MINERAL CONTENT AND BIOMECHANICAL PROPERTIES OF FIBROLAMELLAR BONE

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Hard Tissue I: Tissue Interactions

27/06/2022

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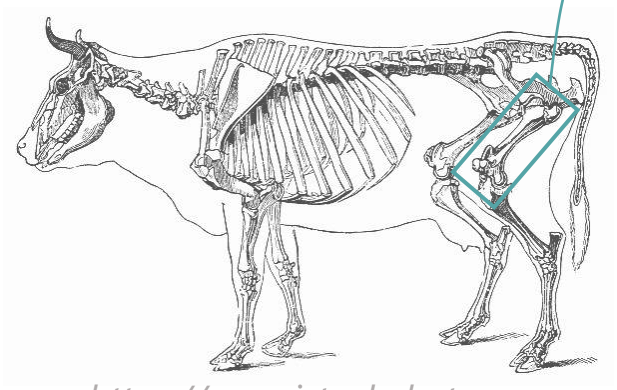
MBBM

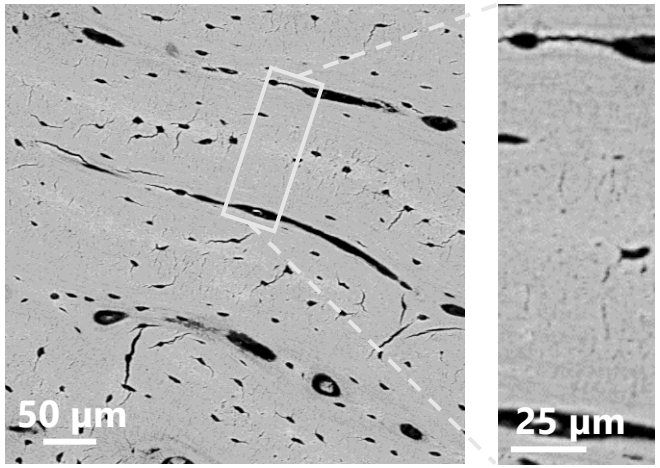
Introduction

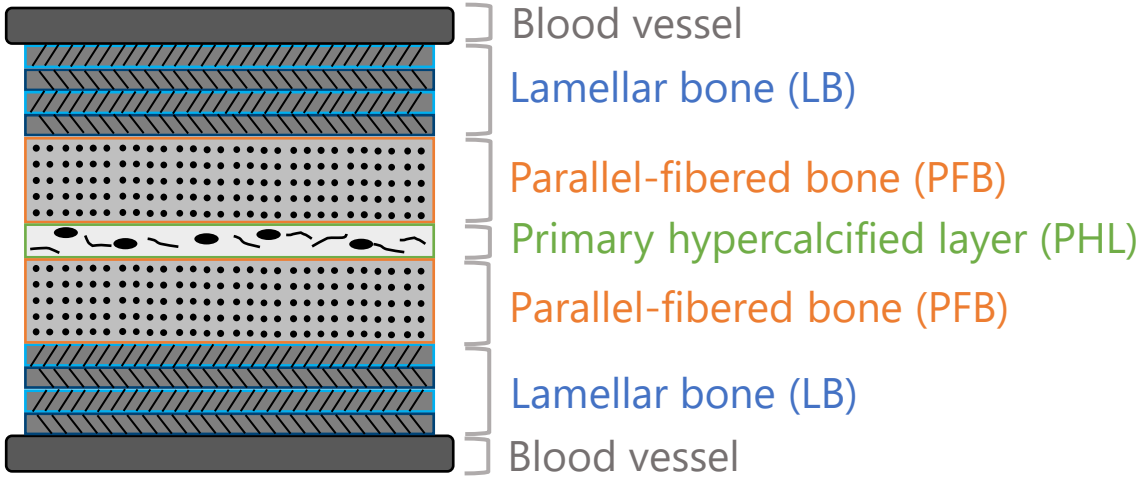
At birth: 30-40 kg → 6-9 months: 220-320 kg → 14-15 months: 550 kg

High stiffness, toughness, fracture resistance >< Fast growth

Introduction: fibrolamellar bone







- Microstructure (fibers orientation, cellular organization) ✓
- Apparent mechanical properties ✓
- Spatial distribution of mineral content ✗
- Local mechanical properties ✗

Clinical relevance: Similarity between deposition and callus formation in bone healing

Kerschnitzki, Cells Tissues Organs, 2011 - Magal, J. Struct. Biol. 2014

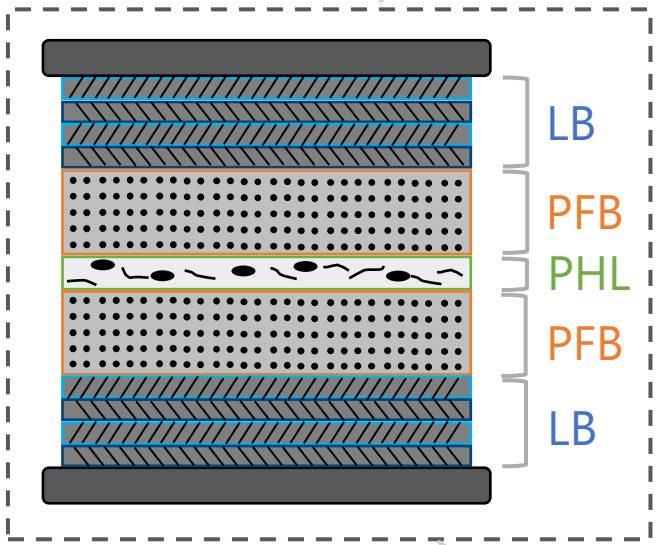
Aims

Combination of imaging & mechanical testings to explore structure-function relationship

Mineral content?
Quantitative backscattered electron imaging (qBEI)

Ludwig Boltzmann Institute Osteology

Microstructure & nanoscale porosity?
Backscattered electron imaging (BEI)



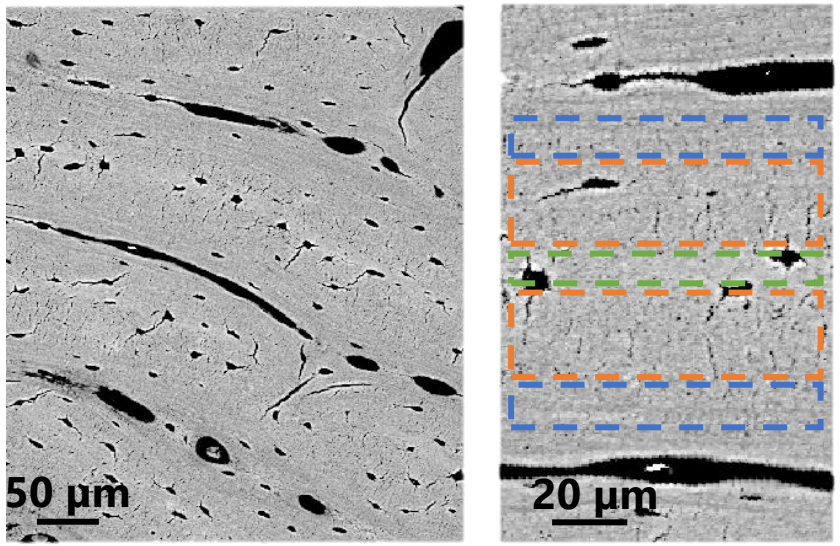
Mechanical properties?
- ▼ Quasi-static nanoindentation (nIND)
- ▼ Nanoscale modulus mapping (NanoMM)

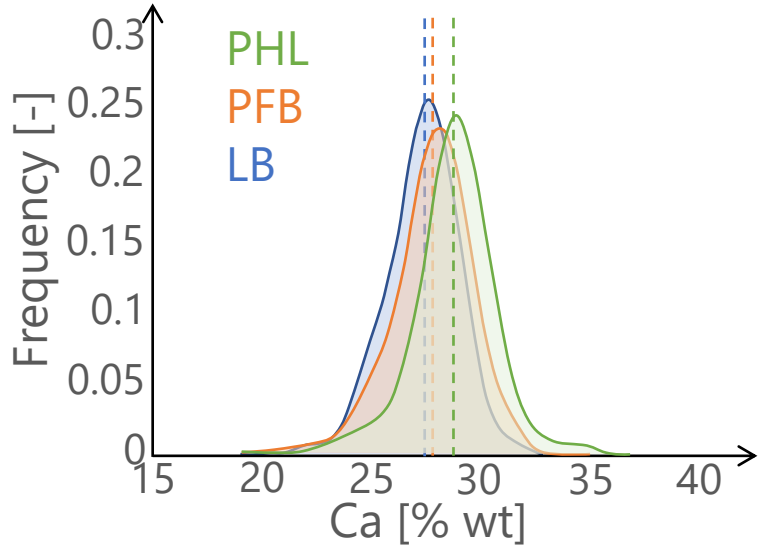
Fracture behavior?
▼ High load indentation
Backscattered electron imaging (BEI)

Mineral content, structure & nanoporosity

Quantification of local mineral content and visualization of microstructure & nanoscale porosity of fibrolamellar bone

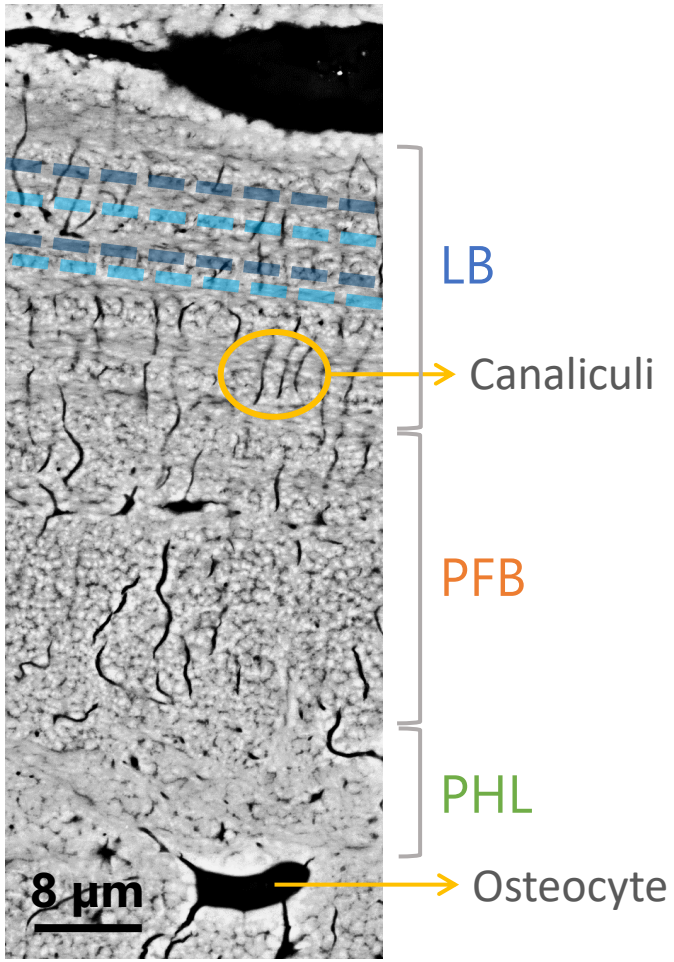
qBEI
Resolution: 0.57 µm





- PHL ~ 5% more mineralized
- LB made of alternating lamellae with ≠ structures
- Osteocytes and less canaliculi in PHL

BEI
Resolution: 56 nm



Magal, J. Struct. Biol., 2014 – Kerschnitzki, Cells Tissues Organs, 2011

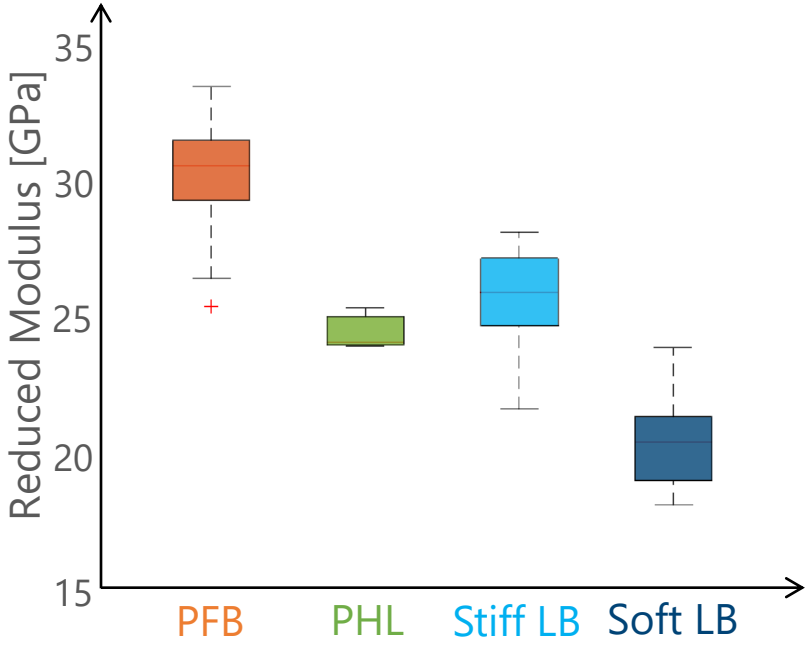
Mechanical properties with nanoindentation

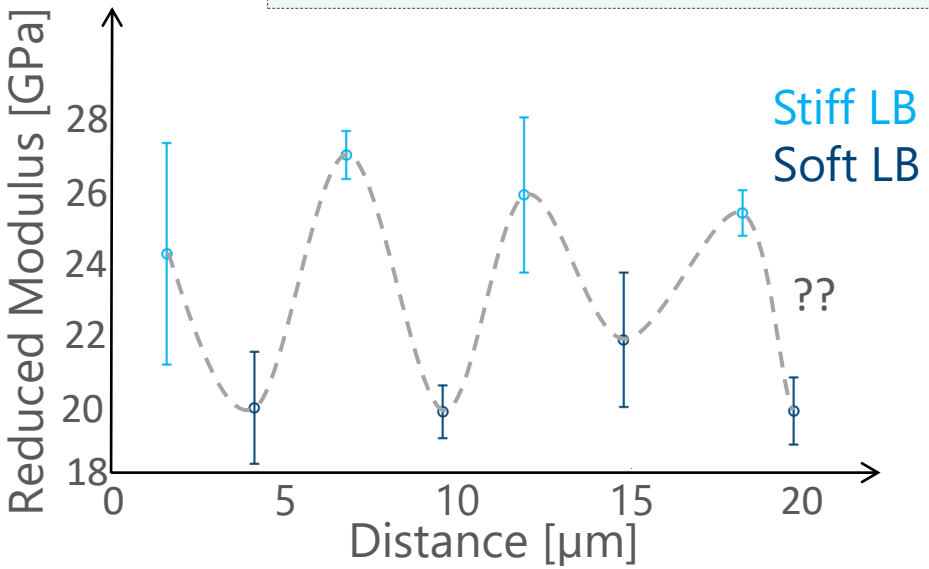
▼ nIND

- Spacing: ~6 µm
- Applied force: 500 µN
- Corresponding penetration depth: ~150 nm

- PHL is softer than PFB
- PFB is the stiffest region
- Clear difference in mechanical behavior between lamellae
- What about their transition?

→ Need of **higher resolution** to characterize submicron mechanical behavior within LB



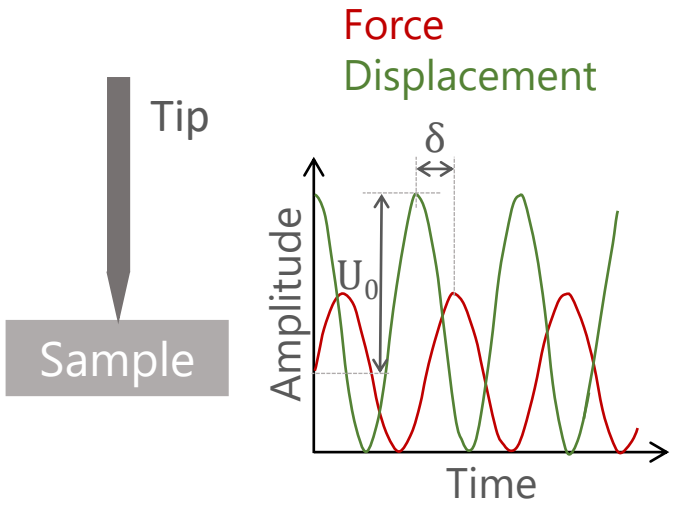


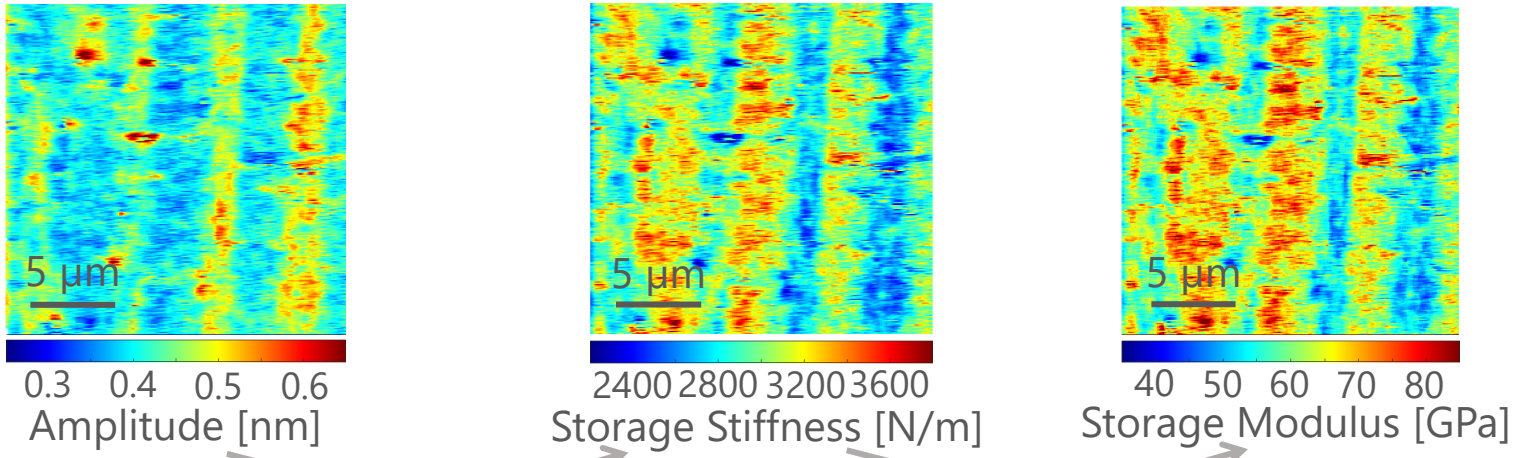
Modulus Mapping based on nanoindentation

Nanoindentation combined with AFM-like piezo-scanner and force modulation system

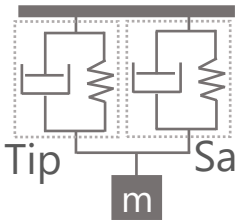
Static force F_s + Periodic modulation force of amplitude F_{AC} and frequency ν → Displacement U

$$m\ddot{U} + C\dot{U} + KU = F_{AC}e^{i\omega t}$$

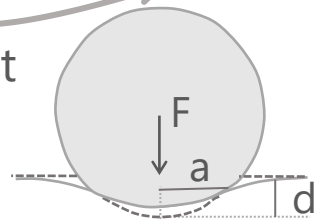




Kelvin-Voigt model assumption



Hertz contact mechanics assumption

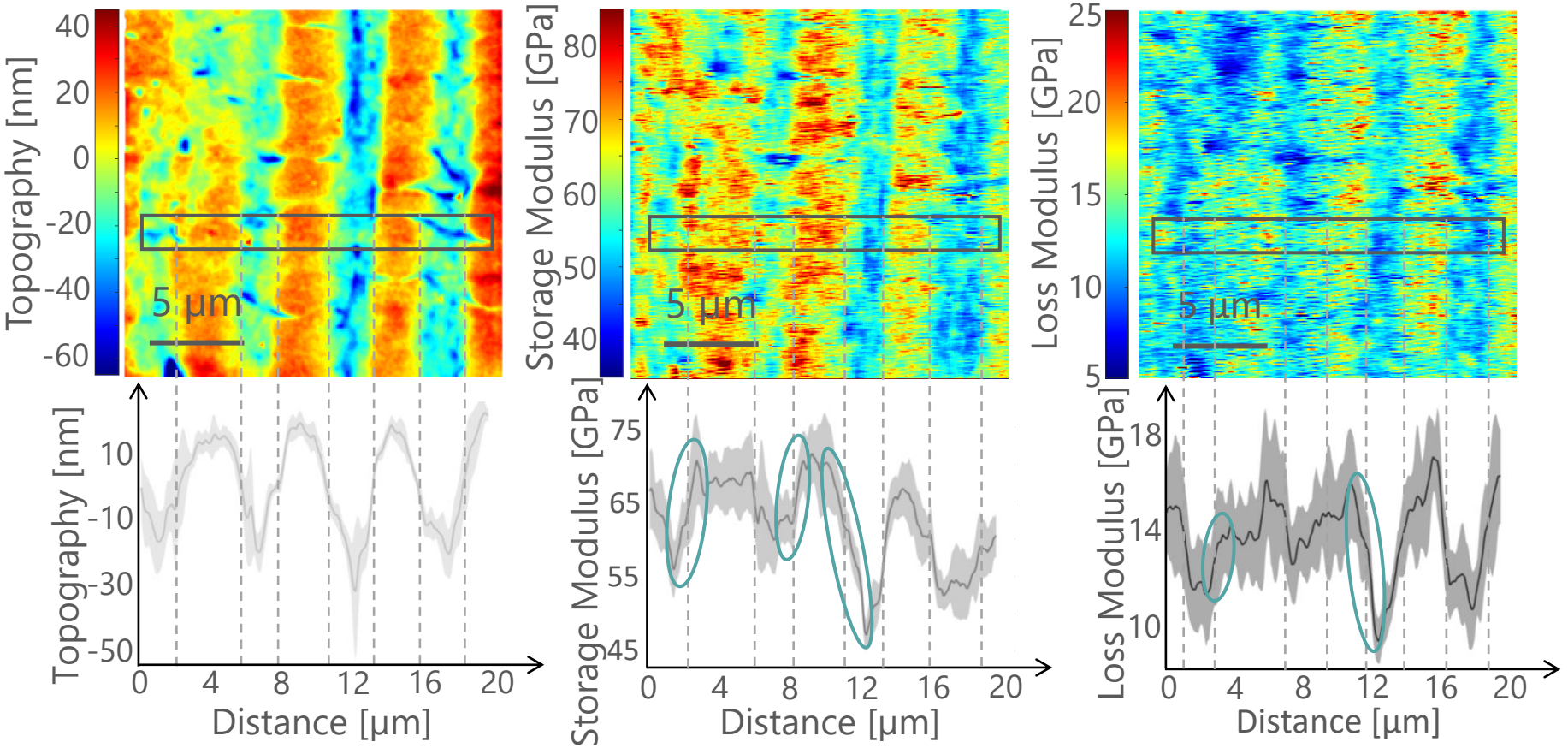


Zlotnikov, Prog. Mater. Sci., 2017

Mechanical properties with modulus mapping

▼ NanoMM

Static force: 3 μN | Dynamic force: 1 μN & 285 Hz | Penetration depth: ~6 nm | Lateral resolution: ~80 nm



- Correlation with topography
- Alternating storage moduli
- Loss modulus with significant contribution
- **Steep** transition between lamellae: 0.8-1.4 μm

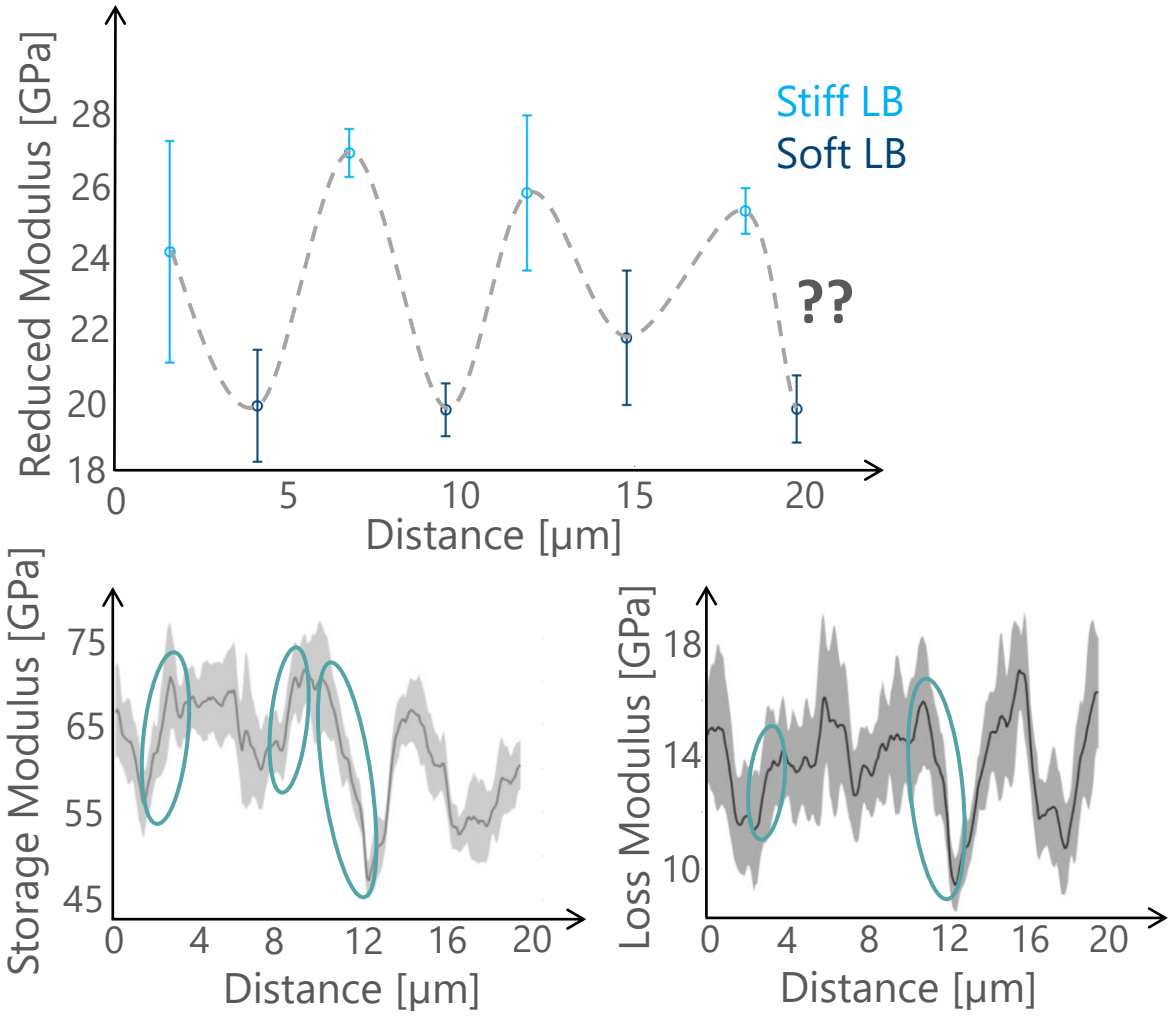
Two techniques, two length scales

▼ nIND

- Large elastic field: 25X larger
- Probing pores & canaliculi
- **~24%** difference between stiff and soft lamellae

▼ NanoMM

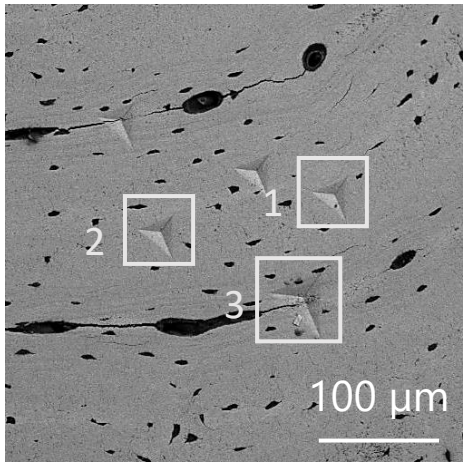
- Small elastic field: 25X lower
- Probing crystal-collagen composite on the surface
- **~25%** difference between stiff and soft lamellae for storage **&** loss

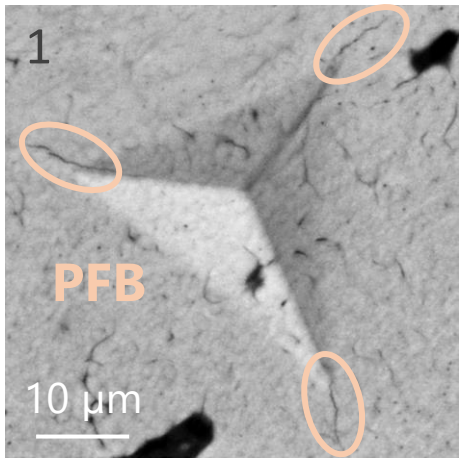


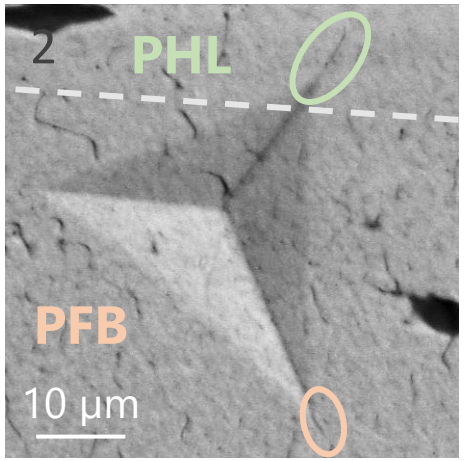
Fracture behavior

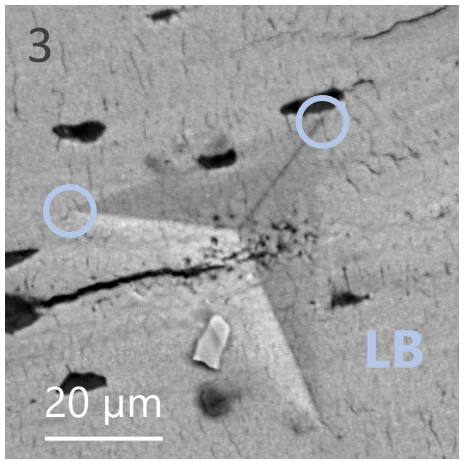
▼ High load indentation
Force applied: 0.5 N
BEI

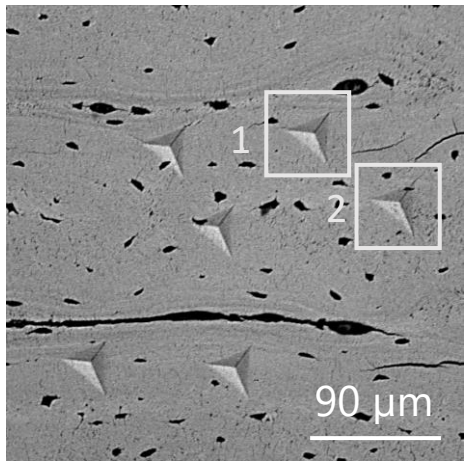
Qualitative analysis of fracture behavior in each region

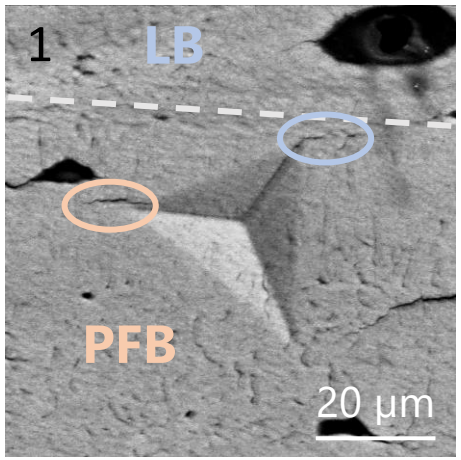


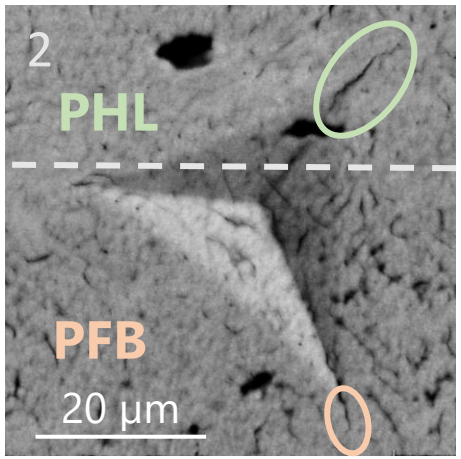












- PFB: Straight cracks
- LB: Deflection at the interface & No cracks within region
- PHL: Longer cracks

Conclusion & Perspective

Mechanical and structural heterogeneity of fibrolamellar bone

- Primary hypercalcified layer: Lower stiffness although higher mineral content and less porosity → Critical role of collagen organization
- Parallel-fibered bone: **Stiffest** region
- Lamellar bone: **Very sharp** modulation of elastic properties → Known to hamper crack propagation [Fratzl, Adv. Mater., 2007]

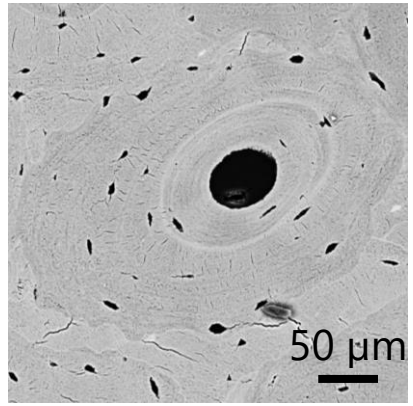
<u>Fibrolamellar bone</u>

Fast growth

High **stiffness**

High **toughness**

NanoMM technique is ideal to characterize small features/**interfaces** such as lamellae or cement lines (1-5 µm)



Thanks to all co-authors!

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Thank you for your attention!

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