Introduction: Traditional histology involves elaborate tissue fixation, decalcification, embedding, sectioning, staining and imaging techniques that allow different cell types, or structural or molecular components, to be preferentially visualized. This process of sample preparation however, may introduce artifacts and is time consuming. In the case of calcified tissue, it can take up to a few weeks. Depending on the resolution required, tissue sections are either viewed in optical microscopic slides or in an electron microscope. Since images are in 2D, it is also difficult to visualize or reconstruct the three-dimensional aspects of the tissue to reveal pertinent biological information. We describe a novel micro-nano x-ray computed tomography (CT) system for rapid virtual histology in 3D of cartilage, soft tissue, bones, scaffold and cells at length scales from mm to sub 30 nm spatial resolution and without the need for elaborate and time consuming histology sample preparation steps. System supports specimen sizes ranging from several cm to microscopic dimensions.

Materials and Methods: The key to this novel Multilengthscale CT lies in proprietary x-ray optics, including Fresnel Zone plates and specialized high resolution detector optics fabricated by Xradia. Depending on resolution required, system can operate in MicroCT mode with resolution to 1 micron or nanoCT mode, with resolution to sub 30 nm. The unique optics also provide exceptionally high contrast imaging for cartilage, soft tissue, bio-composites, scaffold and cells, polymers or higher Z materials such as bones, teeth, medical implants and electronic components.

Results: Micro x-ray computed tomography (microCT) has seen a rapid increase in applications in recent years especially in preclinical small animal in vivo imaging and biomedical applications, driven mainly by cancer and drug efficacy studies in disease treatment. The reported spatial resolution for small animal imaging range from 50 to 100 microns, while those for bones and ex vivo biomedical research can reach spatial resolution to a few microns[1]. The novel Xradia system we are describing provides spatial resolution to < 1 micron under microCT mode[2], and sub 30 nm when operating in the nanotomography model[3,4].

Most microCT for small animals applications can image bones pretty well but very little of the cartilage, soft tissue or internal organs simply because contrast is predominantly determined by the degree of absorption of x-rays. Imaging cartilage and soft tissue for in vivo or ex vivo applications are normally not attempted unless these tissues are stained with contrast enhancing agents. Notwithstanding, with special optics, the Xradia CT provides high resolution images with superb contrast for a variety of these samples which are of interest to the orthopedic research community, often without the need for contrast enhancing agents or elaborate sample preparation (Fig 1 and 2).

Evaluation of cartilage degeneration in osteoarthritis using a mouse model is currently only possible with a conventional microCT provided contrast enhancement agents are used. The Xradia high contrast CT however, can rapidly image cartilage structure and its subchondral bone interface without contrast agent and at high resolution (~1μm). Comparison with a MRI image and conventional histology slide for a rat femur-tibia knee joint is shown (Fig 1) Note: Conventional histology for cartilage typically takes more than 2 weeks to prepare, whereas the novel CT image can be acquired within a few hours. The fast turn around time, higher resolution and the fact there is no contrast agent involved, makes this new imaging technique an exciting tool for monitoring cartilage degeneration and drug efficacy studies in osteoarthritis research.

Figure 2 compares micrographs of AFM, TEM with the non invasive nanoCT at 50 nm resolution for human peritubular dentin, revealing very similar morphology between the different techniques. It also further demonstrates that high resolution x-ray nanotomography which are only currently achieved in synchrotron radiation research, can now be replicated on the novel lab based CT.

The CT results are also comparable to conventional histology utilizing imaging techniques from light microscope, with the advantages of non invasive 3D characterization, versatility and speed of analysis.

Other applications for the novel CT includes bone morphology for osteoporosis or rheumatoid arthritis; porosity evaluation for new orthopedic implant materials and bone-metal interfacial studies. A variety of mechanical loading and crack propagation studies can also be performed on bones, composites and biomaterials.

Discussion: Using proprietary x-ray optics, fresnel zone plates, and detectors, we describe a multilength scale CT capable of imaging a variety of materials from cartilage, calcified, soft tissue to biomaterials in high contrast and with resolution from mm to sub 30 nm. The system offers a rapid technique for virtual histology in 3D without the tedious sample preparation associated with conventional histology in optical or electron microscopy. It is envisioned that such a lab based CT will find widespread adoption in orthopedic research, drug discovery, phenotype characterization, drug efficacies and histopathological evaluation in the years to come.


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