

Noninvasive, Label-Free Imaging of Freely Moving *C. elegans* Nematodes

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Noninvasive, label-free imaging of freely moving *Caenorhabditis elegans* nematodes offers a powerful but technically demanding approach for behavioral and morphological studies. Key challenges arise from their microscopic size, rapid and intricate locomotion patterns, uniform soft-tissue composition with low intrinsic contrast, and the stringent requirements for imaging systems capable of simultaneously achieving high spatiotemporal resolution and exceptional soft-tissue sensitivity. To overcome these challenges, we employed ultrafast phase-contrast x-ray imaging (PCXI) and phase-contrast computed tomography (PCCT). PCXI was conducted at a detector acquisition speed of 500 fps, while PCCT leveraged a synchronized system comprising a fast-acquisition detector (4000 fps), a rapid-rotation stage (1800°/s), and a precision electromechanical shutter (3-ms actuation). This configuration enabled complete three-dimensional (3D) tomographic acquisition (400 projections over 180°) in just 100 ms. Our integrated imaging platform delivers dual capabilities: PCXI enables real-time monitoring of multiple nematodes' natural behaviors, including dynamic irradiation avoidance responses, while PCCT offers comprehensive whole-organism morphometric analysis—quantifying body length, thickness, and volume—all under physiologically natural conditions without the need for anesthesia or contrast agent staining. By synergistically integrating ultrafast PCXI for dynamic two-dimensional functional analysis with PCCT for comprehensive 3D morphological characterization, this multimodal platform establishes an imaging paradigm that significantly advances the study of fast-moving microscopic organisms.

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I. INTRODUCTION

The nematode *Caenorhabditis elegans* serves as a valuable model organism in biological research, owing to its transparent anatomy, short life cycle, and fully sequenced genome [1–4]. The remarkable versatility of *C. elegans* renders it an indispensable model system across multiple research disciplines, particularly in neurobiology, genetics, toxicology, and pharmacological studies [5–8]. Noninvasive, label-free visualization of unrestrained *C. elegans* represents a critical methodological advancement, permitting accurate morphological assessments without compromising the organism's innate behavioral dynamics. This approach circumvents the need for anesthesia, which not only introduces technical complexity but may also induce confounding behavioral alterations. However, capturing high-quality images of freely moving specimens presents substantial technical hurdles. The organism's microscopic dimensions and complex movement behaviors, characterized by rhythmic undulations, sudden directional changes, and axial rotations, necessitate imaging platforms with exceptional spatiotemporal resolution. Since

C. elegans lacks mineralized skeletal structures and consists entirely of soft tissues, effective label-free visualization requires imaging modalities with high soft-tissue contrast sensitivity. This approach eliminates the need for exogenous contrast agents, thereby avoiding both the cumbersome staining protocols associated with chemical treatments and potential confounding effects of contrast media on natural physiological processes.

Synchrotron radiation x rays exhibit three unique advantages: high brilliance, ultrashort pulse characteristics, and superior coherence. These intrinsic properties form the physical foundation for achieving high spatial resolution, exceptional temporal resolution, and phase-contrast x-ray imaging (PCXI), respectively. Recognized as a powerful imaging modality, PCXI provides sufficient spatiotemporal resolution that enables real-time observation of small living specimens while simultaneously capturing both morphological and functional information [9–12]. Although conventional absorption-based micro-computed tomography (CT) can achieve nanometer-scale spatial resolution [13–15], its imaging mechanism relying solely on x-ray absorption significantly limits its applications in soft-tissue visualization. In comparison, phase-contrast CT (PCCT)—an advanced implementation of PCXI—achieves exceptional soft-tissue differentiation by primarily utilizing x-ray phase shifts without requiring contrast agents [16–19]. While PCCT has demonstrated exceptional efficacy in high-resolution microstructural analysis of postmortem *C. elegans* [20], its application to unrestrained living specimens faces two critical

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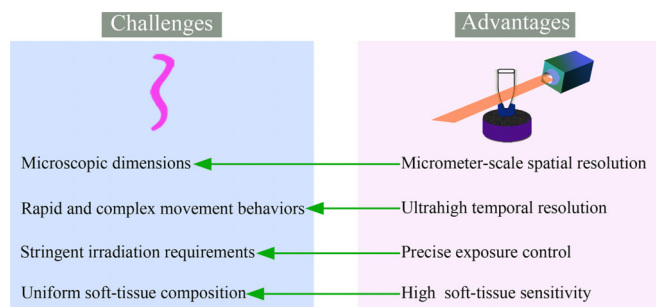


FIG. 1. Challenges in imaging freely moving *C. elegans* nematodes and their solution via ultrafast PCXI and PCCT. The left column outlines the challenges, while the right column highlights the advantages of the imaging system in overcoming these obstacles.

challenges: achieving ultrahigh temporal resolution to minimize motion artifacts and adhering to stringent radiation dose control to ensure organism viability. Achieving this goal will require significant technological breakthroughs in both hardware architecture and software algorithms for PCCT systems.

An ultrafast PCCT imaging system [21] was developed at the BL16U2 beam line of the Shanghai Synchrotron Radiation Facility (SSRF). Through systematic characterization, we assessed the system's imaging performance by visualizing freely moving *C. elegans*, demonstrating its capability for noninvasive, label-free observation of highly dynamic microscopic organisms. The imaging challenges and their corresponding solutions are illustrated in Fig. 1.

II. RESULTS

A. Dynamic behavior of *C. elegans* nematodes under PCXI

PCXI effectively captured the movement dynamics of freely moving *C. elegans* nematodes under different population densities (Figs. 2 and 3), with corresponding continuous video recordings provided in Movies S1 and S2, respectively (movie legends are available in Supplemental Material [22]). The nematodes exhibited characteristic rapid locomotion patterns including undulatory propulsion, directional turns, and rotational movements. At high population density (Fig. 2, Movie S1), imaging revealed significant clustering within the field of view (FOV) due to specimen overlap effects. Time-lapse analysis demonstrated consistent outward migration behavior, with nematodes preferentially moving toward radiation-free areas at the upper periphery of the sample tube, suggesting an avoidance response to prolonged irradiation exposure. At lower specimen densities (Fig. 3, Movie S2), the reduced number of nematodes resulted in significantly less natural aggregation and overlap within the FOV. This optimized condition enabled clearer observation of individual specimens while still demonstrating the peripheral dispersion behavior to escape the x-ray irradiation. The results confirm that enhanced imaging throughput (nematodes per PCXI) comes at the expense of individual organism resolution when imaging live *C. elegans*.

B. Ultrafast PCCT of freely moving *C. elegans* nematodes

Phase retrieval (PR) processing enhances contrast between the low-absorbance plastic sample tube and the soft-tissue

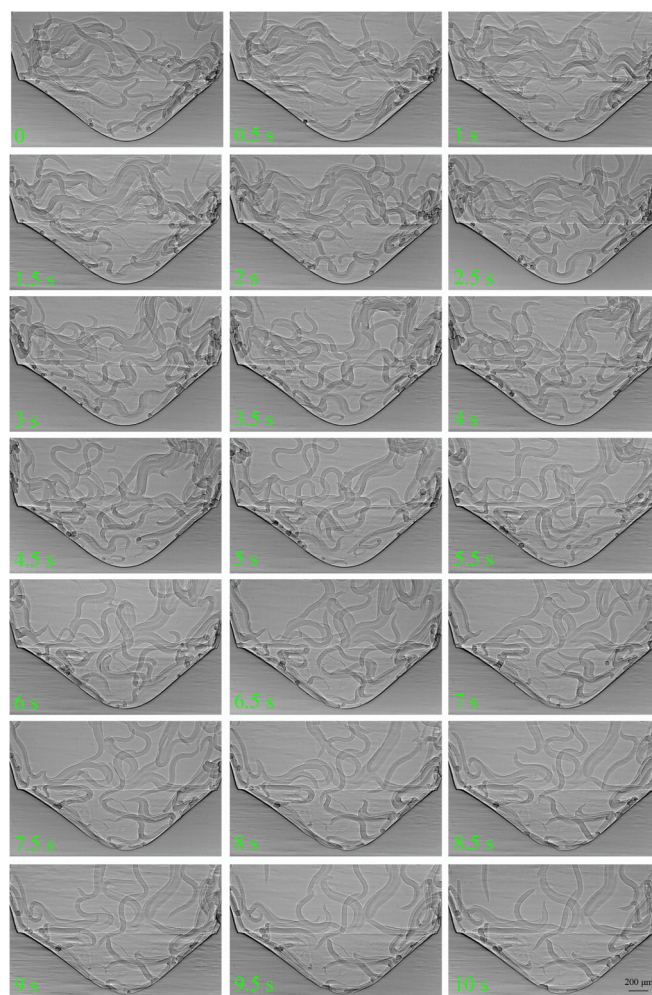


FIG. 2. Dynamic PCXI of multiple freely moving *C. elegans* nematodes. PCXI captured the dynamic behavior of a large population of freely moving nematodes. The results demonstrate that these nematodes exhibit rapid and active movements with diverse behavioral patterns. The nematodes initially presented significant clustering within the FOV. Over time, they displayed coordinated peripheral migration behavior, systematically moving toward and eventually beyond FOV boundaries. This spatial redistribution represents an avoidance behavior in response to prolonged radiation exposure.

nematodes, significantly improving specimen visibility (Fig. 4). Signal-to-noise ratio (SNR) measurements (Fig. 1 of the Supplemental Material [22]) revealed superior performance of the PR-based method (28.977 ± 1.430) versus PR-free (0.257 ± 0.025 ; $P < 0.0001$). Figure 5 presents the PCCT imaging results of multiple *C. elegans* specimens at high population density. The reconstructed images successfully demonstrate visualization of spatially dispersed nematodes, while revealing the technical limitation in resolving individual organisms within clustered aggregates. While simultaneous scanning of multiple specimens enhances imaging throughput, this approach introduces technical challenges related to specimen clustering. Reduced nematode density (Fig. 6) minimized clustering effects apparent at higher densities (Fig. 5), facilitating individual specimen visualiza-

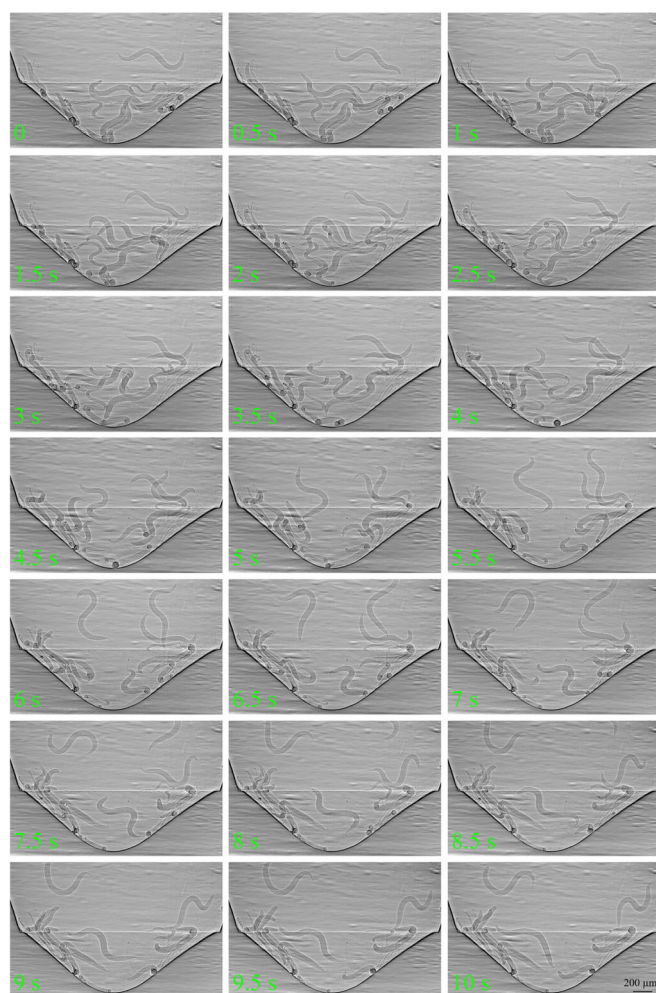


FIG. 3. Dynamic PCXI of freely moving *C. elegans* nematodes at reduced population density. PCXI of freely moving nematodes was performed using a reduced specimen density compared to Fig. 2. The results demonstrate that the nematodes maintained their characteristic rapid and dynamic movements while exhibiting complex behavioral patterns. At this reduced population density, specimen clustering became significantly less pronounced, enabling clearer visualization of individual nematodes. Time-lapse imaging revealed progressive spatial dispersion, with specimens migrating toward the FOV periphery. This behavior suggests an active avoidance strategy to minimize radiation exposure.

tion. This demonstrates that optimizing specimen density is essential for balancing imaging quality and throughput. As illustrated in Fig. 6(c), *C. elegans* morphology was segmented into three regions: the cephalic head (anterior), trunk (midbody), and caudal tail (posterior). Their body shape was elongated and cylindrical, with a thicker middle section and tapered ends at both the anterior and posterior. The acquired images enabled the measurement of key morphological parameters, including body length, body thickness, and volume. Analysis of the 11 fully reconstructed specimens [Fig. 6(e)] yielded the following mean $\pm$ standard deviation (SD) measurements: body length, $811.35 \pm 167.84 \mu\text{m}$; body thickness, $2560.42 \pm 789.23 \mu\text{m}^2$; and volume, $1.359 \times 10^6 \pm 6.817 \times 10^5 \mu\text{m}^3$. Detailed values are provided

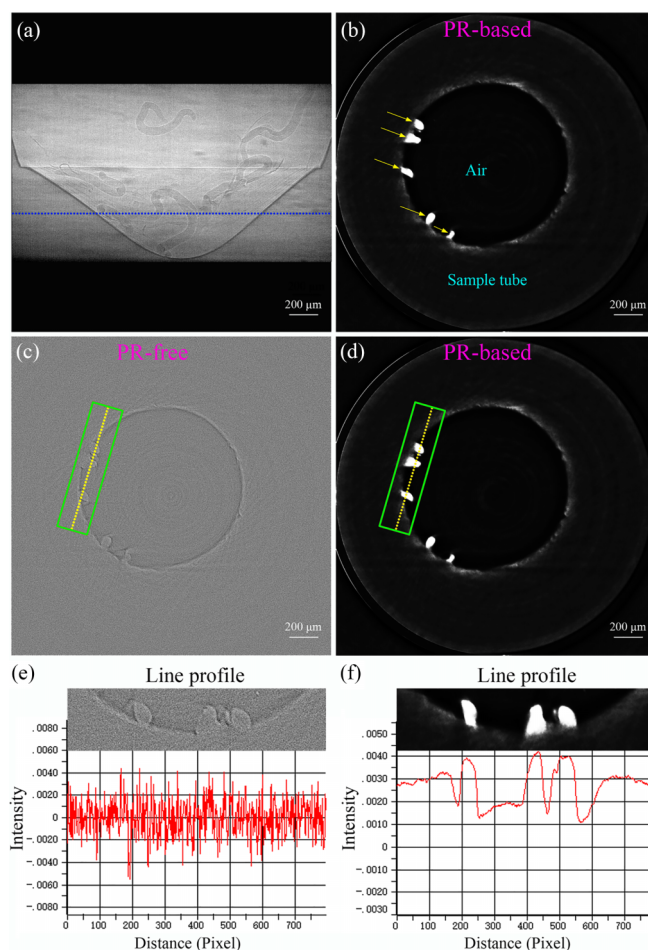


FIG. 4. Enhanced nematode visibility through PR processing. (a) Representative projection image of freely moving *C. elegans* nematodes. (b) PR-enhanced PCCT reconstruction along the dashed blue line in (a) shows distinct contrast between the sample tube, internal air space, and nematodes (yellow arrows). [(c), (d)] Axial cross sections along the dashed blue line in (a) without and with PR processing, respectively. [(e), (f)] Line profile analyses along dashed yellow lines in (c) raw and (d) PR-processed slices, showing enhanced signal characteristics after processing. The raw profile (e) exhibits noise-induced intensity fluctuations, while the PR-corrected profile (f) accurately captures phase-contrast variations across tissue boundaries. Notably, the distinct intensity peaks in (f) correspond to nematode-induced signals. Inset images in (e) and (f) are magnified views of the green-boxed regions in (c) and (d), respectively.

in Table 1 of the Supplemental Material [22]. Statistical analysis showed strong morphological covariation, with body thickness exhibiting an exceptionally high correlation with volume (Pearson's $r = 0.958$, $P < 0.0001$). A similarly significant, though somewhat weaker, positive correlation was observed between body length and volume ($r = 0.838$, $P < 0.01$), indicating proportional scaling across these dimensional parameters.

C. Quantification of absorbed irradiation dose

The x-ray exposure duration ranged from 100 to 106 ms across different sample regions (Fig. 7). Radiation dosimetry

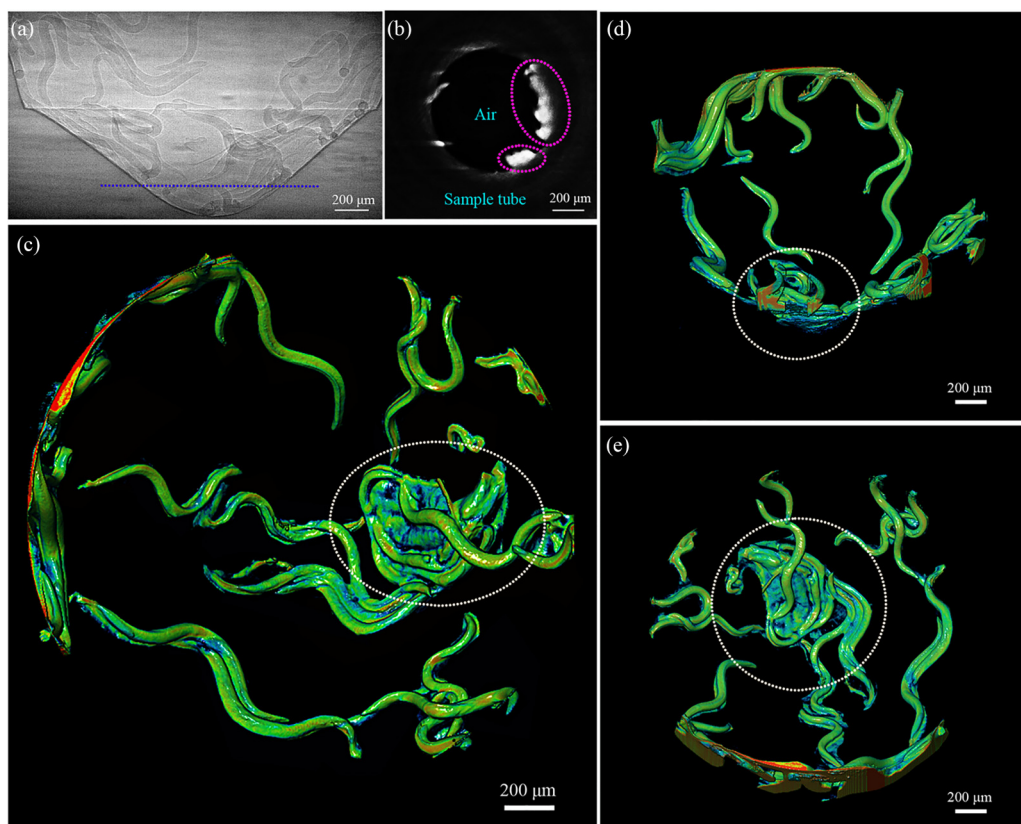


FIG. 5. PCCT of multiple freely moving *C. elegans* nematodes. (a) Representative projection image of nematodes. (b) PR-based PCCT reconstruction of the cross section [dashed blue line in (a)], demonstrating clear contrast differentiation between the sample tube, air, and nematodes. Some specimens (pink circles) appeared clustered, compromising individual resolution due to structural overlap. [(c)–(e)] Different-angle three-dimensional (3D) reconstructions clearly resolved the morphological features of spatially dispersed nematodes. The aggregated specimens (white circles) displayed diminished feature discrimination due to superimposition effects.

measurements showed a dose rate of 230.2852 Gy/s at the ionization chamber position, with the attenuated beam delivering 117.4454 Gy/s at the sample site due to beam path absorption. Quantitative analysis determined the *C. elegans* absorption ratio ($AR_{C. elegans}$) to be 0.02543 (Fig. 2 of the Supplemental Material [22]), resulting in an absorbed irradiation dose (AID) range of 298.7–316.6 mGy per nematode per CT scan.

III. DISCUSSION

Prior to this work, Olendrowitz *et al.* [20] demonstrated the application of PCXI and PCCT for *C. elegans* imaging, achieving exceptional resolution of cellular and subcellular structures in these multicellular organisms. While their methodology provided valuable insights, certain technical considerations should be noted: PCXI necessitated chemical immobilization using sodium azide (limiting observation to stationary specimens), and PCCT was implemented on fixed samples prepared through high-pressure freezing and epon embedding—a standard preparation protocol that alters native structures to some extent. Our PCXI system represents a significant advance by enabling dynamic imaging of freely moving *C. elegans* nematodes through high-speed acquisition. This technological breakthrough allows complete reconstruction of motion sequences that precisely capture the

nematodes' rapid and diverse locomotory patterns without motion artifacts. We observed a striking behavioral response where clustered nematodes progressively dispersed and exited the FOV, suggesting an active avoidance mechanism to x-ray exposure. Cannon *et al.* [23] reported that the endogenous *C. elegans* UV-photoreceptor protein LITE-1 mediates the avoidance response. In their experimental setup, in addition to x-ray irradiation, the nematodes were exposed to back-lighting from a white light-emitting diode (LED) source, with behavioral responses recorded via a complementary metal-oxide semiconductor (CMOS) camera. White LED illumination may induce light-responsive behaviors in *C. elegans*, which confounds interpretation of responses specific to x-ray exposure. Unlike this approach, our method leverages the dual attributes of x rays—both as a stimulant and an imaging source. By integrating stimulation and imaging within a single x-ray-based modality, we eliminate the need for extraneous visible light sources. This integrated design enables precise and unambiguous assessment of nematode behavioral responses specifically to x-ray exposure. The established methodology provides an accurate model for investigating environmental stress responses and radiation-sensing mechanisms in microscopic organisms. PCCT has found broad applications extending beyond nematode imaging to encompass diverse studies of fixed cadaveric specimens [19,24–26]. These studies have demonstrated PCCT's capability for

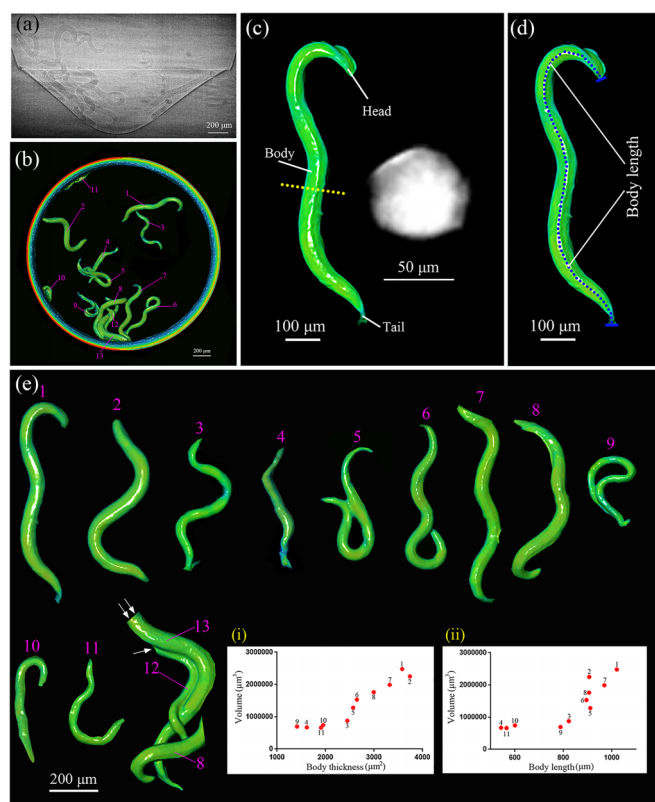


FIG. 6. Morphometric analysis of freely behaving *C. elegans* nematodes. (a) Representative projection image of unrestrained nematodes. (b) Top-view 3D reconstruction demonstrates that reduced population density effectively minimizes specimen overlap, enabling clear visualization of individual organisms. (c) Definition of body thickness. The cross-sectional area at the midbody level (dotted yellow line) of the first nematode specimen in (b) exhibits near-circular geometry, serving as an objective proxy for body thickness—the microscale equivalent of vertebrate waist circumference. Three anatomical regions are demarcated: head, body, and tail. (d) The body length was operationally defined as the longitudinal measurement along the anteroposterior axis (dashed blue line), spanning from the cephalic tip to the caudal terminus. (e) 3D visualization of 11 fully extracted nematodes and their corresponding body thickness versus volume and body length versus volume distribution plots. Two nematodes (individuals 12 and 13) partially moved outside the FOV, as indicated by white arrows. Scatter plots demonstrate significant positive correlations between: (i) body thickness and volume (Pearson's $r = 0.958$, $P < 0.0001$), and (ii) body length and volume ($r = 0.838$, $P < 0.01$), indicating coordinated scaling of these morphological features.

visualizing cellular and subcellular structures through its high spatial resolution and superior soft-tissue contrast. However, live organism imaging poses greater technical challenges yet yields higher scientific value than fixed-sample studies. Successful *in vivo* PCCT imaging requires high-speed scanning to eliminate motion artifacts while maintaining sufficient contrast resolution for biological analysis under strictly controlled radiation doses. Rapid specimen movement can compromise imaging accuracy. To mitigate motion artifacts in highly motile specimens, the scanning speed can be increased by accelerating the rotation of the sample stage to reduce the

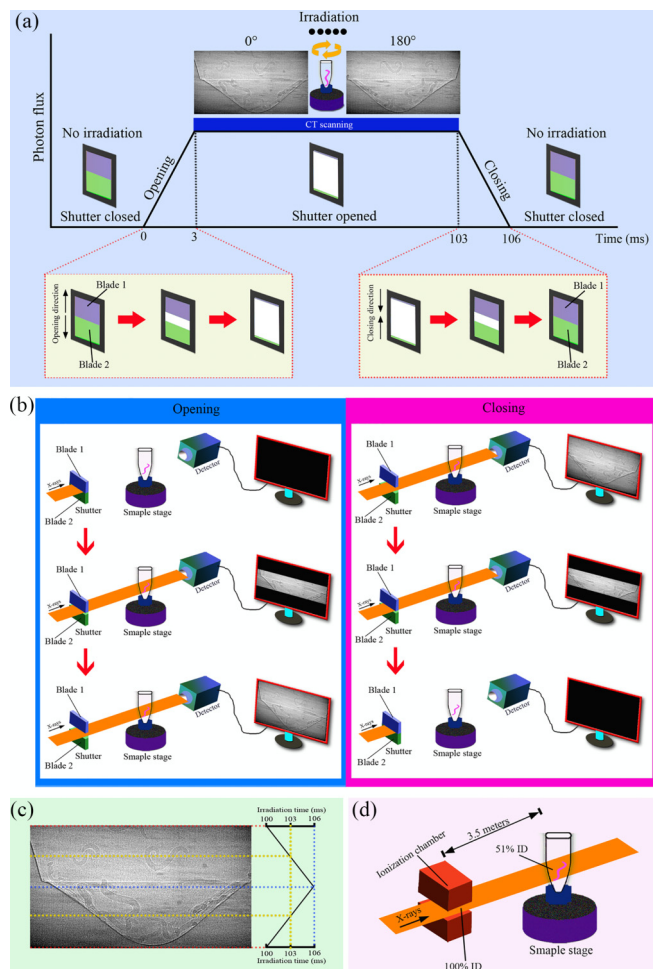


FIG. 7. X-ray irradiation strictly confined to the shutter's opening-to-closure cycle. (a) CT acquisition was precisely synchronized with the shutter operation, initiating scan acquisition upon full shutter opening and triggering shutter closure immediately upon scan completion. The fast-response shutter completed a full opening or closing cycle within 3 ms, ensuring precise temporal control. (b) The shutter comprised two blades driven by a bidirectional mechanism. During the opening phase, the two blades moved in opposite directions (upward and downward, respectively), while closing followed the reverse sequence. This system ensured initial irradiation of the central horizontal zone, followed by radial dispersion to superior and inferior peripheral regions. (c) The irradiation duration displayed a vertically graded distribution, with exposure times decreasing gradually from 106 ms at the central horizontal region (dotted blue line) to 100 ms at both superior and inferior extremities (dotted red lines), establishing a consistent vertical gradient of 6 ms across the sample. (d) After propagating through 3.5 m of air, the x-ray beam intensity underwent 49% attenuation, yielding a 51% transmission rate at the sample position. The abbreviation “ID” denotes the irradiation dose.

total acquisition time. However, this simultaneously shortens the exposure time per projection, resulting in diminished SNR. This inverse relationship establishes a critical trade-off between scanning velocity and image fidelity. Consequently, parameter selection must be carefully optimized to balance radiation dose, SNR, and motion suppression according to specific experimental requirements such as resolution needs

and specimen sensitivity. Though PCCT has been applied in a limited number of live imaging studies [16,27–30], radiation exposure remains a significant concern in these applications. Radiation exposure is known to cause DNA damage and produce free radicals in *C. elegans* [31–34]. The molecular damage can lead to adverse phenotypic outcomes, including reduced lifespan and impaired reproductive capacity. Feng *et al.* [31] demonstrated that a survival fraction exceeding 0.8 is achievable on day 12 for neutron doses up to 1 Gy. Despite differences in biological effectiveness between x-ray and neutron irradiation, the low AID of less than 0.4 Gy serves as a strong indicator of the high biocompatibility of our PCCT system.

The current spatial resolution of 1.1 $\mu\text{m}/\text{pixel}$ does not allow for the resolution of nematode internal organ architecture, which typically demands submicron-level resolution for clear visualization. The spatial resolution of the detector can be enhanced by increasing the magnification of the optical conversion system. However, it is important to note that such improvements involve inherent trade-offs: achieving higher resolution (i.e., a smaller $\mu\text{m}/\text{pixel}$ value) generally requires greater geometric magnification, which reduces the FOV and may necessitate longer exposure times or higher radiation doses to maintain adequate SNR, thereby potentially compromising imaging speed. Conversely, lower-resolution configurations allow faster scanning with reduced radiation exposure, though at the cost of spatial detail. Notably, the PCCT system provides robust acquisition of key whole-organism parameters, including body length, body thickness, and volume. These metrics are critical for precise assessment of developmental and physiological states, with applications spanning developmental biology, aging research, disease modeling, and toxicology. When maintained at optimal densities, multiple *C. elegans* nematodes can be imaged simultaneously, enhancing imaging efficiency.

Owing to its noninvasive nature, optical microscopy serves as the most widely used method for observing *C. elegans* and is particularly well suited for long-term live imaging, enabling researchers to monitor dynamic biological processes in real time throughout the organism's entire life cycle. However, its spatial resolution is inherently limited by diffraction, which prevents detailed visualization of subcellular organelles and ultrastructural details. Additionally, light scattering significantly compromises image clarity when imaging deep internal structures, while the limited depth of field restricts accurate 3D reconstruction and volumetric analysis. Other conventional high-resolution imaging techniques—such as confocal microscopy, electron microscopy, and resolution-optimized PCCT—excel in cellular and subcellular visualization but typically require complex and invasive sample preparation. In contrast, our PCCT system offers complementary capabilities by enabling rapid, label-free, and high-throughput 3D morphological analysis at the whole-organism level. This capability suggests its emerging potential for applications such as large-scale phenotypic screening, particularly in scenarios where speed and whole-organism data are important. Future improvements in detector resolution may help narrow the gap between organ-level and whole-organism imaging, which would expand the system's applicability in integrative biological research.

The successful operation of our ultrafast PCCT system fundamentally depends on the precise integration and synchronization of three core technological components: a fast-acquisition detector, a rapid-rotation stage, and a precision-controlled shutter. This tripartite system operates as an interdependent unit where each element is essential for achieving optimal imaging performance. The electromechanical shutter plays a pivotal role in radiation dose optimization by providing precise temporal control of x-ray exposure, thereby eliminating unnecessary radiation and ensuring protection of the nematodes. PR postprocessing can substantially improve SNR by effectively extracting subtle signals from pronounced phase variations, enabling superior soft-tissue contrast resolution [35–39]. As a result, the nematodes could be clearly visualized in a label-free manner.

IV. CONCLUSION

In summary, we have accomplished noninvasive, label-free dynamic PCXI of multiple freely moving *C. elegans* nematodes and 3D PCCT reconstruction. The system provides comprehensive observation of nematode locomotion patterns and whole-organism dimensional measurements. These results suggest that our platform could provide a valuable complementary approach to existing imaging methodologies for *C. elegans* research, with potential advantages in high-throughput behavioral phenotyping and quantitative morphological analysis.

V. MATERIALS AND METHODS

A. Ultrafast PCCT setup

Synchrotron radiation x rays were produced using a 3.5-GeV electron storage ring at the SSRF, operating at an average beam current of 200 mA. A double-crystal monochromator with Si(111) plane crystals was employed to monochromate the x-ray beam. As depicted in Fig. 8(a), the ultrafast PCCT system consisted of a specialized imaging detector featuring a 10 $\times$ magnification optical conversion system (developed by the SSRF x-ray imaging group) coupled with a fast-acquisition camera (Dimax HS4, PCO, Germany). The scintillator used was a LuAG:Ce crystal (100 μm thickness, 70-ns decay time; CRYTUR Ltd.). By integrating the optical magnification system with the camera's native resolution (11 $\mu\text{m}/\text{pixel}$), an effective pixel size of 1.1 $\mu\text{m}/\text{pixel}$ was achieved. The sample positioning system integrated three precision components: an H-850 hexapod microrobot (Physik Instrumente, Germany) for multiaxis microadjustments, an RSML-200 rotary stage (CORETECH, China) for sample rotation, and an XA10A-R2H01 linear stage (Kohzu Precision, Japan) for precise translational movement. Radiation exposure was precisely controlled via a high-precision shutter (see Fig. 3 of the Supplemental Material [22]) comprising two tungsten blades [21].

B. Ultrafast PCXI and PCCT imaging

The study was conducted in compliance with the guidelines of the Institutional Animal Care and Use Committee at Shanghai Jiao Tong University (SJTU). Wild-type (N2) *C. elegans*

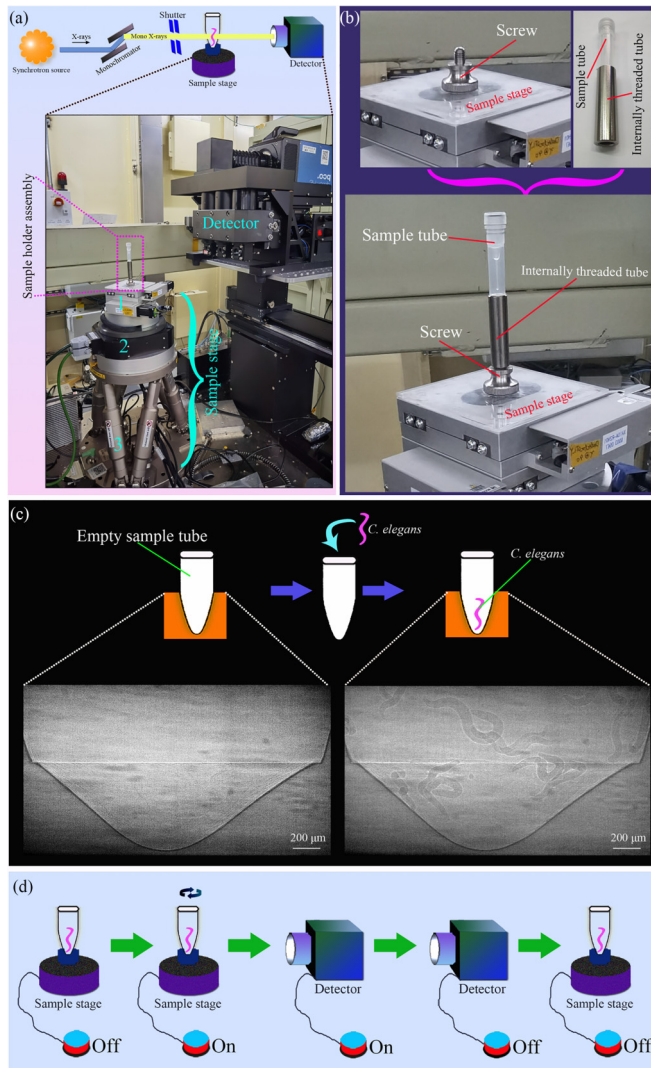


FIG. 8. Imaging system configuration and implementation. (a) Ultrafast PCCT setup. The experimental setup employed a double-crystal monochromator for x-ray beam monochromatization. The inset shows the detector, sample stage, and the sample holder assembly. The detector was integrated with an optical conversion system and a camera. The sample stage consisted of three key components: (1) a horizontal adjustment device, (2) a rapid rotation device, and (3) a multiaxis motion device. (b) Sample holder assembly preparation. A screw was rigidly fixed to the sample stage. A precisely matched threaded tube, featuring a flat end face, was securely coupled to a plastic sample tube. The sample tube was mounted to the stage via the screw-thread interface, allowing stable stage fixation through clockwise rotation (engagement) and release through counterclockwise rotation (disengagement). (c) Sample loading and positioning protocol. First, the empty sample tube was securely attached to the stage by completely engaging the threaded tube with the mounting screw. Under real-time PCXI guidance, the sample tube was carefully aligned to center it within the FOV (orange region) by precisely adjusting the sample stage. Following alignment, the threaded tube bonded with the empty sample tube was disengaged from the screw and transferred to an optical microscope workstation for specimen loading. Upon completion of loading, the assembly was precisely repositioned by fully reengaging the screw-threaded tube coupling system. This optimized protocol provided significant advantages: by maintaining the initial alignment reference, it eliminated the need

nematodes were obtained from the Caenorhabditis Genetics Center (CGC). A custom-designed sample holder was fabricated to position the specimen during scanning procedures [Fig. 8(b)]. After aligning the empty sample tube using PCXI preview [Fig. 8(c)], *C. elegans* nematodes were loaded into the tube under optical microscope observation. Imaging was then performed directly without additional preview steps to minimize radiation exposure: PCXI with a stationary sample stage and PCCT with stage rotation. The PCCT scanning procedure is illustrated in Fig. 8(d). Following imaging, samples were removed from the experimental chamber. For image calibration, ten flat-field and ten dark-field images were acquired. The key imaging parameters for PCXI and PCCT are provided in Tables 2 and 3 of the Supplemental Material [22], respectively.

C. Image processing and analysis

PR-based PCCT slices were generated using PITRE software [35], with the complete processing workflow shown in Fig. 4 of the Supplemental Material [22]. The 16-bit projections were converted to PR projections using the phase-attenuation duality (PAD) method [40], incorporating the δ/β ratio as a critical parameter. Reconstruction with the Gridrec algorithm [41] yielded floating-point PCCT slices that demonstrated substantially enhanced soft-tissue contrast and structural definition. The parameters were entered into the corresponding fields of the PITRE interface (Fig. 5 of the Supplemental Material [22]). The efficacy of PR for enhancing intensity differences on projections is validated in Fig. 6 of the Supplemental Material [22]. The processed slices were imported into Avizo 2020.2 (Thermo Fisher Scientific) for 3D volume rendering and volumetric analysis. Morphometric measurements, including body length and cross-sectional area (body thickness), were performed using ImageJ [42], while intensity summation and line profile analyses were conducted using Image-Pro Plus 6.0 (Media Cybernetics).

D. Calculation of the SNRs

To quantitatively assess contrast enhancement in axial CT images, the SNR was calculated using

$$\text{SNR} = (I - I_{\text{ref}}) / \sigma_{\text{ref}}. \quad (1)$$

Here, I and I_{ref} denote the mean intensity values within circular regions of interest (ROI) placed in the test material and reference material, respectively, while σ_{ref} represents the SD of intensity in the reference material's ROI, which quantifies the image noise.

for additional PCXI preview, thereby minimizing x-ray exposure to sensitive samples while ensuring imaging precision through reproducible mechanical positioning. (d) PCCT data acquisition protocol. The scan was initiated by first accelerating the stationary sample stage to a rotational speed of 1800°/s. Upon reaching the target speed, the detector was activated to acquire projections at 4000 fps. A total of 400 projections were captured during a continuous 180° rotation, completed within 100 ms. The sample stage was promptly decelerated to a stop upon completion of image acquisition.

E. AID determination

The irradiation occurring during the CT scans is illustrated in Fig. 7(a). Irradiation dose was quantified using the ionization chamber (IC) method [43]. The dose at the chamber position (D_{IC}) was calculated according to established methods [39]:

$$D_{IC}[\text{Gy}] = (I_0 \times t \times 33.7) / (S_{\text{slit}} \times n \times \rho_{\text{air}} \times L). \quad (2)$$

Here, I_0 is the ionization current, t represents the total exposure time [100–106 ms, Fig. 7(c)], the constant 33.7 eV/ion pair (as referenced in ICRU Report 1979) corresponds to the mean excitation potential (W value) of dry air, $S_{\text{slit}} \times n$ gives the total slit area, ρ_{air} is air density, and L is the chamber length (Table 4 of the Supplemental Material [22]). Accounting for 51% transmission through the 3.5-m air path [Fig. 7(d)], the dose at sample site (D_{SS}) was derived as

$$D_{SS} = D_{IC} \times 51\%. \quad (3)$$

The $AR_{C. elegans}$ was determined following reference methods [36] and calculated through a two-step analytical procedure (Fig. 2(d) of the Supplemental Material [22]). This enabled the AID to be computed as

$$\text{AID} = D_{SS} \times AR_{C. elegans}. \quad (4)$$

F. Statistical analysis

All statistical analyses (correlation and paired t-tests) were performed in GraphPad Prism software (version 7.04, San Diego, CA). Data are reported as mean $\pm$ SD, with $P < 0.05$ considered statistically significant.

DATA AVAILABILITY

The data that support the findings of this article are not publicly available upon publication because it is not technically feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

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