RADIOBIOLOGICAL EVALUATION OF LASER-PLASMA ACCELERATED VERY HIGH-ENERGY ELECTRONS (VHEEs) AT ULTRA-HIGH INSTANTANEOUS DOSE RATES IN HUMAN LEUKOCYTES

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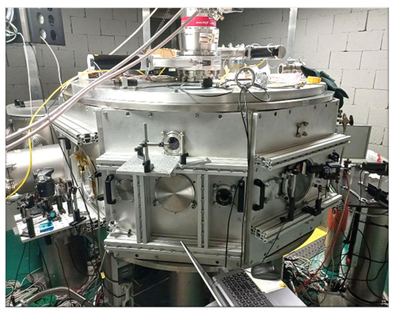
Very high-energy electrons (VHEEs, 50–250 MeV) are emerging as a promising modality for the treatment of deep-seated tumors due to their favorable penetration depth, dose conformity, and reduced sensitivity to tissue heterogeneities. Their potential application in FLASH radiotherapy further increases interest, as ultra-high dose rates may enhance normal tissue sparing while maintaining tumor control.
Laser Wakefield Acceleration (LWFA) offers a compact approach for VHEE generation, producing ultrashort electron pulses (tens of femtoseconds) with instantaneous dose rates far exceeding those achievable with conventional radiofrequency accelerators. However, the biological effects of such irradiation conditions remain poorly understood.

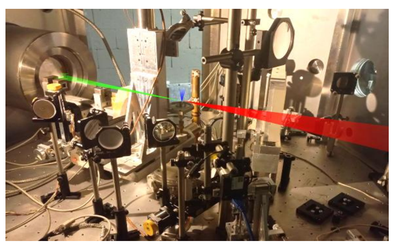
In this study, the LWFA-driven VHEE source available at the Intense Laser Irradiation Laboratory (ILIL) at INO-CNR in Pisa was comprehensively characterized through measurements of beam energy spectrum, charge per shot, and spatial dose distribution using radiochromic films and spectrometric diagnostics. Human blood leukocytes were irradiated with doses ranging from 0 to 4 Gy at instantaneous dose rates exceeding 10^{12} Gy/s. Radiobiological responses were assessed by evaluating both direct and bystander effects through micronucleus formation, telomere length, and mitochondrial DNA copy number.
VHEE irradiation induced measurable biological responses, including increased chromosomal damage and telomere shortening. However, compared with conventional X-rays at equivalent doses, VHEEs produced less DNA damage, an effect that may be attributed to the ultra-high instantaneous dose rate per pulse, which could trigger “FLASH-like” mechanisms influencing radical chemistry and DNA damage processing.
These results provide further evidence supporting the unique radiobiological properties of laser-driven VHEEs and contribute to their preclinical validation for future radiotherapy applications. While additional studies are needed to clarify the underlying mechanisms and support clinical translation, laser-plasma accelerators represent a promising route toward next-generation high-dose-rate precision radiotherapy.

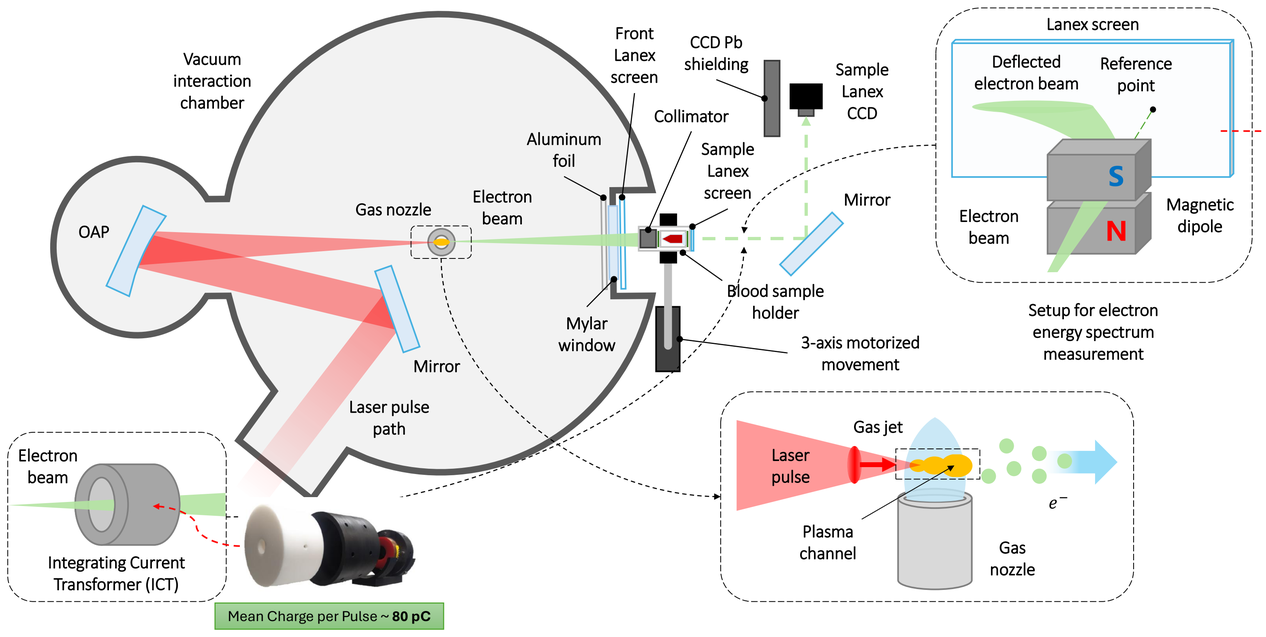
VHEE BEAM CHARACTERIZATION

LWFA experiment set-up: Beam Characterization Activities for Radiobiological Applications

Pulse duration <30 fs; ~ 3 J and 10^{18} -10^{19} W/cm^2 in the focal spot

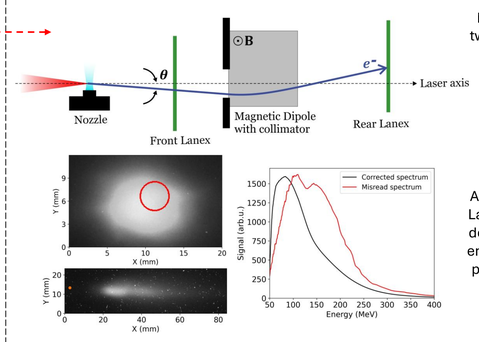






Setup for electron energy spectrum measurement

Energy spectrum reconstruction with pointing and scattering corrections



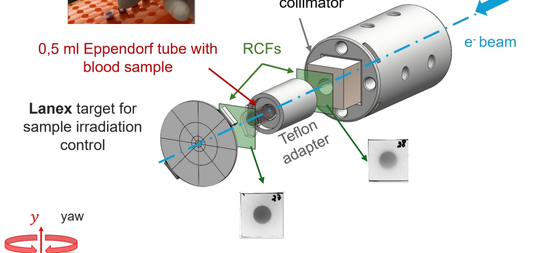
Magnetic spectrometer with two scintillating screens and a 1.06 T dipole

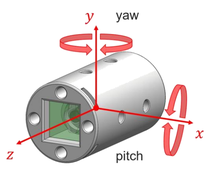
Accelerated electron beam on Lanex screens before and after deflection, with corresponding energy spectra before and after pointing/scattering correction

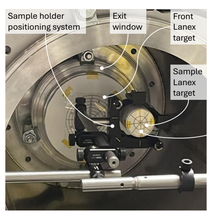
Medium Epeak~ **70-100 MeV** Emax~ **250-300 MeV**

Vlachos, S. G. et al. (2026).. **Nuclear Instruments and Methods in Physics Research Section A,** https://doi.org/10.1016/j.nima.2025.171038

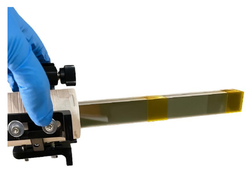
Sample alignment, Irradiation control and monitoring



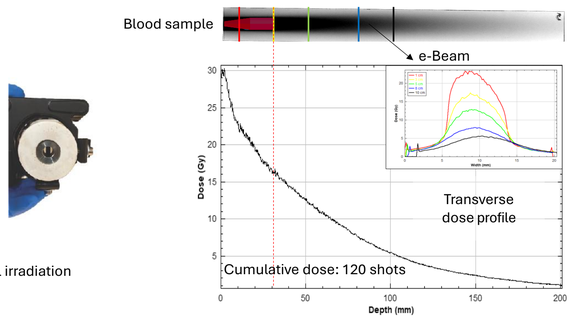




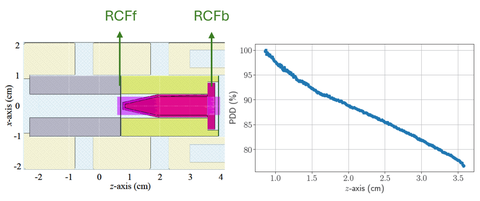
Beam dosimetry and Sample irradiation position in the beamline



2 cm x 20 cm EBT3 stack for longitudinal irradiation



Reconstruction of the dose delivered to the biological sample and Monte Carlo validation



0.3–4 Gy were delivered by multiple laser shots. Sample dose was estimated from the average dose measured with upstream and downstream Gafchromic™ EBT3 films and validated by Monte Carlo (Fluka-Flair) simulations.

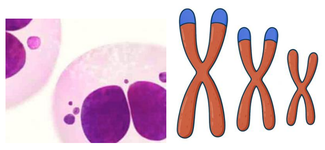
Handling, usage and Calibration Protocol for Radiochromic Films

CASA DI CURA PRIVATA SAN ROSSORE

DNA DAMAGE INDUCTION BY UHDR-VHEE BUNCHES

IFC - Istituto di Fisiologia Clinica Consiglio Nazionale delle Ricerche

Radiobiological Endpoints

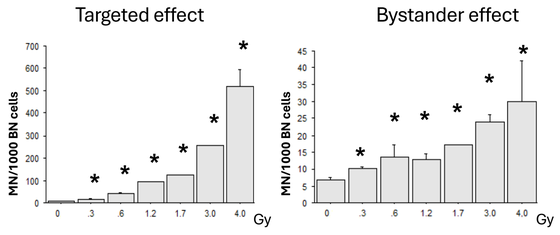


*The **micronucleus (MN)** assay in **normal human lymphocytes** is a validated tool to assess radiation-induced chromosomal damage [Fenech M., Mutat Res. 2000].*
***Telomere length (TL)** has also recently been proposed as an additional biomarker of radiation exposure [Sgura A., Radiat Prot Dosimetry 2006].*

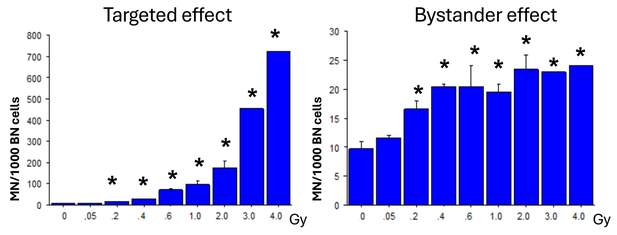
100 MeV VHEE vs 100 kVp X-rays

*** All p<0.05 vs. control value**

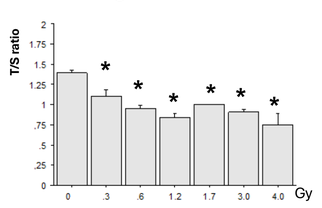
VHEE (10^{12} Gy/s per pulse)



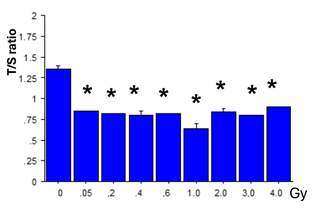
X rays (0.1 Gy/min)

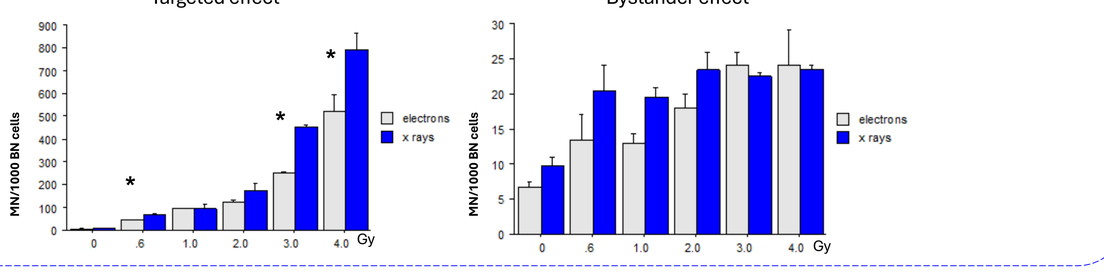


VHEE (10^{12} Gy/s per pulse) - TL



X rays (0.1 Gy/min) - TL





Radiobiological response

- An increase in MN frequency with increasing dose was observed for both targeted and bystander effects.
- Telomere shortening was observed at each dose following irradiation with VHEEs and X-rays compared to baseline values. At each dose, a reduction in telomere shortening was observed after VHEE compared to X-ray irradiation. This reached a statistical significance at 0.3 Gy, 1 Gy, 2 Gy, and 3 Gy ($p < 0.05$).

Instantaneous UHDR VHEE pulses vs CONV X-rays

Decreased chromosomal damage, as indicated by lower micronucleus yields, following VHEE irradiation compared to X-ray beams for both targeted and bystander effects. Possible underlying mechanisms may be associated with the instantaneous UHDR as recently observed. *[Zhu et al. Mol. Med. 31, 79 (2025); McAnespie, Phys. Med. Biol. 2025; H. Kacem, Radiot. Oncol. 2025; C.M. Lazzarini et al. 2026].*

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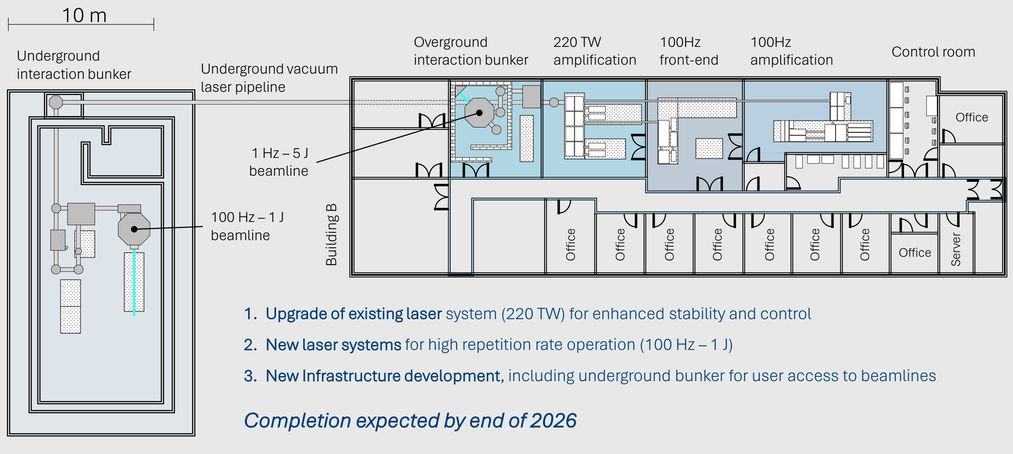
VHEE ILIL FACILITY UPGRADE

100 Hz Beamline at ILIL



1. Upgrade of existing laser system (220 TW) for enhanced stability and control
2. New laser systems for high repetition rate operation (100 Hz – 1 J)
3. New Infrastructure development, including underground bunker for user access to beamlines

Completion expected by end of 2026