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3D Visualization of Reference-Point Indentation in Human and Murine Bone

John Jameson
Marquette University, john.jameson@marquette.edu

Alex Proctor

Carolyne Albert
Marquette University, carolyne.albert@marquette.edu

Gerald F. Harris
Marquette University, gerald.harris@marquette.edu

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3D Visualization of Reference-Point Indentation in Human and Murine Bone

John Jameson
Advanced Light Source (ALS)
Lawrence Berkeley National Laboratory
Berkeley, CA
Alex Proctor
3Active Life Scientific, Inc.
Santa Barbara, CA
Carolyne Albert
Orthopaedic & Rehabilitation Engineering Center (OREC),
Department of Biomedical Engineering
Marquette University
Milwaukee, WI
Gerald Harris
Orthopaedic & Rehabilitation Engineering Center (OREC),
Department of Biomedical Engineering
Marquette University
Milwaukee, WI

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Traditional techniques for assessing bone strength and fracture resistance such as 3 or 4-point bending of whole bones or machined beams are well-developed but destructive. Recently, Reference-Point Indentation (RPI) has been introduced as a minimally destructive method of determining bone tissue properties at physiologically relevant length scales [1-2]. However, to date there is no data showing the 3D damage mechanisms associated with RPI. The purpose of this study was to use high-resolution imaging to visualize RPI tests of human and murine bone, as well as to compare indents from healthy and pathological tissue.

Femora from healthy mice and a mouse model for severe osteogenesis imperfecta (oim; $N_{control}=N_{oim}=3$, Age=12wks) were extracted and tested with repeated RPI measurements (BioDent Hfc, Active Life Scientific, Inc.) along the long axis of the bone using a maximum force of 5 N at 2 Hz for 7 cycles. Each indent was imaged by synchrotron radiation on the X-ray microtomography (SRμCT) beamline at the Advanced Light Source (ALS, Berkeley, CA) with a nominal pixel size of 1.33 μm. Similar imaging was performed on indents from a healthy human tibia donated from an unrelated cadaver study.

Oim femora showed inferior material behavior as assessed by RPI measures (Table 1). On average, first cycle indentation distance (ID), ID increase (IDI), total ID (TID), and creep ID (CID) were higher in oim bones compared to controls, while the slope of the unloading portion of the first cycle (US) was reduced. SRμCT images of representative damage regions revealed an increase in the degree of micro-cracking in oim bone compared to controls (Fig 1-2). Healthy human bone also appeared to display evidence of crack bridging, a common extrinsic toughening mechanism (Fig 3).

The results of this preliminary study suggest that RPI is an effective method for evaluating differences in the material behavior of healthy versus oim mouse bone. Similar trends in RPI parameters have been reported in control versus diabetic rat bone, which is known to exhibit increased fragility [2]. For the first time, we also show how SRμCT can be used to complement RPI testing by providing valuable 3D information on sub-surface fracture behavior. In oim bone,

increased micro-cracking is likely a toughening mechanism required to mitigate the reduced initiation toughness.

[1] Diez-Perez et al, J Bone Miner Res, 2010.
[2] Gallant et al, Bone, 2013.

Table 1: Summary of murine BioDent data.

	Control	Oim
Number of indents	32	14
ID (µm)	28.7 ± 5.2	31.3 ± 9.6
IDI (µm)	4.1 ± 1.3	6.6 ± 4.6
TID (µm)	30.5 ± 5.2	34.9 ± 10.8
CID (µm)	3.0 ± 1.0	3.9 ± 2.6
US (N/µm)	0.74 ± 0.12	0.66 ± 0.10

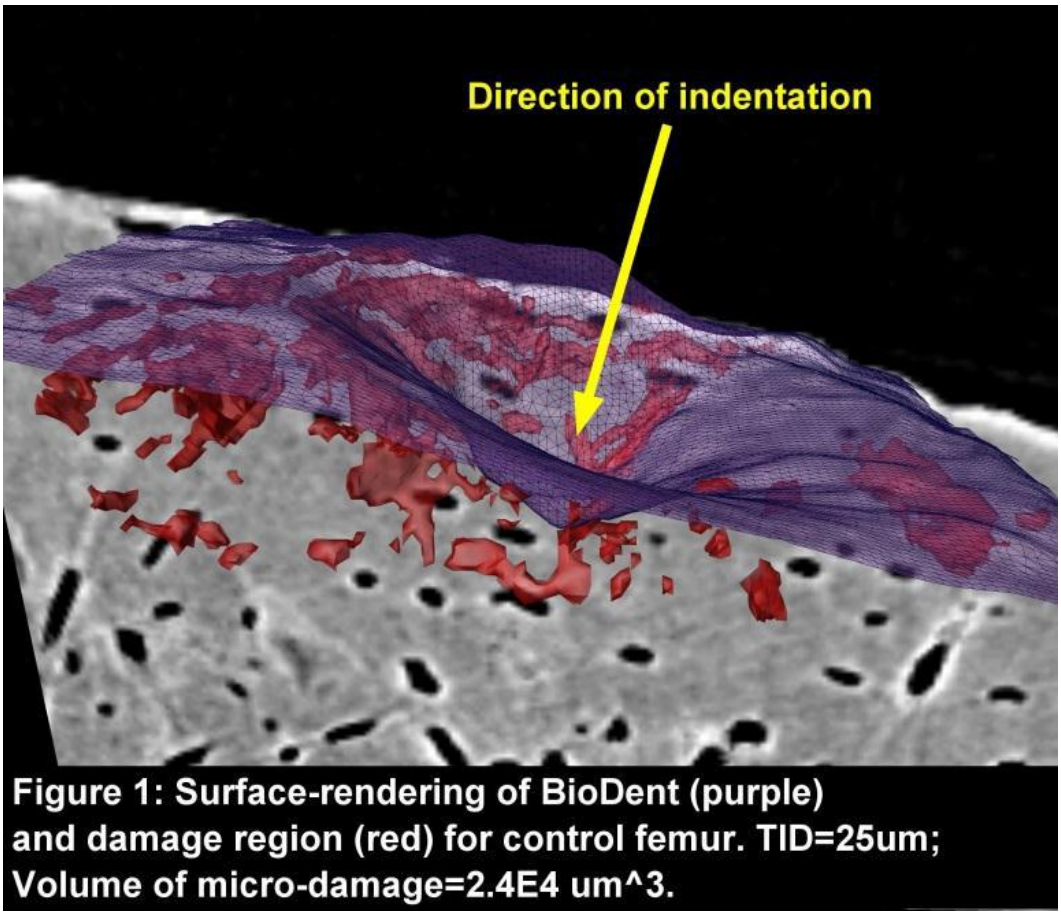

Figure 1: Surface-rendering of BioDent (purple) and damage region (red) for control femur. TID=25um; Volume of micro-damage=2.4E4 um^3.

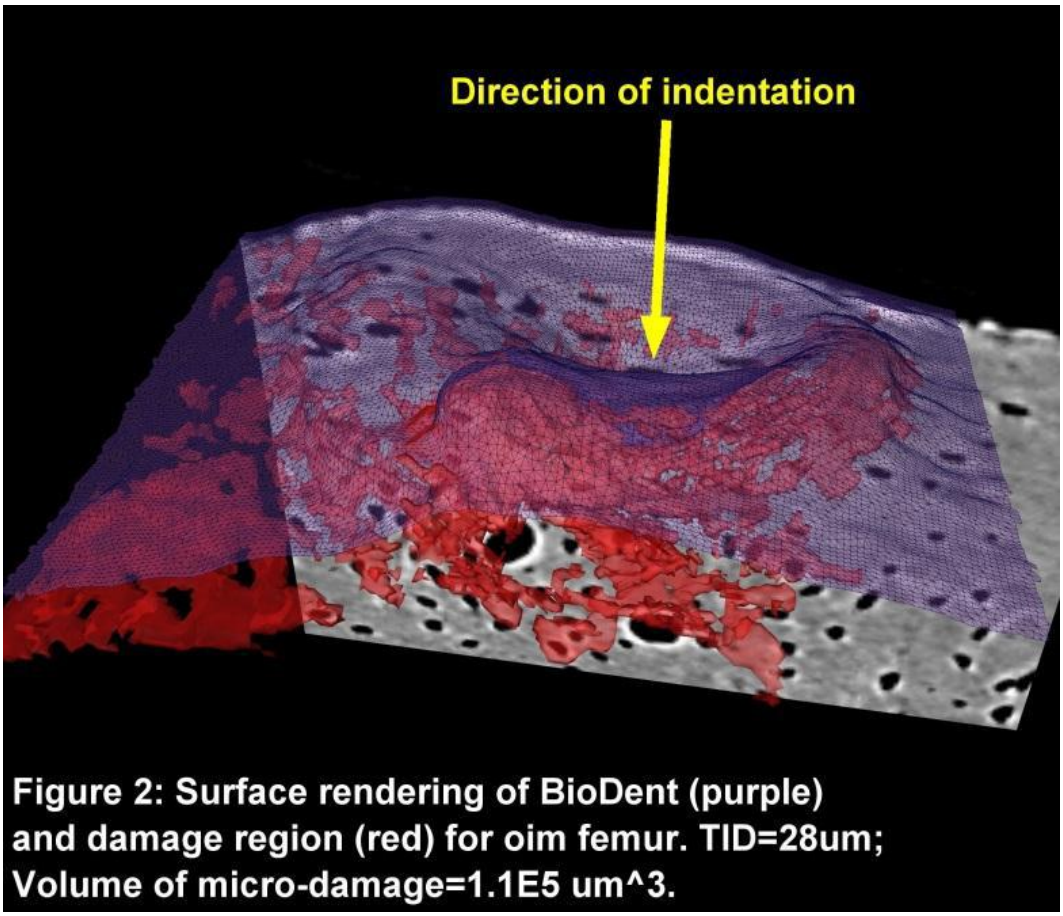

Figure 2: Surface rendering of BioDent (purple) and damage region (red) for oim femur. TID=28um; Volume of micro-damage=1.1E5 um^3.

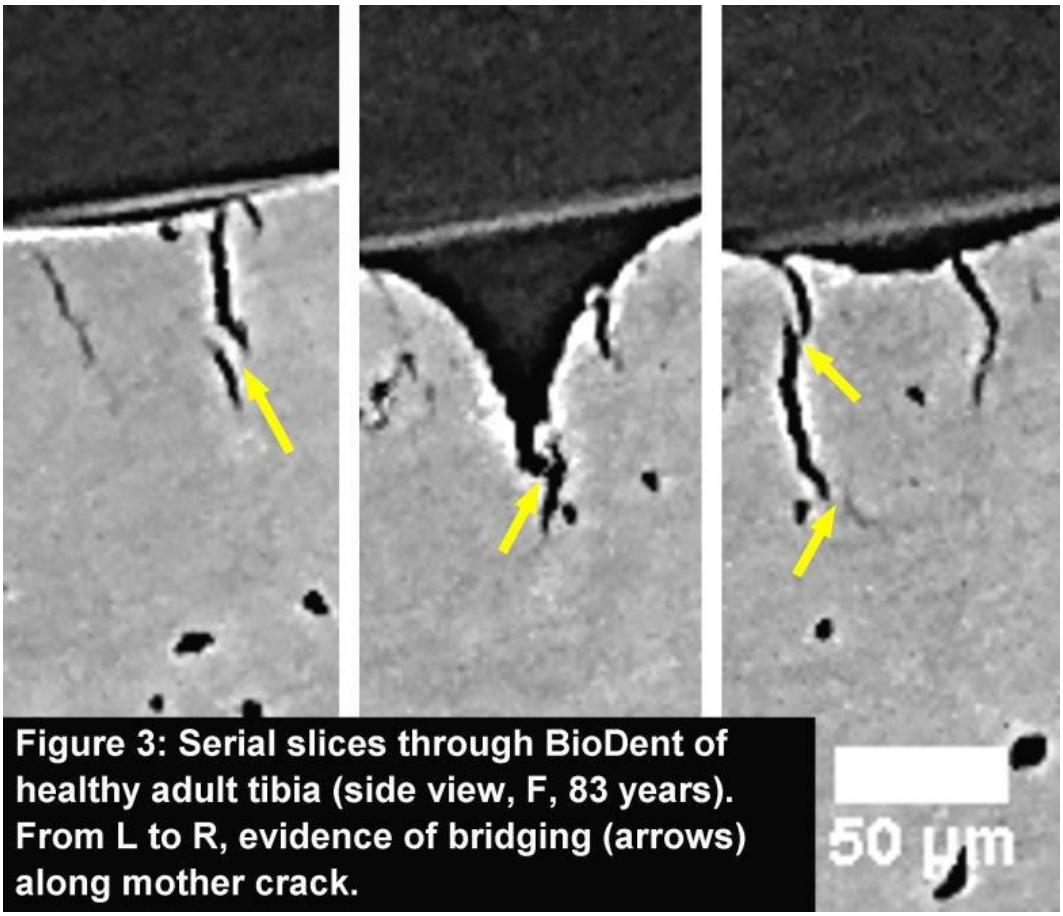

Figure 3: Serial slices through BioDent of healthy adult tibia (side view, F, 83 years). From L to R, evidence of bridging (arrows) along mother crack.