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Volumetric FLASH dosimetry *in vivo* using real-time radiacoustic imaging

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Supplementary material for this article is available [online](#)

Abstract

Objective. The main goal of this research is to verify the spatial fidelity of radiacoustic imaging (RAI) as a quantitative dosimetric monitoring tool for FLASH radiotherapy (FLASH-RT) *in vivo*. FLASH-RT delivers therapeutic radiation at ultra-high instantaneous dose rates ($>10^6$ Gy s⁻¹), offering substantial reductions in normal tissue toxicity while maintaining tumor control. However, clinical translation remains limited by the absence of real-time, *in vivo* dosimetry systems capable of resolving dose delivery at microsecond timescales. **Approach.** Here, we present an RAI platform that enables volumetric, single-pulse mapping of radiation dose deposition during FLASH-RT *in vivo*. The system utilizes a 16×16 ultrasound transducer matrix array with a model-based reconstruction algorithm to generate quantitative, three-dimensional dose maps with single pulse-level temporal resolution. **Main results.** In both water phantoms and *in vivo* murine models, RAI demonstrates high concordance with film dosimetry and TOPAS Monte Carlo simulations (3%/3 mm gamma index pass rates greater than 90% for small fields). **Significance.** This work establishes RAI as a viable technology for real-time, quantitative electron FLASH dosimetry *in vivo* for the first time, with the potential to support adaptive delivery, improve treatment safety, and facilitate the clinical translation of FLASH-RT.

1. Introduction

FLASH radiotherapy (FLASH-RT) is redefining the implementation of radiation oncology by delivering therapeutic doses at ultra-high dose rates, typically exceeding 40 Gy s⁻¹ average dose rates (Esplen *et al* 2020, Soto *et al* 2020, Chaoui *et al* 2025, Yang *et al* 2025). This novel mode of delivery has demonstrated a remarkable ability to reduce normal tissue toxicity while maintaining tumor control across a wide range of preclinical studies (Soto *et al* 2020, Drayson *et al* 2024). The so-called ‘FLASH effect’ has been validated in multiple animal models (including mice (Levy *et al* 2020, Montay-Gruel *et al* 2021, Valdés Zayas *et al* 2023, Drayson *et al* 2024), mini-pigs (Vozenin *et al* 2019, Rohrer Bley *et al* 2022), cats (Vozenin *et al* 2019, Rohrer Bley *et al* 2022), and zebrafish (Beyreuther *et al* 2019, Karsch *et al* 2022)) with observed preservation of gastrointestinal integrity (Levy *et al* 2020, Valdés Zayas *et al* 2023), enhanced crypt regeneration (Levy *et al* 2020), reduced neurocognitive decline (Montay-Gruel *et al* 2021, Drayson *et al* 2024, Jansen *et al* 2025), skin sparing (Soto *et al* 2020), and diminished long-term fibrosis (Vozenin *et al* 2019, Moustafa *et al* 2024, Li *et al* 2025) relative to its conventional dose rate counterparts.

The first human treatment with FLASH-RT, administered to a patient with disseminated CD30+ T-cell cutaneous lymphoma in 2018 at Lausanne University Hospital, demonstrated promising lesion clearance and reduced skin toxicity compared to the patient's previous conventional RT treatments (Bourhis *et al* 2019) though a following study comparing FLASH delivery in two different lesions did not find a difference in toxicity between FLASH and CONV-RT (Gaide *et al* 2022). Further study has been done since to understand which parameters (i.e. dose rate, instantaneous dose rate, number of pulses, number of fractions, etc) are the most important in order to generate the differential FLASH effect. Currently, multiple clinical trials are underway with electron (IMPulse, Flash-Skin I, and LANCE) and proton beams (FAST-01 and FAST-02). FAST-01 findings in their completed first phase suggest clinical feasibility and tumor control comparable to conventional radiotherapy (Daugherty *et al* 2022, Mascia *et al* 2023), allowing for the continuation of study in FAST-02 (Daugherty *et al* 2024). These findings have fueled increased clinical interest in translating FLASH-RT into more widespread use, particularly for radiosensitive and previously irradiated tissues where conventional radiotherapy is often limited by toxicity to normal tissues (Levy *et al* 2020).

However, the clinical implementation of FLASH-RT remains limited by critical technological gaps; one of these is the lack of real-time, *in vivo* dosimetry capable of operating under ultra-high dose-rate conditions (Romano *et al* 2022). Unlike conventional radiotherapy, which delivers dose gradually across hundreds of pulses, electron based FLASH-RT typically delivers the entire therapeutic dose in just a few high-intensity pulses with instantaneous dose rates approximately three orders of magnitude greater than that of conventional delivery (figure 1(a)) (Böhlen *et al* 2022, Subiel and Romano 2023). This condensed delivery leaves little margin for error—each pulse must be spatially and temporally accurate to ensure tumor coverage and avoid overdosing of normal tissue (Dawson *et al* 1998, Meyers *et al* 2025). This challenge is exacerbated by FLASH-RT in clinical scenarios involving respiratory motion or anatomical variability, such as in abdominal or thoracic treatments (Lattanzi *et al* 2000, Wu *et al* 2001, Cai *et al* 2008, Velec *et al* 2012, Yoganathan *et al* 2017).

Existing clinical dosimeters, including ionization chambers (McManus *et al* 2020, Liu *et al* 2024a), diodes (Fontenla *et al* 1996, Jursinic 2013), scintillators (Archer *et al* 2019, Jeong *et al* 2020, Shaharuddin *et al* 2021, Morrocchi *et al* 2022, Vanreusel *et al* 2022, Liu *et al* 2024c), Cherenkov detectors (Ashraf *et al* 2020, Rahman *et al* 2021), and radiochromic films (Villoing *et al* 2022, Liu *et al* 2023a), face varying limitations under FLASH conditions (Pimpinella *et al* 2019, Di Martino *et al* 2020, Subiel and Romano 2023) summarized in table 1. They have been shown to saturate at high dose rates, lack the temporal resolution to resolve micro-scale delivery, or require labor-intensive post-processing that precludes real-time feedback. Furthermore, few of these systems support 3D dosimetry or are suitable for *in vivo* monitoring of dose delivered to the tumor region and not only the surface dose, leaving clinicians without the robust tools needed for verification, quality assurance, or adaptive responses during FLASH treatment *in vivo*.

RAI has recently emerged as a powerful candidate for overcoming these challenges (Oraiqat *et al* 2020, Bjegovic *et al* 2024, Wang *et al* 2024a). RAI is based on the generation of acoustic pressure waves induced by rapid thermoelastic expansion in tissue following radiation absorption (2012, Xiang *et al* 2016), a mechanism similar to the photoacoustic effect (Wang *et al* 2003, Wang and Hu 2012, Xia *et al* 2014, Manohar and Razansky 2016, Li *et al* 2017, Attia *et al* 2019, Lin *et al* 2021, Cao *et al* 2023). The resulting pressure waves can be captured by ultrasound detectors and used to infer dose deposition in real time (Zhang *et al* 2020). Early studies demonstrated that radiacoustic signals scale linearly with absorbed dose and can be detected from both conventional and FLASH electron beams (Oraiqat *et al* 2020, Bjegovic *et al* 2024). Developments extended RAI from 1D (2016, Hickling *et al* 2018) to 2D mapping using linear arrays (Lei *et al* 2018), including single-pulse measurements in *ex vivo* tissues (Bjegovic *et al* 2024), but volumetric imaging and quantitative dose reconstruction *in vivo* remained an unmet challenge.

To address this, we utilized a previously developed prototype matrix-array-based RAI system capable of 3D volumetric imaging (Wang *et al* 2024b). However, the initial system relied on universal back-projection (UBP) algorithms, which are susceptible to artifacts from limited-angle acquisition, measurement noise, finite transducer bandwidth, and geometric simplifications that limit quantitative accuracy. As a result, reconstructed images suffered from non-physical negative pixel values, factors that undermine accurate and quantitative 3D dose mapping in radiotherapy. In this present work, we introduce a next-generation RAI platform that combines our custom-designed 256-element matrix ultrasound transducer array with a model-based (MB) reconstruction framework (Pandey *et al* 2021c) *in vivo* (figure 1(b)). This approach takes into consideration the spatial characteristics of the transducer array, empirically derived calibration data, and the known acoustic properties of the medium to reconstruct quantitative 3D dose maps at FLASH dose rates. In utilizing empirically derived calibration data, this

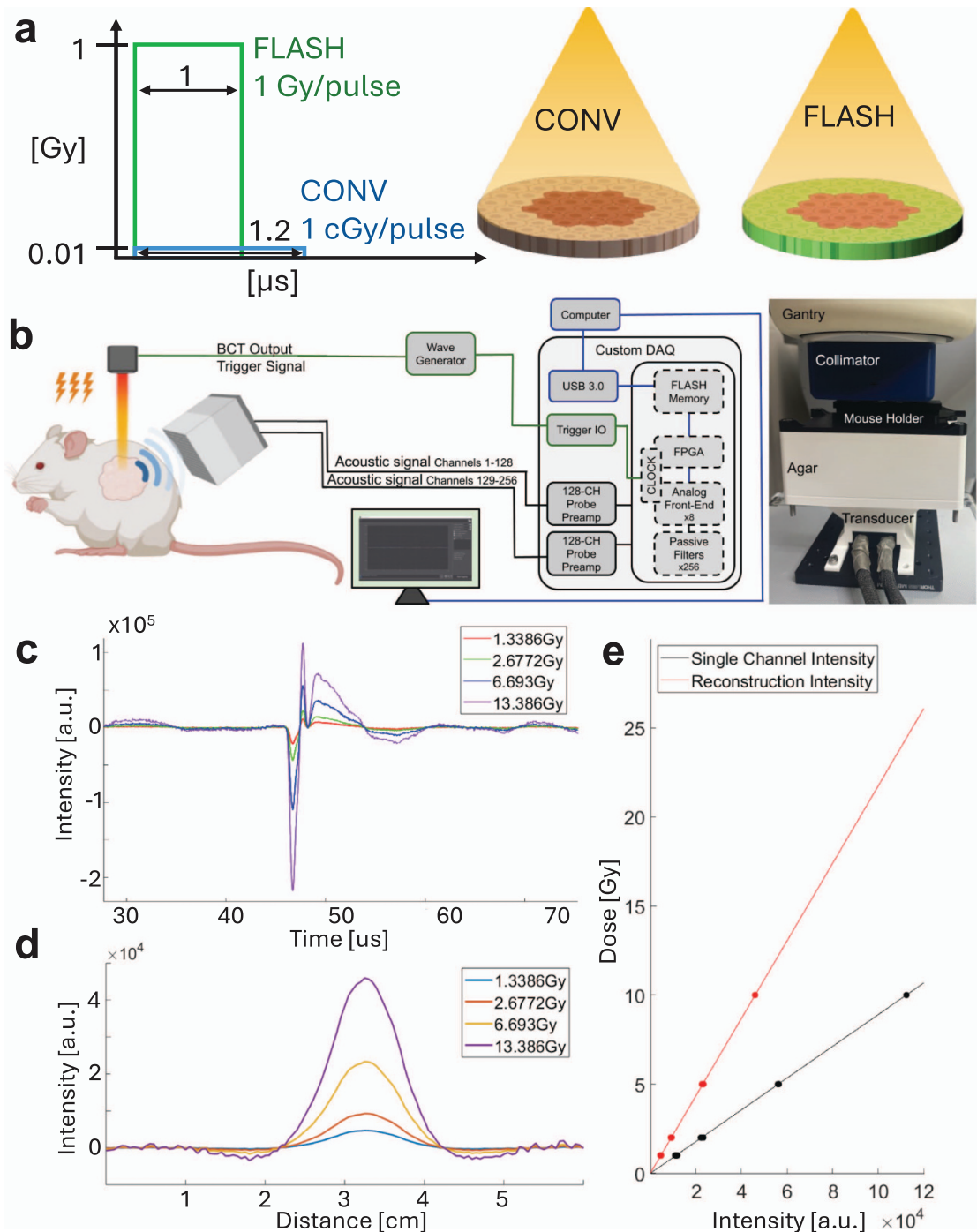


Figure 1. Radiacoustic imaging for *in vivo* dosimetry of FLASH electron therapy. (a) In FLASH radiotherapy, each radiation pulse can deliver on the order of Gray, leading to instantaneous dose rates exceeding 10^6 Gy s^{-1} , as shown with our specific beam delivery parameters, posing significant challenges for real-time dosimetry. FLASH dose delivery allows for maintained tumor kill with relative sparing of normal cells in the margins of beam delivery. (b) Schematic and image of the RAI system used for real-time, volumetric, *in vivo* dosimetry. A 2D matrix array captures radiacoustic signals generated in three dimensions. (c) Representative RA signals recorded by channel 120, a transducer located at the center of the matrix array, demonstrate increases in signal amplitude with escalating total dose: single pulses ($\sim 1.3 \text{ Gy}$ total), two pulse ($\sim 2.6 \text{ Gy}$ total), five pulse ($\sim 6.6 \text{ Gy}$ total), and ten pulse aggregates ($\sim 13.3 \text{ Gy}$). (d) Lateral profiles across the XY plane of representative RA reconstructions demonstrate increases in reconstruction intensity for the 1 cm diameter circle beam with escalating dose. (e) Maximum RA signal amplitude (c) and reconstruction intensities (d) exhibit linear correlations with total delivered dose ($R^2 = 0.99$). Calibration fits (equations (11) and 12 are provided in Methods.

method is not absolute dose but quantified dose, allowing for dosimetry with initial calibration to reference doses.

We validate the system using FLASH electron beams delivered in homogenous water phantoms and *in vivo*, in live murine models. We demonstrate accurate single-pulse volumetric dose mapping across a range of field size and collimator geometries, with high concordance to radiochromic film measurements

Table 1. Comparison of dosimeters currently being used or researched for beam monitoring in clinic. *Applying correction factors to saturated non-FLASH specific ion chamber readings can correct for some recombinant effects, with varying degrees of accuracy (Petersson *et al* 2017, Pimpinella *et al* 2019). Two novel ionization chambers that can handle FLASH dose rates with minimal saturation are now available (Gómez *et al* 2022, Liu *et al* 2024b).

DOSIMETER	IN VIVO	REAL TIME	1D, 2D, OR 3D	FLASH
FILM	No	No	2D	Yes(Villoing <i>et al</i> 2022)
ION CHAMBER	No	Yes	1D–2D	Chamber dependent *(Gómez <i>et al</i> 2022, McManus <i>et al</i> 2020, Di Martino <i>et al</i> 2020, Liu <i>et al</i> 2024a, Pimpinella <i>et al</i> 2019, Liu <i>et al</i> 2024b)
BEAM CURRENT TRANSFORMER (BCT)	No	Yes	1D	Yes, charged particle beams only (Liu <i>et al</i> 2023b, Oesterle <i>et al</i> 2021, Gonçalves Jorge <i>et al</i> 2022)
DIODE	No	Yes	1D	Material dependent(Fontenla <i>et al</i> 1996, Di Martino <i>et al</i> 2020, Pimpinella <i>et al</i> 2019, Jursinic 2013)
SCINTILLATOR	No	Yes	1D–2D (Jeong <i>et al</i> 2020)	Material dependent(Vanreusel <i>et al</i> 2022, Liu <i>et al</i> 2024c, Shaharuddin <i>et al</i> 2021, Archer <i>et al</i> 2019, Morrocchi <i>et al</i> 2022)
ELECTRONIC PORTAL IMAGING DEVICE (EPID)	Yes, only for photon beams(Dragun <i>et al</i> 2013)	Yes	2D	TBD
CHERENKOV-BASED	Yes, shallow depths possible	Yes	2D	Yes(Ashraf <i>et al</i> 2020, Rahman <i>et al</i> 2021)
RAI	Yes	Yes	3D	Yes(Oraiqat <i>et al</i> 2020, Bjegovic <i>et al</i> 2024, Wang <i>et al</i> 2024a)

in water and TOPAS Monte Carlo simulation based treatment plans *in vivo*. Quantitative dose maps were generated with empirical calibration from linearity studies (figures 1(c)–(e)). To our knowledge, this is the first demonstration of real-time, *in vivo*, single-pulse, 3D dosimetry for FLASH radiotherapy. This capability represents a key step toward adaptive FLASH radiotherapy and its clinical translation.

2. Methods

2.1. Generation of FLASH beams

FLASH dose rates were delivered using a FLASH-capable linear accelerator (LINAC) system (Mobetron, IntraOp, Sunnyvale, CA, USA) at MD Anderson Cancer Center. This LINAC is capable of producing electron beams at both conventional and FLASH dose rates (Palmiero *et al* 2025) and has been previously validated in preclinical FLASH studies involving murine models (Levy *et al* 2020).

The Mobetron treatment head contains two beam current transformers (BCTs), produced by Bergoz Instrumentation (Saint-Genis-Pouilly, France). The BCTs are alternating-current current transformers (ACCTs) made from a cobalt-based alloy, with different internal diameters (ACCT-S-028 for the upper BCT and ACCT-S-055 for the lower BCT) (Liu *et al* 2023b), allowing for real-time output monitoring and pulse counting. Beam parameters were optimized by adjusting pulse widths (1–4 μ s) and repetition rates (1–120 Hz), while the delivered dose was controlled by modifying the source-to-surface distance (SSD) and through the use of beam collimation. An average dose rate >40 Gy is typically used to achieve the biological FLASH effect though there is no consensus on the delivery scheme that ensures the biological effect will occur: whether the instantaneous dose rate or the length of single fraction or the number of fractions is still up for debate. For demonstrating the feasibility of RAI to not saturate at high instantaneous dose rates and high doses per pulse, all irradiations were performed with doses per pulse greater than 1 Gy and instantaneous dose rates greater than 1×10^6 Gy s^{−1} (Supplementary tables 1, 2). All irradiations were single fraction deliveries that completed course in 0.3333s.

To simulate preclinical irradiation conditions and evaluate spatial dose conformity, custom collimators were fabricated via 3D printing and filled with a 2 cm layer of aluminum oxide and a 1 cm layer of tungsten powder, consistent with prior preclinical FLASH studies (Valdés Zayas *et al* 2023, Liu *et al* 2025, Saenz *et al* 2025) (supplementary figure S6).

Before experimental delivery, the LINAC output was characterized using EBT3 film, BCT measurements, and ion chamber measurements, enabling accurate calibration and validation of RA signal acquisition against known dose distributions in a single plane (Sun *et al* 2023). Additionally, film was

irradiated during every *in vivo* delivery, placed on the top of the jig (**supplementary figure S5(d), (e)**) and used to calibrate *in vivo* irradiations to prior PDD curves, and BCT signals were acquired for every beam firing.

2.2. RAI system configuration

For acoustic detection, a custom-designed 256-element matrix ultrasound transducer array (Doppler Tech Inc., China) with a center frequency of 1 MHz and -6 dB bandwidth of 60%, optimized for detecting low-frequency radiacoustic waves, was employed. The transducer array is configured in a 16×16 matrix of $3 \text{ mm} \times 3 \text{ mm}$ transducer elements with a 3 mm pitch. Individual element variations include a 3 dB sensitivity homogeneity across elements and an average bandwidth of 79.7 ± 3.5 %, which can contribute to small signal collection differences across the transducer. To mitigate the limited-view challenge intrinsic to matrix arrays, data acquisition was performed with the transducer positioned both perpendicular and parallel to the beam axis in a water tank at 70 mm from beam edges and entrance, enabling 3D reconstruction of dose deposition (figures 2(a)–(d)). For *in vivo* experiments, the same array was positioned perpendicularly to the beam to visualize the beam cross-section within the mouse (figure 1(b), **supplementary figure S6**). Radiacoustic signals from all channels were simultaneously amplified and digitized using a custom-built 256-channel analog front-end Legion ADC system (Photosound Inc., USA), providing 88 dB gain per channel before saving data with a high speed parallel digitizer at a sampling rate of 40 MHz. All post-processing and image reconstruction were performed using MATLAB (2022b, MathWorks).

To synchronize the RAI data acquisition system (DAQ) with beam delivery, the lower BCT signal was split into two channels. One channel was directed to a PicoScope 5000 Series FlexRes Oscilloscope (Pico Technology, St Neots, Cambridge, UK) for conventional beam monitoring, while the other served as a trigger input for the RAI DAQ system. The synchronization signal, initially in the millivolt range, was amplified using a preamplifier (SRS Instruments, USA) and conditioned via a function generator before reaching the DAQ, enabling the capture of radiacoustic signals from individual radiation pulses. After each trigger signal, 4000 samples were collected by each transducer element in parallel at a sampling rate of 40 MHz, allowing for 100 μs of signal collection. Signals were processed and reconstructed offline.

2.3. MB image reconstruction for quantitative dosimetry

Radiacoustic signal generation in homogeneous media under thermal confinement and negligible acoustic attenuation is governed by (Khan and Gibbons 2020):

$$\left(\nabla^2 - \frac{1}{c^2} \frac{\partial^2}{\partial t^2}\right) p(\vec{r}, t) = -\frac{\beta}{C_p} \frac{\partial H(\vec{r}, t)}{\partial t}. \quad (1)$$

Here, $p(\vec{r}, t)$ is acoustic pressure, c is the speed of sound, β is the thermal expansion coefficient, C_p is the specific heat at constant pressure ($\sim 4000 \text{ J Kg}^{-1} \text{ K}^{-1}$), and $H(\vec{r}, t)$ is the heating function. The heating function relates to dose rate, D as (Khan and Gibbons 2020):

$$H = \eta_{th} D \rho \quad (2)$$

where ρ is the tissue density and η_{th} is the fraction of radiation energy that is converted into heat. Substituting, the governing equation becomes (Sun et al 2023):

$$\left(\nabla^2 - \frac{1}{c^2} \frac{\partial^2}{\partial t^2}\right) p(\vec{r}, t) = -\frac{\eta_{th} \rho \beta}{C_p} \frac{\partial D(\vec{r}, t)}{\partial t}. \quad (3)$$

The resulting thermal expansion acts as the acoustic source. The forward solution assuming instantaneous dose deposition is (Xu and Wang 2005):

$$p(\vec{r}, t) = \Phi \frac{\partial}{\partial t} \left(ct \int_{s(\vec{r}, t)} D(\vec{r}') d\Omega \right) \quad (4)$$

where $\Phi = \frac{\Gamma \eta_{th} \rho}{4\pi c}$ is a material-dependent constant, Γ is the Gruneisen parameter, $S(\vec{r}, t)$ represents the spherical surface centered at $\vec{r}$ and radius CT , and the integral variable Ω represents the solid angle integral. Here the integration over the spherical surface of radius CT indicates that at a time t , detector will

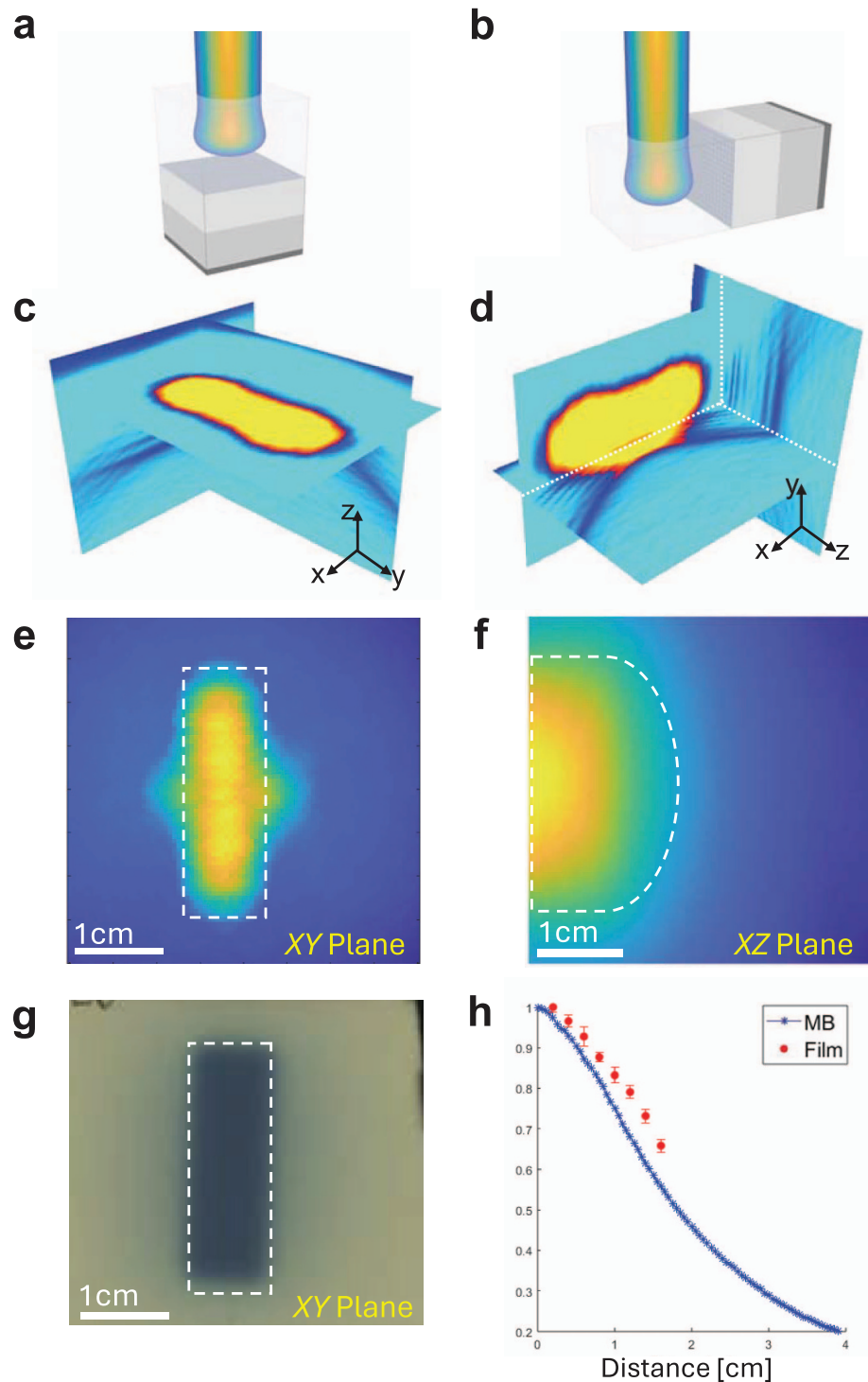


Figure 2. Three-dimensional imaging of a FLASH electron beam using radiacoustic imaging. (a) Perpendicular configuration of the 2D matrix array transducer with respect to the electron beam used to collect signals for beam cross-section imaging. (b) Parallel configuration of the matrix array with respect to the electron beam used to collect depth-dose information along the beam axis. (c), (d) 3D volumetric RAI of FLASH electron beam from a single view, illustrating both the penetration of electrons into water and the cross-sectional geometry of the 1 cm $\times$ 3 cm rectangular beam. (e) RAI-reconstructed image in XY plane, showing lateral distribution. (f) RAI-reconstructed image in XZ plane, showing depth-dose profile. (g) Corresponding Gafchromic film in XY plane. (h) Comparison of percentage depth-dose (PDD) profiles derived from RAI (f) and film dosimetry, demonstrating close agreement.

have the contribution from the sources located at a distance CT —that is the distance traveled by acoustic waves in time t in the medium. Equation (4) represents the semi-analytic solution to the wave equation (equation (1) governing radiacoustic spatio-temporal evolution obtained using the Green's function approach (Wang and Wu 2012) which implicitly enforces the Sommerfeld radiation condition (Schot 1992) as the boundary condition. This simplification for this first demonstration of 3D RAI dosimetry in

small animals, motivated by the fact that the mouse is acoustically coupled through agar and ultrasound gel to the transducer, and that the dominant acoustic path is through a largely water-equivalent medium. Incorporating the heterogeneous wave equation with spatially varying acoustic parameters derived from CT-based tissue segmentation, together with numerically computed medium-specific Green's functions, is a necessary and explicitly planned component of our future work as we extend the methodology toward large animal and human RAI studies.

Discretizing the domain into N_v spherical voxels (radius R), the dose deposition $D(\vec{r})$ can be expressed as (Ding et al 2020):

$$D(\vec{r}) = \sum_{k=1}^{N_v} d(\vec{r}_k) N(\vec{r} - \vec{r}_k),$$

$$N(\vec{r}) = \begin{cases} 1 - \frac{r}{R}, & \text{if } r < R \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

where $d(\vec{r}_k)$ is the dose at location $\vec{r}_k$ in the domain that represents the center of the k th spherical voxel and $N(\vec{r})$ represents the basis function within the voxel. Substituting equation (5) in equation (4), we get

$$p(\vec{r}, t) = \sum_{k=1}^{N_v} \frac{\partial}{\partial t} \left(ct \int_{S(\vec{r}, t)} N(\vec{r}' - \vec{r}_k) d\Omega \right) h(\vec{r}_k) = \sum_{k=1}^{N_v} Q(s_k, t) h(\vec{r}_k) \quad (6)$$

where s_k is the distance of k th node from the transducer. The quantity $Q(s, t)$ is the contribution to the radiacoustic signal at a detector at time t from a spherical voxel located at a distance s . Instead of choosing traditional cubic voxels, the choice of spherical voxels enables a simplified and hence efficient model by getting rid of the dependence of the contribution on the relative source-detector angular location (Ding et al 2020) as shown in **supplementary figure S3A**. For fast evaluation of $Q(s_k, t)$, a look-up table (LUT) based method was employed. The minimum and maximum distances between all detector elements and voxel centers within the region of interest (ROI) were first determined. For a planar detection geometry, the minimum distance ($s_{\min}$) corresponds to the perpendicular distance between the transducer array and the near surface of the ROI, whereas the maximum distance ($s_{\max}$) corresponds to the distance between the corner detector element and the diagonally opposite corner of the ROI as shown in supplementary figure S3A. For 1000 equispaced values of s (between $s_{\min}$ and $s_{\max}$), $LUT \equiv Q(s = [s_{\min}, \dots, s_{\max}], t = [0, N_t - 1] / F_s)$ was computed and stored. More details for this calculation are provided in Supplementary S3A. Appropriate $Q(s_k, t)$ values were evaluated through 2D linear interpolation from this LUT during the reconstructions.

In the discrete domain, equation (6) represents radiacoustic (RA) measurements some detector, at all time steps, can thus be written as:

$$\underline{p}^{(q)} = \mathbf{Q}^{(q)} \underline{d}. \quad (7)$$

The full RA measurement vector $\underline{p}$ (dimension: $(N_d N_t) \times 1$) can thus be expressed as:

$$\underline{p} = \mathbf{M} \underline{d}, \quad \mathbf{M} = \left[Q^{(1)T}, Q^{(2)T}, \dots, Q^{(N_d)T} \right]^T \quad (8)$$

with $\mathbf{M}$ being the model-matrix (dimension: $(N_d N_t) \times N$).

Unlike simple back-projection, which suffers from limited-view artifacts and low-frequency loss, MB reconstruction solves for $\underline{D}$ by minimizing the error between measured and simulated pressure:

$$\hat{\underline{d}} = \arg \min_{\underline{d} \geq 0} L(\underline{d}) : L(\underline{d}) = \|\underline{p}_m - \mathbf{M} \underline{d}\|^2 + \lambda^2 \|\mathbf{R} \underline{d}\|_2^2 \quad (9)$$

where $\underline{D}_{sol}$ is the reconstructed dose map. Regularization was applied using Tikhonov (with identity matrix $\mathbf{R}$) (Pandey et al 2021a):

$$\hat{\underline{d}} = (\mathbf{M}^T \mathbf{M} + \lambda^2 \mathbf{R}^T \mathbf{R})^{-1} \mathbf{M}^T \underline{p}_{meas} \quad (10)$$

where $\mathbf{R}$ is the regularization matrix and λ is the parameter for the regularization. In our study, we use an identity matrix for $\mathbf{R}$ as Tikhonov regularization. For large-scale reconstruction problems (large number of voxels and measurements), evaluating and storing $\mathbf{M}$ takes a lot of time and memory thus making reconstructions computation through equation (9) inefficient. Equation (8) has therefore been solved

using MATLAB's LSQR function which utilizes on-the-fly $\mathbf{M} \mathbf{u}$ and $\mathbf{M}^T \mathbf{v}$ computations, instead of using $\mathbf{M}$ explicitly (Pandey et al 2021a). The regularization parameter λ controls the tradeoff between data fidelity and the regularization terms in the loss functional given in equation (9). The L -curve method (Hansen 2001) is a standard approach for selecting λ , plotting the regularized solution norm against the residual norm across a range of λ values, with the optimal λ corresponding to the corner of the resulting curve. This requires solving equation (9) multiple times for a series of λ s which is unrealistic. An efficient L -curve computation typically, or an explicit SVD of the model-matrix M (Hansen 2007), which is computationally prohibitive for the large-scale model-matrix arising in 3D RAI reconstruction. Since M is never explicitly formed in our matrix-free iterative framework, SVD-based parameter selection methods are not directly applicable. The regularization parameters was selected empirically based on reconstruction quality—specifically, a value that suppressed noise-induced artifacts in the reconstructed dose distribution while preserving the overall magnitude and spatial extent of the solution.

A TOPAS simulation (Perl et al 2012, Faddegon et al 2020) was performed to compute the dose deposition in water by passing a 9 MeV electron beam through a collimator with five rectangular holes of size 5 mm $\times$ 10 mm. The simulation was performed on a 120 $\times$ 120 $\times$ 135 grid with 0.5 mm grid separation. The dose deposition was then exported to MATLAB and k-wave toolbox (Treeby and Cox 2010) was used to perform acoustic simulation. The size of the grid was chosen to be 10 cm $\times$ 10 cm $\times$ 10 cm with 0.5 mm resolution and a 16 $\times$ 16 element transducer array with 3.2 mm inter-element spacing was placed 5 cm from the entrance of the electron beam.

The simulated acoustic signals were then contaminated with white Gaussian noise to get signals with 2 dB SNR. The reconstructions were performed using the UBP as well as the MB algorithms and the results are demonstrated in **supplementary figure S4**. The maximum intensity projection (MIP) images of the true dose deposition and reconstructions from UBP and MB algorithms are shown in **supplementary figure S4a–c**. Both the algorithms yield strong limited view artifacts, which causes loss of the dose information in the direction perpendicular to the ultrasound transducer. However, with respect to the UBP, MB preserves more information of the 3D nature of the dose deposition. It also suppresses the noise in the reconstructions which are present in the UBP counterparts. For dose congruity comparison, a plot along the reconstructed profiles at $x = 8$ mm (depicted by dashed line in x – y MIP image in **supplementary figure S4a**) are compared with the true dose map at $z = 0, 0.5$, and 1 mm from the beam entrance is shown in **supplementary figure S4d** which further signifies the superiority on MB over the UBP algorithm. The better performance of the MB is further reflected in the correlation coefficient with respect to the true dose deposition map which was found to be 0.49 for MB reconstructions as compared to 0.30 for UBP.

All computations were performed on a workstation with AMD Ryzen Threadripper 3960 $\times$ 24-Core Processor (3.79 GHz), 256-GB installed RAM on Windows 10, 64-bit operating system. While the universal backprojection takes about 2 s in evaluating the reconstructions, the MB LSQR optimization algorithm takes about ~ 90 –120 s, depending upon the number of iterations (typically 5–10) taken for evaluating the reconstructions based on the chosen stopping criterion—that is when (a) the loss stopped improving: $\frac{|L(\mathbf{d}_k) - L(\mathbf{d}_{k-1})|}{|L(\mathbf{d}_k)|} < 0.01$ (catches situations where the solution is still oscillating between nearby values but the minimizing cost is essentially flat—meaning further iterations are not meaningful) or (b) the solution stopped changing: $\frac{|\mathbf{d}_k - \mathbf{d}_{k-1}|}{|\mathbf{d}_k|} < 0.01$ (catches the complementary situation where the loss is still technically ticking downward by tiny amounts each iteration, but the actual reconstruction $\mathbf{d}$ has already settled). The regularization parameters as mentioned before, were chosen empirically based on the reconstruction quality. For the simulations, we used $\lambda = 0.5$, while in the experimental reconstructions, we used $\lambda = 0.7$.

Image reconstruction in the current implementation is performed offline on CPU and is not yet real-time. Achieving real-time reconstruction is a recognized challenge in MB tomographic imaging; however, we note that it is not a fundamental barrier. Several acceleration pathways are actively under development in our group and in the broader literature: GPU acceleration of the forward/adjoint model operators, which form the computational bottleneck of iterative reconstruction, has been demonstrated to yield order-of-magnitude speedups in photoacoustic tomography. For example, Wang et al demonstrated GPU-accelerated 3D PAT reconstruction achieving clinically relevant framerates (Wang et al 2013). In our experimental configuration, the Mobetron delivers FLASH electron beams at 1.3 Gy/pulse, 1 μ s pulse width, and 30 Hz pulse repetition rate (PRF), with each pulse generating a fully separable acoustic signal at the transducer confined well within 100 μ s, well before the next pulse at 33.3 ms inter-pulse interval. This establishes a 33 ms per-pulse reconstruction budget. GPU-accelerated MB reconstruction algorithms map naturally onto massively parallel GPU architectures and can approach this budget for

2D and 3D reconstructions. Similar acceleration is directly applicable to the RAI forward model given the shared acoustic wave equation framework. Deep learning-based reconstruction has also been shown to reduce MB PAT reconstruction to real-time framerates. Hauptmann *et al* demonstrated learned iterative reconstruction for limited-view 3D PAT at accelerated rates, and subsequent works have achieved near-real-time performance around 31 ms per frame (Hauptmann *et al* 2018, Dehner *et al* 2023). We intend to adapt this methodology for RAI reconstruction in future work. We therefore view real-time RAI reconstruction as achievable within the near-term development roadmap.

2.4. Film calibration

Calibration curves were generated for each film batch (supplementary figure S2Aa) by fitting red-channel pixel values to minimize uncertainty from a flatbed scanner to dose using a polynomial model (Marroquin *et al* 2016, Liu *et al* 2023a). A reference background film provided the baseline pixel value in the same ROI visually placed at the center of the radiation field on irradiated films, allowing conversion of experimental film pixel values into optical density and subsequently to dose (Liu *et al* 2023a). The dose equation was determined by irradiation EBT3 films with known doses from 0 to 50 Gy under reference conditions using a 16 MeV Varian Truebeam clinical electron beam (Varian Medical Systems, Palo Alto, CA). Experimental films (supplementary figure S2A) were irradiated and scanned after 24 h with the same scanner as the one used to generate calibration curves, and dose maps were calculated for each frame with the established calibration curve, representing both on top of the jig and 10 mm depth positions in solid water to establish reference conditions for RAI comparisons.

2.5. RAI: simulation-experiment agreement

To confirm the equivalence of radiacoustic simulation and experiment, a comparison is shown in **supplementary figure S2B** for the 1 cm circular collimator experiment. The Monte-Carlo simulation-based 3D dose maps were first validated against the film measurements and subsequently imported in MATLAB for radiacoustic simulation using the k-wave toolbox. Representative simulated and experimental RA signals from a randomly chosen element is shown here. While differences exist between the two waveforms, the overall structure and characteristic peak locations are in good agreement. The discrepancies can be attributed to inaccuracies in the system's overall impulse response—specifically, the temporal pulse structure and frequency response, both of which are approximated as Gaussians in the present simulations, the finite transducer element size (~ 3 mm), which is modeled as a point detector, background noise, and small errors in the simulated detection geometry, including source-detector distance and angular alignment. We plan to conduct intensive calibration studies to obtain a more accurate total system impulse response and incorporate it into the reconstruction algorithm in our future studies.

2.6. Water tank validation studies

To validate the performance of the RAI system, a series of controlled experiments were conducted in a water tank using a 9 MeV electron beam delivered by the modified Mobetron LINAC. The 256-element matrix transducer array was mounted at two orthogonal positions (perpendicular and parallel to the beam axis) using a custom-built rotatable frame to capture complementary views and prevent change in position during irradiation. The beam was shaped using 3D-printed collimators (1 cm circle, 1 cm $\times$ 1 cm square, 1 cm $\times$ 3 cm rectangle). EBT3 films were subsequently inserted at various depths in solid water, in place of the water tank, to provide ground truth measurements of spatial dose profiles and PDD. RA signals were acquired from individual pulses (1 μ s pulse width, 30 Hz repetition rate) and reconstructed using both UBP and MB reconstruction. Monte Carlo simulations using TOPAS were also performed for comparison.

2.7. *In vivo* FLASH beam delivery

For *in vivo* studies, FLASH electron beams were delivered using the same Mobetron LINAC setup. Beam parameters (pulse width, repetition rate, SSD) were maintained to deliver ~ 1.3 Gy per pulse (**supplementary table 2**). The beam cross-section was shaped using the previously validated 3D-printed collimators, with alignment verified via a white light field. The mice were placed on a custom-designed stage made of low-scattering materials, positioned such that the beam entrance was orthogonal to the body axis. To ensure proper alignment with the transducer, a custom-built holder was designed and 3D printed (supplementary figure S6c). A mouse holder (shown in green), was modified from prior murine studies, as it has been validated to allow for high repeatability in mouse placement (Valdés Zayas *et al* 2023, Liu *et al* 2025). The bottom of the holder was modified to be 80% open, with small footholds and head-holds for the mouse, in order to allow for coupling with the transducer. Concerns about beam irradiation incident on the transducer due to the small size of the mice were mitigated by a 7 cm high

agar phantom placed in the middle region of the holder. This agar phantom was poured ahead of time and validated after water experiments to ensure good coupling. The mouse abdomen was coupled to the agar phantom with clinical ultrasound gel, replaced between the mice for best recoupling. The agar phantom was coupled to the transducer, located in the perpendicular orientation in the bottom section of the 3D printed holder system. The center of the transducer was thus always centered to the film slot in the lid of the mouse jig, allowing for precise alignment ($<1\%$ uncertainty) during experiments and co-registration between the RAI dose maps and the prior CTs, which included the jig and lid (**supplementary figure S6 e, f**). However, replacement of the mouse in the jig between prior CT and irradiation introduces some uncertainty in the matching of the prior CT to the moment of irradiation. The design of the jig, with the designated slots for the neck, tail, and nose of the mouse attempts to reduce this uncertainty. Dosimetry was confirmed using pre-irradiation film placement and real-time current monitoring through BCTs.

2.8. Treatment plan generation

To enable ground truth comparisons for RA imaging, treatment plans were generated using the TOPAS Monte Carlo simulation framework. A detailed model of the Mobetron LINAC was developed to replicate the electron dose distributions for each of the collimator assemblies designed for these experiments.

The physical collimators used in the *in vivo* experiments—a 1 cm diameter circle, a 1 cm $\times$ 1 cm square, and a 1 cm $\times$ 3 cm rectangle—were modeled in TOPAS using their corresponding STL files, which were originally designed for 3D printing and subsequently filled with a 2 cm layer of aluminum oxide and a 1 cm layer of tungsten powder to shape the electron beam, produce flat dose distributions, and prevent dose leakage around the target in simulation.

Initial TOPAS simulations were conducted in a water phantom to assess beam profiles and depth dose distributions. These results were validated against EBT3 film measurements obtained in solid water phantoms, confirming beam profile and percent depth dose (PDD) alignment (**supplementary figure S7**). The root-mean-square error (RMSE) between simulation and measurement supported the accuracy of the model for each collimator.

Following validation in water, simulations were extended to murine-specific geometries by scoring dose deposition in the CT volumes of two mice. The mouse CT scans were conducted the day prior to the *in vivo* experiments, with the mouse scanned in the treatment jig to enable accurate co-registration. The CTs were calibrated for correct Hounsfield units. These scaled CT DICOM files were then used as input in TOPAS, with geometry aligned according to the experiment design, and the dose was scored inside the CT volumes, to produce treatment plans. The dose volume was further segmented to only include the dose scored within the mouse for isodose line and RMSE comparisons. The 1 cm circular collimated beam was simulated in the CT of mouse 1, while both the 1 cm $\times$ 1 cm square and 1 cm $\times$ 3 cm rectangular beams were simulated in the CT of mouse 2 (figures 4, 5), providing 3D dose distributions for comparison with RAI reconstructions.

2.9. Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Texas MD Anderson Cancer Center. Female C57BL/6 mice (9 weeks old) were used for FLASH irradiation studies. Prior to the experiment, CT scans were acquired of the mice using the Small Animal Radiation Research Platform (SARRP, XStrahl, GA, USA) at MD Anderson cancer center. CT scans were performed in the mouse jig, the same one used for irradiation (**Supplementary figure S6 (e), (f)**), allowing for high repeatability between mouse placements. Additionally, hair removal over the treatment area was conducted on both mice prior to the experiment to ensure accurate coupling with the transducer assembly. Mice were anesthetized with 1.5%–2.5% isoflurane in oxygen and placed in a prone recumbent position. Physiological monitoring was maintained during the procedure. Two mice were used for beam delivery: mouse 1 received a circular (1 cm) beam, while mouse 2 received both square (1 cm $\times$ 1 cm) and rectangular (1 cm $\times$ 3 cm) beams to the abdomen. Each mouse received 20 total pulses of 1–1.5 Gy/pulse, with the total dose delivered to each mouse <30 Gy. The mice were euthanized post irradiation.

2.10. RAI imaging protocols

RAI data acquisition was synchronized with beam pulses using a split signal from the LINAC's lower BCT, which served as the timing trigger. RA signals were acquired using a 256-element matrix array transducer (1 MHz center frequency, Photosound Inc.) positioned in water or in ultrasound-coupled contact with the animal body (*in vivo*). Signals were amplified and digitized using a custom 256-channel DAQ system (Legion ADC, Photosound Inc.). For water tank studies, the transducer was moved to

acquire orthogonal views. *In vivo*, the array was placed perpendicularly below the target area and acoustically coupled using ultrasound gel (**supplementary figure S6(c) and (d)**). Raw data were processed and reconstructed in MATLAB using both UBP and MB inversion methods.

2.11. Quantitative calibration and transferability

An initial calibration of film measurements was generated using films irradiated by 1 cm circle collimated beams in solid water phantom (Supplementary table 1). The film stack was exposed to 10 1 μ s pulses. Using the 10 mm depth film, an ROI was drawn at the center of the irradiated field (Liu *et al* 2023a) and the max value was utilized as the ground truth. RAI signals using the same 1 cm circle collimator were collected from 10 to 1 μ s pulses in a water tank in the orthogonal position at a distance of 70 mm. The center of the transducer was aligned to the isocenter with light field and held in place with a 3D printed component to ensure no movement post-alignment.

Two different methods of creating a calibration curve were created, the first utilizing a single channel from the center of the transducer array as shown in figure 1(c). This channel was used to look at the amplitude of RA signal for single pulse, 2 pulse, 5 pulse, and 10 pulse accumulated signals. This amplitude, was compared to the dose measured by the film exposed to 10 pulses and its 5 pulse, 2 pulse, and single pulse divisions. However, each transducer receives signal from different locations in the 3D irradiated volume. Thus, a conversion using the reconstruction rather than the raw RA signal was also established, which is used for the calibration of the MB pressure maps into relative dose maps. Here, the digitized film was converted from red-channel values to dose using lot specific calibration curves as mentioned above and in supplementary figure 2, following the framework put forth by (Liu *et al* 2023a). The maximum Gy from the center of the same film dose map was taken as ground truth again. The maximum RAI intensity was selected from the corresponding RAI reconstruction for single pulse, 2 pulse aggregate, 5 pulse aggregate, and 10 pulse aggregate reconstructions. This intensity, ($\text{int}_{\text{recon}}$), was compared to the dose measured by the film exposed to 10 pulses and its 5 pulse, 2 pulse, and single pulse divisions. Linear fitting was used on both calibration methods.

The final MB pressure maps were converted to dose distributions using empirical calibration factors derived from film measurements (**supplementary table 3**) and linear fitting models with R2 values >0.999 with 95% confidence bounds and no outlier handling:

$$\text{Dose} = (0.00007692) (\text{int}_{\text{CH120}}) - 0.005915 \quad (11)$$

$$\text{Dose} = (0.0003182) (\text{int}_{\text{recon}}) + 0.0003033. \quad (12)$$

Here, both a single channel measurement and a qualitative reconstruction map can be converted to relative dose, with the amplitude intensity ($\text{int}_{\text{CH120}}$) or the maximum intensity of the dose map ($\text{int}_{\text{recon}}$), using the linearity study in figures 1(c)–(e).

2.12. Evaluation metrics

To quantitatively evaluate the accuracy of the reconstructed dose distributions, we employed three widely used image quality and dosimetric metrics: gamma index (γ), structural similarity index (SSIM), and isodose lines comparisons.

Gamma index (γ): (Low *et al* 1998) The gamma index $\gamma(\vec{r})$ was selected as the primary evaluation metric, as it is a standard metric of quality in radiotherapy for assessing both dose differences (DDs) and spatial agreement between the planned and delivered dose in 2D. It combines distance-to-agreement (DTA) and DD into a single scalar value and is defined as:

$$\gamma(\vec{r}) = \min \left[\sqrt{\left(\frac{\|\vec{r} - \vec{r}'\|_2}{\Delta d} \right)^2 + \left(\frac{\|D_{\text{GT}}(\vec{r}) - D_{\text{pred}}(\vec{r}')\|_2}{\Delta D} \right)^2} \right] \quad (13)$$

where $D_{\text{GT}}(\vec{r})$ is the reference (ground-truth) dose such as the 2D film dose distribution at some depth, $D_{\text{pred}}(\vec{r}')$ is the predicted dose such as the RAI dose map at corresponding depth, $\vec{r}$ is the coordinate in $D_{\text{GT}}(\vec{r})$, $\vec{r}'$ is the coordinate in $D_{\text{pred}}(\vec{r}')$ while Δd is the DTA criterion (in mm) and ΔD is DD criterion (as a percentage of the reference dose). To ensure the D_{GT} and the D_{pred} were

of the same size and well aligned, the film and RAI comparisons were aligned to center using line profiles. Subsequently, mesh grids were used in MATLAB with the resolution of each respective 2D dose map with no interpolation. The DTA and DD were varied between 5 mm/5%, 3 mm/3%, and 2 mm/2% for comparisons RAI dose maps to the reference films. A gamma value $\gamma(\vec{r})$ indicates that the reconstructed dose passes the specified acceptance criteria at location $(\vec{r})$; an overall gamma pass rate is determined for the whole dose distribution being compared. Local dose normalization was utilized. No low-dose threshold or masking was utilized for these comparisons.

SSIM: To assess structural fidelity, SSIM was utilized, which measures perceptual similarity between the reconstructed RAI dose distribution and the reference film dose. SSIM is defined as (Anon n.d.):

$$\text{SSIM} = \frac{(2\mu_R\mu_I + c_1)(2\sigma_{IR} + c_2)}{(\mu_R^2 + \mu_I^2 + c_1)(\sigma_R^2 + \sigma_I^2 + c_2)} \quad (14)$$

where I and R are the reconstructed and reference dose images, respectively while μ_R is an average of R , σ_I^2 is a variance of I , and σ_{IR} is a covariance of I and R . There are two variables that stabilize the denominator that can be further written as $c_1 = (k_1L)^2$ and $c_2 = (k_2L)^2$ where L is a dynamic range of the pixel intensities and k_1 and k_2 are constants (by default $k_1 = 0.01$ and $k_2 = 0.03$).

Isodose Lines (Contour Plots): Lastly, isolines were used to compare the 60% and 80% of max dose delivered in space between RAI and film or treatment plans. Isolines are defined as polylines which represent the boundary of radiation absorption at a specified percentage of max dose within a dose map, ensuring all points on the line receive the same specified total dose (Wigg and Wilson 1981).

Together, these metrics provide a comprehensive evaluation of both the spatial and dosimetric accuracy of our reconstructed dose maps.

3. Results

3.1. 3D volumetric mapping of FLASH dose deposition

To study the spatial accuracy of the 3D RAI dose mapping, a series of water tank experiments were performed. The custom collimator system with field sizes ranging from 1 cm diameter circle to 1 cm $\times$ 3 cm rectangle was installed to shape the beam. To ensure maximal and consistent energy deposition within the FLASH regime, a fixed distance between the end of the collimator to the irradiated sample's surface of 1 cm was maintained across all measurements. Entrance dose was measured using Gafchromic EBT3 film placed at the phantom surface, confirming a dose delivery of ~ 1 Gy per pulse. A total of 10 pulses delivered an entrance dose of approximately 13 Gy, resulting in an instantaneous dose rate exceeding 10^6 Gy s $^{-1}$ (**supplementary table 1, 2**). RAI was performed with the 256 channel matrix array in 2 orientations to reduce the effect of the limited angle problem encountered in previous works.

The transducer was first positioned perpendicular to the beam axis to capture lateral beam profiles during irradiation of homogeneous water (figure 2(a)). Signals from each transducer element were time-synchronized and then reconstructed using a MB inverse solver. The resulting 3D radiacoustic images revealed precise spatial distributions of deposited dose (figure 2(c)). Cross-sectional MIP images in the transverse (X - Y) plane (figure 2(e)) showed beam shapes that accurately matched collimator geometries and exposed radiochromic film (figure 2(g)), such as the rectangular 1 cm $\times$ 3 cm field, with sharp beam edges and uniform intensity. To evaluate depth-dose characteristics, the matrix array was rotated by 90° to align parallel to the beam axis along the Z -direction (figure 2(b)), enabling reconstruction in the sagittal (X - Z) plane (figure 2(d)). Radiacoustic signal waveforms were time-gated to isolate signals from different depths, and reconstructions were performed using a MB algorithm that incorporated known acoustic properties of water and the transducer geometry. To quantitatively validate the reconstructed dose profiles, we acquired a PDD curve using a film stack composed of Gafchromic EBT3 sheets placed at 2 mm intervals in a solid water phantom. Dose profiles obtained from the RAI images were compared directly with the PDD data from film. The beam line profile from the RAI reconstructions in the XZ plane (figure 2(f)) closely matched the measured film-generated PDD curve (figure 2(h)), with the depth of maximum dose (d_{max}) aligning within 0.2 mm accuracy and low RMSE values.

These results demonstrate that our matrix-array-based RAI system enables high-resolution, volumetric, and quantitative mapping of FLASH dose deposition in 3D. The integration of advanced acoustic hardware, optimized signal processing, and MB reconstruction yields dosimetric accuracy comparable to gold-standard film and Monte Carlo methods, but with the added advantage of real-time, *in vivo* capability—critical for adaptive FLASH radiotherapy.

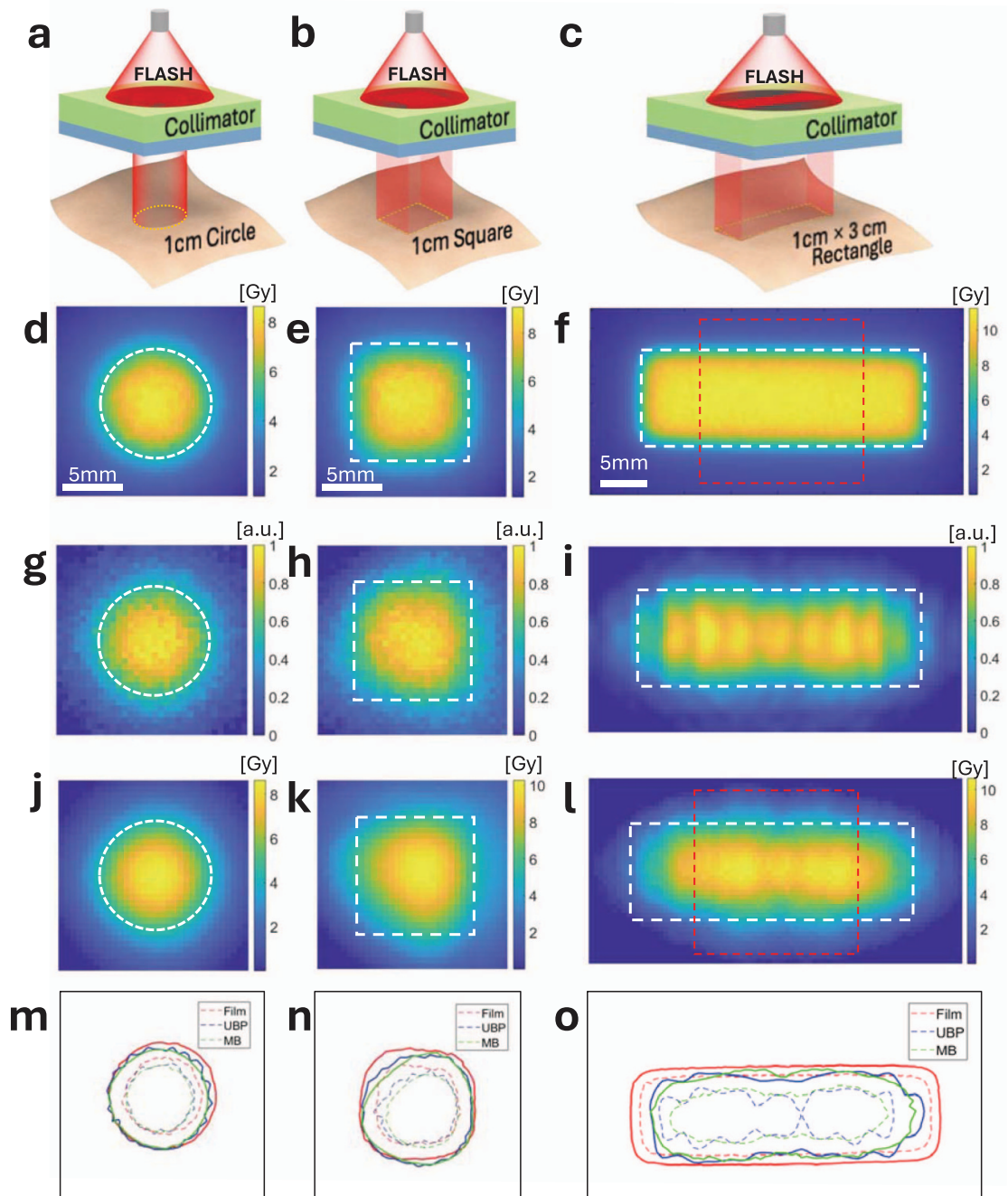


Figure 3. Quantitative radiacoustic for FLASH beams of varying geometry. (a)–(c) Delivery of FLASH electron beams collimated into a 1 cm diameter circular field (a), a 1 cm $\times$ 1 cm square field (b) and 1 cm $\times$ 3 cm rectangular field (c). (d)–(f) Gafchromic film measurements at 1 cm depth, showing absolute dose distributions in Gray (Gy) for the three respective fields. (g)–(i) Radiacoustic imaging (RAI) reconstructions using universal back-projection, providing qualitative dose distributions for each collimated field. (j)–(l) Quantitative dose maps in Gray (Gy) reconstructed from RAI data using model-based algorithms, demonstrating spatially resolved dosimetry. (m)–(o) Isodose contour comparisons between RAI and film measurements; Solid lines indicate 60% isodose lines while dashed lines indicate 80% isodose lines, demonstrating good agreement between the two modalities.

3.2. Quantitative FLASH dosimetry

To enable accurate quantification of dose from radiacoustic signals, we employed a MB image reconstruction framework (Pandey *et al* 2021a, Sun *et al* 2023) across various FLASH beam shapes and sizes. Unlike conventional back-projection, the MB reconstruction integrates a detailed physical model of the imaging system, enforces non-negativity constraints, and applies regularization to mitigate the ill-posedness inherent in the inverse problem. This approach significantly improves spatial resolution, contrast, and quantitative reliability. A pressure-to-dose calibration procedure was also performed to convert reconstructed radiacoustic pressure into absolute dose values in figures 1(c)–(e).

To evaluate the system's performance across different beam geometries, we collimated FLASH electron beams into three distinct shapes: 1 cm diameter circular (figure 3(a)), 1 cm $\times$ 1 cm square

Table 2. RAI comparisons to film. Using the dose maps in figure 3, gamma analysis was performed to compared film to each RAI dose map with 5 mm/5%, 3 mm/3%, and 2 mm/2% criteria. Root mean square error and structural similarity analysis was also performed for each collimator.

COLLIMATOR	5 MM/5% GAMMA INDEX	3 MM/3% GAMMA INDEX	2 MM/2% GAMMA INDEX	RMSE	SSIM
1 cm Circle	97.66%	97.66%	97.46%	0.0423	0.9244
1 × 1 cm Square	97.17%	96.29%	92.19%	0.1038	0.8166
1 × 3 cm Rectangle	72.25%	70.22%	62.44%	0.1378	0.5805
Cropped Rectangle	91.94%	90.88%	77.25%	0.1190	0.6872

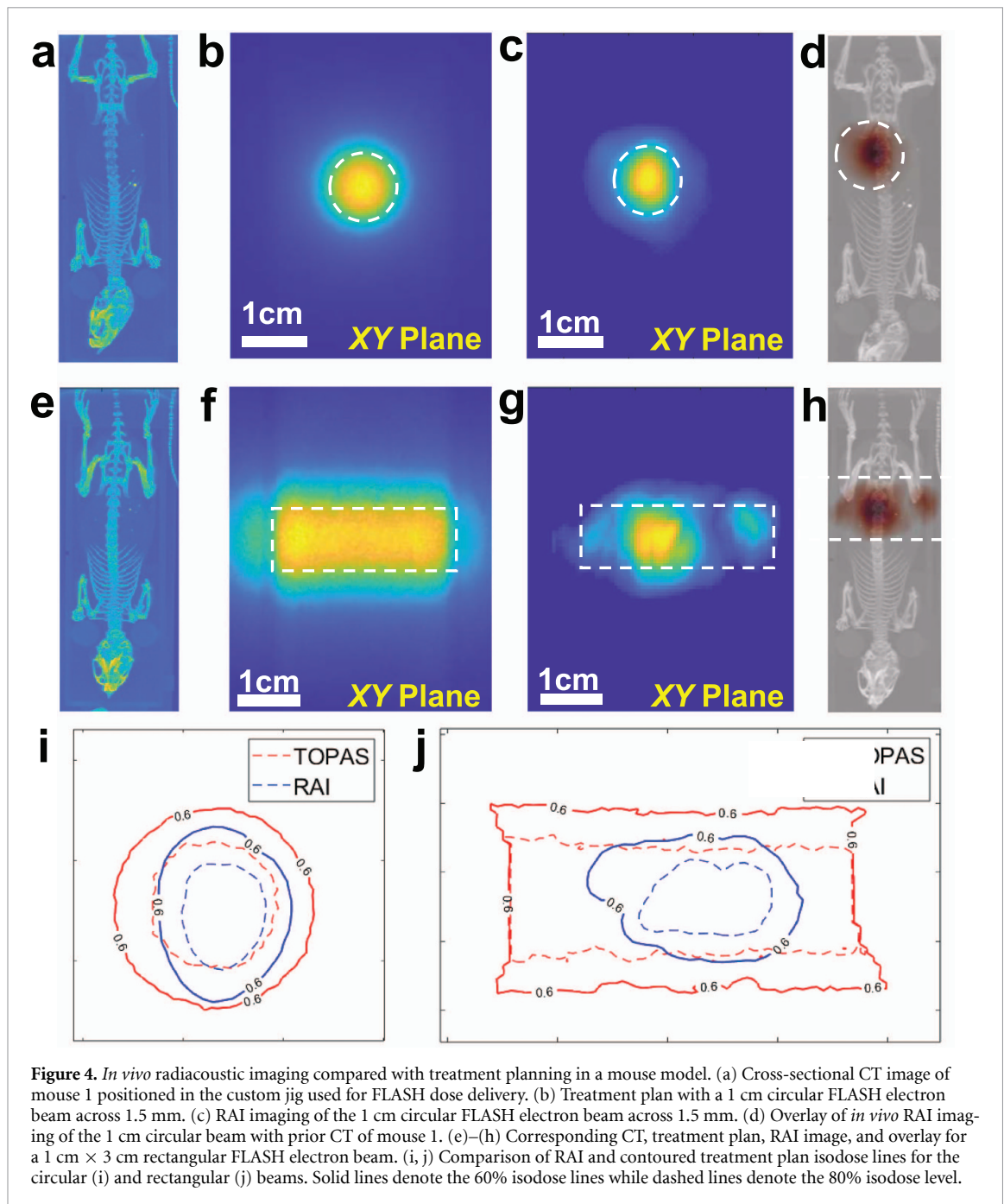
(figure 3(b)), and 1 cm × 3 cm rectangular (figure 3(c)) fields. Representative XY-plane images reconstructed with UBP for the 1 cm circle (figure 3(d)), 1 cm × 1 cm square (figure 3(e)), and 1 cm × 3 cm rectangle (figure 3(f)) are shown qualitatively. Meanwhile, representative XY-plane images reconstructed with MB algorithms for the 1 cm circle (figure 3(g)), 1 cm × 1 cm square (figure 3(h)), and 1 cm × 3 cm rectangle (figure 3(i)) are shown quantitatively, utilizing empirical calibrations. Due to the need for empirical calibration, this quantitative method is not absolute and requires calibration to reference methods. For the calibration here, the 1 cm circle radiacoustic signals, single-pulse, two-pulse, five-pulse, and ten-pulse aggregates were reconstructed and utilized in conjunction with film dosimetry.

The beam line profiles, shown in figure 1(d), demonstrate the increase in reconstruction intensity [a.u.] based on accumulated dose and the linearity shown in figure 1(e) allows for conversion from reconstruction intensity to dose, as shown in equation (12). Both reconstructions were compared to film measurements acquired at 10 mm depth in solid water, demonstrating electron scattering of the beam in homogenous medium. The MB reconstructions produced sharper, artifact-free images that more closely matched the reference film data. Isodose overlays for each collimator with consistent alignment of the 0.6 and 0.8 dose contours of film dose are shown in solid and dashed lines respectively (figure 3(m)–(o)); the isodose lines of the qualitative UBP mapping and quantitative MB dose mapping demonstrated excellent spatial agreement to those of film across all fields.

Quantitative comparisons confirmed the high fidelity of the RAI system. Gamma index analysis (3%/3 mm criteria) showed pass rates above 96% for both the circular and square beams which are above clinical standards for passing, with RMSE <0.11 and SSIM >0.8 (table 2). For the larger 1 cm × 3 cm rectangular field, pass rates were lower, likely due to reduced sensitivity at the array periphery and the limited angle inherent in the transducer geometry. Quantitative analysis performed on the center of the rectangle, with the film and reconstruction cropped to the same size as the 1 cm collimators from the center thus ignoring the beam edges in the 3 cm direction as denoted by the red dashed lines in figures 3(f) and (l), demonstrated much higher pass rates (90% for 3%/3 mm criteria) and improvements in both RMSE and SSIM. Additional validation using larger and more conformal field shapes (e.g., 2 cm × 2 cm square and 1 cm × 2.5 cm conformal rectangle) demonstrated the system's ability to resolve subtle differences in field geometry despite the 5 cm × 5 cm physical aperture of the matrix array (supplementary figure S5), further supporting the system's capability for high-resolution, quantitative FLASH dosimetry—critical for enabling adaptive FLASH radiotherapy.

3.3. *In vivo* dosimetry in animal models

In vivo validation was performed in two mice irradiated using three collimators: a 1 cm circle (Mouse 1), and both a 1 cm × 1 cm square and 1 cm × 3 cm rectangle (Mouse 2). Pre-treatment CT scans were used to generate TOPAS Monte Carlo-based treatment plans (Perl *et al* 2012, Faddegon *et al* 2020). A CT cross-section image for each of the mice is shown in figure 4(a), (e), respectively, along with treatment plans across 1.5 mm at 10 mm depth into the mouse (figures 4(b), (f)). During treatment, radiacoustic signals from each pulse were captured and reconstructed in real time. Dose maps were aligned with CT volumes by registering the matrix array center to the film slot visible in the mouse jig CT, based on the fixed positioning of the transducer to mouse due to the custom designed 3D printed holder (supplementary figure S6), enabling accurate anatomical overlay of RAI reconstructions (figures 4(c) and (g)) onto the prior CTs (figures 4(d) and (h)). Comparison between planned and reconstructed doses revealed slight beam offset (figures 4(d) and (h)) from the mouse center, consistent with observed shifts in beam entry confirmed by light field positioning during setup (supplementary figure S6d). For accurate comparison, RAI dose maps were compared with treatment plans segmented to only the dose within the mouse; as RA signals will only be generated where dose is deposited, electrons in the air surrounding the mouse were disregarded. Isodose line overlays (figure 4(i)) and RMSE analysis (0.0535) confirmed good alignment in shape and location for the 1 cm circle irradiation. For the 1 cm × 3 cm rectangle, isodose



line comparisons (figure 4(j)) demonstrate good alignment in location for the x -axis and RMSE analysis (0.1455) demonstrates greater values than the 1 cm circle delivery. Notably, high-intensity hotspots observed in the RAI reconstructions corresponded with bone structures in the CT, consistent with increased radiacoustic signal from dense tissues. The location of the spine corresponded to most of the 1 cm circular field but only a small portion of the 1 cm × 3 cm rectangular field, creating the difference in isodose lines and RMSE values observed. To ensure the difference in RMSE values for the two deliveries was due to the field size and not mouse coupling or placement, treatment plans and radiacoustic signals of the 1 cm × 1 cm square delivery in mouse 2 were compared (figure 5(f)) and an RMSE value of 0.0444 was found, akin to the 1 cm circle delivery in mouse 1. Future work incorporating the heterogeneity of the mouse, utilizing the prior CT information to incorporate varying Gruneisen parameters and speed of sounds based on different tissues into the MB algorithm will further inform the understanding of the hot spots present in these dose maps, allowing for more robust dose mapping *in vivo*.

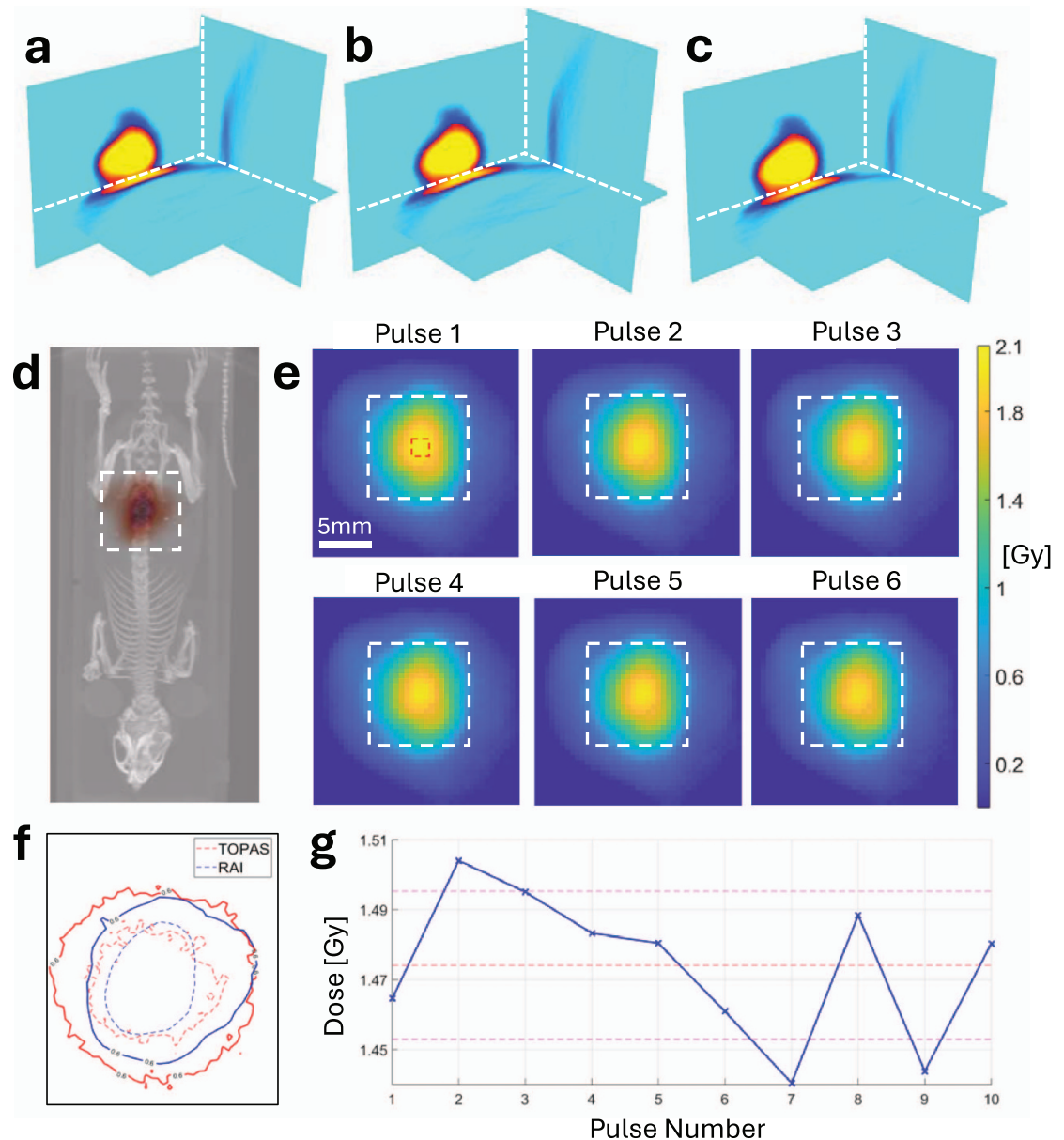


Figure 5. 4D tracking individual pulses during FLASH electron therapy. (a)–(c) 3D volumetric imaging of 1 × 1 cm square FLASH electron beam pulses 2, 5, and 7 respectively, delivered into a mouse. (d) CT cross-sectional image of a mouse with the 1 × 1 cm square RAI image co-registered. The white outline demonstrates beam size and location. (e) XY plane (3 slice aggregates) from reconstructions of 6 consecutive individual pulses of the 1 × 1 cm square delivered within the mouse. (f) Isodose lines showing TOPAS-based treatment plans of the 1 × 1 cm square field in Mouse 2 in red and the corresponding RAI signal in blue. 60% isodose lines are denoted with solid lines while 80% isodose lines are denoted with dashed lines. (g) Averaged dose across a small ROI at the beam center from slices as shown by the red square (e), pulse 1) for all 10 pulses. Red dashed line represents the mean dose while magenta lines represent ± 1 standard deviation. Variance (1.4442 ± 0.0197 Gy) was found to be 1.36% between the 10 pulses for the 1 × 1 cm square.

3.4. Real-time imaging of individual FLASH pulses

One of the key advantages of our system is its ability to resolve and reconstruct individual FLASH pulses in real time. This was demonstrated in Mouse 2, where the full 10-pulse treatment was captured and reconstructed on a pulse-by-pulse basis for the 1 cm × 1 cm square field in 3D; single pulse 3D reconstructions are shown for pulse 2 (figure 5(a)), pulse 5 (figure 5(b)), and pulse 7 (figure 5(c)). Across the 10 pulses, pulse-to-pulse dose variation (1.4442 ± 0.0197 Gy) was measured at 1.36%, indicating consistent delivery. BCT signals measured the dose variance to be between 2.46 and 3.44% in the beam line before dose deposition in mouse. The lower variability in RAI measurements can be accounted for by the small ROI used to measure dose, a method film dosimetry employs, and the electron scattering in air before deposition within the mouse body while the integrated BCT dose captures all electrons in the beam. Variability across collimators, including the 1 cm circle and 1 cm × 3 cm rectangle *in vivo*, was similarly quantified (supplementary figure S8) and corresponding 60% and 80% isodose

lines showed minimal shape variation across pulses *in vivo*. The degree of variability was similar to the in water variation (**supplementary figure S9**). **supplementary videos 10–12** provide real-time playback of radiacoustic reconstructions across all individual pulses, illustrating the feasibility of dynamic dose monitoring *in vivo*. This capability supports future development of adaptive FLASH delivery systems that can respond to motion, positioning shifts, or delivery inconsistencies in real time.

4. Discussion

We report the development and *in vivo* validation of a next-generation RAI system that enables real-time, single-pulse, three-dimensional, quantitative dosimetry for FLASH radiotherapy. Combining a custom-built 256-element matrix-array ultrasound transducer and a MB reconstruction framework and empirical calibration, our platform provides volumetric maps of radiation dose deposition at a microsecond resolution. Comparing against gold standard radiochromic film and Monte Carlo-based treatment planning demonstrated high spatial accuracy and quantitative fidelity across multiple beam geometries.

To our knowledge, this is the first demonstration of *in vivo*, pulse-resolved, quantitative 3D FLASH dosimetry—marking a critical step toward clinical translation of adaptive FLASH therapy.

Accurate 3D dosimetry is essential for verifying beam targeting, assessing delivery accuracy, and adapting treatment in response to anatomical variation. One of the major technical challenges encountered in RAI is the limited angular coverage inherent to with matrix-array ultrasound transducers. While the 256-element matrix enables volumetric imaging, it cannot capture the full spherical wavefronts, leading to reconstruction artifacts particularly at the periphery of large or asymmetric beams. We address this in part by acquiring data from multiple orientations to improve spatial sampling, as shown in figure 2, resulting in PDD curves with close agreement to film-based measurements. RAI PDD curves had high alignment with film-derived PDD curves for the 1 cm × 3 cm collimator, with $d_{\max}$ aligning within 0.2 mm accuracy (figure 2(h)). Film-derived PDD curve comparison to RAI-derived PDD curves for the 1 cm circle (**supplementary figure 1(b)**) and the 1 cm × 1 cm square (**supplementary figure 1(c)**) maintained this high alignment, with $d_{\max}$ accuracy from 0.5 to 2.5 mm.

Our MB reconstruction framework mitigated some of these limitations by incorporating system geometry and acoustic propagation priors, significantly improving spatial resolution and suppressing artifacts compared to traditional UBP (Pandey *et al* 2021a, 2021c). Still, residual errors remain in regions with limited angle visibility especially in off-center or large field configurations (figure 3 (l), (o)). These are further compounded by large distances to the transducer and the impacts of transducer size. Emerging deep learning approaches (Jiang *et al* 2022, Yao *et al* 2022, Lang *et al* 2024a, 2024b) may offer a path forward: by learning to infer missing angular information from training data, these models could enable high-fidelity reconstructions from single-view acquisitions. Currently, new physics informed neural networks, where a physics based constraint is applied to the deep learning model, are showing great promise in improving z-line information and mitigating limited angle issues. Combining MB solvers with these data-driven priors may further enhance reconstruction robustness and generalizability in heterogeneous tissues and clinical scenarios.

This *in vivo* imaging also demonstrates hot spots in all three beam deliveries, across both mice. In all three, the hot spots align with the locations of bones, as shown in the CT overlays (figure 4(d),(h), figure 5(d)). Dense tissues have higher absorption coefficients; the matching of the hot spots with anatomical structures further demonstrates the accuracy of the RAI dose mapping. For the smaller collimator isodose lines, namely the 1 cm circle (figure 4(i)) and the 1 cm × 1 cm square (figure 5(f)), there is higher congruity between the treatment plans and the RAI dose maps due to the size of the hot spot and its location in relation to dose deposition; for both the 1 cm circle (figure 4(d)) and the 1 cm × 1 cm square (figure 5(d)), the hot spot is located approximately at the center of the beam and takes up the majority of the beam. Meanwhile, the larger 1 cm × 3 cm rectangular beam, demonstrates three hot spots (figure 4(h)) that again coincide with bone: the spine and both knees of the mouse. This heterogeneity in RAI dose map corresponds to the tissue heterogeneity *in vivo*, while the treatment plan does not, furthering the importance of *in vivo* dose mapping. It is important to note that treatment plans were generated with prior CTs and mouse replacement in the jig occurred before irradiation, thus introducing small variation mouse positioning during RAI dosimetry. In addition to physiological changes between prior-CT and delivery of radiation, motion can impact both treatment delivery and RAI signals. The delivery of FLASH irradiation (0.333s for 10 pulses) is short. It is akin to 1 breath cycle of a non-anesthetized mouse and longer than the breath cycle of an anesthetized mouse, which should limit the impact of respiratory motion, but further study is needed to determine the impact on RAI accuracy. Additionally, in small animal models where the beam is larger than a single organ or tumor,

this impacts delivery less but does impact RAI overlay onto CT as the CT itself is static. Displacements are small compared to the transducer size, but would create an impact in larger animal study. All *in vivo* experiments were performed in anesthetized mice, which represent a substantially different acoustic and dosimetric environment compared to large animal or human patients, and generalization to large-scale anatomy with deeper targets, larger tissue volumes, and different acoustic propagation characteristics will require systematic validation before pre-clinical and clinical translation. For larger animal models such as porcine, feline, and canine models which have been used in preclinical FLASH studies and for patients, this limitation needs to be addressed. Current research focusing on dual modal US + RAI, where ultrasound monitoring of the tumor site would allow for overlay of the RAI reconstructions on active anatomical information would further address this limitation.

This study also introduces, for the first time, real-time pulse-by-pulse monitoring of FLASH beam delivery *in vivo*. FLASH delivery schemes, where entire therapeutic doses are delivered in just a few pulses, present a unique need for pulse-resolved 3D imaging (Di Martino *et al* 2020, Böhlen *et al* 2022, Romano *et al* 2022). Small anatomical shifts or delivery deviations between pulses can lead to substantial dose misplacement; this underdosing affects tumor control while the resulting overdosing affects normal tissue, especially radiosensitive and re-irradiated tissues (Yoganathan *et al* 2017, Meyers *et al* 2025). The ability to capture and reconstruct each pulse with RAI could allow for adaptive strategies even at FLASH dose rates that dynamically adjust to motion, anatomy, or delivery inconsistencies during treatment. These pulse-resolved dose maps could be fed back to the treatment control system to enable intrafraction correction, halting or modifying delivery if the accumulated dose deviates beyond a predefined threshold before the next pulse is triggered. Within a fraction, the continuously updated cumulative dose map would enable real-time adaptive replanning, where beam parameters can be adjusted on-the-fly in response to measured dose-anatomy mismatches. Over longer timescales, the quantitative dose maps accumulated across fractions would provide a direct measurement-based input to offline adaptive radiotherapy workflows, enabling plan adaptation driven by actual delivered dose distributions rather than solely by anatomical imaging, a capability of particular significance for FLASH regimens where a single ultra-high-dose fraction leaves no margin for undetected delivery error.

The integration of RAI with anatomical imaging modalities, such as B-mode or pulse-echo ultrasound, offers a compelling vision for next-generation image-guided FLASH-RT. While pulse-echo ultrasound can provide structural localization and motion tracking, RAI complements it with quantitative, volumetric dose verification at the single-pulse level to avoid overdosing sensitive structures and reduce the risk of irreversible toxicity, one of the most promising and clinically relevant applications of FLASH therapy. Such a dual-modality system could be particularly impactful in high-risk clinical scenarios, such as FLASH-RT, re-irradiation, or treatment margins including radiosensitive tissues, where precise verification of cumulative dose is essential.

Finally, by co-registering RAI dose maps with prior CTs, we demonstrate the potential for anatomical co-localization of dose *in vivo*. While the RAI hotspots aligned with bone structures, signal enhancement in bone may also be impacted by property-driven amplitude increases and may not be automatically proportional to dose increase by the same factor. Future work will focus on incorporating patient specific anatomical priors into the reconstruction pipeline, enabling personalized compensation for tissue heterogeneity and speed-of-sound variations, allowing for increased accuracy of dose mapping *in vivo*. Gruneisen parameters, for example, can vary widely *in vivo* from water and liver tissue at 0.12 to fat at 0.7 and higher for denser tissues (Chen *et al* 2023), impacting dose absorption and RA signal generation. Speed of sound can also vary drastically, impacting RA signal propagation. Additionally, registering RAI dose maps to the CT-informed treatment plan dose distributions enables more informed assessment of treatment accuracy and supports closed-loop feedback systems for real-time quality assurance (Lyu *et al* 2022).

Together, these results establish RAI as a promising platform for quantitative FLASH dosimetry, with the capacity to transform real-time dose delivery verification and subsequent treatment adaptation in clinical radiation oncology.

5. Limitations and scope of future research

While the studies performed indicate the capability of RAI for 3D dose imaging, discrepancies do exist between the reconstructed and reference dose maps. The observed discrepancies arise primarily due to the limited view problem and transducer frequency bandwidth. Limited-view artifacts arise from the small transducer aperture. The finite angular coverage of the detector array means that not all spatial

frequency components of the dose distribution are captured, leading to characteristic limited-view artifacts in the reconstructions (Deán-Ben and Razansky 2016, Deán-Ben *et al* 2017, Pandey *et al* 2025). This is a well-known challenge in photoacoustic and RAI and is not unique to our system. Secondly, finite frequency bandwidth of the transducer modulates the information in the collected frequency band, which is also a source of inaccuracy. Additionally, radiacoustic frequency components lying outside the transducer passband are attenuated or lost, introducing amplitude scaling errors in the reconstructed dose maps; dedicated impulse response calibration studies with incorporation of a frequency-dependent sensitivity model as a deconvolution operator in the reconstruction, as well as AI-driven bandwidth enhancement approaches (Awasthi *et al* 2020), represent promising avenues for addressing this in future work. Critically, each of these limitations is addressable through established methods: larger or conformal detector arrays to extend angular coverage, hardware improvements to extend bandwidth and SNR. We also plan to conduct systematic calibration studies to characterize the total system impulse response more accurately and incorporate it directly into the reconstruction algorithm. The results presented in figures 2–5, while showing discrepancies, successfully demonstrate that the radiacoustic signal carries quantitative 3D dose information that is recoverable through MB reconstruction. Systematic improvement of reconstruction accuracy through the methods outlined above will be the focus of our future work.

The acoustic wave propagation model employed in this study assumes homogeneous acoustic properties throughout the tissue. Although tissue heterogeneity can introduce reconstruction errors in RAI studies in both animals and humans, this assumption is well-precedented in small animal photoacoustic imaging: a substantial body of PAT literature employing *in vivo* mouse models has adopted the homogeneous tissue approximation in image reconstruction without significant loss (Brecht *et al* 2009a, 2009b, Merčep *et al* 2015, Kalva *et al* 2021). Nonetheless, the assumption of homogeneous medium for *in vivo* dosimetry can affect the accuracy of dose mapping. Conveniently, radiation therapy workflows already require a planning CT prior to treatment, which provides the anatomical and tissue composition information needed to derive spatially varying acoustic parameters. This prior CT can therefore be directly leveraged to incorporate heterogeneous acoustic models (Yu *et al* 2019) with numerically computed Green's functions into the RAI reconstruction pipeline and represents a concrete and planned step toward addressing this limitation in future clinical translation work. Incorporating patient-specific heterogeneous acoustic models will be necessary for pre-clinical and clinical translation, and we intend to address this in the context of further *in vivo* RAI studies as part of our future work.

As mentioned in methods, another limitation of this study is the current implementation of offline image reconstruction utilizing CPUs. For online real-time feedback, the use of GPU acceleration of the forward model operators, the largest source of computation time costs, has shown clinically relevant frame rate reconstructions for 3D PAT (Wang *et al* 2013) and can be applied to RAI as well. With the 33 ms time budget for reconstructions in this experimental configuration, GPU acceleration makes online feedback achievable and is planned future work for this RAI-guided electron FLASH delivery system. Another avenue to be explored is the use of deep-learning neural networks which can reduce computational time to around 31 ms per frame (Dehner *et al* 2023) in 3D PAT as well.

An inherent limitation of RAI is its requirement for a certain temporal structure of the irradiation. As evident from equation (1), radiacoustic signal generation is proportional to $\partial D/\partial t$, which fundamentally couples signal strength to the temporal structure of the irradiation. The radiacoustic signal generation, by physics, as evident from equation (1) requires temporally varying radiation. Radiacoustic signal generation requires that dose be deposited faster than the acoustic transit time (stress confinement) and heat diffusion (thermal confinement) across the heated volume (Wang and Wu 2012). For the FLASH electron beam used in this work, with microsecond-scale pulse widths and heated volumes on the order of centimeters, this condition is well satisfied, yielding strong acoustic emissions amenable to tomographic reconstruction. For longer pulse durations, stress confinement progressively breaks down, reducing signal amplitude and shifting the acoustic frequency content toward lower frequencies. However, complete stress confinement is not a strict requirement for RAI—reasonable radiacoustic signals have been reported in proton-based RAI for pulse durations of up to 26 μs (Wang *et al* 2024a), demonstrating that useful signal can be recovered beyond the strict confinement regime. Furthermore, finite pulse duration effects can be incorporated directly into the forward model through temporal convolution of the ideal impulse response with the known pulse time profile, thereby reducing their impact on quantitative reconstruction accuracy. This approach extends the practical applicability of the RAI framework to delivery conditions where strict stress confinement is not achievable. However, this makes this methodology not suitable for continuous radiation.

Moreover, higher PRFs and quasi-continuous irradiation represent points on a continuum of the same underlying physical challenge. At higher PRFs, acoustic emissions from successive pulses begin to

overlap temporally at the detector, complicating signal separation within the single-pulse reconstruction framework. In the limiting case of quasi-continuous delivery—such as from an isochronous cyclotron, where the nanosecond-scale RF micropulse structure produces a beam that is macroscopically continuous on the acoustic timescale— $\partial D/\partial t$ approaches zero at the macroscopic level, yielding a negligible acoustic source term and consequently very weak radiacoustic signals. RAI in its current form is therefore not directly applicable to quasi-continuous delivery (Montay-Gruel *et al* 2018, Patriarca *et al* 2018, Girdhani *et al* 2019, Diffenderfer *et al* 2020).

6. Conclusion

In conclusion, this paper has demonstrated the spatial fidelity of the RAI system in pulse-resolved, 3D quantitative dose mapping in both homogenous water medium and *in vivo*. To our knowledge, this is the first demonstration of quantitative RAI monitoring of electron FLASH *in vivo*. The RAI system is capable of reconstructing pulse-resolved 3D dose maps of varying geometry *in vivo* with doses up to 1.4 Gy per pulse. The spatial accuracy of mapping different beam shapes was shown with above 90% passing rates at 3 mm 3% accuracy in water compared to radiochromic film with high structural similarity *in vivo* compared to treatment plans. These experiments have shown the feasibility of RAI-monitoring for pre-clinical and clinical FLASH-RT trials, highlighting RAI's potential to track dose deposition in each pulse, and thus monitor instantaneous dose rate, *in vivo*. Further work in larger animal models is ongoing with the integration of a dual modal pulse-echo ultrasound, which will allow for motion tracking of the target site and dose mapping to be anatomically resolved to include the effects of motion.

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Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

Supplementary figures & Tables available at <https://doi.org/10.1088/1361-6560/ae7e38/data1>.

SF10: M1 Single Pulse Circle available at <https://doi.org/10.1088/1361-6560/ae7e38/data2>.

SF12: M2 Single Pulse Rectangle available at <https://doi.org/10.1088/1361-6560/ae7e38/data3>.

SF11: M2 Single Pulse Square available at <https://doi.org/10.1088/1361-6560/ae7e38/data4>.

Ethical statement

Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas MD Anderson Cancer Center (Protocol #00002166-RN01). All procedures were conducted in an AAALAC-accredited facility in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and the PHS Policy on Humane Care and Use of Laboratory Animals (Assurance #A3343-01).

Conflict of interest

The authors declare that they have no competing interests. Dr Xiang is the co-founder of SoundDose and SoundDose is not supporting this work.

Author contributions

The study was conceptualized and initiated by Shawn (Liangzhong) Xiang, who also supervised the overall project design and manuscript preparation. Kristina Bjegovic conducted RAI experiments for FLASH electron beam with Lucy Whitmore and Luke Connell. Kristina Bjegovic performed RAI data analysis. Lucy Whitmore performed film analysis and generated treatment plans with her custom model of the Mobetron. Prabodh Pandey Kumar supported model-based reconstruction algorithm work. Luke Connell created all collimators. Yifei Xu supported 3D printing and design for the transducer holders. Edgardo Aguilar acted as the biologist for the *in vivo* experiments, and Brett Velasquez conducted the CT scans. The manuscript was written as a collaborative effort led by Shawn Xiang and Emil Schueler, with input and contributions from all co-authors.

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References

- Anon n.d. Structural similarity (SSIM) index for measuring image quality—MATLAB ssim (available at: www.mathworks.com/help/images/ref/ssim.html)
- Archer J, Li E, Davis J, Cameron M, Rosenfeld A and Lerch M 2019 High spatial resolution scintillator dosimetry of synchrotron microbeams *Sci. Rep.* **9** 6873
- Ashraf M R, Rahman M, Zhang R, Williams B B, Gladstone D J, Pogue B W and Bruza P 2020 Dosimetry for FLASH radiotherapy: a review of tools and the role of radioluminescence and Cherenkov emission *Front. Phys.* **8** 328
- Attia A B E, Balasundaram G, Moothanchery M, Dinish U S, Bi R, Ntziachristos V and Olivo M 2019 A review of clinical photoacoustic imaging: current and future trends *Photoacoustics* **16** 100144
- Awasthi N, Jain G, Kalva S K, Pramanik M and Yalavarthy P K 2020 Deep neural network-based sinogram super-resolution and bandwidth enhancement for limited-data photoacoustic tomography *IEEE Trans. Ultrason., Ferroelect., Freq. Contr.* **67** 2660–73
- Beyreuther E, Brand M, Hans S, Hideghéty K, Karsch L, Leßmann E, Schürer M, Szabó E R and Pawelke J 2019 Feasibility of proton FLASH effect tested by zebrafish embryo irradiation *Radiother. Oncol.* **139** 46–50
- Bjegovic K et al 2024 4D *in vivo* dosimetry for a FLASH electron beam using radiation-induced acoustic imaging *Phys. Med. Biol.* **69** 115053
- Böhlen T T et al 2022 Normal tissue sparing by FLASH as a function of single-fraction dose: a quantitative analysis *Int. J. Radiat. Oncol. Biol. Phys.* **114** 1032–44
- Bourhis J et al 2019 Treatment of a first patient with FLASH-radiotherapy *Radiother. Oncol.* **139** 18–22
- Brecht H-P F, Su R, Fronheiser M P, Ermilov S A, Conjusteau A and Oraevsky A A 2009a Whole-body three-dimensional optoacoustic tomography system for small animals *J. Biomed. Opt.* **14** 1
- Brecht H-P et al 2009b *Optoacoustic 3D Whole-body Tomography: Experiments in Nude Mice* SPIE BiOS: Biomedical Optics ed A A Oraevsky and L V Wang p 71770E (available at: <http://proceedings.spiedigitallibrary.org/proceeding.aspx?doi=10.1117/12.812319>)
- Cai J, Read P W and Sheng K 2008 The effect of respiratory motion variability and tumor size on the accuracy of average intensity projection from four-dimensional computed tomography: an investigation based on dynamic MRI *Med. Phys.* **35** 4974–81
- Cao R et al 2023 Optical-resolution photoacoustic microscopy with a needle-shaped beam *Nat. Photon.* **17** 89–95
- Chaoui M, Bouhali O and Tayalati Y 2025 FLASH radiotherapy: technical advances, evidence of the FLASH effect and mechanistic insights *Biomed. Phys. Eng. Express* **11** 022003
- Chen F, Sun M, Chen R, Li C and Shi J 2023 Absolute Grüneisen parameter measurement in deep tissue based on x-ray-induced acoustic computed tomography *Biomed. Opt. Express* **14** 1205
- Daugherty E C et al 2022 FAST-01: results of the first-in-human study of proton FLASH radiotherapy *Int. J. Radiat. Oncol. Biol. Phys.* **114** S4
- Daugherty E C et al 2024 FLASH radiotherapy for the treatment of symptomatic bone metastases in the thorax (FAST-02): protocol for a prospective study of a novel radiotherapy approach *Radiat. Oncol.* **19** 34
- Dawson L A, Mah K, Franssen E and Morton G 1998 Target position variability throughout prostate radiotherapy *Int. J. Radiat. Oncol. Biol. Phys.* **42** 1155–61
- Deán-Ben X L, Ding L and Razansky D 2017 Dynamic particle enhancement in limited-view optoacoustic tomography *Opt. Lett.* **42** 827
- Deán-Ben X L and Razansky D 2016 On the link between the speckle free nature of optoacoustics and visibility of structures in limited-view tomography *Photoacoustics* **4** 133–40
- Dehner C, Zahnd G, Ntziachristos V and Jüstel D 2023 A deep neural network for real-time optoacoustic image reconstruction with adjustable speed of sound *Nat. Mach. Intell.* **5** 1130–41
- Di Martino F et al 2020 FLASH radiotherapy with electrons: issues related to the production, monitoring, and dosimetric characterization of the beam *Front. Phys.* **8** 570697
- Diffenderfer E S et al 2020 Design, implementation, and *in vivo* validation of a novel proton FLASH radiation therapy system *Int. J. Radiat. Oncol. Biol. Phys.* **106** 440–8

- Ding L, Razansky D and Dean-Ben X L 2020 Model-based reconstruction of large three-dimensional optoacoustic datasets *IEEE Trans. Med. Imaging* **39** 2931–40
- Dragun A.E. et al 2013 Electronic portal imaging devices (EPID) *Encyclopedia of Radiation Oncology* ed L W Brady and T E Yaeger (Springer) pp 207–13
- Drayton O G et al 2024 A multi-institutional study to investigate the sparing effect after whole brain electron FLASH in mice: reproducibility and temporal evolution of functional, electrophysiological, and neurogenic endpoints *Radiother. Oncol.* **201** 110534
- Esplen N, Mendonca M S and Bazalova-carter M 2020 Physics and biology of ultrahigh dose-rate (FLASH) radiotherapy: a topical review *Phys. Med. Biol.* **65** 23TR03
- Faddegon B, Ramos-méndez J, Schuemann J, McNamara A, Shin J, Perl J and Paganetti H 2020 The TOPAS tool for particle simulation, a Monte Carlo simulation tool for physics, biology and clinical research *Phys. Med.* **72** 114–21
- Fontenla D P, Yarparpalvi R, Chui C-S and Briot E 1996 The use of diode dosimetry in quality improvement of patient care in radiation therapy *Med. Dosim.* **21** 235–41
- Gaide O et al 2022 Comparison of ultra-high versus conventional dose rate radiotherapy in a patient with cutaneous lymphoma *Radiother. Oncol.* **174** 87–91
- Girdhani S, Abel E, Katsis A, Rodriquez A, Senapati S, KuVillanueva A, Jackson I L, Eley J, Vujaskovic Z and Parry R 2019 Abstract LB-280: FLASH: a novel paradigm changing tumor irradiation platform that enhances therapeutic ratio by reducing normal tissue toxicity and activating immune pathways *Cancer Res.* **79** LB-280–LB-280
- Gómez F et al 2022 Development of an ultra-thin parallel plate ionization chamber for dosimetry in FLASH radiotherapy *Med. Phys.* **49** 4705–14
- Gonçalves Jorge P, Grilj V, Bourhis J, Vozenin M, Germond J, Bochud F, Bailat C and Moeckli R 2022 Technical note: validation of an ultrahigh dose rate pulsed electron beam monitoring system using a current transformer for FLASH preclinical studies *Med. Phys.* **49** 1831–8
- Hansen P C 2001 The L-curve and its use in the numerical treatment of inverse problems *Computational Inverse Problems in Electrocardiology* (WIT Press) pp 119–42
- Hansen P C 2007 Regularization tools version 4.0 for Matlab 7.3 *Numer. Algor.* **46** 189–94
- Hauptmann A, Lucka F, Betcke M, Huynh N, Adler J, Cox B, Beard P, Ourselin S and Arridge S 2018 Model-based learning for accelerated, limited-view 3-D photoacoustic tomography *IEEE Trans. Med. Imaging* **37** 1382–93
- Hickling S, Hobson M and El Naqa I 2018 Characterization of x-ray acoustic computed tomography for applications in radiotherapy dosimetry *IEEE Trans. Radiat. Plasma Med. Sci.* **2** 337–44
- Hickling S, Leger P and El Naqa I 2016 On the detectability of acoustic waves induced following irradiation by a radiotherapy linear accelerator *IEEE Trans. Ultrason., Ferroelect., Freq. Contr.* **63** 683–90
- Jansen J et al 2025 Differentiating unirradiated mice from those exposed to conventional or FLASH radiotherapy using MRI (<https://doi.org/10.1101/2025.02.01.636061>)
- Jeong S, Yoon M, Chung K, Ahn S H, Lee B and Seo J 2020 Clinical application of a gantry-attachable plastic scintillating plate dosimetry system in pencil beam scanning proton therapy beam monitoring *Phys. Med.* **77** 181–6
- Jiang Z, Sun L, Yao W, Wu Q J, Xiang L and Ren L 2022 3D in vivo dose verification in prostate proton therapy with deep learning-based proton-acoustic imaging *Phys. Med. Biol.* **67** 215012
- Jursinic P A 2013 Dependence of diode sensitivity on the pulse rate of delivered radiation: dependence of diode sensitivity-to-dose on the pulse rate *Med. Phys.* **40** 021720
- Kalva S K, Dean-Ben X L and Razansky D 2021 Single-sweep volumetric optoacoustic tomography of whole mice *Photon. Res.* **9** 899
- Karsch L et al 2022 Beam pulse structure and dose rate as determinants for the flash effect observed in zebrafish embryo *Radiother. Oncol.* **173** 49–54
- Khan F M and Gibbons J P 2020 *Khan's the Physics of Radiation Therapy* (Wolters Kluwer)
- Lang Y, Jiang Z, Sun L, Tran P, Mossahebi S, Xiang L and Ren L 2024a Patient-specific deep learning for 3D protoacoustic image reconstruction and dose verification in proton therapy *Med. Phys.* **51** 7425–38
- Lang Y, Jiang Z, Sun L, Xiang L and Ren L 2024b Hybrid-supervised deep learning for domain transfer 3D protoacoustic image reconstruction *Phys. Med. Biol.* **69** 085007
- Lattanzi J, McNeeley S, Donnelly S, Palacio E, Hanlon A, Schultheiss T E and Hanks G E 2000 Ultrasound-based stereotactic guidance in prostate cancer—quantification of organ motion and set-up errors in external beam radiation therapy *Comput. Aided Surg.* **5** 289–95
- Lei H, Zhang W, Oraiqat I, Liu Z, Ni J, Wang X and El Naqa I 2018 Toward in vivo dosimetry in external beam radiotherapy using x-ray acoustic computed tomography: a soft-tissue phantom study validation *Med. Phys.* **45** 4191–200
- Levy K et al 2020 Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice *Sci. Rep.* **10** 21600
- Li L et al 2017 Single-impulse panoramic photoacoustic computed tomography of small-animal whole-body dynamics at high spatiotemporal resolution *Nat. Biomed. Eng.* **1** 0071
- Li M et al 2025 Molecular mechanisms of lung injury from ultra high and conventional dose rate pulsed radiation based on 4D DIA proteomics study *Sci. Rep.* **15** 5425
- Lin L et al 2021 High-speed three-dimensional photoacoustic computed tomography for preclinical research and clinical translation *Nat. Commun.* **12** 882
- Liu K et al 2025 Redefining FLASH radiation therapy: the impact of mean dose rate and dose per pulse in the gastrointestinal tract *Int. J. Radiat. Oncol. Biol. Phys.* **121** 1063–76
- Liu K, Holmes S, Hooten B, Schüler E and Beddar S 2024a Evaluation of ion chamber response for applications in electron FLASH radiotherapy *Med. Phys.* **51** 494–508
- Liu K, Holmes S, Khan A U, Hooten B, DeWerd L, Schüler E and Beddar S 2024b Development of novel ionization chambers for reference dosimetry in electron flash radiotherapy *Med. Phys.* **51** 9275–89
- Liu K, Holmes S, Schüler E and Beddar S 2024c A comprehensive investigation of the performance of a commercial scintillator system for applications in electron FLASH radiotherapy *Med. Phys.* **51** 4504–12
- Liu K, Jorge P G, Taylor R, Moeckli R and Schüler E 2023a Comprehensive evaluation and new recommendations in the use of Gafchromic EBT3 film *Med. Phys.* **50** 7252–62
- Liu K, Palmiero A, Chopra N, Velasquez B, Li Z, Beddar S and Schüler E 2023b Dual beam-current transformer design for monitoring and reporting of electron ultra-high dose rate (FLASH) beam parameters *J. Appl. Clin. Med. Phys.* **24** e13891

- Low D A, Harms W B, Mutic S and Purdy J A 1998 A technique for the quantitative evaluation of dose distributions *Med. Phys.* **25** 656–61
- Lyu Q, Neph R and Sheng K 2022 Tomographic detection of photon pairs produced from high-energy x-rays for the monitoring of radiotherapy dosing *Nat. Biomed. Eng.* **7** 323–34
- Manohar S and Razansky D 2016 Photoacoustics: a historical review *Adv. Opt. Photon.* **8** 586
- Marroquin E Y L, Herrera González J A, Camacho López M A, Barajas J E V and García-garduño O A 2016 Evaluation of the uncertainty in an EBT3 film dosimetry system utilizing net optical density *J. Appl. Clin. Med. Phys.* **17** 466–81
- Mascia A E et al 2023 Proton FLASH radiotherapy for the treatment of symptomatic bone metastases: the FAST-01 nonrandomized trial *JAMA Oncol.* **9** 62
- McManus M, Romano F, Lee N D, Farabolini W, Gilardi A, Royle G, Palmans H and Subiel A 2020 The challenge of ionisation chamber dosimetry in ultra-short pulsed high dose-rate very high energy electron beams *Sci. Rep.* **10** 9089
- Merčep E, Burton N C, Claussen J and Razansky D 2015 Whole-body live mouse imaging by hybrid reflection-mode ultrasound and optoacoustic tomography *Opt. Lett.* **40** 4643
- Meyers T, Alsou N, Ahmad S and Ali I 2025 A four-dimensional dosimeter and quality assurance phantom to quantify respiratory motion effects on the dose delivery for adaptive radiation therapy *J. Radiother. Pract.* **24** e2
- Montay-Gruel P et al 2018 x-rays can trigger the FLASH effect: ultra-high dose-rate synchrotron light source prevents normal brain injury after whole brain irradiation in mice *Radiother. Oncol.* **129** 582–8
- Montay-Gruel P et al 2021 Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice *Clin. Cancer Res.* **27** 775–84
- Morrocchi M et al 2022 Experimental characterization and Monte Carlo simulation of scintillator detectors in online electron FLASH radiotherapy dosimetry *J. Inst.* **17** 09005
- Moustafa L et al 2024 Surprising dose dependent sparing effects of fractionated flash electrons in experimental radiation induced lung fibrosis model *Int. J. Radiat. Oncol. Biol. Phys.* **120** e390
- Oesterle R, Gonçalves Jorge P, Grilj V, Bourhis J, Vozenin M, Germond J, Bochud F, Bailat C and Moeckli R 2021 Implementation and validation of a beam-current transformer on a medical pulsed electron beam LINAC for FLASH-RT beam monitoring *J. Appl. Clin. Med. Phys.* **22** 165–71
- Oraiqat I, Zhang W, Litzenberg D, Lam K, Ba sunbul N, Moran J, Cuneo K, Carson P, Wang X and El Naqa I 2020 An ionizing radiation acoustic imaging (iRAI) technique for real-time dosimetric measurements for FLASH radiotherapy *Med. Phys.* **47** 5090–101
- Palmiero A et al 2025 On the acceptance, commissioning, and quality assurance of electron FLASH units *Med. Phys.* **52** 1207–23
- Pandey P K, Gonzalez G, Bjegovic K, Sun L, Chen Y and Xiang L 2025 3D photoacoustic radiography: a proof of principle study *Appl. Phys. Lett.* **126** 074102
- Pandey P K, Wang S, Aggrawal H O, Bjegovic K, Boucher S and Xiang L 2021a Model-based x-ray induced acoustic computed tomography *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* **68** 3560–9
- Pandey P K, Wang S and Xiang L 2021c Model based reconstruction algorithm for x-ray induced acoustic tomography *Proc. SPIE* **11595** 115953V
- Patriarca A et al 2018 Experimental set-up for FLASH proton irradiation of small animals using a clinical system *Int. J. Radiat. Oncol. Biol. Phys.* **102** 619–26
- Perl J, Shin J, Schumann J, Faddegon B and Paganetti H 2012 TOPAS: an innovative proton Monte Carlo platform for research and clinical applications *Med. Phys.* **39** 6818–37
- Petersson K, Jaccard M, Germond J, Buchillier T, Bochud F, Bourhis J, Vozenin M and Bailat C 2017 High dose-per-pulse electron beam dosimetry—A model to correct for the ion recombination in the advanced Markus ionization chamber *Med. Phys.* **44** 1157–67
- Pimpinella M, Andreoli S, De Angelis C, Della Monaca S, D'ariento M and Menegotti L 2019 Output factor measurement in high dose-per-pulse IORT electron beams *Phys. Med.* **61** 94–102
- Rahman M, Ashraf M R, Zhang R, Gladstone D J, Cao X, Williams B B, Jack Hoopes P, Pogue B W and Bruza P 2021 Spatial and temporal dosimetry of individual electron FLASH beam pulses using radioluminescence imaging *Phys. Med. Biol.* **66** 135009
- Rohrer Bley C et al 2022 Dose- and volume-limiting late toxicity of flash radiotherapy in cats with squamous cell carcinoma of the nasal planum and in mini Pigs *Clin. Cancer Res.* **28** 3814–23
- Romano F, Bailat C, Jorge P G, Lerch M L F and Darafsheh A 2022 Ultra-high dose rate dosimetry: challenges and opportunities for FLASH radiation therapy *Med. Phys.* **49** 4912–32
- Saenz F R et al 2025 FLASH radiotherapy spares lymphocytes in tumor-draining lymph nodes and increases infiltration of immune cells in tumors (<https://doi.org/10.1101/2025.04.07.647544>)
- Schot S H 1992 Eighty years of Sommerfeld's radiation condition *Historia Math.* **19** 385–401
- Shaharuddin S et al 2021 Real-time dosimetry of ultrahigh dose-rate x-ray beams using scintillation detectors 2021 *IEEE Sensors* (IEEE) pp 1–4 (available at: <https://ieeexplore.ieee.org/document/9639825/>)
- Soto L A et al 2020 FLASH irradiation results in reduced severe skin toxicity compared to conventional-dose-rate irradiation *Radiat. Res.* **194** 618–24
- Subiel A and Romano F 2023 Recent developments in absolute dosimetry for FLASH radiotherapy *Br. J. Radiol.* **96** 20220560
- Sun L et al 2023 Towards quantitative in vivo dosimetry using x-ray acoustic computed tomography *Med. Phys.* **16476**
- Treeby B E and Cox B T 2010 k-Wave: MATLAB toolbox for the simulation and reconstruction of photoacoustic wave fields *J. Biomed. Opt.* **15** 021314
- Valdés Zayas A et al 2023 Independent reproduction of the FLASH effect on the gastrointestinal tract: a multi-institutional comparative study *Cancers* **15** 2121
- Vanreusel V et al 2022 Point scintillator dosimetry in ultra-high dose rate electron “FLASH” radiation therapy: a first characterization *Phys. Med.* **103** 127–37
- Velec M, Moseley J L, Craig T, Dawson L A and Brock K K 2012 Accumulated dose in liver stereotactic body radiotherapy: positioning, breathing, and deformation effects *Int. J. Radiat. Oncol. Biol. Phys.* **83** 1132–40
- Villoing D et al 2022 Technical note: proton beam dosimetry at ultra-high dose rates (FLASH): evaluation of GAFchromic™ (EBT3, EBT-XD) and OrthoChromic (OC-1) film performances *Med. Phys.* **49** 2732–45
- Vozenin M-C et al 2019 The advantage of FLASH radiotherapy confirmed in mini-pig and cat-cancer patients *Clin. Cancer Res.* **25** 35–42
- Wang K, Huang C, Kao Y-J, Chou C-Y, Oraevsky A A and Anastasio M A 2013 Accelerating image reconstruction in three-dimensional optoacoustic tomography on graphics processing units *Med. Phys.* **40** 023301
- Wang L V and Hu S 2012 Photoacoustic tomography: in vivo imaging from organelles to organs *Science* **335** 1458–62

- Wang L V and Wu H 2012 *Biomedical Optics: Principles and Imaging* (Wiley)
- Wang S, Gonzalez G, Owen D R, Sun L, Liu Y, Zwart T, Chen Y and Xiang L 2024a Toward real-time, volumetric dosimetry for FLASH-capable clinical synchrotrons using photoacoustic imaging *Med. Phys.* **51** 8496–505
- Wang S, Pandey P K, Lee G, van Bergen R J P, Sun L, Xu Y and Xiang L 2024b x-ray-induced acoustic computed tomography: 3D x-ray absorption imaging from a single view *Sci. Adv.* **10** eads1584
- Wang X, Pang Y, Ku G, Xie X, Stoica G and Wang L V 2003 Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain *Nat. Biotechnol.* **21** 803–6
- Wigg D R and Wilson B C 1981 Isodose versus isoeffect in radiotherapy planning as applied to parallel opposed fields *Austral. Radiol.* **25** 205–12
- Wu J, Haycocks T, Alasti H, Ottewell G, Middlemiss N, Abdoell M, Warde P, Toi A and Catton C 2001 Positioning errors and prostate motion during conformal prostate radiotherapy using on-line isocentre set-up verification and implanted prostate markers *Radiother. Oncol.* **61** 127–33
- Xia J, Yao J and Wang L H V 2014 Photoacoustic tomography: principles and advances *Electromagn. Waves* **147** 1–22
- Xiang L, Han B, Carpenter C, Pratz G, Kuang Y and Xing L 2012 x-ray acoustic computed tomography with pulsed x-ray beam from a medical linear accelerator: x-ray acoustic computed tomography *Med. Phys.* **40** 010701
- Xiang L, Tang S, Ahmad M and Xing L 2016 High resolution x-ray-induced acoustic tomography *Sci. Rep.* **6** 26118
- Xu M and Wang L V 2005 Universal back-projection algorithm for photoacoustic computed tomography *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **71** 016706
- Yang X-X, Luo H, Zhang J-J, Ge H and Ge L 2025 Clinical translation of ultra-high dose rate flash radiotherapy: opportunities, challenges, and prospects *World J. Radiol.* **17** 105722
- Yao S, Hu Z, Xie Q, Yang Y and Peng H 2022 Further investigation of 3D dose verification in proton therapy utilizing acoustic signal, wavelet decomposition and machine learning *Biomed. Phys. Eng. Express* **8** 015008
- Yoganathan S, Maria Das K, Agarwal A and Kumar S 2017 Magnitude, impact, and management of respiration-induced target motion in radiotherapy treatment: a comprehensive review *J. Med. Phys.* **42** 101
- Yu Y, Li Z, Zhang D, Xing L and Peng H 2019 Simulation studies of time reversal-based photoacoustic reconstruction for range and dose verification in proton therapy *Med. Phys.* **46** 3649–62
- Zhang W, Oraiqat I, Lei H, Carson P L, EI Naqa I and Wang X 2020 Dual-modality x-ray-induced radiation acoustic and ultrasound imaging for real-time monitoring of radiotherapy *BME Front.* **2020** 1–10