Imagerie tomographique 3D du tissu osseux : du micro au nano

F Peyrin[1,2]

1 : CREATIS, INSERM U1206, UMR CNRS 5220,
INSA Lyon, Univ Lyon, France
2 : ESRF, Grenoble, France

& LabEx PRIMES (Physics, Radiobiology, MEdical Imaging, Simulation)

Outline

- Introduction
- CT and Quantitative X-ray CT
- Absorption monochromatic synchrotron CT
  - Microstructure scale
  - Cellular scale : Imaging osteocyte lacunae and canaliculi
- Phase synchrotron CT
  - Principle
  - Application to bone at the nanoscale
- Conclusion

Introduction

Bone hierarchical structure

- Macro scale
  - Compact and Cancellous bone (> 1 mm)
- Micro scale
  - Osteon and trabecula (20 ~ 500 μm)
  - Lacunae / Lamellae (1 ~ 20 μm)
- Nano scale
  - Canaliculi/ Collagen fibers (100 nm ~ 1 μm)
  - Crystal / Collagen molecule (1 nm ~ 100 nm)

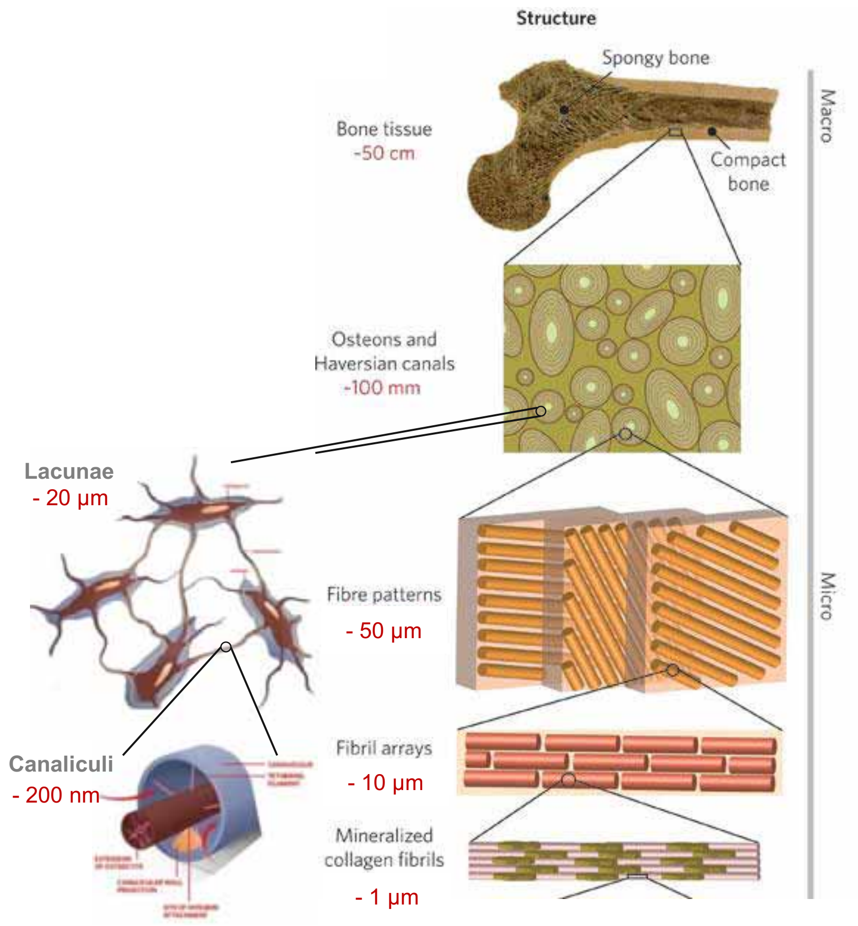
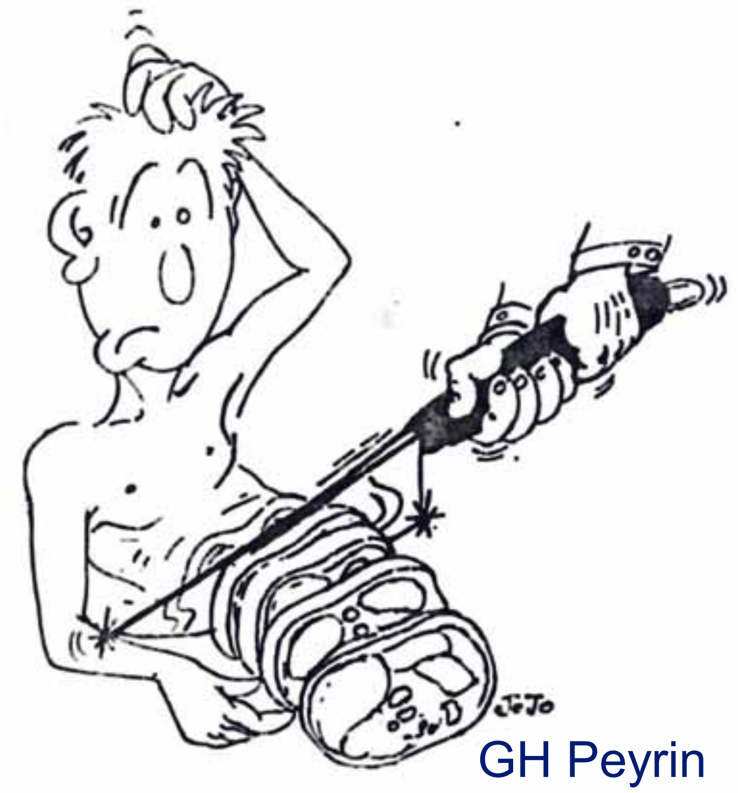


Image modified from (Ritchie 2011)

CT and Quantitative X-ray CT



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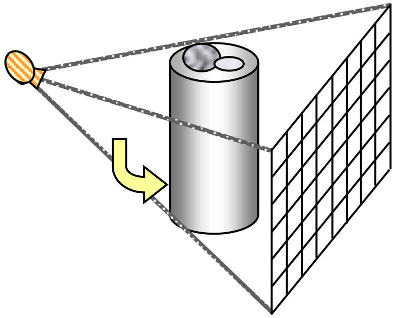
Development of HR and micro-X-ray CT

1980

1990

2000

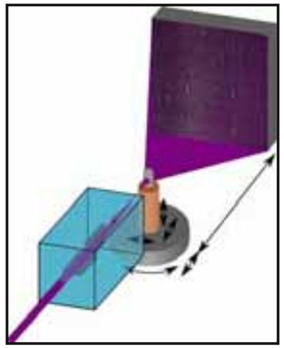
2010



Prototype µCT

Synchrotron µCT

Commercialized µCT



Synchrotron nCT

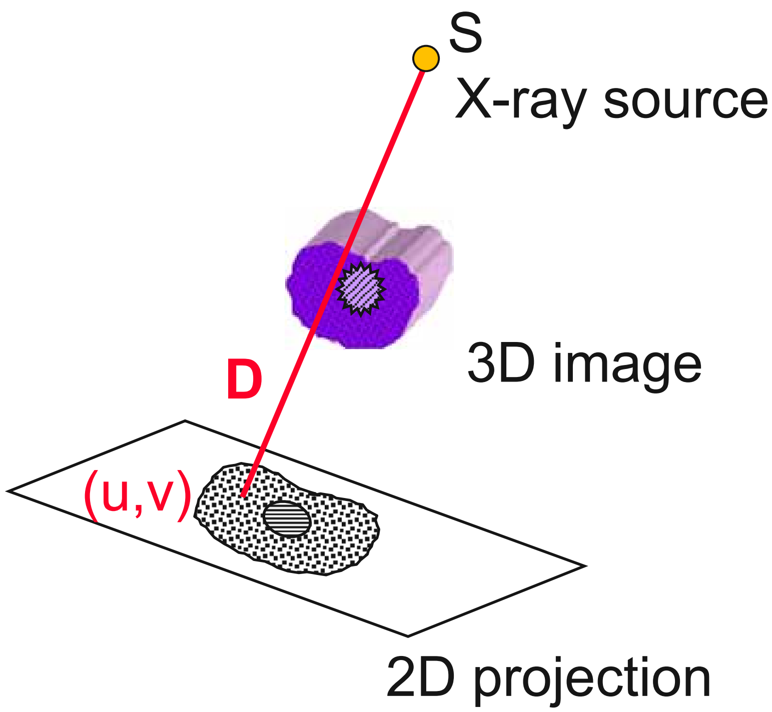
HR-pQCT

Image formation in CT

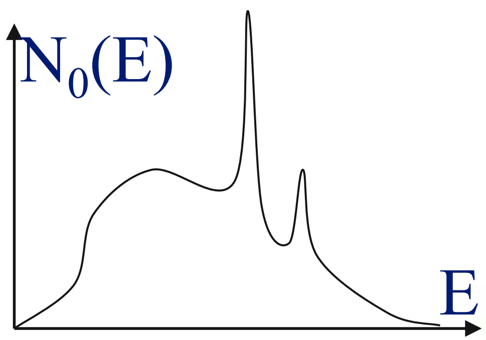
- Theory :
  - Assumption : monochromatic X-ray beam
  - Measurements :

$$N(E) = N_0(E)\exp(-\int_l \mu(x,y,z;E)\,dl)$$

  - Reconstruction μ(x,y,z; E) : map of the linear attenuation coefficient for energy E



- Limitations in standard CT
  - Polychromatic beam $N = \int_E N_0(E)\times\exp(-\int_l \mu(x,y,z;E)\,dl)\,dE$



  - Solutions for quantitative CT :
    - Synchrotron source
    - Bi energy techniques
    - Spectral CT

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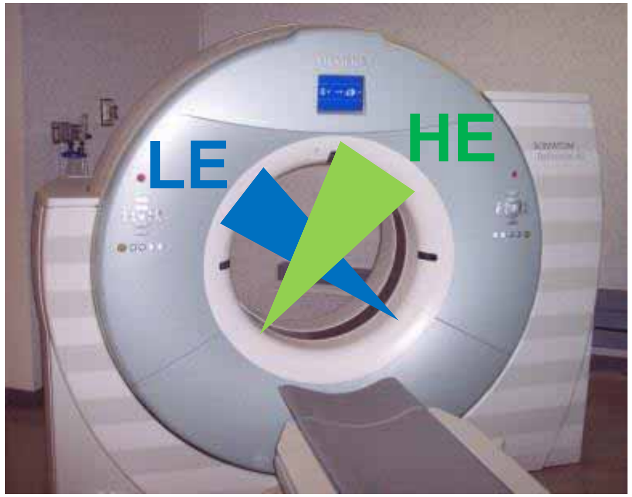
Quantitative CT

- Bi energy techniques (Dual X-ray absorptiometry or QCT)
  - Measurements at Low and High energy
  - Reconstruction of μ at Low and High Energy

  -> Estimation of the mass concentration of bone



Siemens Somatom CT

- Spectral X-ray CT
  - Energy resolved detectors
  - Measurement in different energy bins

$$\{ \ N(E_k) / k = 1, P \ \}$$

  -> Recovery of the concentration in different materials

-> Prototype of first small animal spectral CT in Lyon (France Life Imaging)

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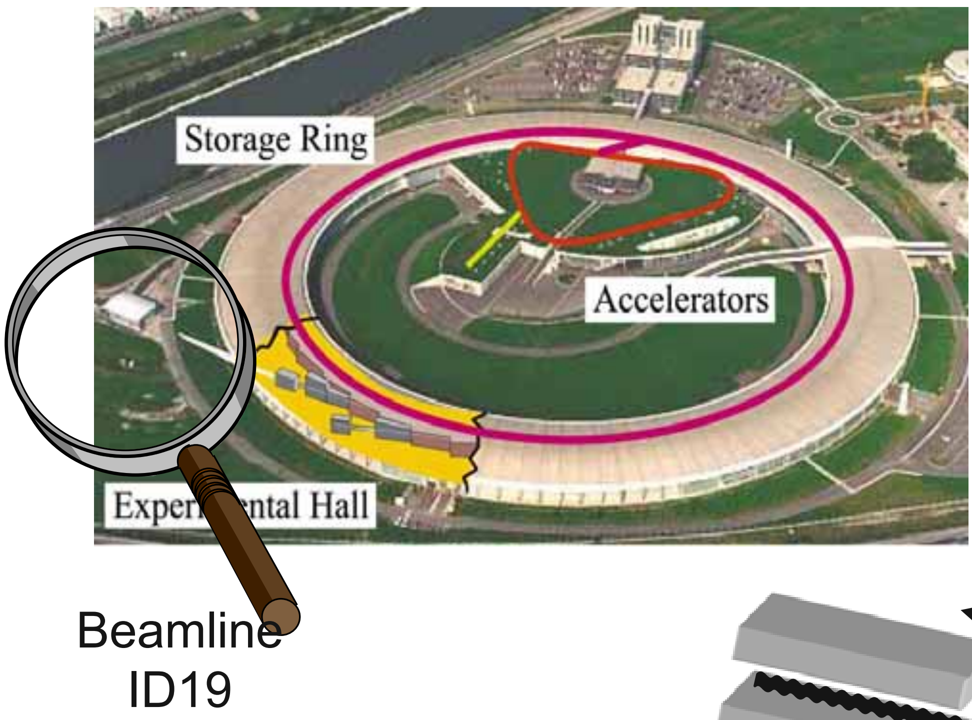
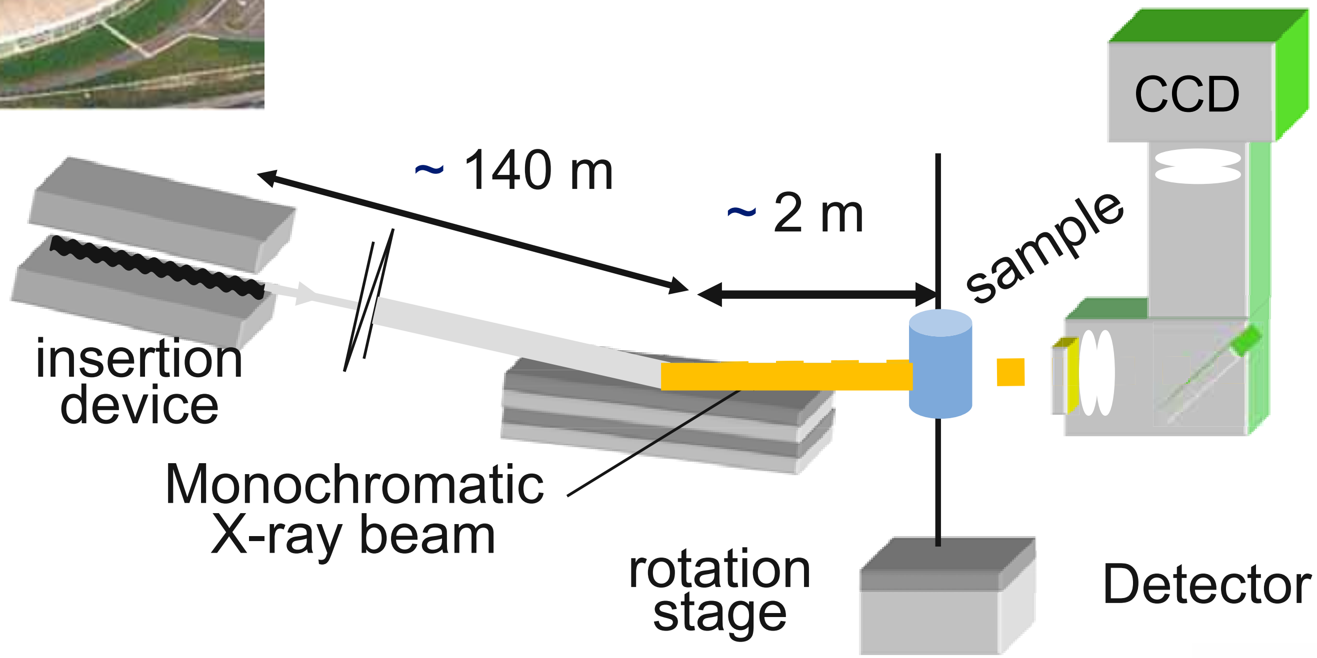
Synchrotron radiation micro-CT

3D SYNCHROTRON RADIATION (SR) MICRO-CT



<u>ESRF micro CT setup, ID19</u>

- Energy : 8 to 80 KeV
- Voxel size : 10 µm to 0.2 µm
- Images : $(2048)^3$



[Salomé et al., Med phys, 1999]

Why SR micro-CT ?

- Monochromatic beam
  - ⇒ No beam hardening artifacts
  - Ø optimal energy for a series of sample
- Very high photon flux
  - Ø High Signal to Noise Ratio (SNR)
  - Ø improve segmentation & quantification
  - Ø possible to reach nanometric resolution while keeping sufficient SNR
- 3D Parallel CT
  - Ø exact reconstruction (no Cone Beam artifacts)

Avoids various sources of blurs

➔ Quantitative imaging

➔ Quantify structure AND mineralization

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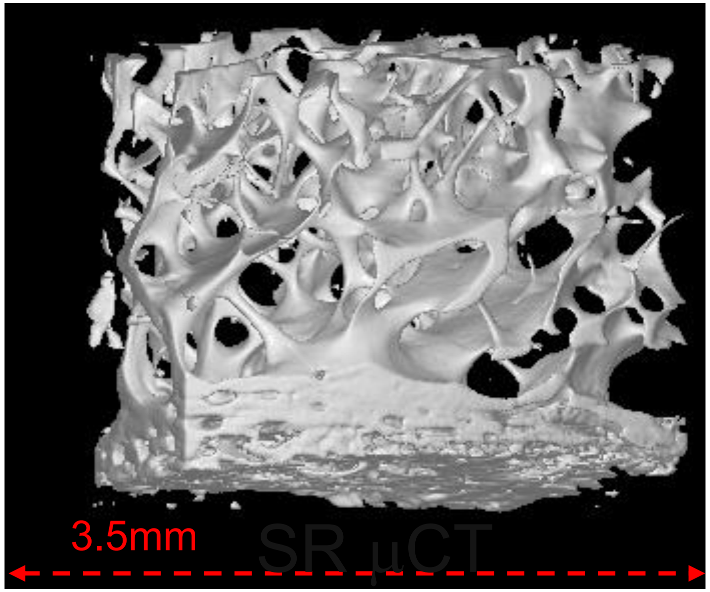
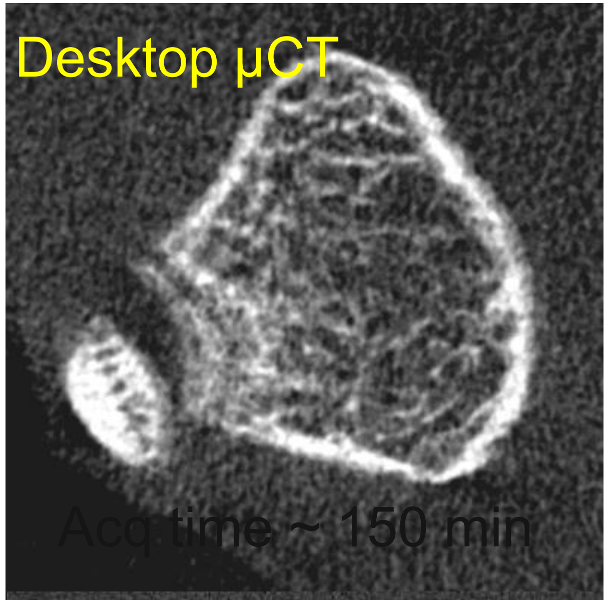
EXAMPLE : SR MICRO-CT vs DESKTOP MICRO-CT

Human iliac crest biopsy

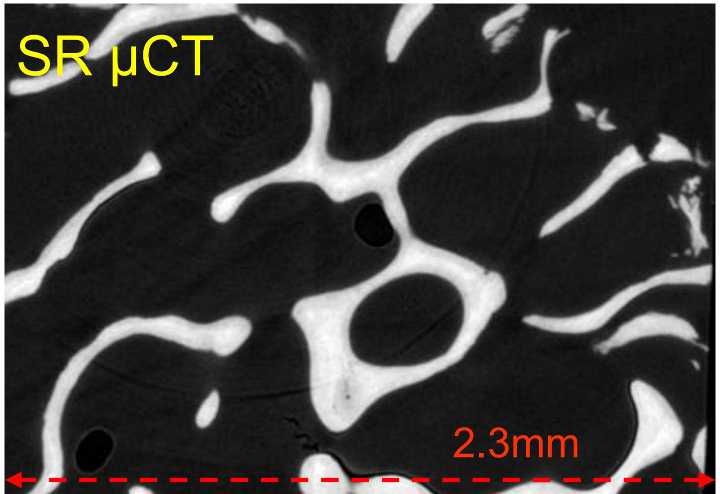


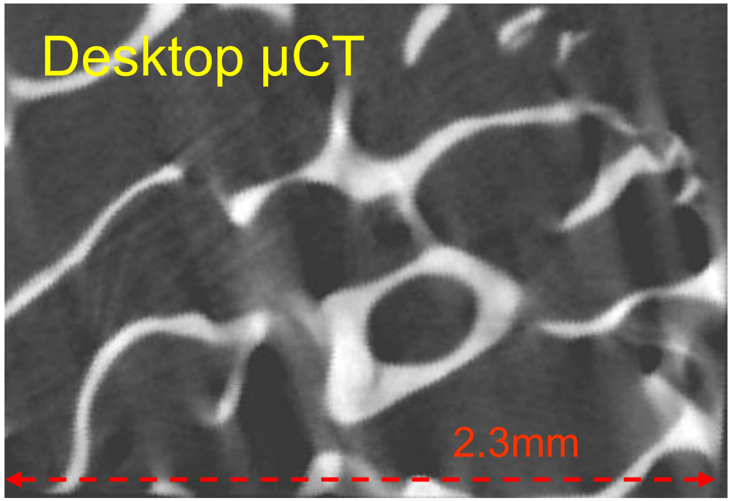
Mice in vivo

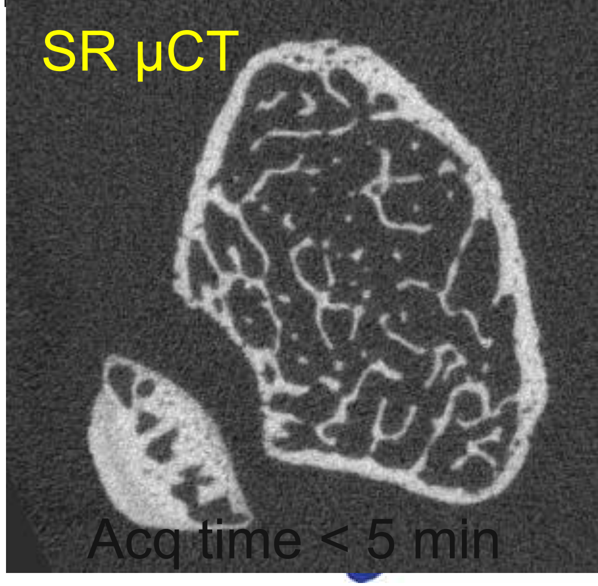


Same conditions

Voxel: 10 µm



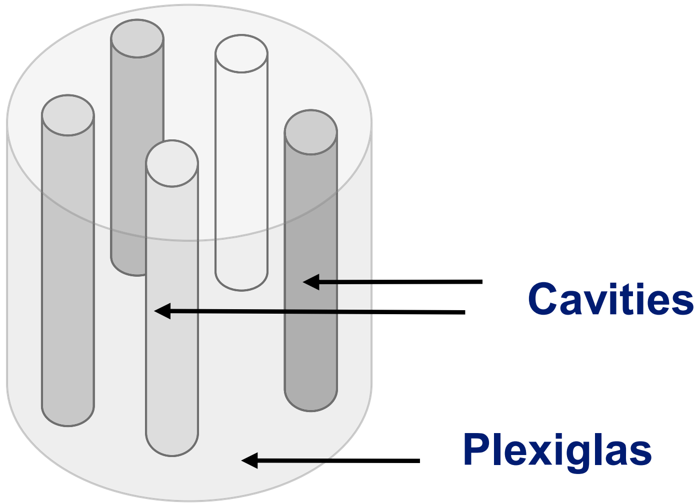


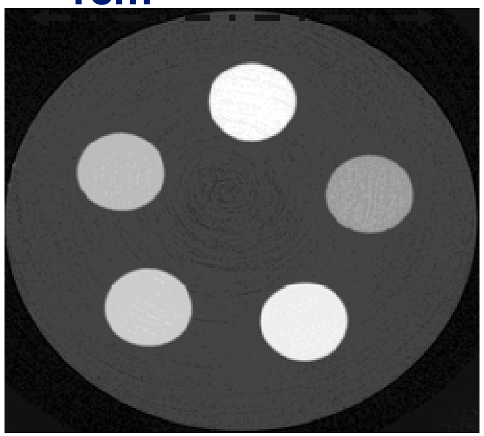


[Nuzzo et al., Med Phys, 2002]

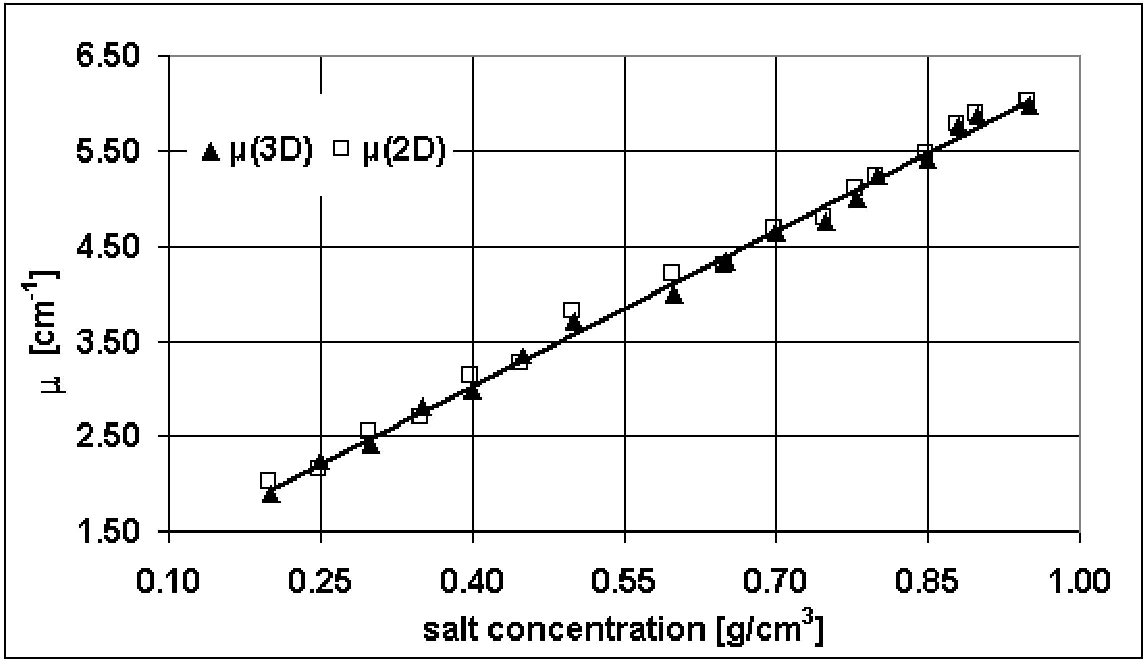
MONOCHROMATIC SYNCHROTRON CT

- Quantitative imaging
- Phantoms: homogeneous water solutions of various known K2HPO4 concentrations





2D Slice



gray level ⇔ mineral concentration

[Nuzzo et al., Med Phys, 2002]

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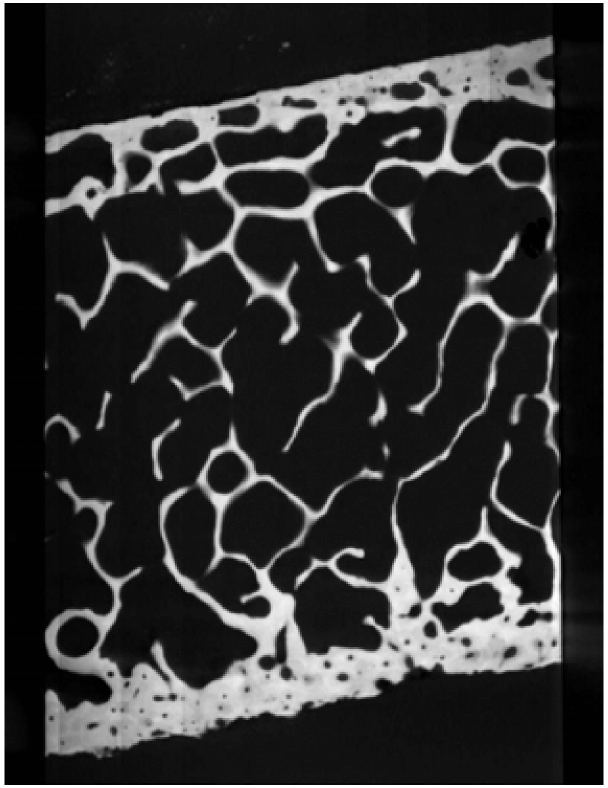
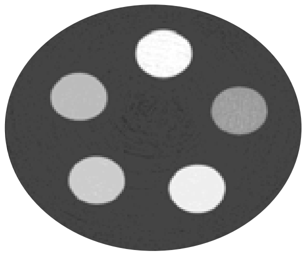
COMPARISON WITH MICRORADIOGRAPHY

SR µCT [ESRF - Grenoble]

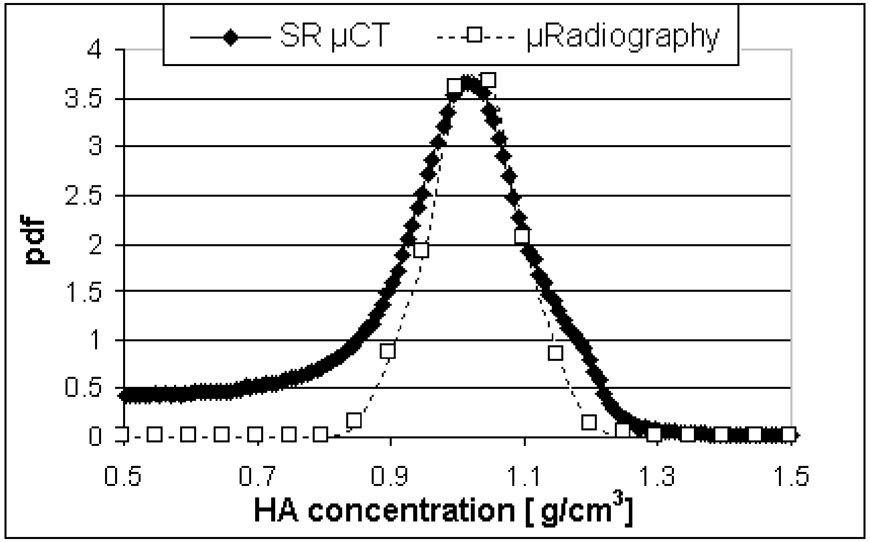


SR calibration: HA phantoms



2D Slice

Slice of bone : Thickness ~ 100 µm

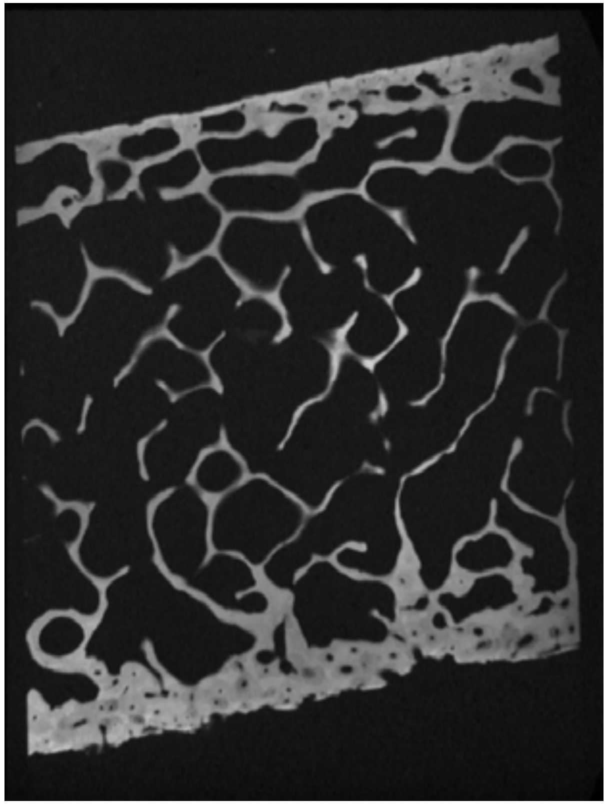


4 BIOPSIES

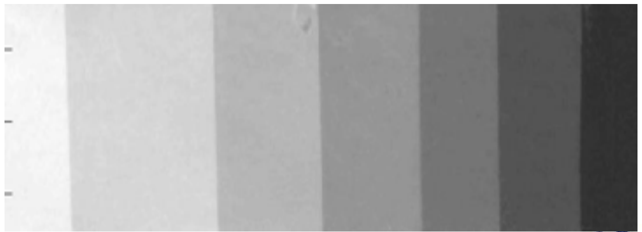
Mean difference = 4.7 %

Slightly higher in trabecular than in cortical

Radio [Dr G.Boivin - Lyon]



Al step-wedge calibration



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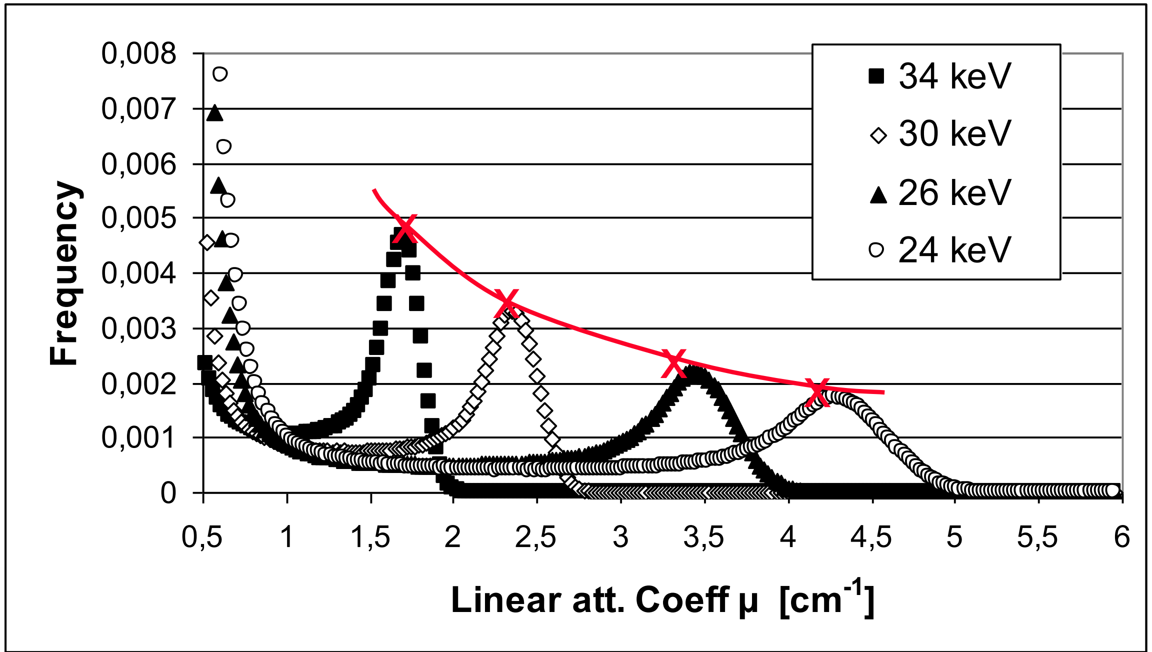
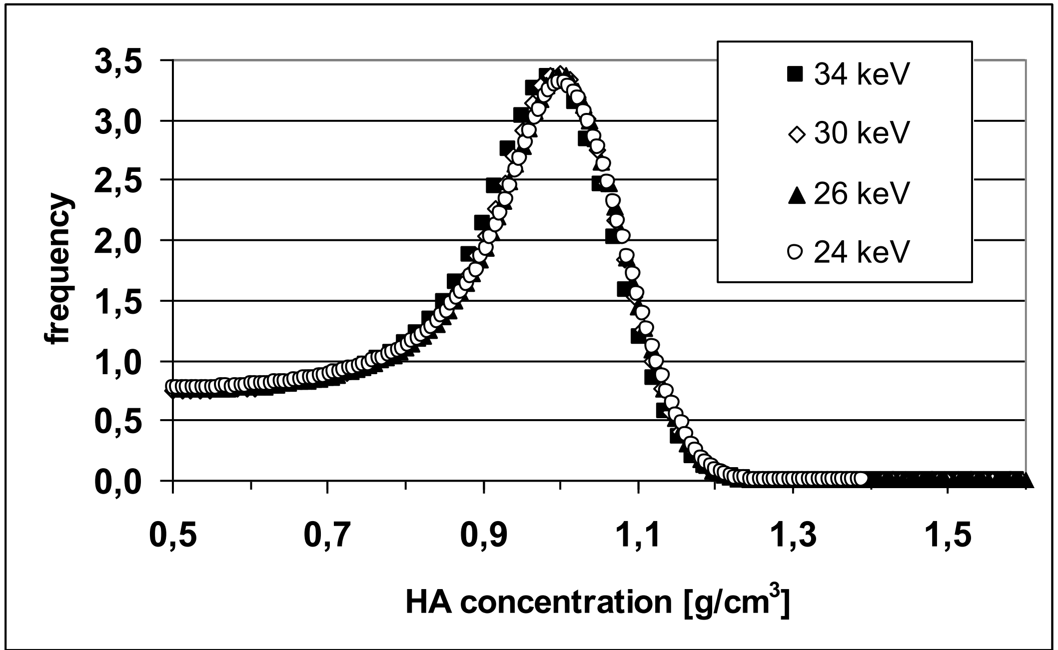
Quantitative CT : monochromatic CT

- Same biopsy imaged at various energies between 18 and 32 keV
  - E_1 : $\mu (E_1) = f_1 (c_{HA})$
  - E_2 : $\mu (E_2) = f_2 (c_{HA})$ but μ(E)same HA distribution expected …………
  - E_n : $\mu (E_n) = f_n (c_{HA})$

Reproducibility = 0,26%





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[Nuzzo, MedPhys 2002]

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Absorption monochromatic synchrotron CT

Microstructure scale

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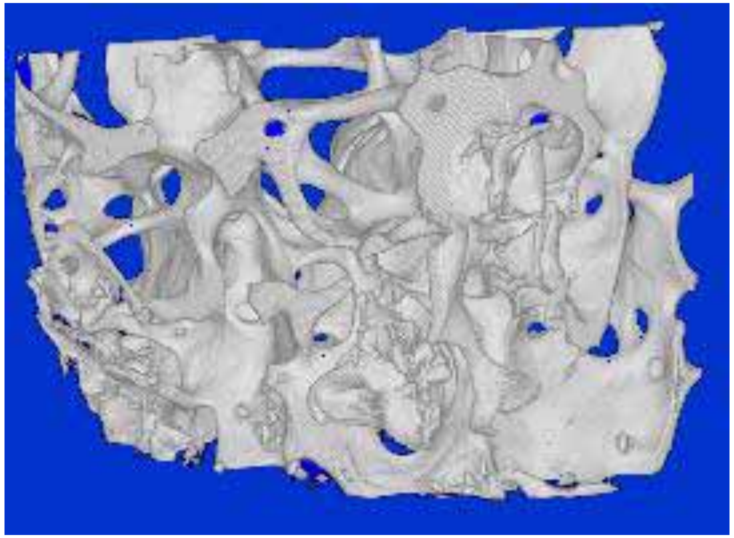
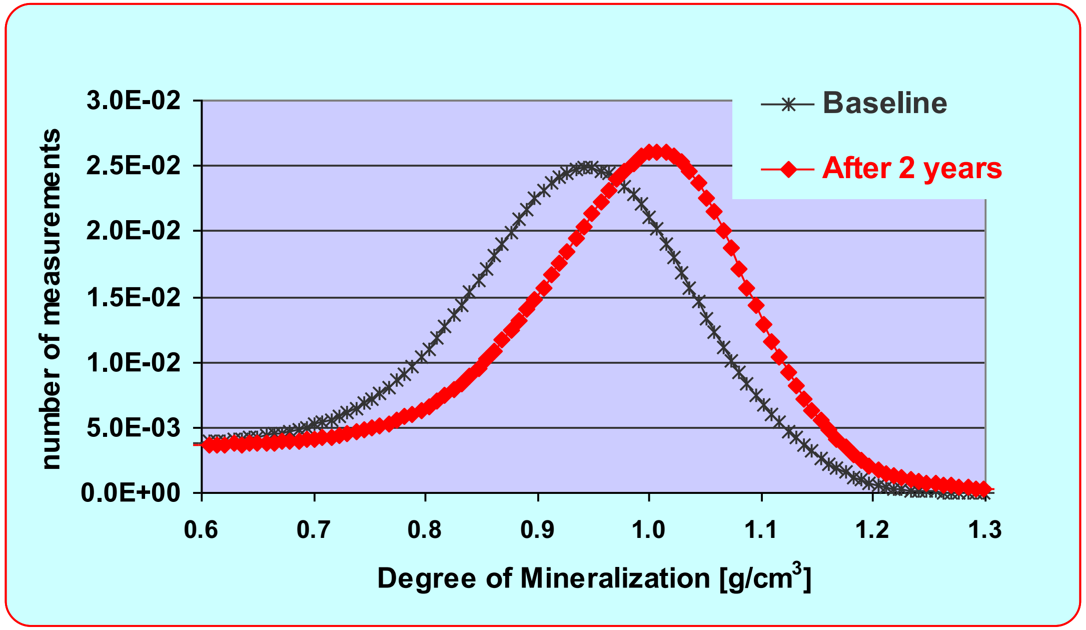
ANALYSIS OF AN ETIDRONATE TREATMENT

- Biphosphonate Treatment repeated every 3 months :
  Etidronate (400 mg/day for 14 days) + Ca (1g for 11 weeks)





[S. Nuzzo and al., JBMR, 2002]

Coll LBTO, Lyos

- **Baseline and After 2 years :**
  - **HA concentration 12% (cortical)**
  - **HA concentration 8% (trabecular)**

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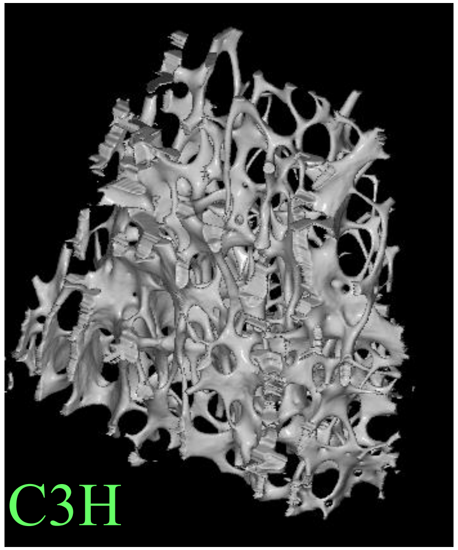
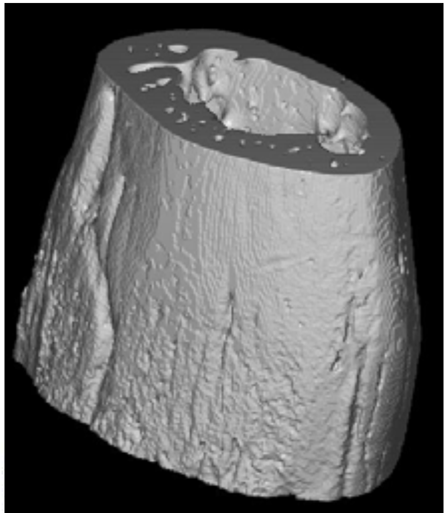
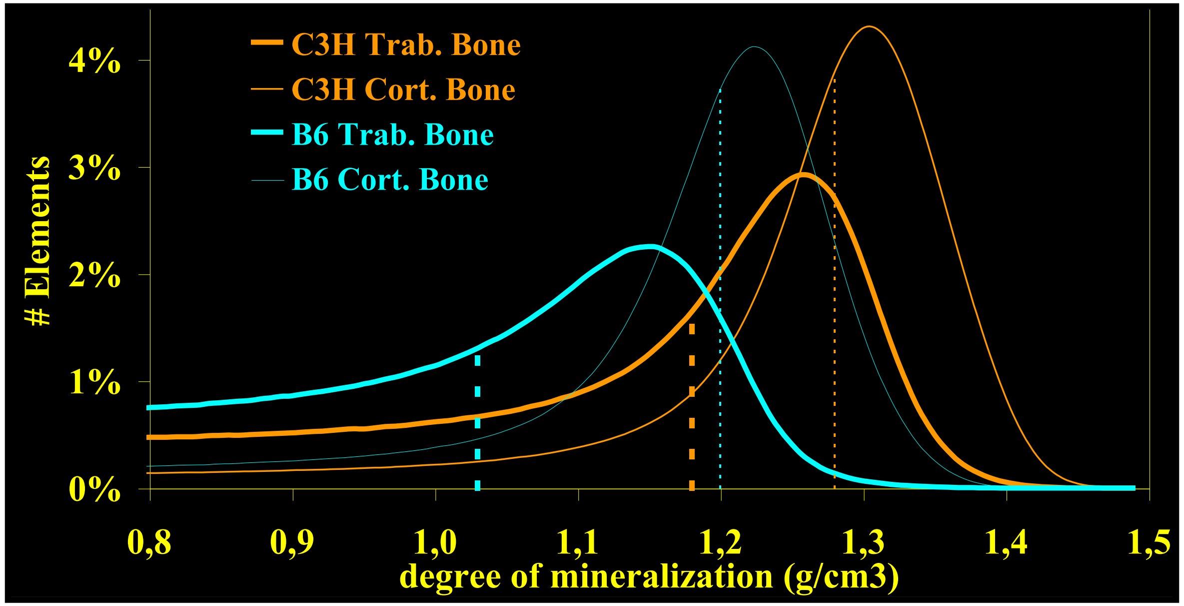
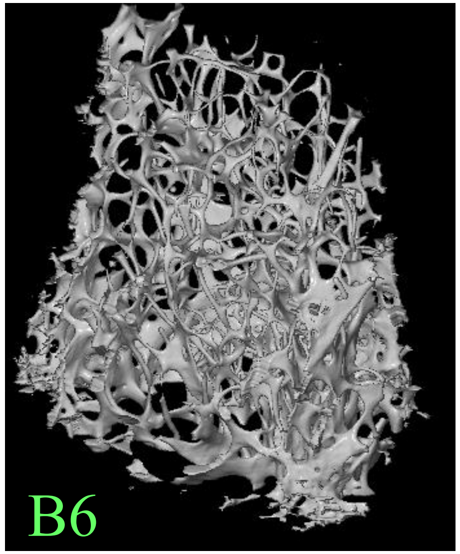
MINERALIZATION in 2 MICE STRAINS : C3H/HeJ / C57BL/6J

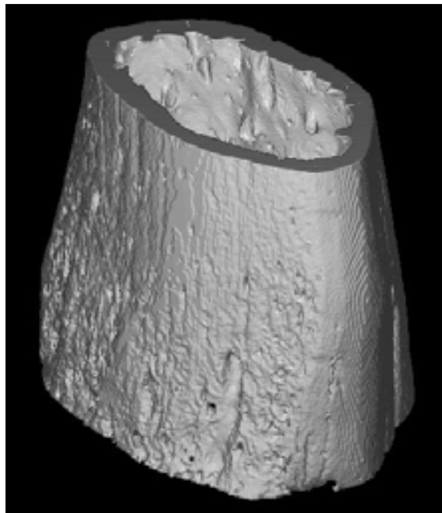
- Significant differences between strains, coll LBTO











g/cm^3	B6	C3H
Trab	1.03±0.01	1.18±0.01
Cort	1.20±0.01	1.28±0.01

[Martin-Badosa et al., 2003, Radiology]

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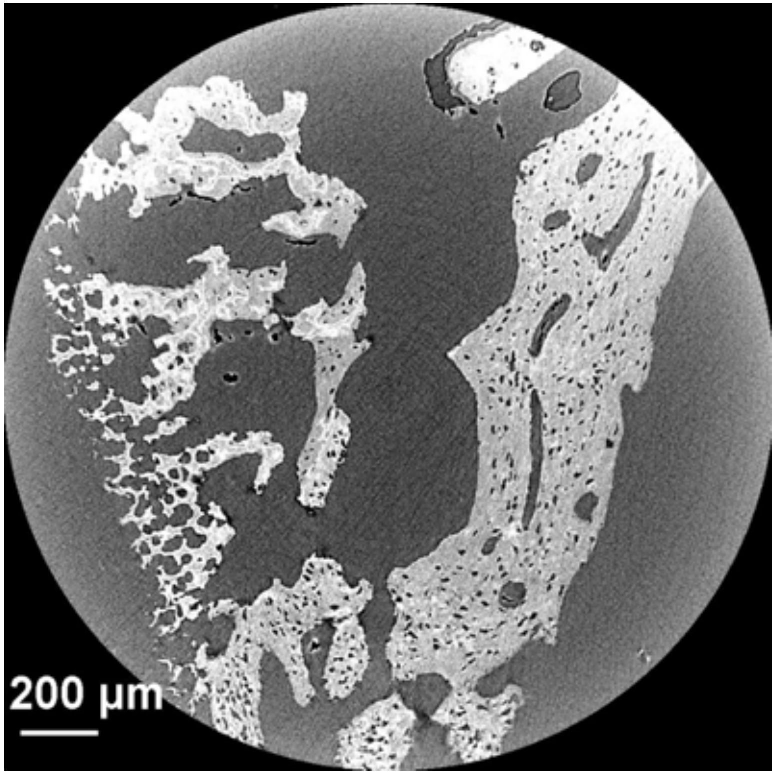
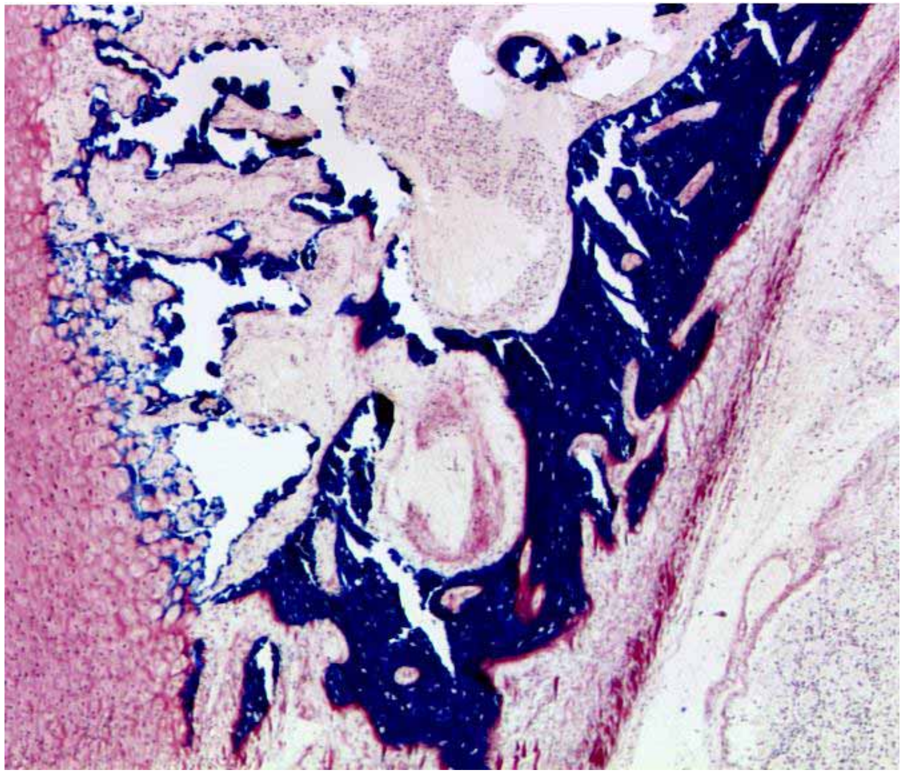
SR MICRO-CT : Fetal bone – 1.8 µm





Gestation weeks	19	21	27
Mean DMB (g/cm^3)	0.827	0.839	0.803
CV (%)	18 %	17 %	20 %

[Nuzzo et al., JBMR, 2004]

[Peyrin et al., Clin Cases, 2005]

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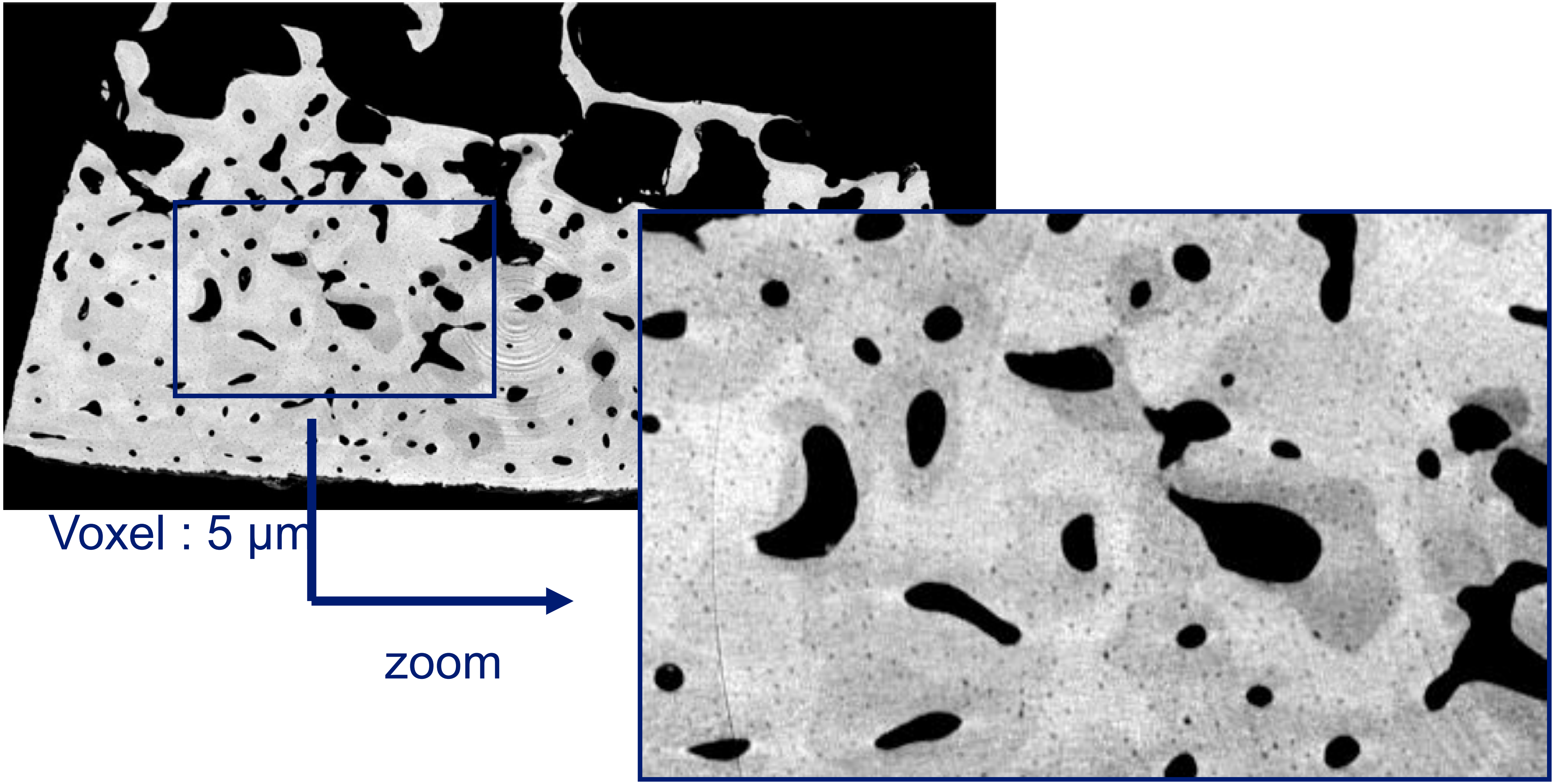
SR MICRO-CT : cortical bone – 5 µm

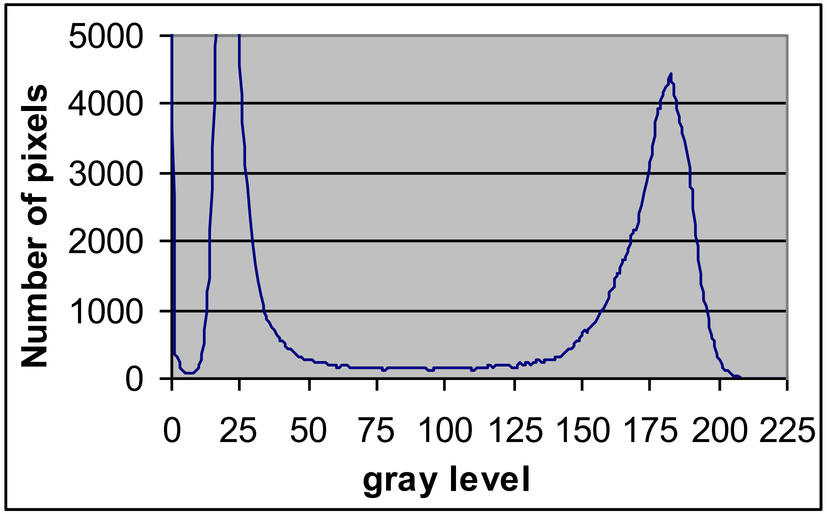


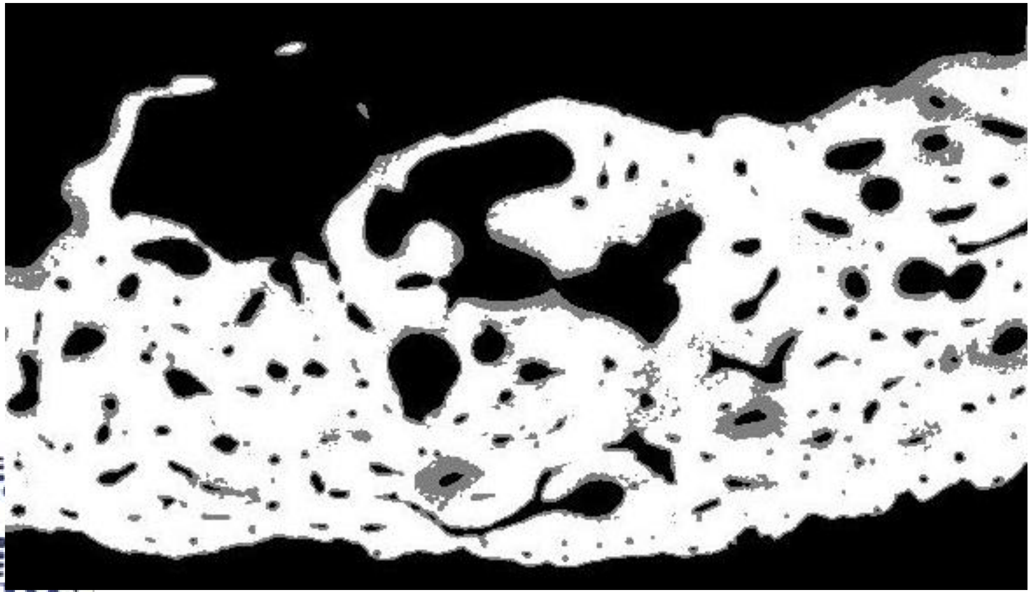
[Bousson et al., JBMR, 2004]

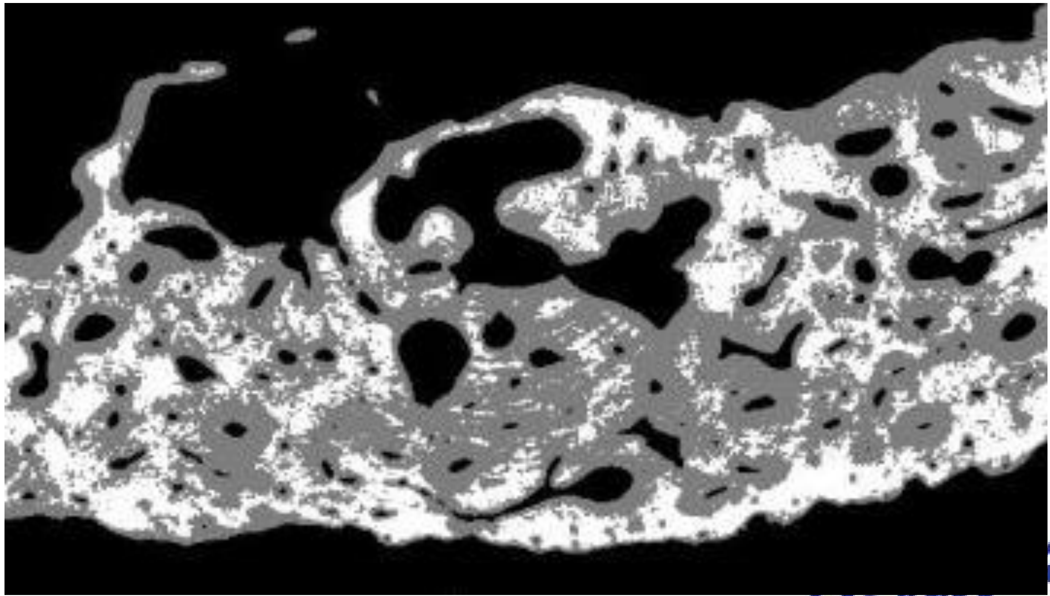
[Chappard et al., Med Phys, 2006]

QUANTIFICATION OF OSTEONS

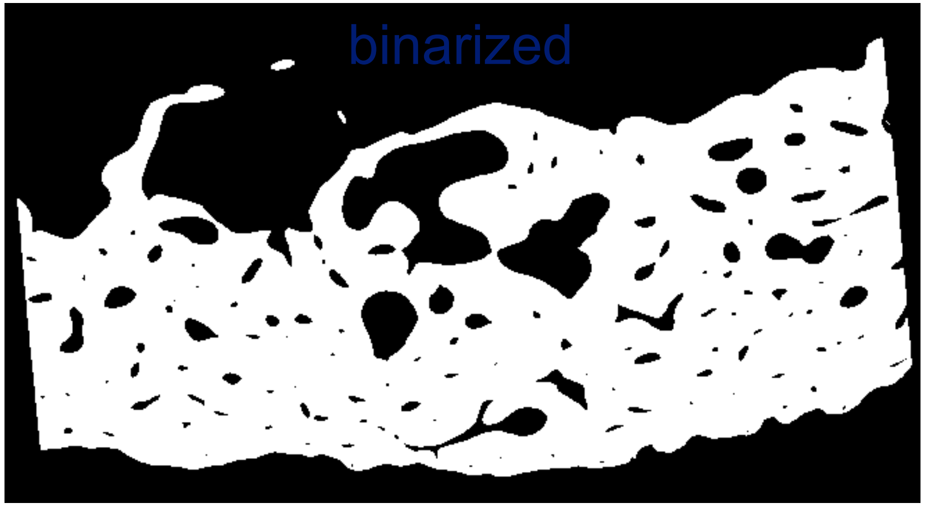
- Osteons are visible but with low contrast
- Histogram of images
  - Bimodal : background / bone
  - But only one peak in bone
- Thresholding methods fail

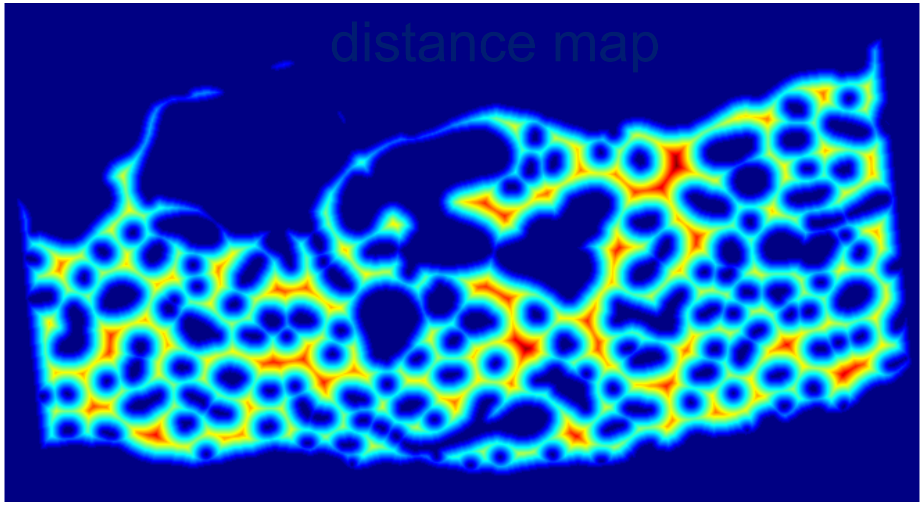


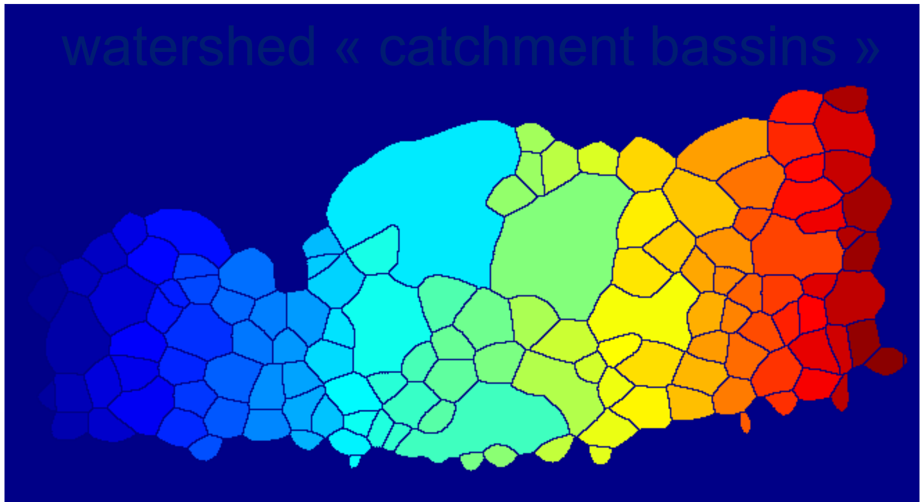


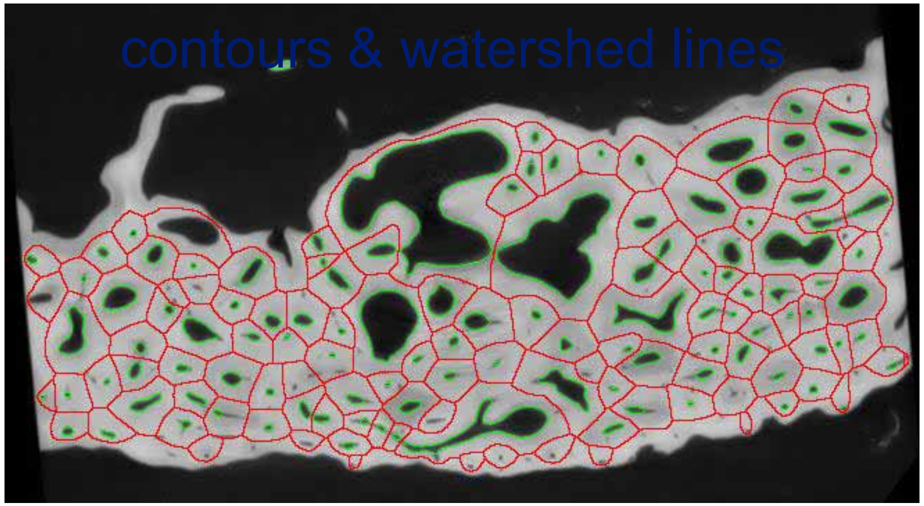


SEGMENTATION METHOD : CRGW









[Peter et al., Pattern Recognition 2008]

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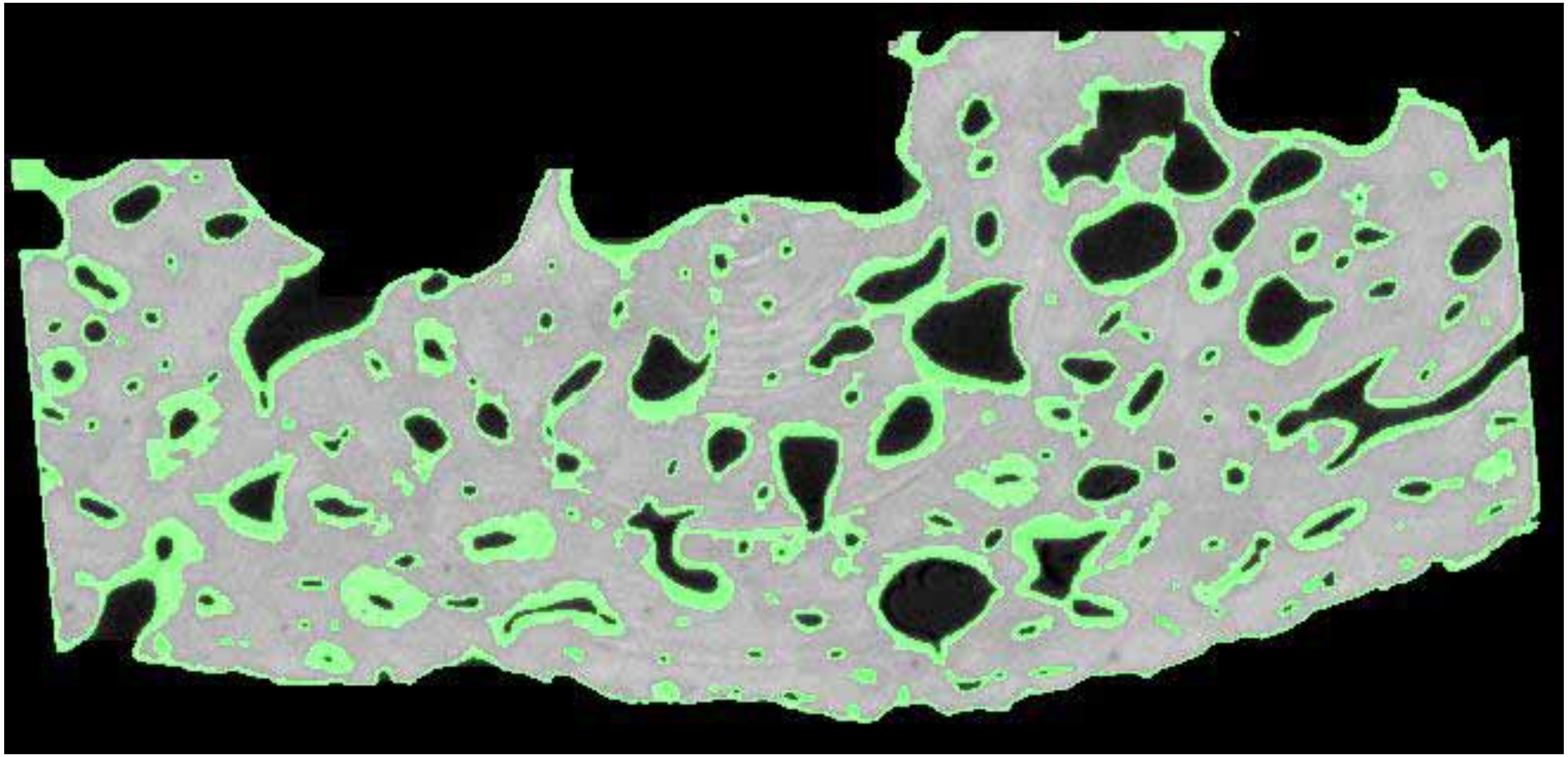
SEGMENTATION METHOD : CRGW

- Application to a volume data set



[Peter et al., Pattern Recognition 2008]

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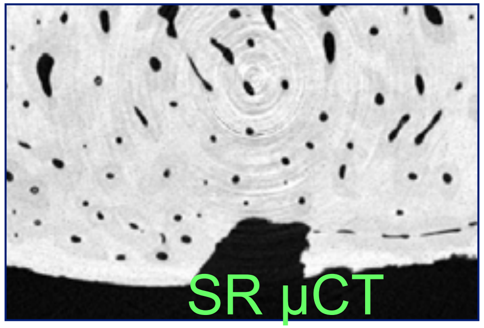
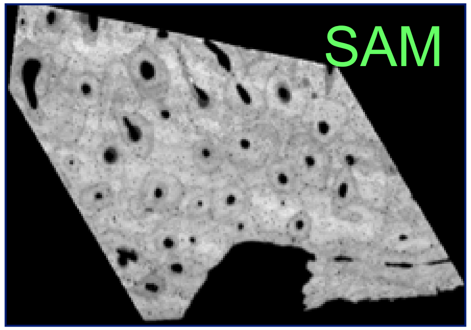
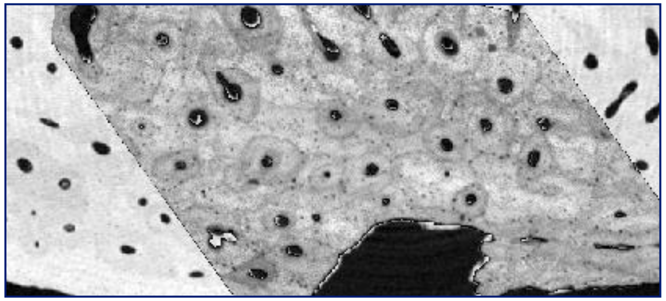
Mineralization – SR CT and SAM







Registration

- ESRF µCT, Voxel size = 5 µm, minéralisation
- SAM (Raum K, Laugier P) pixel size = 4 µm
- Application to mice and human data

µ(x,y) → DMB → Density

SAM → Acoustic impedance (Z)

Density + Acoustic impedance (Z) → Elastic coefficient c33 → Young Modulus E3

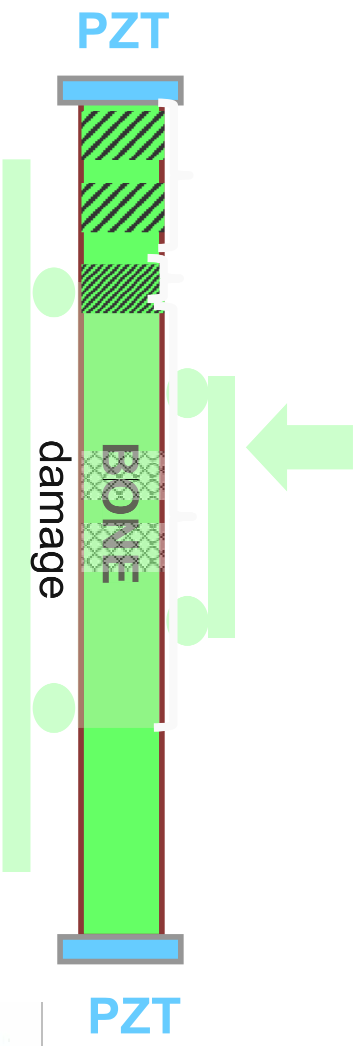
[Raum, et al, Bone, 2007]

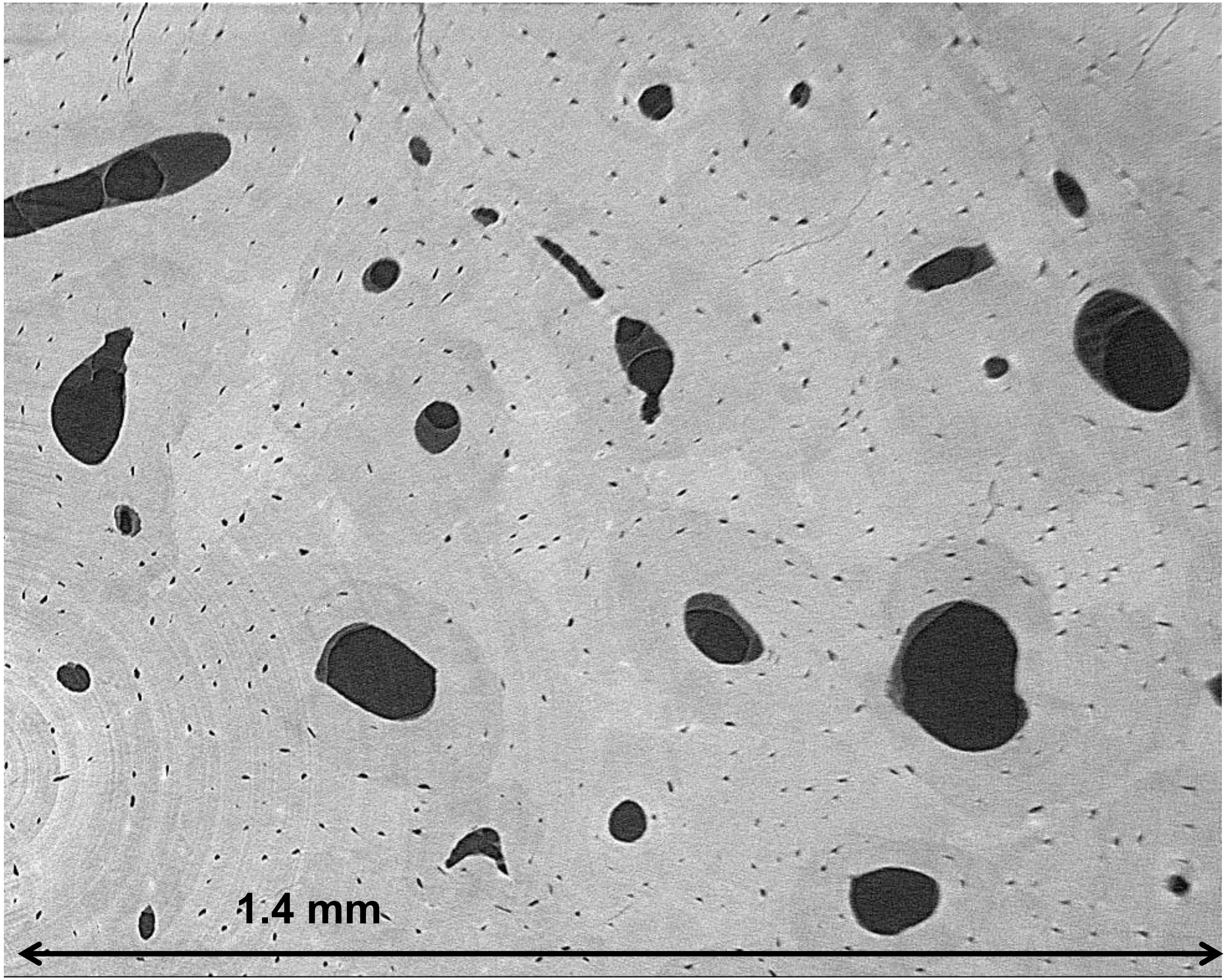
[Raum, Cleveland, Peyrin, Laugier, Phys Med Biol, 2006]

Absorption monochromatic synchrotron CT

Cellular scale : lacunae and canaliculi

Parallel SRµCT at 1.4µm – Human cortical bone





in coll with the LIP, S Haupert, P Laugier

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Analysis of lacunae : descriptors

- Analysis of each lacunae Xn

$$X_n = \{\mathbf{x} = (x, y, z) \in \Omega / L(\mathbf{x}) = n\}$$

- 2nd order centered moments

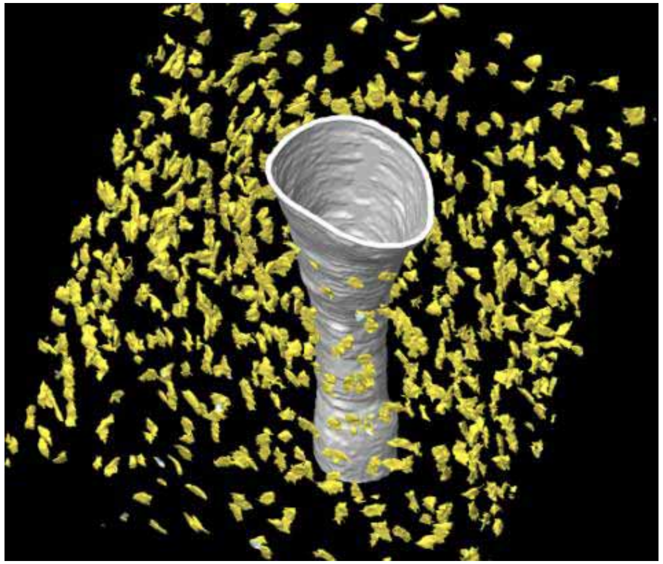
$$\mu_{pqr} = \sum_{(x,y,z)\in X_n} (x - \bar{x}_n)^p \cdot (y - \bar{y}_n)^q \cdot (z - \bar{z}_n)^r$$

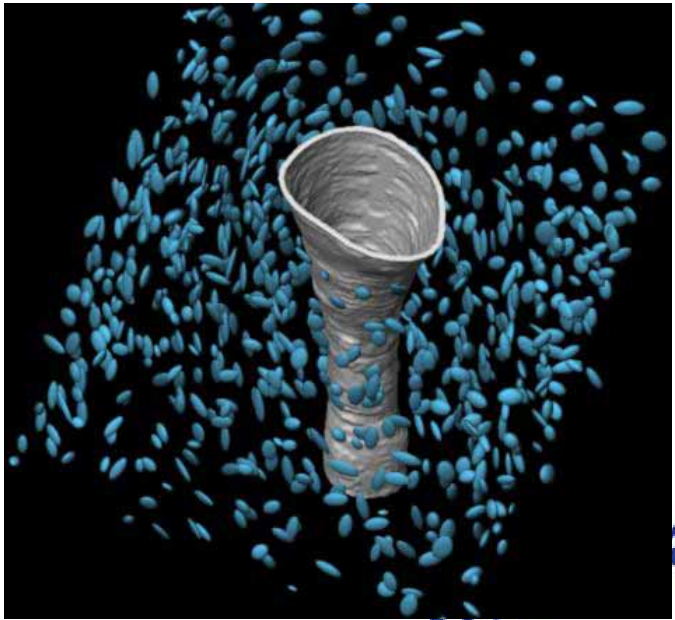
$$M(O_n) = \begin{pmatrix} \mu_{200} & \mu_{110} & \mu_{101} \\ \mu_{110} & \mu_{020} & \mu_{011} \\ \mu_{101} & \mu_{011} & \mu_{002} \end{pmatrix}$$

Lengths, orientation, anisotropy

- Intrinsic volumes
  - Discretized Crofton formula [J. Ohser, 2009]

$$SMI(X_n) = \frac{3\pi\, Vol(X_n)\, \hat{V}_1(X_n)}{(\hat{V}_2(X_n))^2}$$





[Dong, Bone 2014]

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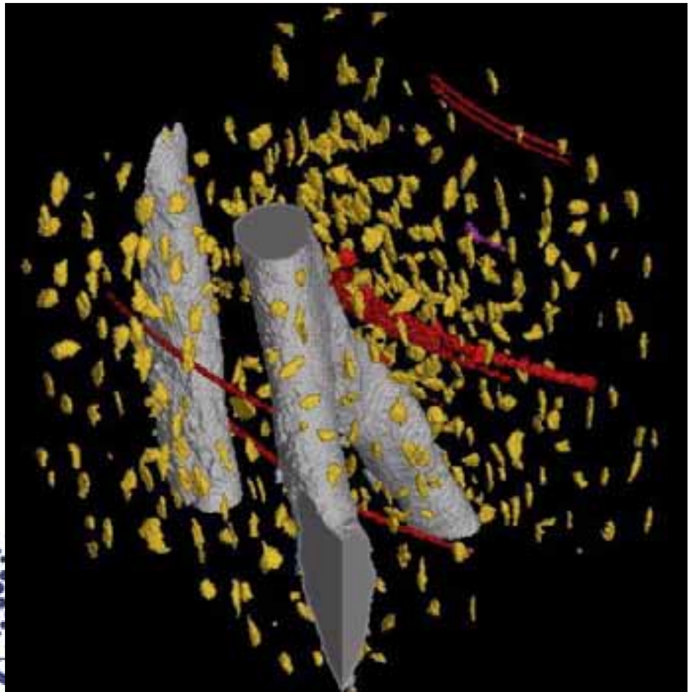
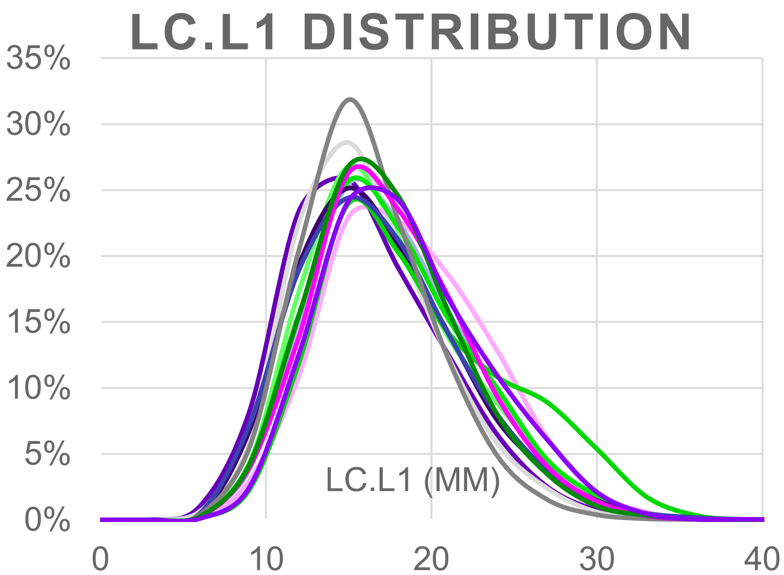
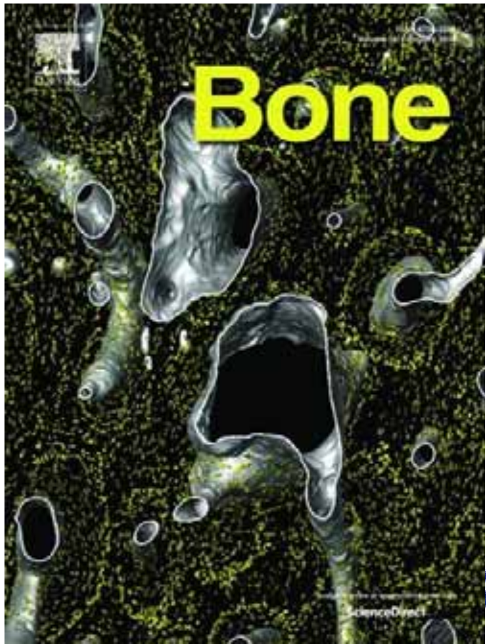
Validation - Artifacts elimination

- Validation vs "Manual" segmentation (ground truth)
  - Filtering based on descriptors: Volume, Anisotropy: Length/Width, Euler #
  - Results : / Ratios~ 98%
- Application to human cortical bone femur (n=12)
  - Large population of cells (>13000/sample)
  - Average : Density ~20694/mm^3, Sizes ~ 18.8, 9.3, 4.8µm, Lc.Vol ~405 µm^3



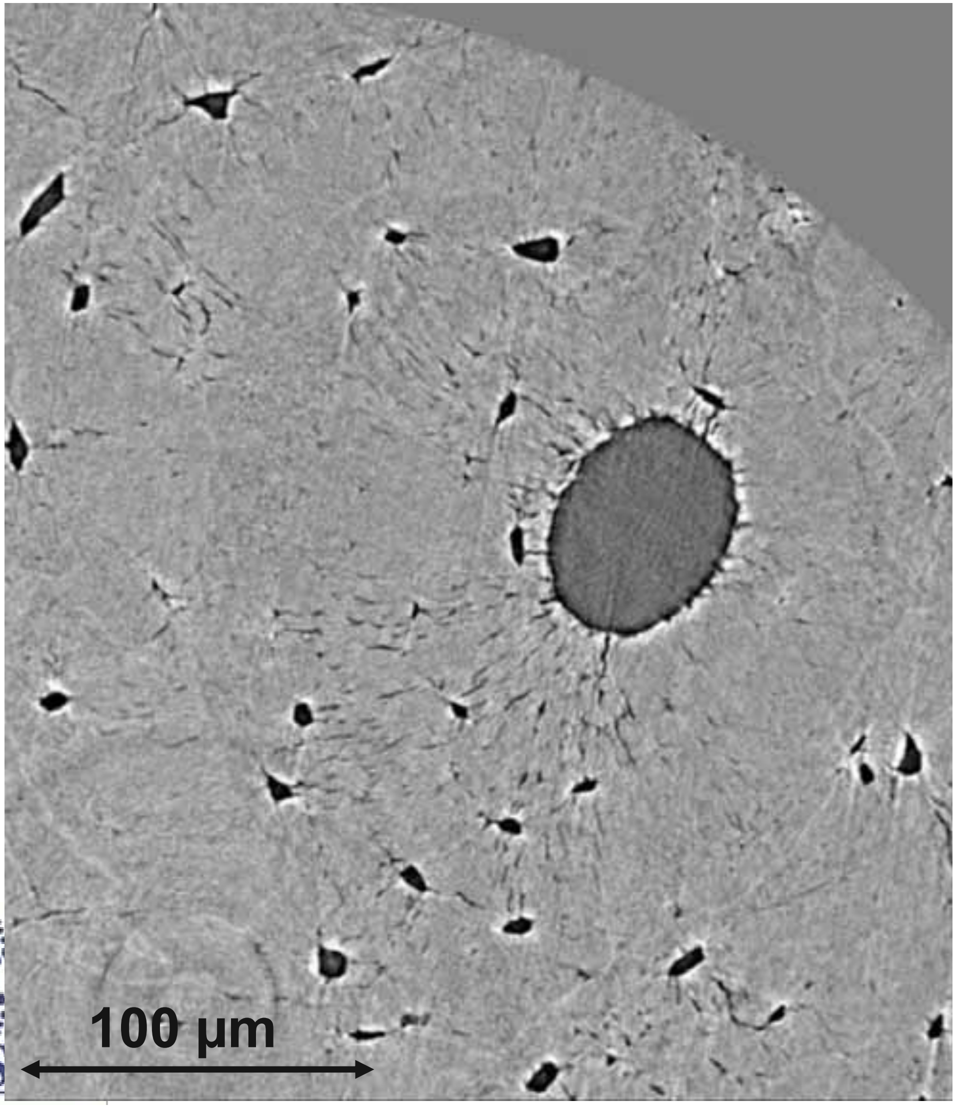


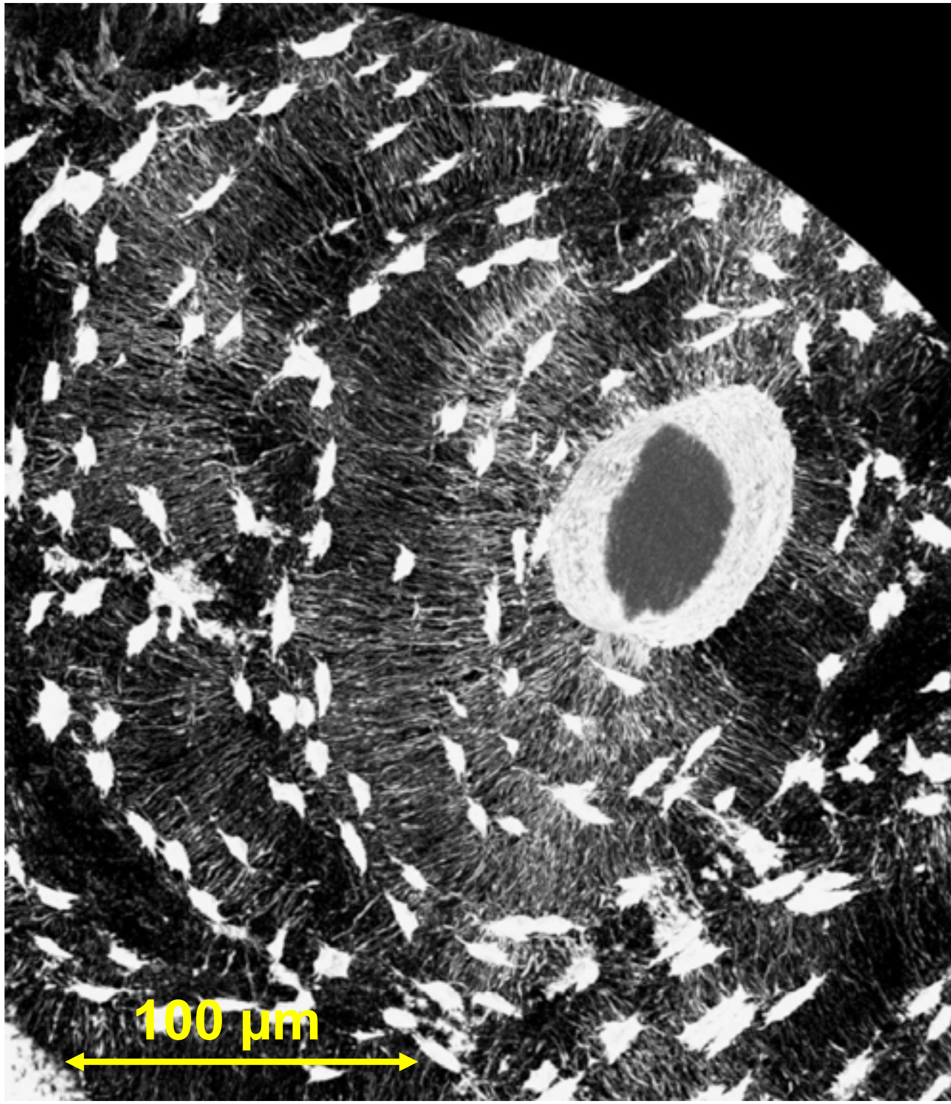


[Dong, Bone 2014]

Parallel SRμCT at 300nm – Human cortical bone

- Optimization of acquisition system (dose versus noise)
  - Human femoral cortical bone (diaphysis)





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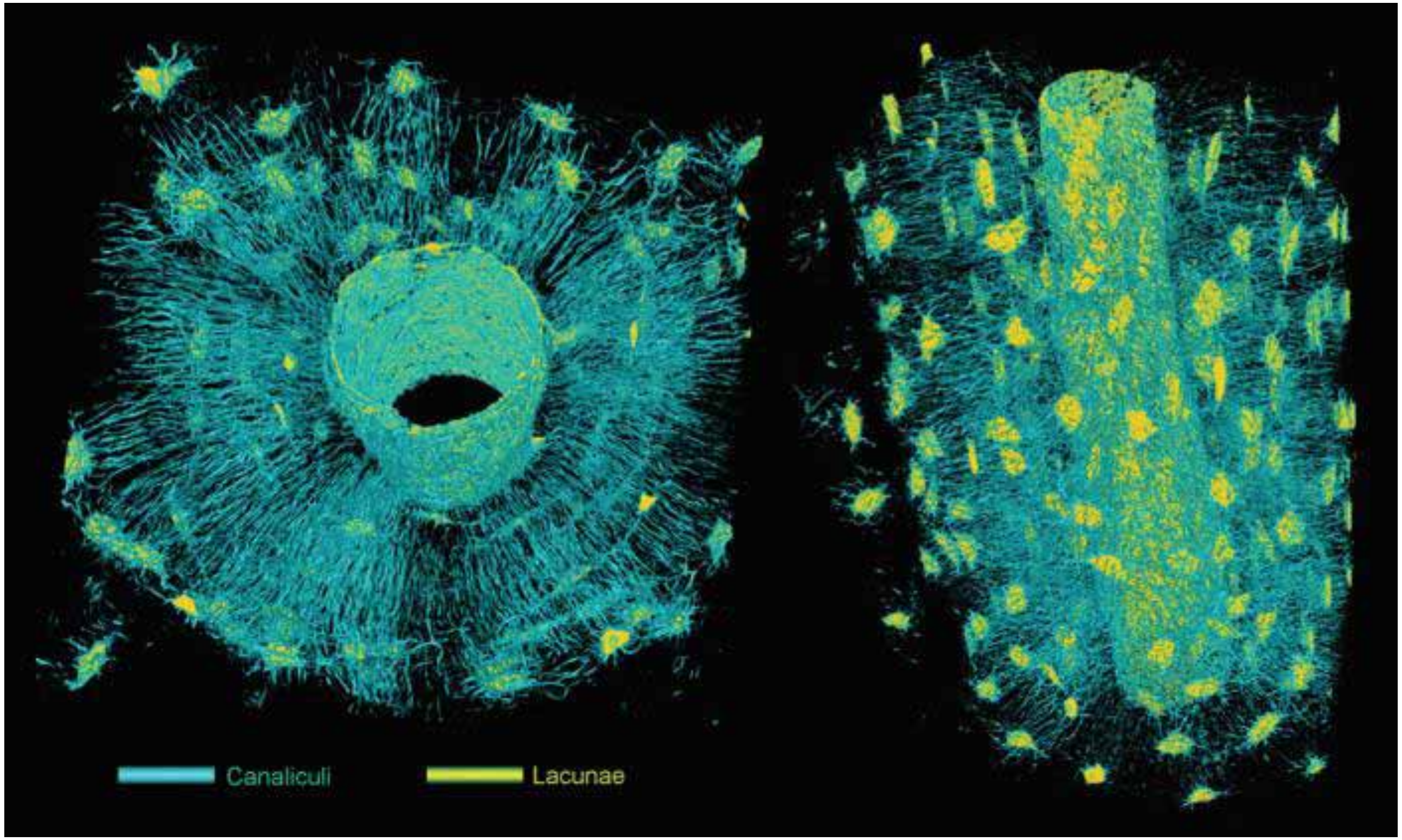
Parallel SRμCT at 300nm – Human cortical bone

- Human femoral cortical bone :
  - 3D rendering around an Haversian canal

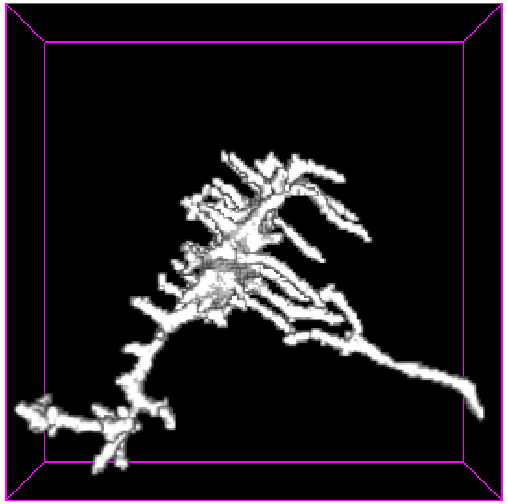


[Pacureanu et al., Med Phys 2012]

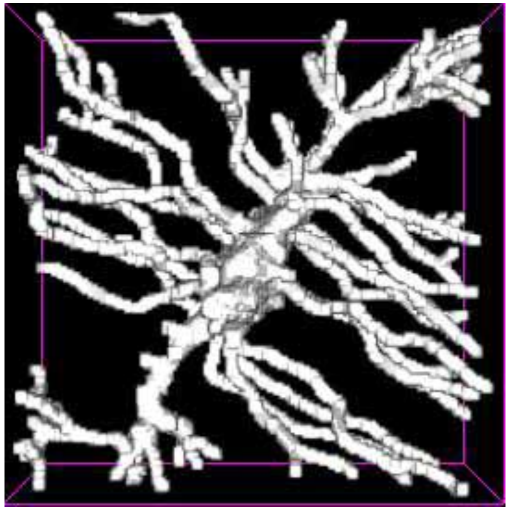
Segmentation of the lacuna canalicular network

- The extraction of canaliculi from images at 0.3µm :
    - Standard segmentation methods fail

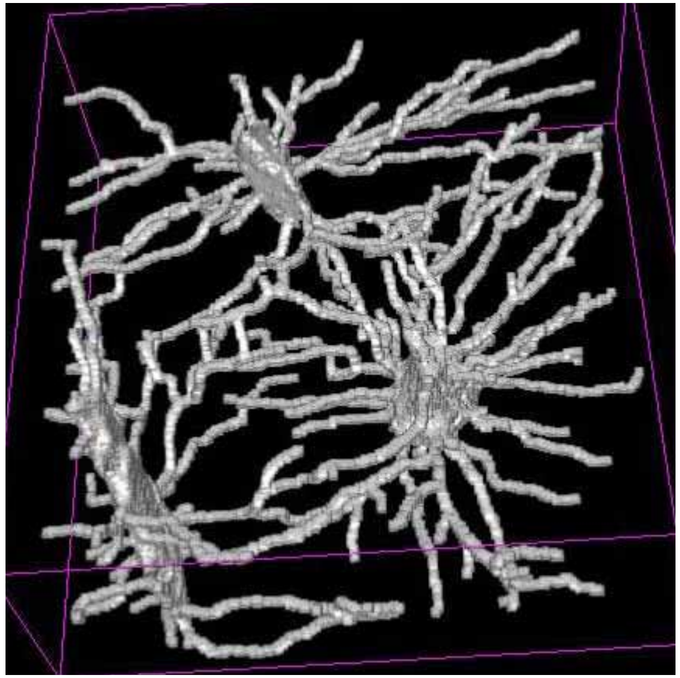
- Derivation of a new 3D method based on 3D geodesic voting



Thresholding after Non-linear filtering



Minimal path :
More canaliculi are detected
The paths are connected



Segmentation of 1-5 cells and their canaliculi

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[Zuluaga et al., Phys Med Biol 2014]

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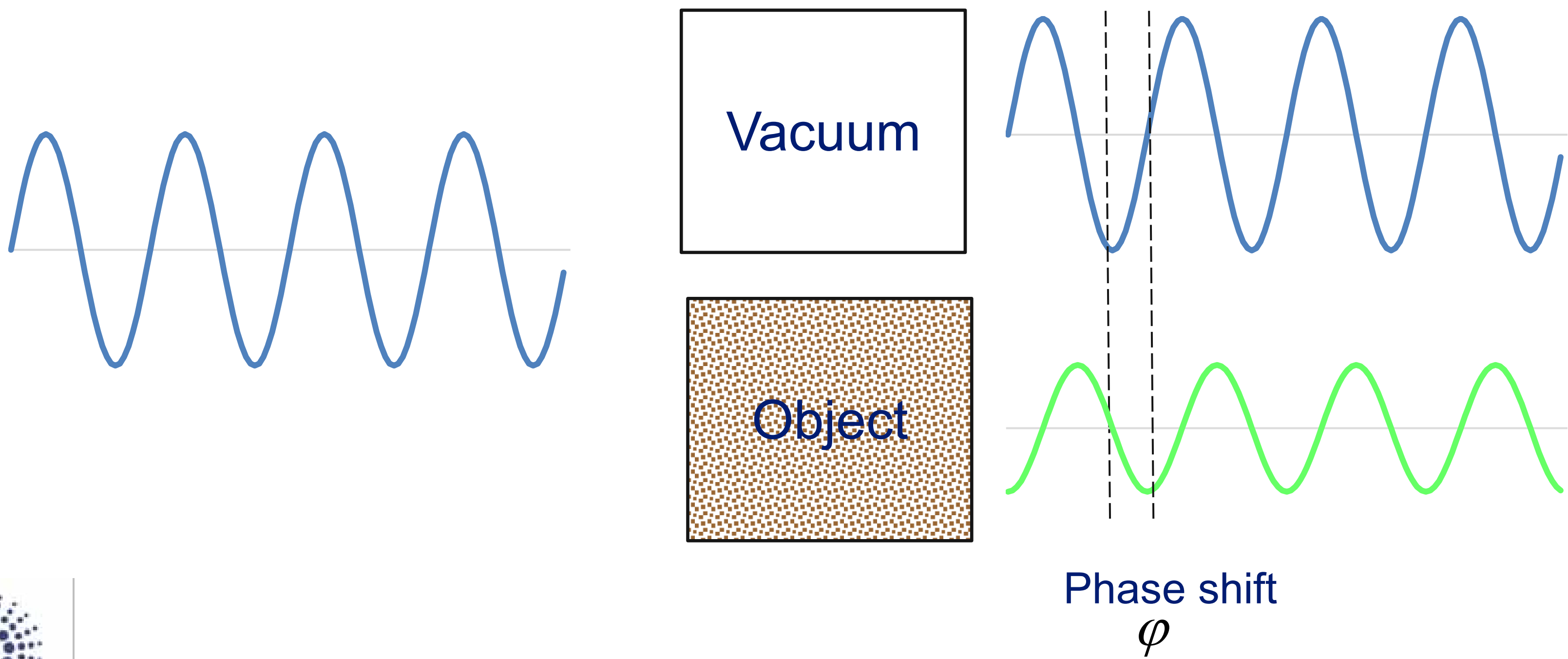
Phase CT

Applications in bone research

SYNCHROTRON CT : ABSORPTION vs PHASE

- Physical principle of X-Ray CT : Attenuation
  - Map of linear attenuation coefficient μ
  - Well adapted for tissue with high attenuation (like bone)

- Another mode of contrast : Phase contrast



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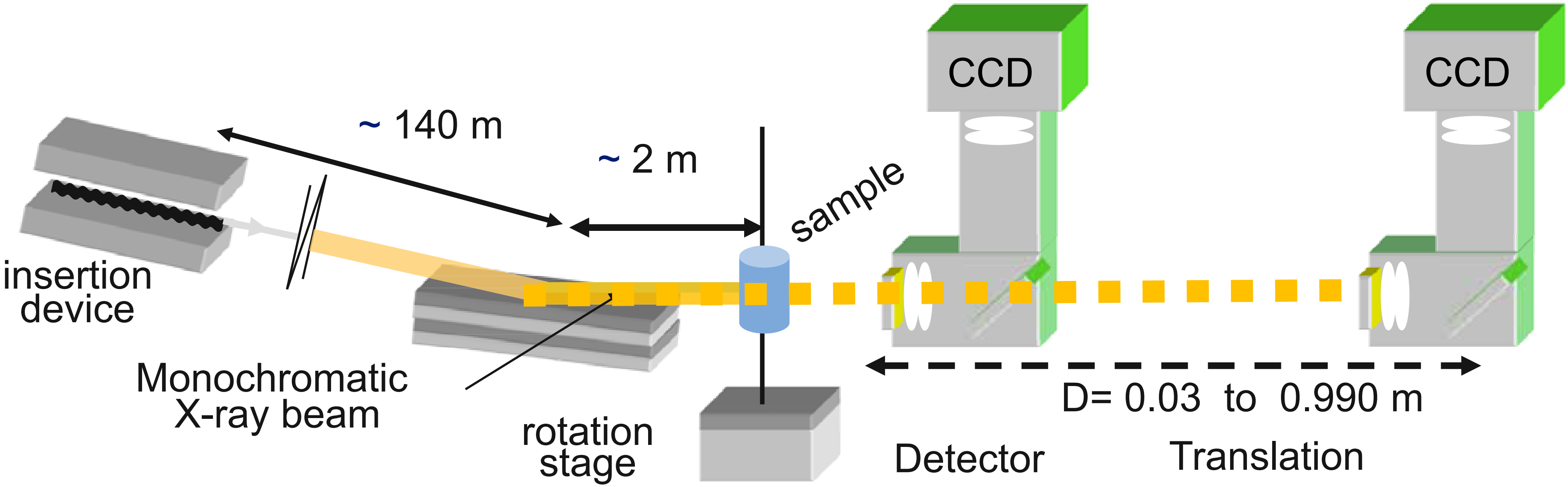
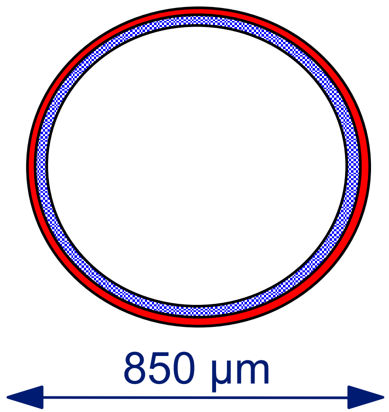
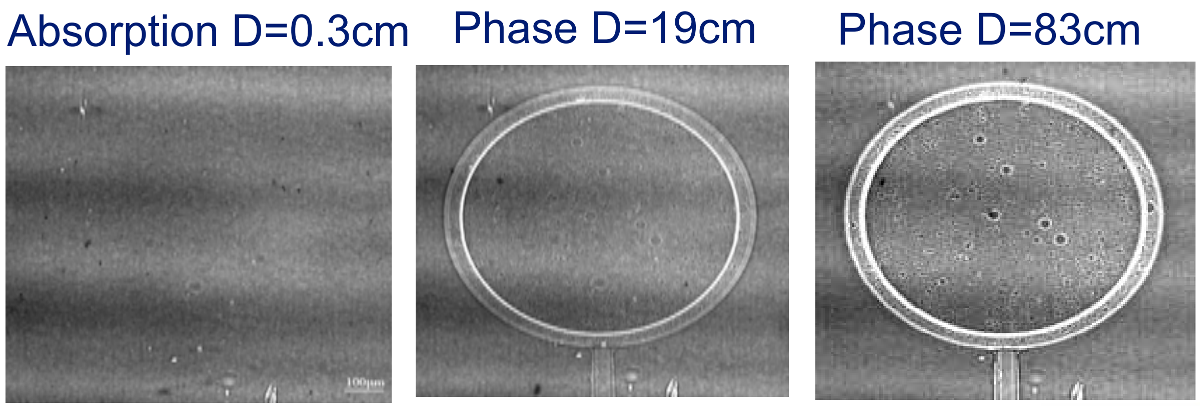
SYNCHROTRON PROPAGATION BASED PHASE CT :

- A coherent source propagation generates phase contrast







[Cloetens et al., J. App Phys, 1997]

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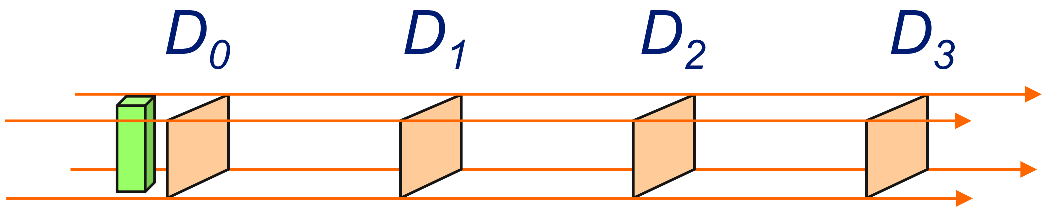
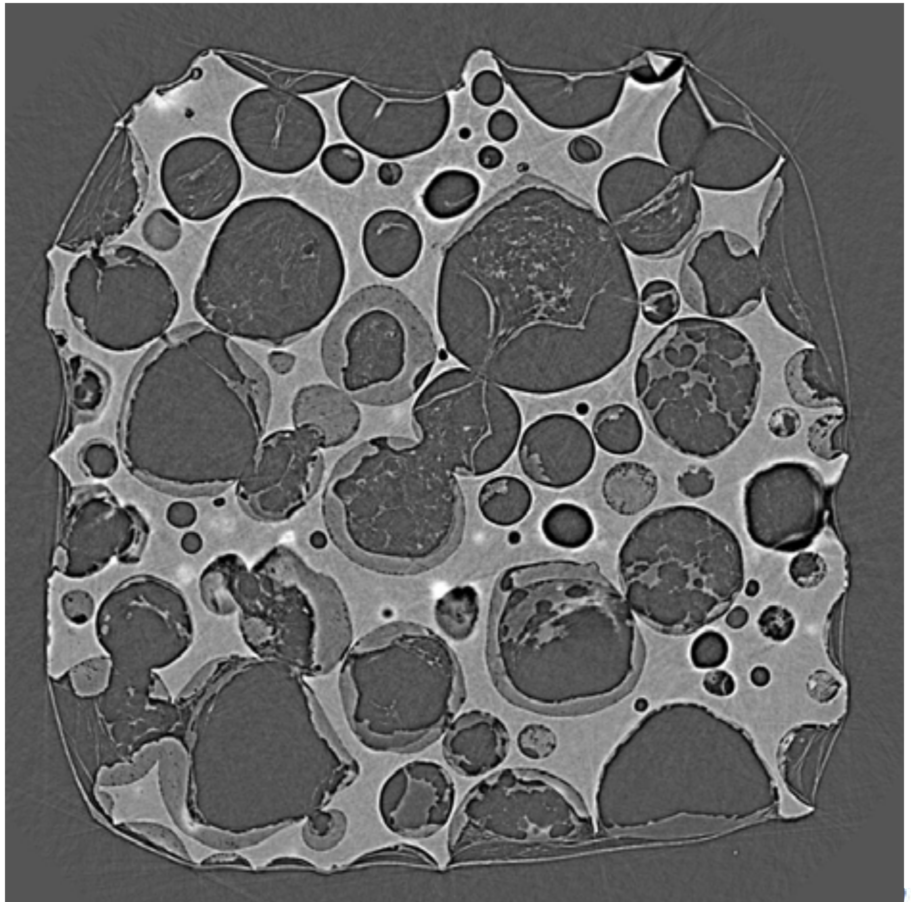
PHASE CONTRAST TOMOGRAPHY

- Phase contrast & tomography
  - Repeat CT acquisitions at the different distances





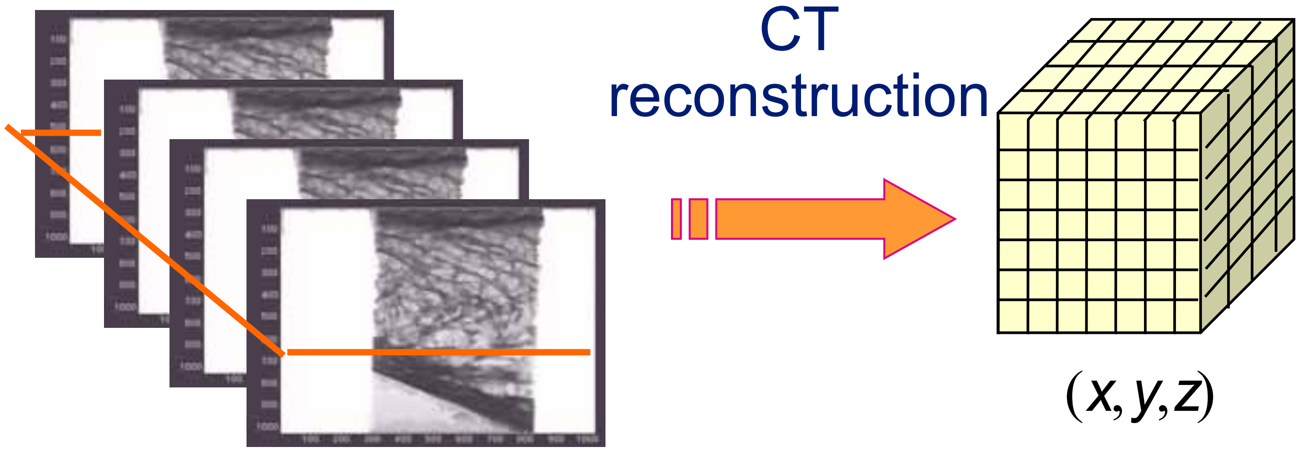
- Reconstruction
  - Usual FBP algorithm (Filtered Backprojection Algorithm)
  - $\mu(x,y,z) + d \quad ^{2}(1-\delta)(x,y,z)$

  [Cloetens et al., J. App Phys, 1997]

  - Edge enhancement effect

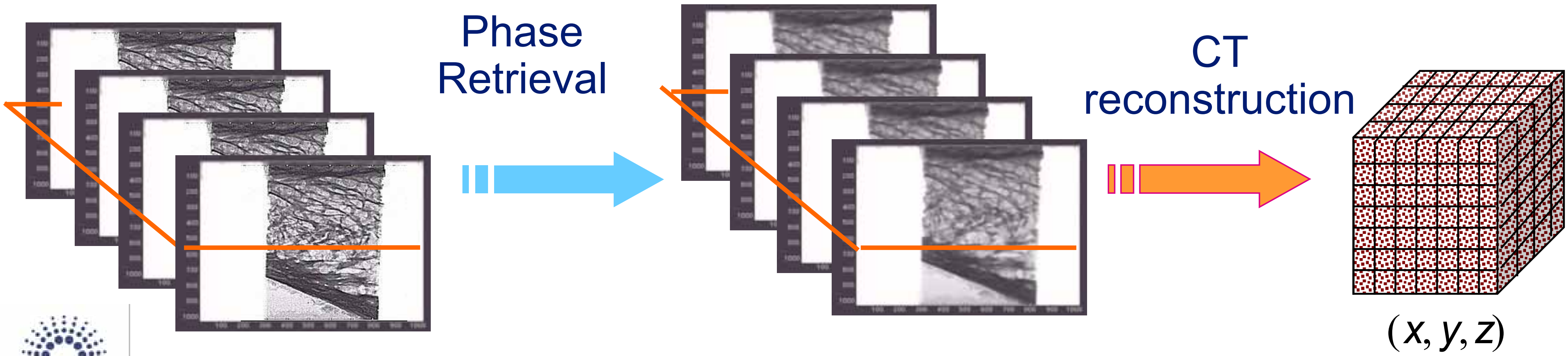
(QUANTITATIVE) PHASE TOMOGRAPHY

- Absorption CT



- Phase CT

  (one or several distances)



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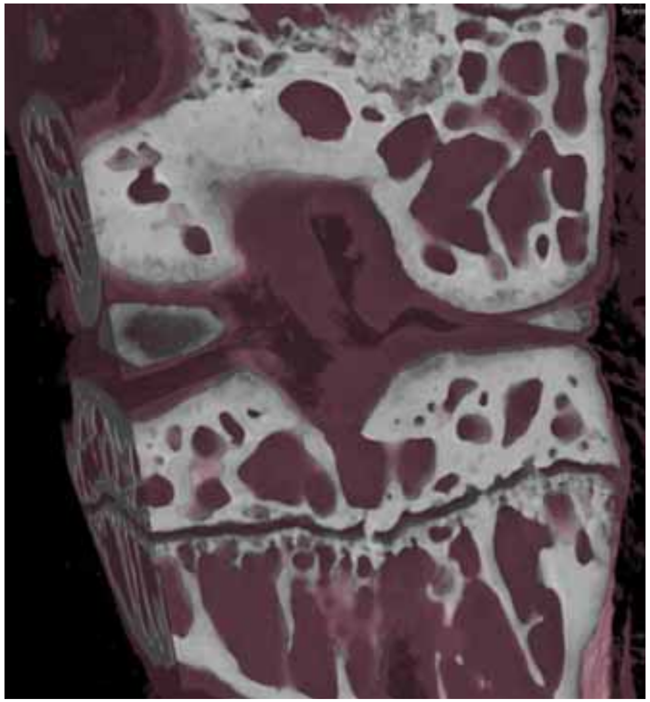
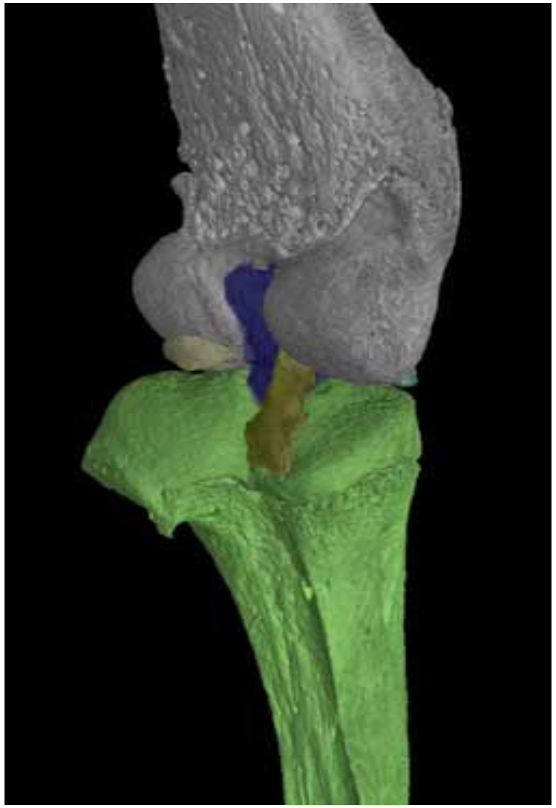
Phase CT

Applications in bone research

PHASE RETRIEVAL

- Extension of the previous approach to heterogeneous objects *[Langer et al., Optic L,et 2012] [Langer et al., Phil Trans Royal Soc, 2014]*
- Taking into account the non linearity of the problem *[Davidoiu et al., Optics Express, 2012]*





[Langer et , ISBI 2012]

- Work in progress : solving the joint phase retrieval/CT problem

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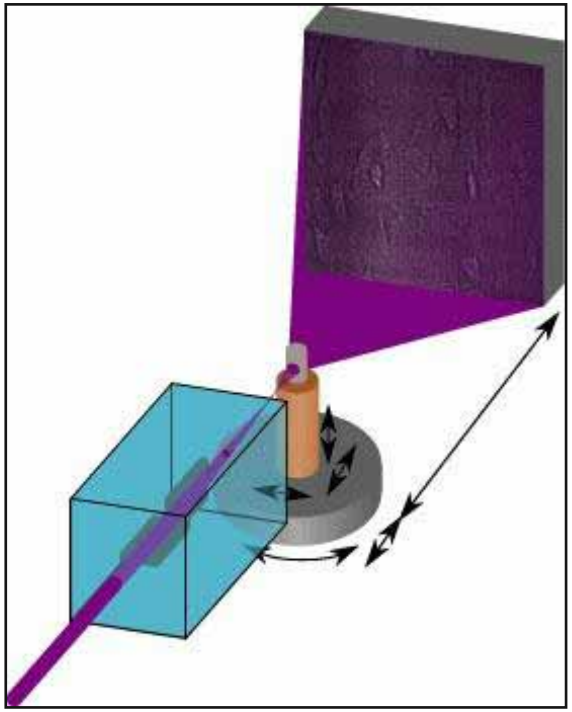
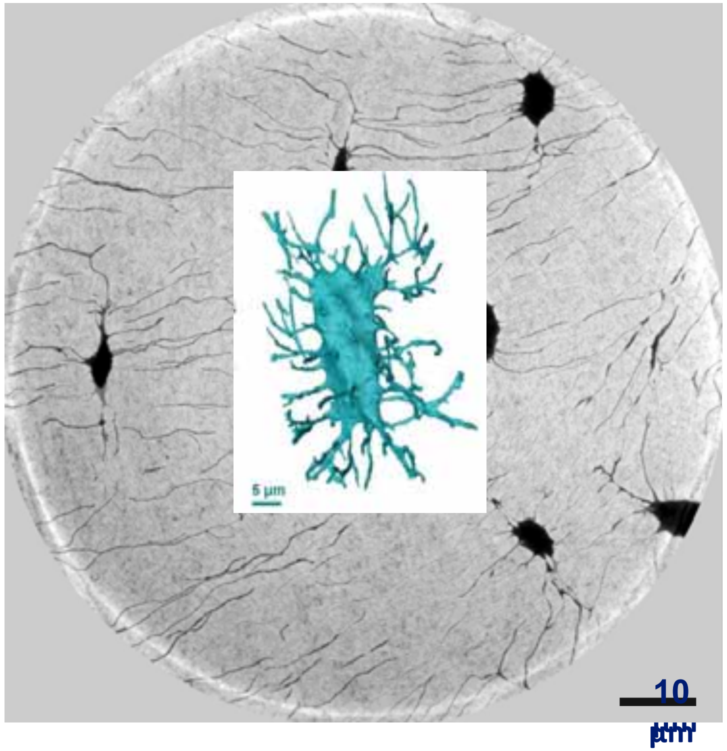
Phase Contrast Imaging of Bone at nano scale

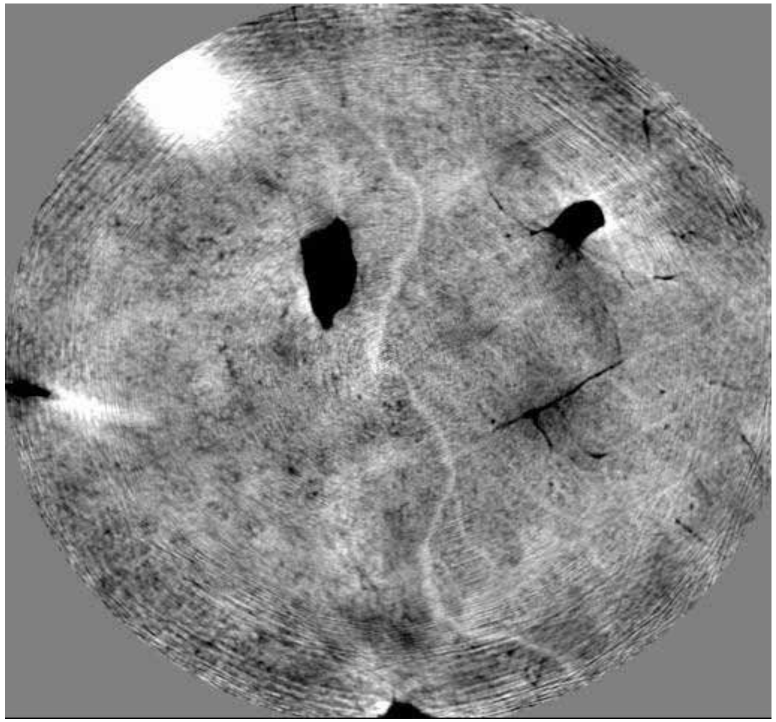
- Magnified Phase nano CT
  - Divergent X-ray beam
  - Acquisition of 4 data sets at 4 distances

Images voxel size : 60nm

[Langer et al., PlosOne, 2012]







- New device 2015 : ID16A, isotropic spatial resolution up to 20nm

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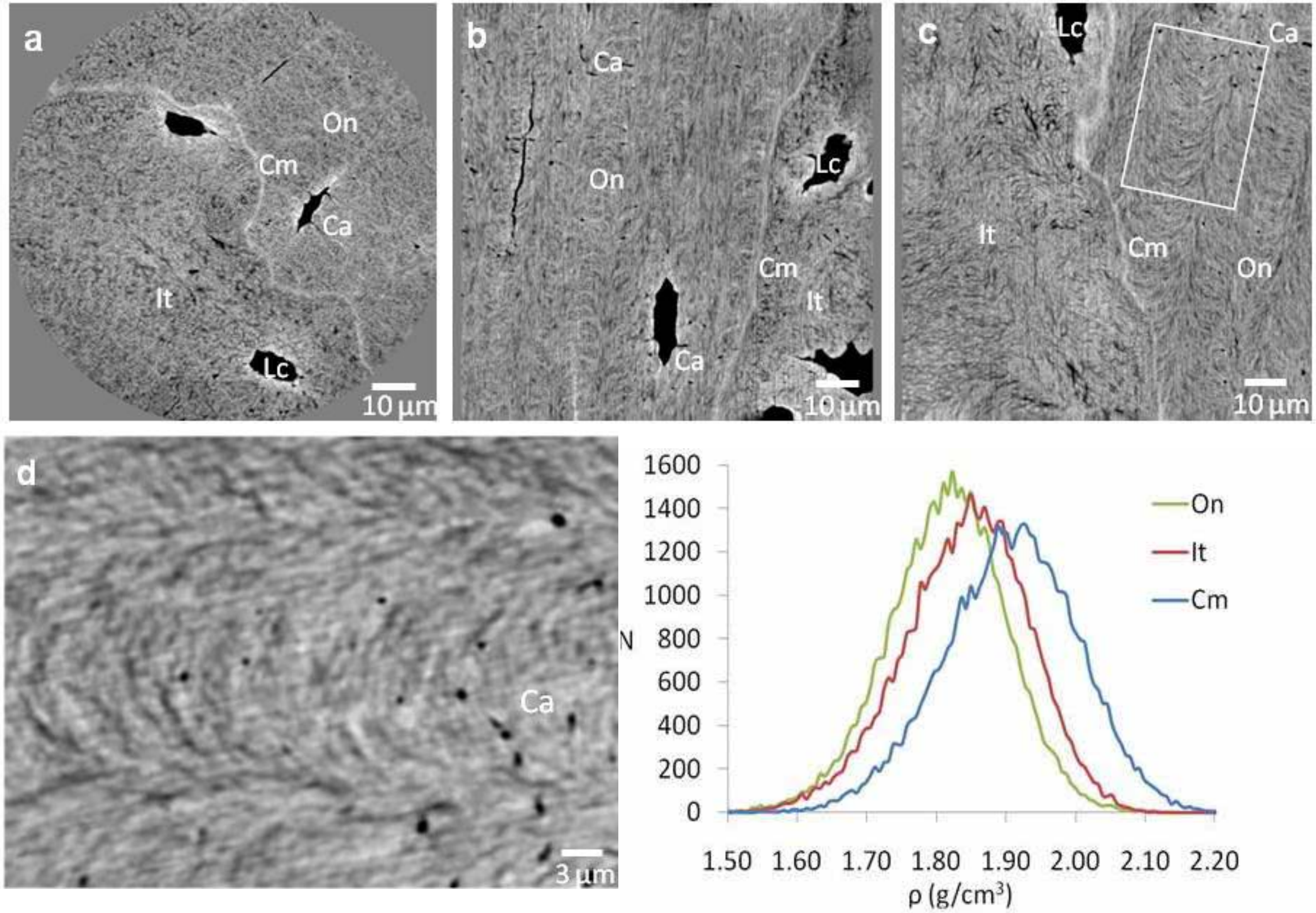
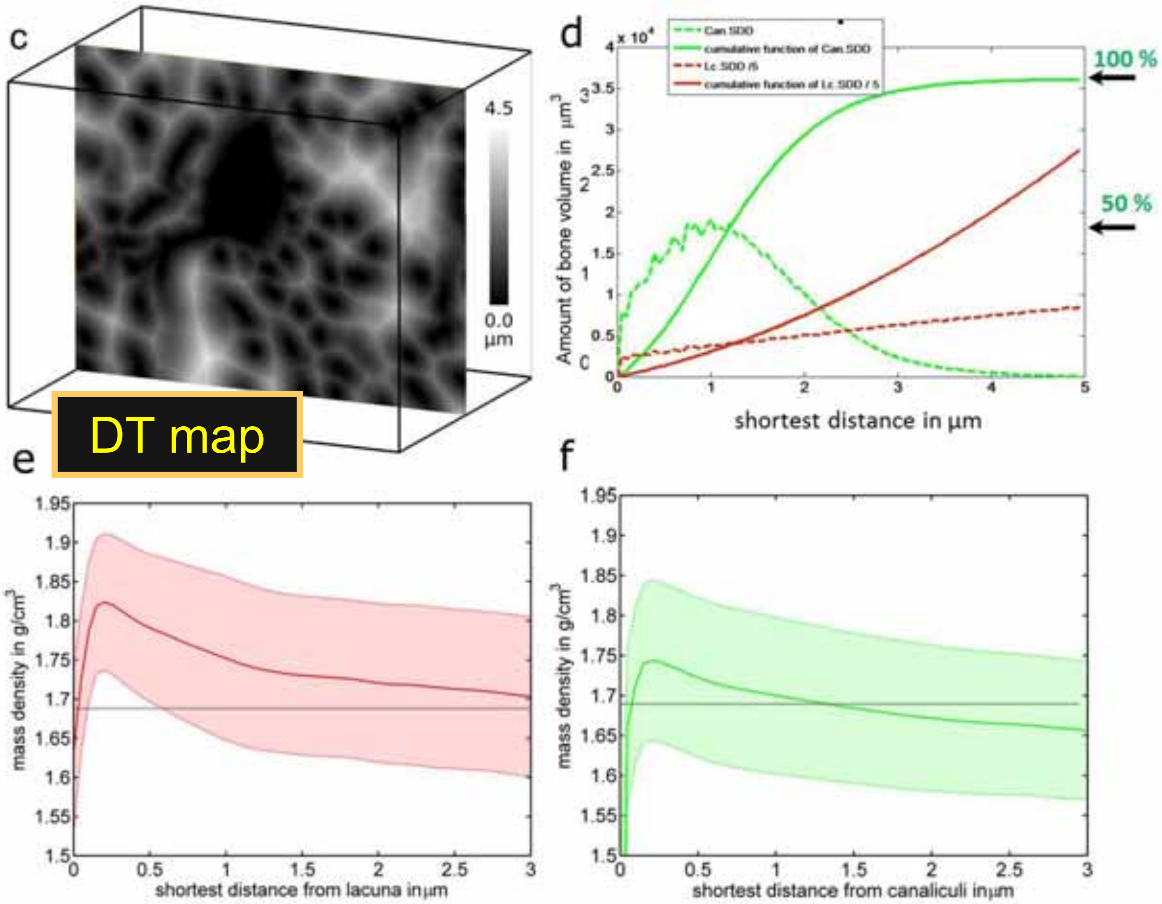
QUANTIFICATION of ELECTRONIC DENSITY

- Relationship between δ and ρ $1.3\,10^{6} \quad ^{2} \quad \Leftrightarrow \quad E^{2} / 1.99\,10^{4}$



[Langer et al., PlosOne, 2012]

Mineralization in the LCN



c) DT : shortest distance from each point of the matrix to the LCN.

d) Histogram of the DT : Green : only canaliculi, Red : only lacunae

50 % of bone tissue is within ≈ 1.2 µm from canaliculi.

e) and f), mean density and std error bands as a function of the shortest distance to the osteocyte lacuna (Red) and canaliculi (Green). Gray : mean bone tissue density of the entire ROI.

-> mass density gradient at the vicinity of the lacunar and canalicular boundary

-> support recent discussions about the ability of the LCN to remodel their environment in terms of mineral deposition or resorption.

[Hesse et al., JBMR 2014]

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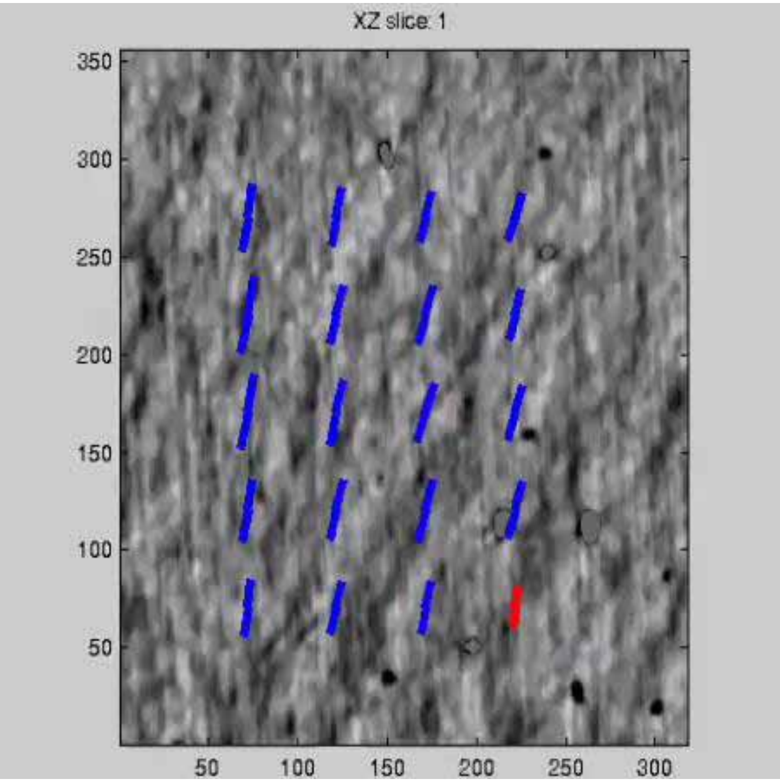
COLLAGEN FIBERS

- Extraction of fiber directions from PCT images



[Varga et al. Biomaterials 2013]

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New device at ESRF : X-ray nano-CT on ID16A

- ESRF ID16A new long beamline for X-ray nano-CT with high resolution

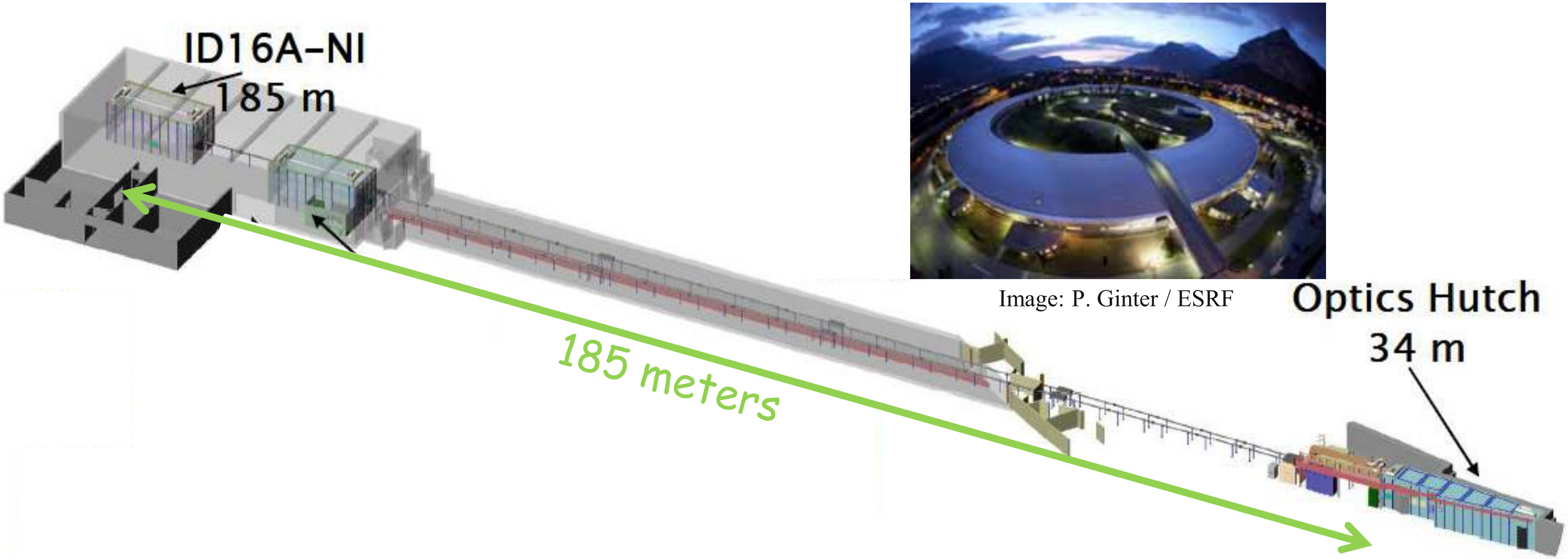


Image: P. Ginter / ESRF

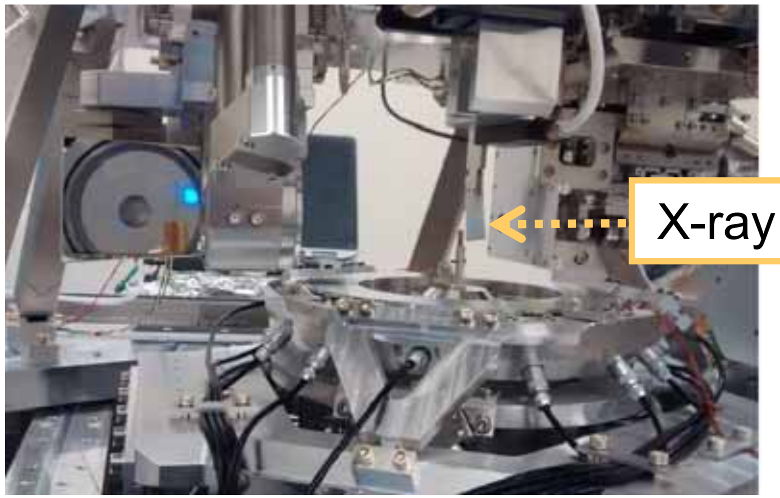
(1) Two beam energies: 17keV and 33keV

(2) Long beamline and few optics: better coherence

(3) Nano-focused beam by KB mirrors: high resolution (up to 15nm)

(4) Nano-positionning of the sample: high resolution

(5) High resolution imaging detector



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CONCLUSION

- 3D X-ray CT imaging
  - allows to span a large range of spatial resolution with new contrasts
  - Quantitative CT : more challenging
- Challenging data processing problems
  - Phase reconstruction : non linear inverse problem
  - Challenging Image segmentation : canaliculi, osteons…
  - Image quantification : canaliculi, collagen fibers in 3D

ACKNOWLEDGMENTS

- CREATIS
  - Cécile Olivier, Max Langer, Pierre Jean Gouttenoire,
  - Stefania Nuzzo, Zolt Peter, Alexandra Pacureanu, Pei Dong, Maria Zuluaga
- ESRF
  - Elodie Boller, Peter Cloetens, José Baruchel
- Collaborators
  - Georges Boivin, Laurence Vico, MH Lafage-Proust, Pascal Laugier, Quentin Grimal, Kay Raum

  et al…