Irradiation of cell cultures with laser-generated protons and X-rays

M. Seimetz[1] A. Reija[1,2], A. Alejo[2], A. Bembibre[2], J. Benlliure[2], M.E. Aguado-Barrera[3,4], A. Crujeiras[3,4], A. Vega-Gliemmo[3,4], M. Alfonso[5], O. Calvo[5], J.I. Apiñaniz[6], M. Ehret[6] and M.D. Rodríguez Frías[6,7]

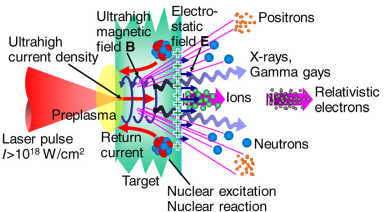
[1]Instituto de Instrumentación para Imagen Molecular (i3M), CSIC - Universitat Politècnica de València, Valencia
[2]Instituto Galego de Física de Altas Enerxías (IGFAE), Universidade de Santiago de Compostela
[3]Fundación Pública Galega de Medicina Xenómica (FPGMX), Instituto de Investigación Sanitaria de Santiago de Compostela
[4]Hospital Clínico Universitario de Santiago, Rúa Choupana s/n, Santiago de Compostela
[5]Instituto de Biología Funcional y Genómica (IBFG), CSIC - Universidad de Salamanca, Salamanca
[6]Centro de Láseres Pulsados (CLPU), C/ Adaja 10, Salamanca
[7]Universidad de Alcalá (UAH), Madrid

7th European Advanced Accelerator Conference (EAAC 2025)
Isola d'Elba, Italy
September 21-27, 2025

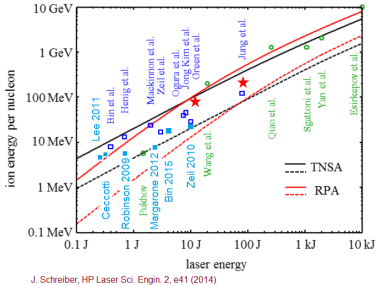
Laser-based sources of ionizing radiation

Interactions of femtosecond laser pulses with (solid) targets:

- Expanding preplasma interacts with main pulse
- $I > 10^{16}$ W/cm^2 ($E_L \sim$mJ): 10-100 keV X-ray generation
- $I > 10^{18}$ W/cm^2 ($E_L \sim$J): MeV proton/ion acceleration
- Ultra-short, ultra-intense (ps-ns) radiation bunches
- Pulse dose rates $> 10^9$ Gy/s
- Radiobiology experiments at several laser facilities: P. Chaudhary *et al.*, Front. Phys. 9 (2021).



H. Daido, Rep. Prog. Phys. 75, 056401 (2012)

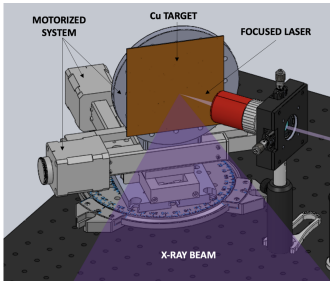


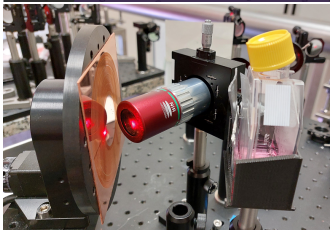
J. Schreiber, HP Laser Sci. Engin. 2, e41 (2014)

At Laser Laboratory for Acceleration and Applications (L2A2, Santiago de Compostela):

First exp. with laser-X-ray source:

- Ti:Sa laser, 35 fs
- 0.8 mJ on Cu target in air
- 1 kHz shot rate; 3D axis movement to refresh target at focal spot
- $<$ 100 keV, 28 mGy/s at 10 cm
- Sterile cell culture flasks (25 cm^2)
- Cells grown on 70 µm peel-off foil
- Dose measurement with RCF.

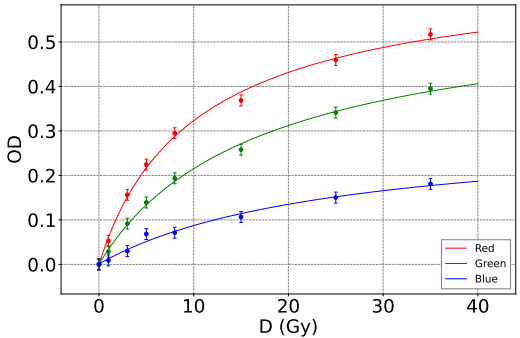




Absolute dose measurement

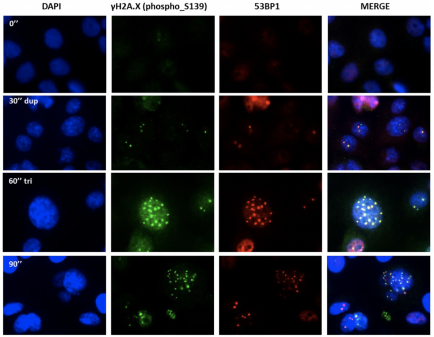
Radiochromic film (EBT3-Unlaminated) calibrated up to 35 Gy with clinical X-rays from 6 MV linac, Santiago de Compostela: A. Reija, PhD thesis (2025)

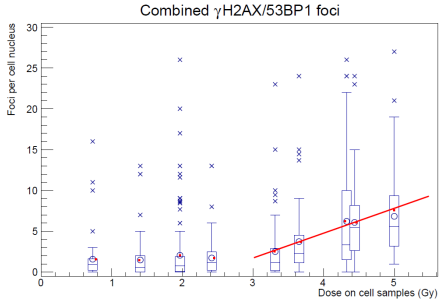
- RCF sandwiched between PMMA plates
- Absolute dose in water measured with ionization chamber
- Specific corrections applied in laser-based experiments.



Preparation and analysis of cell cultures at Health Research Institute of Santiago de Compostela (IDIS) (Ana Vega, Miguel Aguado):

- Cell line: human lung adenocarcinoma (A549)
- Visualization of γ-H2AX/53BP1 foci by immunostaining and microscopy: 60 cells/sample
- Not ideal: poor reproducibility of dose; only 10 samples.



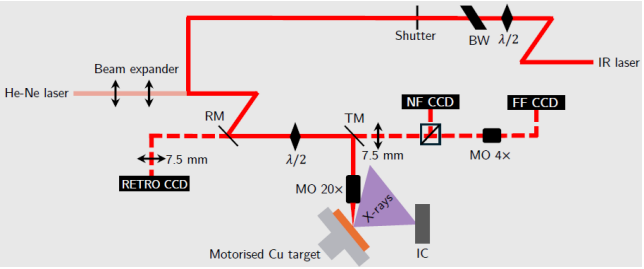


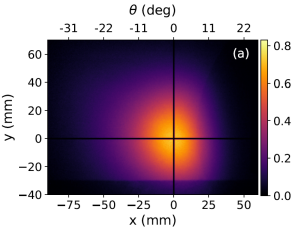
A. Reija, EuNPC 2022; EPJ Web of Conf. 290, 08001 (2023)

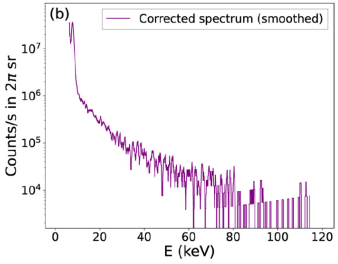
Improved X-ray source

Revised version:

- Focal spot: 1.5 µm FWHM; Rayleigh length: 8.4 µm
- $I_L = 2 \cdot 10^{17}$ W/cm^2
- Emission in target normal direction (LANEX screen)
- $2.3 \cdot 10^9$ photons/s; CE $= 3.6 \cdot 10^{-6}$
- $E_{\max} \simeq 80$ keV.







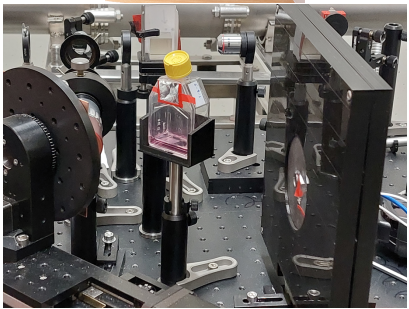
A. Reija, A. Coathup *et al.*, Optics Express **33**, 16125 (2025)

Improved X-ray source

2nd experiment with better performance:

- Focal stability $\Rightarrow$ less fluctuations, 70-100 mGy/s
- Ionization chamber for real-time dose control
- RCF for control, shielded from UV light with Al foil
- Dose on cells obtained from RCF and calibrated loss in PE foil
- 100 Gy/target disk; use 2 Cu plates, 2×30 min
- 36 samples at 3/5/8 Gy + control samples
- Colony formation and cell proliferation.

Comparison with X-rays from clinical, 6 MV linac.



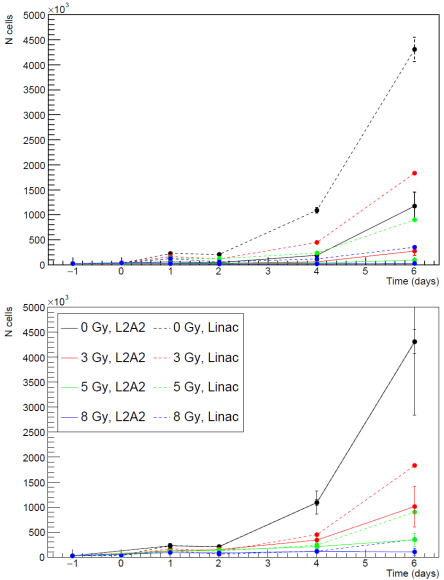
Improved X-ray source

Cell proliferation assay:

- Seed 25k cells one day before irradiation
- Count cells 0/1/2/4/6 days after irradiation.

Observations:

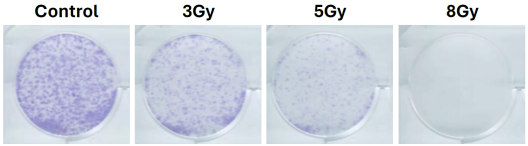
- Final dose values: $D_1 = (3.4 \pm 0.4)$ Gy, $D_2 = (5.9 \pm 0.5)$ Gy, $D_3 = (8.4 \pm 0.6)$ Gy
- At linac or laser X-ray: higher dose $\Rightarrow$ less cells
- Difference in control samples $\Rightarrow$ possible influence of flask
- At same dose, less cells with laser X-rays than at 6 MV linac $\Rightarrow$ cell growth slower after laser X-rays.

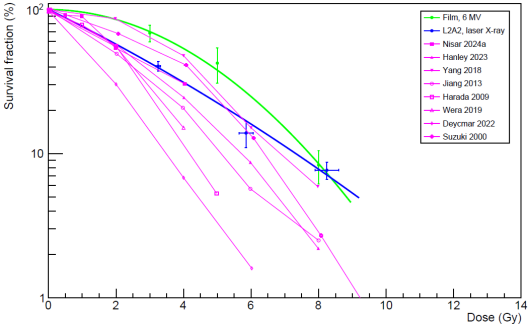


Improved X-ray source

Colony formation assay: 7 days post-IR

- Final dose values: $D_1 = (3.3 \pm 0.1)$ Gy, $D_2 = (5.9 \pm 0.2)$ Gy, $D_3 = (8.2 \pm 0.4)$ Gy
- Clonogenic survival depending on dose
- Lower SF as compared to 6 MV linac; coherent with proliferation
- SF values within range of data from low-energy (200 kV) X-ray tubes with same cell line (A549)
- In LQ-model fit, linear term dominant for laser X-rays.





Radiobiological effects of laser-accelerated ions:

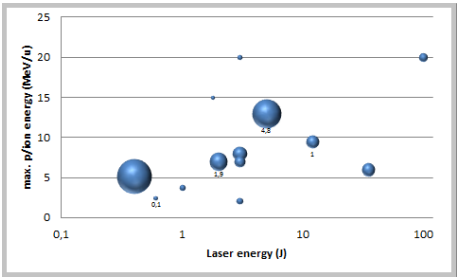
- Ultra-short (ns), ultra-intense pulses; peak dose $\sim 10^9$ Gy/s
- Single-shot dose $\sim 0.1 - 5$ Gy
- At ~ 10 Hz rep. rate: FLASH regime.

Requirements (for monolayer cell cultures):

- Stable particle source, 5-10 MeV/u
- $\Phi_p \Leftrightarrow D \Rightarrow$ narrow energy interval

$$D = \frac{dE}{dx}\left[\frac{\text{keV}}{\mu\text{m}}\right] \cdot \Phi\left[\frac{1}{\text{cm}^2}\right] \cdot 1.602 \cdot 10^{-9}\,\text{Gy}\,.$$

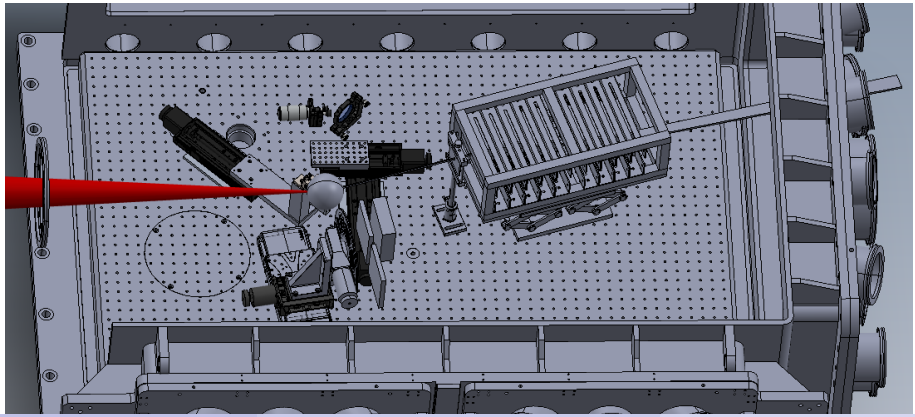
- Shot-to-shot dose control.



Radiobiology with laser-accelerated protons

Experimental setup at the Spanish Pulsed Laser Center (CLPU, Salamanca):

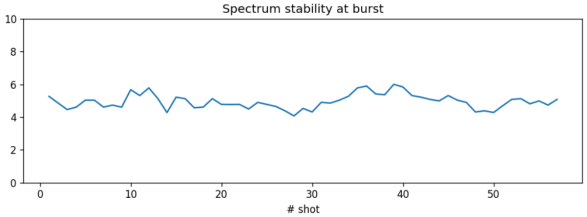
- VEGA-3 laser, 1 PW, 30 J/30 fs
- Target wheel, focal diagnostics $\Rightarrow$ energy selector 30 cm behind target
- Cell irradiation outside vacuum chamber.

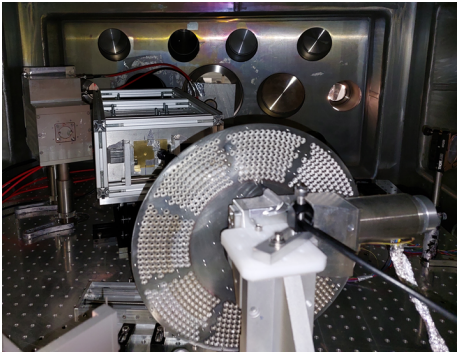


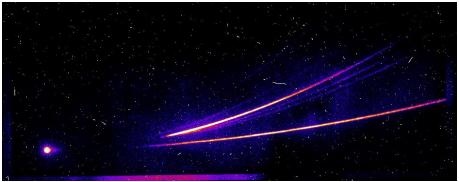
Laser-proton source at VEGA-3

Proton source, developed by USC:
J. Peñas *et al.*, HPLSE 12, e22 (2024)

- Target wheel on 3-axes stages
- 808 membranes per vacuum cycle
- At VEGA-3, up to 1 Hz shot rate
- Particle detectors: Thomson parabola (CLPU), time-of-flight (TOF, i3M).



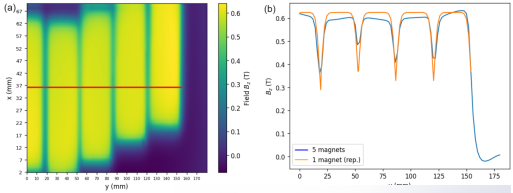


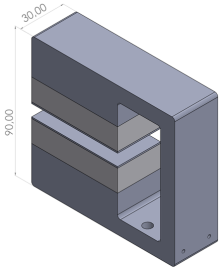


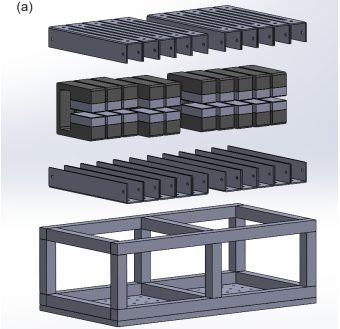
Energy selector: design scheme

Basic components:

- Up to 10 identical C-magnets
- NdFeB N40 blocks, $60 \times 30 \times 15$ mm^3
- Lateral position adjustable (rails)
- Mag. field $\simeq$ sum of single magnets.





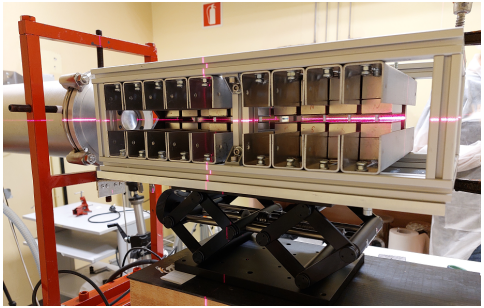


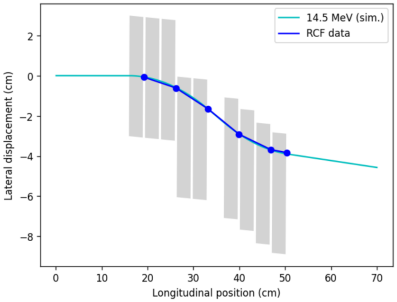
A. Reija, D. Esteban *et al.*, Instruments **8**, 36 (2024)

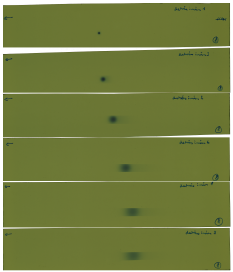
Test with mono-energetic proton beam

Measurement of proton trajectories through energy selector:

- CNA cyclotron, external beamline; $E_p = 14.5$ MeV at vacuum window
- 1 mm + 3 mm collimators, $\sim 0.7°$ half angle
- Place RCF behind each magnet (one by one)
- Hit coordinates coincide with simulations.



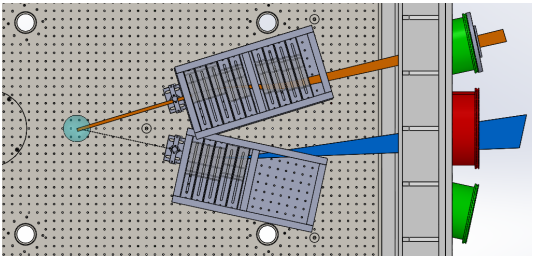


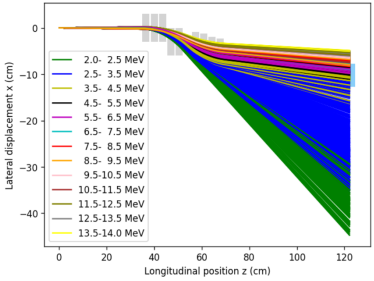


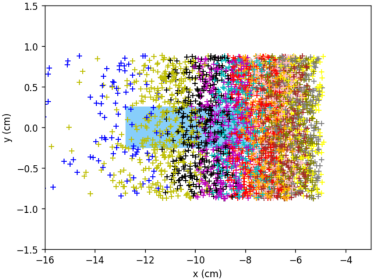
Simulated proton spectra

Simulate spectral separation with ray-tracing code (Python):

- Energies at target: 2-14 MeV
- Solid angle determined by slit collimator (5 mm)
- Hits on vacuum exit window (50 mm wide slit)
- Spectra at different horizontal positions.



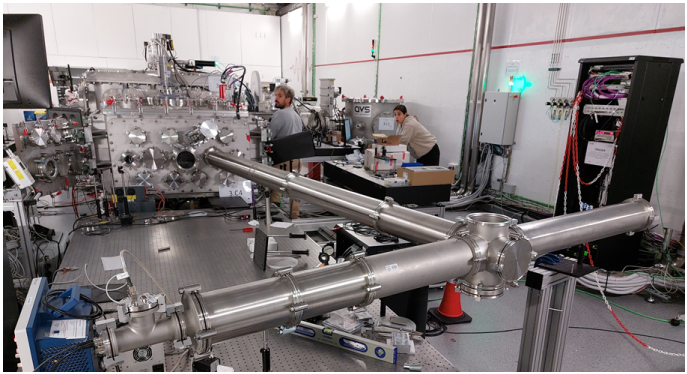


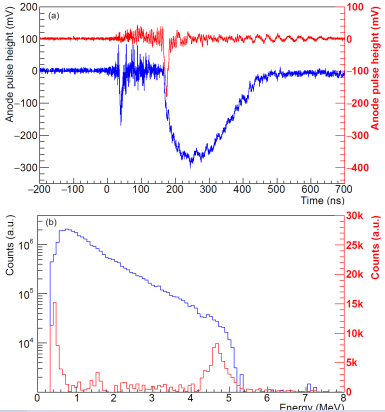


Measurement with time-of-flight detector

Energy selector implemented into VEGA-3; protons detected with TOF

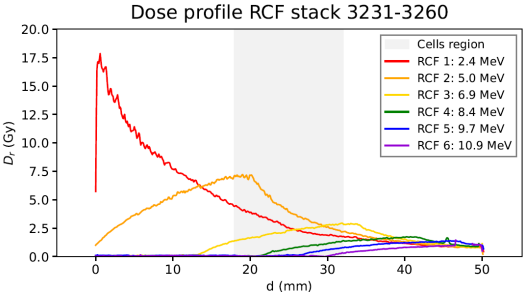
- Timing reference by γ peak and photodiode
- Without selector: broad spectra, exponential slope
- With selector: narrow peak at 4.8 MeV.

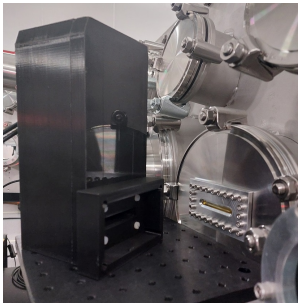


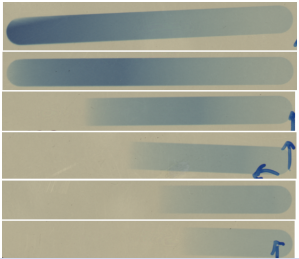


Proton beam in air:

- Kapton window, 50×5 mm^2, 12.7 µm thickness
- Horizontal dose profile with RCF
- RCF stack with Al foils for depth-dose profile.







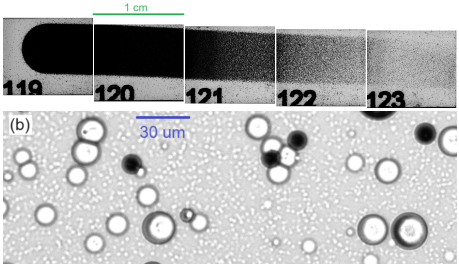
Deflection of ions

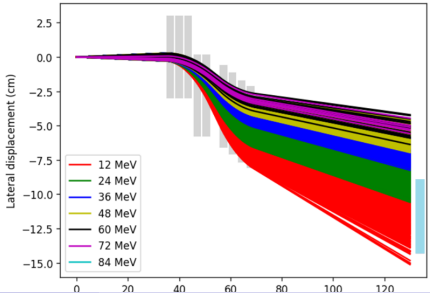
Simulations of C ion trajectories from laser-plasma interaction:

- 12 MeV min. energy to penetrate 12.7 µm kapton foil
- due to lower q/m lateral deflection is smaller than for p
- C^{4+} ions can reach $x_w \sim 5$ mm
- C^{6+} ions spread over entire window.

Observations on PADC plates (Tastrak) outside kapton window:

- High density of large tracks (ions) at low deflection
- Small tracks (protons) visible at large deflection.

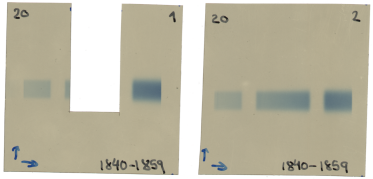




Dose measurement

Dose measurement with RCF EBT3-U:

- Daily check of dose distribution with RCF strip on kapton window
- Use U-shaped RCF in front of cell flask
- Single-shot dose ~ 0.1 Gy assumed
- Accumulate 30-80 shots per sample.



Real-time dose monitoring not reliable due to strong electromagnetic pulse.

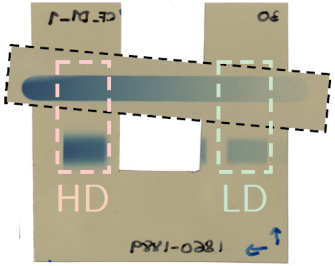
Dose measurement

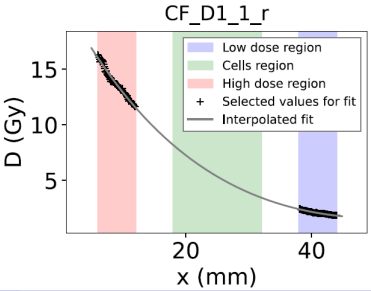
To obtain the dose on cells:

- Convert OD to dose with 6 MV linac calibration
- Apply quenching correction (14%) in analysis of proton data

  D. Sánchez-Parcerisa *et al.*, Med. Phys. Biol. 66, 115006 (2021)
- Get dose profile on both sides of irradiated area
- Interpolate to cells area
- Consider change of spectral distribution within 70 µm PE foil
- Calculate mean value over 15 mm width.

Real dose rate: 0.2-0.3 Gy/shot.





Cell culture irradiation with protons

Cell samples for CLPU prepared and analysed by Instituto de Biología Funcional y Genómica (IBFG, Salamanca). Irradiation procedure:

- 3 days at end of 4 weeks beamtime
- 808 shots per vacuum cycle; samples with 3/5/8 Gy envisaged
- Time per sample: 10s of laser shots at ≤ 0.5 Hz + lab access
- Max. 9 samples/day + control samples
- Colony formation, proliferation and protein extraction.

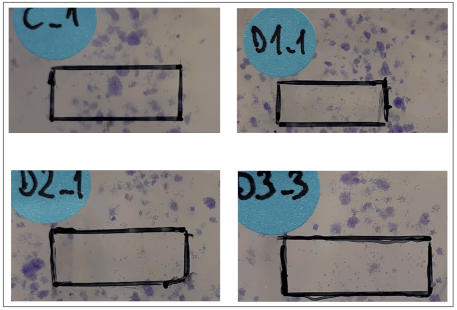
Observations during data analysis (ongoing):

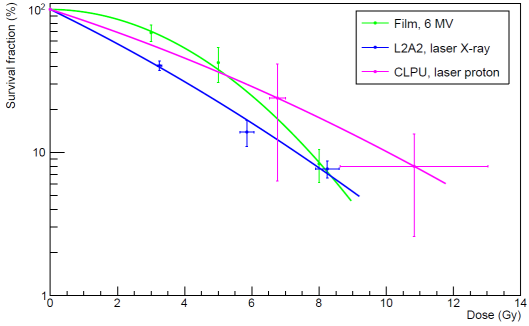
- Small irradiated area, difficult to handle
- Actual dose much higher than expected
- Dose variation within one sample $\sim 25\%$
- Dose variations among samples, lack of online monitor.

Cell culture irradiation with protons

Preliminary results:

- Colony formation (3 samples/dose): similar to X-rays from linac
- Final dose values: $D_1 = (6.8 \pm 0.2)$ Gy, $D_2 = (10.8 \pm 2.2)$ Gy, $D_3 = (16.5 \pm 5.0)$ Gy
- Very few colonies in irradiated area $\Rightarrow$ large uncertainty
- Proliferation (2 samples/day and dose): inconclusive assay, too many cells seeded.





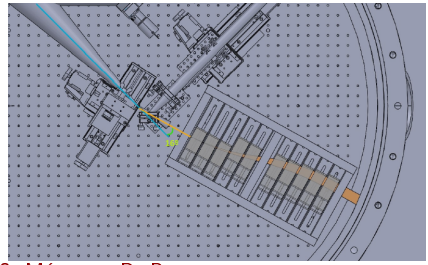
Next steps

With laser X-rays:

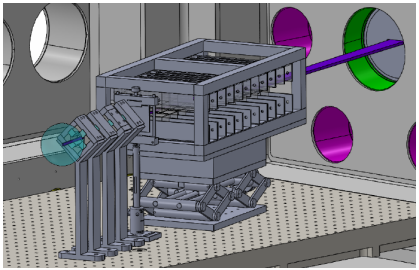
- Systematic comparison between cell types/radiation fields.

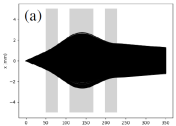
Ongoing improvements with protons:

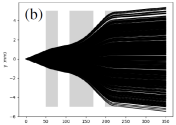
- Increase single shot dose and mean dose rate on cells:
  - Shorter flight paths (7×)
  - 10 Hz rep. rate (VEGA-2)
  - Focalization with PQMs (14×)
- Real-time monitoring
- Better match with biological endpoints
- Comp. with CNA cyclotron.



S. Márquez, D. Pastor







A.M. Salinas

Conclusions

1. Experiments with laser X-ray source (L2A2):
   - Laser: 1 mJ, 1 kHz. Dose rate: 100 mGy/s
   - Real-time dose monitoring with ionization chamber
   - High throughput: 18 samples/hour
   - Cell growth stronger inhibited as compared to 6 MV linac.
2. Laser-proton source at CLPU:
   - Stable source operation for 100s of laser shots
   - Monoenergetic proton pulses, 5 MeV
   - Single shot dose: 200-300 mGy
   - Reasonable trend for colony formation
   - Increase single shot dose/sample throughput (ongoing)
   - Need online monitor $\Rightarrow$ EMP suppression
   - Small area; check for suitable biological endpoints.

Close collaboration between Physics (i3M, IGFAE, CLPU) and Biology labs (IDIS, IBFG).

Acknowledgements

Proyecto CIAICO/2022/008 financiado por Generalitat Valenciana en el programa Grupos de Investigación Consolidados.

Co-financed by the European Union through Programa Operativo del Fondo Europeo de Desarrollo Regional (FEDER) of the Comunitat Valenciana 2014-2020, IDIFEDER/2021/004.

The Spanish Center of Pulsed Lasers (CLPU) and the members of the Consortium consider experiment 00562-0101 as a strategic proposal that develops new potentialities for CLPU and, therefore, finance the access to its facilities, as well as to acknowledge the results obtained with its petawatt laser system (VEGA-3) and its scientific and technical assistance.

Thank you for your attention!