to the cells of the human body.

Current state of knowledge

The cell response to DNA damage is a multistage and highly ordered process (Wojcik and Harms-Ringdahl, 2019). The first step involves the recognition of DNA damage, which is followed by the activation of a complex cascade of signals responsible for finding the damaged site in the cell nucleus and activating the appropriate repair mechanism (Dantuma and van Attikum, 2016). Although the general mechanisms of repair are well-known (Nobel Prize in Chemistry in 2015), it is still unknown how cells react to damage of varying complexity, especially in cases when DNA strand breaks are concentrated in a small volume of the cell nucleus. Such clustered DNA damages form clusters, i.e. spatial objects in which there are two or more changes within one or two turns of the DNA strand.

Ionising radiation is a unique tool that can be used to induce damage to genetic material in a strictly controlled way. We can control both the time of exposure to radiation leading to the induction of damage and the energy deposited in cells (which is described by the dose, measured in Gy). The characteristics of radiation (charge, energy) and the mechanism of its interaction with living matter are the main determinant of the biological consequences of this interaction (Goodhead, 2007). DNA damage induced by ionising radiation can be classified into three main types (Wojcik and Martin, 2014):

- single-strand breaks (SSB),
- double-strand break (DSB),
- base damage (BD).

The interaction of ionising radiation with a biological system takes place in three stages: (i) physical processes (lasting no longer than a few picoseconds), (ii) chemical reactions (approximately hundreds of seconds), and (iii) biological processes (early effects occurring within a few up to several dozen hours after irradiation of the cellular material). Physical processes are the first and shortest stage of the interaction of ionising radiation with biological systems, but they determine the complexity of damage and thus the cellular response. This answer also depends on the characteristics of the chemical processes that follow the physical phase, in which i.a. ionised and excited water molecules and cascades of biological processes related to damage recognition and repair (Hall et al., 2006).

One of the methods of macroscopic description of the interaction of ionising radiation with matter is the use of linear energy transfer (LET), which quantifies the average energy deposited per unit length of the particle's path. High-energy photons are an example of low LET ionising radiation. They only interact electromagnetically with the electrons of atoms and can travel long distances inside the cell nucleus before an atom is ionised or excited (Wojcik and Martin, 2014). For this type of radiation, considering the length of the particle trajectory comparable to the size of the cell nucleus, the number of ionisation acts along this trajectory is small. High-LET radiation includes e.g. high-energy helium nuclei (alpha particles), which, according to the Bethe-Bloch formula, successively lose their kinetic energy as a result of ionisation of the atoms of the medium. If the medium is the cell nucleus, the densely ionising alpha radiation causes mainly DSB concentrated in a small volume of genetic material, which leads to its greater effectiveness in inducing DNA damage, e.g. because of the spatial distribution of ionisation acts.

The level of cell damage depends on the dose, but the quality of the damage depends on the LET. To study the relationship between cellular response and LET in the context of proton radiation therapy, we use easily available densely ionising alpha particles. For both protons and alpha particles the characteristic method of energy deposition is described by the Bragg curve. The shape of this curve is of great importance for the precise killing of cancer cells in the tumour and for the protection of normal tissues. At the same time, the fact that protons, alpha particles, and heavier charged particles ionise locally makes it difficult to ensure dose uniformity in the irradiation volume.

In parallel with the experimental approach, the distribution of hits inside the nucleus and the structure of the particle track can be calculated with high precision using MC methods (Incerti et al., 2018; Friedland et al., 2011; Nikjoo et al., 2001; Nikjoo and Girard, 2012). Here, we use the aforementioned unique feature of ionising radiation, which allow us to accurately estimate the number of hits for a given dose in a given volume in a given time. The analysis of the track structure in the MC approach is based on an 'event-by-event' description of the physical processes that occur as a result of the interaction of ionising radiation with the atoms of the medium. Each type of interaction for the tracked particle is described by the energy deposited and the position at which the interaction occurs. In addition, we can also model the indirect acts of radiation interaction with the cellular microenvironment caused by radicals emerging as a result of water radiolysis. The time characteristics of these processes are an important part of these models. The combination of these two steps (direct and indirect interactions) allows the study of damage within the DNA molecule as well as between the different chromosomes in the cell nucleus. Proper quantification of physical phenomena at the nanometre level is a crucial initial step in any attempt to predict the biological response to given irradiation conditions. It is of the utmost importance that damage complexity hypotheses be tested using a single biological system (a given cell line), yielding both radiobiological and purely physical results for the same or at least equivalent irradiation parameters, which has been highlighted in (Briden et al., 1999; O'Neill and Wardman, 2009; Conte et al., 2017; Rucinski et al., 2021).

The research results presented here are described in three subsections, corresponding to the individual stages of the interaction of ionising radiation with a biological system. Research on the physical stage, which involves direct interactions between the ionising particle and the DNA molecule, is discussed in section 4.3.2. We have recently started studies on the chemical phase involving the indirect interaction that takes place between the products of the radiolysis of water and genetic material. Therefore, the research results are under discussion and are being prepared for publication. However, for the completeness of the description, a short discussion on the simulations of the production of reactive oxygen species, which are carried out in our research group, is included in section 4.3.3. The last element of the process is the cellular response during the biological phase, which has been studied in various ways using radiobiological assays and is described in detail in section 4.3.4. Section 4.3.4 consists of two parts: (A) a description of research on DNA damage caused by photon radiation and (B) a description of research on the synergy of mixed X and alpha radiation.

4.3.2 Physical phase of irradiation

As pointed out above the LET is the standard parameter for the description of interaction at the macroscopic level. It may not be sufficient to describe the stochastic nature of radiation and its effects, especially at the subcellular (nm) level. Instead of using such average values, it is worth considering nanodosimetric quantities which provide insight into the details of ionisation at the nanometric level and thus allow understanding of the cellular response to ionising radiation in the context of DNA damage. The number of ionisation acts in the volume of individual DNA nucleotides is the main factor determining the relative biological effectiveness (RBE) of ionising radiation. Based on the spatial distribution of the ionisation acts, the probability of lethal cell damage can be calculated (Conte et al., 2017), which is closer to the description of phenomena at the nanoscale than the average LET parameter.

Photons interact with matter indirectly, producing positively charged ions and electrons through the photoelectric process, Compton scattering, and pair creation. Therefore, both the photon radiation and the generated electrons ionise the medium in a homogeneous way throughout the volume. While the LET may describe well the ionising effect of photons and electrons, for heavy charged particles (such as alpha) such an average statistical description is insufficient. Therefore, because of the local nature of the interactions, nanodosimetric quantities should be considered in the study of the biological effects of heavy charged particles. In such cases, the same average doses may give large differences in local doses near and beyond the particle trajectory, which is not observed in the interaction of photons and electrons. Ionisation acts, which are generated relatively sparsely by photon and electron radiation, result mainly in SSB, and only at high radiation doses DSB appear. On the contrary, for ra-

diation with high LET (protons, alpha particles, heavy ions), numerous DSB appear even at low doses.

Modelling of the particle track structure

Ionisation density is a crucial parameter in radiobiological experiments, where biological material, e.g. in the form of a monolayer of cells seeded in Petri dishes, is exposed to ionising radiation. The complexity of DNA damage and the distribution of the dose delivered by alpha particles to cells make alpha particles an interesting subject of radiobiological research. Therefore, in the research described in the publication (**BB10**) we estimated the level of dose heterogeneity and its uncertainty using MC methods for four different experimental systems containing a planar source of alpha particles, ^{241}Am. The LET and RBE values depend on the source characteristics, the configuration of the irradiation system, the biological system, and the biological endpoint. MC methods are helpful not only in characterising the configuration of the experimental setup, but also in determining the factors affecting the beam characteristics, thanks to which errors in the correct dose estimation during experiments, which are costly and time-consuming, can be avoided. Appropriate simulations, using the PARTRAC (Friedland et al., 2011) and Geant4 (Agostinelli et al., 2003; Allison et al., 2006) codes, were intended to demonstrate the importance of irradiation system characteristics in reporting experimental results, especially when biological effects are studied at the molecular level. The modelled systems took into account situations where cells grown on a flat disc were placed over a surface ^{241}Am source, with alpha particles penetrating from below, and when the source was positioned above the cells, i.e., alpha particles reached the cells from above. The latter configuration was simulated with and without a collimator, as well as with a rotating collimator. The configurations were selected as a result of the cell culture conditions and the specificity of the cell material irradiation systems used in radiobiological experiments. The use of a collimator makes it possible to obtain a high homogeneity of the LET distribution while reducing the dose rate, while its absence results in wide LET distributions of alpha particles. Using a collimator is obviously beneficial for studying the relationship between LET and RBE. The RBE is highest at a LET of about 100 keV/μm (Barendsen, 1994) and decreases rapidly as the LET increases. On the basis of the obtained LET distributions, it can be expected that lower RBE values will be obtained in the case of irradiation with alpha particles without the use of a collimator compared to collimated alpha particles. The result of our simulations, worth emphasising in the context of planning and performing radiobiological experiments, is the low-dose uniformity in cell nuclei irradiated using a stationary collimator. This effect results from the elimination of most alpha particles that leave the source at an angle that deviates from 90° and from the absorption of particles by the walls of the collimator. Dose uniformity can be improved by wobbling the collimator.

Nanodosimetry

The most effective experimental method used to count the number of ionisation acts induced by a particle passing through the medium in nanometre volumes and to study its spatial and temporal track structure is nanodosimetry. This method uses highly sophisticated ion detection systems that are formed in a gas-filled active volume at low pressure. The study of single acts of ionisation in rarefied gas is easier than an analogous measurement in a dense water medium that simulates the cellular environment. The volume of active gas in the nanodosimeter is equivalent to the cylindrical volume of a biological object (the object may be, for example, a DNA strand). Due to the structure of the DNA molecule, 1-2 strand turns of 10-20 base pairs (bp) are often considered, where the distance between bp is about 0.34 nm. The results of nanodosimetric measurements in gas cannot be directly applied to biological systems; therefore, a method of interpreting the results obtained in gas in the context of liquid-water simulating subcellular structures is needed. To determine the size of the cellular structure, the scaling procedure between the surface density of the gas used in the nanodosimetric measurement and the surface density of water should be used, described by the formula below:

$$(D\rho)^{(\text{water})} = \eta \frac{(\lambda\rho)^{(\text{water})}}{(\lambda\rho)^{(\text{gas})}} (D\rho)^{(\text{gas})} \cdot K_e,$$

where η is the efficiency of ion registration in the experiment, $(D\rho)^{(\text{water})}$ and $(D\rho)^{(\text{gas})}$ are the surface

densities of the sensitive volume in water and gas, respectively, ($\lambda\rho$) is the mean free path length of the primary particle in a given medium, and K_e is a correction for the difference in the interaction of electrons between the medium ($K_e \approx 1$). For example, the nitrogen density in the sensitive volume of the nanodosimeter at the National Centre for Nuclear Research equal to 0.5 μg/cm^3 corresponds to 2 nm of the subcellular structure. The scaling procedure is well established and described in (Grosswendt, 2004). Nanodosimeters make it possible to measure the size distributions of ionisation clusters that are formed as a result of a particle passing through a gaseous medium (ICSD, Ionisation Cluster Size Distributions) and to determine the average size of such a cluster (first moment of distribution).

So far, only four prototype nanodosimeter systems have been developed (Casiraghi et al., 2014; Bantsar et al., 2018)), and one of them is the Jet Counter (JC) designed at the National Centre for Nuclear Research. A detailed description of the structure and principles of operation of the JC can be found, e.g. in (Pszona et al., 2000), and the ICSD obtained with JC and their interpretation in the context of the complexity of DNA damage are the main subject of the research described in the (**BB9**) publication. For the measurements, we used a radioactive source of alpha particles (^{241}Am) with an energy of 4.6 MeV, which is lower than the nominal energy of 5.5 MeV due to the fact that the alpha particles must pass through a layer of the source and gold that covers the source before leaving the source. However, the key novelty in this work was not the experimental data, but ICSD simulations performed using Geant4-DNA codes (Incerti et al., 2018) based on MC methods. In (**BB9**) we quantified for the first time the uncertainty of ICSD, taking into account:

(i) three main physics models of Geant4-DNA using cross sections for liquid water,

(ii) low pressure gas ionisation scaling procedures to water environment using PTra codes (Kling et al., 2014) containing ionisation cross sections for alpha particles in nitrogen,

(iii) the efficiency of ion extraction and the presence of passive detector elements, i.e. the Mylar wall,

(iv) different sizes of the sensitive volume.

We found that the uncertainty of determining the performance of the JC nanodosimeter introduces greater differences between the ICSD obtained from the Geant4-DNA simulation and those obtained experimentally than taking into account different physics models. This confirms the legitimacy of further work towards modernising the apparatus and proposing new measurement methods. We also confirmed that the density scaling procedure used to simulate the Geant4-DNA ICSD in liquid water allows the modelling of nanoscopic quantities as accurately as in the PTra simulation performed in nitrogen gas. Using the ICSD parameters, we also calculated the value of F_4, which is the cumulative probability of creating a cluster of size 4 or larger. We assumed that this parameter, which is a physical quantity, corresponds to the probability of complex, irreversible DNA damage induction. Our results indicate that the prediction of ICSD and their parameters based on Geant4-DNA simulations is accurate enough to be applied to treatment planning based on nanodosimetric quantities (Ramos-Méndez et al., 2018; Rucinski et al., 2021).

4.3.3 Influence of the chemical phase on radiation damage

Apart from the amount of energy deposited by radiation (the dose D), the biological effects are determined also by the manner in which the dose is delivered to the cells (described by the dose rate, dD/dt). It has been shown that the use of ultra-high dose rates, of the order of 500 Gy/s, improves the protection of healthy tissues while killing cancer cells. This mode of rapid dose delivery was called FLASH therapy (Wilson et al., 2020). Although the biological mechanism of the FLASH effect is still not understood, oxygen depletion in the cellular environment, which arises as a result of the radiolysis reaction, appears to be one of the plausible hypotheses (Labarbe et al., 2020). Most computer simulations are based on the assumption that the cell nucleus consists of pure liquid water. This is too much of a simplification because of the importance of chemical reactions that occur as a result of the interaction of ionising radiation with the cellular environment. In our research, which is carried out under my supervision in cooperation with scientists from Loma Linda University (USA),

we compare calculations based on the radiolysis of pure water with models that take into account the radiolysis of the cellular environment containing DNA, RNA, proteins and amino acids. We have implemented two models of water radiolysis with chemical reactions of oxygen and biologically relevant molecules that can help to understand the FLASH effect in healthy tissues. Simulations of the time evolution of the concentrations of the resulting radicals for these models were performed using TOPAS-nBio codes (Schuemann et al., 2019)). The simulation results show that the oxygen concentration in the water environment differs from that obtained in the cellular environment, which is due to the introduction of competing scavengers in both models that react with free radicals. Additionally, differences in oxygen concentration are also visible when comparing two irradiation techniques: the FLASH technique with a high dose rate of 500 Gy/s and conventional radiotherapy with a low dose rate of 0.29 Gy/s. These results are being prepared for publication. Independently, measurements of H_2O_2 and O_2 concentrations in the phantoms for which simulations will be carried out in cooperation with the Institute of Nuclear Chemistry and Technology in Warsaw. The concentrations of radiolysis products measured using gas chromatography and titration will be used to verify the calculations made in the Kinetiscope programme (Wiegel et al., 2015) based on MC methods. and the results will be the content of the manuscript that we are currently working on.

4.3.4 Biological consequences of ionizing radiation

(A) DNA damage induced by photon radiation

The source of photon radiation can be either an X-ray tube, which enables the production of X-rays, or radionuclides (e.g ^{137}Cs, ^{60}Co), which emit gamma radiation as a result of nuclear transformations. Photon radiation, passing through the cell nucleus, ionises atoms uniformly throughout the volume. Due to the nature of this interaction, the damage that will occur as a result of irradiation of the cellular material will be mainly SSB, although, at high doses of gamma radiation, DSB damage will occur.

Colony size of surviving cells

The study of the survival of cells exposed to ionising radiation is the basic method of determining the cytotoxic effect of radiation and chemical toxins. One of the last steps in performing this assay is to count the colonies (clusters containing a minimum of 50 cells) that result from the division of cells that survived irradiation. In large experiments, manual counting of colonies is time-consuming and error-prone, resulting, among others, from the inexperience of the counting person. Moreover, it is often interesting to quantify the size of individual colonies, which has not been included in the data analysis so far. Such calculations are largely performed using computer image analysis systems. While there are many such systems, they all focus on colony counting rather than colony size analysis. To address this problem, we developed a new software package for colony counting and size distribution analysis, described in (**BB5**). The software called countPHICS consists of two parts:

1. a tool written for the ImageJ image processing system, enabling the analysis of images of scanned Petri dishes, determining the number of colonies on the dish, estimating their size (area occupied by individual colonies);

2. Plotting HIstograms of Colony Size (PHICS) programme with a graphical user interface using portable Qt libraries, which allows visualisation of colony size distribution histograms based on results from the previous point and parameterisation of this distribution by fitting the best function (Gaussian or Weibull distribution).

To present an example application of the programme, we analysed the data from (Lundholm et al., 2014). In the original publication a decrease in the growth rate of non-small cell lung cancer was observed relative to the control conditions, but it was not quantified. In the cited article, lung cancer stem cells were exposed to three factors: gamma radiation, mitogen-activated protein kinase (MEK; when MEK is inhibited, cell proliferation is blocked and apoptosis, or controlled cell death, is induced), and a combination of these two factors. In (**BB5**) we analysed the size colony

using countPHICS software and we showed for the first time a trend towards a reduction in colony size for cells pretreated with the MEK inhibitor prior to irradiation. This means that the colonies have been reduced not only in terms of their numbers, but also in terms of growth rate (i.e. colony size).

Changing the structure of the chromatin

Photon radiation induces DNA strand breaks uniformly in the volume of genetic material packed in the cell nucleus. The degree of chromatin packing in the cell nucleus plays a role in controlling gene expression. While chromatin in a densely packed state (heterochromatin) is usually genetically inactive, less condensed euchromatin is genetically active. The way in which the chromatin is packaged can also lead to different efficiencies of DNA damage generation by ionising radiation. In the work (**BB1**), we used lung cancer stem cells to study the effectiveness of histone deacetylase inhibitors, which are responsible for changing the structure of the chromatin. Cells modified in this way were exposed to X-rays and/or cisplatinum, which is used in chemotherapy for cancer patients. Both the measured survival curves and colony size distributions indicate lower cell survival and smaller colony sizes when chromatin condensation inhibitors were added to the cells 16 hours before irradiation. Based on our results, it can be assumed that unlike euchromatin, where X-rays penetrate and induce DNA strand breaks, the heterochromatic (i.e. densely packed) structure protects the deeper layers of genetic material and thus plays a role in the resistance of lung cancer stem cells to radiotherapy. These results indicated that clinically available inhibitors can be potentially used to enable the treatment of cancer patients who are resistant to radiotherapy.

(B) Mixed beam synergy

Ionising radiation from natural sources usually does not contain only one type of particle emitted. The alpha and beta decay (i.e. nuclear transformations with the emission of alpha particles and electrons/positrons, respectively) is usually accompanied by gamma emission. The interaction of the proton beam used in proton radiotherapy with elements of the collimation system or with the patient's body leads to the production of neutrons. Also, the cosmic rays we are exposed to during flights are mainly protons converted into heavy ions in the aircraft's shell.

The principles of radiological protection are based on the assumption that the total biological effect of various radioactive sources is the sum of the effects of their individual components. The results of the studies described in this section show that the response of cells as a result of exposure to mixed beams of photons and alpha particles is different from that predicted based on the assumption of additivity.

53BP1 repair foci

While in the clonogenic survival assay, the effectiveness of ionising radiation is studied at the cellular level, the observation of repair foci is possible at the submicrometre scale. Repair foci appear in the DNA molecule at the site of DSB formation, the recognition of which initiates the repair mechanism of radiation damage, e.g. by recruiting appropriate repair proteins. In the studies described below, we investigated the 53BP1 protein. When bound with the green fluorescent protein, accumulations of 53BP1 are visible under a fluorescence microscope.

We used the study of 53BP1 repair foci in publications (**BB2**) and (**BB3**) to answer the question of whether their induction was caused by exposure of cells to radiation with different LET (about 1.5 keV/μm for X-rays and 100–172 keV/μm for alpha radiation) results from the additivity or synergy of the interaction of both types of radiation. For this purpose, osteosarcoma cancer cells were irradiated with alpha particles, X-rays, and their combination, i.e. mixed beams (50% of the total dose from alpha radiation and 50% of the total dose — from X-rays). We observed qualitative differences in the induction of repair foci: while photon-induced foci are small, most alpha-particle-induced foci are large. The study of the synergy of X-ray and alpha radiation was possible thanks to the calculation of additivity envelopes for selected biological effects, e.g. induction of 10 small foci in the cell nucleus, as shown in the Fig. 1.

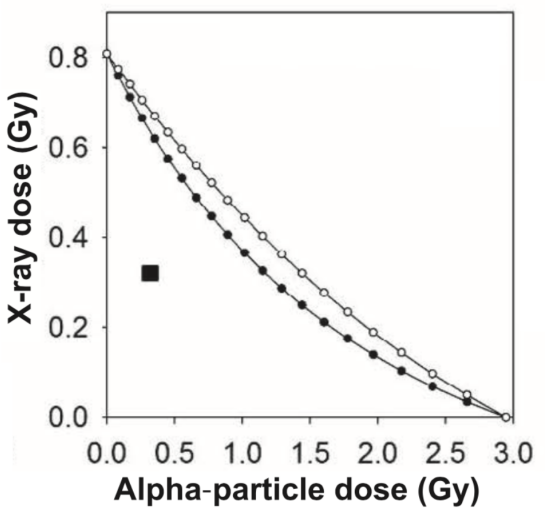

Figure 1: Example of an additivity envelope for induction of 10 small foci (**BB2**). The edges of the envelopes (the points marked as full and empty circles in the figure) correspond to two isobolograms created for the heteroadditive and isoadditive forms of interaction between the two types of radiation studied. The isoadditive response (open circles) is calculated on the basis of the assumption that X-rays and alpha have the same mechanism of action and that the combined effect is superadditive. The heteroadditive response (full circles) is calculated on the basis of the assumption that the two types of radiation have different mechanisms of action, but the combined effect is additive. If the data from the mixed-beam experiment (square point in the figure) are within the envelope of the additivity envelope, then the effect is additive. If the data are outside the envelope, the effect is synergistic (point to the left of the envelope) or antagonistic (to the right of the envelope).

In the work (**BB2**) the additivity envelopes were calculated on the basis of the response curves (i.e. the focus frequency as a function of the dose) for three irradiation scenarios, and the results clearly showed that the induction of foci by mixed beams is a synergy effect, not additivity, as is assumed in the radiation protection.

In addition, in the work (**BB3**), we studied the kinetics of the formation and repair of these foci. We observed that the behaviors of small and large foci differ significantly: small foci appear and disappear quickly, whereas large foci form and disappear slowly. These results support the assumption that complex or small-volume DNA damage is a serious problem for the cell in the DNA repair process. The repair of foci induced by mixed-beam is slower than the decay of foci induced independently by either X-rays or alpha particles. The use of a fluorescence microscope for real-time imaging allowed to observe the movement of foci within the cell nucleus and to calculate their mean square displacement (MSD) in the Brownian motion formalism. Our results indicate that foci induced by mixed beams exhibit a low degree of mobility, possibly contributing to the increased repair of erroneous damage, especially in clusters of DSB. These results confirm our previous observation of a high frequency of complex chromosome aberrations in cells exposed to mixed beams.

Chromosome aberrations: experiment and modelling

Dose assessment plays an important role in dealing with the effects of radiation accidents and can be performed by assessing structural changes in chromosome morphology (chromosome aberrations) caused in cells by ionising radiation. The aim of the (**BB8**) study was a comparative analysis of previously published experimental results on the induction of chromosomal aberrations in human peripheral blood lymphocytes exposed to mixed beams of alpha particles and X-rays with computer simulations using the PARTRAC framework based on MC methods. The advantage of simulation over experimental research is the lack of potentially misleading influence of environmental factors (temperature fluctuations, acidity) and factors such as efficiency and repeatability of the result counting process on the obtained result. PARTRAC codes were used to simulate DNA damage in the form of SSB and DSB (both complex and single) and the number of aberrations of the chromosomes that resulted from these damages. The number of SSB and DSB induced by a given dose is different for

X-rays (more SSB than DSB are induced) and for alpha particles, which, due to dense ionisation, induce more DSB than SSB. The total number of SSB and DSB caused by mixed beams reveals an additive effect, i.e. the sum of SSB and DSB from photons and alpha particles. However, the situation is different at the level of chromosomal aberrations, where the simulations indicate a synergistic effect, which is in line with the experimental results. This result shows that the synergistic effect of the mixed beams is due to the way the cell handles the SSB and DSB repair (biological phase). Using the PARTRAC codes, we checked whether the occurrence of a synergistic effect depends on the ratio of alpha and X-rays. The level of synergy depended on the composition of the mixed beams, with the highest level observed at a dose ratio of 50:50 alpha particles to X-rays.

Contrary to the classification of chromosomal aberrations, which are modelled on the basis of MC methods, counting chromosome aberrations in an experiment is difficult for a person who is not experienced in microscopic analysis of biological material. This is confirmed by the results of our research, described in (**BB6**). In a situation where a quick analysis of a large amount of data is needed (e.g. after a radiation accident), it is recommended to use easy-to-learn radiobiological assays. Therefore, we decided to compare the performance of two radiobiological tests: chromosome aberrations and the micronucleus test. Each participant in a two-week EU-funded radiobiology course at Stockholm University was asked to assess cytogenetic damage to Chinese hamster ovary cells exposed to 0-4 Gy of gamma radiation. Comparative analysis of assay results obtained by inexperienced people (students) with reference values generated by an expert (experienced cytogeneticist) was performed for each dose and for the parameters of the dose-response curve fitting between students. The micronucleus assay was usually faster and easier to learn than assessing chromosomal aberrations. However, both assays performed by inexperienced students showed reasonable dose-dependent relationships between the number of chromosome aberrations and the micronuclei. In the case of a major radiological accident with multiple victims, even inexperienced persons could support the selection and classification process of people exposed with cytogenetic biological dosimetry.

Peripheral blood lymphocytes

When it comes to the use of biological dosimetry in radiation accidents, the cellular material collected from injured people is peripheral blood lymphocytes (PBL). Since people are exposed to mixed radiation during an accident, the objective of the human PBL study reported in (**BB4**) was to examine the mechanism of the interaction, particularly with regard to the question of whether it is due to an increased level of initial damage or to impaired DNA repair. PBL were exposed to alpha particles, X-rays, and mixed beams using different dose values. DNA damage and damage repair kinetics were quantified by an alkaline comet assay using an electrophoresis method. Levels of phosphorylated key DNA Damage Response (DDR) proteins: ATM, p53, and DNA-PK were measured by Western blotting, and mRNA levels of six selected DNA damage active genes were measured by RT-qPCR (real-time quantitative polymerase chain reaction). Research results show that alpha particles and X-rays interact with cellular material to cause DNA damage beyond the level predicted under the additivity assumption, with the delayed repair of the damage. The activation levels of the DDR protein and the mRNA levels of the above-mentioned genes were highest in cells exposed to mixed beams. This confirms that exposure of PBL to mixed beams is a challenge for a cellular DDR system. Additional evidence of a synergistic effect in PBL exposed to mixed alpha particles and X-ray radiation was provided by the extended genetic response analysis described in (**BB7**). In this study, qPCR was used to measure the levels of FDXR, GADD45A, BBC3, MDM2, CDKN1A, and XPC mRNA 24 hours after irradiation. Alpha particles and mixed beams increase the level of gene expression more than X-rays, which was evident from the dose-response curve. In three out of four blood samples taken from the tested donors (healthy, non-smoking men) we observed a synergy effect on the mixed beams of radiation. When two donors were sampled again a year later, the previous additive effect in one donor was now synergistic. Furthermore, based on the micronucleus assay performed using PBL exposed to gamma radiation, no significant differences in intrinsic radiation sensitivity were observed. Overall, synergy was present in all donors, but the results of (**BB7**) suggest individual variability due to lifestyle factors.

4.3.5 Summary

The coherent series of research underlying this work has enabled a deeper understanding of the processes and mechanisms of interaction of ionizing radiation with biological systems. Understanding these issues is crucial for the effective assessment of the risks caused by irradiation of victims of radiation accidents and for effective planning of radiotherapy.

A popular assay for examining the cellular response to ionizing radiation is the clonogenic survival test, based on determining the number of colonies formed as a result of cell division that survived irradiation. This is not sufficient to assess the complexity of the damage, so in the paper (**BB5**) it was proposed to extend this test by assessing the size of the colony, thanks to which we can determine not only how much damage was created, but also their quality, which affects the effectiveness of radiotherapy of cancer patients.

In order to increase the effectiveness of induction of DNA damage caused by photon radiation, used in conventional radiotherapy, chromatin condensation inhibitors were used in the work (**BB1**). Thanks to the modification of the chromatin packing in the cell nucleus, the spatial distribution of ionization acts changed, and thus the DNA damage increased, which is of key importance in the effectiveness of killing cancer cells in patients who respond worse to radiotherapeutic treatment.

The effect, which was not fully explained and was the motivation for further research, is the issue of the synergistic cellular response to mixed radiation doses, in particular low ionization density photon radiation administered simultaneously with densely ionizing alpha particles. Papers (**BB2**), (**BB3**), (**BB4**), (**BB7**) and (**BB8**) show experimental results confirming that the studied biological effects do not result from the additive effect of both types of radiation. These results are of key importance in determining the dose when assessing the exposure of victims of radiation accidents, where we always deal with mixed radiation, and in radiotherapy planning systems, in which the knowledge of non-linear summing of effects allows for the optimization of radiotherapy treatment of oncological patients.

This non-linearity is due to the specific mechanism of complex DNA damage induction, as shown in (**BB8**) and (**BB10**) using MC simulations. It has been shown that the effectiveness of radiation depends directly not only on the number of induced lesions in the genetic material, but also on their spatial structure. The number of single-stranded and double-stranded DNA chain breaks depends on the spatial distribution of ionization acts, which in turn results from the structure of the tracks of particles emitted from a radioactive source. The summary results of these works also allowed for the estimation of the number of SSB, DSB, and distributions of LET values.

Based on spatial information about the distribution of SSB and DSB in the paper (**BB8**), the number of chromosome aberrations that arise from incorrect repair of SSB and DSB was determined using MC methods. Experimental detection of the mentioned chromosome aberrations is a difficult procedure and is burdened with an error depending, among others, on the experience of the person performing the test. On the other hand, this procedure is crucial in assessing the dose received by victims of radiation accidents. Therefore, this paper (**BB6**) compared the chromosome aberration assay with another radiobiological test — the micronucleus assay. The results of this comparison clearly indicated that the recognition of chromosomal aberrations in biological samples is difficult. Still, at the same time, the estimation of the dependence of the number of aberrations on the dose by inexperienced people after a two-week course is sufficient to assess the exposure of victims of radiation accidents, when the key parameter is time.

The study of the basic physical processes causing the ionization of the atoms of the medium, causing a cascade of cellular response signals to ionizing radiation, contained in the work (**BB9**), crowns a coherent series of publications, which is the basis for the habilitation procedure. The most detailed description of the physical processes leading to the biological effects described above is possible at the nanoscale, through MC simulations and unique nanodosimetric measurements. By combining the results of these two approaches, in the paper (**BB9**) it was proposed a new descriptor that takes into account local energy deposits and describes the stochastic nature of the interaction of radiation with living cells more precisely than the LET parameter. The nanodosimetric characterization of the structure of the particle track is not only of cognitive importance. The nanodosimetric parameters discussed in the paper (**BB9**) are the basis for a more accurate description of the mechanism of DNA

damage and repair, which opens the way to a significant improvement of dose estimation, necessary both in radiological protection and in radiotherapy planning.

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5 Presentation of significant scientific activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

5.1 Medical Physics at the Faculty of Physics, University of Warsaw

When I started working at the Faculty of Physics at the University of Warsaw in 2011, the two most important tasks I was given were to supervise first- and second-degree students of the Medical Physics (MP) specialty and to create a research group that would undertake studies in this field for the first time at the University of Warsaw. An additional difficulty was the change of the subject of research, because as part of my doctoral project, I was dealing with a deep inelastic scattering of electrons on protons, i.e. high energy physics. At the time, the University of Warsaw did not conduct independent research in the field of MP, which is necessary to award titles in this field. Thanks to Dr. hab. Zygmunt Szefliński and Prof. Piotr Durka (then head of the Biomedical Physics Division), in the first years of my work as an adjunct, I managed to establish cooperation with clinical units in Warsaw, where students could carry out internships and diploma theses. It is also Dr hab. Zygmunt Szefliński who put me in touch in 2015 with Prof. Andrzej Wójcik from the Department of Molecular Biosciences at Stockholm University, where I spent 1.5 years of postdoctoral fellowship. Prof. Wójcik taught me the methods used in radiobiological research and enabled me to go to Dr. Werner Friedland, the father of PARTRAC codes, during which I mastered the basics of simulation of the interaction of ionising radiation with biological material based on MC methods. The experience in performing simulations, which I gained during my PhD project, enabled me to quickly master the use of PARTRAC codes. Undoubtedly, my internship at Stockholm University motivated me to create a space for conducting *in silico* experiments, in addition to *in vitro* experiments, which became the subject of my research after returning to the Faculty of Physics. Therefore, I devoted the first years — apart from creating and consolidating the research group — to the construction of the experimental facilities described in section 6.2.1, including the radiobiology laboratory, a class Z isotope laboratory and an X-ray laboratory. In 2019, in the radiobiology laboratory, I created at the Faculty of Physics, we started studying DNA damage caused by ionising radiation. In addition to bachelor's and master's theses, we have started several doctoral projects in which I act as an assistant supervisor, and thus a research group was established, which, to a greater or lesser extent, continues the research I present in section 4.3. The circumstances described above forced me to move relatively quickly to the stage when the basis of my activity became the coordination of research of a newly established scientific group and the promotion of young scientists entering the field (almost exclusively my graduate and doctoral students), which can be seen in the lists of authors of some of the publications described, in whose name is often in the last position, usually as PI (Principal Investigator). Currently, the group led by me consists of six doctoral students (two of them are just finishing their dissertation) and two students of the Inter-faculty Individual Studies in Mathematics and Natural Sciences of the University of Warsaw.

My involvement in scientific work was appreciated by the Authorities of the University of Warsaw in the form of the 2nd degree individual award of the Rector of the University of Warsaw in 2021 and the distinction of the Rector of the University of Warsaw for achievements influencing the development and increase of the prestige of the University of Warsaw in 2022.

5.2 Radiation-induced DNA damage with different LET

I use my experience in the study of radiation damage to DNA using both radiobiological tests and computer simulations to supervise doctoral projects conducted in our group.

Adrianna Tartas, working on a PhD project in our group, analysed NBS1 repair foci (a protein whose expression appears during the start of the DNA damage repair mechanism earlier than the 53BP1 protein I studied) in osteosarcoma cells. Thanks to the invitation of Prof. Wójcik, the experimental part could be carried out using a fluorescence microscope for live imaging at Stockholm University. In 2022, we equipped our laboratory with a fluorescence microscope for real-time imaging. This equipment will enable further development of radiobiological research at the Faculty of Physics and will additionally improve the quality of the medical physics teaching programme.

Due to the fact that the structure of the particle track is of great importance for the response of irradiated cells, our next research topic is an attempt to correlate the physical description of the radiation interaction, including chemical reactions in the cellular environment, with biological endpoints (cell survival, induction of repair foci). Monika Mietelska, a PhD student, studies nanodosimetric quantities in the context of their application to the interpretation of radiobiological data. In her work, she will use the parameters of the probability distributions of the induction of ionisation clusters with a size equal to or greater than a fixed number, as potential quantities that describe the complexity of biological damage.

Using the possibilities offered by our radiobiology laboratory, we have also started research aimed at answering the question of whether there is a mechanism for cell adaptation to ionising radiation. To this end, PhD student Mateusz Filipek analyses the response of prostate cancer cells, previously irradiated with low doses from the ^{90}Sr source (low-LET beta emitter), to densely ionising alpha particles (^{241}Am). In addition to survival curves, Mateusz will analyse repair foci using our fluorescence microscope.

5.3 Exosomes and their role in radiotherapy

In cooperation with scientists from the Medical University of Warsaw (MUW), we started research on exosomes in the context of optimising radiotherapy in cancer patients. In these works, apart from Dr. Wioletta Olejarz and Dr. Tomasz Lorenz from the Medical University of Warsaw, two PhD students from the Biomedical Physics division participate. One of them is Beata Pszczółkowska, who under my supervision conducts research as part of her doctoral project using prostate cancer cells (PC3 and DU145, differing in radiosensitivity). Her research topic concerns the sensitisation of cancer cells to X-ray and alpha radiation by adding to the medium exosomes, responsible for intercellular communication. The effectiveness of this procedure will be analysed using fluorescence microscopy to observe induced repair foci.

5.4 The use of 3D printing in radiotherapy of patients with breast cancer

As part of cooperation with the National Oncology Center in Warsaw, Edyta Dąbrowska-Szewczyk's doctoral project is being carried out under the supervision of Prof. Paweł Kukołowicz (head of the Medical Physics Department in the National Oncology Centre in Warsaw) and mine. Edyta's research concerns the testing of new materials used in 3D printing to create boluses for the irradiation of patients with breast cancer. The bolus is used to increase the surface dose delivered during classical photon radiotherapy. 3D printing technology makes it possible to prepare a bolus individually for each patient, but requires the characteristics of the material used as the filament and the print structure, which contains air spaces. Therefore, for the correct calculations of the treatment planning system and the correct reconstruction of computed tomography images, dosimetric measurements are necessary for 3D-printed boluses.

6 Presentation of teaching and organizational achievements as well as achievements in the popularization of science

6.1 Teaching achievements

6.1.1 Student supervision

When I was employed at the Faculty of Physics of the University of Warsaw, my task was to take care of the students from the medical physics (MP) specialty, which was one of the five specialties available as part of the first- and second-degree studies in the field of Applied Physics in Biology and Medicine (ZFBM in Polish). Since September 2011, I have been the supervisor (or co-supervisor) of 20 bachelor's theses (including the 2nd prize of the Polish Nuclear Society for Maria Szoła) and 28 master's theses (including a distinction from the Polish Nucleonic Society for Kinga Żelechowska-Matysiak). In the current academic year (2022/23) I am the supervisor of 1 bachelor's thesis and 4 master's theses. Due to the interdisciplinarity of the course, I also tutor the students from the Inter-Faculty Individual Studies in Mathematics and Natural Sciences. Currently, I am a tutor for a student who, apart from physics, chose chemistry (and after a year of cooperation, she decided to change the chemical profile to a physical profile as her main field of study) and a student whose additional field is mathematics and computer science.

In response to the great commitment and activity of MP students, in 2012 I took care of the Student Club of Medical Physics of the Faculty of Physics at the University of Warsaw and held this position until 2019. This year, Dr. Józef Ginter, employed at the Faculty of Physics of the University of Warsaw, took care of the Club, and I became the supervisor of the newly established *LEThal Dose* Doctoral Students' Club. Thanks to the great work of students, we managed to organise several editions of conferences and popular science events mentioned in Appendix 3.

During the 11 years of my work at the Faculty, I was/am an assistant supervisor of 7 PhD students. Currently, one doctoral student has defended his doctorate, three are writing down the results of their research in the form of a doctoral dissertation, and three are at the stage of data collection and analysis.

6.1.2 Organisation of new classes

The classes I teach mostly focus on specialised subjects in the ZFBM major for students choosing the MP specialty. In the years 2011–2014, I took an active part in the work on the preparation of the first and second-degree study programme in the field of ZFBM and in the coordination of apprenticeships. A special achievement was the launch of exercises in Radiotherapy Planning; we were the only university that, in the MP study programme, teaches students to plan treatment using the treatment planning system — in 2013 we obtained a license for the PlanUNC software (http://planunc.radonc.unc.edu).

6.1.3 Distinctions and awards for teaching activities

My commitment to teaching has been appreciated by both the authorities of the University of Warsaw and students. In 2013, I received the 2nd-degree Individual Award of the Rector of the University of Warsaw for teaching and organisational achievements supporting the development of the MP specialty and the 3rd-degree Individual Award of the Rector of the University of Warsaw in 2017. On the basis of the questionnaires completed by the students, I was awarded a distinction by the Dean of the Faculty of Physics of the University of Warsaw for conducting classes in Statistical Inference (the academic year 2016/17) and for conducting classes on a Physical Basis Of Radiotherapy in the academic year 2017/18.

6.1.4 Classes conducted for foreign students

During the postdoctoral fellowship at Stockholm University in April 2016, I conducted laboratory classes on ionising radiation dosimetry as part of the CELOD (Cellular effects of ionising radiation —

introduction to radiation biology) course organised for biology students at the Department of Molecular Biosciences, The Wenner-Gren Institute.

After returning to Poland, I was asked to give a lecture on MC modelling of DNA damage caused by ionising radiation for second-cycle students who chose radiobiology at Stockholm University. Since 2018, I travel to Stockholm (except during the pandemic) and have had the pleasure of giving a lecture entitled *The dose concept and Monte Carlo methods in radiation biology.*

6.2 Organisational achievements

6.2.1 Radiobiology laboratory at the Faculty of Physics, University of Warsaw

MC codes and a computing unit are sufficient to implement projects related to DNA damage modelling in cells exposed to ionising radiation. To experimentally validate the simulation results, it is necessary to perform radiobiological experiments. Thanks to funding from the FID projects in 2018 (about PLN 197,000) and 2021 (about PLN 177,000), of which I was the project leader, a radiobiology laboratory was established at the Faculty of Physics of the University of Warsaw. Conducting radiobiological research in our group is possible thanks to the equipment the laboratory is equipped with; these include incubators and a laminar chamber for culturing and working with cell material, a freezer, and a Dewar vessel that allows us to store cells during experiments and create a cell base after their completion. Currently, we have the ability to work with five cell lines: two lines of cancer cells and one line of normal prostate cells; osteosarcoma cells and a non-small cell lung cancer line. The laboratory is also equipped with two microscopes: an optical microscope, which was purchased as part of one of the FID projects and is used for everyday work with cellular material, and a fluorescent microscope, which enables real-time imaging of cells.

In addition, we have equipped our class Z isotope laboratory with point sources of beta radiation and the necessary detectors to perform dosimetric verification. Both the radiobiology laboratory and the mentioned radiation sources enable us to carry out projects with scientists from other units, such as the Medical University of Warsaw, the Institute of Experimental and Clinical Medicine of the Polish Academy of Sciences, and with pharmaceutical companies.

In 2021, another radiobiology laboratory was established at the Heavy Ion Laboratory of the University of Warsaw. I had only a small part in this project as part of a grant, the funds of which enabled the purchase of an incubator.

6.2.2 Innovation

Using my experience gained while working on the MP study programme, I took part in the creation of a new second-cycle programme in radiogenomics at the Faculty of Chemistry of the University of Warsaw. My involvement in this programme includes also support in the care of students in the form of conducting classes, master's theses, and student projects.

In my work, I try to enable students to participate in research that we conduct in a group, through the implementation of exercises as part of the Physics Laboratory of the 2nd degree and the Specialised Laboratory — both open to all students from the Faculty of Physics, not only MP. To this end, I created a set of exercises that students perform in our Radiobiology Laboratory, created and equipped with the financial support of the Teaching Innovation Fund (FID in Polish, projects in 2018 and 2021).

6.2.3 National and international cooperation

In addition to PhD projects in the field of MP, which are carried out at the Faculty of Physics of the University of Warsaw, I have established cooperation with several Polish and foreign institutions, which resulted in ongoing scientific projects:

- National Institute of Oncology in Warsaw: a study of the relationship between the probability of developing breast and ovarian cancers and BRCA1 and BRCA2 gene mutations (in cooperation with Dr. Krzysztof Fornalski of the Faculty of Physics of Warsaw University of Technology);

- Bronowice Cyclotron Centre: a comparative analysis of treatment plans for patients with lung cancer using photon and proton radiotherapy;
- Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Sweden: (i) studies of DNA damage in the area of eu- and heterochromatin of cancer cells exposed to mixed beams of gamma and alpha radiation, (ii) characteristics of a silicon dosimeter in radiobiological applications;
- Institut für Radiobiologie der Bundeswehr, Munich, Germany: image analysis of radiobiological data (repair foci, chromosomal aberrations);
- Danish Centre for Particle Therapy, Aarhus Hospital University, Denmark: research into the response of cancer cells to mixed proton and photon beams;
- Loma Linda University, USA: computer simulations of the concentration of reactive oxygen species formed as a result of high-dose electron radiation.

These collaborations enable us to conduct research at a high level and follow international trends.

6.3 Popularising activities

The promotion of research was an important task for me in order to reach talented students who would like to continue scientific research as part of a PhD project in the future and thus attract young people to the field of MP at the Faculty of Physics of the University of Warsaw. My popularising activities devoted to the issues of medical physics are dedicated to several groups:

- high school students:
  - lectures given in high schools in the Mazowieckie Voivodship (*How to win hide and seek with cancer cells* on medical imaging and *How we study DNA damage and repair, or what radiobiology does*); several lectures a year since 2017;
  - Summer School of Physics organised at the Faculty of Physics of the University of Warsaw: conducting workshops *Do cancer cells survive radiotherapy?* on the impact of ionising radiation on human cells, annually with breaks since 2012;
  - individual supervision: a project by Kacper Waluk and Jakub Pietrzak from the 18th LO of Jan Zamoyski in Warsaw entitled *Dosimetric verification of an oncological patient's treatment plan using an anthropomorphic 3D printed phantom*; this work was qualified for the final of the EUCYS competition and the semifinal of the E(x)plory competition in 2019/20;
- primary school students:
  - Physical carousel: running a workshop on magnetism *What is the difference between magnesium and magnet?* several workshops in 2022;
  - Summer School of Physics organised at the Faculty of Physics of the University of Warsaw: conducting workshops *Do cancer cells survive radiotherapy?* adjusted to the level of knowledge of the course participant, annually from 2021;
- co-creation of the website of the Biomedical Physics Division (brain.fuw.edu.pl), where we share teaching materials and research results;
- creating promotional videos for the MP speciality (short version https://youtu.be/tUScaXwdlz0, long version: https://youtu.be/C7j4xrZ-f2c).

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