Willkommen
Welcome
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BIOMEDICAL ENGINEERING RESEARCH

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Combining bone proteotype and multiscale extracellular matrix properties for improved clinical fracture risk prediction

Dr. Jakob Schwiedrzik, Group Leader Architectured Materials
Laboratory for Mechanics of Materials and Nanostructures

Strategic Focus Area
Personalized Health and Related Technologies

FNSNF
SCHWEIZERISCHER NATIONALFONDS
ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

Motivation

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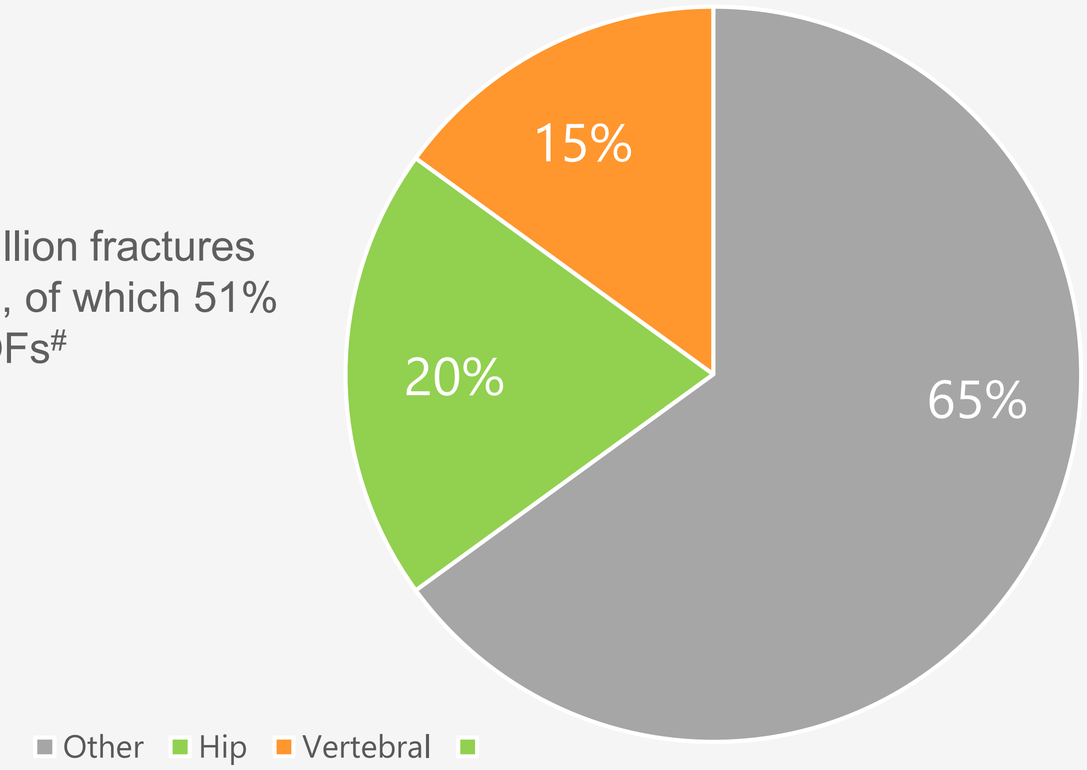
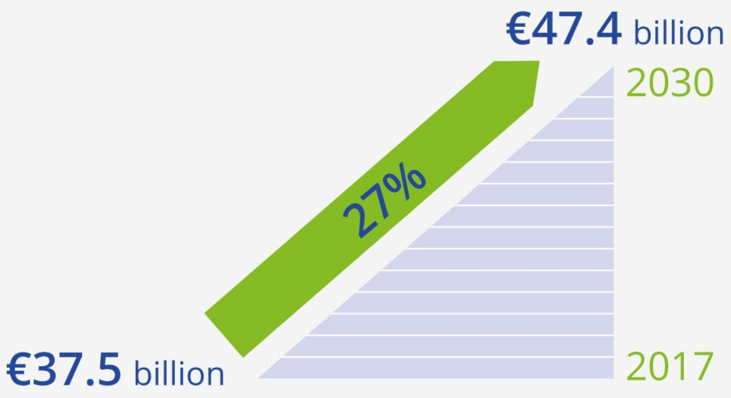
Total *fragility fracture** number across *EU6***:

~2.7 million fractures in 2017, of which 51% are MOFs#



Associated healthcare cost per year increase:

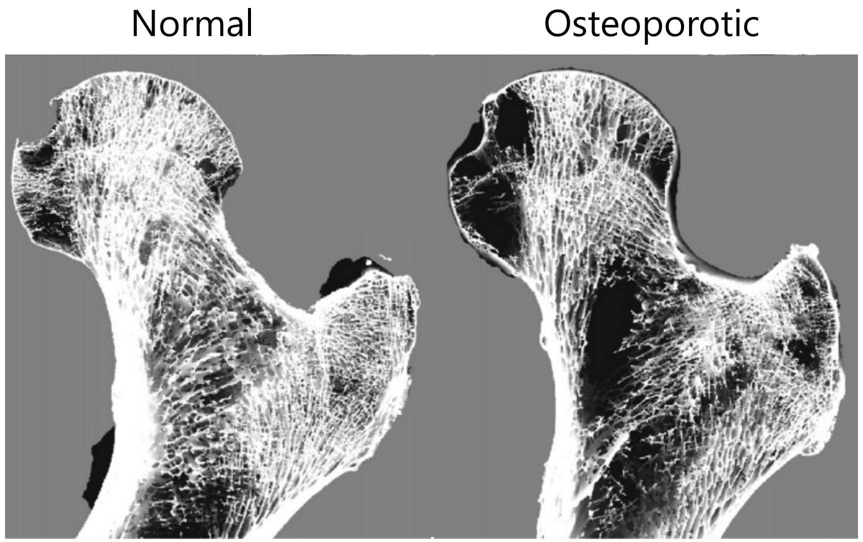


Data from International Osteoporosis Foundation (IOF)
www.iofbonehealth.org

* fractures that result from low-level mechanical forces that would not ordinarily result in fracture
** EU6 nations: France, Germany, Italy, Spain, Sweden, and the UK
\# Major osteoporotic fracture (hip, spine, humerus, or forearm fractures)

Motivation

- Imbalance of bone remodeling
- Loss of bone mass
- Changes in *bone quality**:
  - Microstructure
  - Microcrack density
  - Tissue organization and properties



Van Rietbergen et al., J Bone Min Res (2003)

* bone quality is the combination of various parameters contributing to fracture resistance

Fracture risk assessment in clinics

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Current methods of assessing *bone density and fracture risk* in clinics:

Bone parameters	Methods	Disadvantages
Bone mineral density (BMD), geometry, microarchitecture	• Dual-energy X-ray absorptiometry (DXA) • Quantitative computer tomography (QCT) variations: HR-QCT, μ-CT	• Scans are affected by many artefacts • High radiation doze: from 5 μSv (HR-QCT) up to 1.2 mSv during hip QCT *Choksi et al. Clin Diabetes Endocrinol. (2018)* *A. Marques et al., Ann. Rheum. Dis. (2015)* *R. Krug et al., Radiol. Clin. North Am. (2010)*
BMD + Medical history	• FRAX	• Same as DXA/QCT *J.Kanis et al., Bone (2009)*
BMD, structure, and estimated strength	• QCT-based Finite Element models (FE)	• High radiation dose, average material properties used in simulations *F. Johannesdottir et al., Curr Osteoporos Rep (2018)*

State-of-the-art of multiscale bone characterization

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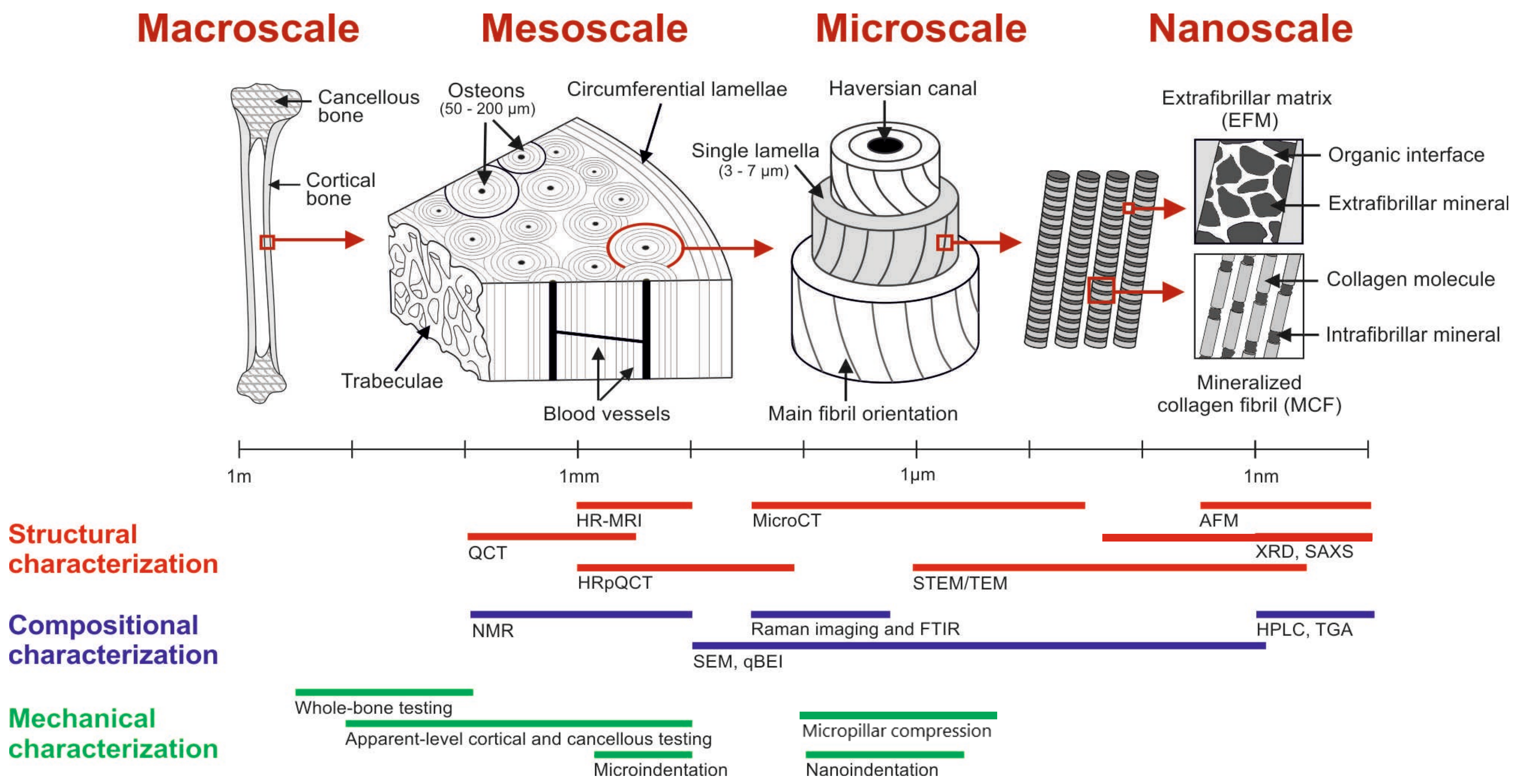
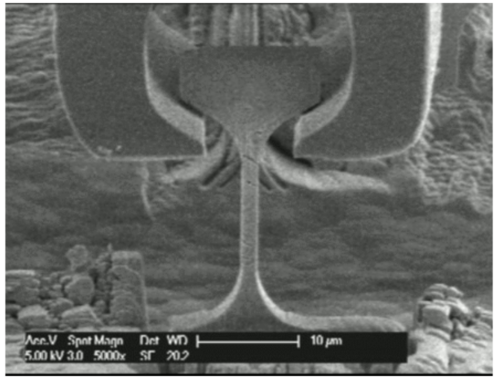


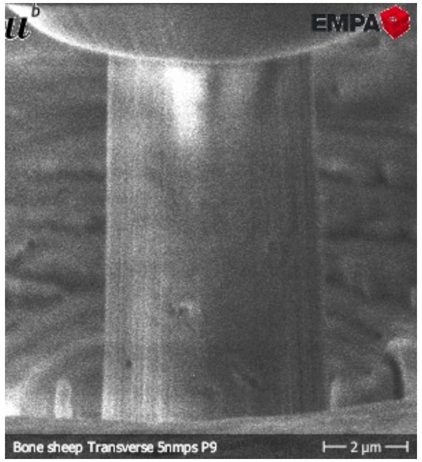
Figure modified from D.Casari et al. (2020) and Donnelly (2011)

Bone from a materials science perspective

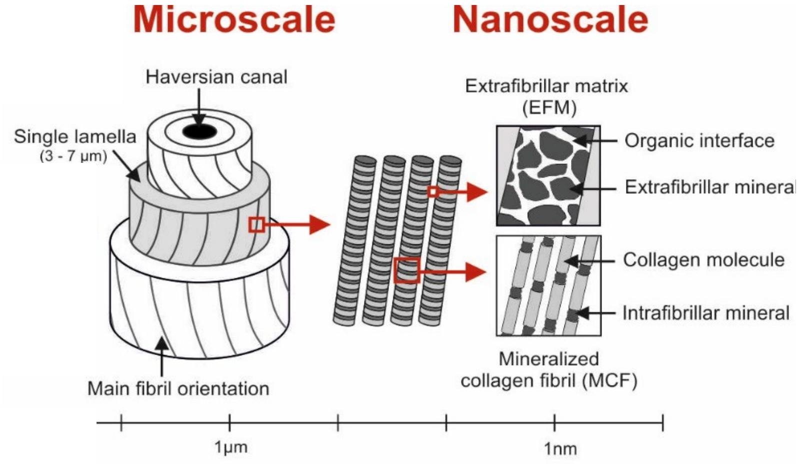
Mechanics



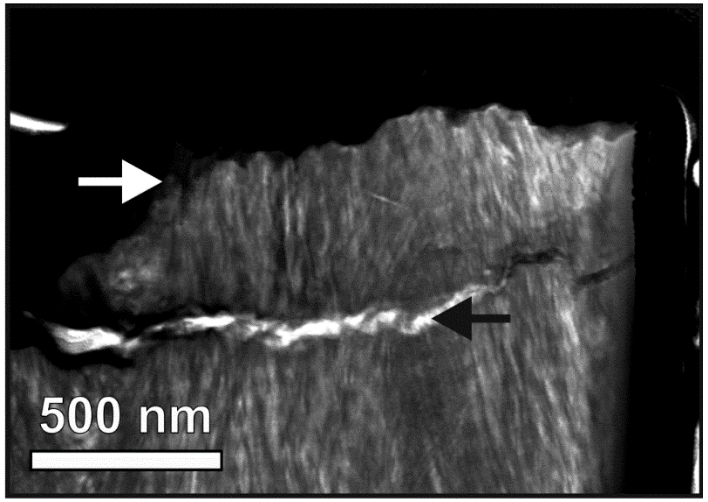
Casari et al., Acta Biomat., 2020



Schwiedrzik et al., *Nat Mat. (2014)*

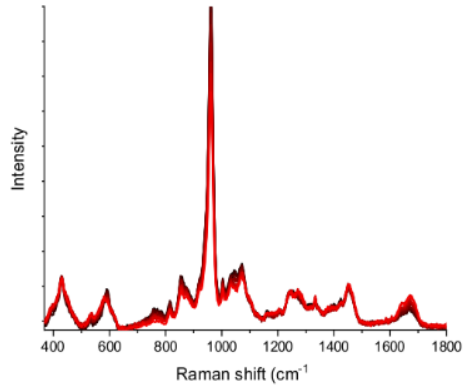


Failure mechanisms

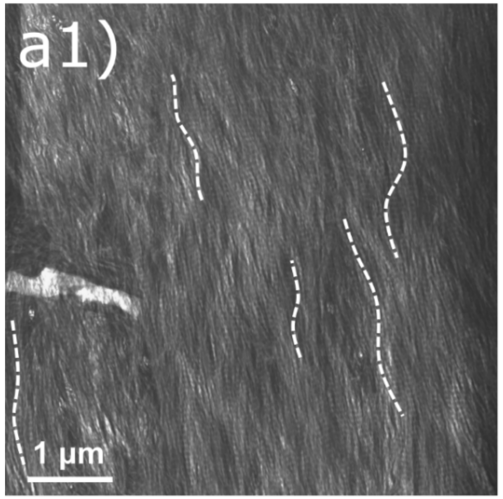


Casari et al., Acta Biomat., 2020

Composition and microstructure



Kochetkova & Peruzzi et al., Acta Biomat., 2021



Schwiedrzik et al., Acta Biomat., 2017

Materials science meets personalized health

- **Can we use materials science methods to quantify bone quality?**

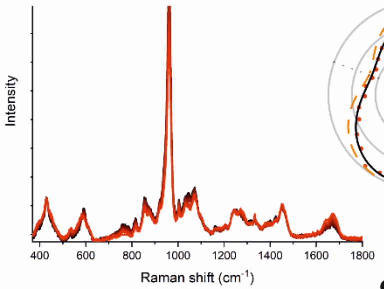
  - Examine human bone
    - multiscale morphology and composition,
    - proteotype,
    - and micromechanical properties

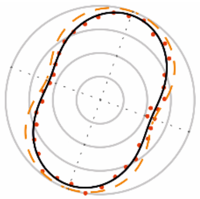
  of patients who underwent total hip arthroplasty (THA).
  - Correlate multimodal dataset with clinical information of each individual patient

Hypothesis: integration of microscale properties and proteotype data can help to assess fracture risk and tailor treatments to **personal** needs based on screening of bone biopsies taken during surgery.

Study overview

- **Polarized Raman spectroscopy & micro-CT** for fast and non-destructive structural and compositional analysis





Composition & Fibril orientation

- **Femto-second laser ablation** for high throughput experiments

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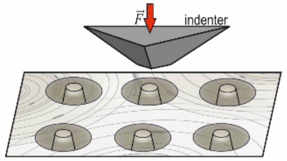
Berner Fachhochschule

Micromechanics



- **Nanoindentation & Micropillar compression** to study microscale elastic and post-yield bone properties

Data mining

Improved clinical diagnostics

- **60 biopsies** from total hip arthroplasty (THA)

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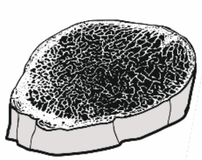
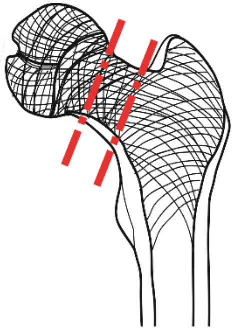
SPITALTIEFENAU
INSELGRUPPE





Bone biopsies & Patient information

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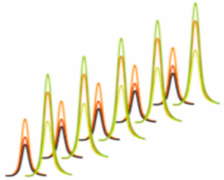
- **SharePoint platform** for data collection (age, gender, disease, …)

Proteomics analysis



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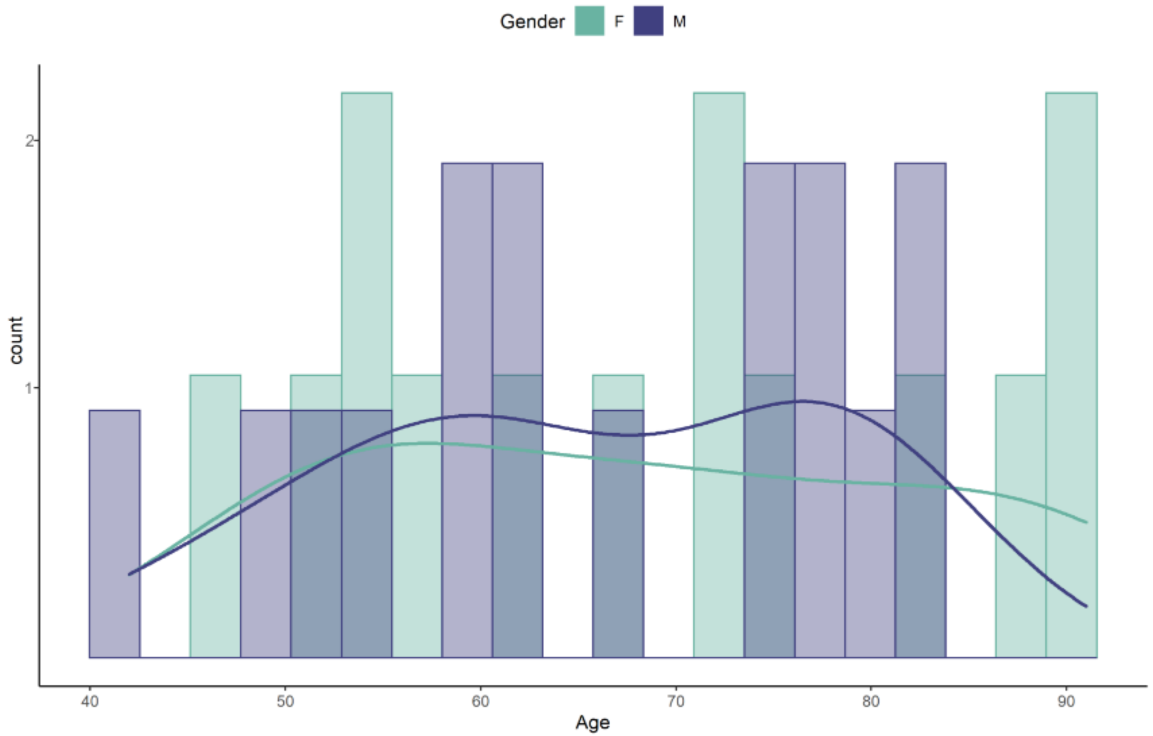
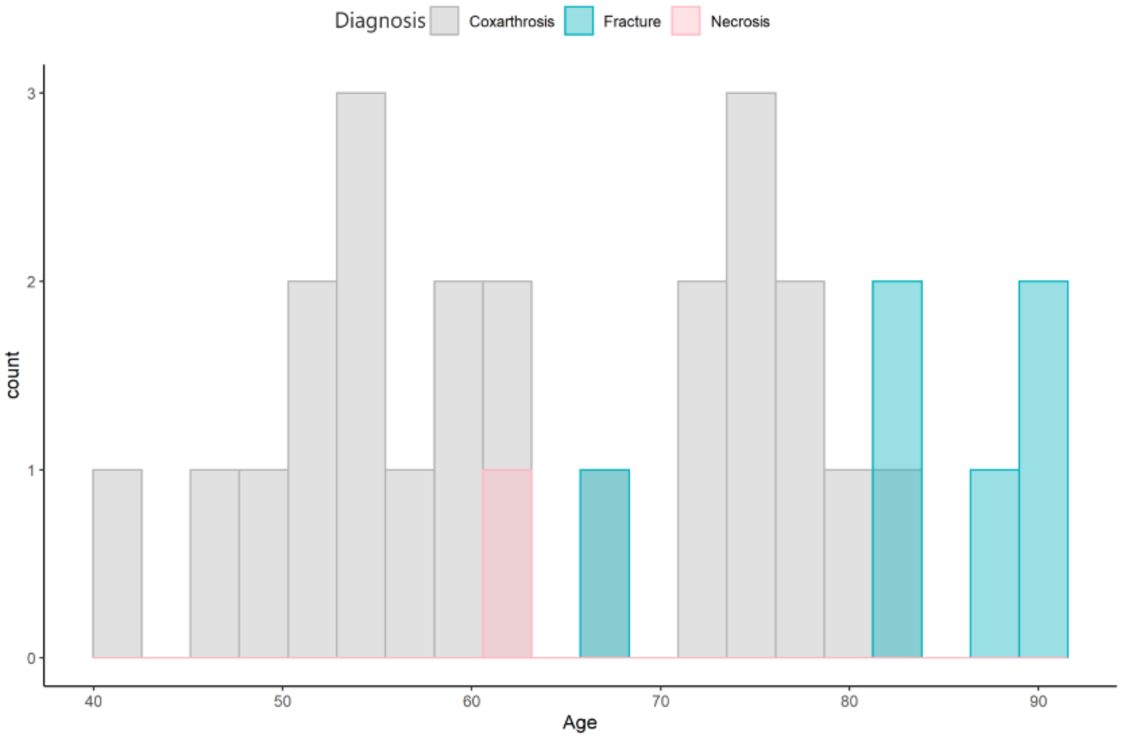
- quantitative protein matrices collection via **Data-Independent Acquisition (DIA) MS/MS workflow**

Patient cohort

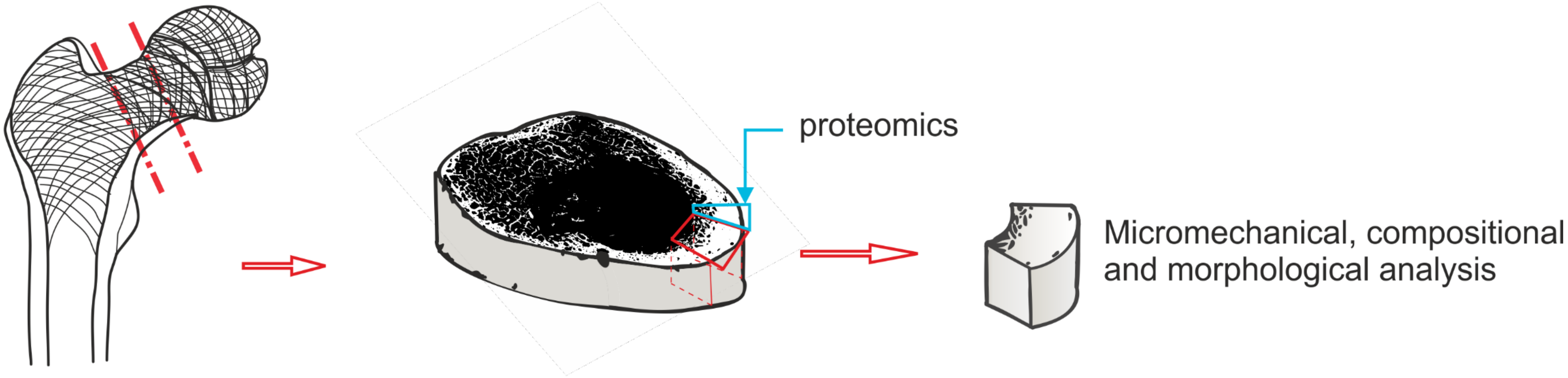
- Indicators:
  - Age, Gender, Femur side
  - Primary, and secondary diagnoses
  - Blood tests results: blood type, electrolytes, metabolites, hematogram
- Primary diagnoses: coxarthrosis, fall fracture.
- Secondary diagnoses: Obesity/Cachexia, Hypertension, Diabetes Type 2.





Biopsy collection

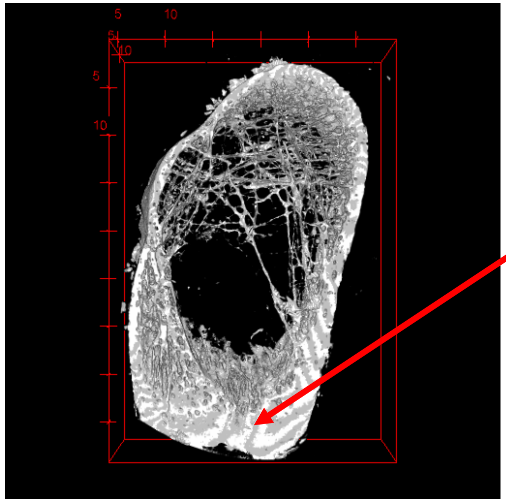
- **60 biopsies** from total hip arthroplasty (THA)



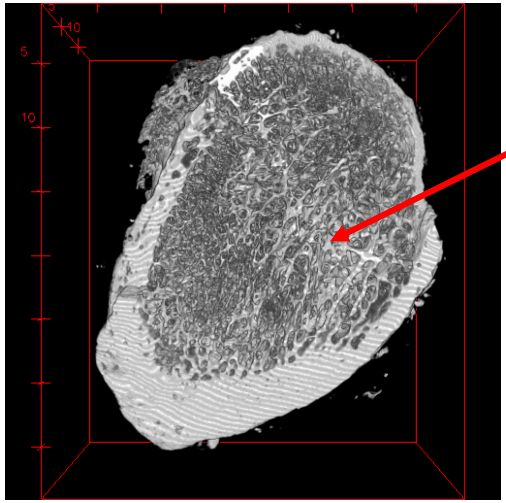
Human femoral neck

Inferomedial region of the neck slice

Bone morphology by micro-CT



- Cortical region:
  - Cortical thickness
  - Tissue mineral density
  - Bone mineral density
  - Bone volume/total volume
  - Anisotropy

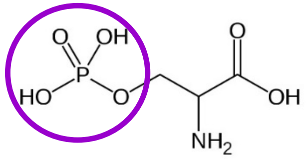


- Trabecular region:
  - Trabecular thickness
  - Trabecular separation
  - Connectivity

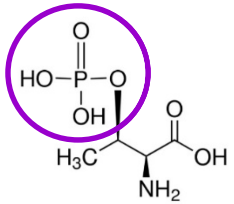
→ Comparison to clinically available techniques (QCT, DXA)

ECM proteotype analysis

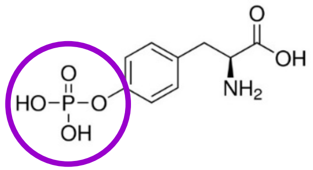
- Quantitative label-free proteomics analysis of bone extracellular matrix proteins
- Post-translational modifications:
  - serine/threonine/tyrosine phosphorylation,
  - proline hydroxylation



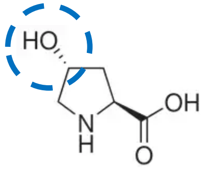
Phospho-serine



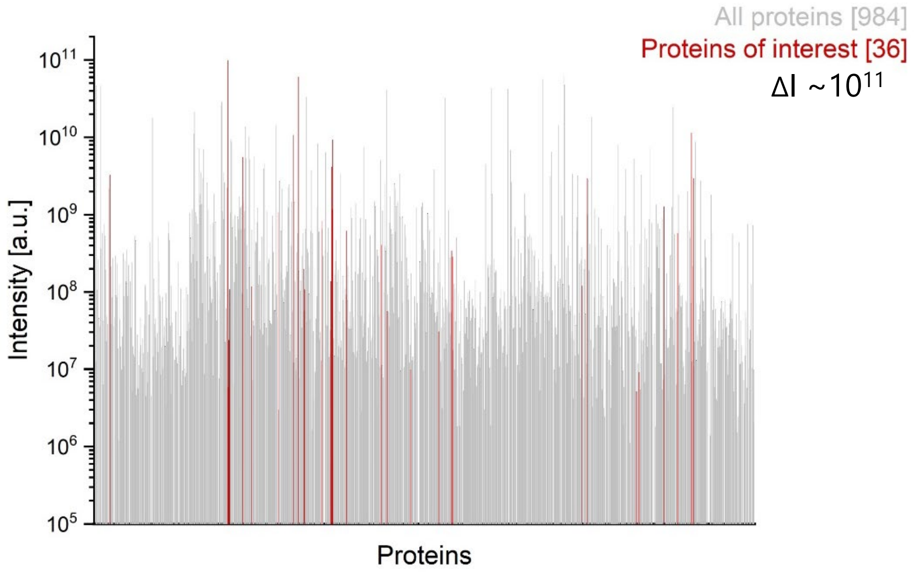
Phospho-threonine



Phospho-tyrosine



hydroxyproline



Proteins of interest:

- <u>Collagen:</u> Type I, III, V
- <u>Non-collagenous proteins (NCP)</u>: Osteocalcin, Osteopontin, Fibronectin, Thrombospondin-2, Matrix gla protein, Bone sialoprotein II, decorin

→ Role of collagen and NCP in bone quality

Polarized Raman Spectroscopy

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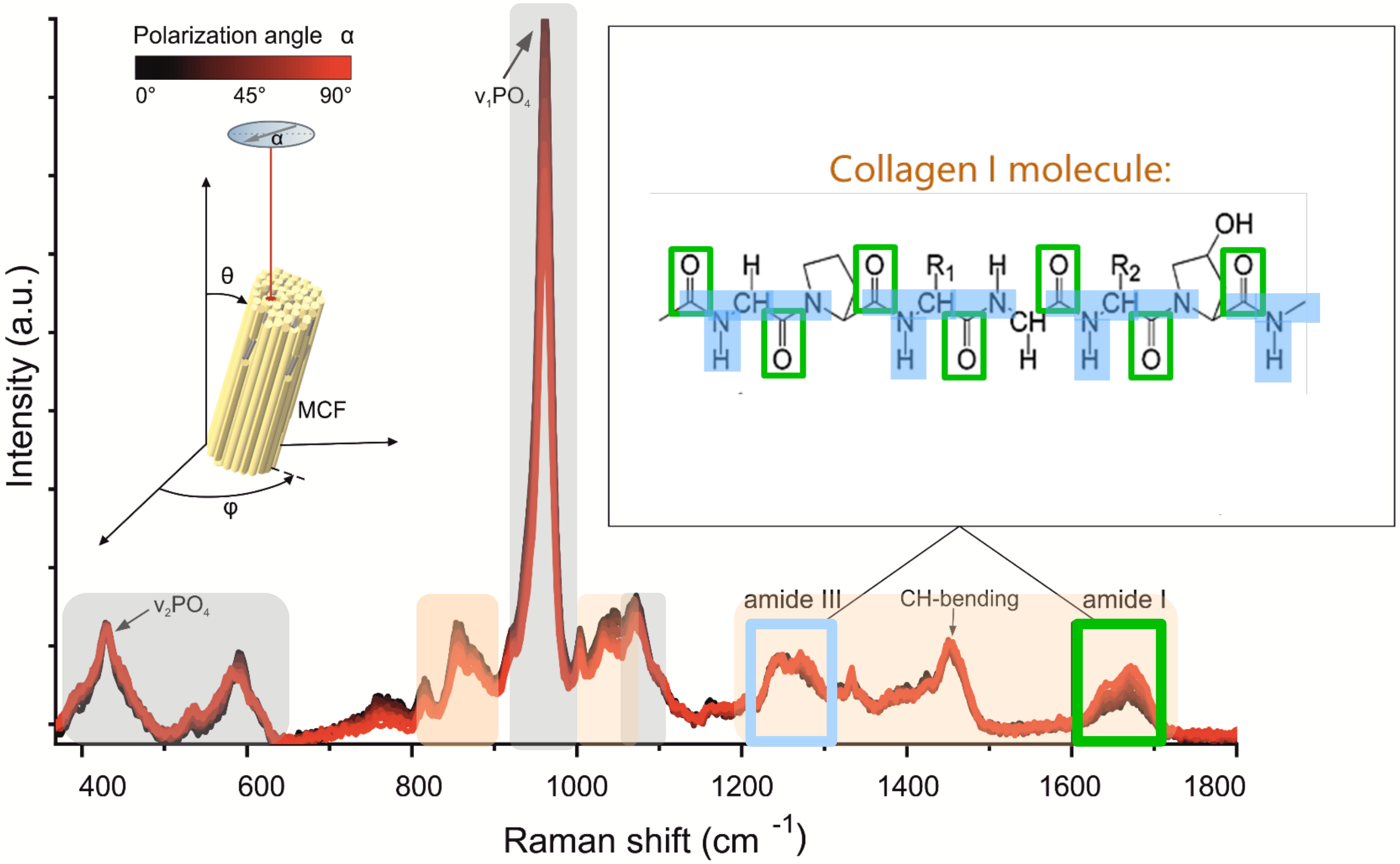
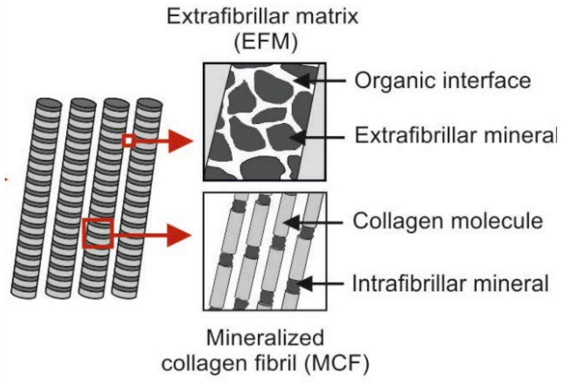




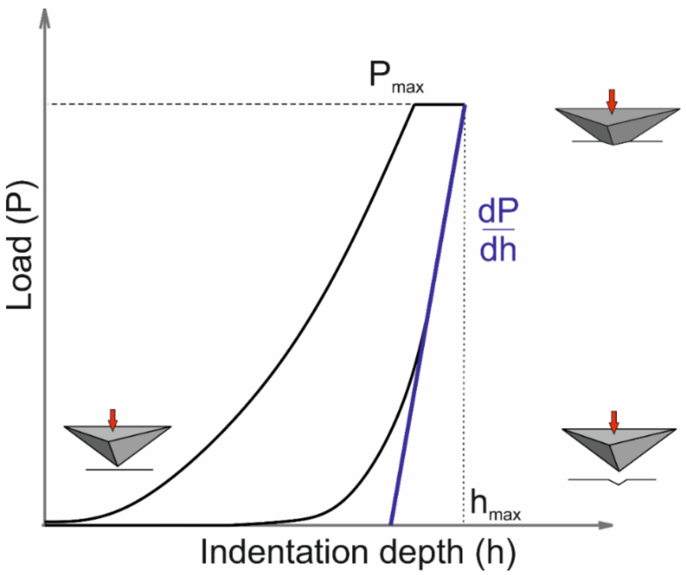
- Mineral
- Collagen

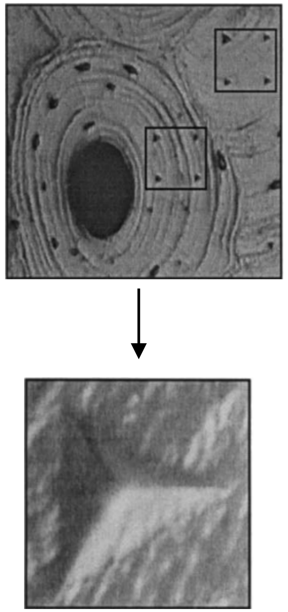
→ Bone matrix composition and microstructure

Micromechanical experiments

Nanoindentation

- Allows measuring local hardness and modulus
- Complex stress field below the tip
- Contact area function assumptions

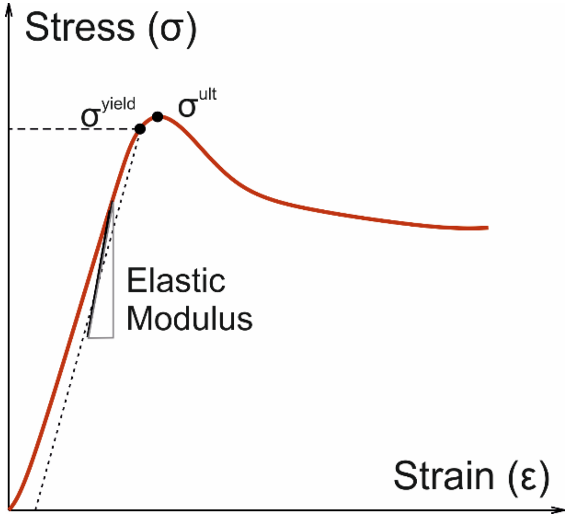


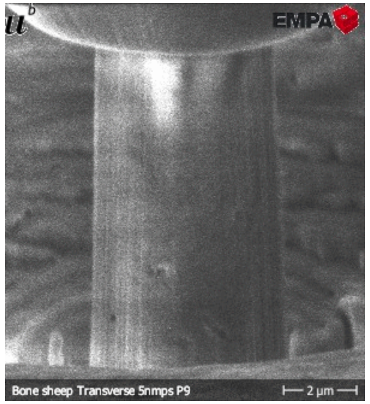


P.K. Zysset et al., J. Biomech. (1999)

Micropillar compression

- Flat punch indenter tip, micropillar with known geometry
- Uniaxial stress field in the volume of interest
- Elastic and post-yield properties



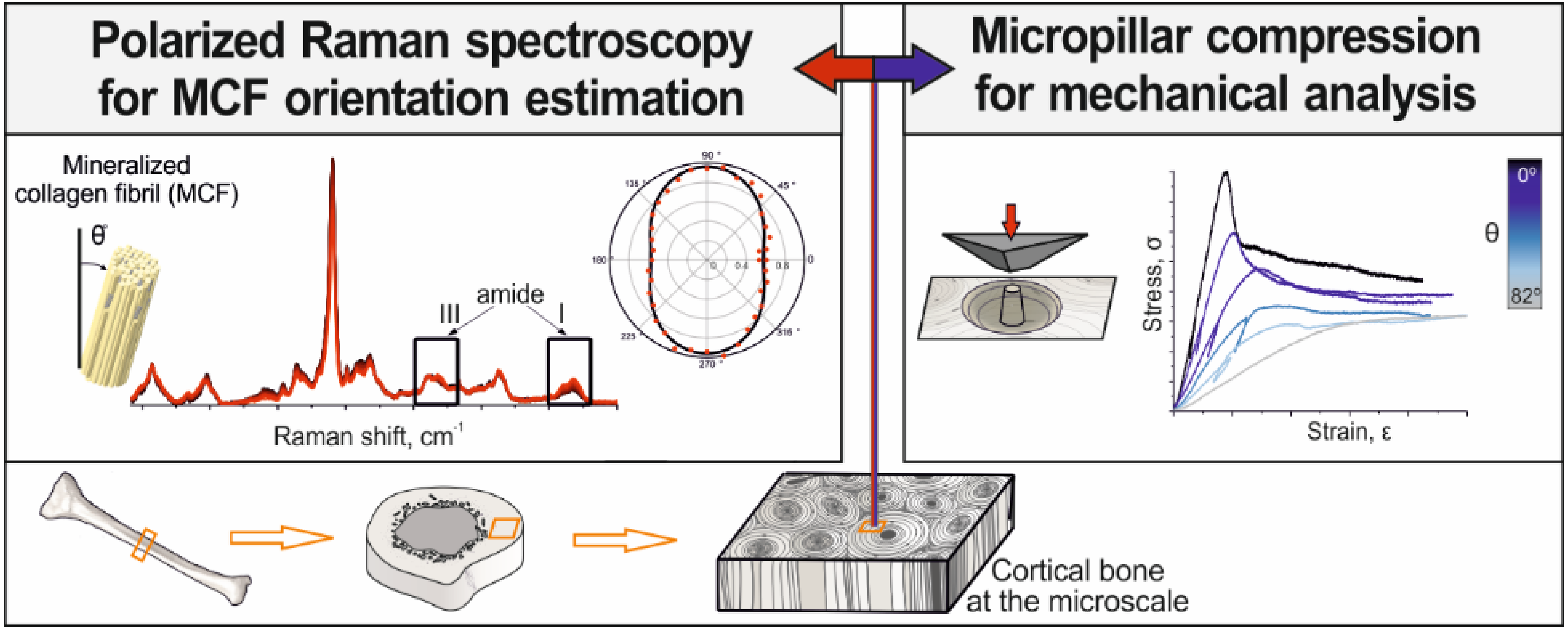


Schwiedrzik et al., *Nat Mat. (2014)*

→ Mechanical properties of individual bone lamellae

Combining polarized Raman spectroscopy and micropillar compression to study microscale structure-property relationships in mineralized tissues

- **Development of the qPRS method** that allows measuring local MCF angle with uncertainty <10°
- Unique **spectrum of microscale mechanical data** for MCF orientations from 0° to 82°



Kochetkova & Peruzzi et al., Acta Biomat. 2021

Compression tests on bovine cortical bone micropillars with known mineralized collagen fibril angles

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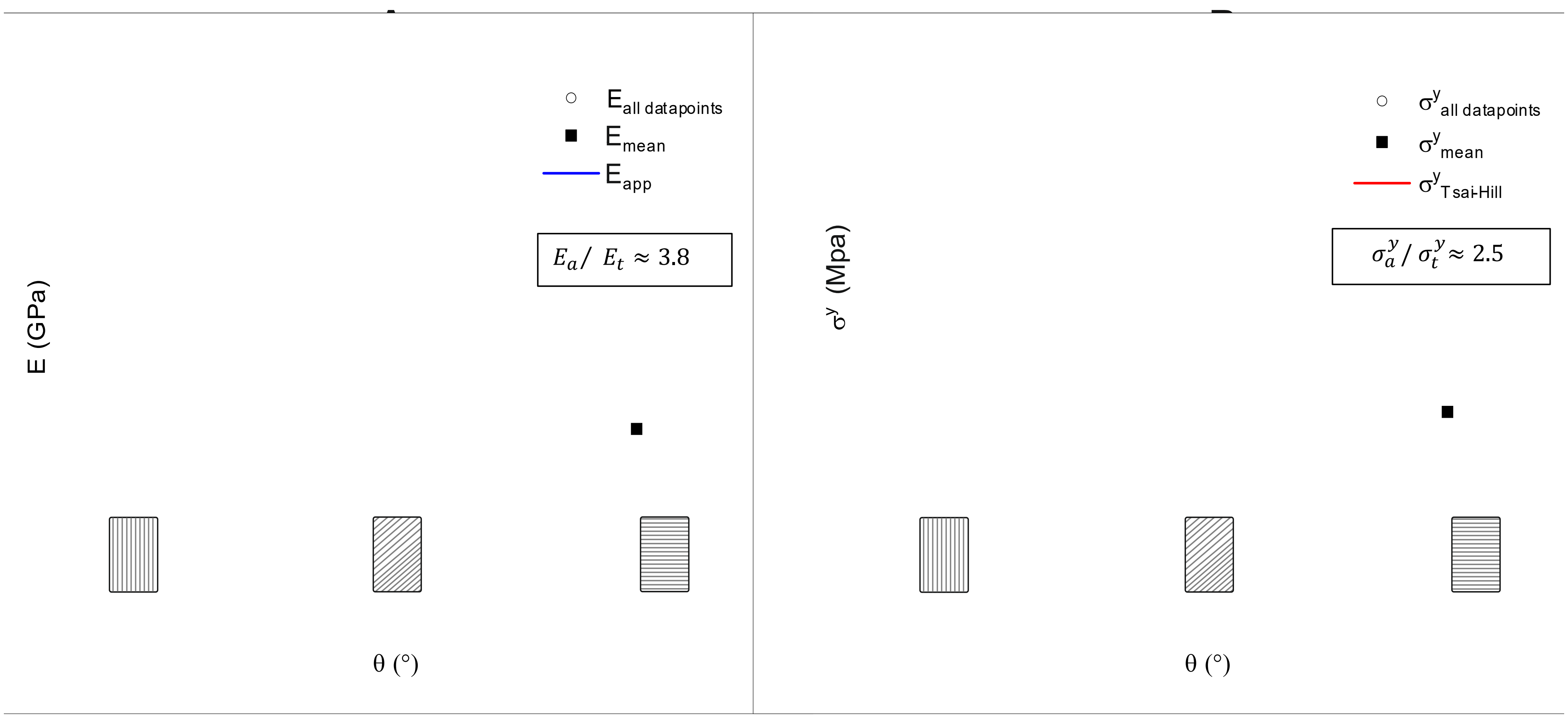
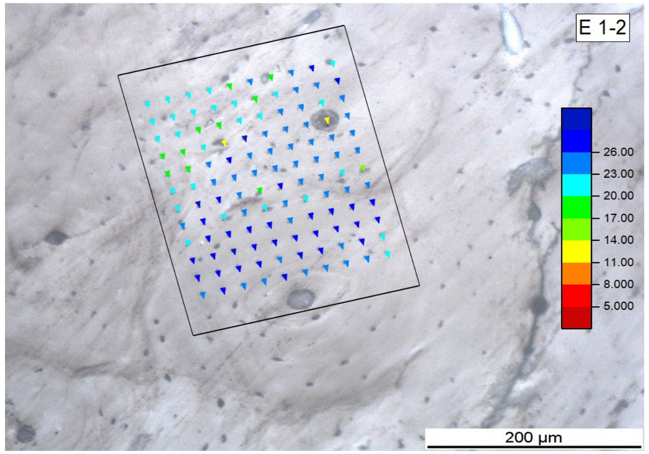
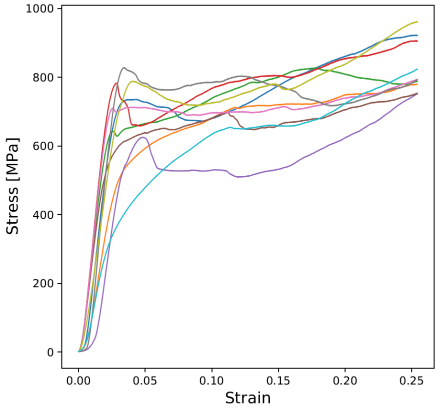
Elastic modulus (A) and yield stress (B) vs. collagen fibril orientation θ:

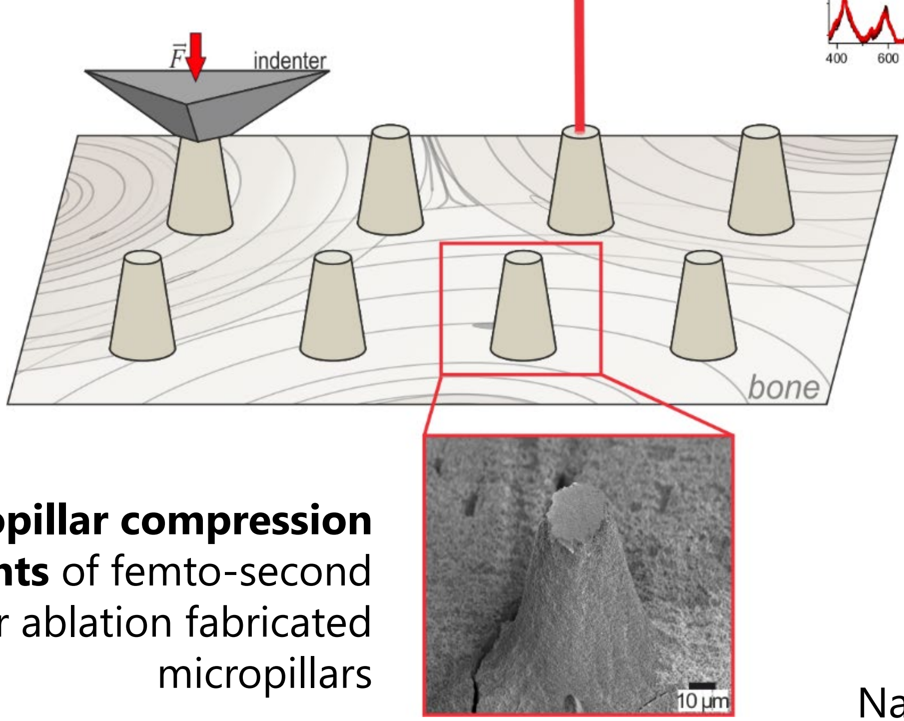


High-throughput experiments on human biopsies

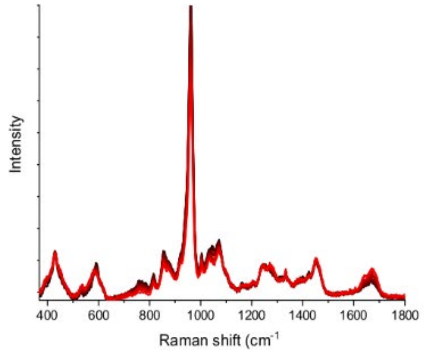
12000 nanoindentations



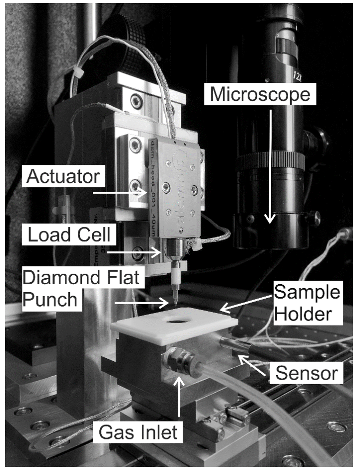




30000 site-matched polarized Raman spectroscopy measurements



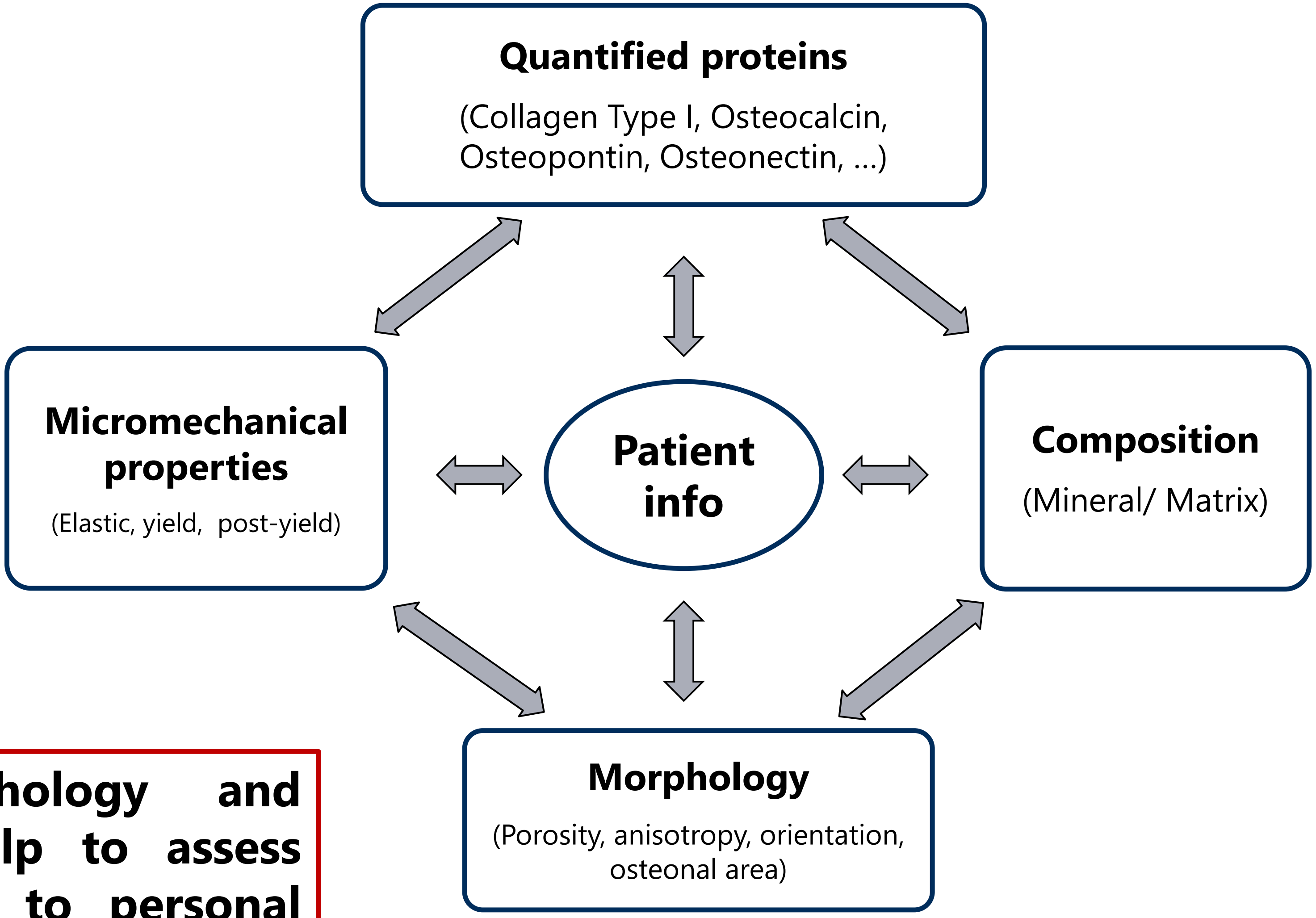
3000 Micropillar compression experiments of femto-second laser ablation fabricated micropillars



Nanoindenter w/ controlled humidity for **near-physiological conditions**

Data analysis

- Database including multimodal experimental and clinical patient information.
- Identification of significant differences between patient groups
- Search for trends in the multimodal dataset using principal component analysis and other unsupervised machine learning methods.



Integration of multiscale morphology and properties with proteotype can help to assess fracture risk and tailor treatments to personal needs based on a single bone biopsy.

Conclusion

- Understanding bone’s multiscale mechanical behaviour aims at predicting patient-specific fracture risk more accurately
- We use high throughput biopsy screening using Raman and micromechanics analyses to build up a database of tissue properties for different pathologies
- Normalizing the micromechanical properties by average fibril angle and mineralization helps to reduce apparent data scatter
- Adding information on the proteotype allows investigating the role of specific proteins in bone quality and fracture risk.
- In the long run, alternative non-invasive predictors of the tissue properties are sought to improve clinical diagnostics

Thank you for your attention!

Acknowledgements:

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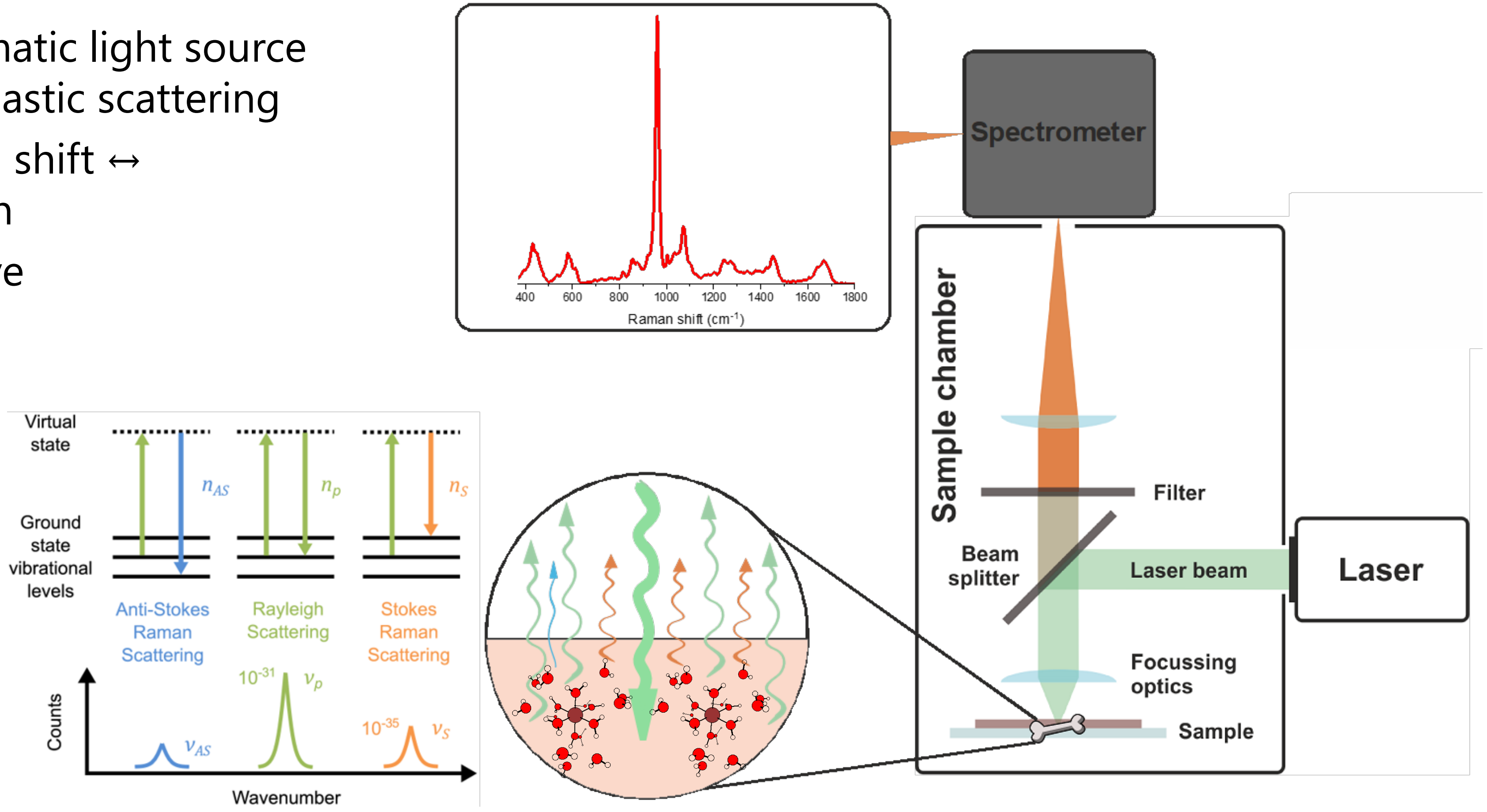
Prof. Dr. Jörn Dengjel, University of Fribourg
Dr. Markus Hanke, Inselspital
Prof. Dr. Bernd Wollscheid, ETH Zürich

Composition & morphology analysis: Polarized Raman Spectroscopy (PRS)

- Monochromatic light source induces inelastic scattering
- Wavelength shift ↔ composition
- Non-invasive



Calibrating PRS on model material for quantitative microstructural analysis

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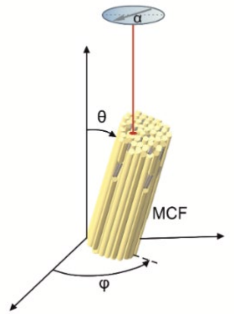
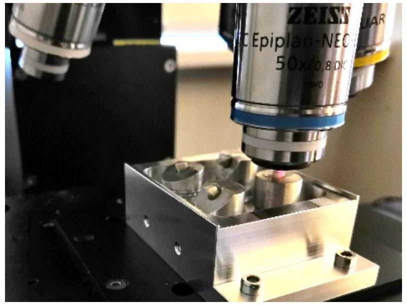
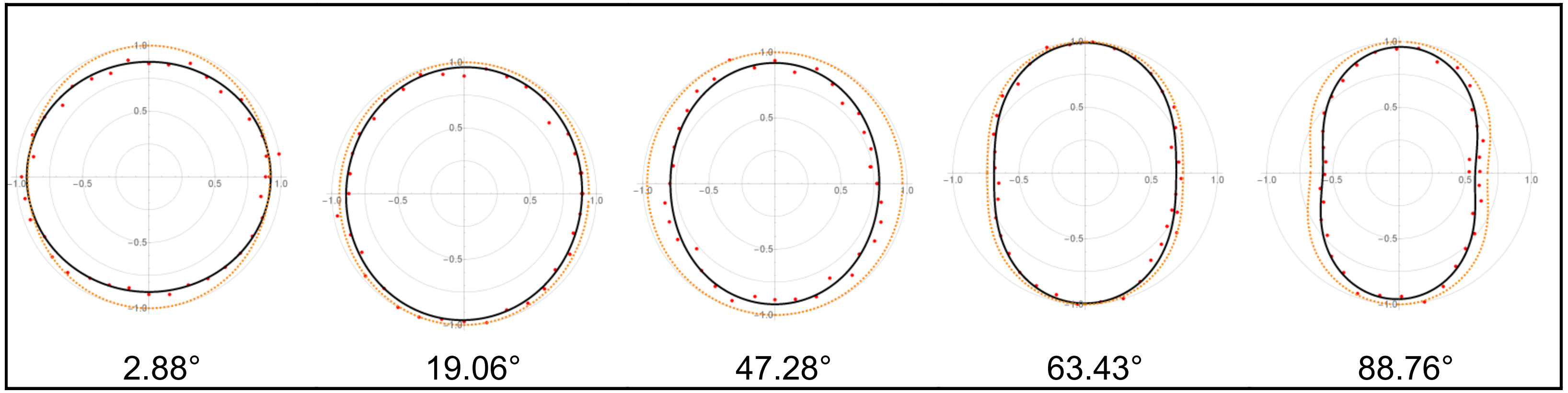
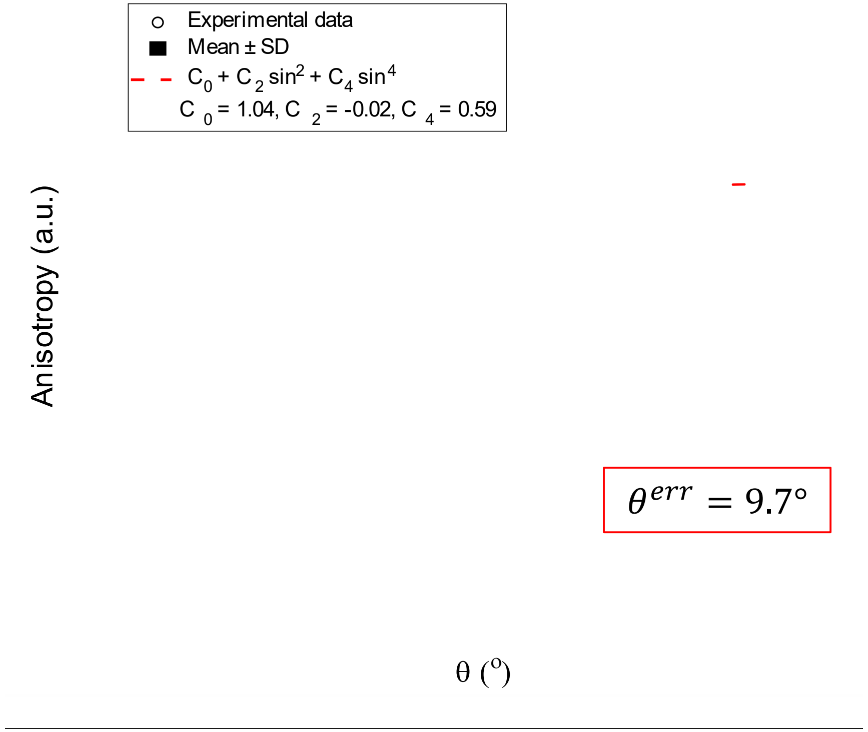
- Mineralized turkey leg tendon (MTLT) – nature model material
- Out-of-plane angles θ of mineralized collagen fibrils were measured with SAXS





Spectral anisotropy parameter vs polarization angle for different MTLT orientations:





Bone ECM	Expressed from	Function in bone tissue	Reference
Organic ECM			
Collagenous protein			
Type I collagen	Osteoblast	–Scaffold for bone cells –Maintain bone strength –Promote bone formation –Regulate collagen fibrillogenesis	(Saito and Marumo, 2015) (Fonseca et al., 2014)
Types III and V collagen	Bone	–Promote bone	(Garnero, 2015)
Noncollagenous protein			
Proteoglycans			
Biglycan	Osteoblast	–Promote collagen fibrillogenesis –Promote bone formation	(Moorehead et al., 2019)
Decorin	Osteoblast	–Promote collagen fibrillogenesis –Promote bone formation	(Coulson-Thomas et al., 2015)
Keratocan	Osteoblast	–Promote mineral deposition rates	
Asporin	Articular cartilage or periodontal tissue	–Promote collagen mineralization	(Kalamajski et al., 2009)
γ-carboxyglutamic acid-containing proteins			
Osteocalcin	Osteoblast	–Regulate calcium metabolism –Indicate bone formation	(Mizokami et al., 2017)
Matrix Gla Protein *(MGP)*	Osteoblast, osteocyte, and chondrocyte	–Inhibit bone formation and mineralization	(Kaipatur et al., 2008)
Periostin	Osteoblast and precursor cells	–Regulate collagen fibrillogenesis -Maintain bone strength	(Wen et al., 2018)
Glycoproteins			
Osteonectin	Osteoblast	-Promote bone formation and mineralization -Regulate collagen fibrillogenesis -Maintain biomechanical properties	(Rosset and Bradshaw, 2016) (Delany et al., 2000)
Thrombospondins	Osteoblast	-Promote bone formation -Regulate collagen fibrillogenesis	(Delany and Hankenson, 2009)
R-spondins	Bone	-Promoter Wnt/β-catenin signaling -Regulate bone development	(Shi et al., 2017)
Small integrin-binding ligand *N*-linked glycoproteins/SIBLINGs			
BSP	Mineralized tissues	–Promote bone formation and mineralization	(Marinovich et al., 2016)
OPN	Osteoblast, odontoblast and osteocyte	–Promote bone formation and mineralization –Regulate bone remodeling	(Singh et al., 2018)
DMP1	Osteocyte and dentin	–Regulate phosphate metabolism –Promote bone mineralization	(Jani et al., 2016)
MEPE	Osteocyte and dentin	–Regulate phosphate metabolism –Promote bone mineralization	(Zelenchuk et al., 2015)
Inorganic ECM			
Hydroxyapatite	Bone	–Biomineralization	(Tavafoghi and Cerruti, 2016)

Table from Lin et al., 2020