

## Determination of micro scale tissue mechanical behavior using Reference Point Indentation

*Musculoskeletal Structure and Strength Core B*

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### Background

**What is Reference Point Indentation (RPI) testing?** A technique for measuring the local mechanical properties of a material. It uses the material's surface as a reference point which enables measurement in previously impossible environments, such as: non-uniform surfaces and in vivo environments. An indentation on the order of 50-200 $\mu$ m in diameter is created on the sample surface. Because of the small testing area required, multiple measurements can be made on a single sample while preserving the sample for other assays such as histology.

**How RPI works:**

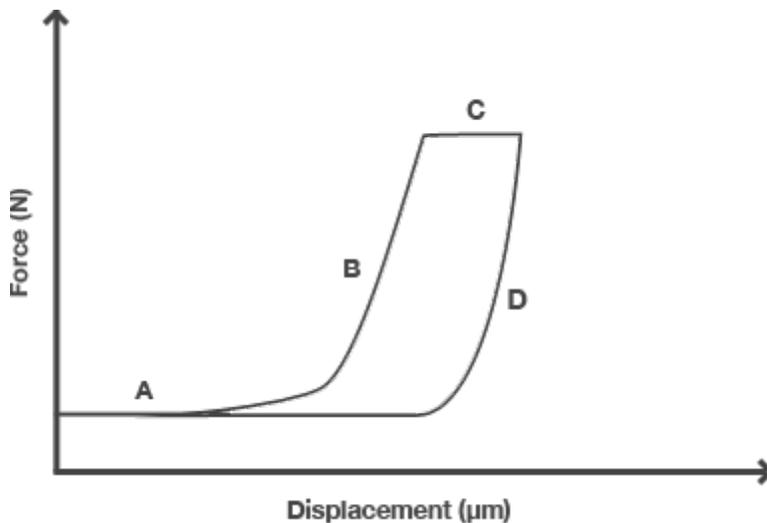
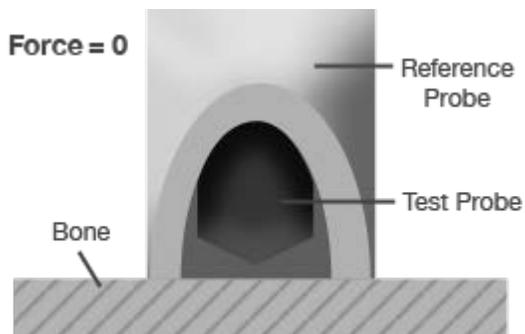
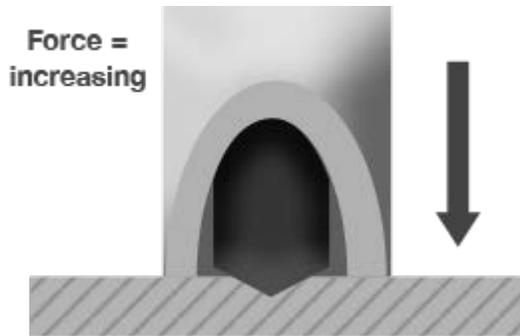


Figure- One Cycle of an idealized RPI test.



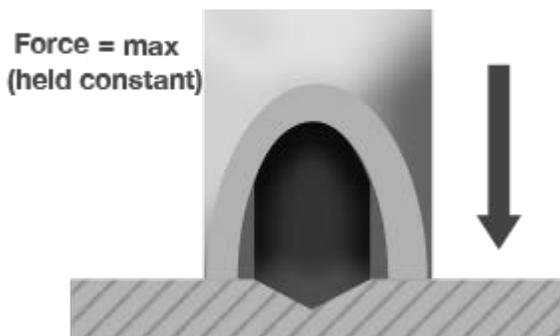
1) Reference probe establishes local reference point

The reference probe establishes a reference point at the sample's surface and is secured in place. Next, the test probe moves towards the sample's surface (section "A" of graph above).



- 2) Test probe identifies surface, ramp up force, observe loading

The test probe identifies the surface and the ramps up the applied force in a controlled manner and the depth of test probe penetration (displacement) is measured relative to the local reference point (section "B" of graph above).



- 3) Max force reached, then held constant to observe creep effects

The test probe applied force reaches the protocol-determined maximum where it is held constant for a set period of time to observe potential creep effects within the sample (section "C" of graph above).



- 4) Force is decreased, material causes test probe to retract, observe unloading

The test probe applied force is decreased in a controlled manner causing the probe to retract according to the force it experiences from the sample "pushing back" or "recovering from deformation" (section "D" of graph above).

(Text/images taken from Active Life Scientific website.)

## Outcomes

During a typical mouse bone test, the bone is held in position under the RPI indenter. Mid-diaphyseal bone is indented in 4 different sites at least 1 mm apart. At each site the force applied is 2N and the indenter is cycled 10 times at 2 Hz (compared to the 10 N, 20 cycle graph on human bone below).

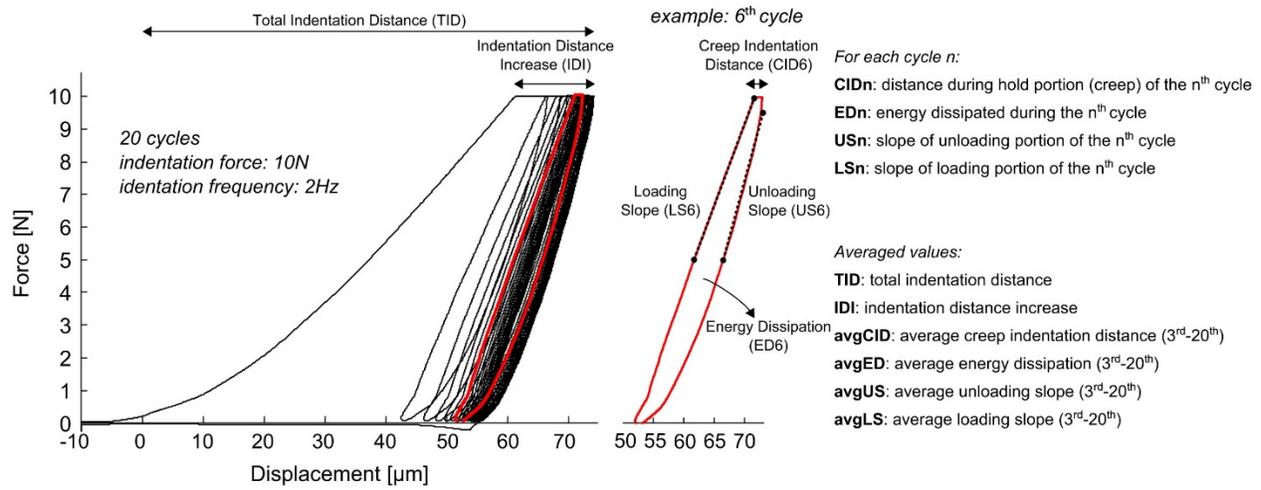


Figure- Typical RPI outcome graph loading at 10N for 20 cycles. (Taken from: Nyman JS, J Mech Behav Biomed Mater. 2014 Sep;37:174-85.)

### **Parameters to report for publication include:**

ID1 (First Indentation Distance)- Test probe penetration depth for the first test cycle ( $\mu\text{m}$ )

TID (Total Indentation Distance)- Total test probe penetration depth ( $\mu\text{m}$ )

IDI (Indentation Distance Increase)- Increase in penetration depth from the first test cycle to the last test cycle ( $\mu\text{m}$ )

US1 (First Unloading Slope)- Unloading slope for the first test cycle ( $\text{N}/\mu\text{m}$ )

AvgCID (Average Creep Indentation Distance)- Average of the creep indentation distance over all test cycles ( $\mu\text{m}$ )

(Text adapted from: Jasiuk I , J Mech Behav Biomed Mater. 2014 Jun;34:57-65.)

## Caveats and Data Interpretation

RPI is a new method of testing bone and it does have some limitations. The first is that it is a newer method of testing the mechanical properties of bone. Although the RPI measurements are relatively simple to conduct and obtain, the interpretation of this data can be nuanced and may require further

investigations to tease out the differences reflected by RPI. For example, AvgCID appears to correlate with collagen changes, while US1 may correlate with the tissue mineral density of the bone<sup>3</sup>. Further experiments confirming these changes are recommended.

Mouse bones pose an additional challenge in RPI testing because of their small size. For example, the very thin cortices of a mouse bone may flex during RPI measurements, and this could introduce variability in the outcomes. It is recommended that users work with Core staff to ensure testing is done with as maximum confidence as possible.

RPI measurements are best interpreted when comparing differences between two groups. Unlike traditional mechanical testing parameters that are comparable between different experiments, the boundary conditions for RPI testing are not as tightly controlled, and variations can occur due to differences in bone quality, bone thickness, and testing protocol. Because of these variables, the absolute values obtained by RPI are not directly comparable across species, although trends due to the same treatments/pathologies may be similar.

Nevertheless, RPI measurements, when coupled with other experiments can reveal important insights at the micro- and tissue- level scales of bone

<sup>3</sup> Abraham AC, Agarwalla A, Yadavalli A, Liu JY, Tang SY. 2016. [Microstructural and compositional contributions towards the mechanical behavior of aging human bone measured by cyclic and impact reference point indentation](#). Bone. 2016 Mar 25. pii: S8756-3282(16)30081-3. doi: 10.1016/j.bone.2016.03.013.