Uporaba nanotomografije v biomedicinski znanosti in medicini

The application of computed nanotomography in biomedical sciences and medicine

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Izvleček

Namen: *Računalniška nanotomografija (nano-CT) je napredna slikovna tehnika, ki omogoča visoko ločljivo tridimenzionalno (3D) vizualizacijo na celični in subcelični ravni, ter tako bistveno presega zmogljivosti tradicionalnih tehnik računalniške tomografije (CT) in drugih slikovnih metod. Ta pregledni članek obravnava tehnološke novosti nano-CT, njegove trenutne zmogljivosti in potencialno vključitev v klinično medicino.*

Metode: *Izvedli smo pregled novejše literature in študij s področja tehnologije nano-CT, s poudarkom na njegovi uporabi v biomedicinskih raziskavah ter potencialni integraciji v klinično prakso.*

Rezultati: *Nano-CT se je izkazal kot zelo uporabna tehnika v različnih vejah medicine, vključno*

Abstract

Background: *Computed nanotomography (nano-CT) is a cutting-edge imaging technology that provides ultra-high-resolution three-dimensional (3D) visualization at the cellular and subcellular levels, significantly surpassing conventional computed tomography (CT) and other imaging modalities. In this review, we describe the technological development of nano-CT along with its current capabilities and potential for integration into clinical medicine.*

Methods: *We reviewed the existing literature relating to nano-CT technology, emphasizing biomedical research applications and evaluating potential for future clinical integration.*

Results: *Nano-CT has demonstrated exceptional utility across various*

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s kardiologijo, vaskularno medicino, nevrologijo, pulmologijo, onkologijo in zobozdravstvom. Omogoča edinstven vpogled v arhitekturo tkiv, celične podrobnosti in patološke procese, kar bistveno izboljšuje razumevanje bolezni in podpira napredek v diagnostiki, načrtovanju zdravljenja ter novih terapevtskih strategijah.

Zaključki: *Kljub izjemnemu potencialu na področju biomedicinskih raziskav je klinična uporaba nano-CT še vedno soočena z več izzivi, kot so omejitve v hitrosti slikanja, kontrastu mehkih tkiv, sevalni obremenitvi in velikosti vzorcev. Nadaljnje tehnološke inovacije, validacija s kliničnimi študijami ter razvoj multimodalnih slikovnih pristopov so nujni za uspešno integracijo nano-CT v rutinsko medicinsko diagnostiko in personalizirano medicino.*

biomedical disciplines, including cardiology, vascular medicine, neurology, pulmonology, oncology and dentistry. This imaging technique provides unprecedented insights into tissue architecture, cellular details and pathological processes, providing significant enhancement in our understanding of diseases and supporting advancements in diagnosis, treatment planning, and therapeutic strategies.

Conclusions: *Despite remarkable potential for biomedical research, the clinical adoption of nano-CT faces several challenges, including limitations in imaging speed, soft tissue contrast, radiation dose and sample size. Continued technological innovations, clinical validation, and the development of multimodal imaging approaches are essential for the successful transition of nano-CT into routine medical diagnostics and personalized medicine.*

INTRODUCTION

Despite the accelerated technological development of X-ray-based computed tomography (CT) over recent years, especially with regards to temporal resolution; the spatial resolution of this cross-sectional imaging method in the clinical setting still lies between 500 µm and 625 µm (1). Spatial resolution in CT depends on several factors, such as X-ray focal spot size, the number of projection views per X-ray tube rotation, the size of the detector cell, and reconstruction algorithms (1). Recent advances in detector technology, iterative reconstruction algorithms, and X-ray source optimization have improved resolution capabilities, although fundamental limitations remain due to the physical properties of X-ray sources and detector elements (2-4). The first in vivo scanners with a spatial resolution of 105 to 55 µm (high-resolution peripheral quantitative CT scanners, HR-pQCTs) are already being used to image microscopic bone structures (5, 6). Structures smaller than this resolution cannot be imaged with this CT technology. To image structures smaller than 200 µm, histological and pathohistological techniques still remain the gold standard. However, histological methods have their own disadvantages, including labor-intensive sample preparation, irreversible tissue destruction, and potential artifacts introduced during processing (7). Computed microtomography (micro-CT) is a well-established complementary technique to histological examinations as a non-destructive three-dimensional (3D) imaging technique with micrometer resolution (8). The non-destructive nature of the CT technique is one of the most important advantages over histology. Tissues used as examination specimens in micro-CT devices can subsequently be used as samples for further pathohistological studies after the examination has been completed.

There is no uniform definition of micro-CT in the existing literature. Kalender (2011) suggested a threshold spatial resolution of at least 100 µm and reported that CT devices with better spatial resolution can be referred to as micro-CT devices, irrespective of other device specificities (9). Micro-CT systems, typically achieving spatial resolutions between 5 and 50 µm, provide essential insights

into the 3D microarchitecture of various biological tissues, bridging the gap between clinical imaging and microscopy. This technique is gradually creating opportunities for virtual histology, live cell imaging, subcellular imaging, and correlative microscopy (10). This combination of histology and radiological techniques was first applied to study the morphology of spongy bone (6). Over recent years, with the development of radiocontrast agents, micro-CT has been applied in many other fields, such as [1] the visualization and quantification of pathological vascular lesions in animal models (8); [2] generating pulmonary and cardiac function parameters from animal models (8) and [3] the evaluation of local biomechanical behavior under complex loading conditions (11, 12). Nevertheless, micro-CT, when performed both in vivo and ex vivo) is still limited by spatial resolution. This limitation is particularly evident when imaging structures such as terminal vessels, the components of arteriosclerotic plaques, and cellular lacunes in calcified hard connective tissue, which represent structures that are too small to discern with the use of micro-CT devices (8).

Building upon the advancements of micro-CT, computed nanotomography (nano-CT) has emerged as a cutting-edge imaging modality that offers sub-micrometer spatial resolution that significantly surpasses the capabilities of micro-CT. Nano-CT systems employ specialized transmission-target X-ray tubes featuring a focal spot size < 400 nanometers, achieving spatial resolutions down to approximately 50–400 nm, thus enabling detailed visualization of structures at the cellular and even subcellular level (8). One of the primary advantages of nano-CT is its ability to provide non-destructive and 3D imaging of biological specimens at the cellular level in a manner that preserves the integrity of samples for subsequent analyses. This feature is particularly beneficial for certain fields, such as pathology, for which traditional methods require labor-intensive sample preparation and result in tissue destruction. Nano-CT facilitates virtual histology, allowing for the detailed examination of tissue architecture without physical sectioning (13-18).

In the realm of vascular imaging, nano-CT has demonstrated exceptional utility by revealing microvascular networks and intricate plaque compositions in atherosclerotic models. The high spatial resolution of this method enables the detection of intraplaque hemorrhages and calcifications that are not discernible with micro-CT, thereby enhancing our understanding of vascular pathologies (19). Moreover, nano-CT has proven invaluable in dental research, particularly in evaluating root canal morphology and the quality of endodontic treatments. The ultra-high resolution of nano-CT allows for the clear visualization of complex root canal systems and the assessment of treatment efficacy, thus providing insights that are critical for improving dental procedures (20, 21).

Despite its numerous advantages, nano-CT is primarily limited to ex vivo applications due to both technical and physical constraints. The high radiation doses, prolonged scanning times, and limited field-of-view associated with nano-CT imaging currently restrict its application in vivo. However, recent advancements, such as phase-contrast imaging and improved detector efficiency, are gradually reducing both radiation exposure and scan duration, thus enabling limited in vivo applications, particularly for research involving small animals (22). It is important to note that nano-CT necessitates the use of contrast agents to enhance the visualization of soft tissue, as soft tissues inherently exhibit low X-ray absorption (8). Thus, nano-CT represents a significant step forward in imaging technology, offering a better resolution that bridges the gap between traditional micro-CT and histological methods. Furthermore, the ability of nano-CT to visualize biological structures at the cellular level in a non-destructive manner holds significant promise for the advancement of research in various medical fields, including histopathology, vascular medicine, and dentistry.

THE NANO-CT TECHNIQUE

Nano-CT, often referred to as nanotomography, is an advanced imaging technique that enables 3D visualization of objects at the nanometer scale. Building upon the principles of traditional CT, nano-CT employs highly focused X-ray beams and

sophisticated detectors to achieve superior spatial resolution, often reaching sub-400-nanometer levels. This exceptional spatial resolution is primarily attributed to the use of specialized transmission-target X-ray tubes that possess focal spot sizes < 400 nanometers, as well as advanced detector technology and precise sample positioning systems, significantly surpassing the capability of conventional micro-CT systems (8, 23).

Nano-CT imaging involves rotating the specimen and capturing multiple two-dimensional (2D radiographic images from various angles. These images are then reconstructed computationally to produce a detailed 3D representation of the internal structure of a given specimen. The reconstruction algorithms, including iterative and filtered back-projection methods, play a crucial role in achieving high-quality volumetric data from the acquired projections. This non-destructive technique is invaluable when investigating the intricate architecture of biological tissues, materials, and chemical compounds without altering their inherent properties (23, 24).

Recent advancements in nano-CT technology have expanded the application of this technique across various scientific domains. For instance, the development of stress nanotomography allows us to map internal structures and stress distributions in materials at the nanoscale, achieving resolutions approximately 100-fold higher than traditional X-rays and neutron tomography (25, 26). Furthermore, developments in phase-contrast imaging and synchrotron-based nano-CT have significantly improved contrast resolution, particularly in soft tissues and materials with low absorption differences, thus broadening the applicability of nano-CT to a diverse range of scientific disciplines (27, 28). This innovation holds significant promise for nanotechnology and materials science as it could provide deeper insights into material properties and behaviors (23, 29).

In the field of medicine, nano-CT offers unprecedented opportunities for the detailed visualization of biological specimens. The high-resolution imaging capabilities of nano-CT facilitate the investigation of cellular and subcellular structures and can contribute to a better understanding of disease mechanisms and the development of targeted therapies. Furthermore, nano-CT holds promise for detailed investigations of pathological processes such as tumor angiogenesis, microcalcifications in cancerous tissues, and neurovascular remodeling in neurological diseases, thereby facilitating the discovery of novel biomarkers and therapeutic targets (8, 30-32). As nano-CT technology continues to evolve, this method is poised to become an integral tool in both research and clinical settings to enhance our ability to diagnose and treat various medical conditions with greater levels of precision (23).

General aspects and working principles of nano-CT

In many languages, X-radiation is referred to as Röntgen radiation, after the German scientist Wilhelm Conrad Röntgen after his initial discovery in 1895 (33). X-rays are produced in a vacuum tube that contains two main electrodes: a cathode and an anode, positioned at opposite ends of the tube. The cathode typically consists of a coiled filament, while the anode is located directly across from the cathode. In conventional X-ray machines, the anode is usually made of copper, with a tungsten target at the focal area that can withstand high temperatures. When the cathode filament is heated, typically by a low firing voltage of 8–12 V, it emits electrons by thermionic emission. These electrons are then accelerated towards the anode by a high direct current (DC) voltage ranging from 10 to 150 kV, thus creating the conditions necessary to generate X-rays (34-36). The accelerated electrons, also known as cathode rays, strike a small, designated area of the anode known as the focal spot. Upon impact, these electrons are rapidly decelerated, resulting in the release of energy. The majority of this energy, approximately 99%, is converted into heat, while only approximately 1% is emitted as electromagnetic radiation in the form of braking radiation, commonly known as X-rays (35, 36). Figure 1 depicts how X-rays are formed.

The resulting narrowly confined beam of X-ray photons, which is rotated around the subject/patient in conventional CT machines in clinical practice,

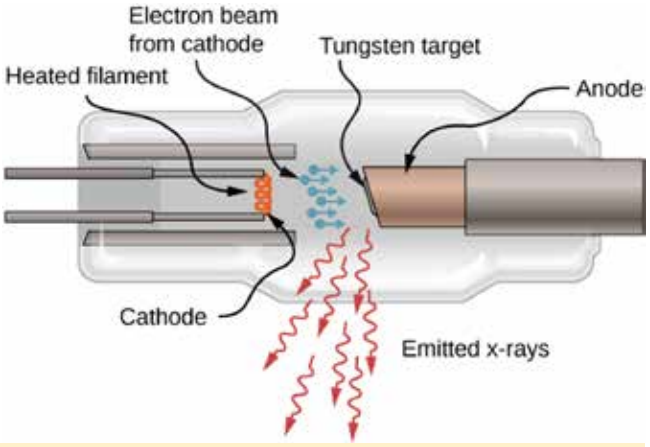

Figure 1. *A schematic showing the formation of X-rays with the most important components annotated. LibreTexts. Licensed under CC BY-NC-SA 3.0. Photo via Fotoeins Fotografie: https://fotoeins.com/2019/06/10/wuerzburg-roentgen-memorial-xrays/xrays-libretexts/*

travels towards the object and attenuates as it passes through the object. The light passed through an object is measured by a system of detectors, and the signals derived from the individual detectors are assembled into a profile (projection). These projections originate from various angles and undergo computational reconstruction, typically via filtered back-projection or iterative reconstruction algorithms, to generate a cross-sectional image of the specimen or patient. Each spatial element in the reconstruction is assigned an attenuation coefficient (μ), which is specific to the density and atomic composition of the substance. Instead of the raw attenuation coefficient, CT imaging utilizes relative values compared to water, known as Hounsfield Units (HU). This scale is named after Sir Godfrey Hounsfield, inventor of the X-ray CT scanner (37). Thus, each pixel in a final CT image represents the two-dimensional projection of the X-ray attenuation from a voxel (volume element) within the observed object (37).

Nano-CT originates from the further development of micro-CT technology and has achieved superior resolution via the use of transmission-target X-ray tubes with significantly smaller focal spots (< 400 nm), advanced high-resolution detector arrays, precise geometric magnification, optimized angular scanning protocols, and noise reduction techniques that involve repeated imaging sequences (8). These enhancements allow imaging at the sub-micron scale, hence the term nano-CT, coined specifically to differentiate these advanced systems from micro-CT technology. The unprecedented ability of nano-CT to detect detailed subcellular features can allow researchers to investigate biological questions that were previously beyond the capabilities of conventional micro-CT, thus exerting significant impact on fields such as histology, developmental biology and materials science.

Development of nano-CT and differences between nano-CT and other CT technologies

Until the late 1980s, CT scanners operated by a process known as axial scanning in which data were acquired sequentially in discrete slices. However, newer techniques have been developed, most prominently spiral or helical CT, in which the observed object translates through a gantry while the scanner rotates continuously around the object. This allows for new options in reconstruction. Spiral CT provides significant advantages compared to axial CT, particularly in terms of minimizing motion artifacts, reducing radiation exposure essential for in vivo imaging, improving spatial resolution along the Z-axis, and enhancing 3D image rendering capabilities (37).

During the early 1980s, the first micro-CT scanner was developed for the automotive industry by physicist Lee Feldkamp from Ford Motor Company (11). In 1984, Steven Goldstein from the University of Michigan replicated the micro-CT system in his own laboratory, establishing the first university micro-CT system that was built to allow the investigation of biomechanics (11).

High-resolution CT at the sub-micron level has primarily been considered the domain of synchrotron-based micro-CT (synMCT) (8). This technology is regarded as the gold standard for sub-micron imaging due to its exceptionally high photon flux, monochromatic beam capabilities, and enhanced

phase-contrast imaging performance, which can significantly increase image contrast and resolution when compared to laboratory-based CT systems. Unfortunately, synMCT is not a stand-alone device but rather a method that is implemented in large synchrotron centers, such as "The German Electron Synchrotron" (DESY), the "Conseil Européen pour la Recherche Nucléaire" (CERN) and "The National Synchrotron Light Source" (NSLS). Conversely, nano-CT systems represent stand-alone high-resolution devices that are designed for laboratory use and provide comparable resolutions but significantly greater accessibility and convenience when compared to synchrotron-based methods (8).

One primary operational difference between conventional medical CT and nano-CT is that in nano-CT systems, the specimen rotates around its own axis rather than the X-ray source and detectors rotating around the specimen. Once the sample is illuminated, the X-rays can reach the detector (8). X-ray absorption and diffraction patterns in the sample provide detailed structural information that are critical for high-resolution imaging. Unlike the microfocus X-ray tubes (reflection/direct-beam tubes) used in micro-CT, nano-CT systems utilize transmission tubes in which the X-ray window and focal spot coincide. This configuration allows the specimen to be positioned much closer to the focal spot, substantially reducing the effective focal spot size to approximately 500 nm and significantly enhancing achievable spatial resolution, often down to the sub-micron range (8).

A comparison of nano-CT and other imaging techniques in medicine: advantages and limitations

Advantages and limitations of imagine techniques in general

Computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) are all widely used imaging techniques in medicine, each with its own strengths and limitations. CT scanning, and PET scanning that uses 18F-fluorodeoxyglucose, involve ionizing radiation, which presents a critical consideration for repeated in vivo studies, especially in pediatric and longitudinal research contexts (38). MRI, on the other hand, has a key advantage in that it does not involve ionizing radiation.

CT is valued for its rapid acquisition times (39), making it ideal in emergency settings, such as the assessment of trauma or stroke. MRI and PET generally have longer acquisition times, which can cause discomfort (39) and anxiety in patients with claustrophobia, often necessitating sedation for MRI studies (40, 41). CT excels in providing high levels of spatial resolution; this is particularly useful for detecting small lesions, and provides excellent bone contrast, thus allowing detailed evaluation of skeletal structures. However, CT is not without its drawbacks. The primary disadvantage of this technique is exposure to ionizing radiation, raising concerns relating to cumulative radiation doses, particularly in pediatric populations or in patients requiring repeated scans. Furthermore, CT involves limited soft tissue contrast, often requiring the use of contrast agents to enhance the visualization of organs and vascular structures.

MRI, on the other hand, provides superior soft tissue contrast, making it particularly useful for brain, musculoskeletal and abdominal imaging (39). Furthermore, MRI offers multi-parametric capabilities, including diffusion-weighted imaging (DWI), functional MRI (fMRI), and spectroscopy, enabling comprehensive structural and functional evaluations. A major advantage of MRI is the absence of ionizing radiation (40), rendering this technique a safer option for repeated imaging, particularly for young patients. Moreover, the contrast material used for MRI is safer for the kidneys than that used for CT (40). In addition, MRI has certain limitations, such as longer acquisition times, that can be problematic for critically ill or restless patients. Also, the loud noises generated during scanning necessitate the use of hearing protection (40). Another consideration is that MRI is more expensive and less widely available than CT, and certain patients, particularly those with metal implants such as pacemakers, may not be suitable for MRI scans.

PET imaging, frequently combined with CT or

MRI (as in PET/CT or PET/MRI), is invaluable for functional imaging, especially in the fields of oncology, neurology and cardiology (39). The high sensitivity of PET to metabolic and molecular activity enables the early detection of disease and the precise assessment of physiological processes such as glucose metabolism and cerebral blood flow. Nevertheless, PET imaging is generally associated with lower spatial resolution when compared to CT or MRI (39), thus limiting its anatomical localization capabilities. PET involves exposure to radiation; although this is typically lower than the dose used for diagnostic CT scans (38, 42), the dose of radiation used in PET can vary significantly based on scan protocols, resolution requirements, and the specific type of radiotracer used (42). PET is also associated with high costs and logistical challenges due to the need for specialized radiotracers with short half-lives. In addition, acquisition times are generally longer than in CT (39).

In conclusion, while CT, MRI, and PET each offer unique strengths, they also present specific limitations that dictate their suitability in clinical and research contexts. The selection of an appropriate imaging modality depends critically on the clinical or research question, the requirement for anatomical or functional data, radiation safety considerations, and individual patient factors such as age, health status and the presence of implants.

A comparison of high-resolution 3D imaging techniques

High-resolution 3D imaging plays a crucial role in medical diagnostics and research as it can allow the detailed visualization of anatomical structures and physiological processes. Although CT, MRI, and PET all provide 3D imaging capabilities, these methods differ significantly with regards to spatial resolution, contrast sensitivity, acquisition speed, functional assessment capabilities, and levels of radiation exposure (Table 1).

CT, MRI, and PET each offer distinct advantages and limitations with regards to high-resolution imaging tailored to specific clinical or research requirements. CT, notably in micro-CT and nano-CT formats, delivers superior high-resolution 3D structural imaging, with nano-CT achieving spatial resolutions down to sub-400 nm levels. This high spatial resolution, combined with rapid acquisition times, makes CT particularly suited for the detailed visualization of hard tissues, such as bone and calcified structures. Nevertheless, CT is associated with significant limitations, including comparatively poor intrinsic soft tissue contrast, necessitating the use of contrast agents for adequate soft tissue differentiation, and exposure to ionizing radiation, which limits its frequent in vivo use, especially in longitudinal studies.

MRI is renowned for its exceptional soft tissue contrast and provides detailed 3D anatomical images without the need for ionizing radiation, rendering this technique particularly advantageous for repeated longitudinal imaging studies and safer for application in pediatric patients. MRI also supports advanced multi-parametric imaging modalities, such as functional MRI (fMRI), diffusion-weighted imaging (DWI), and spectroscopy, enabling

Table 1: Comparison of computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET). fMRI – functional MRI; DWI – diffusion-weighted imaging.

Modality	Spatial Resolution	Soft Tissue Contrast	Functional Imaging	Radiation Exposure	Acquisition Time
CT (micro/nano)	High (50–100 µm for micro-CT, sub-400 nm for nano-CT)	Moderate (improved with contrast agents)	No	Yes	Fast
MRI	Moderate to High (50–100 µm for high-field MRI)	Excellent	No (except fMRI, DWI)	No	Long
PET	Low (1–2 mm)	Poor (relies on CT/MRI fusion)	Yes	Yes	Long

both structural and functional assessments at relatively high resolutions. However, MRI typically achieves spatial resolutions in the micrometer range that are lower than those of nano-CT. The limitations of MRI include prolonged scan durations, susceptibility to motion artifacts (which are problematic for non-compliant or critically ill patients), high operating costs, and the limited accessibility of high-field MRI equipment.

PET imaging primarily provides functional information rather than detailed anatomical structures. This method exhibits high levels of sensitivity for metabolic and physiological processes at the molecular level and is therefore invaluable for the detection of disease in the early stages, especially in oncology and neurology. The combination of PET with CT or MRI (PET/CT, PET/MRI) can significantly improve anatomical localization by integrating functional and structural data. However, PET imaging typically exhibits lower spatial resolutions (approximately 1–2 mm) compared to CT and MRI. Furthermore, PET involves radiation exposure from radiotracers, often requiring short half-life radiopharmaceuticals; this introduces logistical complexity, higher costs, and practical limitations.

In summary, for high-resolution 3D anatomical imaging, nano-CT provides the highest achievable spatial resolution, and is particularly beneficial for ex vivo research settings. MRI excels in soft tissue differentiation, yet its spatial resolution remains limited compared to nano-CT. PET offers indispensable functional imaging capabilities but lacks the spatial resolution necessary for fine structural detail. Hybrid imaging modalities (PET/CT, PET/MRI) effectively integrate complementary functional and anatomical information, overcoming the inherent limitations of standalone modalities and significantly enhancing diagnostic and research capabilities in both clinical and preclinical settings.

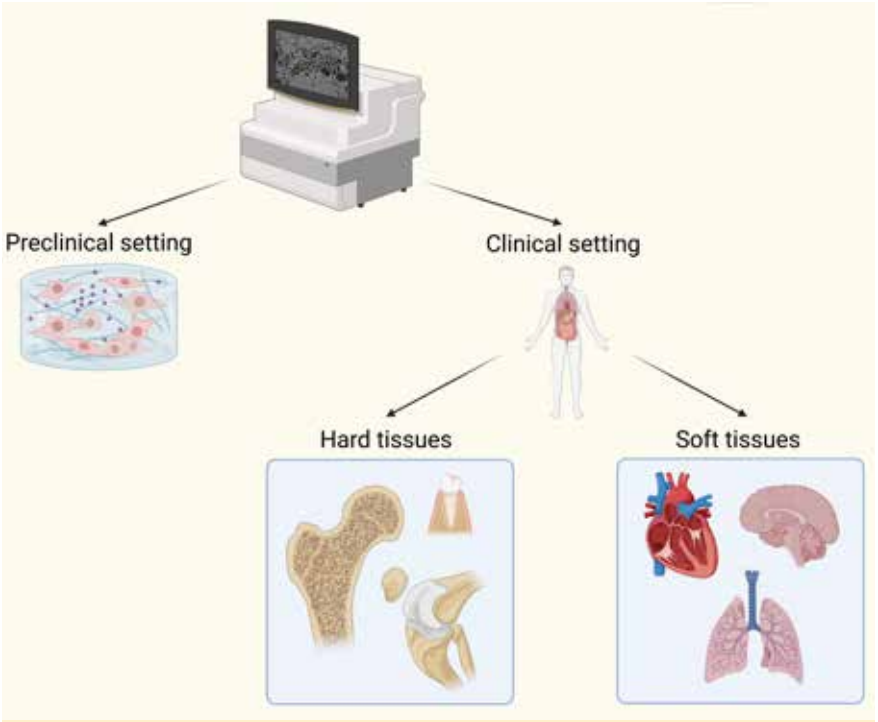

Figure 2. *Schematic showing the potential uses of nano-CT in both preclinical and clinical settings.*

SPECIFIC APPLICATIONS

Figure 2 depicts the potential applications of computed nanotomography (nano-CT) in the field of medicine.

Preclinical setting: research

Nano-CT has emerged as a powerful tool in preclinical and biomedical research as it provides ultra-high-resolution 3D imaging at the nanometer scale (50–400 nm). While conventional clinical CT is routinely used for diagnostic purposes, nano-CT is predominantly employed for ex vivo and studies due to current limitations. Nano-CT enables researchers to investigate intricate structural details in biological tissues, disease models, and biomaterials, and can provide precise volumetric and structural measurements. A key advantage of this technique is

its non-destructive nature, which preserves sample integrity for subsequent analytical methods (8).

Biomaterials and tissue engineering

Nano-CT plays a crucial role in the evaluation of biomaterial properties and their interaction with biological tissues. This technique can facilitate the detailed characterization of biomaterial scaffolds by evaluating a range of microstructural features, including porosity, pore interconnectivity, degradation patterns, and cellular infiltration within engineered tissues (43-48). Imaging quality can be further enhanced by contrast-enhanced nano-CT which is particularly beneficial for visualizing soft tissues. This technology can make significant contributions to nanoparticle distribution studies within drug delivery systems and enables the high-resolution visualization of pathological alterations in models of experimental disease (49).

Imaging is primarily conducted ex vivo, with limited in vivo applications constrained by prolonged scan times, radiation dose concerns, and restricted sample sizes. Soft tissue visualization typically necessitates the use of contrast agents. However, advances in phase-contrast imaging, synchrotron-based nano-CT, and multi-modal imaging approaches are poised to significantly enhance the capabilities of nano-CT. With continued improvements in acquisition speeds, reduced radiation doses, and the performance of soft tissue imaging, nano-CT is expected to increasingly bridge preclinical research and clinical translational applications.

Clinical settings

While nano-CT is currently predominantly utilized in preclinical research, recent technological advancements suggest promising potential for future clinical applications. Currently, nano-CT remains largely unimplemented in routine clinical care due to a number of constraints, including a small field-of-view, extensive scan durations, and concerns relating to radiation exposure. However, as technology progresses, the high-resolution 3D imaging capability of nano-CT may exert profound impact on diagnostics, surgical planning, and personalized medicine. Despite promising prospects, nano-CT is not yet applicable for routine clinical practice due to radiation safety issues, prolonged imaging times, and the necessity for small sample sizes. Nevertheless, future developments in phase-contrast techniques, synchrotron nano-CT methodologies, and artificial intelligence (AI)-driven image reconstruction algorithms could overcome these limitations, potentially facilitating the integration of nano-CT into precision medicine workflows (50, 51).

Hard tissues

Nano-CT provides an ultra-high-resolution and non-destructive imaging modality for bone and dental tissues. Compared to conventional micro-CT and clinical CT, nano-CT delivers sub-micrometer resolution, enabling detailed visualization of bone microarchitecture, dental structures and implant integration (6, 16, 50). In addition, nano-CT facilitates high-resolution 3D imaging of trabecular and cortical bone, surpassing micro-CT with regards to the detection of microscopic bone porosities and mineralization patterns (2). Furthermore, nano-CT provides detailed structural insights into fracture healing processes, including callus formation and bone remodeling in complex fractures. Nano-CT has also proved to be invaluable for the early detection of trabecular bone loss in osteoporosis and in the analysis of microstructural changes associated with metabolic bone diseases and rare skeletal disorders. Furthermore, nano-CT can significantly enhance the evaluation of bone metastases, tumor invasion into osseous structures, and implant osseointegration, thus providing detailed insights into bone-implant contact and peri-implant bone density. These capabilities are instrumental in optimizing orthopedic implants and investigating the mechanisms that underlie implant wear and failure (16, 52).

Nano-CT represents a significant advancement in the visualization of mineralized structures in dentistry and oral surgery, and can achieve levels of detail that are unattainable by conventional radiography. The sub-micrometer imaging capability of nano-CT enables researchers to investigate early-stage caries, enamel demineralization, and microfractures prior to clinical manifestation to provide insights into dental

tissue aging, wear, and erosion processes (26). Nano-CT also enhances the 3D visualization of root canal morphology and can elucidate complex variations in root systems (12). In addition, nano-CT can facilitate the assessment of endodontic therapy, evaluate the adaption of filling materials, periapical healing, and the osseointegration of dental implants. This imaging tool could also facilitate the development of biocompatible dental prosthetics, restorative materials, and veneers, and permit the investigation of adhesion properties and material degradation under realistic conditions (20, 53).

Challenges and future perspectives in the use of nano-CT for the imaging of hard tissue

Current nano-CT systems are unsuitable for live human imaging, thus restricting its application primarily to ex vivo analyses. The high-resolution imaging capability of this technique is constrained by narrow fields-of- view, limiting applicability to whole-bone or large joint evaluations. However, despite these limitations, nano-CT offers unparalleled resolution in hard tissue imaging, proving invaluable for bone pathology research, dental diagnostics, and biomaterials assessments. Ongoing technological advancements are expected to broaden the applications of nano-CT in the fields of clinical diagnostics and personalized medicine via faster imaging protocols, AI-enhanced reconstruction techniques are improving our ability to visualize soft tissue and facilitating integration with conventional clinical imaging methods such as CT and MRI. These developments will likely facilitate a comprehensive, multi-resolution approach to diagnostic evaluations and therapeutic planning in the future (50).

Soft tissues

Thus far, nano-CT has proved to be more effective for the imaging of hard tissue due to the high X-ray absorption properties of bone and dental structures. However, recent developments in contrast-enhancement methods, phase-contrast imaging, and synchrotron-based nano-CT have markedly improved the capability of nano-CT to visualize soft tissues at nanometer-scale resolution (54-56). Nano-CT is increasingly being integrated into histopathological workflows to enhance tissue analysis. This integration provides comprehensive 3D histological insights without the necessity for the sectioning of physical tissue, thereby preserving sample integrity and facilitating subsequent specialized analyses. These advancements open promising opportunities for clinical applications across various medical disciplines, including histopathology, oncology, neurology, cardiovascular medicine, and organ-specific research (50).

Nano-CT could represent a significant contributor to oncology research by facilitating the detailed assessment of tumor microarchitecture, vascularization, tumor-stroma interactions, and cellular heterogeneity. The ultra-high-resolution imaging capabilities of nano-CT enable the precise visualization of microstructural tumor characteristics, thus facilitating the early detection of disease, prognostic assessment, and personalized therapeutic planning (31). In addition, nano-CT facilitates the highly detailed imaging of microvascular networks in a manner that surpasses the capabilities of conventional 2D histology and other imaging modalities. This makes nano-CT particularly beneficial for applications in cardiovascular and vascular medicine. Potential applications include the comprehensive analysis of atherosclerotic plaque composition, microvascular remodeling within arterial tissues, the three-dimensional visualization of capillary networks, the detailed assessment of stent and vascular graft integration, and the characterization of heart tissue, including myocardial fibrosis and damage assessment in ischemic heart disease (8, 57).

Nano-CT can significantly enhance the evaluation of lung tissue architecture in pulmonary diseases such as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and lung damage related to COVID-19 infection. Furthermore, this technique allows for the precise characterization of alveolar structures, microarchitectural changes, collagen deposition, lung tissue remodeling, and inflammation-associated vascular alterations, thus facilitating detailed investigations into disease progression and pathophysiological mechanisms (8, 15, 58).

Another consideration is that nano-CT offers considerable potential for the field of neuropathology due to its ability to resolve subcellular features within neural tissues. This technique could significantly enhance research, diagnosis, and therapeutic approaches for neurodegenerative disorders by enabling detailed analyses of the microstructural alterations associated with Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Moreover, nano-CT can facilitate the precise examination of brain microvasculature, synaptic architecture, and neuronal structures, providing deeper insights into the pathology of neurological disease (8, 59, 60).

Challenges and future perspectives in the application of nano-CT for soft tissue imaging

Soft tissues inherently exhibit low X-ray contrast; this poses challenges for nano-CT imaging. However, several strategies are being explored to enhance soft tissue visualization. First, contrast agents, such as heavy-metal-based staining agents (e.g., iodine, osmium and phosphotungstic acid) could improve detail at the cellular-level by increasing X-ray contrast. Second, phase-contrast nano-CT is under development; this technique aims to exploit variations in X-ray phase shifts to achieve label-free, high-contrast imaging of soft tissues. Third, synchrotron radiation-based nano-CT is under development. This technique aims to utilize the highly intense and coherent X-rays available at specialized synchrotron facilities to deliver superior soft tissue contrast and higher spatial resolution.

Although nano-CT is currently limited to ex vivo imaging for the analysis of clinical soft tissue, the application of this technique is rapidly expanding within the fields of histopathology, cardiovascular research, neurology and pulmonology. Ongoing advancements in contrast-enhancement techniques, multi-modal imaging integrations that combine nano-CT with functional imaging modalities (MRI, PET), along with further technological improvements, are anticipated to significantly broaden the clinical utility of nano-CT in the future.

CONCLUSIONS

Nano-CT represents a significant advancement in 3D imaging technology, providing unprecedented resolution at both the cellular and subcellular levels. The ability of this method to visualize intricate biological structures in a non-destructive manner has transformative implications for preclinical research, particularly in tissue engineering, the evaluation of biomaterials, and detailed pathological analyses across numerous medical fields, including oncology, cardiovascular medicine, pulmonology, neurology, and dentistry. Despite its evidential strengths and potential for significant clinical impact, the routine application of nano-CT in clinical settings remains limited due to several technological and practical constraints. These limitations include prolonged scan times, limited fields-of-view, concerns related to the dose of radiation, and challenges associated with soft tissue contrast. Nevertheless, ongoing technological innovations, particularly advancements in phase-contrast imaging, contrast agent development, synchrotron-based imaging techniques, and AI-driven reconstruction methods, are steadily overcoming these limitations. Future research and clinical validation are essential to fully realize the integration of nano-CT into routine diagnostic and treatment planning workflows, ultimately supporting precision medicine and personalized therapeutic strategies.

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