

STUDIES ON THE IMPACT OF THE BEAM TIME STRUCTURE ON VHEE/FLASH IRRADIATION BIOLOGICAL EFFECTS

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Abstract

The CERN Linear Electron Accelerator for Research (CLEAR) is a versatile user facility delivering electron beams for multidisciplinary research. Recently, CLEAR has been instrumental in advancing studies on the feasibility of employing Very High Energy Electrons (VHEE, 100–200 MeV) at Ultra-High Dose Rates (UHDR, $> 10^8$ Gy/s) for cancer radiotherapy. These beam conditions enable the exploration of the FLASH effect, a biological phenomenon where normal tissue toxicity is reduced while maintaining tumor control when the entire therapeutic dose is delivered within milliseconds. Using the CLEAR beam's adjustable microstructure—based on picosecond-scale electron bunches—recent experiments have investigated the influence of short time scale temporal beam parameters on the physico-chemical and biological responses to irradiation.

In collaboration with radiobiology laboratories, in particular from the Hôpitaux Universitaires de Genève (HUG), systematic studies using aqueous systems, plasmid DNA, and zebrafish embryo (ZFE) models have shown that beam intensity and bunch structure may critically affect biological outcomes, with evidence of preserved ZFE morphogenesis at ultra-high instantaneous dose rates ($\approx 10^{11}$ Gy/s), even when the total dose is delivered over longer time scales, up to a few minutes.

These findings establish the CLEAR facility as a unique platform for defining the beam characteristics required to trigger the FLASH effect and guide the development of future clinical VHEE-based FLASH radiotherapy systems. Some of these recent radiobiological experiments performed at CLEAR are summarized in this paper.

INTRODUCTION

The CERN Linear Electron Accelerator for Research (CLEAR) is a 200 MeV electron linac followed by an experimental beam line, operated at CERN as a user facility [1,2]. CLEAR serves a wide and diverse scientific community covering a broad array of experimental applications, including R&D on beam instrumentation, high-gradient acceleration and components for existing and future accelerators and tests

of novel concepts as the use of plasma or THz radiation to manipulate and accelerate electrons.

In this context, the investigation of medical applications of electron beams with energies above 100 MeV has become over time one of its main activities, covering in the last few years more than 30 % of the user beam time.

Radiotherapy at Ultra-High Dose Rate (UHDR), in which the total dose is delivered in a fraction of a second, might offer a new modality for cancer treatment. The so-called FLASH biological effect that takes place in such regime, observed in several experiments [3–5], significantly increases the differential response between healthy and tumour tissue broadening the therapeutic window.

Very-high-energy electron (VHEE) beams with energies above 100 MeV are promising candidates for FLASH Radiotherapy due to their favorable dose distributions and accessibility of ultra-high dose rates. Linear electron accelerators can indeed easily provide the high intensity beams needed for UHDR, and have the additional advantage, thanks to recent advances in high-gradient technology, of being rather compact and relatively cheap. The CLEAR user facility covers a large range of electron beam parameters, as detailed in Table 1, offering therefore great flexibility for experiments [6–10]. In particular, CLEAR beam energy range (20–220 MeV) and high charge per pulse (up to 80 nC) have made it so far the only facility widely available to users for complete VHEE/UHDR studies.

Table 1: CLEAR Beam Parameters, 2026

Parameter	Value
Beam Energy	20 – 220 MeV
Beam Energy Spread	< 0.2 % rms (< 1 MeV FWHM)
Bunch length RMS	0.1 – 10 ps
Bunch frequency	1.5 or 3.0 GHz
Bunch charge	0.005 – 1.6 nC
Norm. emittance	1 – 20 μ m
Bunches per pulse	1 – 150
Max. pulse charge	80 nC
Repetition rate	0.8333 – 10 Hz

Early experiments with zebrafish embryos in collaboration with the Hôpitaux Universitaires de Genève (HUG) have

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demonstrated for the first time the FLASH sparing effect with a VHEE beam and have revealed that the therapeutic FLASH effect might depend on the instantaneous dose rate rather than the average dose rate [11]. Findings suggest that the timing of radiation delivery, particularly at the picosecond and nanosecond time scales, has a large impact in preserving biological integrity of the samples. The importance and role of these time scales can provide precious hints in understanding the chemical and biological mechanisms underlying the FLASH effect. Such a discovery could also largely simplify future medical technologies.

EXPERIMENTAL SETUP

The CLEAR team has developed methods and data analysis techniques adapted to VHEE/FLASH studies, including detailed procedures for sample handling, beam delivery and passive dosimetry techniques. It also explored different new methods for real-time dosimetry in UHDR conditions, and recently put in routine operation a fast dosimetry system based on beam charge measurement coupled with imaging from a YAG scintillations screen.

For these studies, sample irradiations are performed in the In-Air Test Area of CLEAR, using a dedicated set-up, developed to support high-throughput radiobiology and chemistry experiments. The set-up enables automated handling by a robotic system (the so-called C-Robot [12]) and irradiation of a large number of samples while minimizing the need for manual intervention. A remote camera image of the whole system is shown in Fig. 1. The C-Robot consists of a three-axis linear motion system equipped with a custom-designed grabber, allowing precise positioning of samples within the irradiation field with an accuracy of approximately 50 μm . It operates in conjunction with two water tanks: a storage tank and an irradiation tank (water phantom), both temperature-controlled at $(28 \pm 0.1)^\circ\text{C}$ using dedicated heating and circulation systems. The robot is capable of handling up to 32 individual 3D-printed sample holders, each designed to accommodate PCR tubes containing the biological or chemical samples. The modular design of the holders allows adaptation to specific experimental requirements. In order to provide a transversely uniform irradiation over a round field with a diameter up to 1 cm, a dual-scattering system — consisting of a pre-scatterer and a scatterer [13] is installed in the beam line, and a movable round collimator is placed at the beam line exit before the set-up.

Two beam charge monitors (Integrated Current Transformers - ICTs) are placed there, before and after the collimator, to measure the incoming and delivered charge. Accurate dosimetry under both conventional and ultra-high dose rate (UHDR) conditions is a key challenge in VHEE and FLASH studies. To address this, a combination of passive and active dosimetry systems is employed. Fast beam monitoring and dose estimation are obtained by using two YAG scintillating screens: a 50 μm thick screen installed in air upstream of the irradiation tank (in-air YAG), and a 200 μm thick screen that can be positioned in water at the sample location (in-

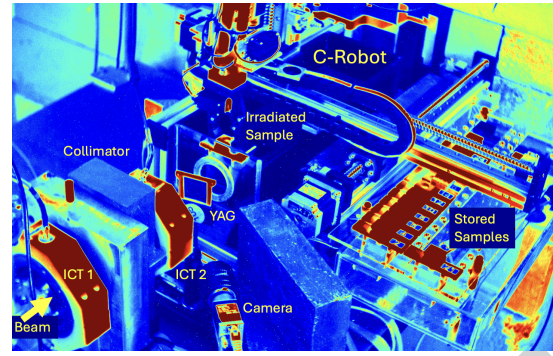


Figure 1: Camera image of the CLEAR irradiation setup.

water YAG) using the robotic system. The in-air YAG screen provides online monitoring during irradiation, while the in-water screen enables fast spatially resolved measurements at the irradiation point. Local charge density can thus be calculated at both locations using charge measurement from the ICTs. A dedicated analysis framework is used to correlate the measured charge density with the delivered dose [14]. Active dosimetry is used during irradiation to target and assess delivered doses, while passive measurements are used for precise post-irradiation dose evaluation [15]. For this EBT-XD radiochromic films are employed, due to their high spatial resolution and dose-rate independent response. For each sample holder, two films are placed—one upstream and one downstream of the sample—to measure the absolute delivered dose and assess dose uniformity. Following irradiation, the films are scanned and analysed after a 24-hour stabilization period, using calibration curves obtained from conventional clinical linear accelerators at the Centre Hospitalier Universitaire Vaudois (CHUV) and HUG. This combined dosimetric approach ensures reliable dose determination across the wide range of dose rates explored.

IRRADIATION RESULTS

Systematic studies are ongoing in CLEAR studying aqueous systems, plasmid DNA, and zebrafish embryo (ZFE) models in collaboration with HUG. In the following we will concentrate on the results obtained using ZFE. Wild-type Zebrafish are bred to produce embryos at CHUV in Lausanne or at HUG. Embryos are typically irradiated at 4 hours post-fertilization in water at 28°C using the set-up previously described. The irradiation of all the samples of one batch are normally performed in less than 30 minutes to ensure the ZFE are all in the same developmental stage. Radiation-induced effects are quantified by measuring embryo length at 5 days post fertilization after fixation. The relative length deficit is calculated by comparing irradiated embryos to non-irradiated controls, as follows:

$$LengthDeficit = \left(1 - \frac{l_{RT}}{\langle l_{CTRL} \rangle}\right) * 100$$

where l_{RT} is the length of the irradiated embryo while $\langle l_{CTRL} \rangle$ is the average length of the non-irradiated control embryos.

Total doses between 5 and 12 Gy and two distinct dose rate ranges were used for this study: 0.125 - 0.2 Gy/s (Conventional Dose Rate - CDR) and 10^8 - 10^{11} Gy/s (UHDR). For CDR irradiation the target dose was accumulated using several pulses of a single 3.0 ps long bunch with a repetition rate of 10 Hz. This corresponded to a dose of 20 mGy per bunch sent every 0.1 s. The UHDR irradiations were performed with a single pulse of 6 to 40 bunches, with a bunch separation of 666 ps, corresponding to a dose of 333 mGy per bunch, in a single train with bunch separation of 666 ps. The main result of the initial experiments is shown in Fig. 2. A clear difference in the length deficit is observed between the ZFE irradiations undertaken at CDR and at UHDR (left). The ZFE irradiated at UHDR are on average around 20 % longer than the ZFE irradiated at CDR, showing for the first time a dose-rate dependent sparing of ZFE development with VHEE irradiations. However, when the total dose was delivered in CDR mode (accumulating bunches at 10 Hz with an average dose rate of 0.125 Gy/s) but using a high dose in the bunch of 150 mGy (right), no differential effect w.r.t. the UHDR mode was observed, leading to the hypothesis that the instantaneous dose rate, i.e. the dose delivered with each bunch, might play a relevant role in the sparing effect [11].

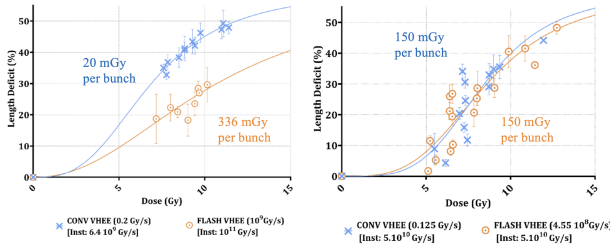


Figure 2: Length Deficit of ZFE irradiated at CLEAR with a VHEE beam, in CDR (CONV) and UHDR (FLASH) conditions, from [11]. Left - different dose/bunch, right - same dose/bunch.

In order to confirm this, other irradiation tests were carried out [16]. In one case, “standard” CDR and UHDR irradiation modes with similar dose rates as the ones of Fig. 2 (left) were compared with “long” irradiations in single bunch mode with high dose per bunch. Results are summarised in Fig. 3. Sparing is observed for both the “standard” UHDR mode and long irradiation times (up to 2 minutes) with high instantaneous dose rates (240 mGy/bunch, delivered in 3 ps). In a second experiment, irradiation was carried out comparing “standard” CDR to fast irradiation using a single bunch train, changing the dose per bunch and thus the instantaneous dose rate (Fig. 4). For low dose per bunch no sparing effect is observed even for very fast irradiation, while sparing is gradually recovered increasing the instantaneous dose rate, up to saturation beyond ≈ 150 mGy per bunch.

Both experiments show the importance of the beam time structure in triggering the FLASH effect, with the main parameters appearing to be the dose per bunch, and therefore the instantaneous dose rate.

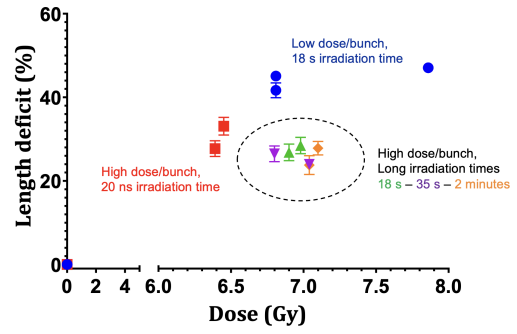


Figure 3: Length Deficit of ZFE irradiated at CLEAR with VHEE in different conditions. Blue dots correspond to “standard” CDR with 40 mGy/bunch, red squares to “standard” UHDR (240 mGy/bunch). Other data were taken with high dose per bunch (240 mGy/bunch), but using single bunches as in standard CDR, and long irradiation times.

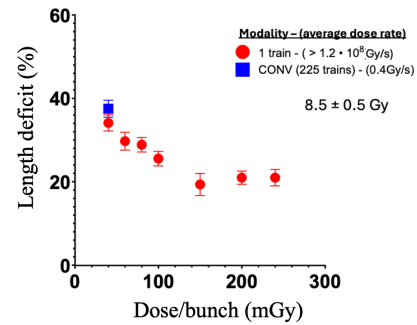


Figure 4: Length Deficit of ZFE irradiated at CLEAR with VHEE in different conditions. Blue square is “standard” CDR with 40 mGy/bunch, red dots correspond to single trains with varying dose per bunch.

CONCLUSIONS

The studies reported here provide the first direct evidence of the FLASH sparing effect with VHEE beams and highlight the key role of high instantaneous dose rate in triggering it. Investigating the dependence on beam time structure enables identification of the relevant time scales of the underlying chemical and biological processes, offering important insight into the mechanism.

Furthermore, the observation that high instantaneous dose rates preserve the FLASH effect even for irradiation times of the order of minutes could significantly relax the requirements for dedicated FLASH RT accelerators, while enabling fully conformal treatments with sufficient time to vary the irradiation angle.

The reported experiments strengthen the position of VHEE beams as strong candidates for the clinical implementation of FLASH radiotherapy, combining precision, biological selectivity, and technological compatibility for future cancer treatment advancements. Further studies are however needed in order to extend and confirm these results, in particular using different biological models, including mammalian models available in other facilities than CLEAR.

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