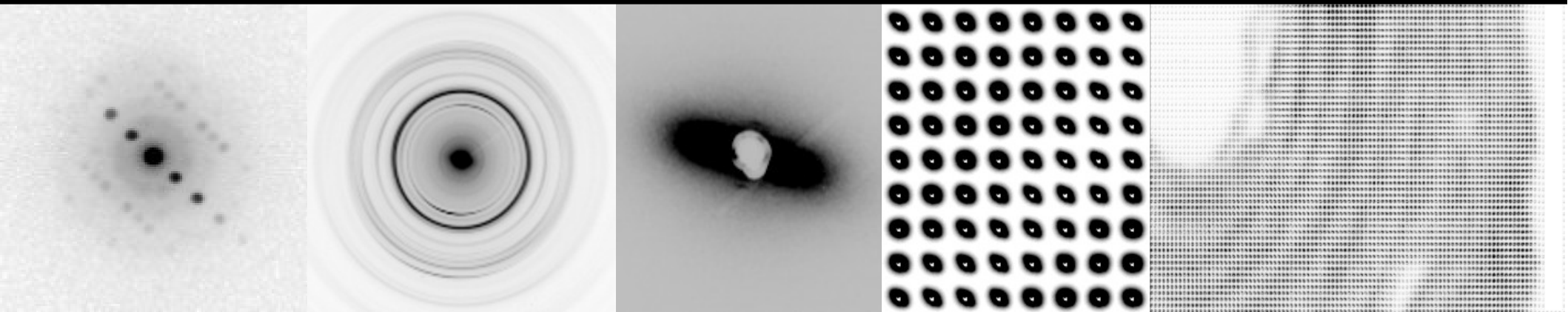
Ordre et désordre nanocristallin dans les tissus osseux.

A. Gourrier

Laboratoire Interdisciplinaire de Physique (LIPHY)



Laboratoire Interdisciplinaire de Physique
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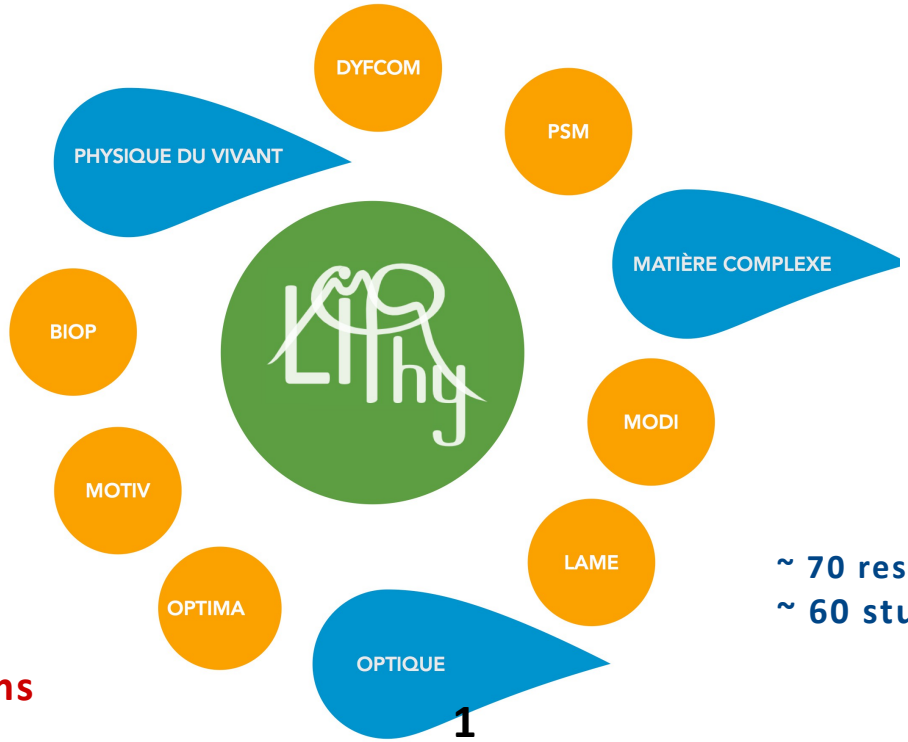
General presentation.

published on 5 February 2015, updated on 20 March 2018 at 21:56



Research topics
- General presentation.
- Physics of living systems
- Complex Matter
- Optics

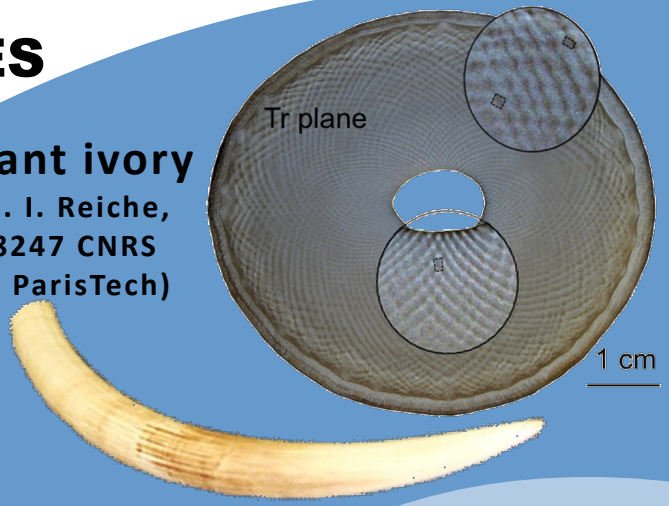
Bioimaging
Life sciences
Complex systems

~ 70 researchers & faculty members
~ 60 students

SYNCHROTRON µFOCUS X-RAY SCATTERING OF BIOLOGICAL MATERIALS

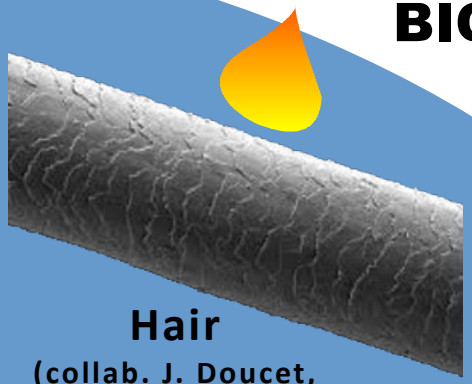
FOR BIOMEDICINE & ARCHAEOLOGY

BIOCOMPOSITES

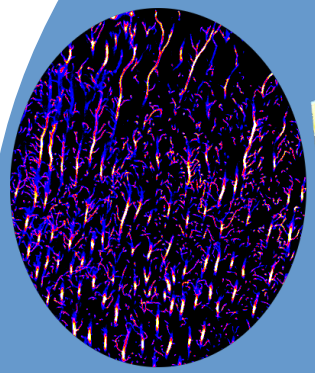


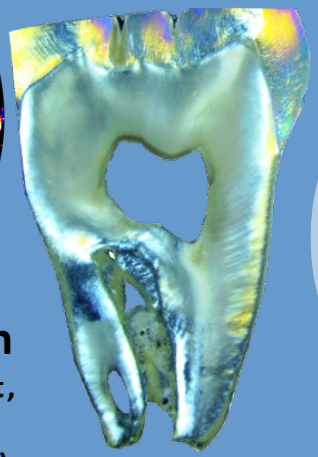
Elephant ivory
(collab. I. Reiche, UMR 8247 CNRS Chimie ParisTech)

BIOPOLYMERS

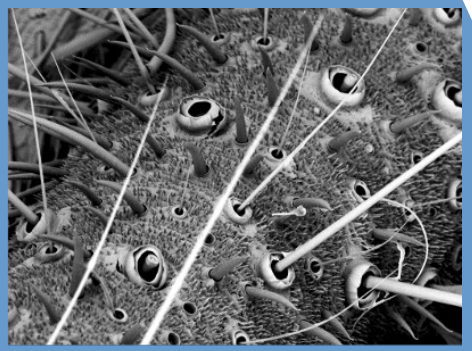


Hair
(collab. J. Doucet, UMR 8502 CNRS UPSud)

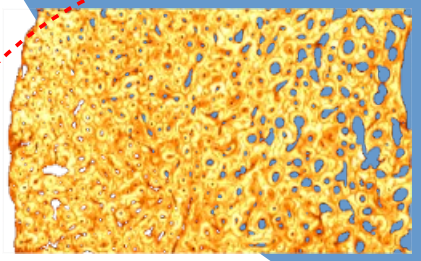


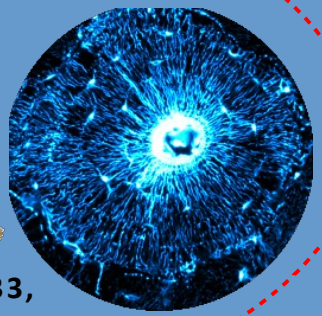


Tooth dentin
(collab. E. Vennat, UMR 8579 CNRS Centrale Supelec)

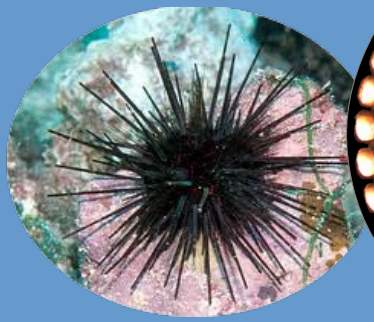


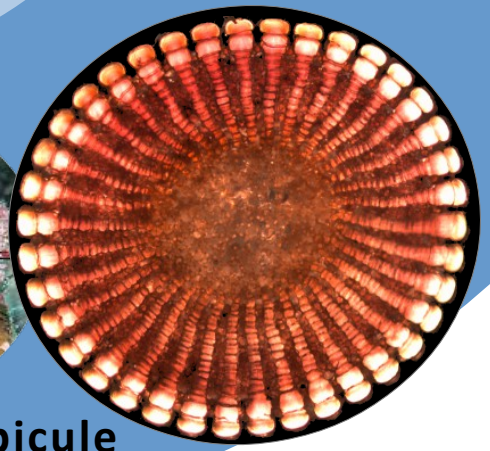
Cricket appendix
(collab. O. Paris, MPIKG, Potsdam, Germany)





Bone
(collab. INSERM U1033, UMR S 1146 / UMR 7371 CNRS INSERM UPMC,...)





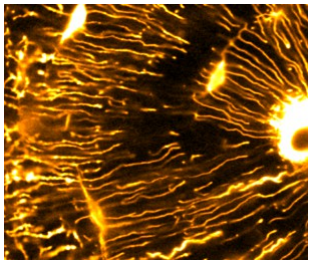
Sea urchin spicule
(collab. H. Coelfen, U. Konstanz, Germany)

BIOMINERALS

ORDER/DISORDER | MULTISCALE | DYNAMICS/ CIRCULATORY PATHWAYS

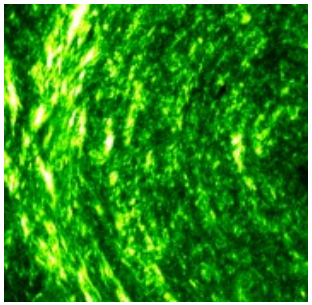
Fluorescence microscopy imaging of the cellular network in bone and dentin

Collab. D. Débarre (DYFCOM@LIPhy) & D. Rousseau (LARIS, Angers)



SHG (second harmonic generation) imaging of the microfibril organization

Collab. D. Débarre (DYFCOM@LIPhy), I. Wang, J.C. Vial (MOTIV@LIPhy), E. Beaurepaire, M.C. Schanne-Klein (LOB, Palaiseau)



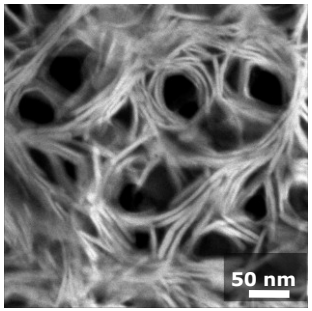
R. Genthial

PhD thesis 2013-16

now @McGill

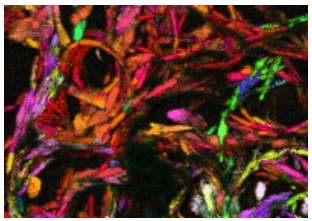
FIB-SEM (focused ion beam) analysis of mineral nanoparticle organization

Collab. K. Grandfield (McMaster, Ontario, CA) & E. Vennat (Centrale-Supelec)



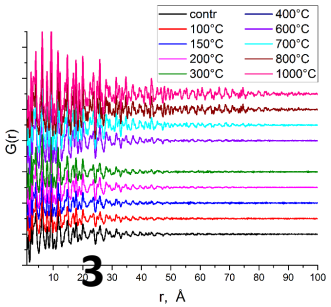
ACOM-TEM (electron diffraction) analysis of mineral crystal structure at the individual nanocrystal level

Collab. E. Rauch, M. Véron (SIMAP) & J.L. Puteaux, C. Lancelon-Pin (CERMAV)



Pair distribution function (PDF) analysis (X-ray diffraction) of atomic-scale disorder

Collab. P. Bordet (Institut Néel)



M. Verezhak

PhD thesis 2013-16

now @PSI (cSAXS)

Facts and figures

Here are some facts and figures about osteoporosis in the European Union provided by the World Health Organization in October 2001 (1):

- A fracture from osteoporosis occurs every 30 seconds in the European Union
- The disease affects 20 to 30% of postmenopausal women, 50% of the women over 60 years old, and 13% of the men over 50 years old, the fracture risk increasing sharply with the age.
- This is the second most important public health problem for women after breast cancer.
- the number of hip fractures, estimated to near ly 400 000, is estimated to reach one million within 50 years.
- The induced hospital costs are estimated to 23 Billion Euros per year

The impact of osteoporosis is becoming more and more important everyday with population aging and increasing impact of sedentary lifetsyle. Osteoporosis figures are alarming and show that the disease has become a public health priority.

(1) Sources OMS Press Release published in October 2001 around the international osteoporosis day
You can also have more information thanks to the links page.

http://www.medes.fr/home_fr/applications_sante/osteoporose/eristo/osteoporosis.html *from* W.H.O.

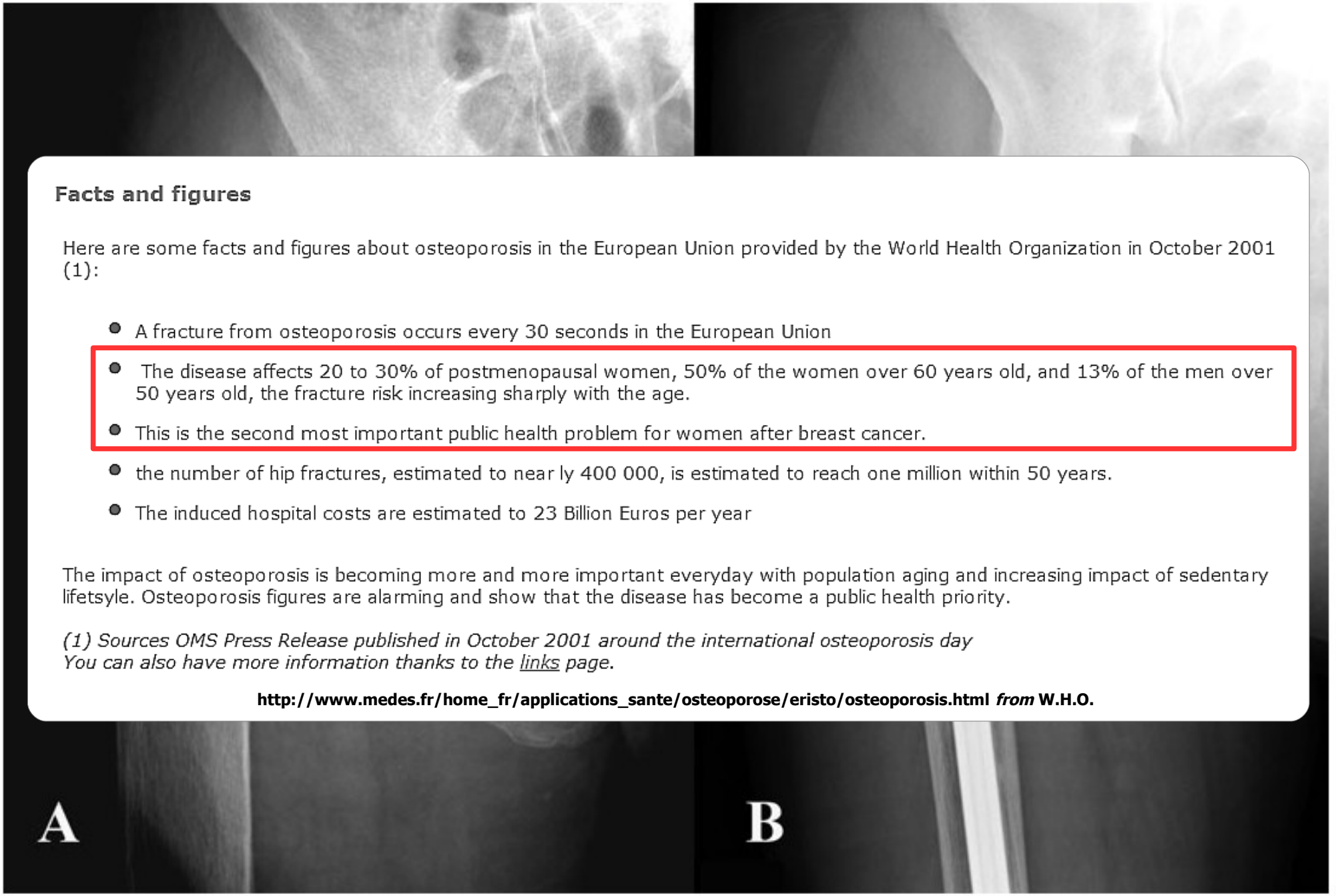
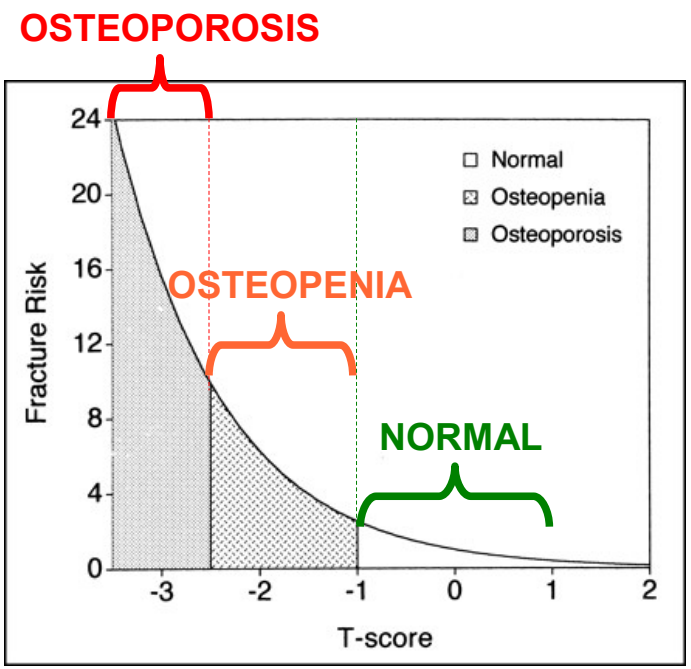


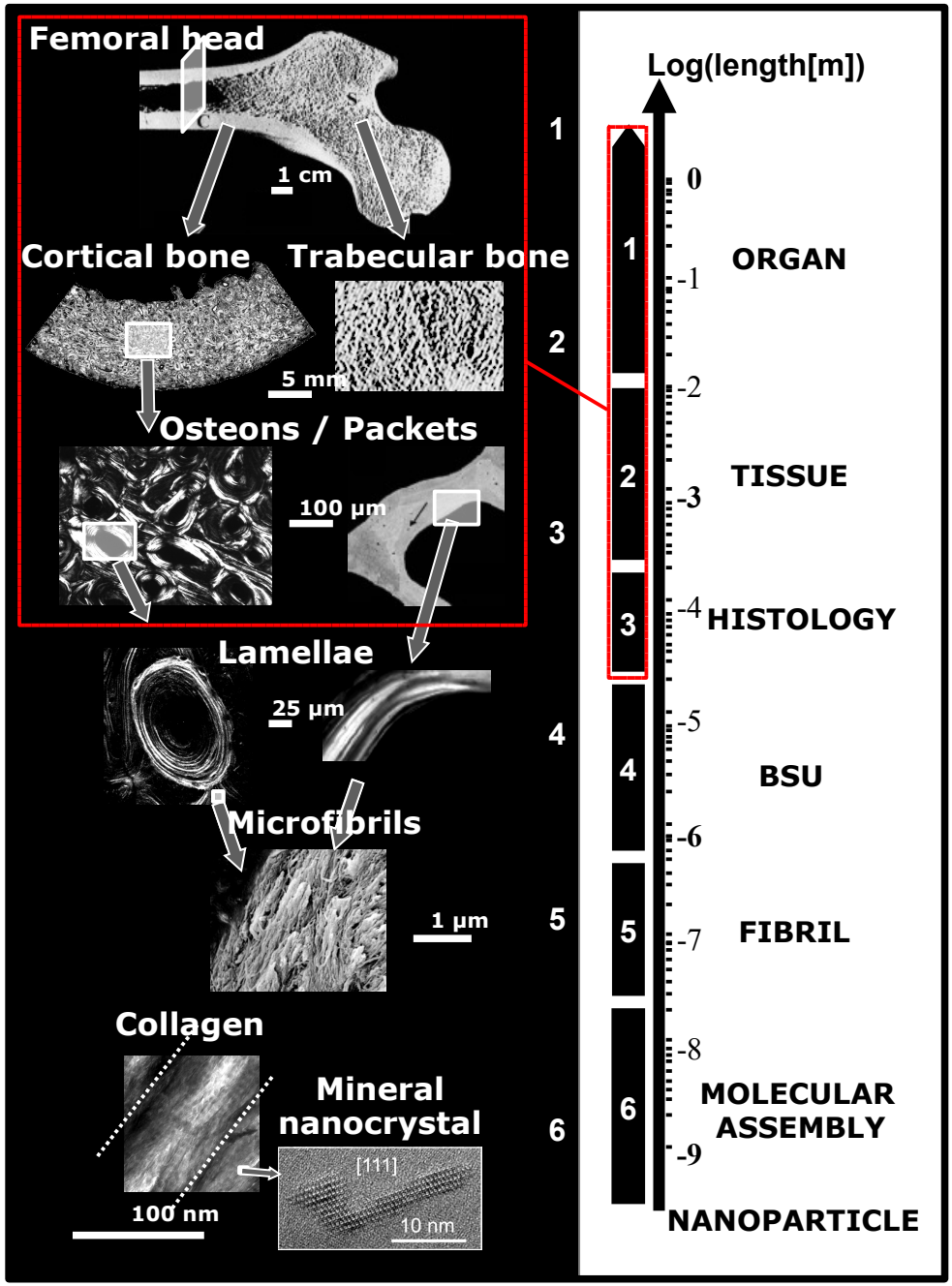
Fig. 1 -- Fig. 1-A Preoperative anteroposterior hip radiograph showing a right basicervical femoral neck fracture after the patient experienced a low-energy fall (arrows). Fig. 1-B Postoperative radiograph made at twenty-one months following closed reduction and internal fixation with an intramedullary nail

Image 1 of 6

Based on current diagnostic tests (DEXA), **50 % of osteopenic fractures are unexplained** !



Fogelman *et al.* (2008) J Nuc Med 12, 2000



Osteoporos Int (2008) 19:1251–1265
DOI 10.1007/s00198-008-0579-1

REVIEW

The effect of the microscopic and nanoscale structure on bone fragility

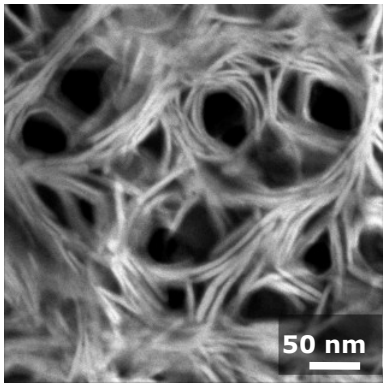
M. E. Ruppel · L. M. Miller · D. B. Burr

What lies beyond BMD ?

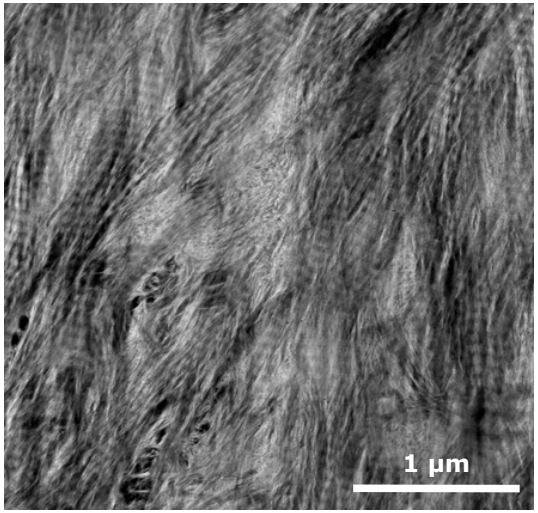
Can we identify **nanoscale structural markers** of bone diseases ?

More specifically, can the **size** and **organization** of the mineral nanocrystals be used as such ?

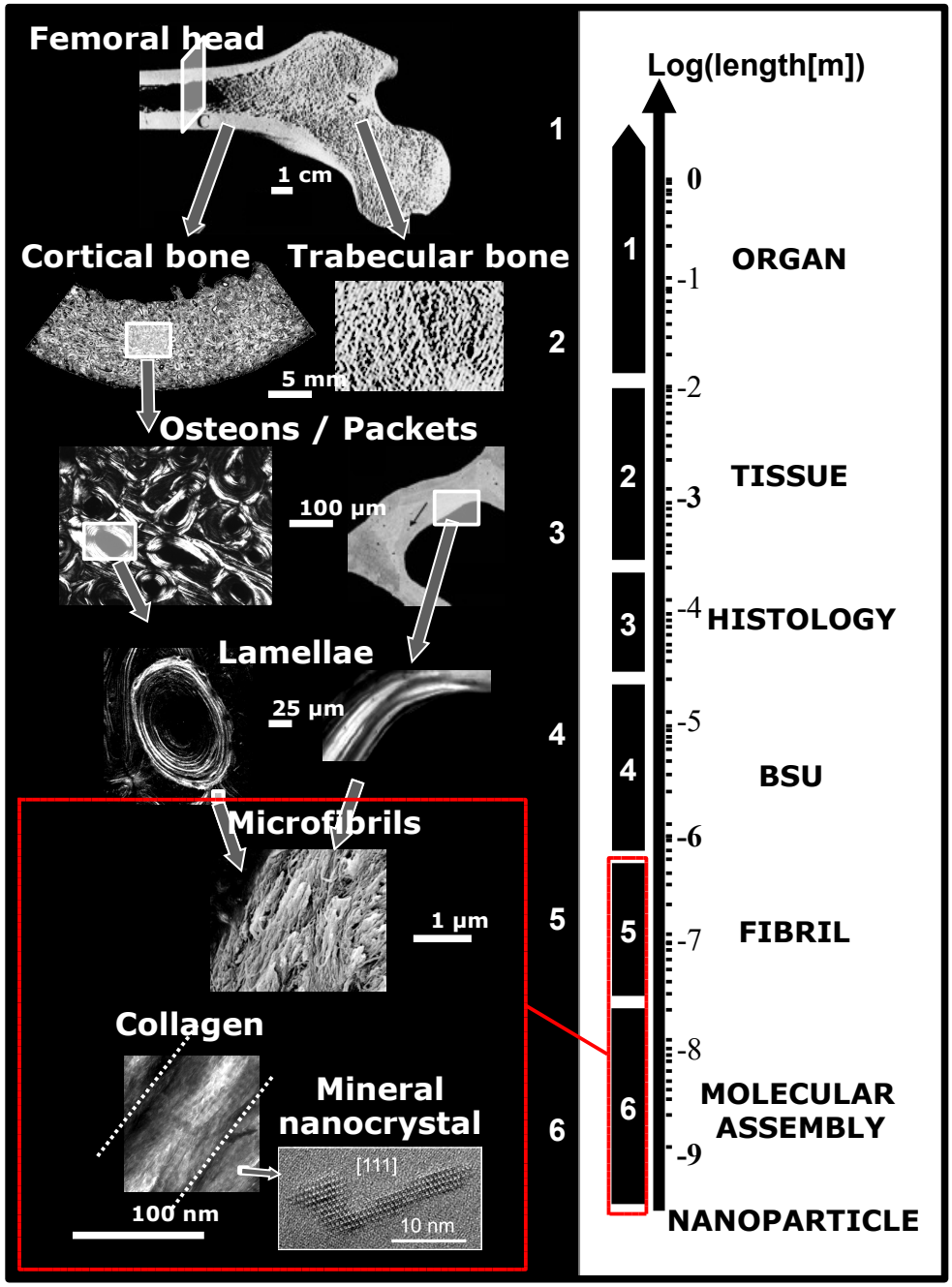
(HR)TEM:
mineral nanoparticles



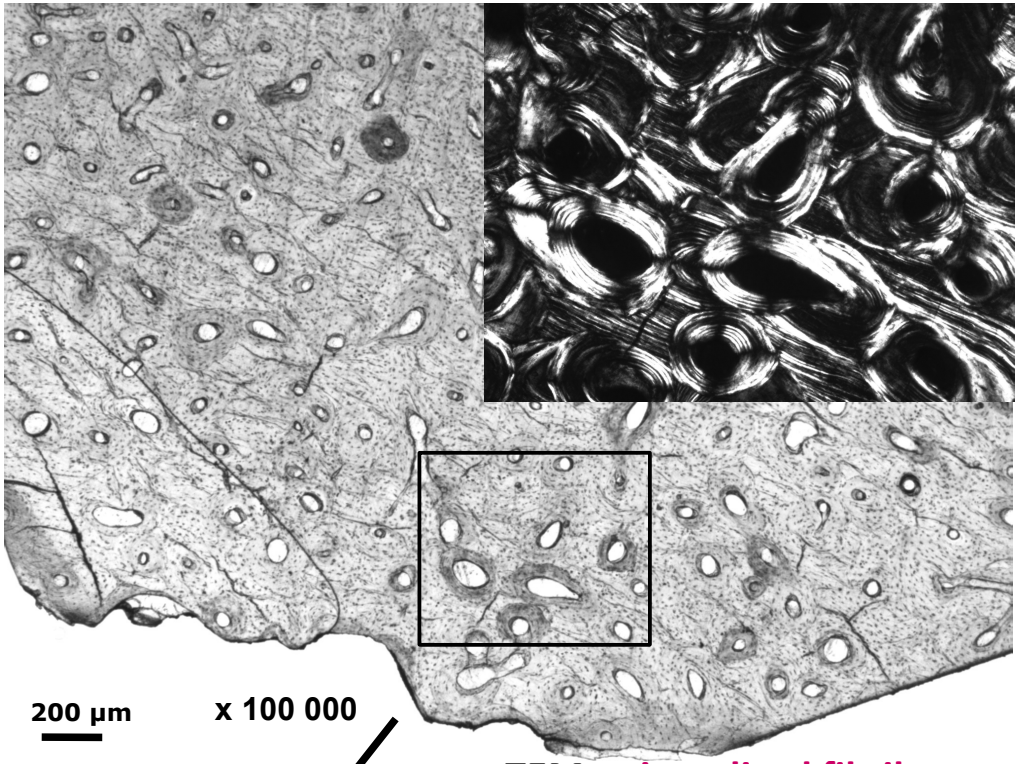
TEM: mineralized fibrils



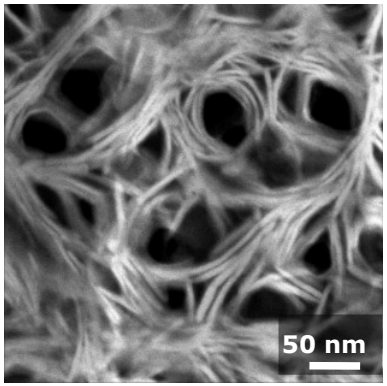
Courtesy K. Grandfield@McMaster



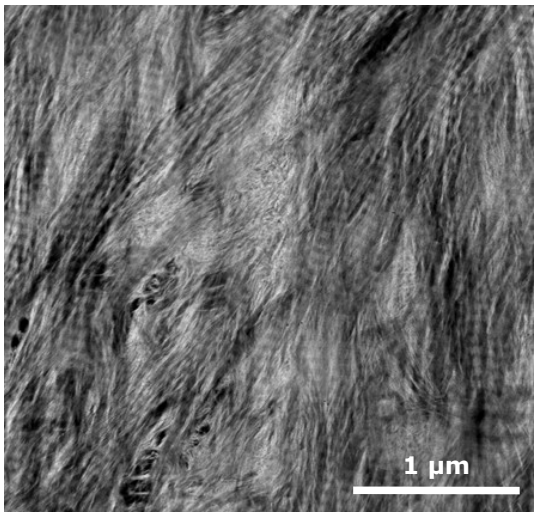
Probing the nanostructure of bone requires **nanoscale resolution** while achieving **4-5 orders of magnitude higher fields of view...**

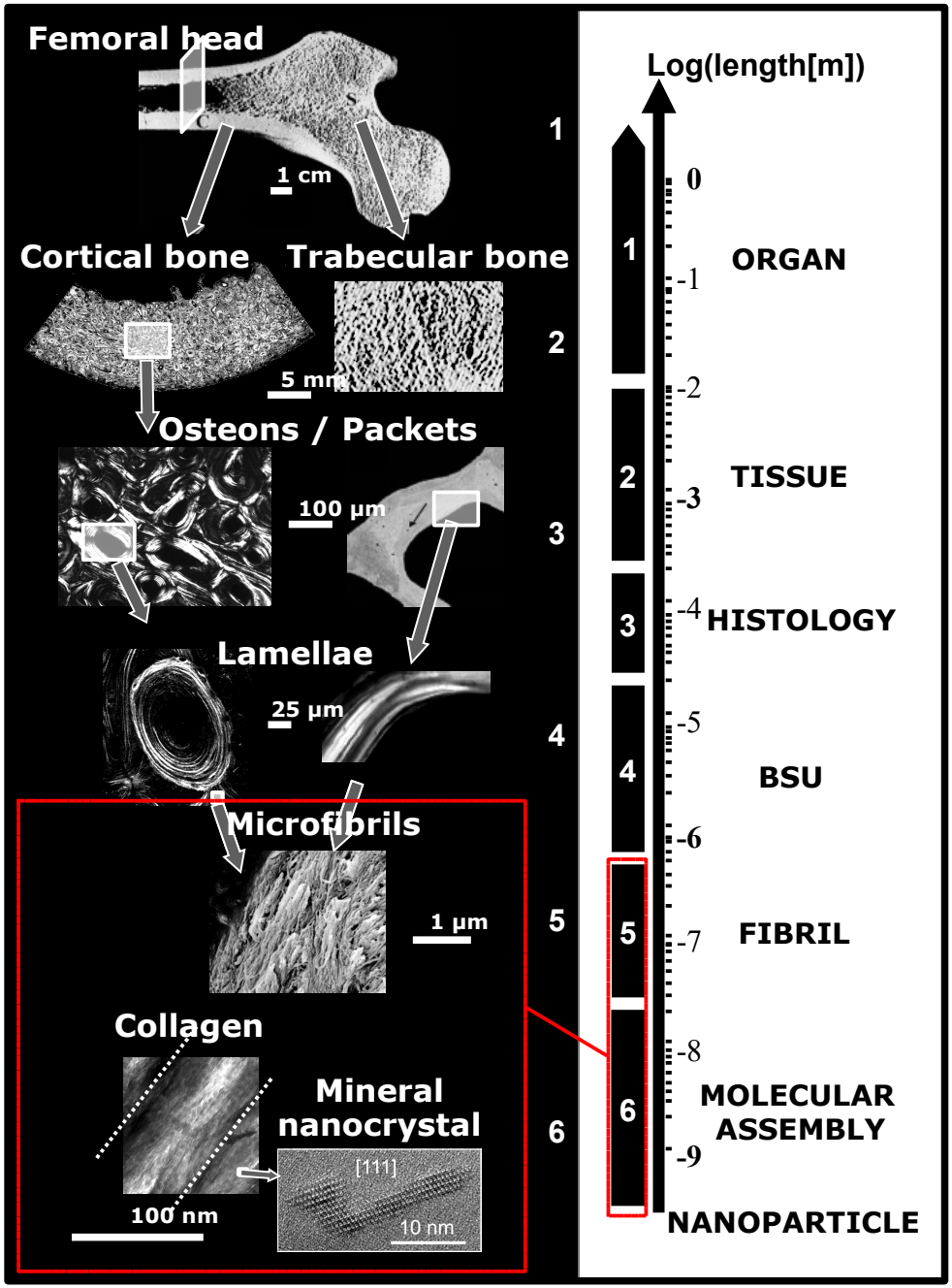


(HR)TEM:
mineral nanoparticles



TEM: mineralized fibrils

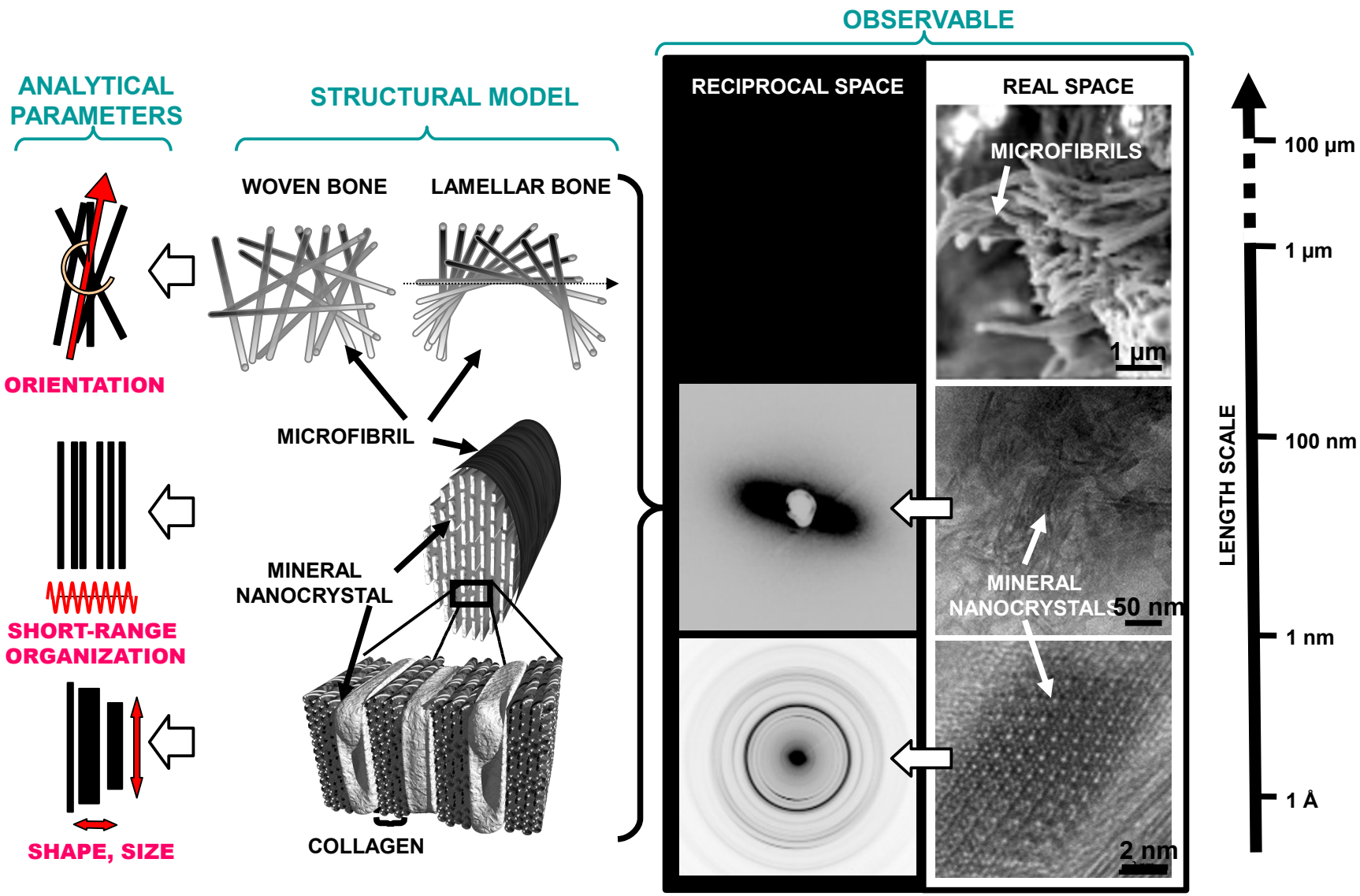




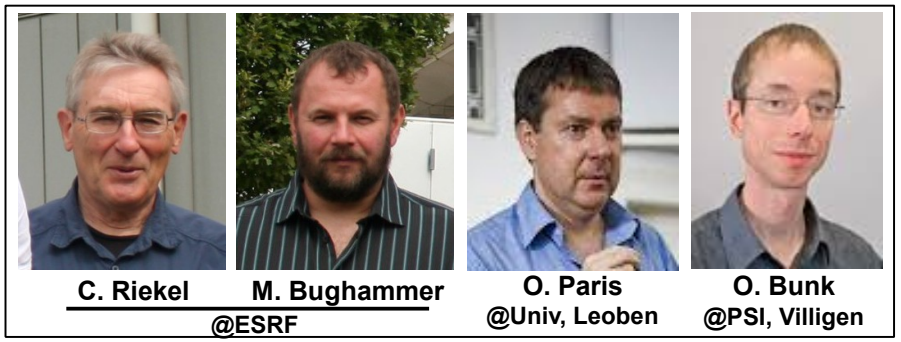
Courtesy K. Grandfield@McMaster

In bone, the diffraction peaks/rings result from the **mineral crystal structure**, while **the SAXS signal arises from the electron density difference between the mineral nanoparticles and the collagen matrix**.

⇒ SAXS analysis of bone provides **average** structural information on the mineral nanoparticles and their collective organization within the collagen matrix **in the volume probed by the beam**.



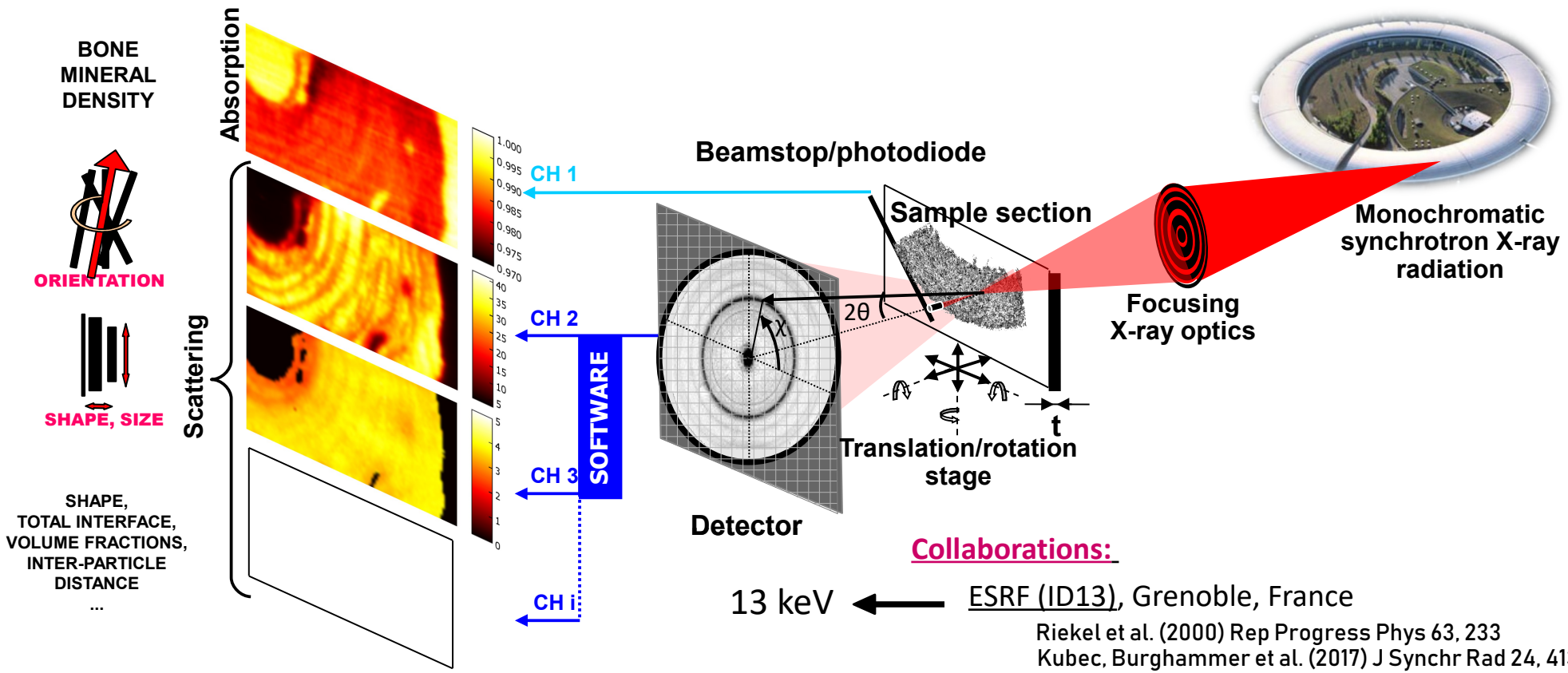
(*quantitative*) scanning-SAXS imaging (*q*sSAXSI) is the process by which maps of structural parameters are derived from 2D SAXS patterns acquired in scanning mode using X-ray micro/nanobeams.



Pixel size: focal spot of the X-ray beam.

Pixel intensity: scalar parameter derived from the 2D SAXS at each scan point.

Field of view: ***f*** (photon flux, detector sensitivity & speed, sample scattering power)



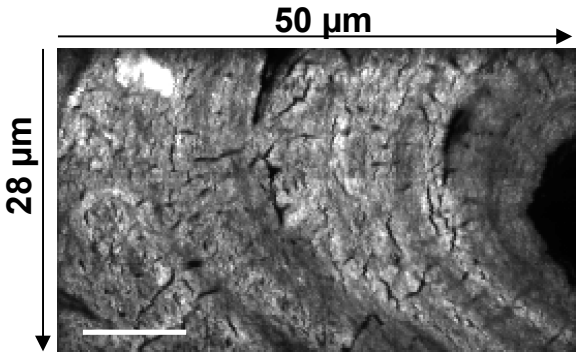
Collaborations:

13 keV ← ESRF (ID13), Grenoble, France
Riekel et al. (2000) Rep Progress Phys 63, 233
Kubec, Burghammer et al. (2017) J Synchr Rad 24, 413

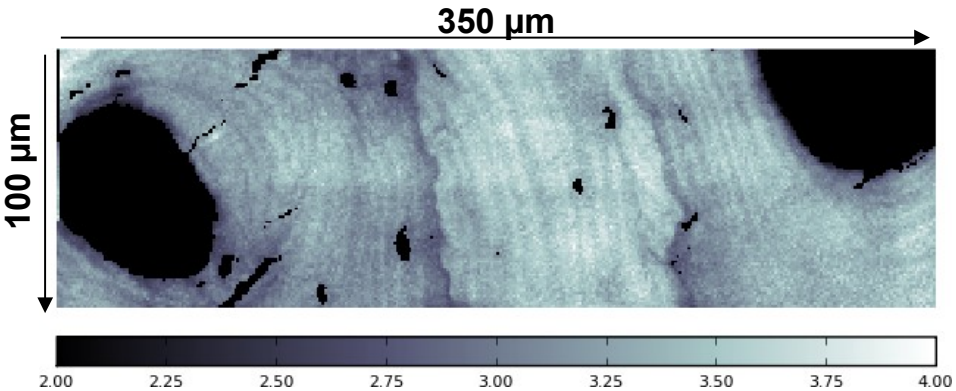
17 keV ← BESSY II (µSPOT), Berlin, Germany
Paris (2008) Biointerphases 3, FB16

18 keV ← SLS (PSI, cSAXS), Villigen, Switzerland
Bunk (2009) New J Phys 11, 123016

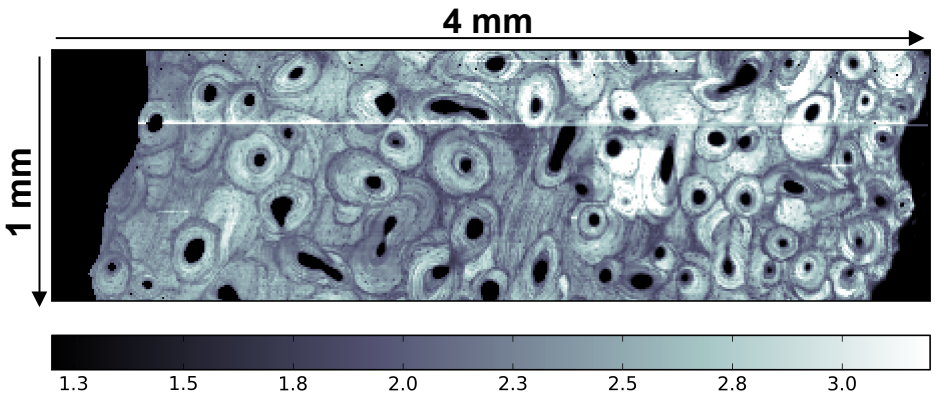
SAXS → nanoscale structural parameters
qsSAXSI → maps of nanoscale parameters
⇒ **provides a histological perspective of the nanoscale structure**



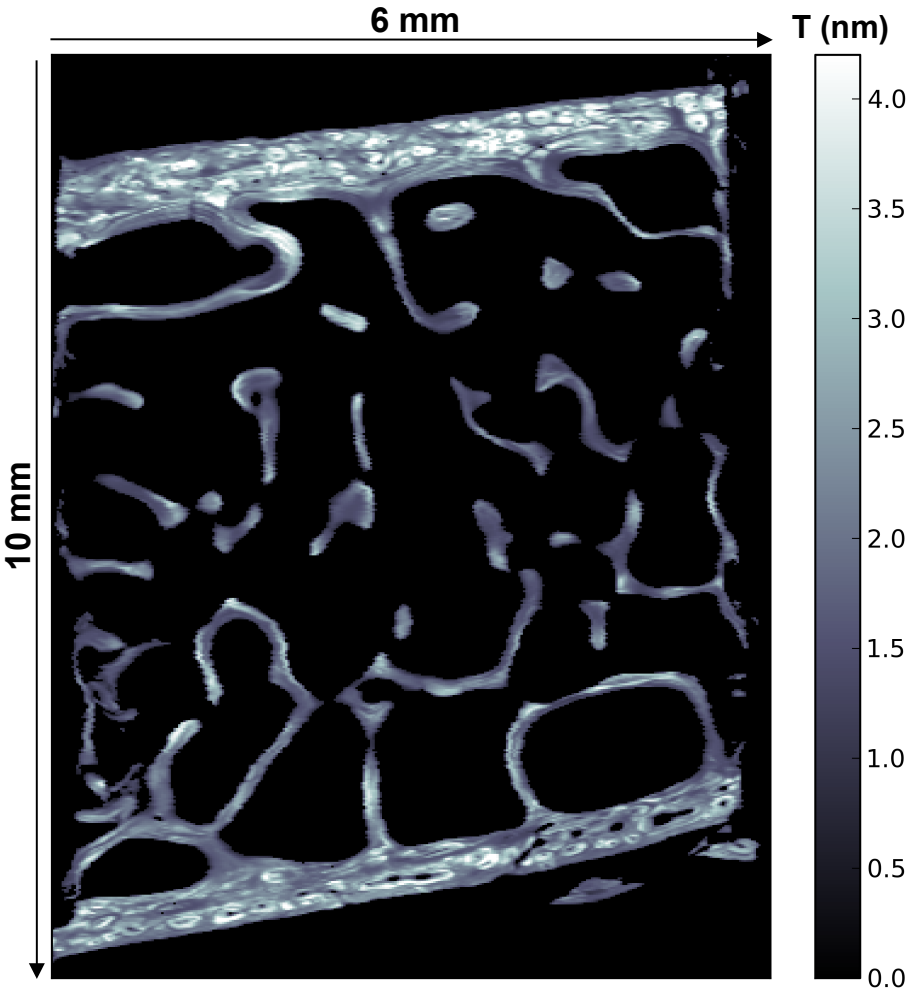
T (nm); $Ø_{BH,V}$ = 80 nm; $s_{H,V}$ = 100 nm



T (nm); $Ø_{BH,V}$ = 1 µm; $s_{H,V}$ = 1 µm



T (nm); $Ø_{BH,V}$ = 5 µm; $s_{H,V}$ = 8 µm



$Ø_{BH}$ = 20 µm; $Ø_{BV}$ = 12 µm; s_H = 40 µm; $s_{H,V}$ = 20 µm

W. Wagermaier, A. Gourrier, B. Aichmayer in *Bio-inspired Materials Design: Function through Inner Architecture*, RSC publishing, 2013.

PLOS | ONE

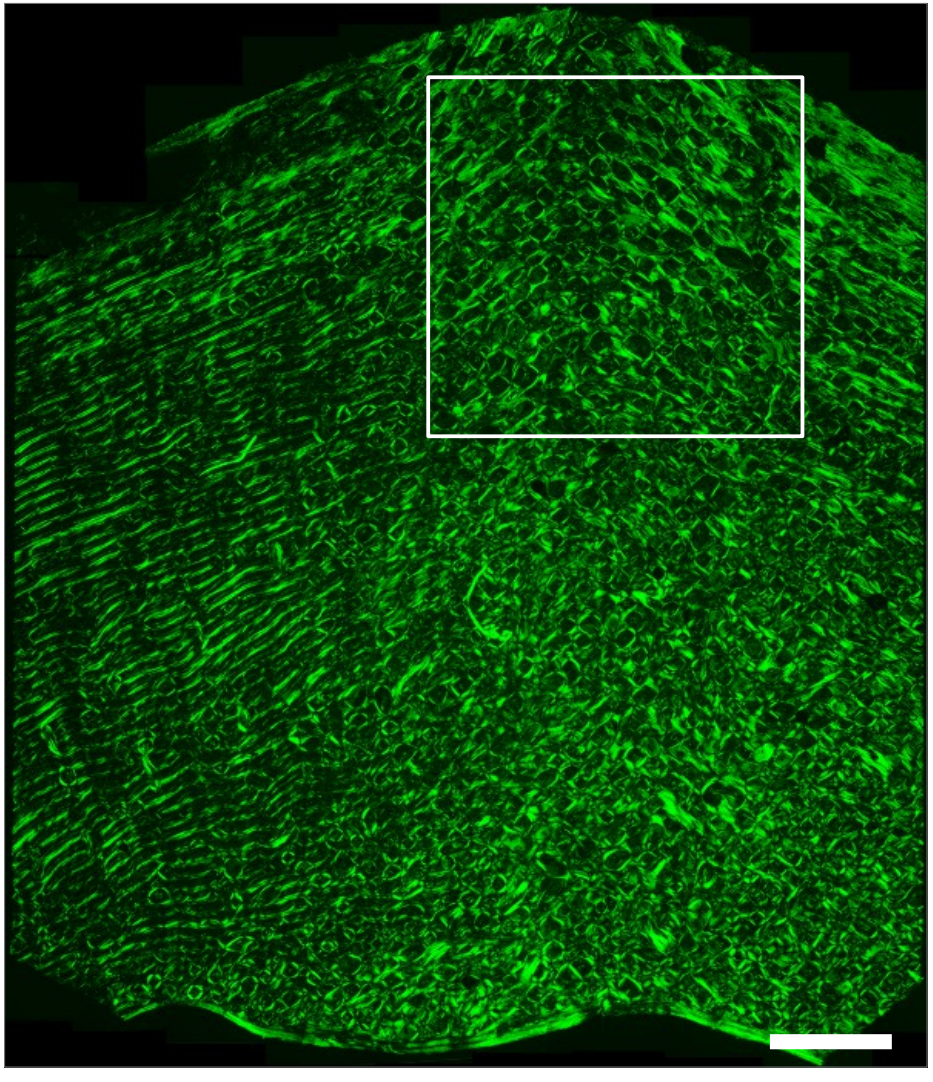
RESEARCH ARTICLE

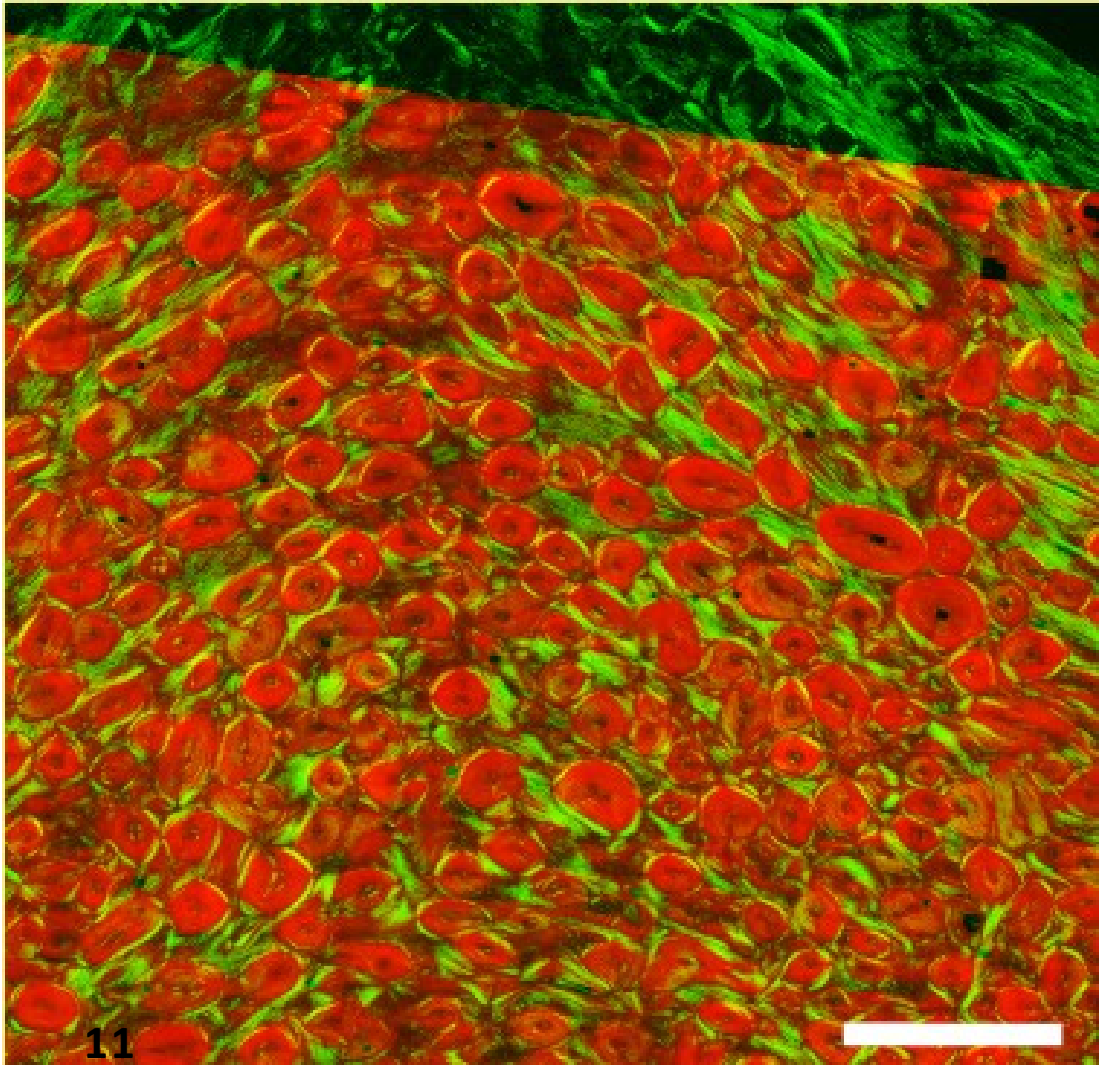
Nanoscale modifications in the early heating stages of bone are heterogeneous at the microstructural scale



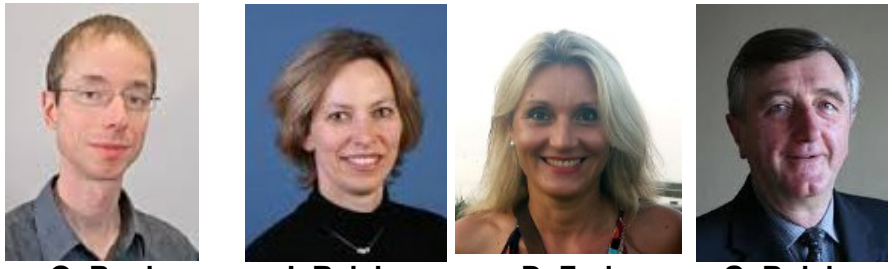

O. Bunk @PSI, Villigen | I. Reiche @IRCP, Paris | D. Farlay @INSERM, Lyon | G. Boivin

Aurélien Gourrier[1,2,3]*, Céline Chadefaux[4], Estelle Lemaitre[1,2], Ludovic Bellot-Gurlet[5], Michael Reynolds[3], Manfred Burghammer[3], Marie Plazanet[1,2], Georges Boivin[6], Delphine Farlay[6], Oliver Bunk[7], Ina Reiche[4,8]

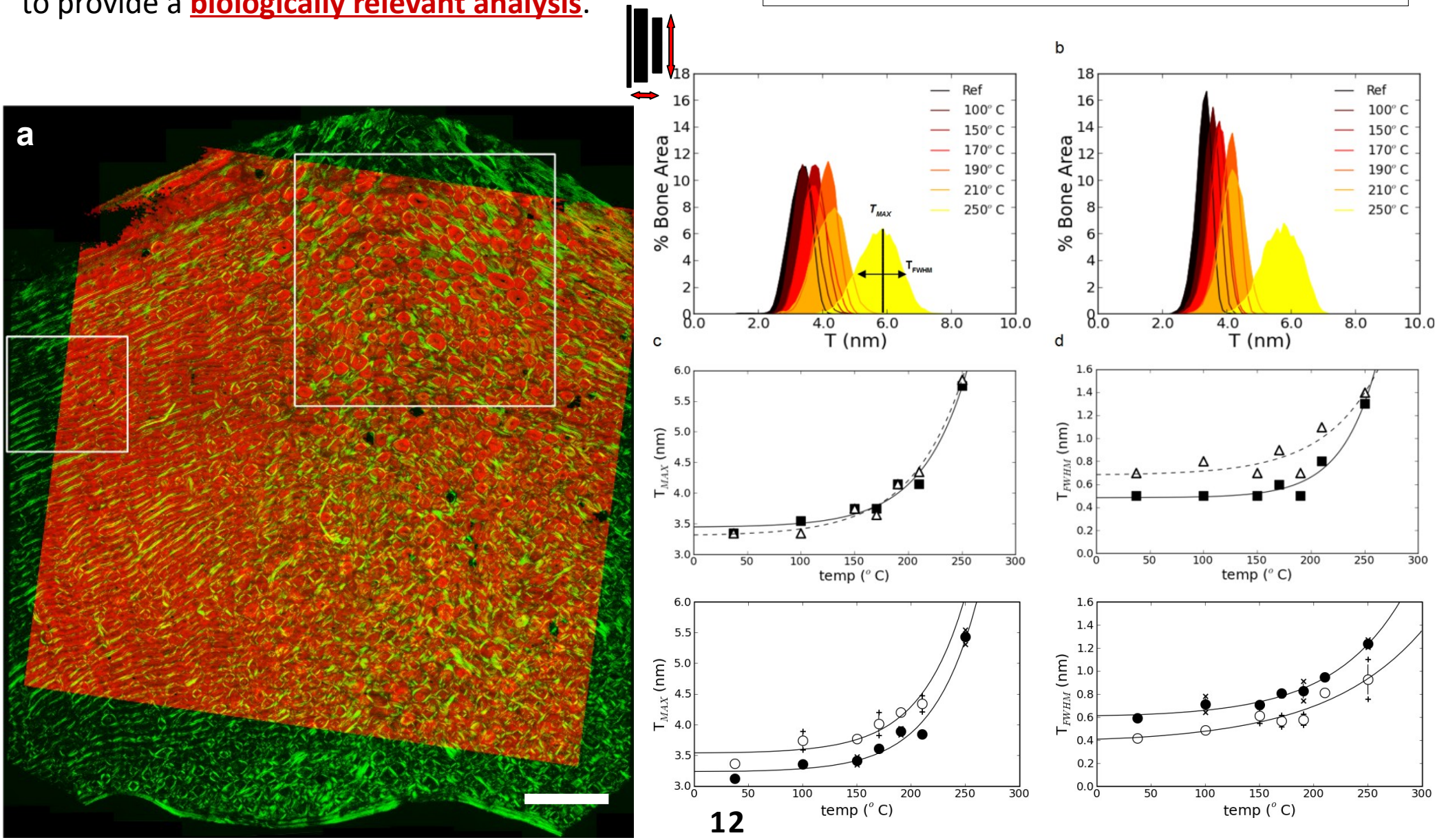




Analysis of the T distribution (~ nanoparticle thickness) shows that the **mineral nanoparticle characteristics** provide **valuable proxies of ultrastructural change** <u>but</u> it is mandatory to take into account the **sample histology** (microstructure) to provide a **<u>biologically relevant analysis</u>**.



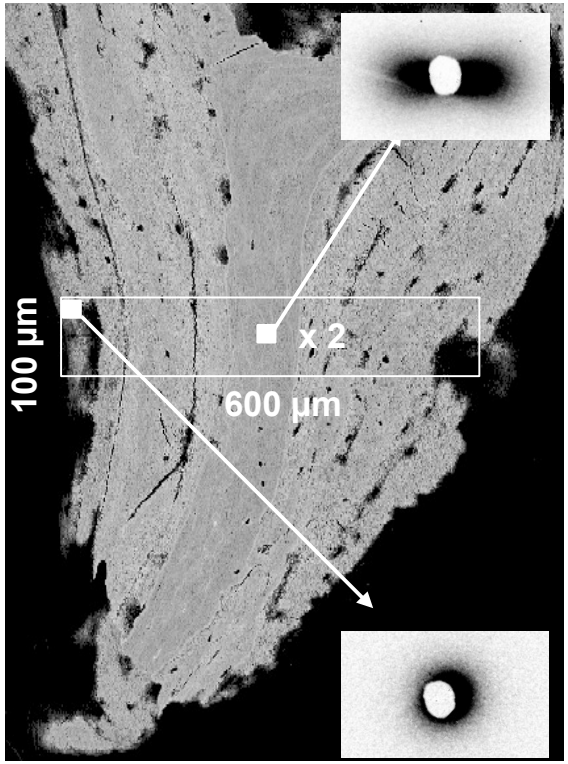
O. Bunk @PSI, Villigen | I. Reiche @LAMS, Paris | D. Farlay @INSERM, Lyon | G. Boivin

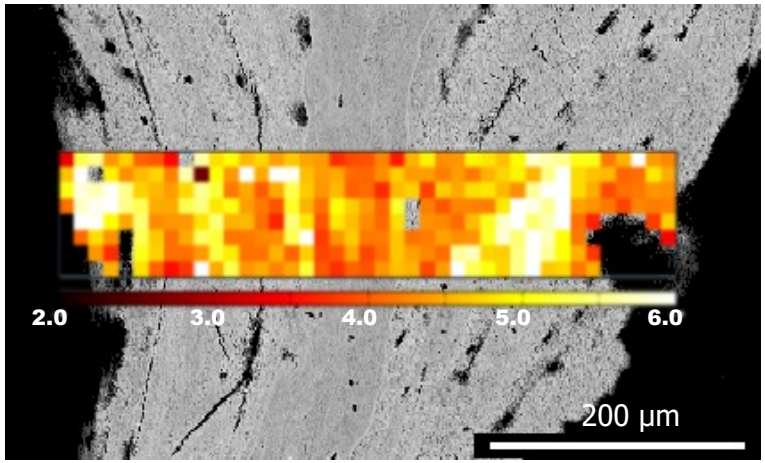


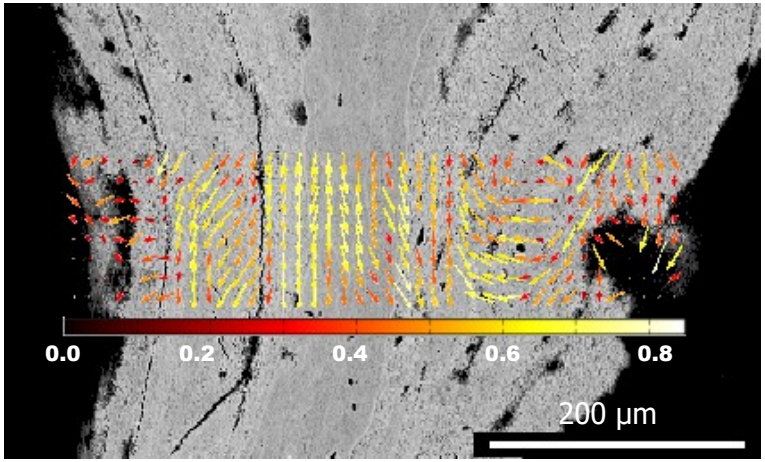
Transiliac biopsy of an 82 years old woman with **post-menopausal osteoporosis treated with NaF** ⇒ **iatrogenic fluoride overdose** (150 g NaF in 10 years) ⇒ **acute pain** and **increased fracture risk**.

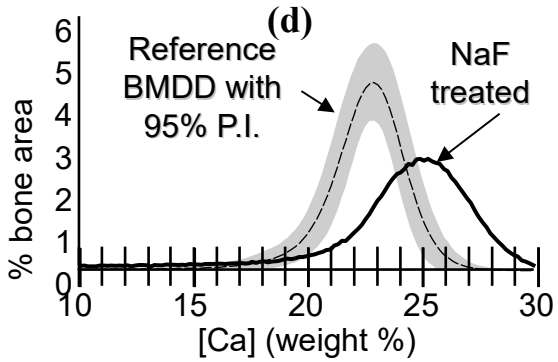
O. Paris @Univ, Leoben | P. Roschger @Osteologie, Vienna | P. Fratzl @MPIKG, Potsdam

⇒ **T increases by ~ 25 % (1 nm**, intra-individual); additionally, there is a **strong perturbation of the mineral nanocrystal organization.**

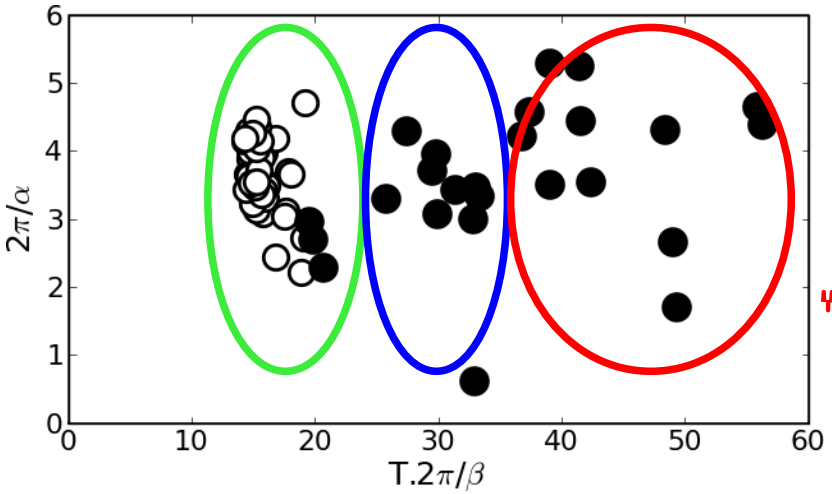








Fratzl, Roschger *et al.* (1996) J Bone Min Res 248, 11



Gourrier *et al.*,(2010) J Appl Crystallogr 43, 1385

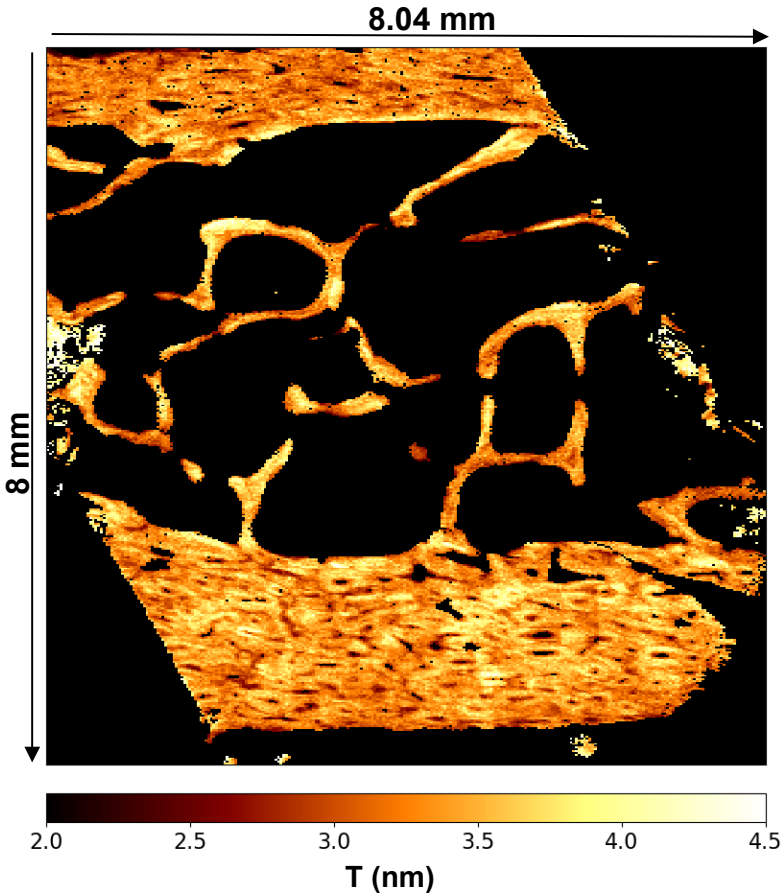
<u>Conclusion1:</u> the stack-of-cards model seems to pick up fluctuations much better than T-parameter fluctuations.

<u>Conclusion 2:</u> the fluorosis biopsy shows that one needs to take the spatial heterogeneity into account.

D. Farlay G. Boivin

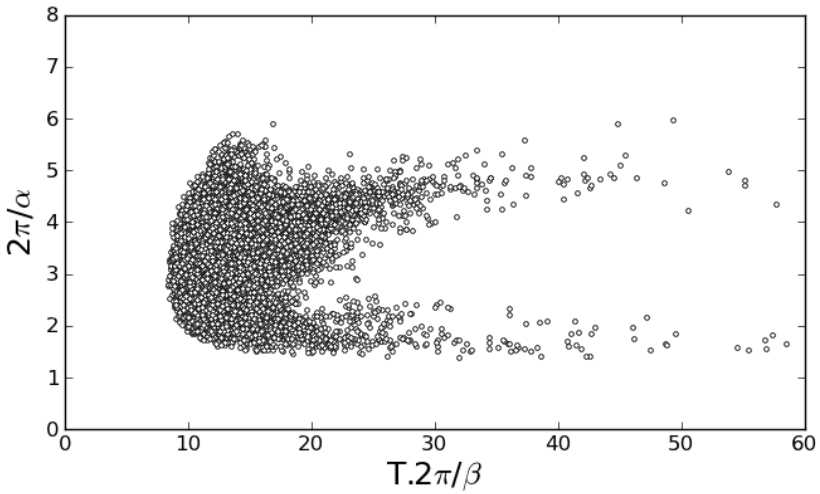
@INSERM Lyon

⇒ case study: Transiliac biopsy (M) **0.94 % ash weight of Fluoride** (normal 0.1 %)

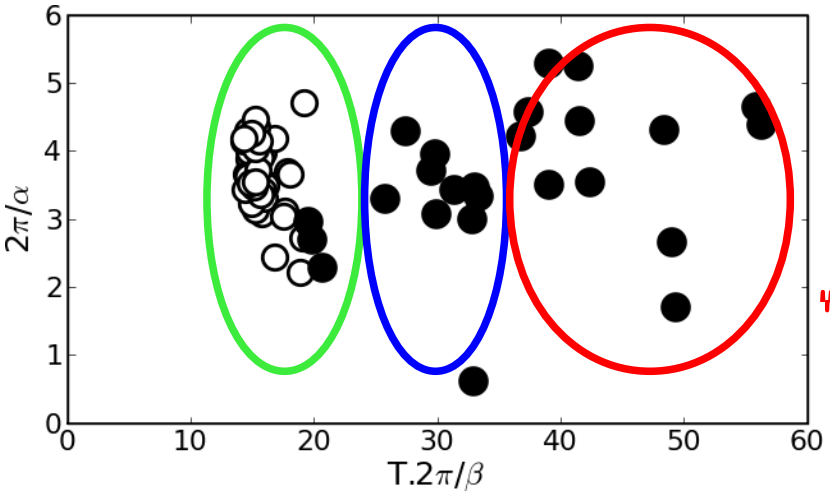


$Ø_{BH}$ = 25 µm; $Ø_{BV}$ = 6 µm; s_H = 30 µm; s_V = 20 µm

= **107 468** 2D SAXS frames

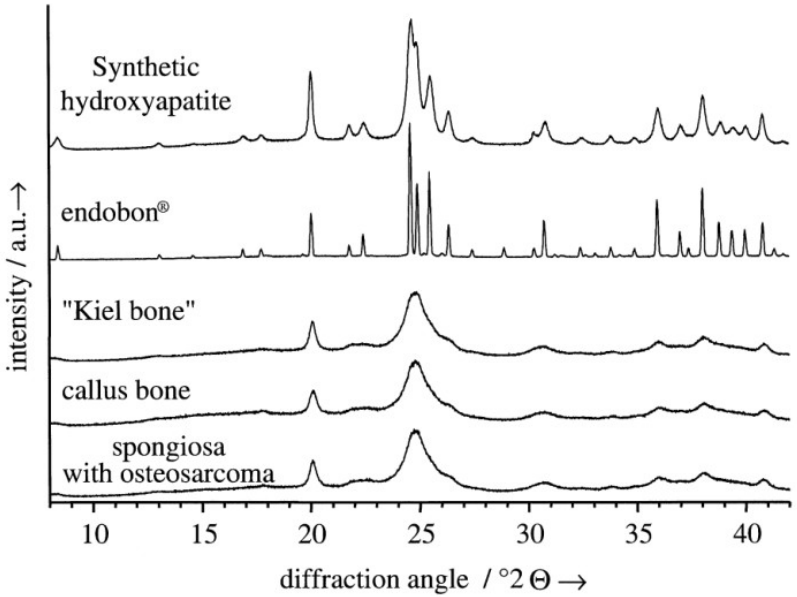


Gourrier, Farlay, Boivin *et al.*, *in preparation*

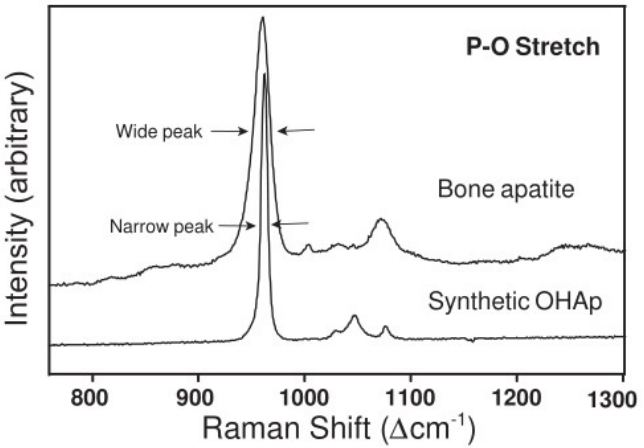


Gourrier *et al.*,(2010) J Appl Crystallogr 43, 1385

Bone mineral is “poorly crystalline”, i.e. contains a **high degree of crystalline disorder**.
→ gives rise to peak broadening & overlapping in spectroscopy and diffraction



Petters *et al.* (2000) Thermochim Acta 361, 131



Petters (2000) Thermochim Acta 361, 131
Wopenka & Pasteris (2005) Mater Sci Eng C 25, 131

DeJong (1926) Rec Trav Chim Pays Bas, 45, 445
Posner (1969) Physiol Rev 49, 760
Boskey (1975) J Dent Res 76, 1433
Rey *et al.* (2009) Osteoporos Int 20, 1013

Résumé.

Il existe un minéral de la série des apatites dans les os, probablement $3\,Ca_3(PO_4)_2 . CaCO_3$ [$3\,Ca_3(PO_4)_2 . CaO$, hypothétique, serait possible aussi]. Le radical CO_3 peut être remplacé — comme dans la fossilisation — par F_2, Cl_2 ou SO_4 et peut-être par d'autres radicaux encore, le Ca par Mg et peut-être par Fe et Al.

Le minéral colloïdal collophanite, cité par Dana (A System of Mineralogy, 6 Ed. p. 108): $Ca_3P_2O_8 . H_2O$, ne peut pas être décelé dans les os.

Les cristallites ne contiennent que quelques dizaines ou centaines de molécules: le roentgenogramme montre des lignes diffuses; en incinérant les os on fait accroître les cristaux et les lignes deviennent nettes. Nous nous attendons à ce que cela soit d'importance pour les préhistoriens.

Note: **Laue & Bragg(s): 1912 !!!**
Debye: 1913

Materials Science and Engineering C 25 (2005) 131–143

A mineralogical perspective on the apatite in bone

Brigitte Wopenka*, Jill D. Pasteris

Department of Earth and Planetary Sciences, One Brookings Drive, Campus Box 1169, Washington University, St. Louis, MO 63130, USA

American Mineralogist, Volume 99, pages 16–27, 2014

MINERALS IN THE HUMAN BODY

Molecular water in nominally unhydrated carbonated hydroxylapatite: The key to a better understanding of bone mineral†

JILL DILL PASTERIS[1,*], CLAUDE H. YODER[2] AND BRIGITTE WOPENKA[1]

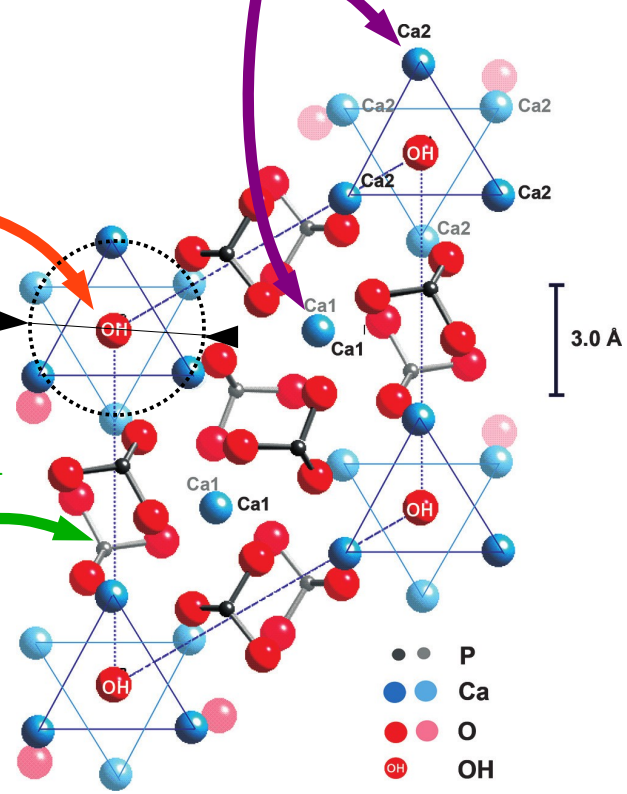
[1]Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, Missouri 63130-4899, U.S.A.
[2]Department of Chemistry, Franklin and Marshall College, Lancaster, Pennsylvania 17603, U.S.A.

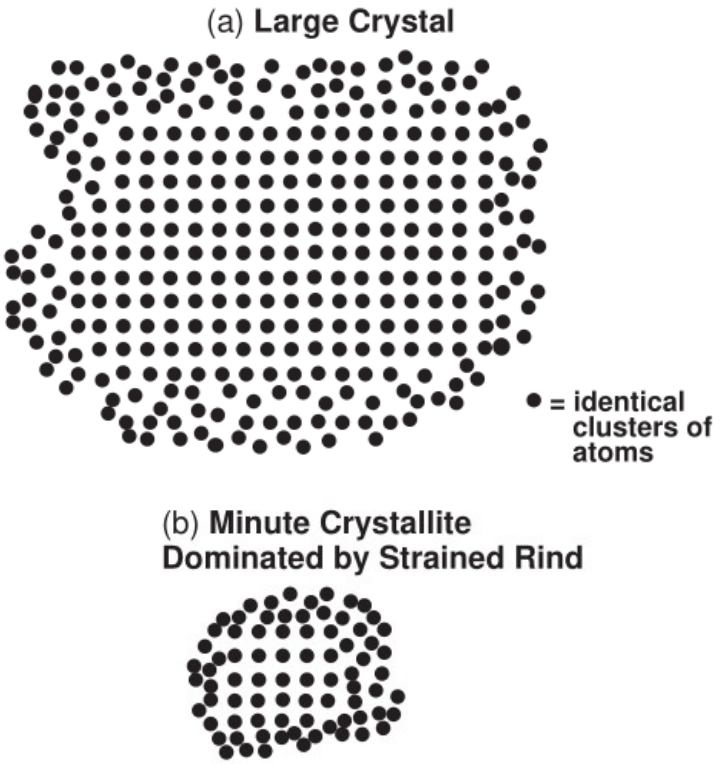
Apatite minerals have a **very flexible structure** that can accommodate at least half the perodic table of elements.

K^+, Na^+, Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Cd^{2+}...

OH^-, Cl^-, F^-

CO_3^{2-}, SO_4^{2-}, AsO_4^{3-}, SiO_4^{4-}





See also C. Rey, C. Combes et al. @CIRIMAT, Toulouse

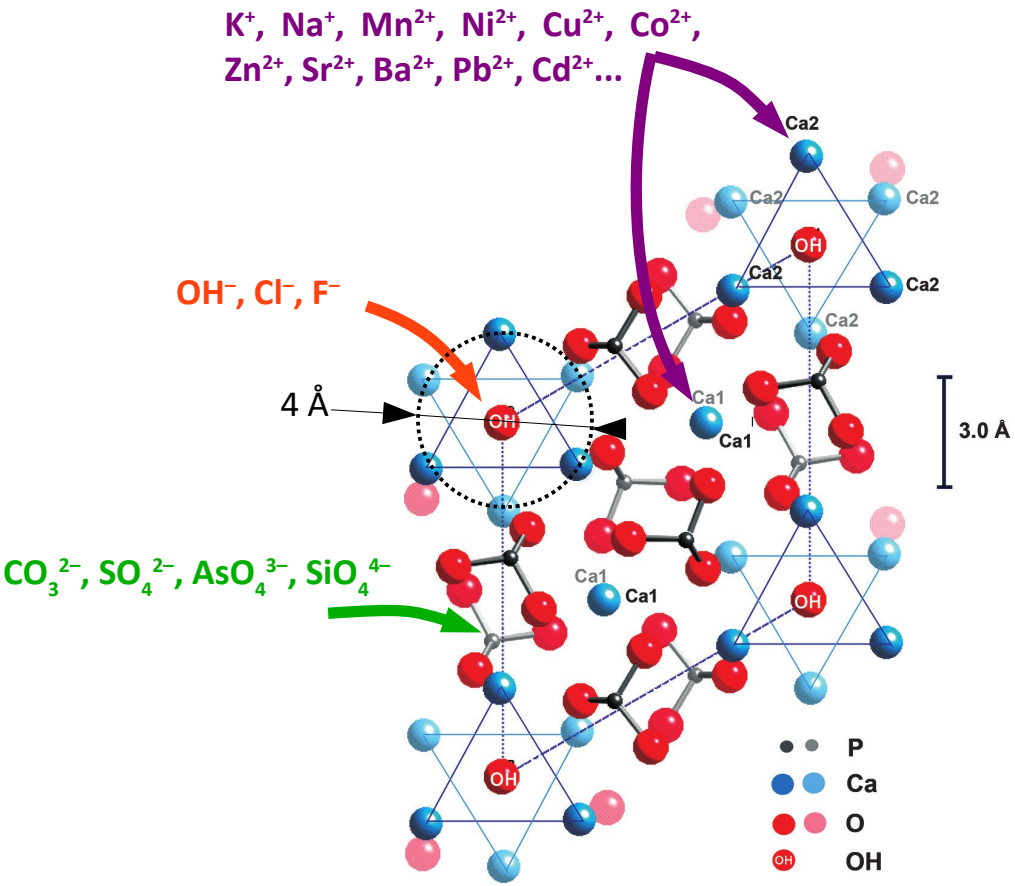
Model structure: $Ca_{10}(PO_4)_6(OH)_2$

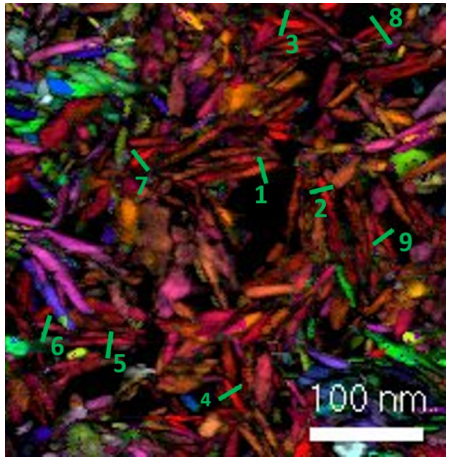
Bone apatite (?): $Ca_{10-x}[(PO_4)_{6-x}(CO_3)_x](OH)_{2-x} \cdot nH_2O$

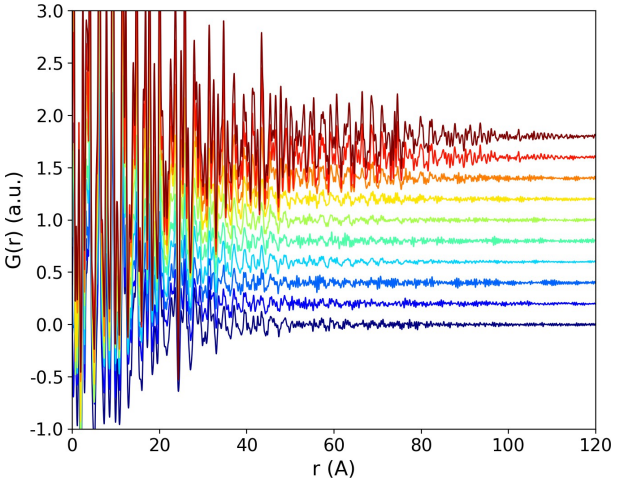
Project: beyond SAXS: from mineral nanoparticles to mineral nanocrystals: what drives the nanoscale structure of bone mineral ?

OPTION 1: quasi-direct space analysis: ACOM-TEM

OPTION 2: reciprocal space analysis: PDF analysis



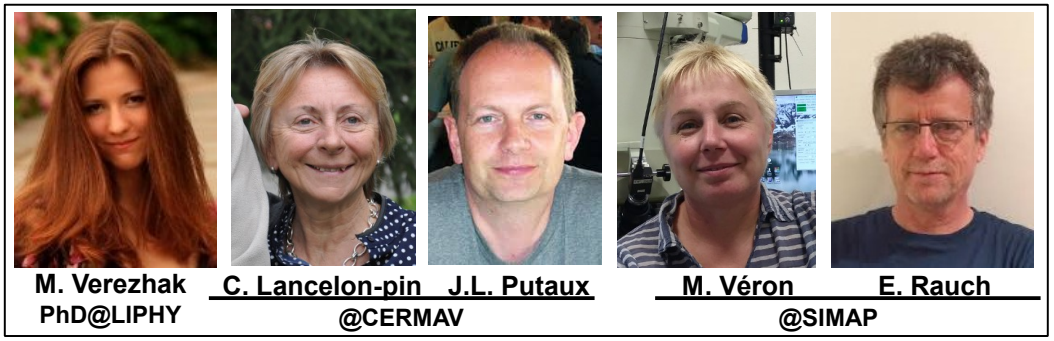




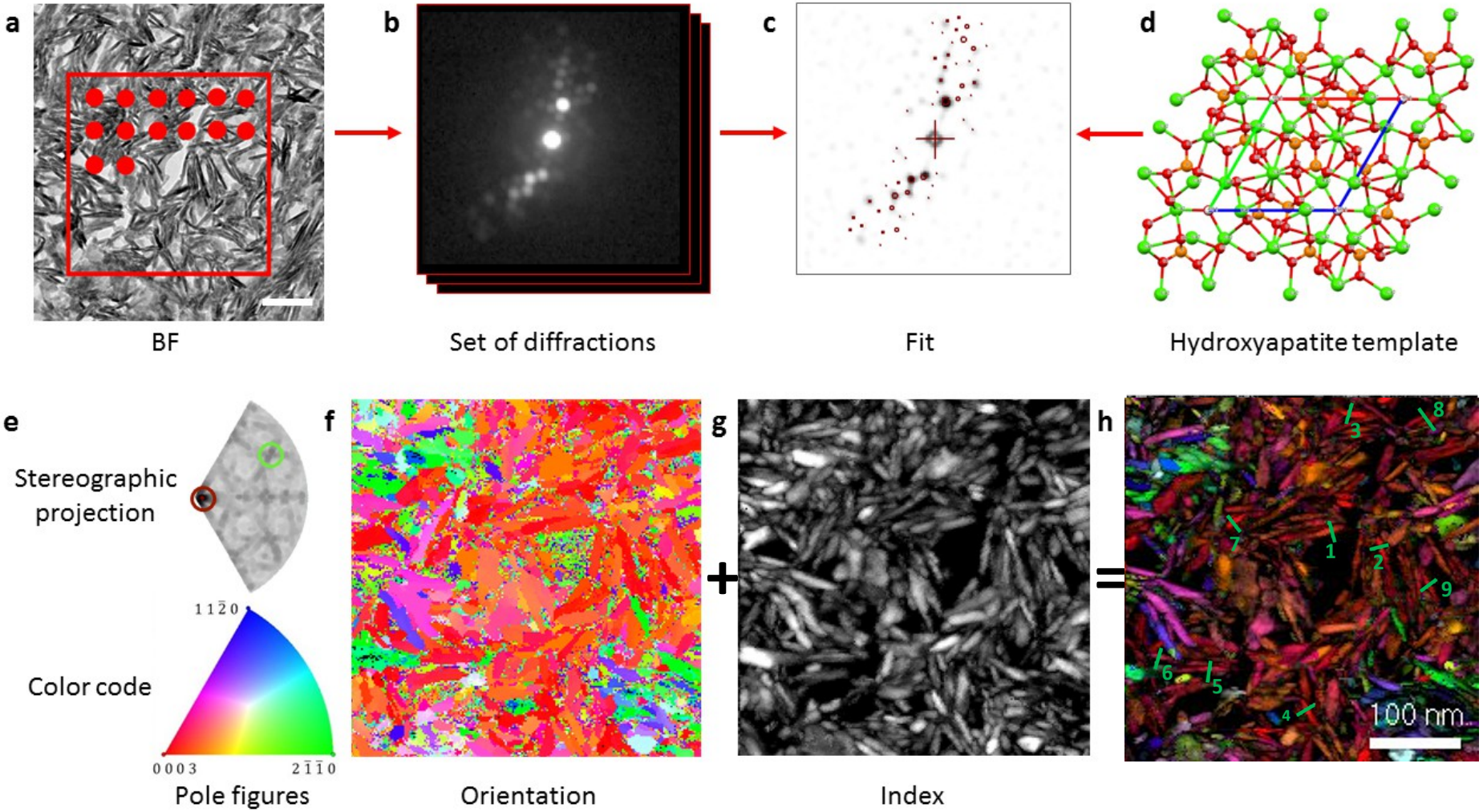
Model structure: $Ca_{10}(PO_4)_6(OH)_2$

Bone apatite (?): $Ca_{10-x}[(PO_4)_{6-x}(CO_3)_x](OH)_{2-x} \cdot nH_2O$

In **automated crystal orientation mapping (ACOM) TEM**, an ultrathin sample is scanned with an electron nanobeam (> **2 nm**) to record 2D electron diffraction patterns at each scan position (similar to scanning-XRD).



⇒ **maps of crystal orientations** are derived by correlating the measured diffraction patterns with simulated ones from an ideal crystal structure in all orientations.

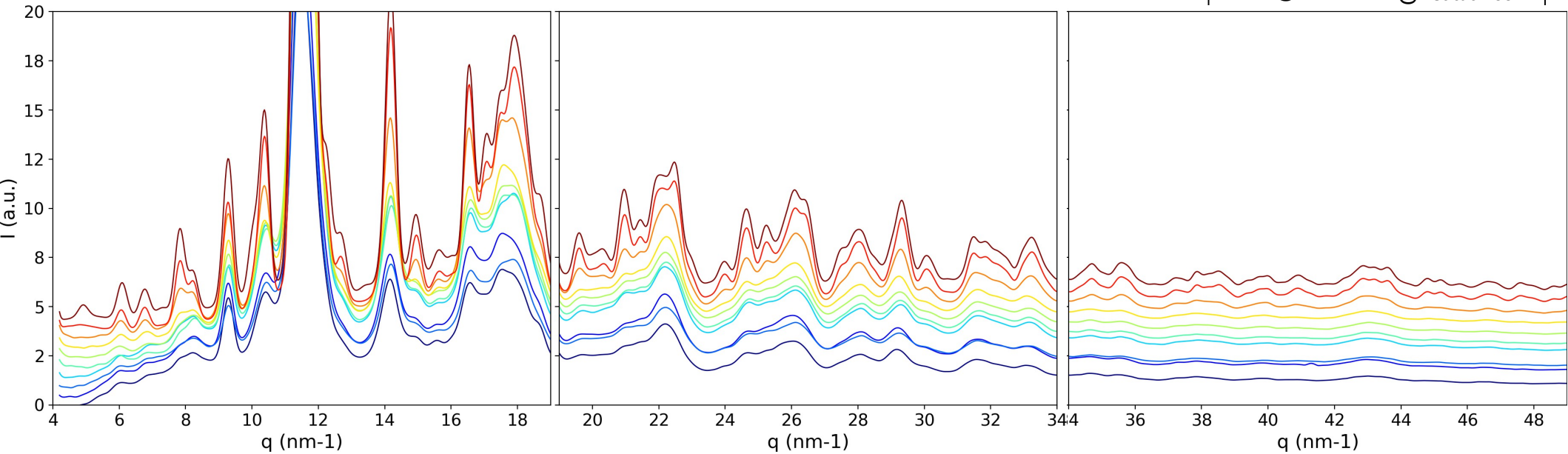


Verezhak *et al.* (2018) Acta Biomater 73, 500

Although widely used, “traditional” X-ray diffraction analysis of bone fails in many cases due to the crystalline disorder. **Pair distribution function (PDF)** analysis seems to be an ideal tool to analyse this disorder.

M. Verezhak
PhD@LIPHY

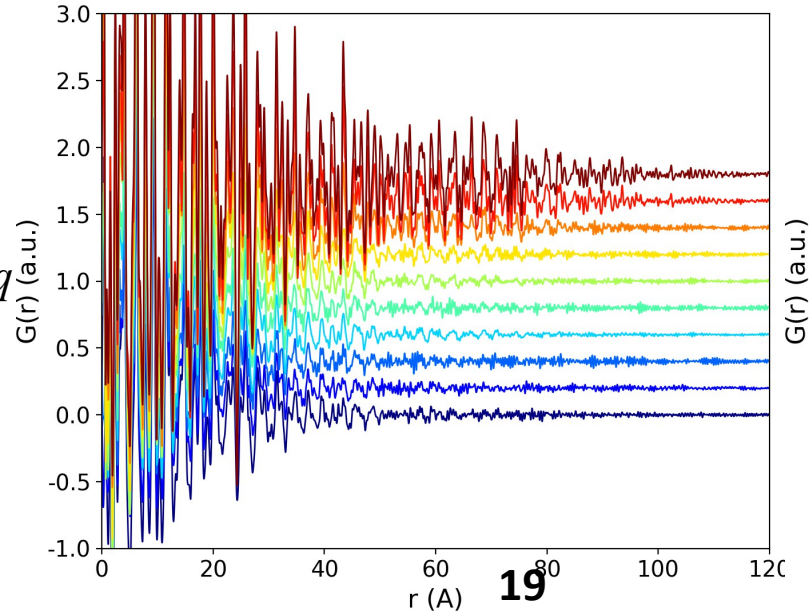
P. Bordet
@Institut Néel

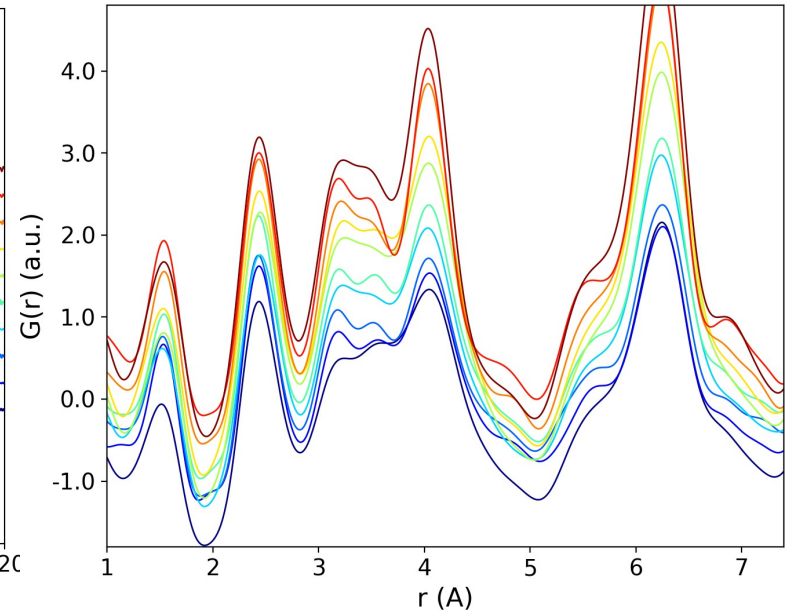


$$G(r) = 4\pi r \rho_0 \left(g(r) - 1\right)$$

$$= \frac{2}{\pi} \int_0^\infty q\left(S(q) - 1\right) \sin(qr)\, dq$$

$$S(q) = \frac{I(q)}{\langle b \rangle^2}$$

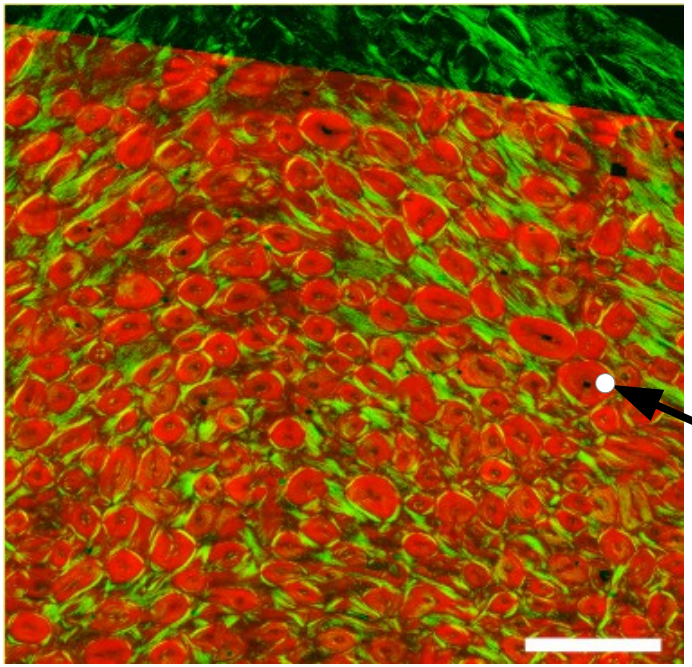




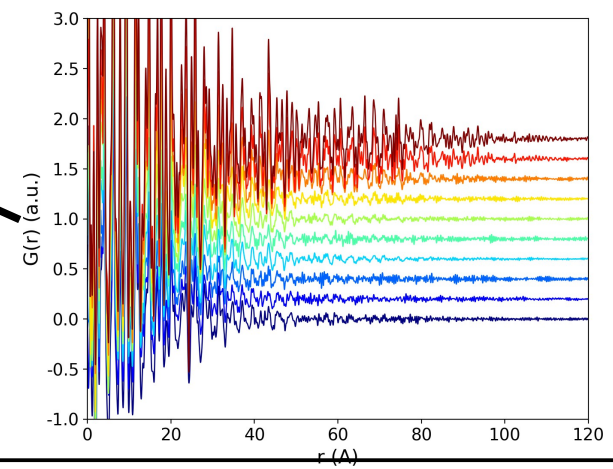
CONCLUSION: Pair distribution function (PDF) @ ThomX <u>for fundamental biomedical analysis of bone ultrastructure ?</u>

⇒ minimum requirements:

- **<u>high energy monochromatic beam</u>**: tested 78.25(78) keV @ ID11 (ESRF)
- high flux (> 10^{10} photons.s^{-1}) [or months of beamtime...]
- **<u>small beam size</u>** (< 20 µm) with low divergence (< 1 mrad)
- **<u>scanning stage + synchronization</u>** (e.g. shutter)
- efficient detector, e.g. Pilatus/Eiger = high sensitivity *+ high collection frequency*



⇒ additional wishlist:
- chemical sensitivity (e.g. fluorescence, EXAFS)
- long-time instrument design

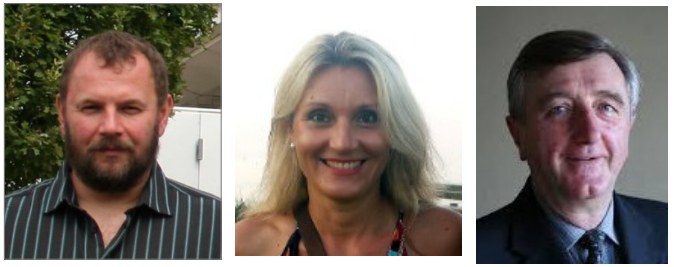


ANR MULTIPS
2013-2018

This work is supported by:

PROJET FINANCÉ PAR L'ANR - PROJECT FUNDED BY THE ANR

(*quantitative*) scanning-SAXS imaging (*q*sSAXSI) is the process by which maps of structural parameters are derived from 2D SAXS patterns acquired in scanning mode using X-ray micro/nanobeams.



M. Bughammer @ESRF

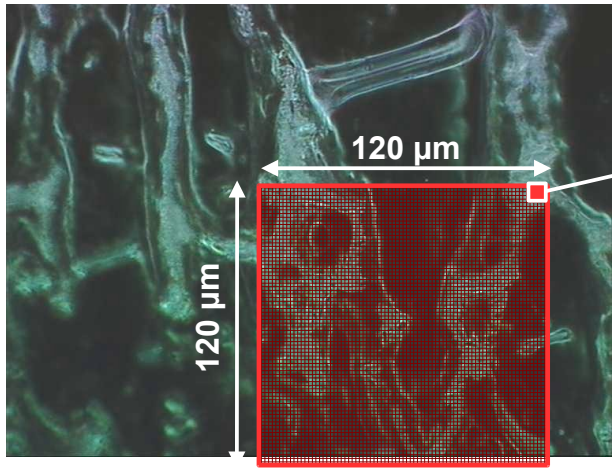
D. Farlay, G. Boivin @INSERM, Lyon

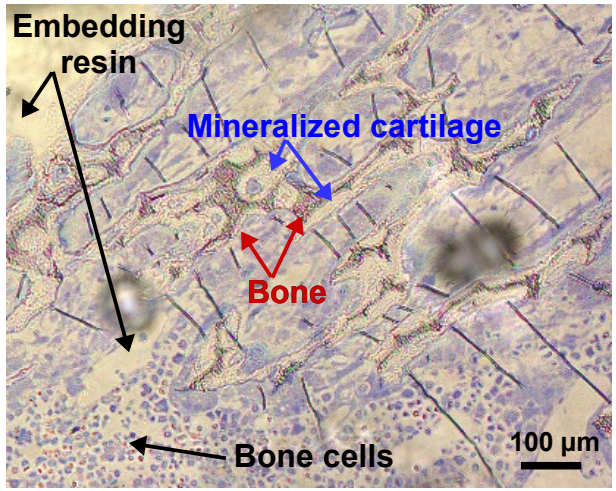
4D (space) problem:

→ 2D real space scan coordinates

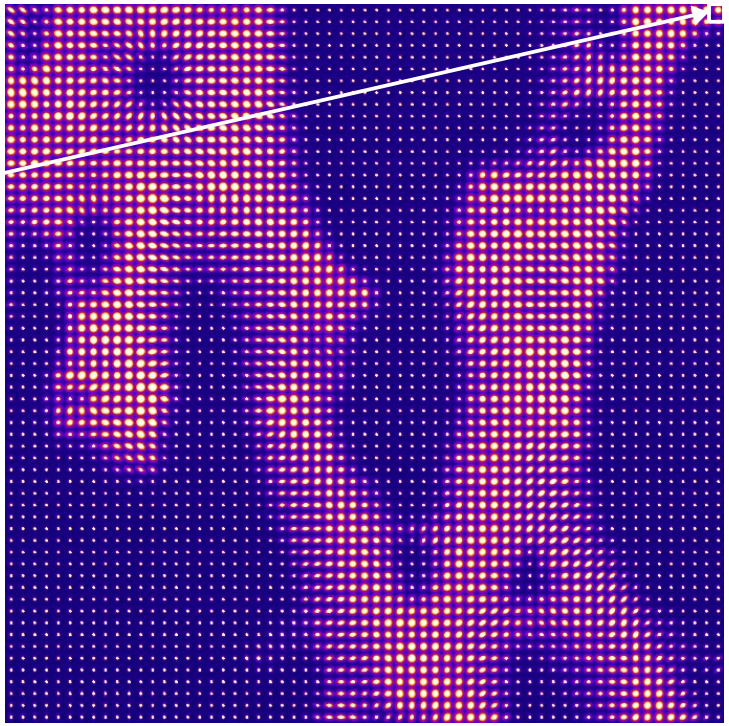
→ 2D reciprocal (diffraction) space coordinates

Online microscope

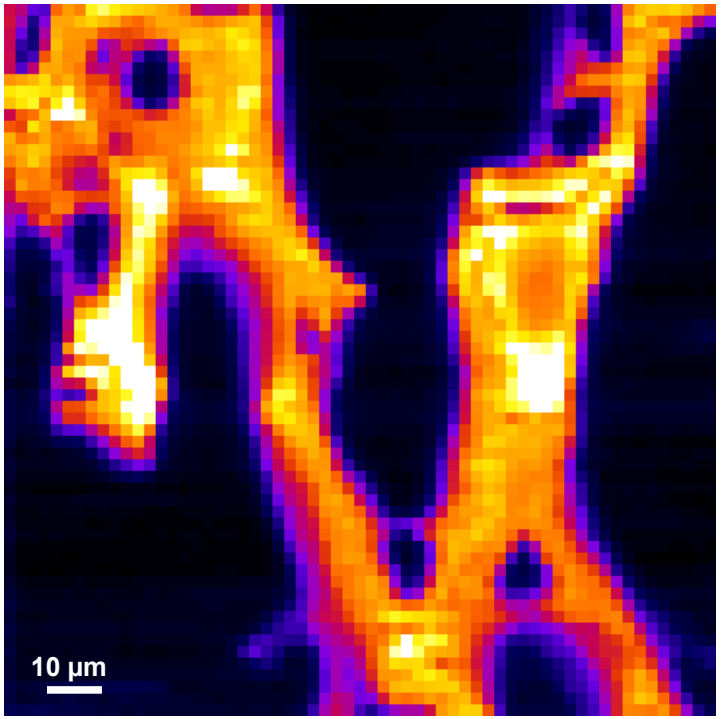


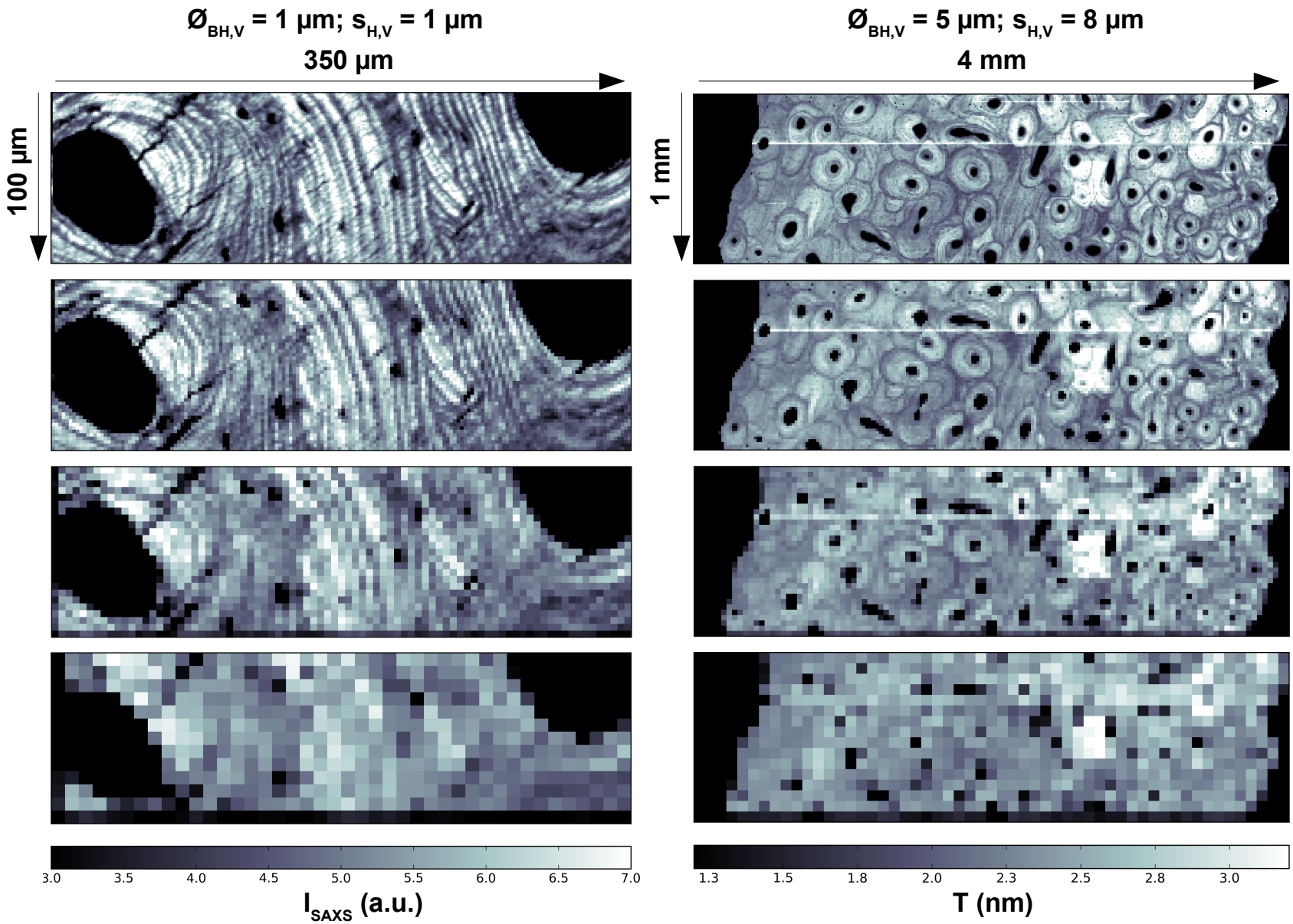


Mosaic image of 2D SAXS patterns



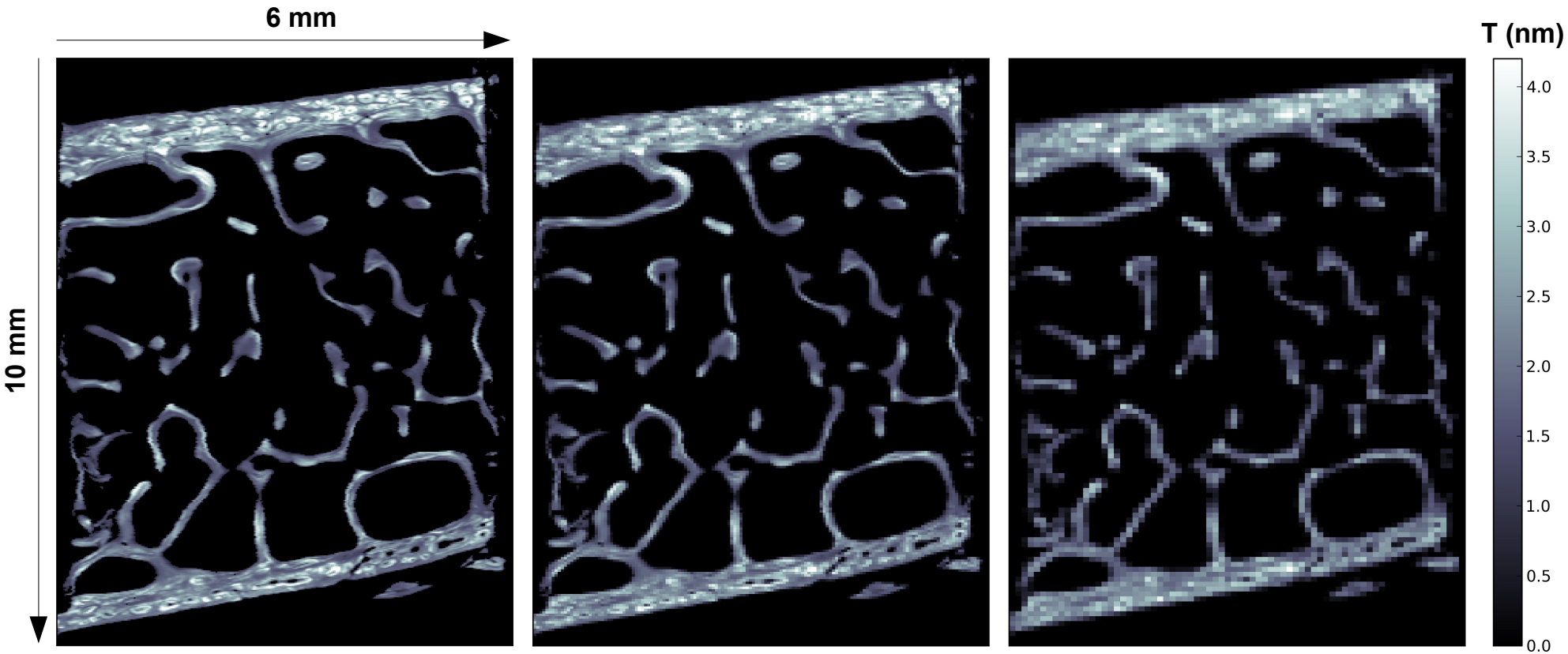
Reconstructed SAXS image





W. Wagermaier, A. Gourrier, B. Aichmayer
in *Bio-inspired Materials Design: Function through Inner Architecture*.
P. Fratzl, J.W.C. Dunlop, R. Weinkamer eds, RSC publishing *in press*.

from reciprocal to real space imaging...



$Ø_{BH}$ = 20 µm; $Ø_{BV}$ = 12 µm; s_H = 40 µm; $s_{H,V}$ = 20 µm

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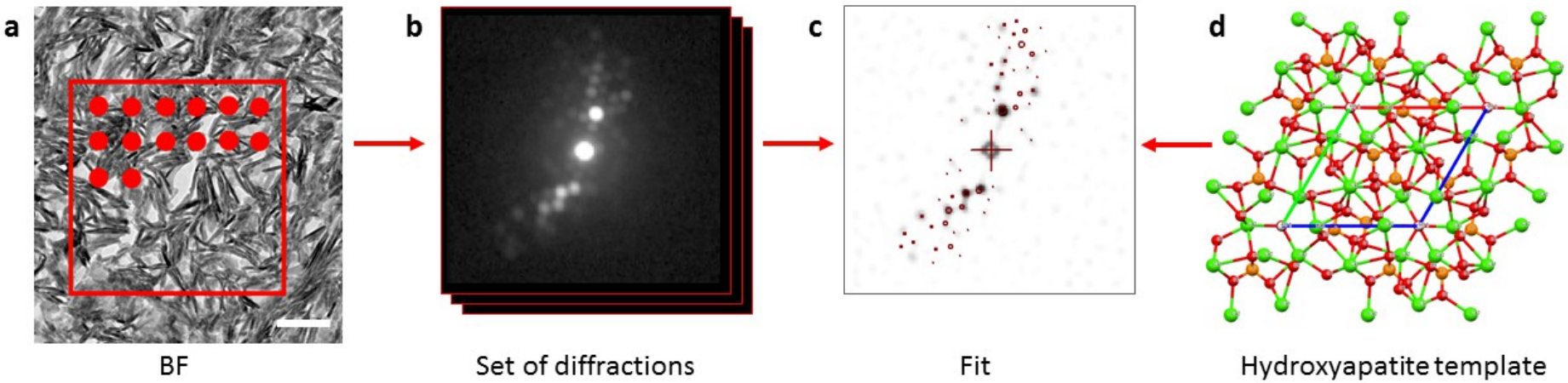
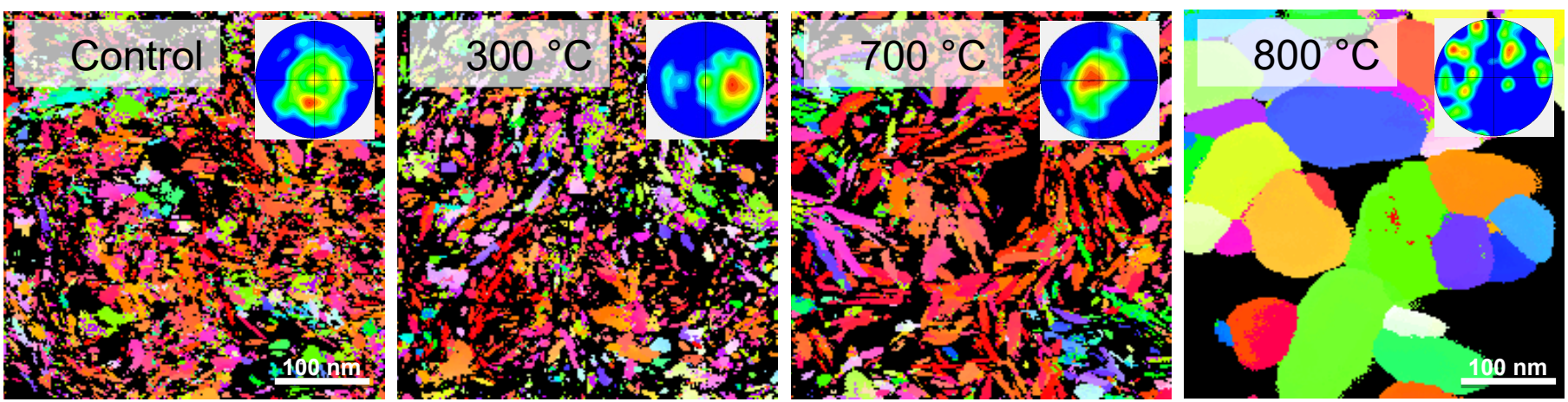
Due to the unusually small nanocrystal size in bone, a **heated cortical bovine bone model** with well controled size distributions was studied as a **resolution object**.

⇒ **1st proof of concept of the potential of ACOM-TEM for structural analysis at the single nanocrystal level**

but... main limitation = sample preparation...





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Molecular water in nominally unhydrated carbonated hydroxylapatite: The key to a better understanding of bone mineral†

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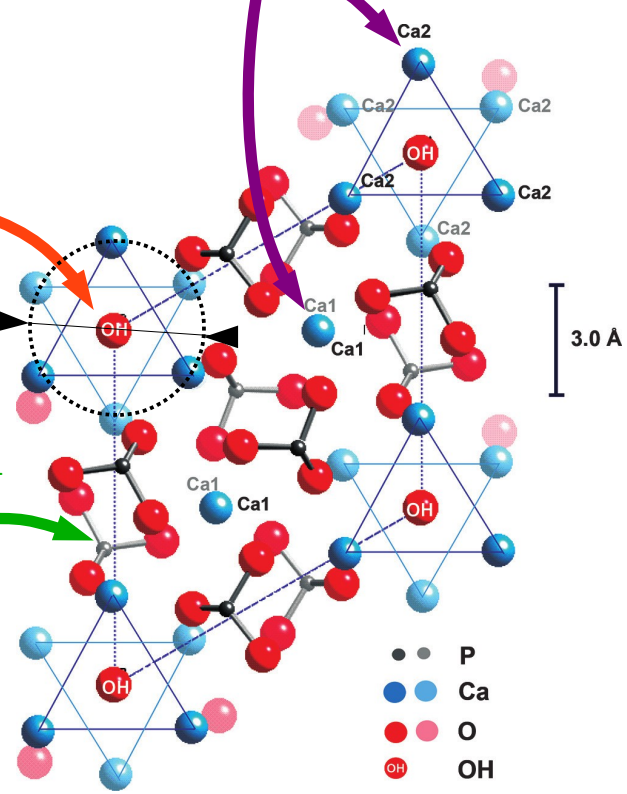
K^+, Na^+, Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Cd^{2+}...

OH^-, Cl^-, F^-

CO_3^{2-}, SO_4^{2-}, AsO_4^{3-}, SiO_4^{4-}



Model structure: $Ca_{10}(PO_4)_6(OH)_2$

Bone apatite (?): $Ca_{10-x}[(PO_4)_{6-x}(CO_3)_x](OH)_{2-x} \cdot nH_2O$

Possible stoichiometry for apatitic phosphates[a]

Chemical formula	Name	Ca/P ratio
$Ca_{10}(PO_4)_6(OH)_2$	end-member hydroxylapatite	1.67
$Ca_{10}(PO_4)_6F_2$	end-member fluorapatite	1.67
$Ca_{10}(PO_4)_6(OH,F)_2$, e.g., $Ca_{10}(PO_4)_6(OH)_{0.4}F_{1.6}$	mixed hydroxyl-fluorapatite	1.67
$Ca_{10}(PO_4)_6Cl_2$	end-member chlorapatite	1.67
$Ca_{10}(PO_4)_6(Cl,F)_2$ e.g., $Ca_{10}(PO_4)_6Cl_{1.2}F_{0.8}$	mixed chlor-fluorapatite	1.67
$Ca_{10}(PO_4)_6CO_3$	end-member A-type carbonated apatite, unhydroxylated	1.67
$Ca_{10-x}[(PO_4)_{6-2x}(CO_3)_{2x}]F_2$	end-member B-type carbonated fluorapatite old mineral name: francolite	≥1.67
$Ca_{10-x}[(PO_4)_{6-2x}(CO_3)_{2x}](OH)_2$	end-member B-type carbonated hydroxylapatite old mineral name: dahllite	≥1.67
$Ca_{10-x}[(PO_4)_{6-2x}(CO_3)_{2x}]CO_3$	mixed A-type and B-type carbonated apatite	≥1.67
e.g., $Ca_{9.75}[(PO_4)_{5.5}(CO_3)_{0.5}]CO_3$, x=0.25		1.77
$Ca_{10-x}[(PO_4)_{6-x}(CO_3)_x](OH)_{2-x}$	Ca- and OH-deficient B-type carbonated apatite	≥1.67
e.g., $Ca_9[(PO_4)_5(CO_3)](OH)$, x=1		1.8
e.g., $Ca_8[(PO_4)_4(CO_3)_2]$(empty), x=2		2.0
$Ca_{10-x}[(PO_4)_{6-x}(HPO_4)_x](OH)_{2-x}$	HPO_4-containing apatite	≤1.67
e.g., $Ca_9[(PO_4)_5(HPO_4)](OH)$, x=1		1.5
e.g., $Ca_8[(PO_4)_4(HPO_4)_2]$(empty), x=2		1.33
e.g., $Ca_8[(PO_4)_4(CO_3)(HPO_4)$(empty)		1.6