3D BONE CELLS QUANTIFICATION FROM 3D MICRO-CT IMAGES

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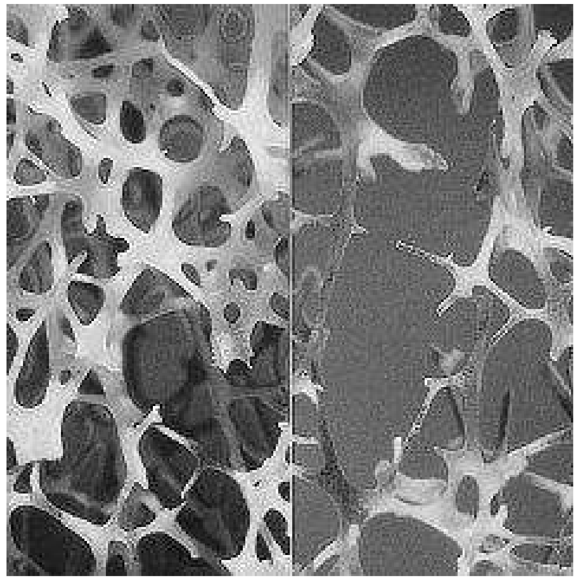
Background

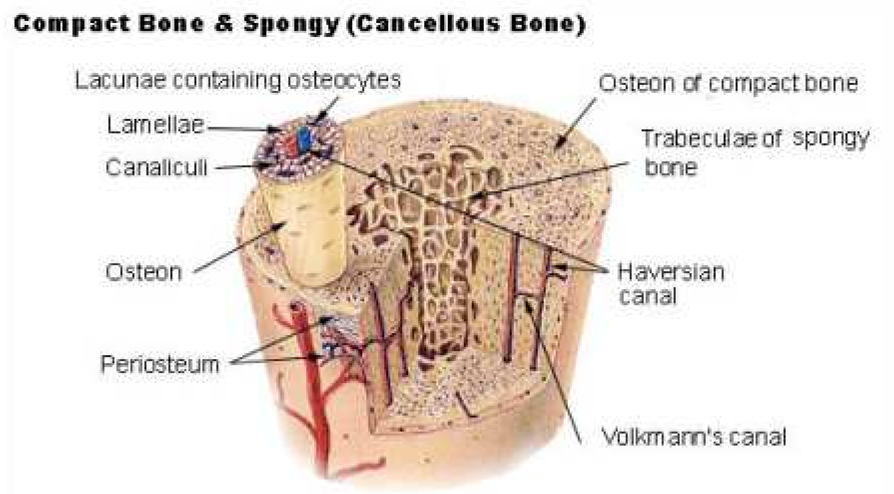
- over **200 million** women have osteoporosis

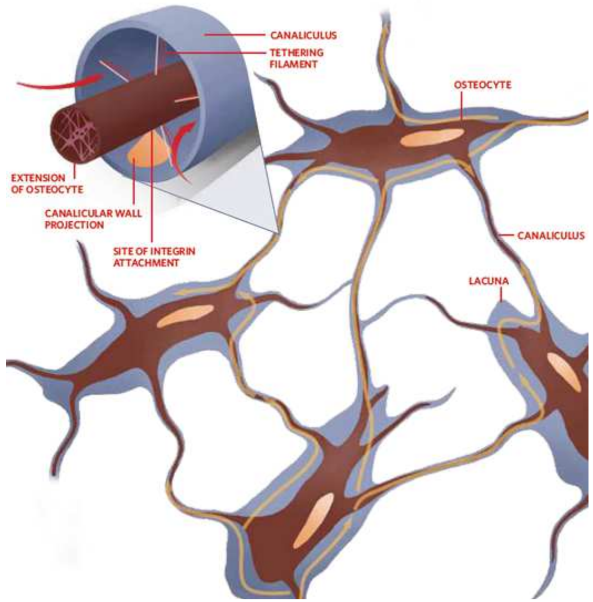
- Silent disease
  - Not fully understood
  - Difficult to predict in early stage



Background

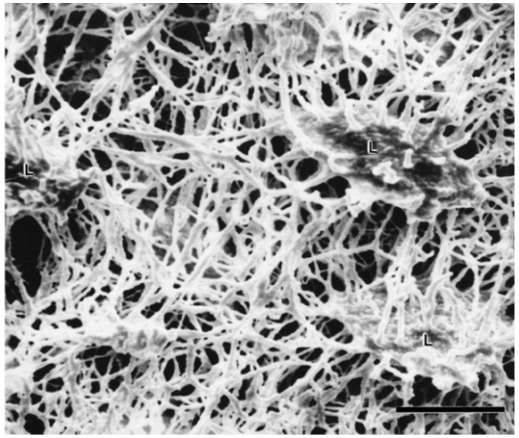
- Osteocytes
  - The most numerous bone cells
  - Fundamental role in bone remodeling
- Lacuno-canalicular network (LCN)
  - Encapsulating the osteocytes
- Imaging
  - Deeply embedded in bone matrix
  - Highly complex network
  - Lacunae density ~ 20000 / mm^3
  - Canaliculi : diam. ~ 100-500 nm



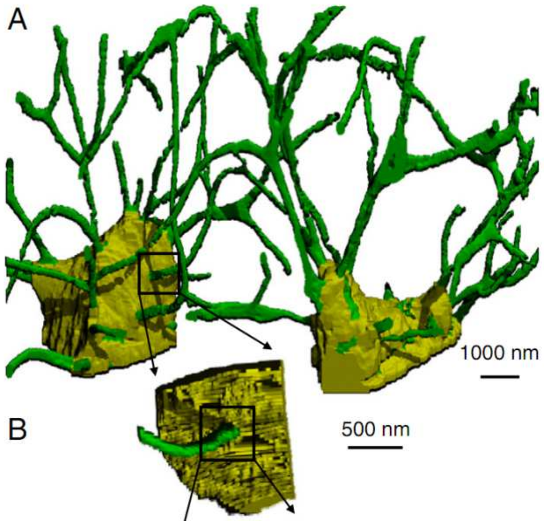


State of the arts

- 2D imaging methods
  - Optical microscopy, SEM, TEM …
  - Drawbacks : sensitive to slice cutting,
    manual quantification
- 3D imaging methods
  - Confocal microscopy [Sugawara 2005]
  - X-ray Nano-CT [Van Hove 2009]
  - Synchrotron X-ray CT [Peyrin, 1998] [Thomas, 2010]



SEM



FIB/SEM

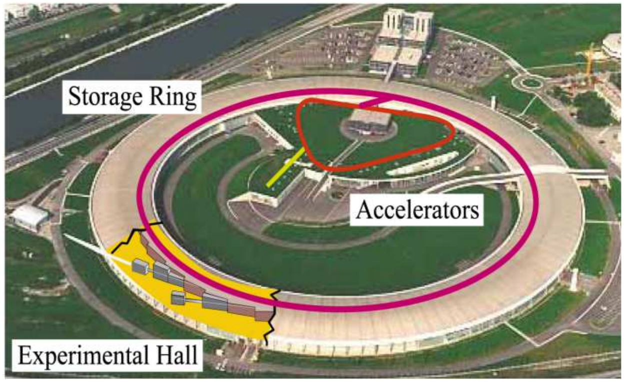
Purpose of the work

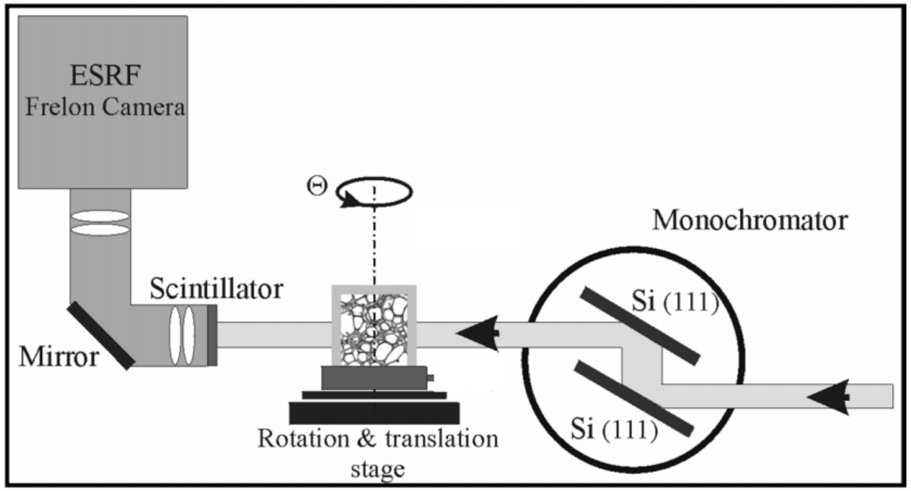
- Limitations:
  - Image quality
  - Lack of statistics on 3D descriptors

- Aim of the work :
  - propose an automated method based on synchrotron radiation micro-computed tomography (SR-μCT) to quantify the distribution of the 3D morphology of osteocytes lacuna properties
  - Application to female femoral cortical bone.

Material and methods

- Synchrotron micro-CT at ESRF
  - 3D parallel beam micro-CT, ESRF, Grenoble [Salomé, MedPhys, 1999]





- Advantages
  - High spatial resolution → from 10µm down to 0.3 µm
  - High intensity → high signal–to-noise ratio
  - Parallel beam → no image magnification
  - Monochromatic beam → no beam hardening

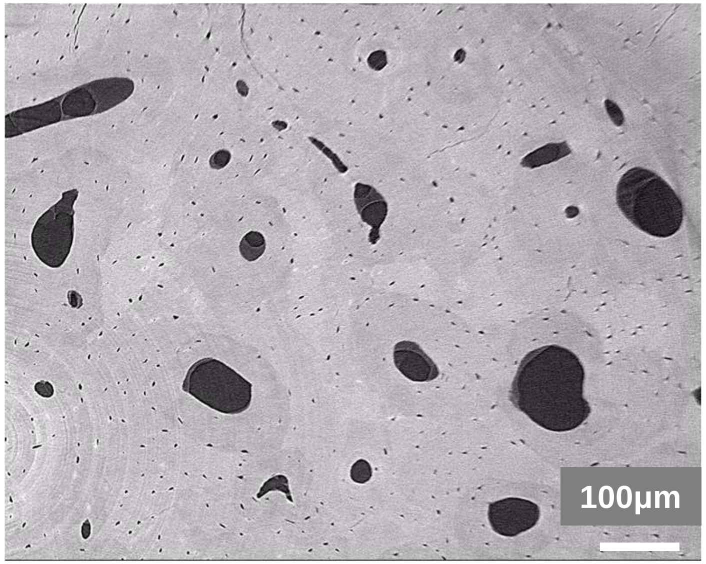
Material and methods

- Samples
  - Human cortical bone: femur, Sex: Female
  - Number:12 Samples, Age: 79

- Image Acquisition conditions
  - Voxel size: 1.4µm, FOV: $2.9 \times 2.9 \times 1.4 mm^3$
  - Energy: 25KeV, Projections: 3000

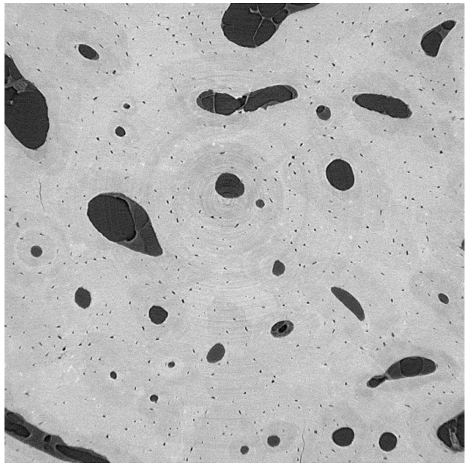
- 3D image analysis
  - Segmentation
  - Extraction of 3D descriptors
  - Challenges : Image size $2048 \times 2048 \times 1024$

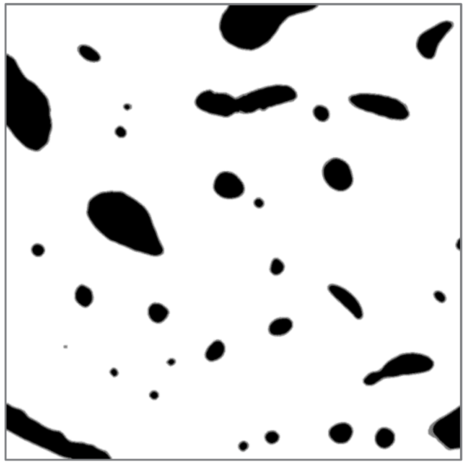
    Large population of cells: $10^5 \sim 10^6$

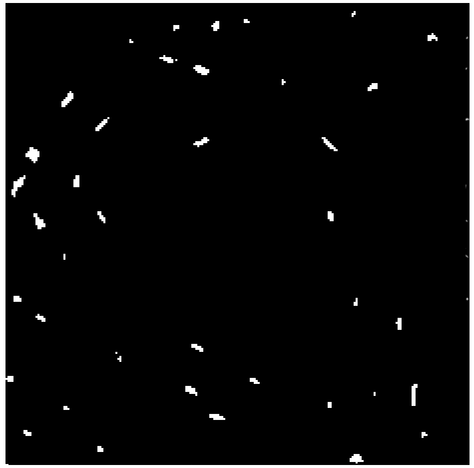


Reconstructed image

Segmentation and labeling of lacuna



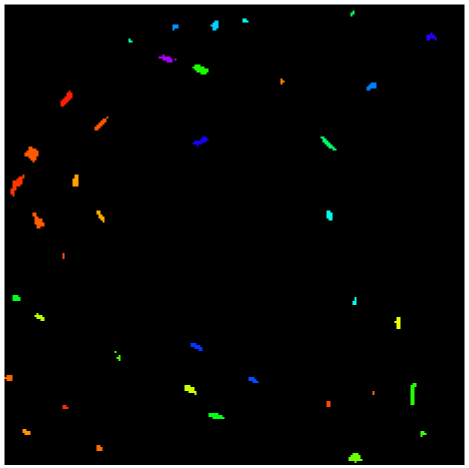




Gaussian Lowpass Filter

Simple Threshold

Hysteresis Thresholds



Connected Component Analysis

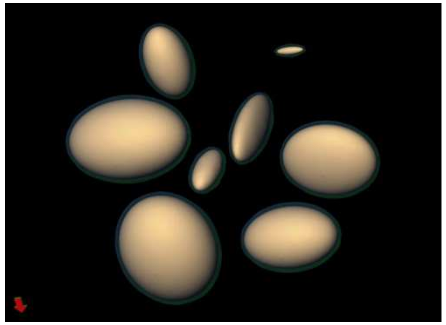
#13000 lacunae

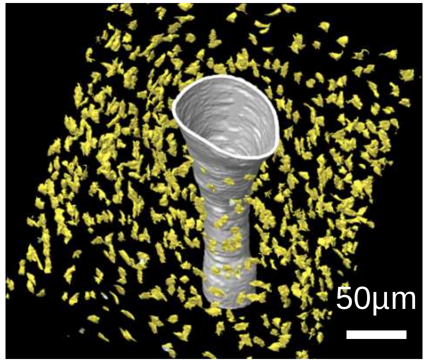
Lacunae Descriptors

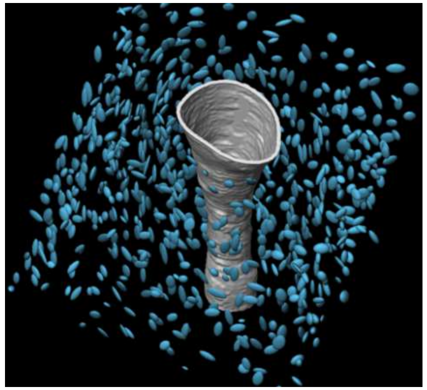
- **Moment based method**

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

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







Volume	μ_{000} : zero−th order moment
Axes lengths	$L_1 \quad L_2 \quad L_3$
Orientation	$V_1(\theta_1,\varphi_1) = \begin{pmatrix} \cos\theta_1 \sin\varphi_1 \\ \sin\theta_1 \sin\varphi_1 \\ \cos\varphi_1 \end{pmatrix}$
Anisotropy	$L_1(O_n)/L_2(O_n)$ *and* $L_1(O_n)/L_3(O_n)$

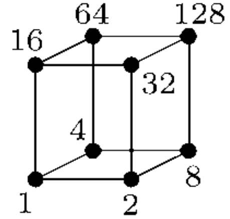
Lacunae Descriptors

- Intrinsic Volumes
  - The intrinsic volumes are important characteristic functions serving as a basis of object features
- Efficient computation
  - 3D Crofton formula

$$\frac{1}{2}V_{3-k}(X_n) = \int_{\mathcal{L}^k} \underbrace{\int_{\perp L} \chi(X_n \cap (\mathrm{L}+\mathrm{y}))d\lambda_{\perp L}(y)}_{p^k_{X_n}(L)}\, d\mu(L),$$

  - Discretized Crofton formula

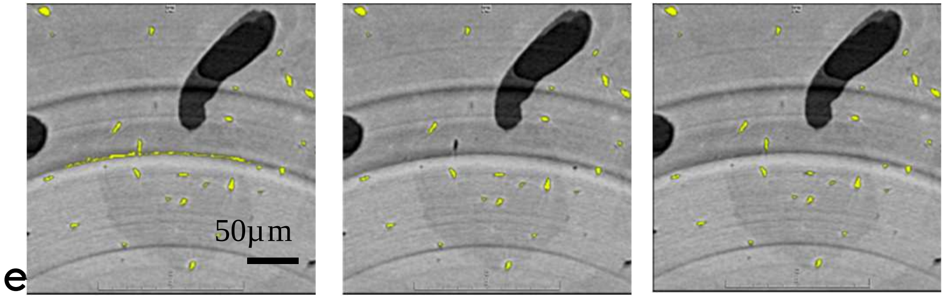
$$\hat{V}_{3-k}(X_n) = 2a^{3-k}\sum_{\ell=0}^{v} v_\ell^{(k)} h_\ell\,(X_n)$$

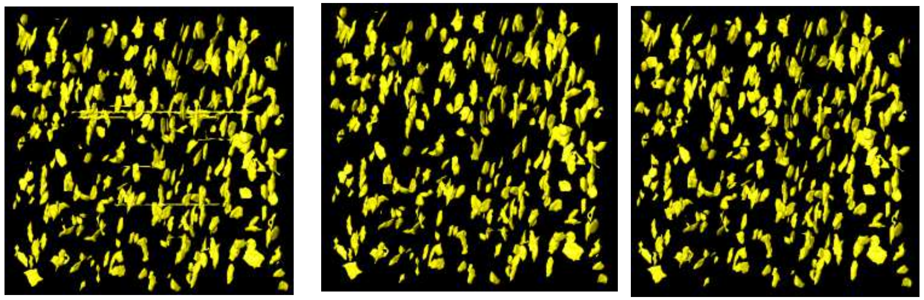


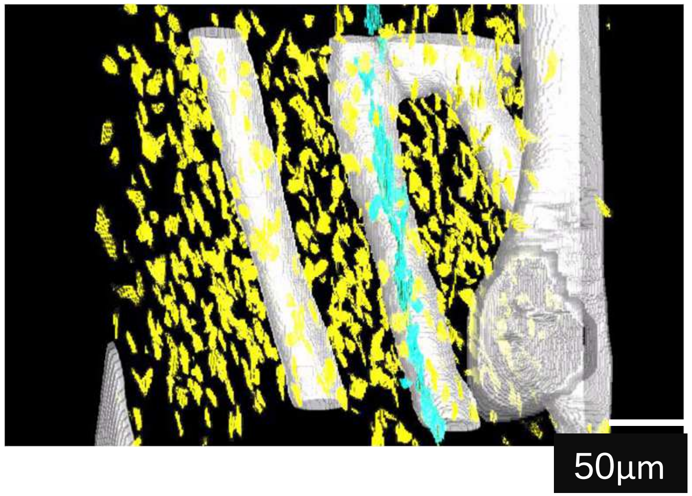
Surface	$Lc.S(X_n) = 2\hat{V}_2(X_n)$
Mean curvature	$Lc.M(X_n) = \pi\hat{V}_1(X_n)$
Eurler Number	$Lc.\chi(X_n) = \hat{V}_0(X_n)$
SMI	$Lc.f_{SMI}(X_n) = 12Lc.V(X_n)Lc.M(X_n)/Lc.S(X_n)^2$

Artifacts elimination

- Artifacts:
  - Ring artifacts
  - Micro cracks
  - Clustered and ill-shaped lacunae
  - Fragments…
- Elimination Criterion:
  - Volume size
  - Anisotropy: Length/Width
  - Mean curvature
  - Euler number
- Validation
  - "Manual" segmentation
  - Measurement of errors





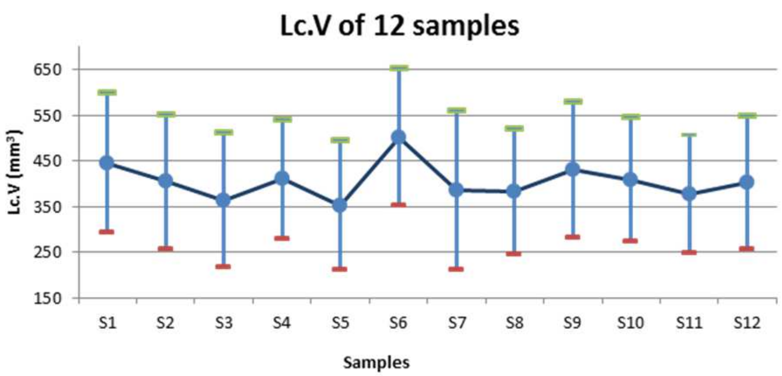


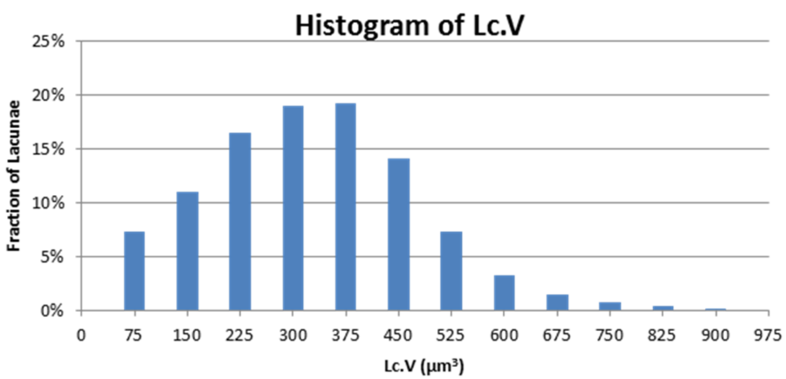
Lacunae Descriptors : results

- Application to 12 samples
- 13000 lacunae /sample data
- Fast quantification
  - ~ 20 seconds for each data
  - ~ 700 objects/s

TABLE 1 MEAN VALUES OF 12 SAMPLES

Descriptors	Mean±std	Descriptors	Mean±std
N.Lc	12953	Lc.L1 (μm)	18.82±4.75
BV(mm^3)	0.62	Lc.L2 (μm)	9.27±2.04
BV/TV (%)	90.2%	Lc.L3 (μm)	4.79±1.03
N.Lc/BV(mm^{-3})	20693.89	Lc.L1/Lc.L2	2.14±0.76
Lc.V (μm^3)	405.81±145.79	Lc.L1/Lc.L3	4.12±1.37
Lc.S (μm^2)	334.51±92.76	Lc.M	0.078
τ	92.8%	Lc.f_{SMI}	0.0033





Conclusion & Perspective

- Conclustion:
  - Automated method to extract direct 3D characteristics of the osteocyte lacunae geometry from 3D SR-μCT images at the micrometer scale.
  - Unbiased estimation of the lacunae parameters compared to 2D evaluation.
  - Efficient analysis over large populations.
  - Statistical results in agreement with existing reports.
  - Provide biologically relevant data to get a better understanding of the role of osteocytes in bone diseases.
- Perspective:
  - Deliver automated method for 3D descriptors on canaliculi network

Thanks for your attention!