Proton Therapy beyond cancer: Potential benefits for neurodegenerative disorders

C.M. Coelho[1,2,3], L. Pereira[1,2,3], P. Teubig[1,2], F. Murtinheira[3], P. Santos[4], F. Mendes[4], S. Viñals[5,6], D. Galaviz[1,2], F. Herrera[1,3]

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IGFAE workshop on technologies and applied research at the future Galician proton-therapy facility

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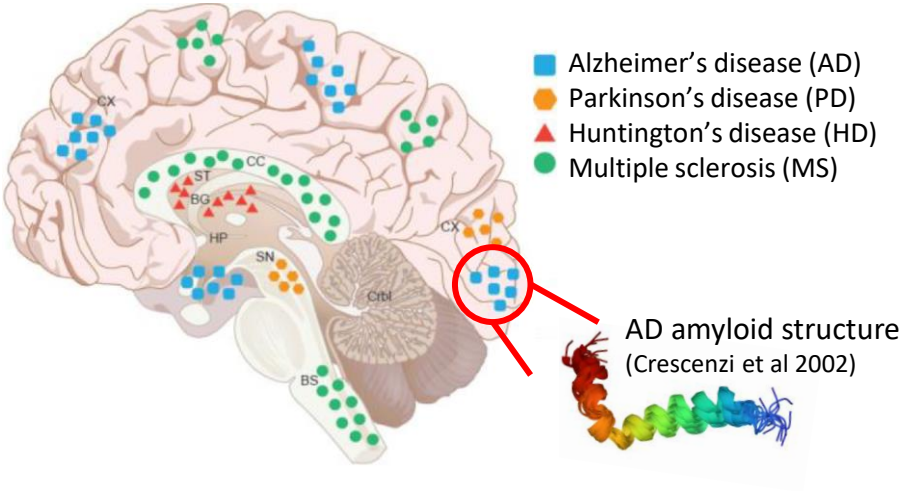
OVERVIEW

- **Introduction and objectives**
- **Gamma-irradiations**
- **Proton beam**
  - Development of the IMP beam line for cell irradiation
  - Homogeneous irradiation for pT studies @ IMP
  - Proton irradiation of cells
- Future work
- Summary
- References

INTRODUCTION

Neurodegenerative disorders

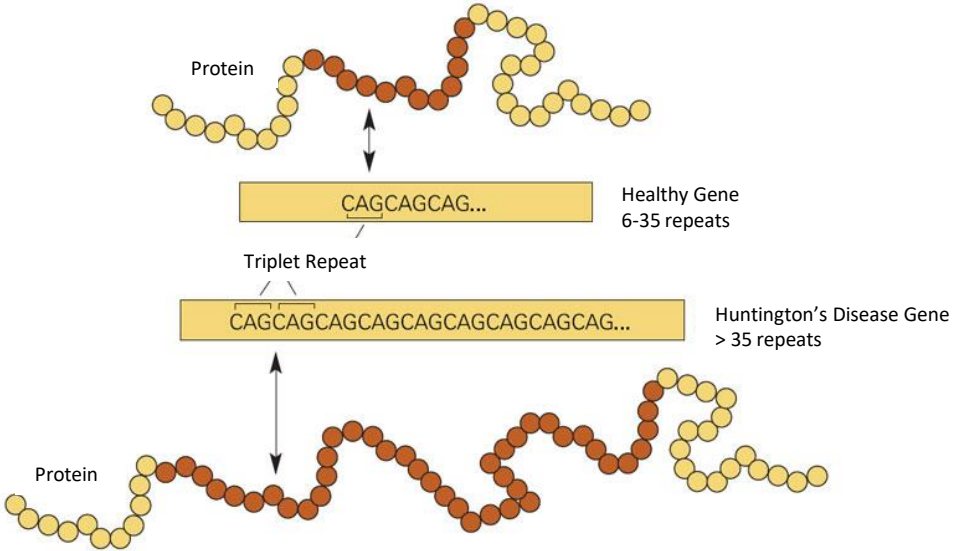
- Increased prevalence
- **Progressive loss of neurons**
  - Unclear mechanisms
- **No cure**
- Histopathological hallmarks
  - Oxidative stress
  - Neuroinflammation
  - **Alterations in the homeostasis of proteins** → **Amyloids**

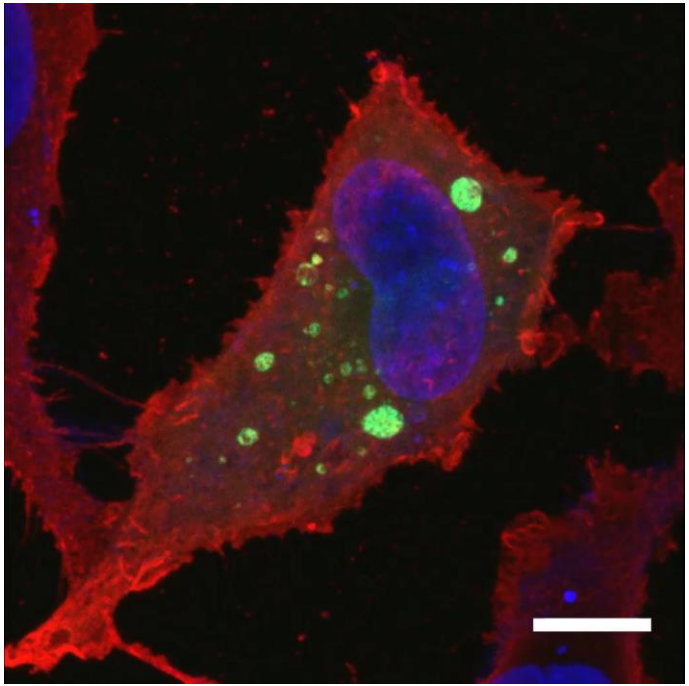


Most common degenerative cognitive diseases and their position in the brain (Hussain et al 2018)

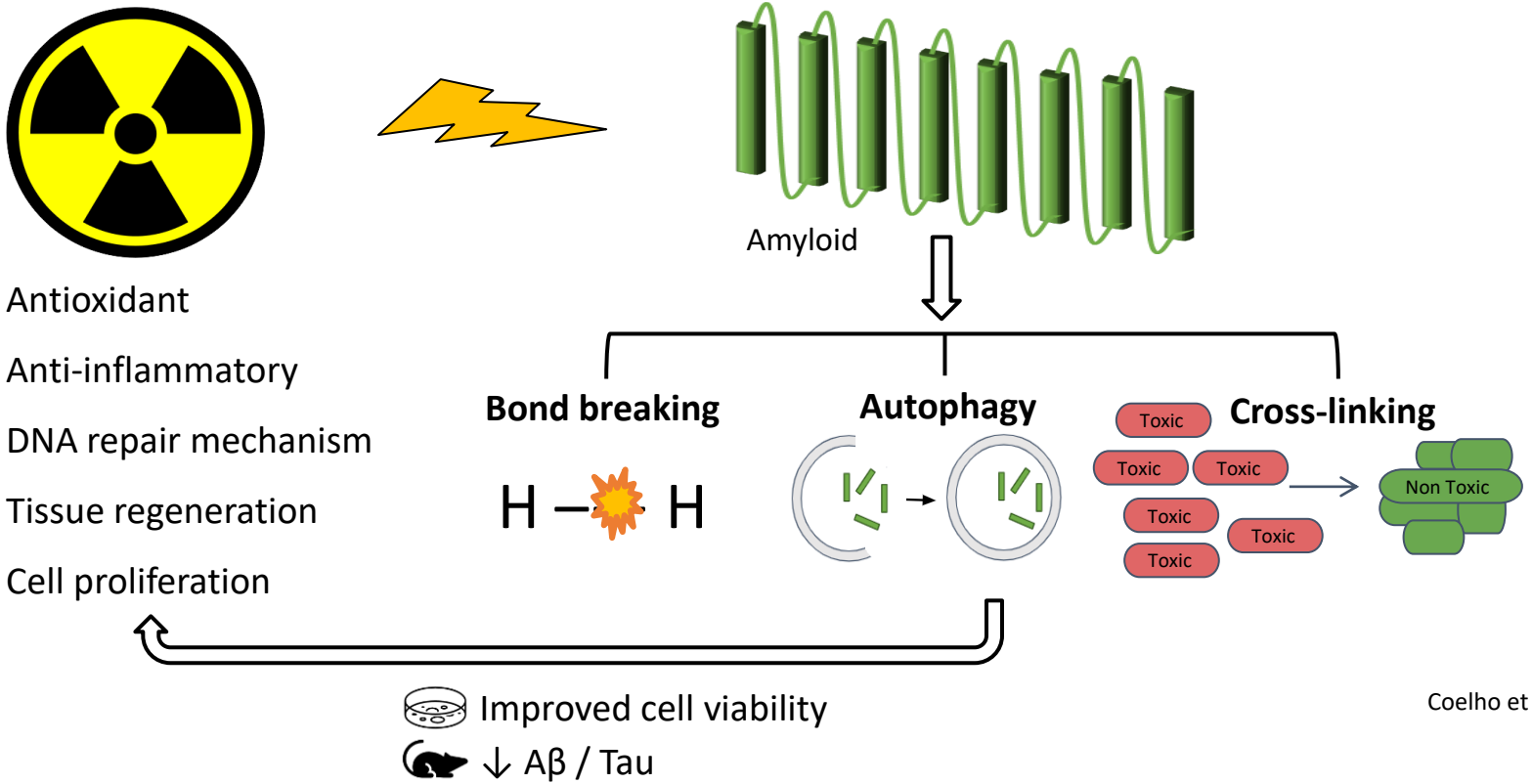
Huntington Disease

- **Mutations** in exon 1 of the gene that encodes the Htt **protein**
  - **Abnormal long polyglutamine tract**
    - Greater expansion leads to a bigger aggregation of the **mutant form** of Htt





Impact of LDRT in amyloidosis



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Impact of LDRT in amyloidosis

International Journal of *Molecular Sciences*

MDPI

Review

Radiation as a Tool against Neurodegeneration—A Potential Treatment for Amyloidosis in the Central Nervous System

Carina Marques Coelho [1,2,†], Lia Pereira [1,2,†], Pamela Teubig [1], Pedro Santos [3], Filipa Mendes [3,4], Sílvia Viñals [5], Daniel Galaviz [1,6] and Federico Herrera [2,7,*]

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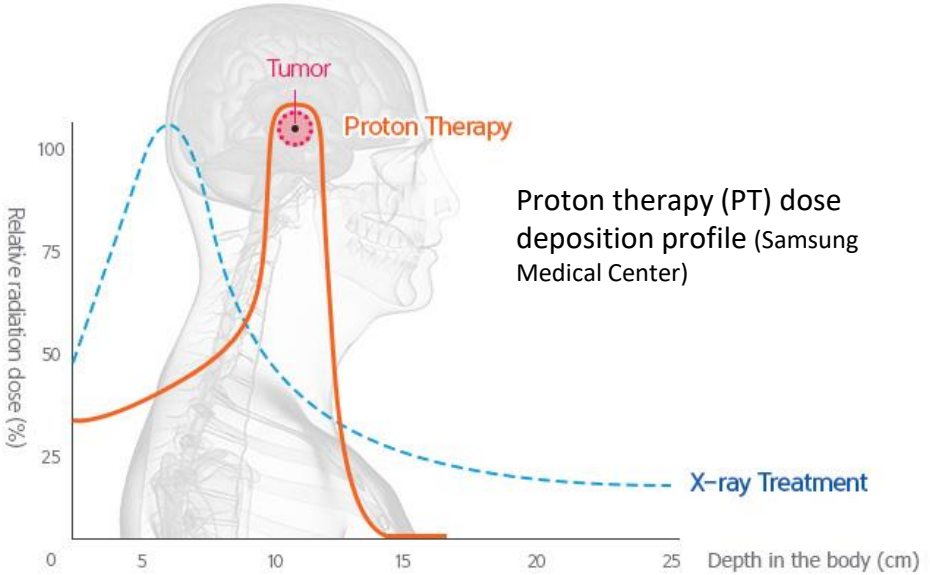
Proton Therapy

- Finite range
- Minimal lateral scattering
- **Higher dose conformity**
- Advanced dose shaping capabilities
- **Higher precision**
- Lower integral dose
- **Reduced toxicity**



Proton therapy (PT) dose deposition profile (Samsung Medical Center)

OBJECTIVES

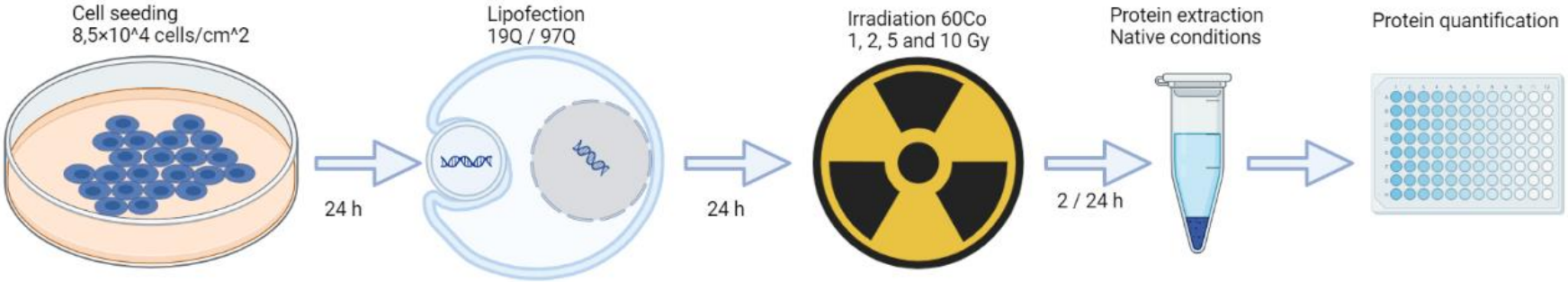
- Evaluate the capability of **PT** to **disrupt or diminish** the formation of **toxic protein amyloids** associated with neurodegenerative disorders
  - Study the effects of different types of **radiation** on the structure and conformation of those **toxic protein amyloids**
    - **Experimental validation** of the predicted results on abnormal deposits of protein amyloids
  - Optimize experimental conditions and develop an **irradiation protocol**
  - Characterization of the **biochemical** and **biophysical** mechanisms underlying the optimal **PT** conditions for **disruption of amyloid deposits**

GAMMA-IRRADIATIONS
Irradiation facility

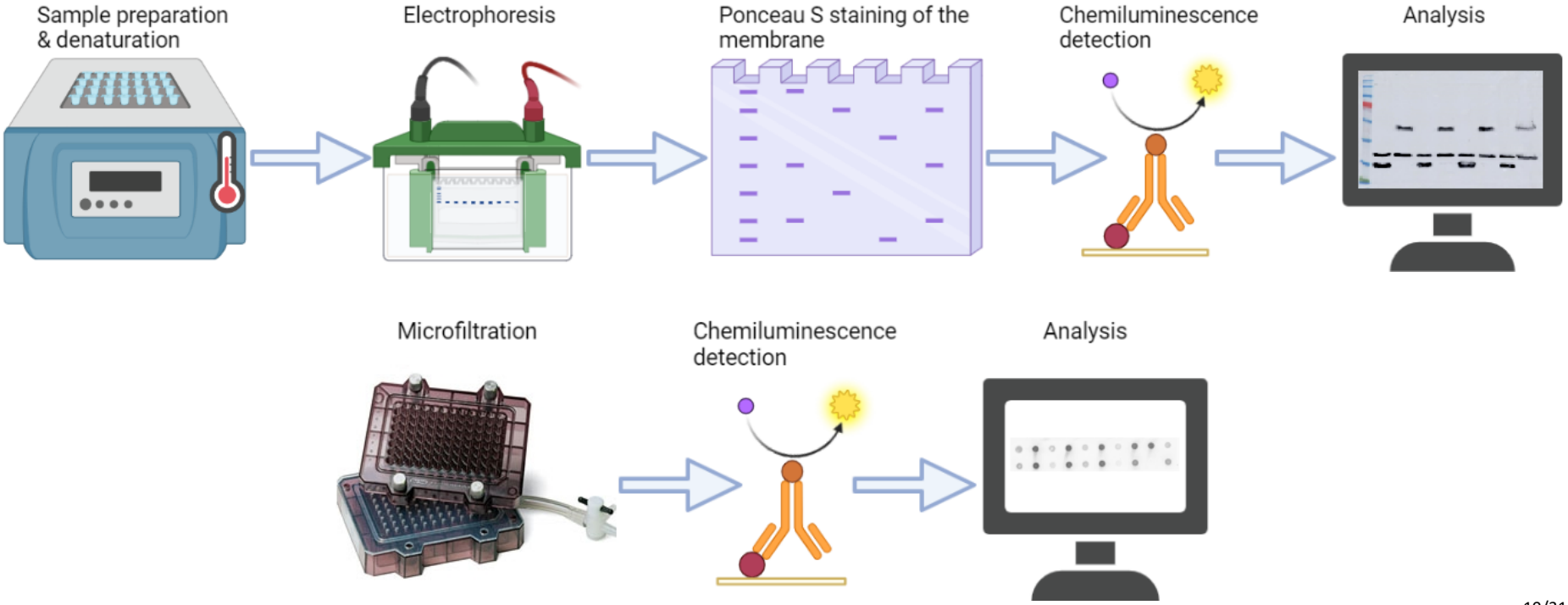
PRECISA22 - ^{60}Co irradiator used in the first experiments.

Dosimetric measurements of PRECISA22 performed with an ionizing chamber.

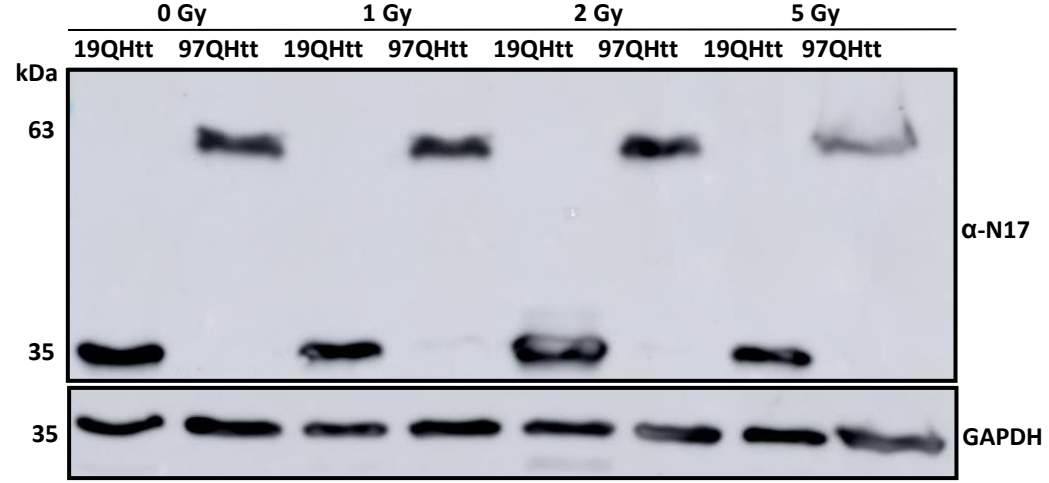
Irradiation and protein extraction



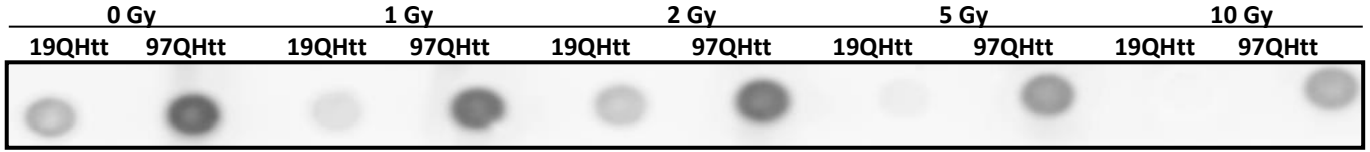
Detection of protein levels and aggregation



Irradiation reduces the expression and aggregation of huntingtin

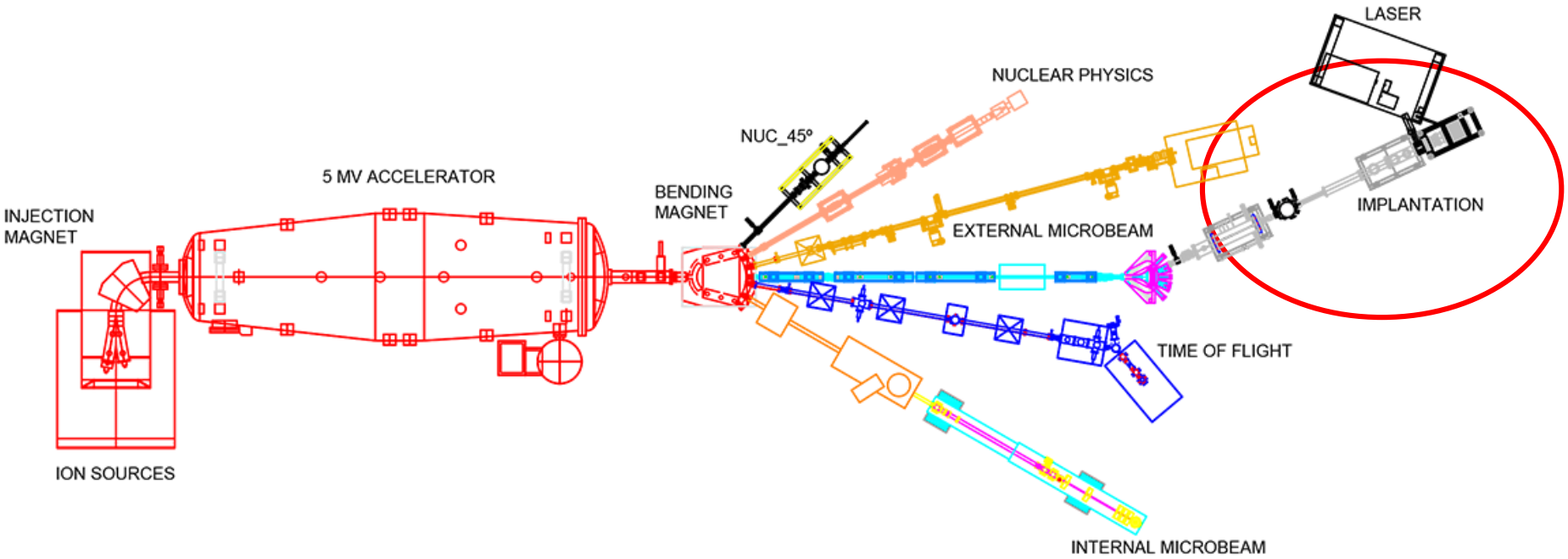


Representative Western blot of samples extracted from transfected HeLa cells 24 h after the irradiation.



Representative filter trap assay of 19QHtt or 97QHtt transfected HeLa cells after the irradiation with the referred doses.

DEVELOPMENT OF THE IMP BEAM LINE FOR CELL IRRADIATION



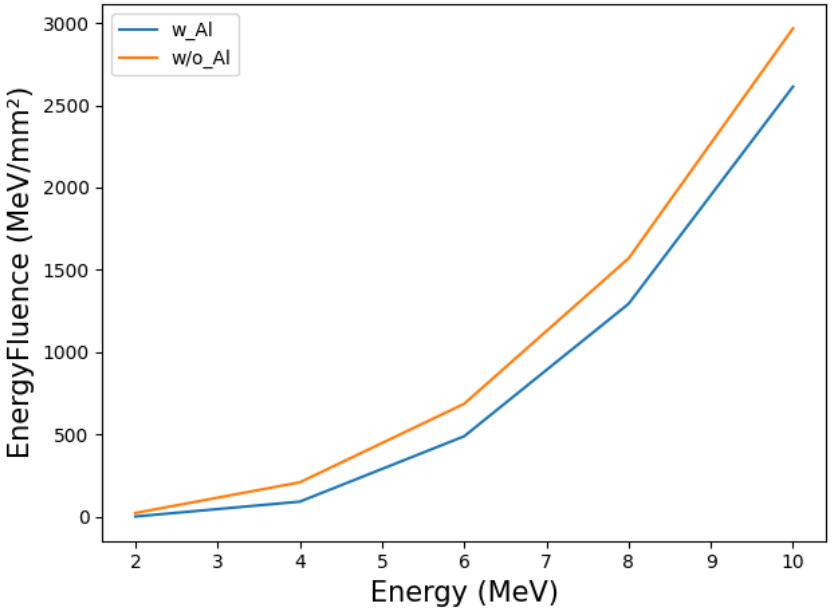
Exit window

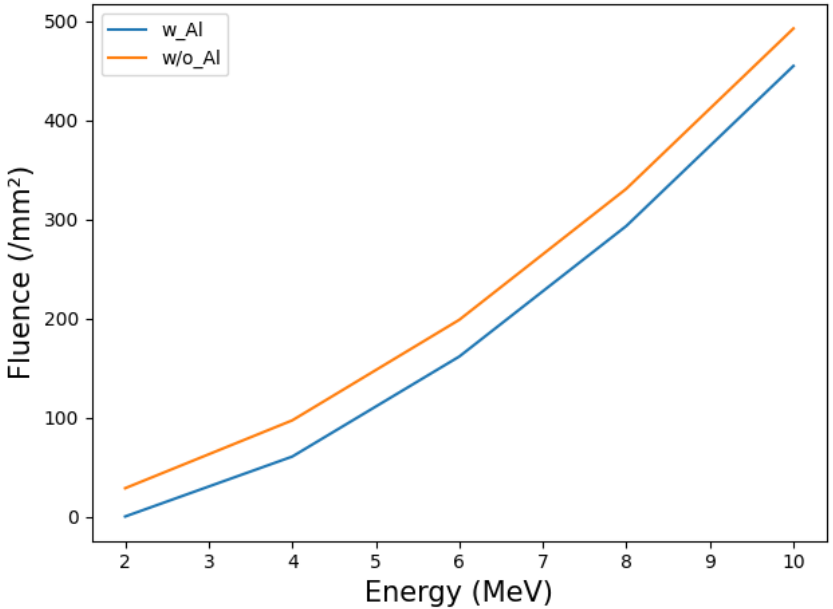
- Ø 35 mm
- Al foil
  - 99% purity
  - 50 µm thickness
- Vacuum test

Vacuum test of the larger exit window.

Exit window

- TOPAS simulations





Results from the MC simulations with and without the aluminum foil in terms of fluence and energy fluence depending on the initial beam energy.

HOMOGENEOUS IRRADIATION FOR PT STUDIES @ IMP

RCF dosimetry system

Matte Surface Clear Polyester Base, 125 µm
Active Layer, 28 µm
Matte Surface Clear Polyester Base, 125 µm

EBT3 RCF's

$$D(Gy) = a + \frac{b}{PV - c}$$

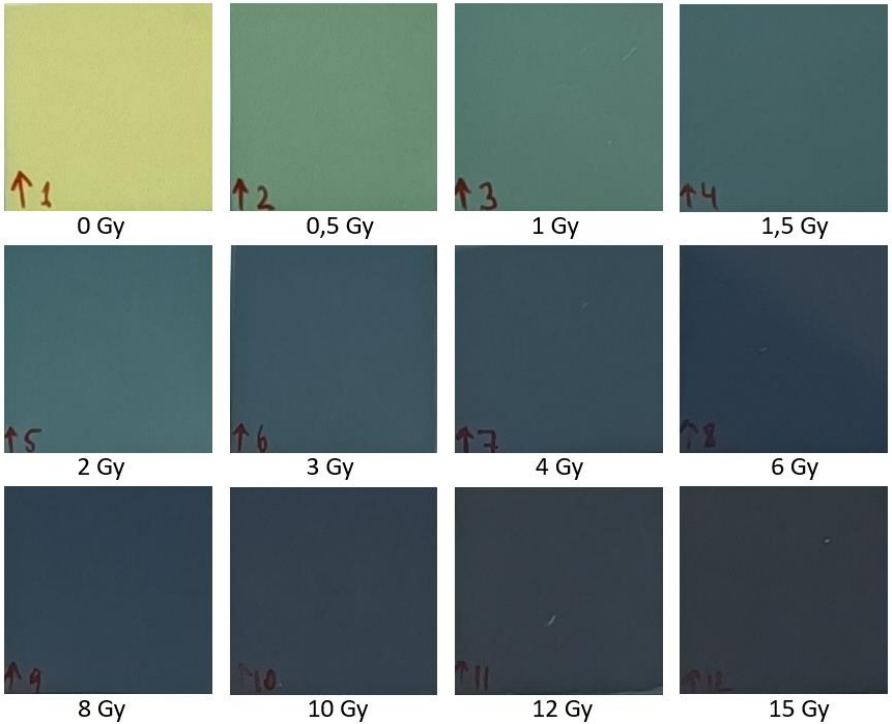
Calibration equation (Sanchez-Parcerisa et al 2021)

RCF dosimetry system

- Calibration

Parameter	<Red>	<Green>	<Blue>
a	-1.59 ± 0.88	-0.497 ± 0.642	-3.12 ± 0.60
b	89.4 ± 23.0	78.9 ± 18.8	227 ± 33
c	58.6 ± 1.3	26.9 ± 4.2	27.1 ± 1.6

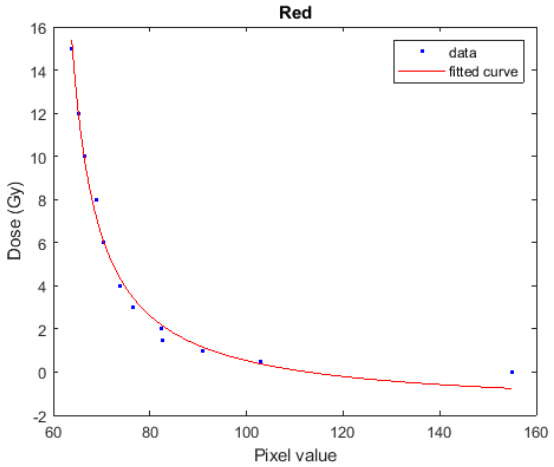
Calibration parameters.

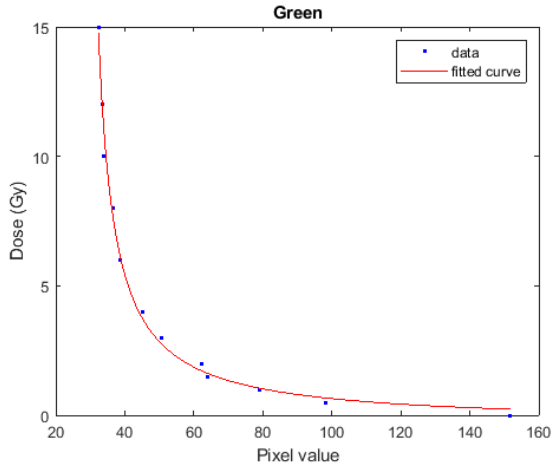


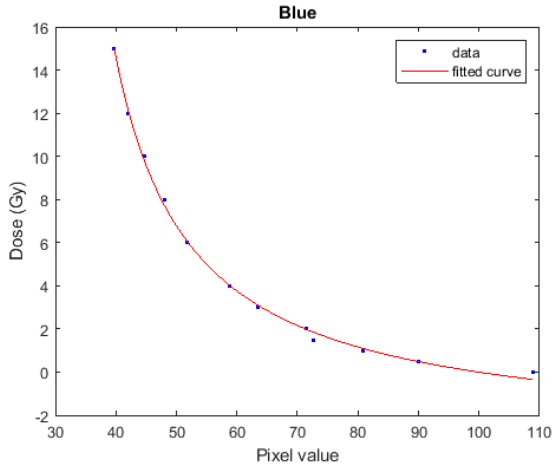
Irradiation pattern of the calibration RCFs.

RCF dosimetry system

- Calibration

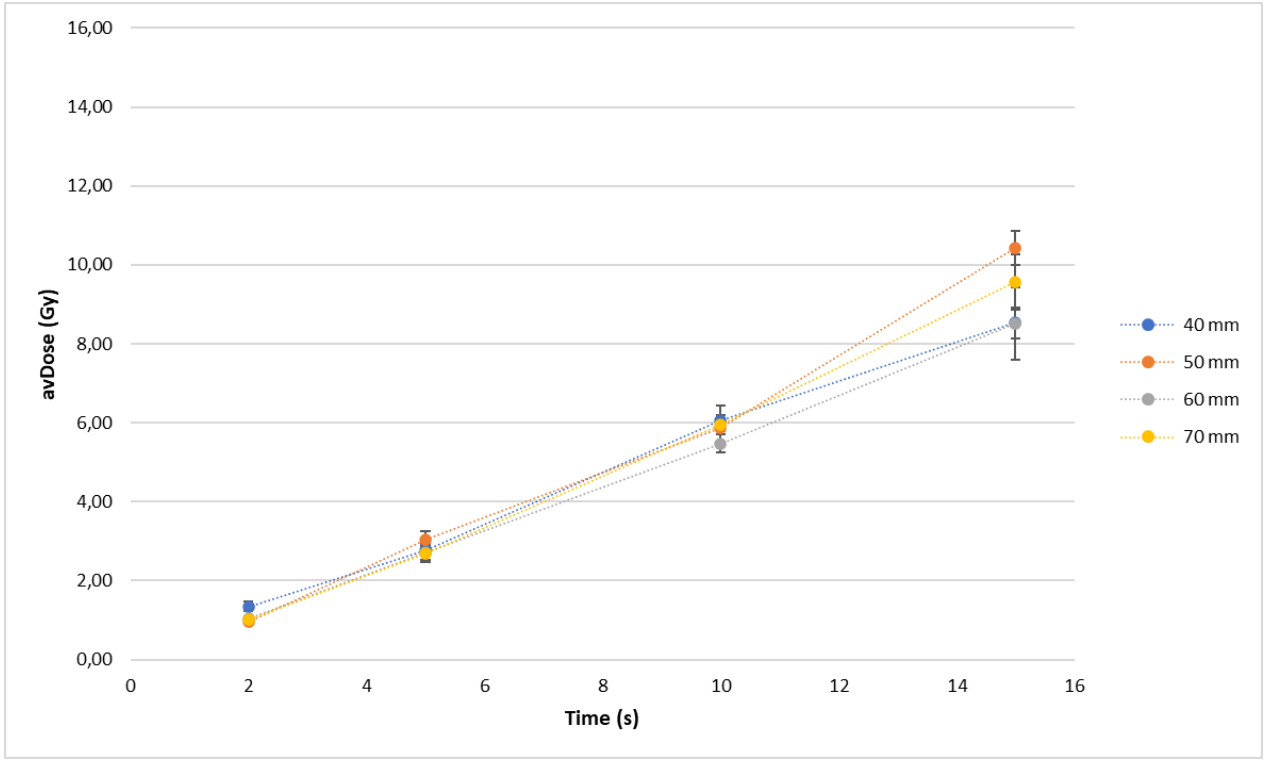






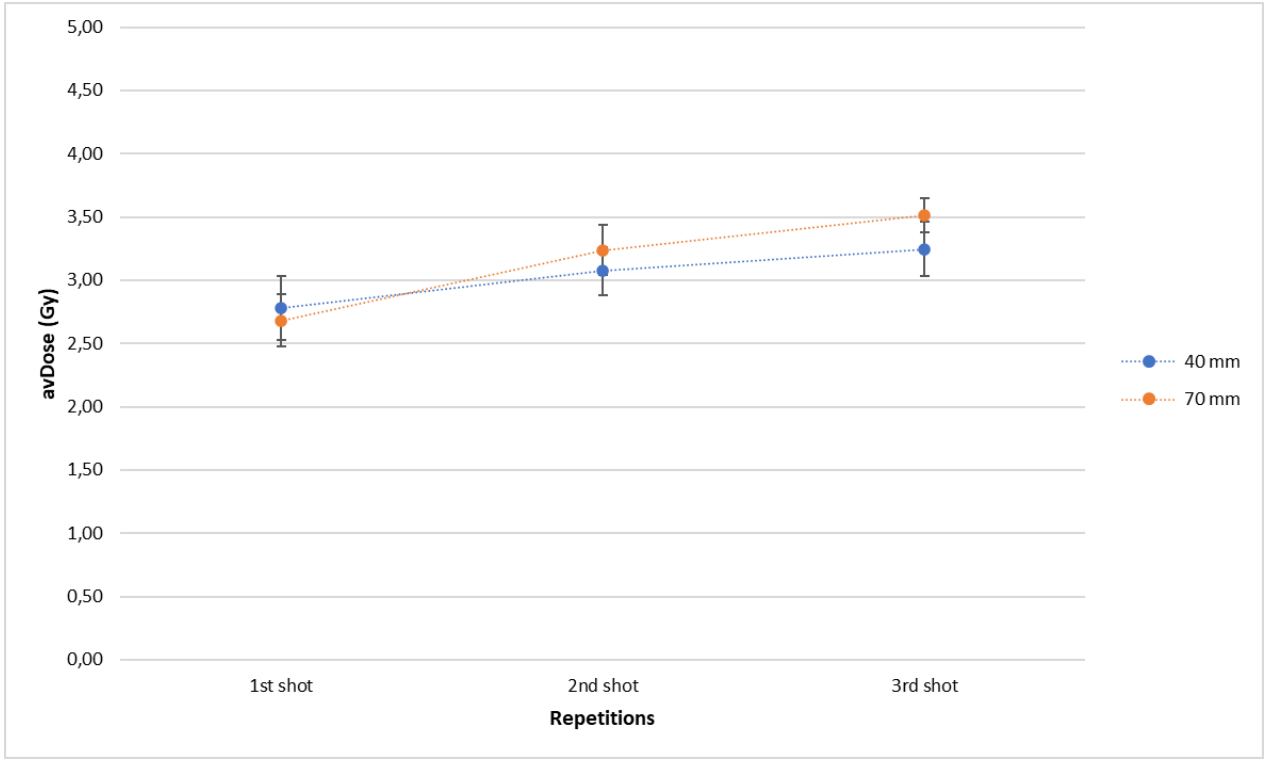
Fitting of the pixel values (PV) in RGB with the defined calibration parameters

RCF dosimetry system – time and distance



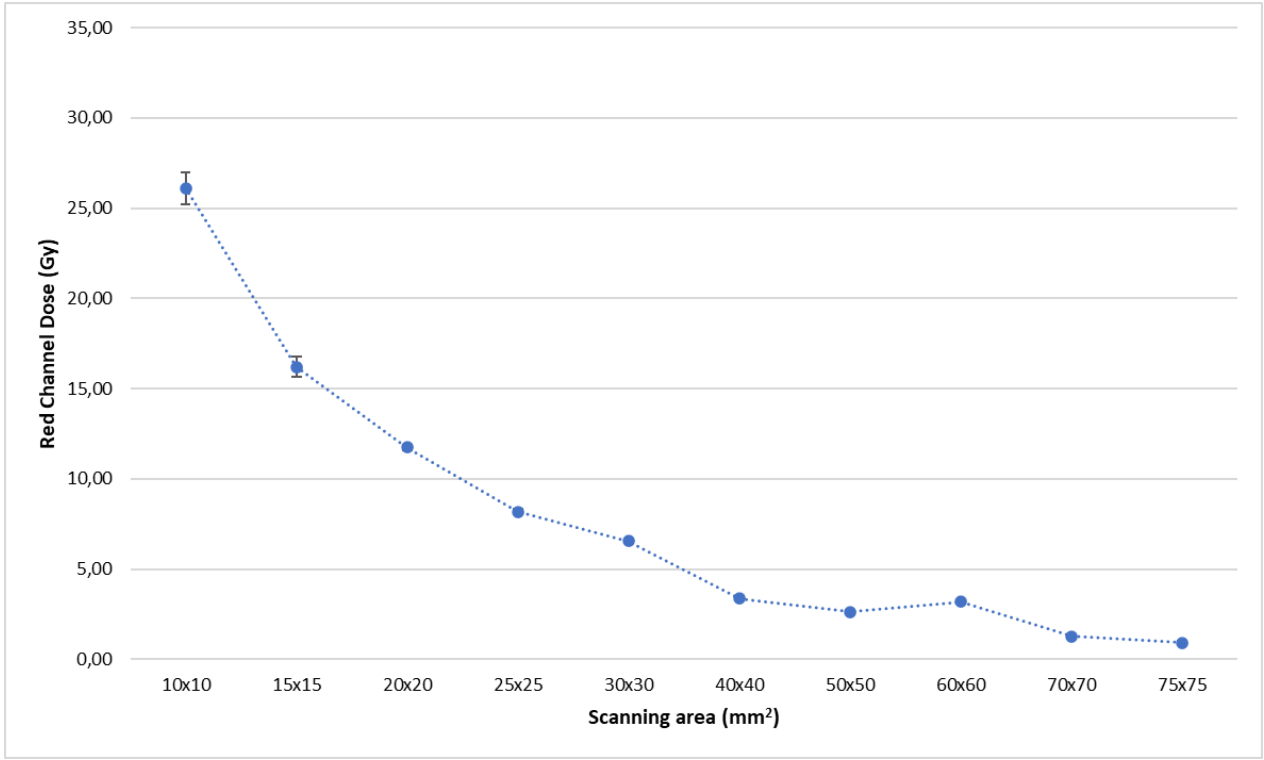
Calculated avDose in function of the exposure time and the distance to the exit window

RCF dosimetry system - reproducibility



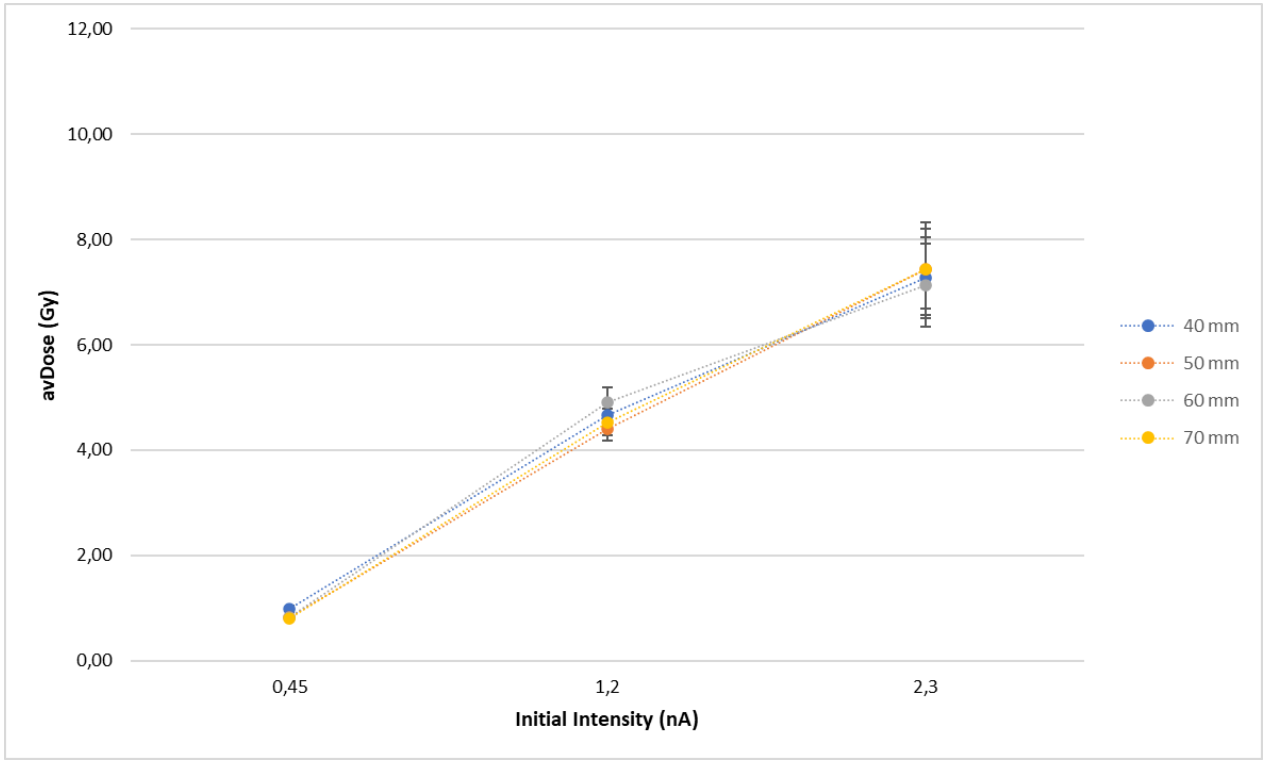
Calculated avDose for RCFs irradiated in the same conditions

RCF dosimetry system – scanning area



Calculated Red Channel Dose in function of the scanning area

RCF dosimetry system – beam intensity



Calculated avDose in function of the initially measured beam intensity for different distances to the exit window

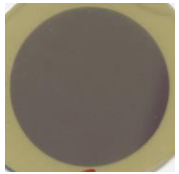
PROTON IRRADIATION OF CELLS

Experimental conditions

- IMP beam line
- 10 MeV H+
- Large Al exit window (Ø = 35 mm)
- Intensity = 0.9(1) nA
- Distance to the sample = 60 mm
- Area scanned with the raster = 75x75 mm^2

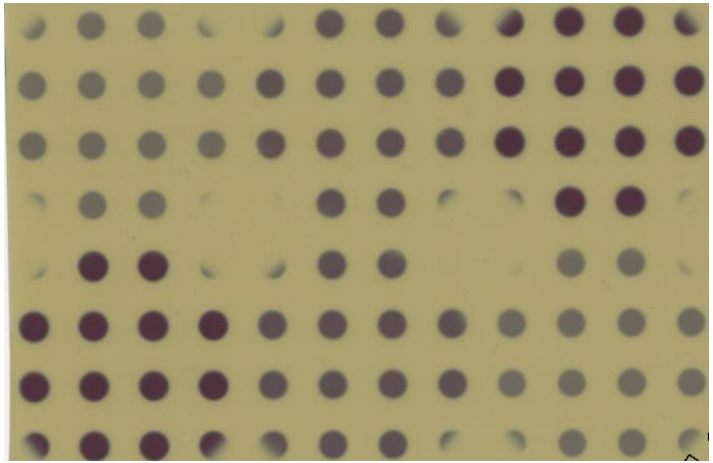
Online RCF analysis

- RCFs placed inside the 35 mm cell culture dish
  - t = 5, 10 and 20 s
- RCF placed inside the 96well plate
  - t = 5, 10 and 20 s
- Dose rate ≈ 0.1 Gy/s
  - 10 s to deliver 1 Gy


RCF05


RCF06

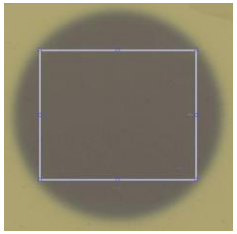

RCF07


RCF12

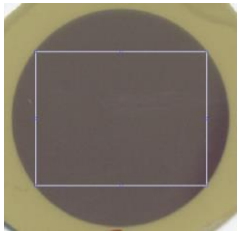
RCF 24 h analysis

RCF ID	Placement	Planned time (s)	Real time (s)	Intensity (nA)	Dose rate (Gy/s)
RCF04	Outside 35 mm dish	5	7	0.91	0.06
RCF05	Inside 35 mm dish	5	7	0.93	0.1
RCF06		10	11	0.93	
RCF07		20	22	0.94	
RCF10	Outside 96 well plate	5	6	0.93	0.06
RCF12_1st	Inside 96 well plate	5	7	0.93	0.06
RCF12_2nd		10	12		0.1
RCF12_3rd		30	32		
RCF12_4th		30	32		
RCF12_5th		10	12		
RCF12_6th		5	7		0.06

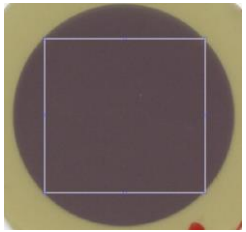
RCF 24 h analysis



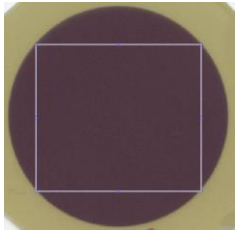
RCF04



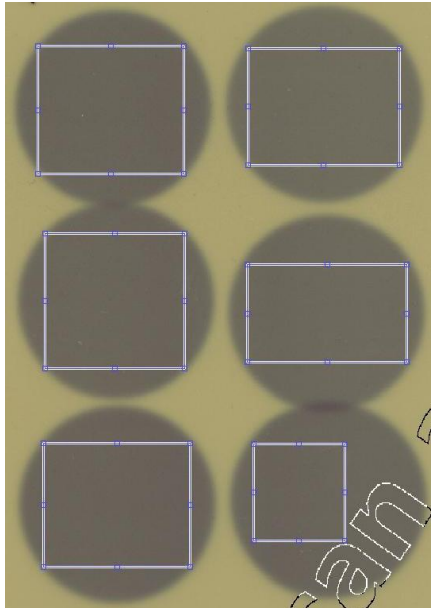
RCF05



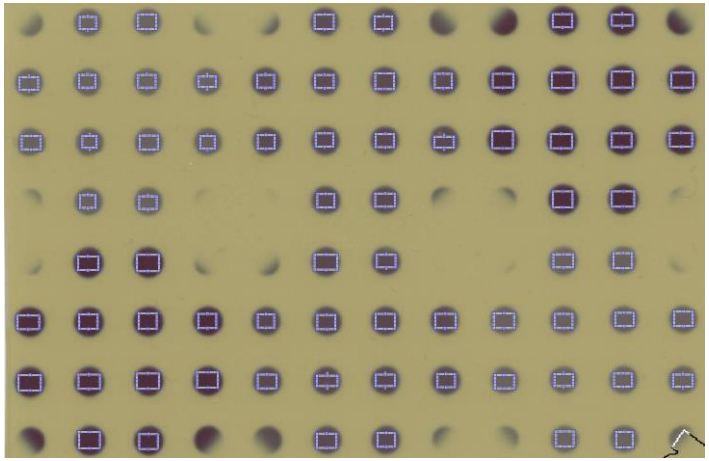
RCF06



RCF07



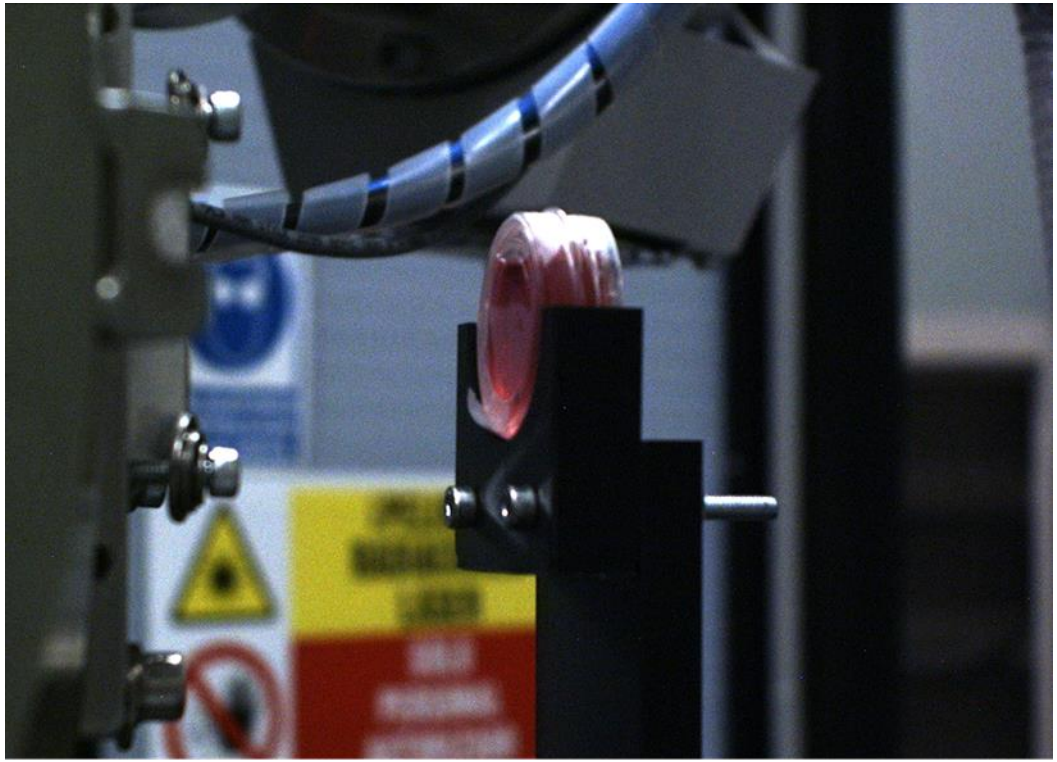
RCF10



RCF12

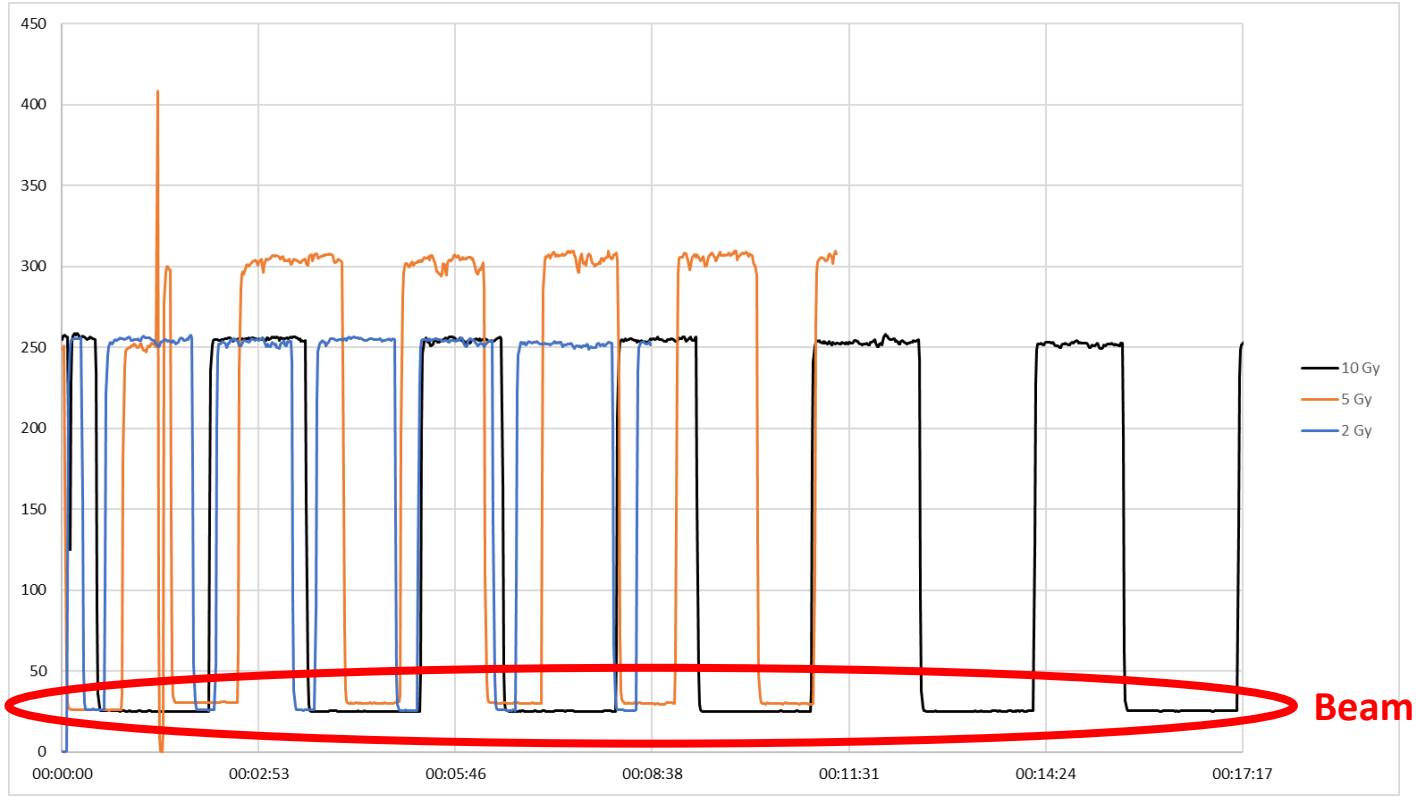
Biological samples irradiation

- 3 cell lines
  - STHdh Q7, STHdh Q111 and STHdh Q7/111
- 3 doses of radiation
  - 2, 5 and 10 Gy
- Irradiation in duplicate



Setup of the biological samples irradiation

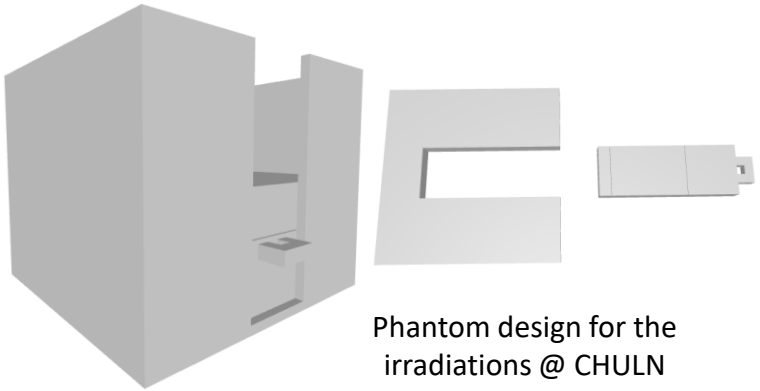
Biological samples irradiation



Internal charge measurements during the irradiation of the biological samples with 2, 5 and 10 Gy

FUTURE WORK

- Analysis of the biological samples irradiated with the proton beam
- Irradiation of biological samples with a commercial linear accelerator used for mec purposes



Phantom design for the irradiations @ CHULN

- Monte Carlo simulations of radiation effects on the protein aggregates

SUMMARY

- Effects of RT demonstrated with the preliminary experiments performed using γ-rays
  - Reduction in the expression and aggregation of proteins
- RCF dosimetry system is a reliable and easy to use method to calculate the dose rate
- Proton irradiation conditions with higher influence in dose delivery
  - Beam intensity
  - Scanning area
  - Time of exposure

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Thanks!

Any questions?

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Support slides

Institution	Patients	Treatment	Outcome measures	Reference
University Hospital of Geneva	10 patients (observation) + 10 patients (LDRT)	10 Gy in 5 x 2 Gy (5 consecutive days)	PET scan (8-12 weeks) Neurocognitive tests (6 and 12 months) Treatment toxicity (12 months)	Zilli, T. 2021 (NCT03352258)
William Beaumont Hospitals	15 patients (arm 1) + 15 patients (arm 2)	10 Gy in 5 x 2 Gy (arm 1) 20 Gy in 10 x 2 Gy (arm 2)	PET scan (4 months) Neurocognitive tests and Treatment toxicity (6 weeks, 3, 6 and 12 months)	Fontanesi, J. 2022 (NCT02359864)
Kyung Hee University Hospital	5 patients (arm 1) + 5 patients (arm 2)	9 Gy in 5 x 1,8 Gy (arm 1) 5,4 Gy in 3 x 1,8 Gy (arm 2)	PET scan and neurocognitive tests (6 months)	Kyung Hee University Hospital at Gangdong 2020 (NCT04203121)
Virginia Commonwealth University	15 patients (arm 1) + 15 patients (arm 2)	10 Gy in 5 x 2 Gy (arm 1) 20 Gy in 10 x 2 Gy (arm 2)	Neurocognitive tests (6 weeks, 3, 6 and 12 months) Treatment toxicity (48 months)	Virginia Commonwealth University 2021 (NCT02769000)

Support slides

Patient	Disease	Age (years)	Nr. of scans	Total administered dose (Gy)	Reference
1	AD	81	11	0,447	Cuttler et al. 2016, 2017, 2018, 2021
2	PD	n.a.	6	0,240	Cuttler et al. 2017
3	AD	88	4	0,165	Cuttler et al. 2021
4	AD	90	4	0,175	Cuttler et al. 2021
5	AD	84	4	0,162	Cuttler et al. 2021
6	AD	82	4	0,161	Cuttler et al. 2021

Support slides

- RCF dosimetry system
  - LET quenching effect (Sanchez-Parcerisa et al 2021)
    - Surface energy at the film active layer

$$E_s(z, E_0) = \left[(E_0 - sE_0^q)^p - \frac{z}{r} \right]^{1/p}$$

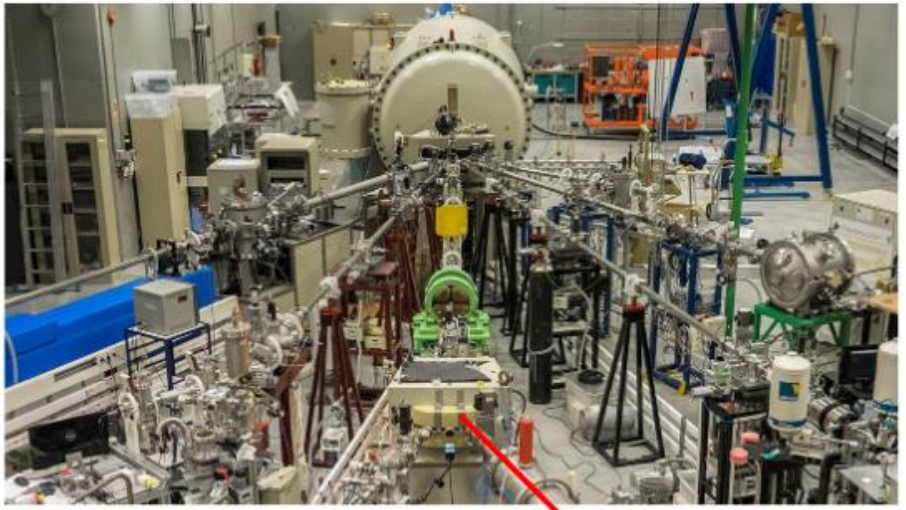
    - Proton LET in film active layer

$$LET_{AL} = ae^{-bE_s} + ce^{-dE_s}$$

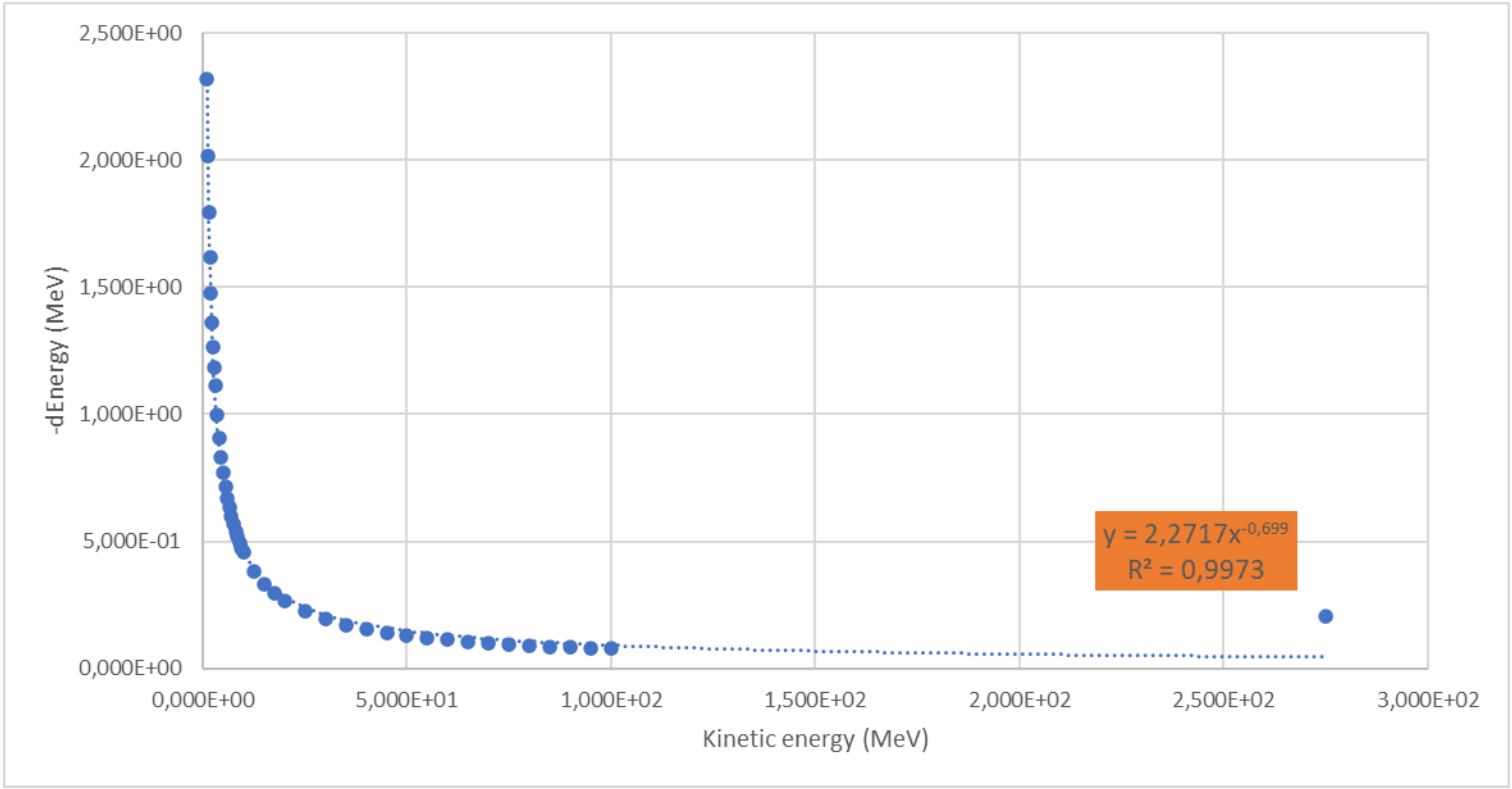
    - Relative efficiency

$$RE(LET_{AL}) = 1 - A \cdot LET_{AL}^B,$$

Support slides



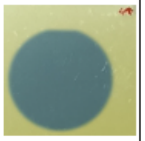
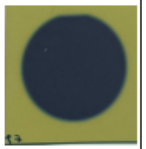
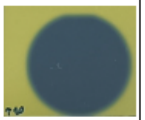
Support slides



Support slides

	$a\,(\mathrm{keV}\ \mu\mathrm{m}^{-1)}$	$b(\mathrm{MeV}^{-1})$	$c\,(\mathrm{keV}\ \mu\mathrm{m}^{-1})$	$d\,(\mathrm{MeV}^{-1)}$
EBT3	$(4.1 \pm 1.5) \times 10^5$	2.88 ± 0.12	22.5 ± 1.9	0.142 ± 0.013

Support slides

Picture	avDose (Gy)	Irradiation time (s)	Dose rate (Gy/s)	Beam intensity (nA)	Distance to beam exit (mm)	Scanning area (mm^2)
	1.34 ± 0.12	2	0.67	0.8	40	40x40
	2.78 ± 0.25	5	0.556	0.8	40	40x40
	6.07 ± 0.37	10	0.607	0.8	40	40x40
	8.53 ± 0.40	15	0.569	0.8	40	40x40
	1.04 ± 0.06	2	0.52	0.8	60	40x40
	2.71 ± 0.22	5	0.542	0.8	60	40x40