Bone and osteoporosis research has played a role in driving the development of microCT systems with high resolution and throughput for analysis of preclinical and clinical bone samples. Bone research continues to create requirements that test the frontiers of performance of micro/nanoCT technology. It does so in two different directions. First, in the direction of submicron nano-CT imaging: interest is growing into the role of osteocytes within the bone matrix. MicroCT can measure in 3D the size, shape and distribution of osteocyte lacunae, providing information about the history of lacunar formation and remodeling—a four-dimensional time-stamp of the activity of the osteoblasts-osteocytes in growing and remodeling bone. Osteocyte lacunae have a diameter range of 5–9 microns, meaning that microCT voxel sizes of near or below a micron are ideal for accurate measurement of osteocyte lacunar morphology. Good image signal to noise ratio is also essential to resolve the lacunae from noise dots. Second, in the direction of imaging of metal orthopedic implants which pose a particular difficulty for microCT due to strong Z-proportional X-ray absorption and consequent extreme beam hardening in metals. It is not the imaging of metals per se that is the problem, but more the imaging of much lower density materials surrounding the metal—and in this context bone itself becomes a “very low density material”. This is a different technical challenge for microCT requiring innovations in camera and source technology and reconstruction software.

A new nanoCT instrument, the SkyScan2211, represents a step toward solving both the above requirements, with two X-ray cameras optimized respectively for both the above imaging challenges. The first is an 11 megapixel CCD, precisely coupled without geometric distortion to a scintillator optimized for submicron resolutions, it also has a high top flat panel detector with special radiation-hardened electronics, optimized for submicron resolution. The second camera is a megapixel CCD, precisely coupled without geometric distortion to a scintillator optimized for submicron resolution. The second camera is a flat panel detector with special radiation-hardened electronics, optimized for submicron resolution.

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**Fig. 1.** Submicron pixel resolution allows visualisation and analysis of osteocyte lacunae as well as mineralisation heterogeneity.
osteocytes utilize [Ca\textsuperscript{2+}] oscillations to activate pulsing, muscle-like contractile mechanical behavior. In this study, we used a quasi-3D imaging technique to simultaneously measure the contractile behavior of the actin networks and [Ca\textsuperscript{2+}] in single osteocytes. [Ca\textsuperscript{2+}] spikes were induced using fluid flow, ATP, or ionomycin in MLO-Y4 osteocytes. Contraction in the actin networks was measured immediately upon onset of [Ca\textsuperscript{2+}], influx in all groups, indicated by a decrease in the strain value. Microtubule networks did not display a similar contractile response. Longer imaging of actin contractions displayed reversible, phasic contractions in the actin networks over a period of ~180 s.

As non-muscle and smooth muscle myosin II isoforms are regulated by myosin light chain kinase (MLCK) and skeletal and cardiac myosin II isoforms by troponins, we sought to determine the myosin responsible for the observed contraction. Under ATP stimulation, MLCK inhibition by ML-7 drastically altered the kinetics of contraction, but skeletal and non-muscle myosin II inhibition by blebbistatin had no effect. This pointed towards a smooth muscle myosin mediated contraction. Furthermore, we verified the presence of smooth muscle myosin heavy chain (SMMHC) in primary osteocytes and MLO-Y4 osteocytes.

Here, we demonstrate a novel osteocyte mechano- and transduction behavior where [Ca\textsuperscript{2+}], oscillations activate dynamic actomyosin contractions. Future studies will verify this mechanism in ex vivo osteocytes, as well as investigate downstream behaviors of contractility in osteocytes, such as contractility-mediated vesicle exocytosis.

**Brief CV**

Dr. Guo received his M.S. in 1990 and Ph.D. in 1994 in Medical Engineering and Medical Physics from Harvard University-MIT. In 1994–1996, Professor Guo did his postdoctoral fellowship in the Orthopaedic Research Laboratories at the University of Michigan at Ann Arbor with Professor Steven A. Goldstein in orthopaedic bioengineering. In 1996 he joined the Department of Mechatanical Engineering and then Department of Biomedical Engineering at Columbia University. He directs the Bone Bioengineering Laboratory in the Department of Biomedical Engineering at Columbia focusing his research interests in micromechanics of bone tissue, computational biomechanics, and mechanobiology of bone. His past honors include Young Investigator Recognition Award from the Orthopaedic Research Society, National Science Foundation (NSF), Funds for Talented Professionals (Joint Research Fund for Overseas Chinese Young Scholars) from the National Natural Science Foundation of China. He was elected as a fellow to the American Institute for Medical and Biological Engineering. He was one of the founders and served as co-Editor-in-Chief of Cellular and Molecular Bioengineering (CMBE), an international journal of US Biomedical Engineering Society (BMES). He served as President of International Chinese Musculoskeletal Research Society, the Society for Physical Regulation in Biology and Medicine, Member of Board of Directors of Orthopaedic Research Society, and Member of Board of Directors of American Institute for Medical and Biological Engineering.

**Technical Expertise:** Bone Mechanics, Imaging and Finite Element Analyses of Human Bone Microstructure, Mechanobiology of Bone, Mechanotransduction, and Cell Mechanics.

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**Calcium-dependent Actomyosin Contractility in Osteocytes Under Mechanical Loading**

Edward Guo

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Intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]) oscillations, mediated by mechanosensitive Ca\textsuperscript{2+} channels and the ER Ca\textsuperscript{2+} store, have been observed in osteocytes in response to mechanical loading in vitro and ex vivo in our laboratory. While many distant downstream gene expression pathways of [Ca\textsuperscript{2+}], signaling have been studied, the immediate, temporally-regulated effects of these numerous [Ca\textsuperscript{2+}] oscillations in osteocytes have not been elucidated. A recent study in the gene expression of primary osteocytes has suggested high levels of muscle contraction-related proteins. A hallmark of muscle is its [Ca\textsuperscript{2+}]-dependent actomyosin contractility. We hypothesized that osteocytes utilize [Ca\textsuperscript{2+}], oscillations to activate pulsing, muscle-like contractile mechanical behavior. In this study, we used a quasi-3D imaging technique to simultaneously measure the contractile behavior of the actin networks and [Ca\textsuperscript{2+}], in single osteocytes. [Ca\textsuperscript{2+}] spikes were induced using fluid flow, ATP, or ionomycin in MLO-Y4 osteocytes. Contraction in the actin networks was measured immediately upon onset of [Ca\textsuperscript{2+}], influx in all groups, indicated by a decrease in the strain value. Microtubule networks did not display a similar contractile response. Longer imaging of actin contractions displayed reversible, phasic contractions in the actin networks over a period of ~180 s.

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**Tracing Stem Cells Using In Vivo Imaging Techniques in Bone Repair and Cancer Research**

Gang Li

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