Institute of Micro- and Nanostructure Research

CENEM Center for Nanoanalysis and Electron Microscopy

Empa Materials Science and Technology

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Electron Tomography in a correlative approach to multimodal characterization of human bone

Master Thesis of Tatiana Kormilina[1,2,*]

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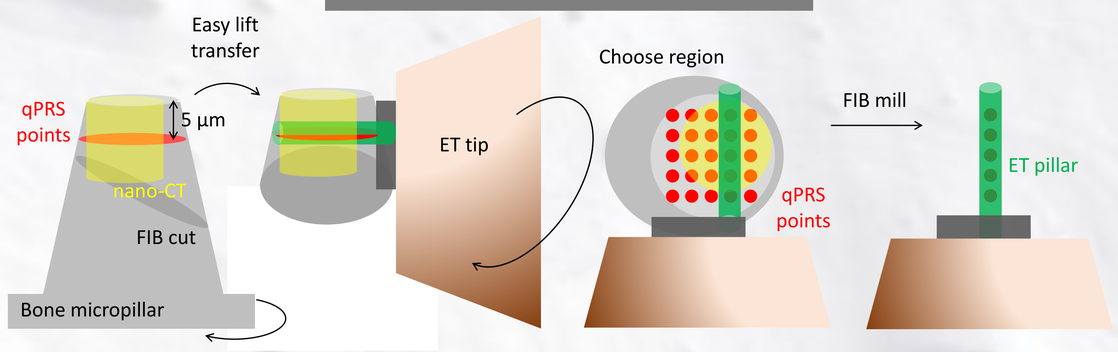
Abstract

MCF – mineralized collagen fibrils
qPRS – quantitative polarized Raman spectroscopy
nanoCT – nanoscale X-ray computed tomography
ET – electron tomography

Pursuing the task of creating a correlative approach to quantitative characterization of MCF orientation, we combined the efforts of tree techniques, bridged by clever sample preparation, 3D data processing algorithms and intimate understanding of the bone structure, provided by ET.

Sample preparation scheme

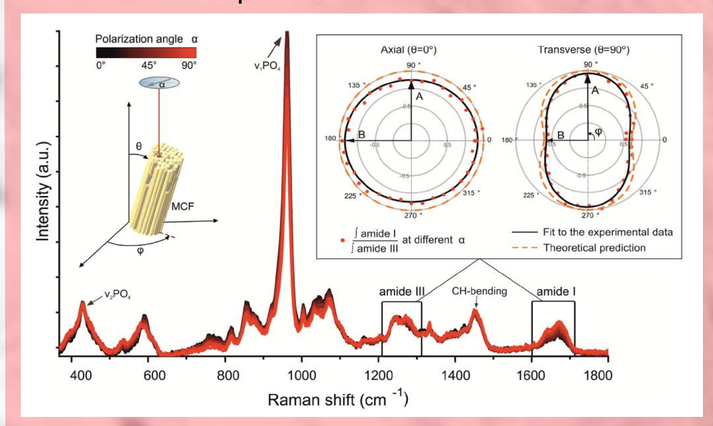


qPRS

PHOTO BY WITEC

WiTec Alpha 300 R

- 5 µm below the surface
- Spot size elliptical ~1 µm diameter
- 785 nm laser

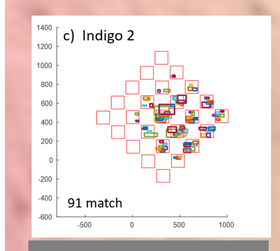


Kochetkova T. et al. *Acta biomaterialia* 119 (2021): 390-404.

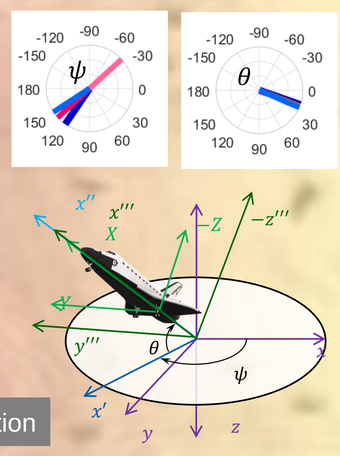
QPRS is a new method of MCF orientation from the ratio of orientation-dependent amide I and independent amide III signal intensities in each spot of the 5x5 map.

Correlation qPRS-nanoCT

Segmented objects in nano-CT are matched with qPRS measurement positions. Averaged angles from two independent tomographies (indigo 1 and 2) are compared with those from qPRS (pink)



Segments matched to qPRS spots



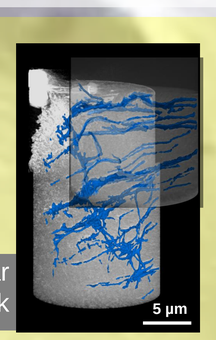
Angle notation

Nano-CT

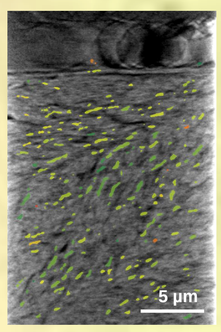
PHOTO BY ZEISS

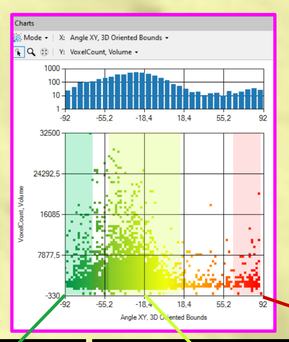
Xradia 810 Ultra

- Phase contrast
- ROI volume (16 µm)³
- Resolution 50 nm
- Energy 5.4 keV

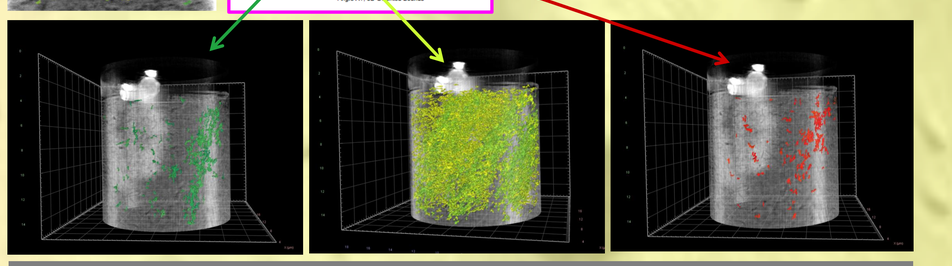


Canalicular network



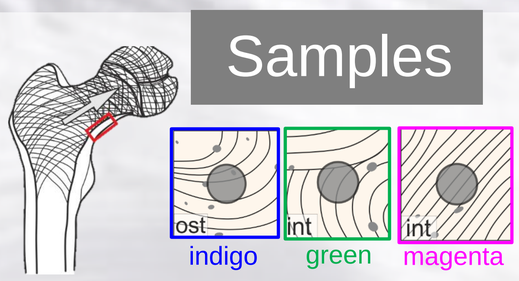


Feature segmentation with machine learning is used to reveal canaliculi networks and segments pointing the MCF orientation, which can be quantified.

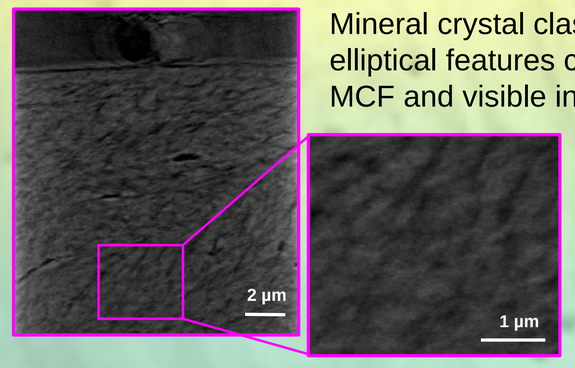


Segments colored according to their orientation

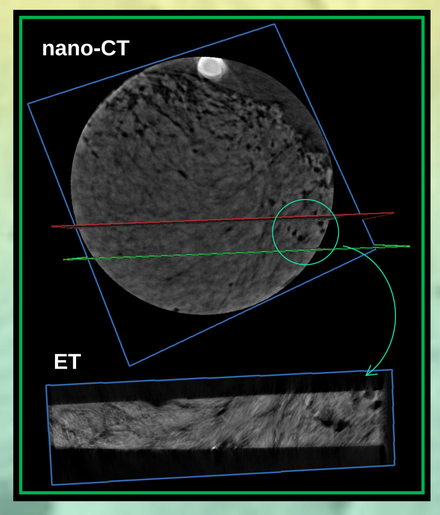
Samples



Correlation nanoCT-ET



Mineral crystal clasters form bright elliptical features cooriented with MCF and visible in nanoCT.



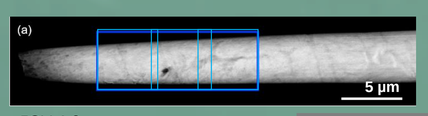
ET volume can be identified and correlated with respective region in the nanoCT.

Electron Tomography

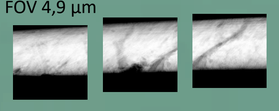
PHOTO BY MINGJIAN WU

FEI Titan Themis³ 300

- Acceleration voltage 300 kV
- Z contrast
- Micro-probe regime
- Voxel size (2,39 nm)³

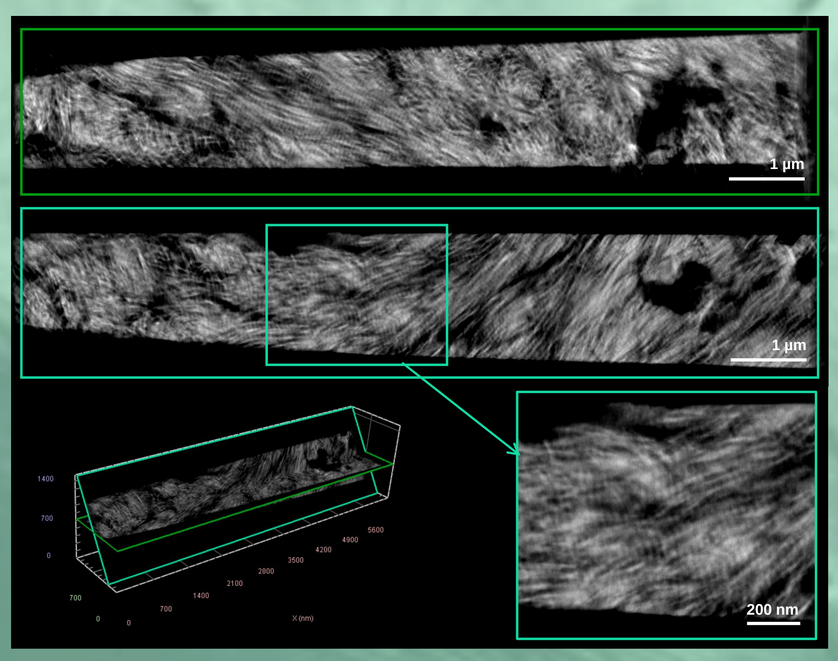


FOV 4,9 µm



Stitching three tomography series

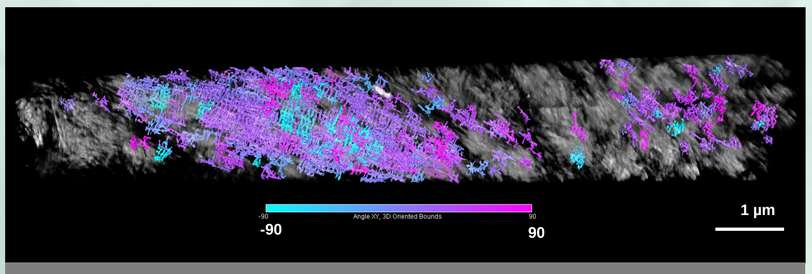
FOV ~12 µm



Virtual slices cut at different angles show both mineral crystals forming bright elliptical features and d-spacing ‘zebra’ pattern, indicative for MCF. Inset shows change in the orientation of MCF.

Breakthrough

Simultaneous study of both mineral and organic compounds of the bone structure is often impossible. We achieved resolution, that allows to inspect pristine organomineral arrangement, and give direct assessment of the MCF orientation without demineralization.



MCF segmented out using the d-spacing pattern

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