Nano-Computed Tomography: Technique and Applications
Nanocomputertomografie: Technik und Applikationen

Abstract
Nano-computed tomography (nano-CT) is an emerging, high-resolution cross-sectional imaging technique and represents a technical advancement of the established micro-CT technology. Based on the application of a transmission target X-ray tube, the focal spot size can be decreased down to diameters less than 400 nanometers (nm). Together with specific detectors and examination protocols, a superior spatial resolution up to 400 nm (10 % MTF) can be achieved, thereby exceeding the resolution capacity of typical micro-CT systems. The technical concept of nano-CT imaging as well as the basics of specimen preparation are demonstrated exemplarily. Characteristics of atherosclerotic plaques (intraplaque hemorrhage and calcifications) in a murine model of atherosclerosis (ApoE\(^{-/-}\)/LDLR\(^{-/-}\)-double knockout mouse) are demonstrated in the context of superior spatial resolution in comparison to micro-CT. Furthermore, this article presents the application of nano-CT for imaging cerebral microcirculation (murine), lung structures (porcine), and trabecular microstructure (ovine) in contrast to micro-CT imaging. This review shows the potential of nano-CT as a radiological method in biomedical basic research and discusses the application of experimental, high resolution CT techniques in consideration of other high resolution cross-sectional imaging techniques.

Citation Format:

Zusammenfassung
**Introduction**

Regardless of the rapid technical development of multidetector CT in relation to high temporal resolution, this cross-sectional imaging method used in the clinical routine has a maximum spatial resolution of 240–600 μm. First in-vivo scanners, so-called high-resolution peripheral quantitative CT scanners (HR-PQCT), with a spatial resolution between 105 and 55 μm at 10% MTF (XtremCT II), SCANCO Medical, Brüttisellen, Switzerland) are already in use for the visualization of the fine structure of peripheral bone [1]. Structures exceeding this achievable resolution currently cannot be visualized in detail in clinical cross-sectional CT imaging. Therefore, histology/pathology is still considered the gold standard for the visualization and quantification of structures with a size of less than 200 μm. This means that in addition to the time and personnel-intensive specimen preparation there are disadvantages regarding inexact or incomplete three-dimensional structure visualization and the destructive character of sample analysis. Micro-CT has become established as a complement to histological examination as a non-destructive three-dimensional imaging technique on the microscopic level. The following should be noted: There is no standard definition of the term “micro-CT”. Kalender et al. [2] suggest a spatial resolution of at least 100 μm. CT scanners with higher spatial resolution can be referred to as micro-CT scanners regardless of the specific model. As a result of the acquisition of high-resolution image datasets with isotropic voxel geometry and an average spatial resolution of 5 – 50 μm, this technique provides a three-dimensional view of different organ systems and can provide information in addition to histomorphometry. This method was used primarily as part of osteological issues regarding the morphometry of compact and spongy bone [3]. The development of new contrast enhancement techniques could significantly expand the spectrum of possible applications. By using intravascular polymerizing contrast agents, knowledge of basic vascular research can be attained and pathological vascular changes can be visualized and quantified with high accuracy [4]. In-vivo micro-CT became established as a functional diagnostic method for pulmonary and cardiac performance parameters in small animal models [5, 6] and allows examination series with a longitudinal study design. Nonetheless, micro-CT, both in-vivo and ex-vivo, is limited by spatial resolution. These limits become evident in particular in fine structure imaging, e.g. in the visualization of terminal vessels, components of atherosclerotic plaques and cellular lacunae in calcified hard tissue. Building on the further development of micro-CT technology, nano-CT systems that differ partially with respect to design and performance (spatial resolution, tube voltage, and tube power) could be introduced (Xradia 800 Ultra; Zeiss, Oberkochen, Germany; nano-CT 2011, nano-CT 2211: Bruker MicroCT, Kontich, Belgium; Nanotom S, Nanotom M; GE/Phoenix X-ray, Wunstorf, Germany). By reducing the focal spot size and using suitable detectors and examination protocols (geometric enlargement, angular scanning, noise reduction by repetitive image acquisition), these systems allow imaging in the submicrometer range which is why the term nano-CT was introduced to differentiate from micro-CT. The goal of this overview is to describe technical device fundamentals, sample mounting, and application examples of nano-CT by way of example.

**Basics of nano-CT technology**

**X-ray generation**

High-resolution computed tomography in the submicrometer range has primarily been a domain of synchrotron-based micro-computed tomography (synMCT) [7, 8]. Based on the high achievable photon density and the monoenergetic X-ray radiation, the image quality is to be considered the gold standard due to the high signal-to-noise ratio and the lack of beam-hardening artifacts. However, synMCT does not represent an independent device class. Rather it is a method that is practiced at synchrotron facilities, i.e. major research institutions, such as the German Electron Synchrotron (DESY), the Conseil Européen pour la Recherche Nucléaire (CERN), and the National Synchrotron Light Source (NSLS). In contrast, the nano-computed tomography system (nano-CT 2011) presented in the following can be referred to as a prototype of an independent high-resolution device class. It is characterized by compact individual machines (standalone technology).

In the presented system the X-ray tube does not have a constant vacuum which is why a vacuum (6 × 10⁻⁴ Pa) is created via a suction pump and must be kept consistently stable (“open pumped type X-ray source”). The coil of the filament cathode is made of lanthanum hexaboride (LaB₆). Two horizontally and vertically connected condenser/objective electron lenses focus the emitted electrons. The anode is comprised of a tungsten-vaporized beryllium glass plate on which X-ray radiation is generated at acceleration voltages of 20 – 80 kVp and a tube current of 100 – 200 μA. Due to the lack of collimation, no useful beam with a detector-adapted form is generated in the presented system and the aperture angle of the radiation is approx. 170° (manufacturer information: Mars-Tohken, Tokyo, Japan). The horizontal and vertical divergence of the radiation results in coverage of the detector whose exposure geometry is analogous to that of flat detector/cone beam CT. Depending on the targeted spatial resolution/magnification, focal spot sizes between < 400 and 600 nm can be selected, applications with a voxel side length in the submicrometer range typically being realized with the smallest possible focus (< 400 nm). Compared to most microfocus X-ray tubes (classic reflection/direct beam tubes) used in micro-CT, these are transmission tubes, i.e., the X-ray window and focal spot coincide (Fig. 1). Since the object to be examined can be positioned closer to the focal spot and the diameter of the nano-focused focal spot is significantly smaller than in conventional micro-CT, a maximum spatial resolution in the submicrometer range is achieved. However, the thermal energy resulting from X-ray generation cannot be actively deflected from the focal spot which is why the tube current is significantly lower than in micro-CT and also differs between the selectable focal spot sizes. As a result, the photon density is lower than in micro-CT. Greater image noise occurs particularly at the smallest focus. In addition, greater enhancement of the detector signal is necessary due to the lower space-charge effect of the X-ray tube at a lower voltage, which also causes increased noise. Based on Kalender et al. [9], Table 1 shows major differences between in-vivo and ex-vivo micro-CT and nano-CT.
The vacuum in the tube is maintained by the connection (3) of a downstream vacuum pump (not shown). The X-ray window and the X-ray focus are comprised of a beryllium glass plate with a vapor-deposited tungsten layer (4). The generated radiation (5) is not collimated. The emitted radiation therefore does not have a cone geometry that is adapted to the detector but rather an opening angle of approx. 170° (manufacturer specifications). The nano focusing of the X-ray beam is made possible by controllable pairs of electromagnetic condenser lenses (6) and electromagnetic objective lenses (7). A rotating platform (3) with mounted sample holder (4) positioned between X-ray tube (partially shown) with X-ray window (1) and camera unit (2). By shifting along the Z-axis the desired geometric magnification is achieved, while the sample can be centered in the field of view by lateral and vertical movements.

**Image creation**

The sample fixed to an air-suspended rotating platform via a sample holder can be adjusted in all three spatial directions with a precision of up to 100 nm. Depending on the selected magnification, the rotation movement can be freely defined in angle increments up to 0.1° and scanning is performed over 180° plus horizontal fan angle (= 185.15°). Projection images are acquired using the “step and shoot” technique. This procedure is necessary to allow noise reduction by additional frame averaging at exposure times of 2 f/s. At a fixed focus-detector distance (FDD) of 152 mm, the rotating platform is mounted on a base that can move in the Z-direction between the focal spot/X-ray window and detector (Fig. 1) which determines the focus-object distance (FOD). The resulting geometric magnification (FDD/FOD) is between M_max = 52.1 at 2.92 mm FOD and M_min = 1.1 at 138.4 mm FOD. The image generation system is comprised of a terbium-doped gadolinium oxysulfide scintillator layer (GD_2O_2S:Tb) with a thickness of 15 µm, an image intensifier of a terbium-doped gadolinium oxysulfide scintillator layer (138.4 mm FOD). The image generation system is comprised of a beryllium glass plate with a vapor-deposited tungsten layer (4). The generated radiation (5) is not collimated. The emitted radiation therefore does not have a cone geometry that is adapted to the detector but rather an opening angle of approx. 170° (manufacturer specifications). The nano focusing of the X-ray beam is made possible by controllable pairs of electromagnetic condenser lenses (6) and electromagnetic objective lenses (7). A rotating platform (3) with mounted sample holder (4) positioned between X-ray tube (partially shown) with X-ray window (1) and camera unit (2). By shifting along the Z-axis the desired geometric magnification is achieved, while the sample can be centered in the field of view by lateral and vertical movements.

**Table 1** Technical characteristics of in-vivo and in-vitro micro-CT and presented nano-CT based on Kalender et al. [9].

<table>
<thead>
<tr>
<th></th>
<th>in-vivo micro-CT</th>
<th>in-vitro micro-CT</th>
<th>nano-CT</th>
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<tbody>
<tr>
<td>tube design</td>
<td>reflection tube</td>
<td>reflection tube/</td>
<td>transmission tube</td>
</tr>
<tr>
<td>focus size</td>
<td>50 – 200 µm</td>
<td>1 – 50 µm</td>
<td>&lt;400 nm</td>
</tr>
<tr>
<td>tube voltage</td>
<td>20 – 90 kVp</td>
<td>20 – 130 kVp</td>
<td>8 – 190 kVp</td>
</tr>
<tr>
<td>tube power</td>
<td>10 – 300 W</td>
<td>1 – 50 W²</td>
<td>8 – 25 W</td>
</tr>
<tr>
<td>spatial resolution</td>
<td>50 – 200 µm</td>
<td>5 – 100 µm</td>
<td>50 – 600 nm</td>
</tr>
<tr>
<td>scan times</td>
<td>0.1 – 30 min</td>
<td>10 – 300 min</td>
<td>60 – 180 min</td>
</tr>
<tr>
<td>detector</td>
<td>CCD/flat panel detector</td>
<td>CCD/flat panel detector</td>
<td>CCD/flat panel detector</td>
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</table>

The tube voltage¹ or power² used in biomedical in-vitro diagnosis is significantly lower than the power used for industrial measurement purposes that can exceed 1 MVp (linear accelerator, not microfocussed) or 500 W. Significant differences between consecutive scans due to the filament properties, i.e., electron emission that fluctuates over time. Therefore, calibration based on one scan does not allow reliable transferability to subsequent examinations. The cross-sectional image datasets are therefore reconstructed and stored without calibration from a preselected gray value range of the projection dataset with gray value scaling of 8 bits as a bitmap (BMP) or 16 bits as tagged image file format (TIFF).

**Sample mounting**

To minimize truncation artifacts, the projection image of the sample should only minimally laterally exceed the detector. For optimal utilization of the detector, this means that the axis of rotation of the object moves near the vertical center of the detector. Axis deviations can be corrected by incremental fine adjustment of the rotating platform during examination preparation which allows continuous centering of the sample in front of the detector. Since detector size, object centering, and the required spatial resolution limit the maximum diameter of the sample, imaging in the submicrometer range requires preparation of samples in the order of magnitude of ≤ 1 mm diameter. As technical tools, a binocular reflected-light microscope or magnifying glasses, preparation instruments, a cutting mat, cylindrical punches, and wax paper (to protect against drying out and for securing on the sample holder) are virtual necessities for the preparation of samples.
Application spectrum of nano-CT

Examination material

The image material presented in the following comes from approved animal experiments (aorta experiment number: GI 20/9 no.106/2011; brain experiment number: GI 20/9 no.107/2011) or killings for non-scientific purposes (porcine lung, ovine bone). Examinations for comparative purposes were performed on a multidetector CT scanner (SOMATOM Definition, Siemens, Erlangen) or two micro-CT scanners (type 1072 and 1173) and a nano-CT scanner (type 2011, Bruker microCT, Kontich, Belgium). The technical differences among high-resolution systems are summarized in Table 2.

Imaging of atherosclerotic plaques in a small animal model

The animal model of the ApoE(-/-)/LDLR(-/-) double knockout mouse (AL mouse) has become established in the last decade as an essential small animal model in atherosclerosis research [11]. Already 15 weeks after birth, atherosclerotic plaques can be seen in the descending aorta and in the aortic arch [12]. The first cup-shaped calcified plaques in the aortic arch and in the supraaortic vessels are seen after 20 weeks. Features of vulnerable lesions (plaque hemorrhage and increased vasa vasorum (VV) neovascularization) can be primarily seen in the descending aorta after 80 weeks. A quantitative evaluation of VV neovascularization could be repeatedly performed in micro-CT [13, 14]. Plaque hemorrhages were primarily detected in synMCT [15]. In previous studies regarding plaque differentiation of atherosclerotic vascular wall lesions, quantitative statements regarding plaque composition differentiating fibrous, calcified, and lipid-rich plaques could be made in micro-CT studies [16]. The distribution, size, and type of atherosclerotic plaque can also be detected and histologically verified via nano-CT equivalent to micro-CT. Calcifying, fibrotic, and hemorrhagic plaques can be reliably differentiated on the basis of the vasa vasorum density, the X-ray opacity, and the object size [17]. Based on the improved spatial resolution in nano-CT, post-hemorrhagic iron accumulations can be identified on the basis of their fine-granular morphology (Fig. 2). In contrast to calcified plaques, grouped, point-shaped hyperdensities are seen in soft plaques located apart from the vasa vasorum neovascularization. While in-situ micro-CT ensures reliable detection of calcified plaques, there are clear deficits in the visualization of the internal structure. Imaging in the submicrometer range via nano-CT makes it possible to detect non-calcified recesses of single cells within the plaques (Fig. 3) whose histopathological correlate corresponds to matrix-producing chondrocyte-like cells.

Imaging of cerebral vascularization

Vascularization of the cerebral cortex shows hierarchical structuring in micro-CT. Radial blood conduits (cortical veins) drain into superficial veins (superficial cerebral veins) that are then directed into the sinus via bridging veins. Depending on the spatial resolution, horizontally situated fine parenchymal vessels in the sense of capillaries (nano-CT, Fig. 4) can be detected in addition to radially running larger parenchymal blood conduits (micro-CT). In addition to the improved detectability of the details of the smallest structures, the edge sharpness of the visualized objects increases regardless of the individual size. This results in more precise binarization which is mandatory for morphometry techniques for the automatic calculation of vessel volume, diameter, separation, etc. [18].

### Table 2

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Tube Size</th>
<th>Focus Voltage</th>
<th>Tube Voltage</th>
<th>Detector</th>
<th>Spatial Resolution (10% MTF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1072</td>
<td>Reflection tube</td>
<td>8 µm</td>
<td>80 kVp</td>
<td>CCD</td>
<td>15 µm</td>
</tr>
<tr>
<td>Type 1173</td>
<td>5 µm</td>
<td>40–130 kVp</td>
<td></td>
<td>Flat panel detector</td>
<td>7 µm</td>
</tr>
<tr>
<td>Nano-CT 2011</td>
<td>Transmission tube</td>
<td>&lt;400–600 nm</td>
<td>20–80 kVp</td>
<td></td>
<td>400 nm</td>
</tr>
</tbody>
</table>

Fig. 2 Maximum intensity projection (MIP) of the descending aorta of a 78-week-old AL mouse: a Micro-CT (type 1072), imaging at 13.6 µm isotropic voxel side length, b nano-CT: Enlarged image at 2.5 µm isotropic voxel side length. Improving the spatial resolution makes the vasa vasorum neovascularization clearer. In addition, the smallest points of opacity can be detected (histologically verified as iron content) as proof of plaque stage V or VIII, i.e., fibromuscular or fibrotic [30] after a hemorrhage (stage VIIb), in terms of an advanced lesion.
Imaging of fixed porcine lungs
Micro-CT and synMCT are well established techniques in lung imaging and structure analysis [19]. By using mono-energetic, high-intensity synchrotron radiation, the fine structure of the lung can be visualized with minimal artifacts and high image quality, thus allowing anatomical fine structure analyses, e.g., of acinar lung structures [20, 21]. While micro-CT requires the use of metal-containing contrast agents (e.g., osmium tetroxide) for contrast enhancement of parenchymal structures, high-resolution nano-CT allows visualization of alveolar structures without additional contrast enhancement and suppresses interfering partial volume effects. This facilitates binarization that is then the foundation of software-based structure analysis or morphometry techniques. However, this advantage is not applicable as a result of shifting of the spatial resolution into the application range of micro-CT. Use of the nano-CT technique does not show any advantages here (Fig. 5).

Imaging of trabecular cell structures
As opposed to clinically established multidetector CT, micro-CT is highly suitable for the visualization and characterization of trabecular networks and represents an established standard in preclinical osteoporosis research [22]. By also examining calcium hydroxyapatite phantoms, the bone mineral density (BMD) or the tissue mineral density (TMD) can be determined analogously to clinical QCT. For cellular regulation of bone metabolism, osteoblasts and osteoclasts as well as the population of osteocytes are increasingly becoming the focus of scientific interest [23–25]. In the case of an average osteocyte lacunae volume of approx. 400 µm³ [26, 27] and under the assumption of a spherical shape, i.e., a diameter of approx. 9 µm, it quickly becomes clear that conventional micro-CT systems (see Table 1) reach their limit here. The increase of the spatial resolution as a result of using nano-CT allows detection and morphometry of individual osteocyte lacunae and description of the spatial distribution in mineralized bone (Fig. 6, 7).

Conclusion
Imaging methods including the classic imaging techniques of radiology (CT/MRI) are an inherent component of basic biomedical research. The improved detection of details demonstrated for nano-CT makes it possible to address issues that could not be or could only be insufficiently examined under the previous device-specific resolution limits in computed tomography. The visualization of soft cellular lacunae in calcified tissues [17, 28] provides information that characterizes the interaction between cells and an extracellular matrix in the three-dimensional space. In addition, it shows the necessity for the development and researching of new contrast agents that allow differentiation of cells and the extracellular matrix in soft tissue, i.e., in the low contrast range [29]. The imaging of plaque hemorrhages indicating complicated or unstable atherosclerotic plaques was previously not possible via tube-based polychromatic micro-CT and was reserved for synchrotron-based monochromatic micro-CT. Thanks to the improved resolution in nano-CT, the visualization of histopathologically verifiable plaque hemorrhages in the form of ultra-fine iron particles...
is now possible and provides information for the imaging of vulnerable plaques. The following is seen: Clinically relevant issues, e.g. precise plaque characterization [30], can already be addressed today from a technical standpoint, i.e., via nano-CT. Nano-CT is part of a spectrum of high-resolution non-destructive examination methods. In particular, optical and acoustic techniques such as confocal laser scanning microscopy (LSCM), optical coherence tomography (OCT), or ultrasound (ultrasound biomicroscopy – UBM) also allow cross-sectional imaging-based three-dimensional visualization and characterization of microscopic structures. As a result of rasterized scanning, LSCM acquires a three-dimensional dataset that shows the distribution of biomolecules, e.g., via temporary fluorescent marking. Thanks to examination times in the (sub-) second range, dynamic processes in living cells can be visualized, but other basic requirements than for nano-CT are valid for a sufficient examination. This includes a lower maximum thickness (approx. 100 µm) and lowest possible light scattering of the examination object. The surface topography, but not the internal structure, of opaque and light-scattering samples can be evaluated [31]. Since the best possible lateral spatial resolution and the Z-resolution (200 nm vs. 700 nm) differ [32], voxel anisotropy occurs at maximum resolution.

In contrast to LSCM, the axial resolution of OCT is defined by the capacity, i.e., bandwidth and central wavelength of the light source, and today achieves resolution in the range of 500 nm and examination times in the sub-second range (swept source optical coherence tomography – SS-OCT) [33]. There are limitations for the examination of opaque, highly reflective structures, such as metallic stents [34, 35] that do not apply for micro-CT or nano-CT [29, 36]. As a real-time procedure, ultrasound biomicroscopy (UBM) achieves a spatial resolution of approx. 15 µm (axial) or 30 µm (lateral) [9, 37] at sound frequencies of up to approx. 100 MHz with a tissue-dependent penetration depth of approx. 1 – 2 mm which corresponds to a significantly lower spatial resolution compared to nano-CT at a comparable FOV. For the spectrum of higher sound frequencies (scanning acoustic microscopy – SAM – up to 2 GHz), mapping of mechanical properties [38, 39] with spatial resolution of less than 100 nm can be achieved. Sample preparation is more complex. While UBM can be applied in vivo, slice creation prior to examination is recommended for SAM [40]. In addition, the use of suitable coupling media (degassed water) has a certain disadvantage since dry preserved samples can be damaged.
Typical limitations of the method must also be taken into consideration for nano-CT. The relatively long examination times compared to optical and acoustic methods allow a throughput of two to three examinations per day. Moreover, the size of samples must be small when a spatial resolution in the sub-micrometer range is targeted (see sample mounting). Solutions for this were developed in previous years: As a result of the use of powerful tubes and detectors, the examination in nano-CT at 600 nm isotropic voxel side length allows a look at the trabecular microstructure and visualizes osteocyte lacunae and trabecular vascular canals.
(flat panel detectors to $3072 \times 2400$ pixels: Nanotom M) and techniques for enlarging the field of view by lateral shifting of the detector (offset technique: SkyScan 2211), relatively large objects can be examined in nano-CT. Moreover, the improvement of existing reconstruction techniques makes it possible to suppress truncation artifacts which makes artifact-free examination of defined regions within an object (ROI scanning) possible [41–43]. The associated improvement for fine structure analysis of larger objects highlights the non-destructive examination character of the method since the preparation effort, i.e., manipulation of the samples, can be reduced in advance.

Limitations
In contrast to micro-CT, in-vivo use of nano-CT does not seem promising for now. The necessary suppression of motion artifacts from physiological movement patterns, e.g. from heartbeat, breathing, and intestinal movement, would require significantly shorter image acquisition times and higher tube power. Thus the technique is to be viewed also prospectively as an imaging method of ex-vivo analysis.

Future possibilities
The application of nano-CT in experimental ischemia diagnosis and therapy allows improved visualization of cerebral microcirculation [44] to the capillary level. Use for fine structure imaging of the osseous [28, 45], renal [46, 47], and dental [48] anatomy and pathophysiology as well as scaffold imaging [29] are examples for the potential of this radiological technique and expected uses in coming years.

Acknowledgment
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References

Fig. 7 Nano-CT of the osseous microstructure: a Grayscale image of a 150 m x 150 m image section, isotropic voxel size of 600 nm in the case of an image matrix of $250 \times 250$ pixels. The osteocyte lacunae and an included intracortier vessel have soft internal structures. b Image fusion of the mineralized bone substance (grayscale image) and the non-mineralized bone segments identified by adaptive threshold adjustment and displayed in black. c Inverted, binarized cross-sectional image with visualization of mineralized (black) or non-mineralized (white) bone segments. d Derived three-dimensional visualized dataset with segmentation of the osteocyte lacunae (magenta) or of a vascular canal (yellow) in volume-rendering technique, VRT.
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