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Ostéoporose - Cancérologie osseuse - Lyon

MINERALISATION OSSEUSE DE QUOI PARLONS-NOUS ?

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Minéralisation tissulaire

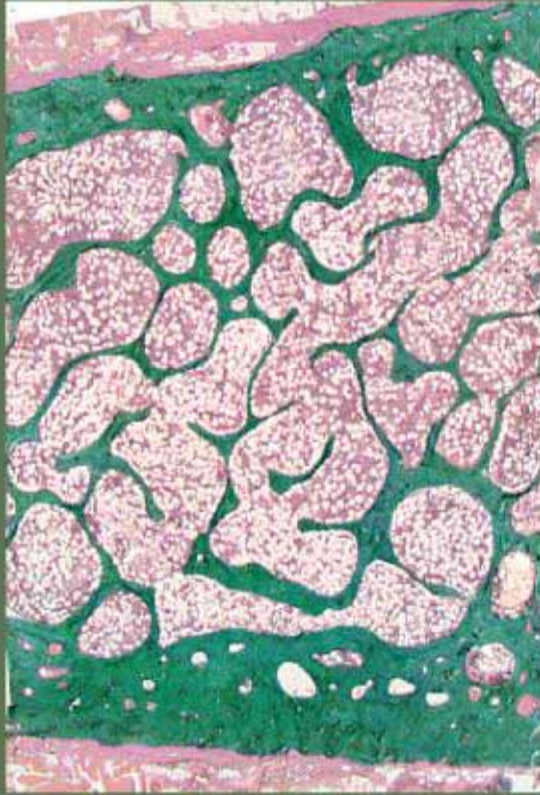
Dépôt d'une substance minérale dans une matrice organique extracellulaire. Chez l'homme c'est une **calcification** (phosphate de calcium normalement dans tissus osseux, cartilage calcifié, dents).

Des **dépôts pathologiques**, intra- et/ou extracellulaires, peuvent être de l'apatite, du pyrophosphate de calcium, de l'oxalate de calcium.

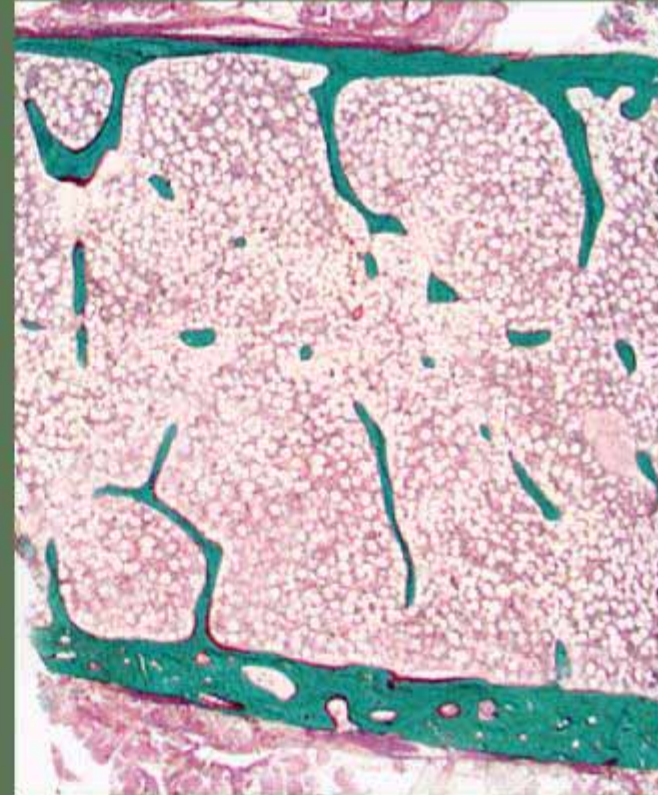
Ces dépôts pathologiques se rencontrent aussi parfois dans des tissus mous (**calcifications ectopiques d'origine métastatique ou dystrophique**).



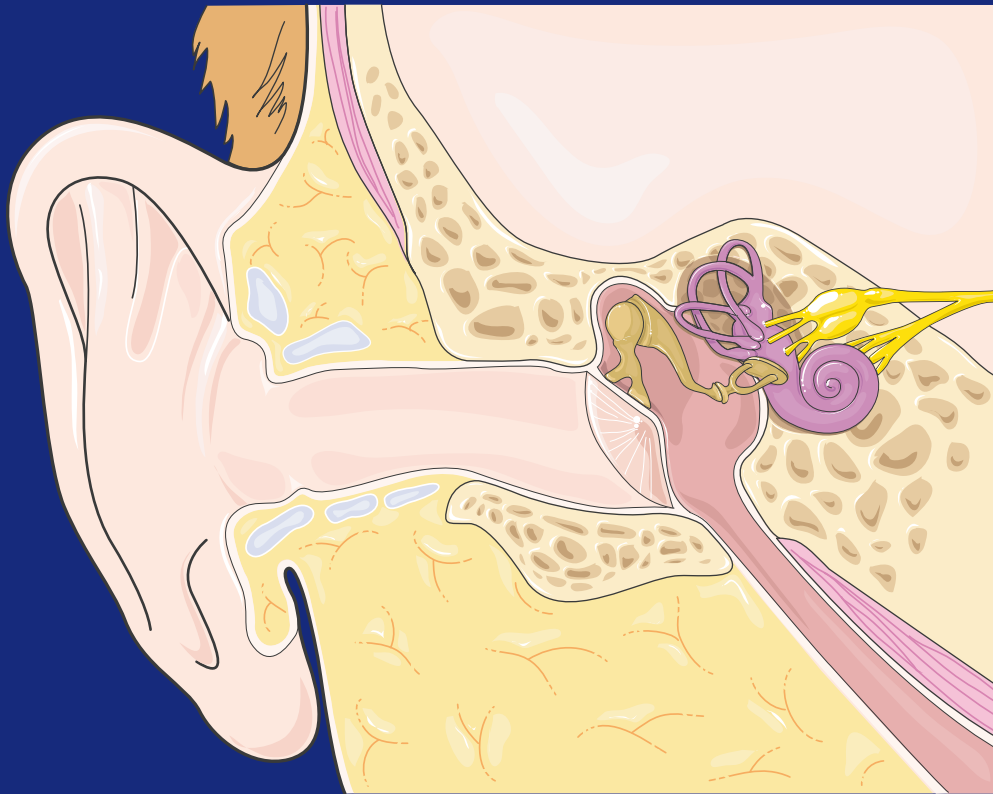
Osteoporosis - Histology



Control



Osteoporosis



Ossicles are more compact, more mineralized and harder than iliac cortical bone.

Bone quality of ossicles correspond to a tissue rapidly modeled after birth then not remodeled.

Bone quality of ossicles is well adapted to their major mechanical function (transmission of vibrations).

Biomaterials: titane or teflon.

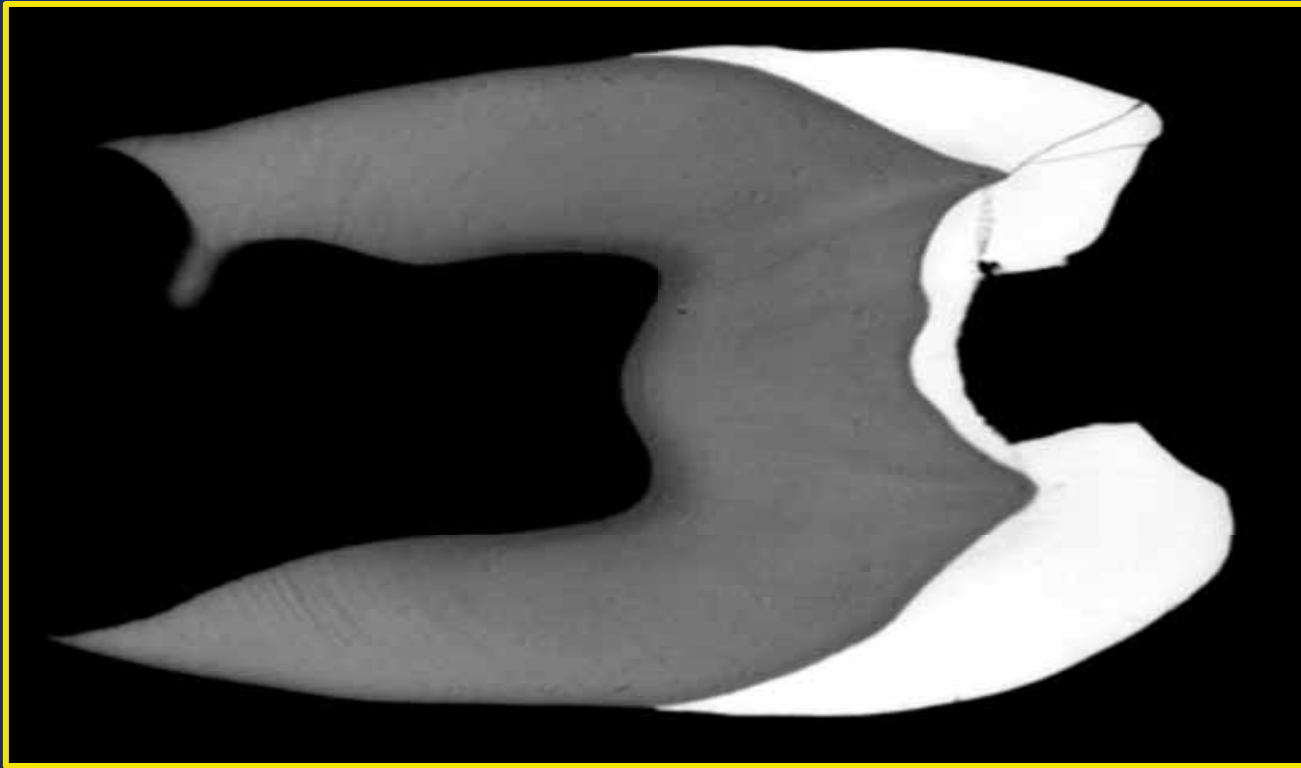
Duboeuf et al. 2015, Bone 73: 105-10.

Minéralisation tissulaire

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microradiographie
numérique d'une coupe
longitudinale de dent.

Variables reflétant la minéralisation

	(g/cm ³)	
	Dentine	Email
DMB ± SD	1.32 ± 0.09	2.22 ± 0.20
DMB Freq. Max.	1.37	2.29
HI	0.085	0.148

Microdureté (kg/mm²)

Dentine : 65.4 ± 10.4
Email : 274 ± 45.6

Minéralisation tissulaire

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Pyrophosphate de calcium (chondrocalcinose)



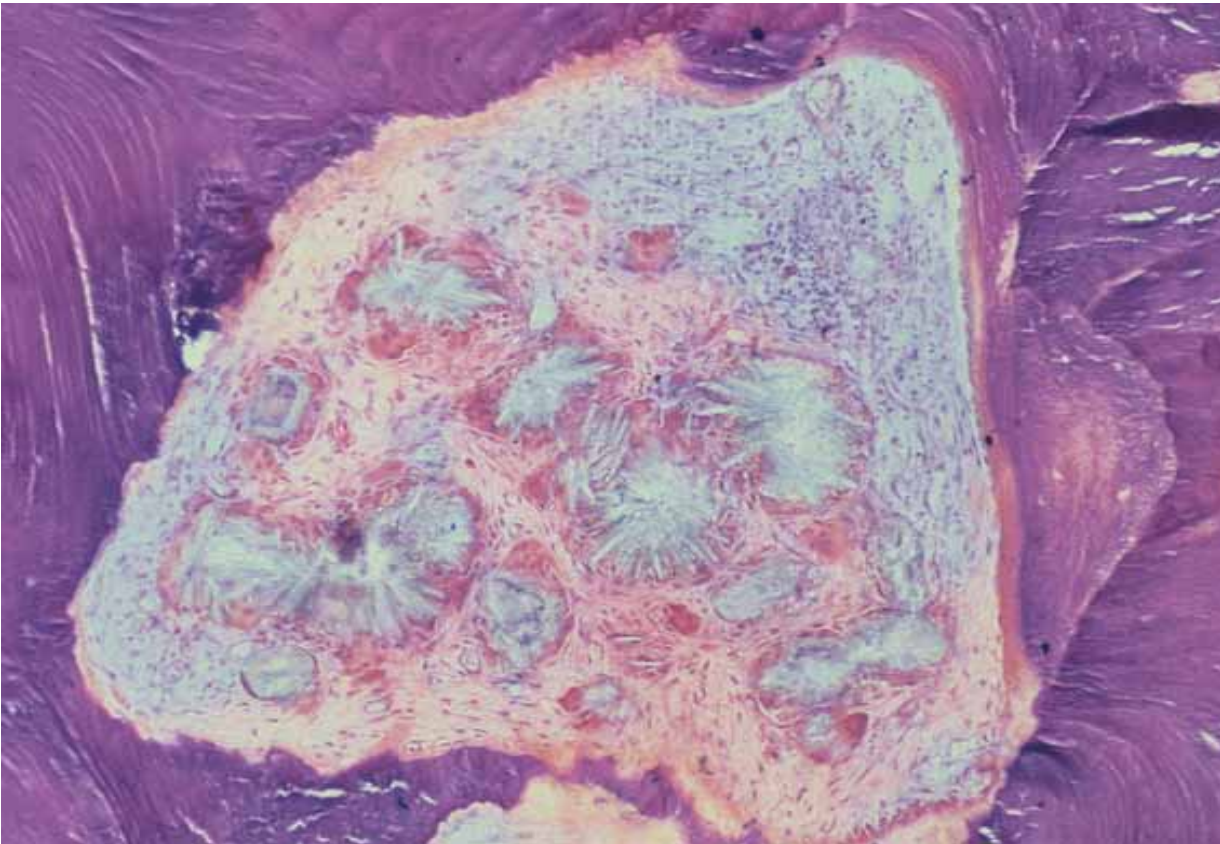
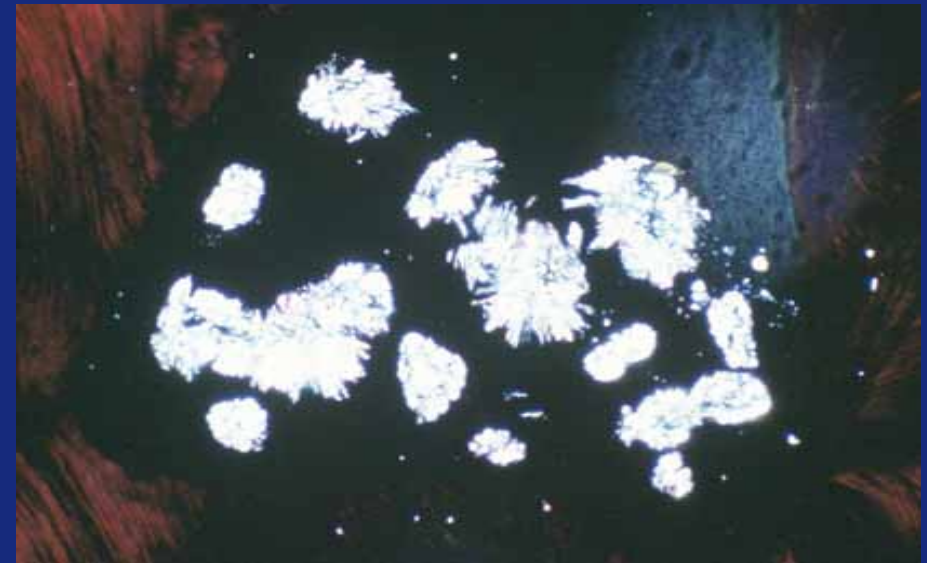
Bone oxalosis : mineral deposits

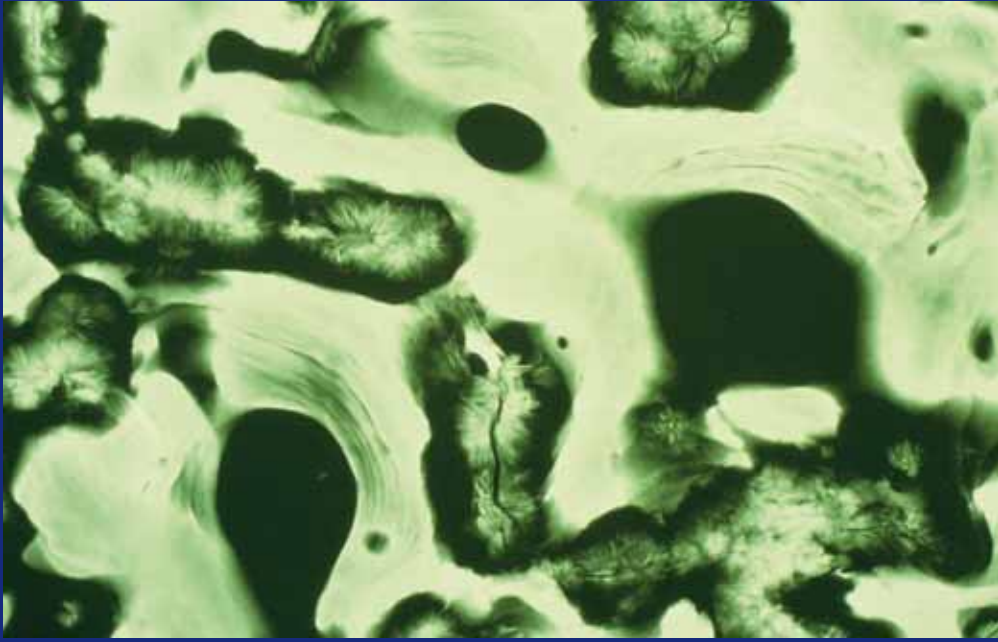
They are mainly situated in bone marrow space in the vicinity of or at a short distance from bone.

Bone mineral is constituted of biological apatite, the usual mineral component of bone tissue.

Pathological deposits are composed of more or less numerous birefringent crystals appearing as needle-like, elongated, straight-edged structures. These crystals are identified (X-ray diffraction + FTIRM) as calcium oxalate monohydrate (whewellite).

Bacchetta et al. 2015, Bone 81: 161-7; Bacchetta et al. 2015, Pediatr. Nephrol. 6p





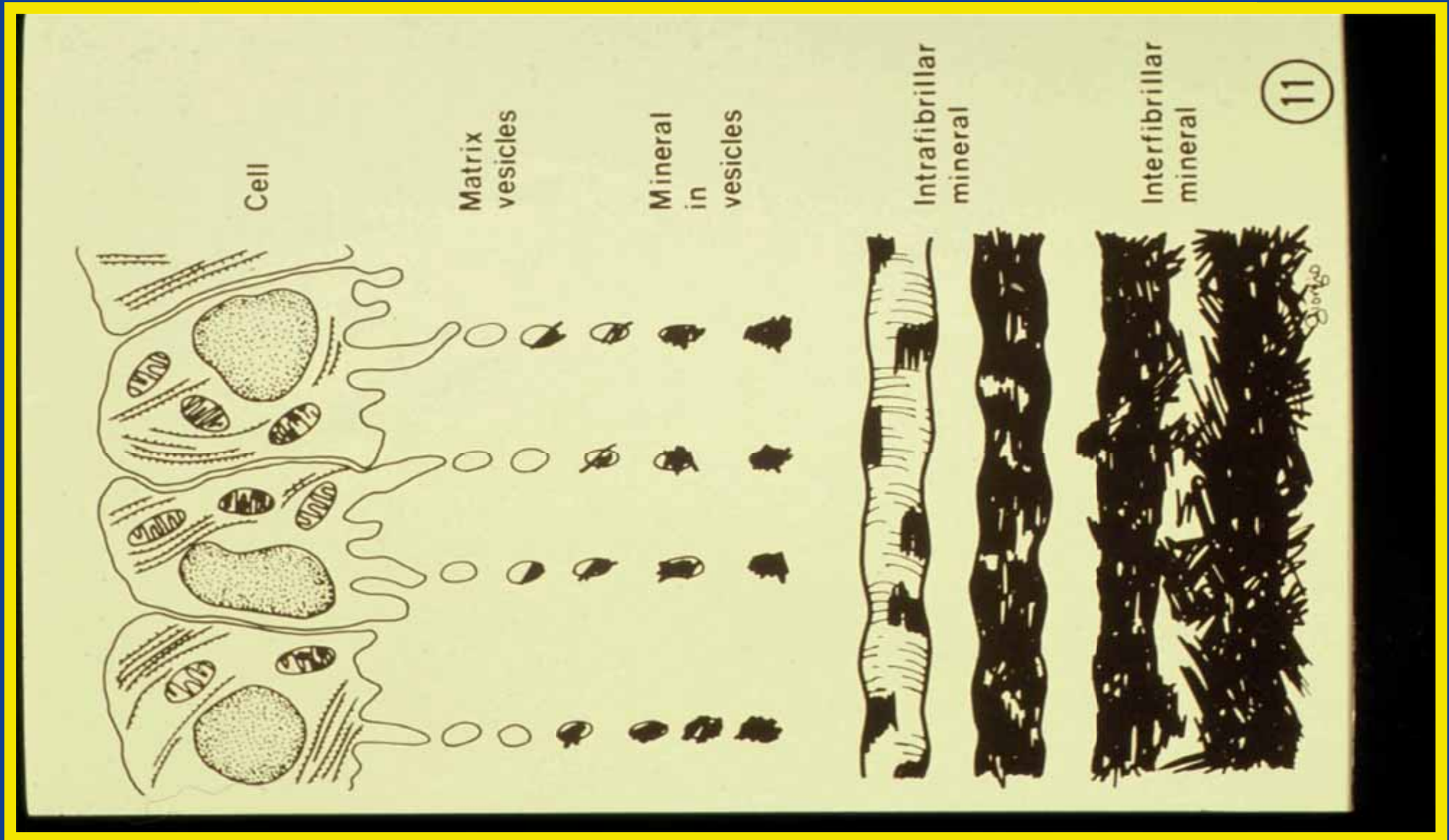
Bone Oxalosis: microradiographs (left) and electron micrographs (right)

Transformation de phase, pas réaction chimique

Une calcification commence à partir d'une solution **métastable** (concentration en Ca^{++} et PO_4^{---} supérieure à la normale mais pas suffisante pour créer une précipitation spontanée (**pas de nucléation homogène**)).

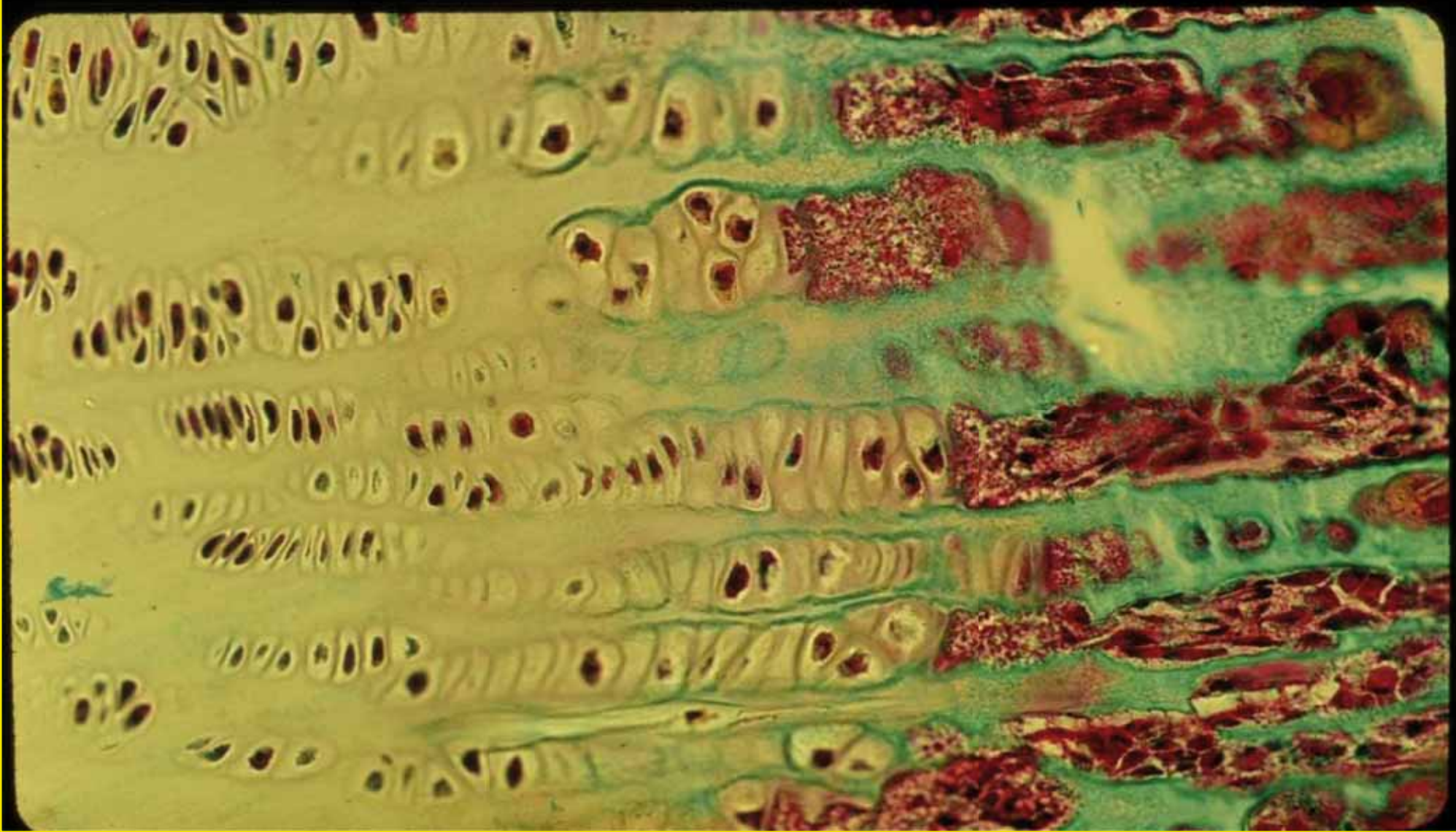
Il y a **nucléation hétérogène** avec des sites initiateurs (vésicules matricielles, collagène) permettant le passage d'une phase liquide (solution métastable) en phase solide (cristal d'apatite).

Mécanismes initiaux de la calcification



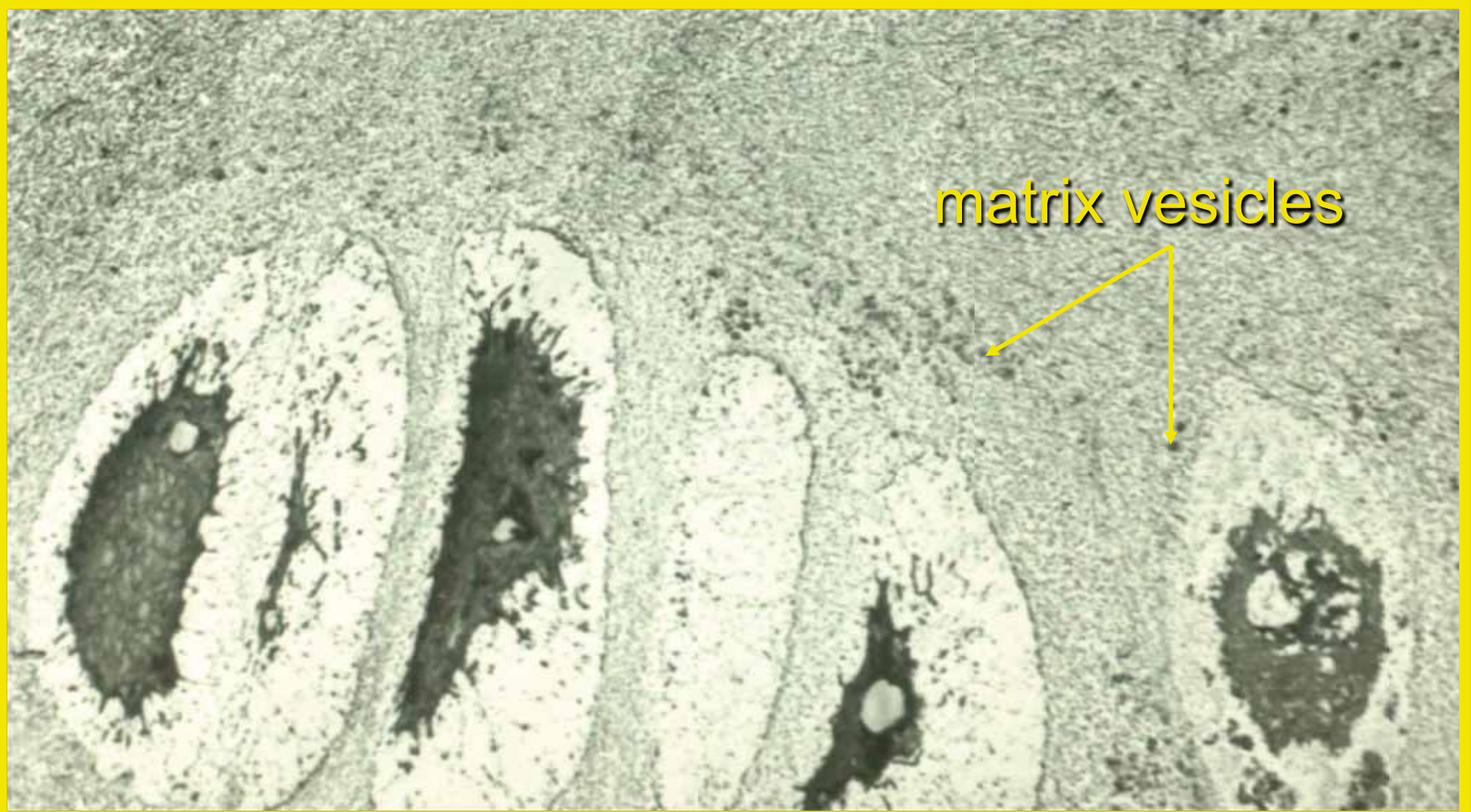
Boivin & Anderson 1996, In: Maladies Métaboliques Osseuses de l'Adulte, D. Kuntz ed, Médecine-Sciences Flammarion, Paris, pp. 36-48

Endochondral ossification (growth plate)



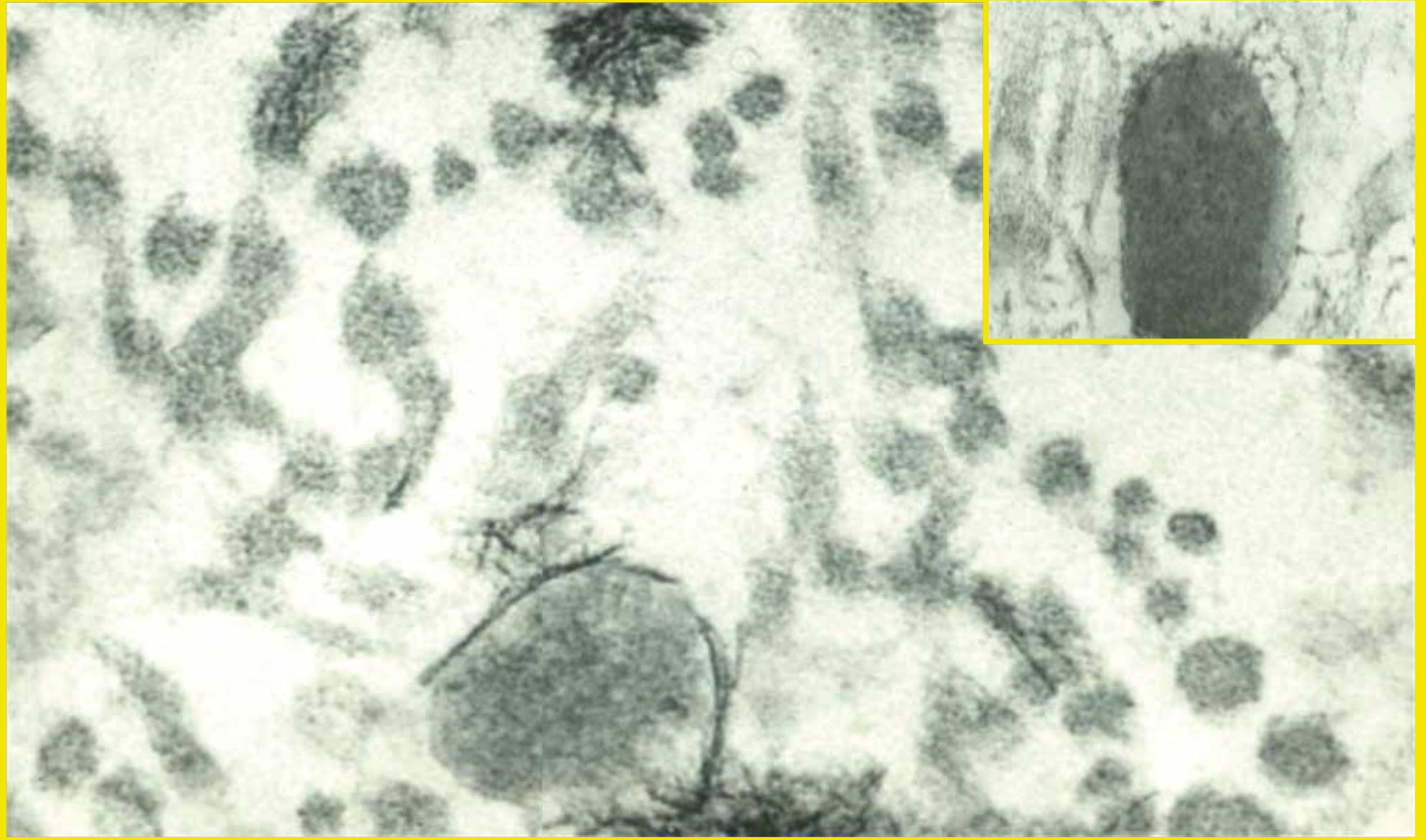
Boivin & Anderson 1996, In: Maladies Métaboliques Osseuses de l'Adulte, D. Kuntz ed, Médecine-Sciences Flammarion, Paris, pp. 36-48

Initial calcification of cartilage (heterogeneous nucleation)



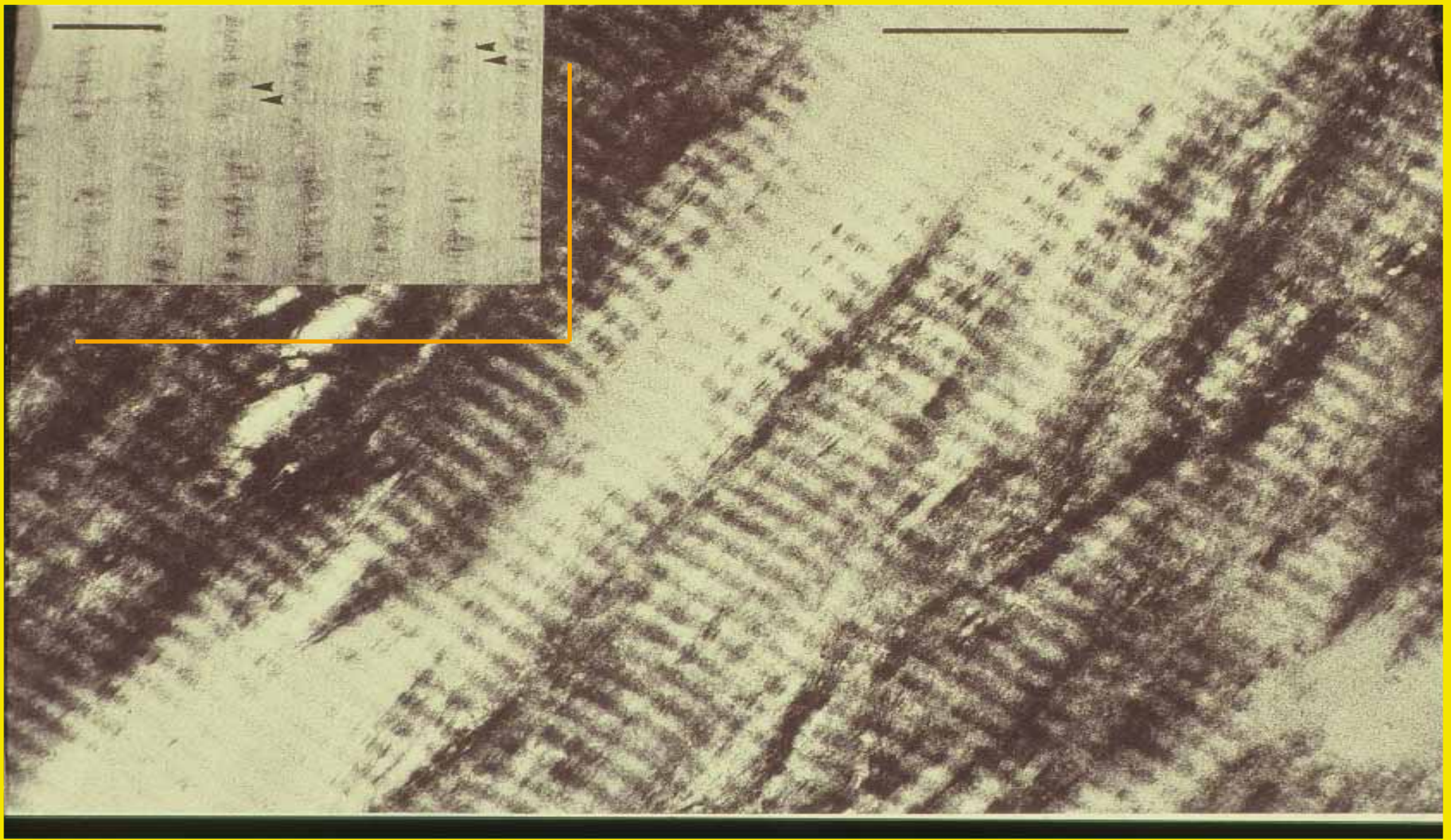
Boivin & Anderson 1996, In: Maladies Métaboliques Osseuses de l'Adulte, D. Kuntz ed, Médecine-Sciences Flammarion, Paris, pp. 36-48

Initial calcification via matrix vesicles



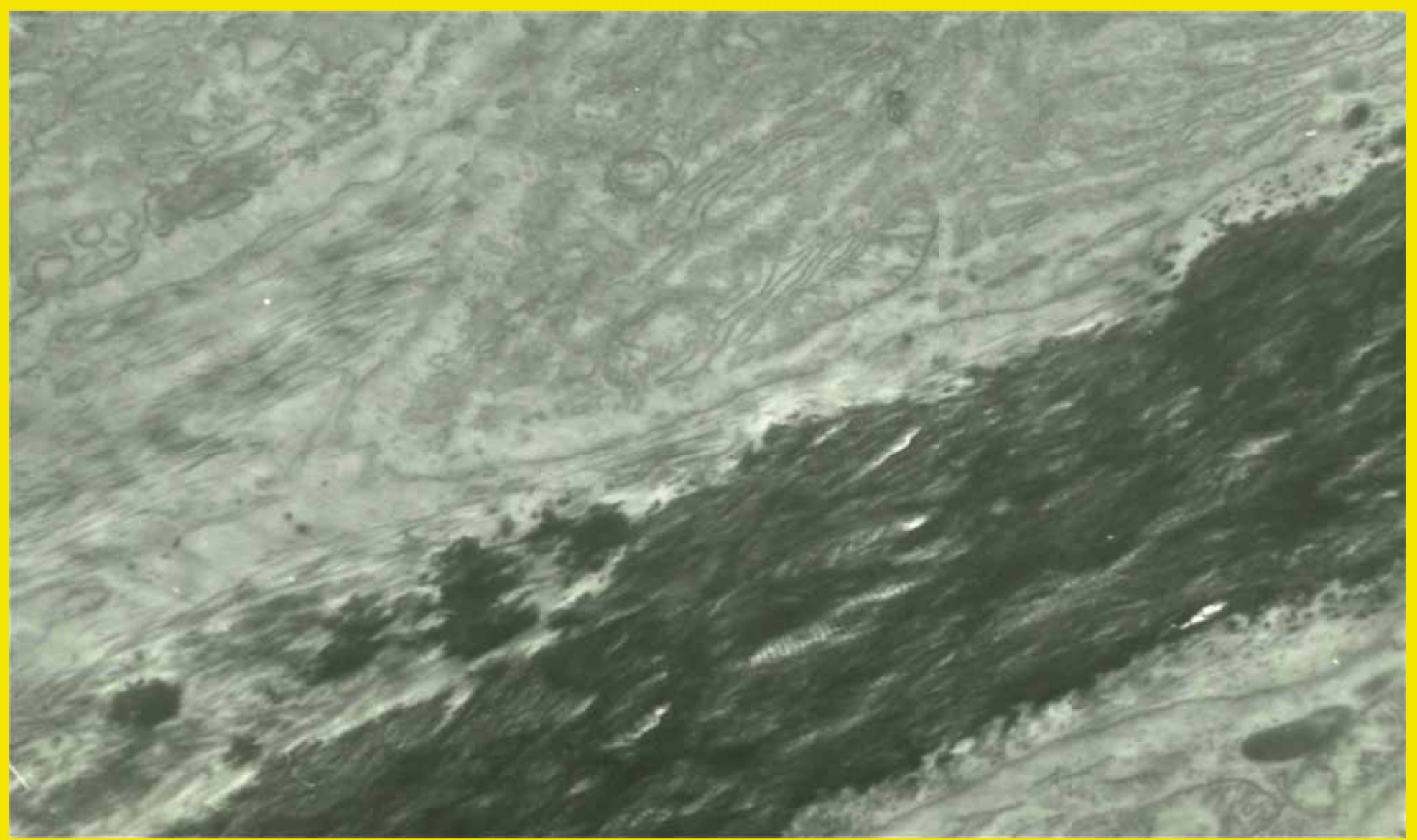
Boivin & Anderson 1996, In: Maladies Métaboliques Osseuses de l'Adulte, D. Kuntz ed, Médecine-Sciences Flammarion, Paris, pp. 36-48

Calcification initiale dans les bandes à trous des fibrilles

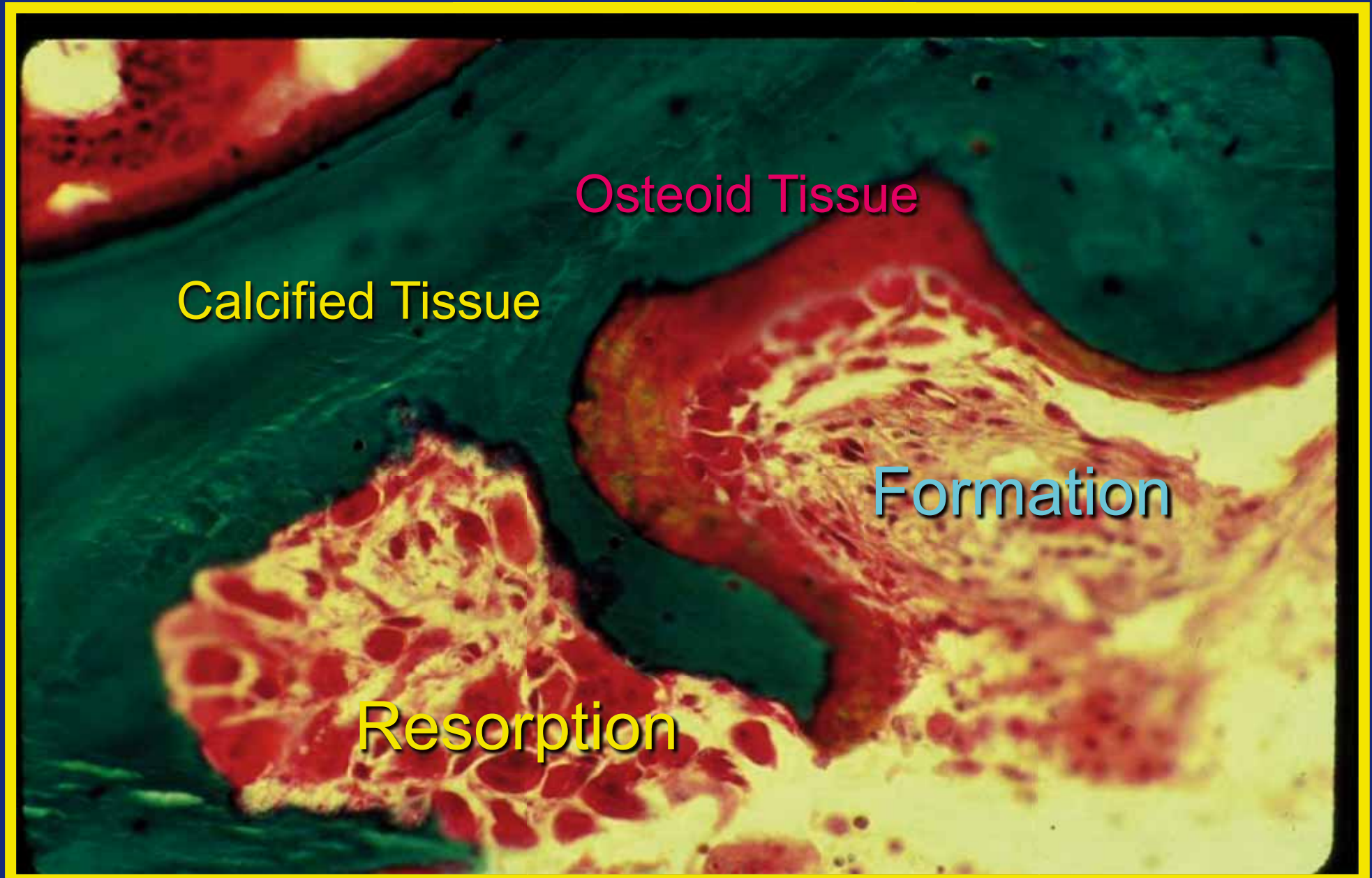


Boivin & Anderson 1996, In: Maladies Métaboliques Osseuses de l'Adulte, D. Kuntz ed, Médecine-Sciences Flammarion, Paris, pp. 36-48

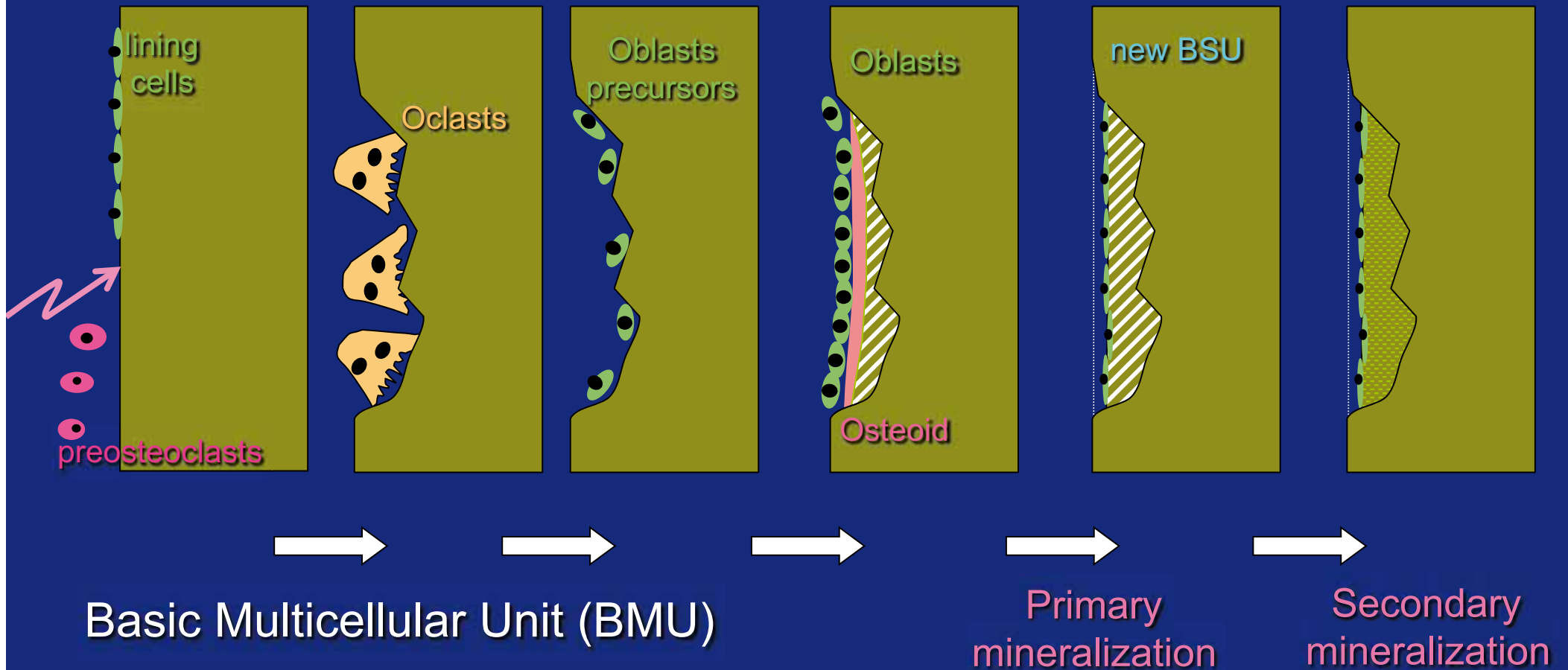
MATRICE OSSEUSE CALCIFIÉE



Bone remodeling



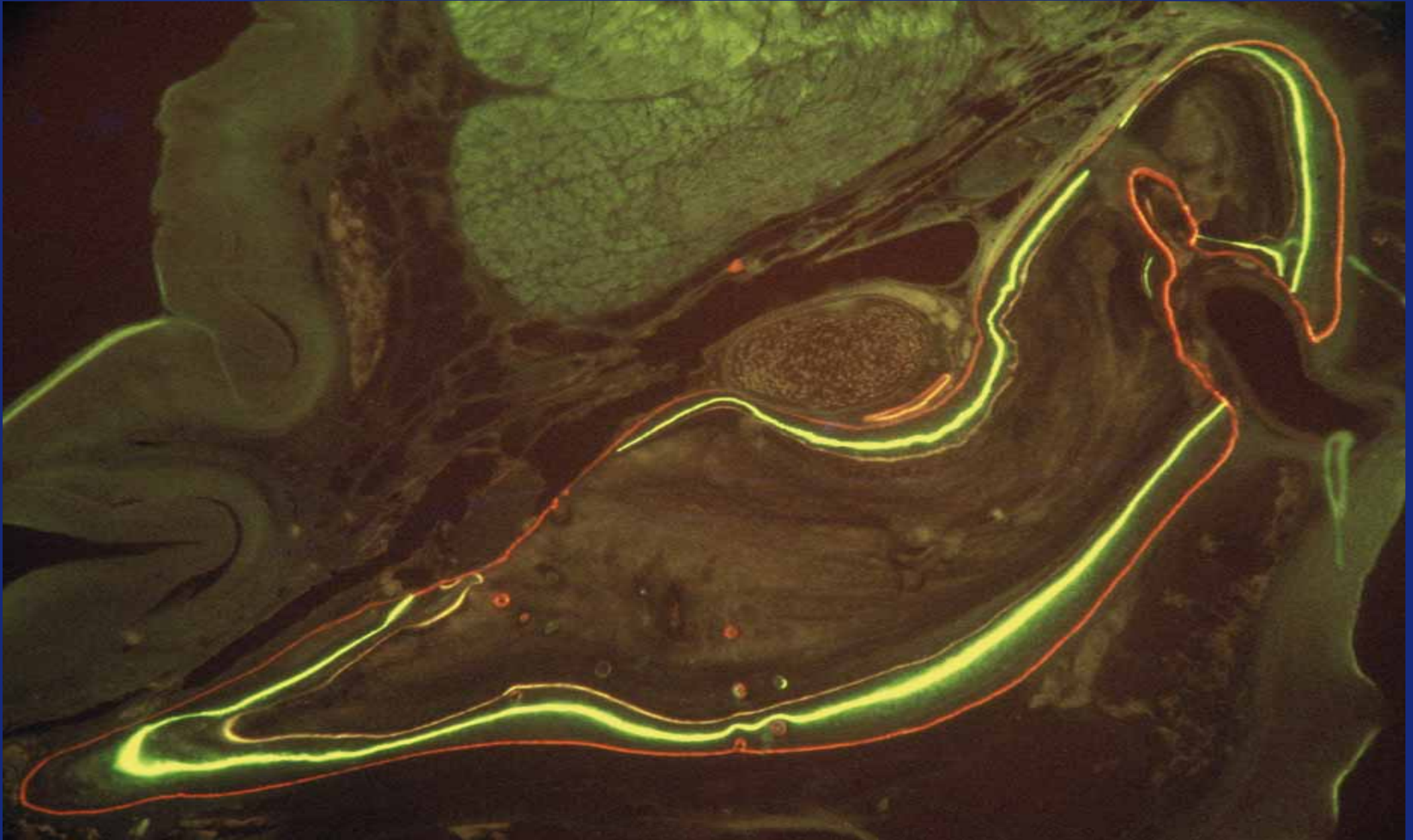
Cascade of events in normal bone remodeling



The degree of mineralization of bone (DMB) depends on:

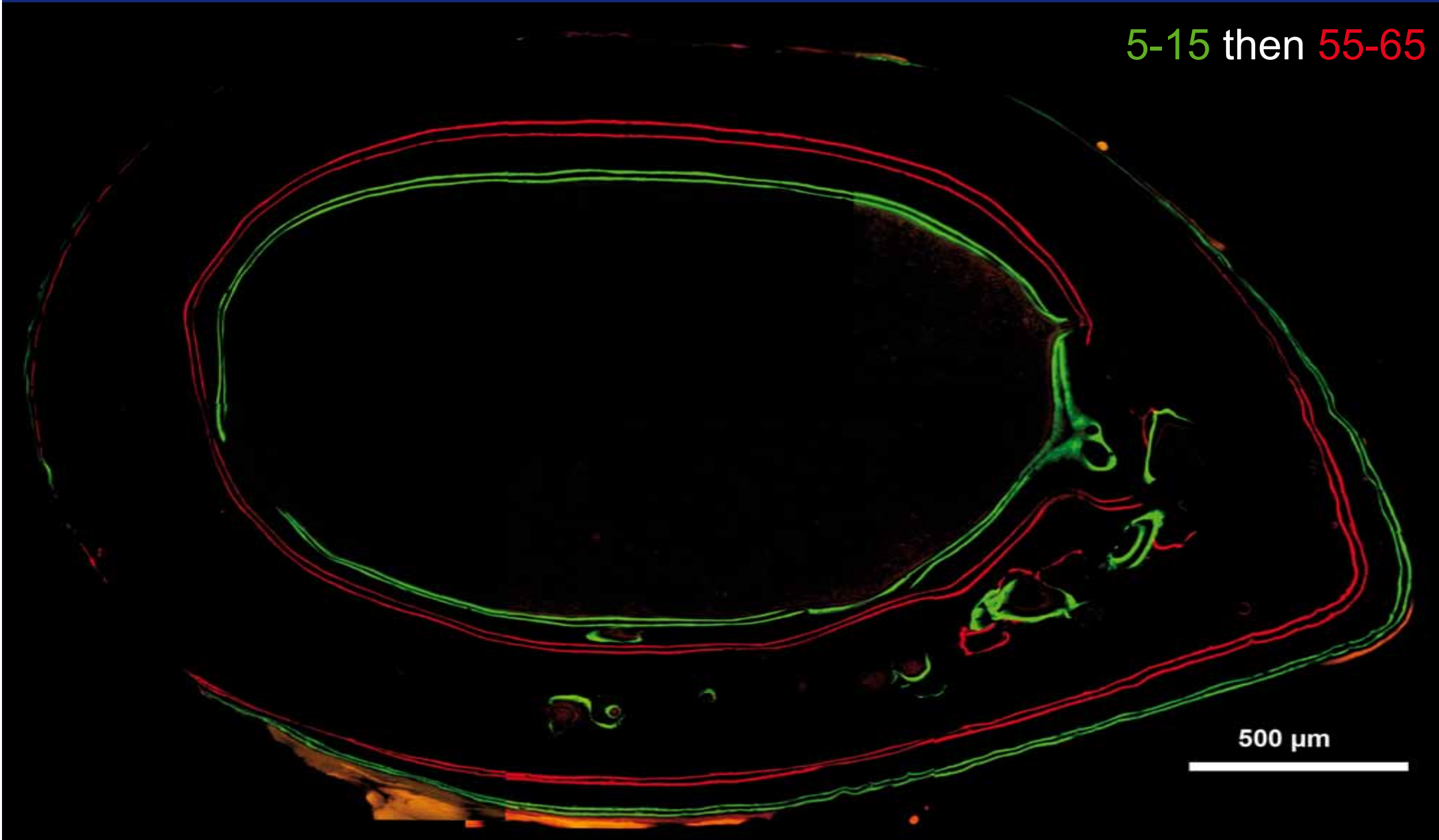
- 1) the *primary mineralization* measured at calcification front after double tetracycline labeling (deposition of only 50 to 60 % of the mineral substance).
- 2) the duration of the *secondary mineralization* necessary for a complete maturation of the BSU and consisting in a slow augmentation in the number, size and perfection of apatite crystals.

Bone labeling in a mandible of fish



Meunier & Boivin 1972, Bull Soc Zool France, 97: 539-40

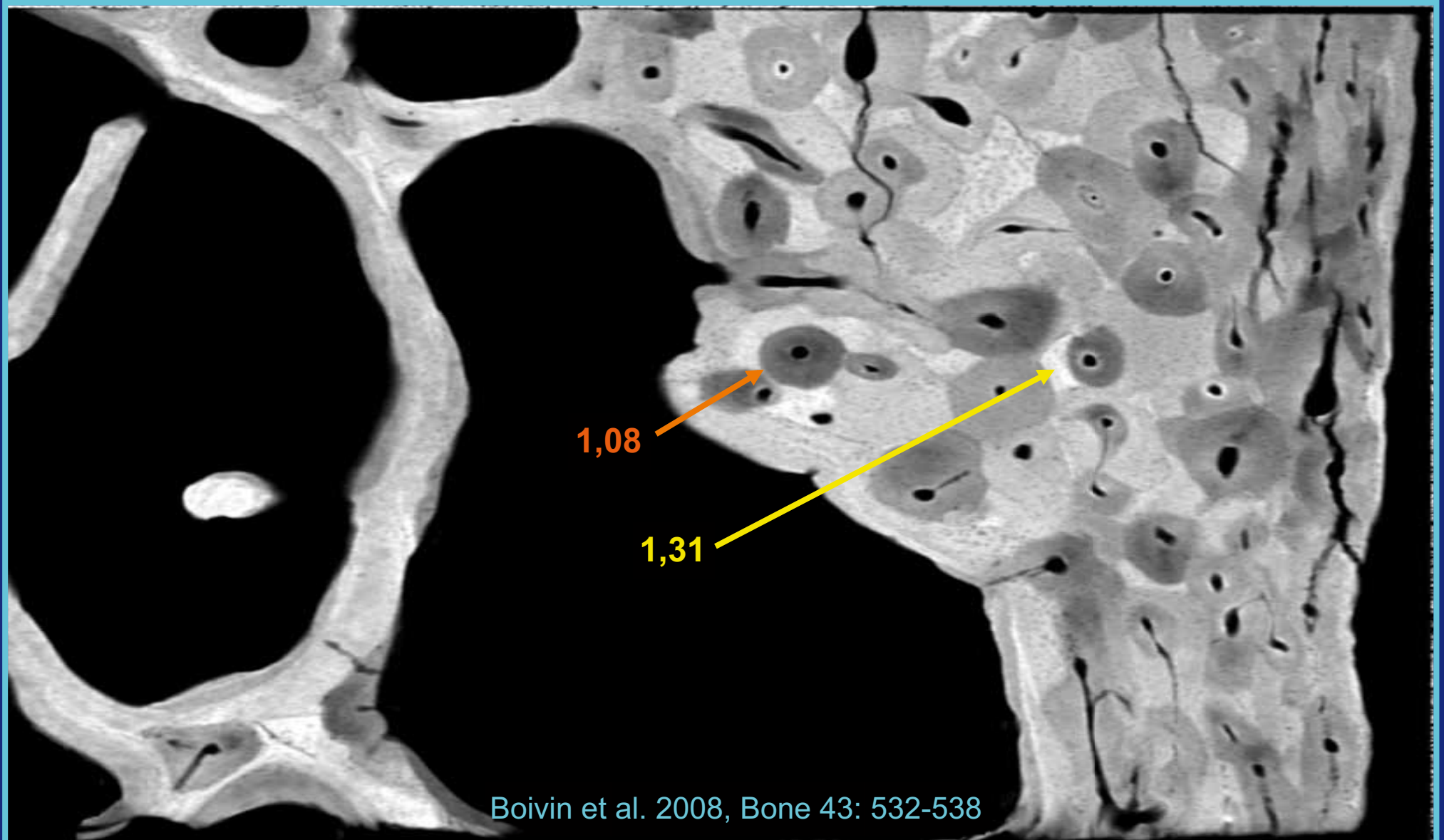
5-15 then 55-65



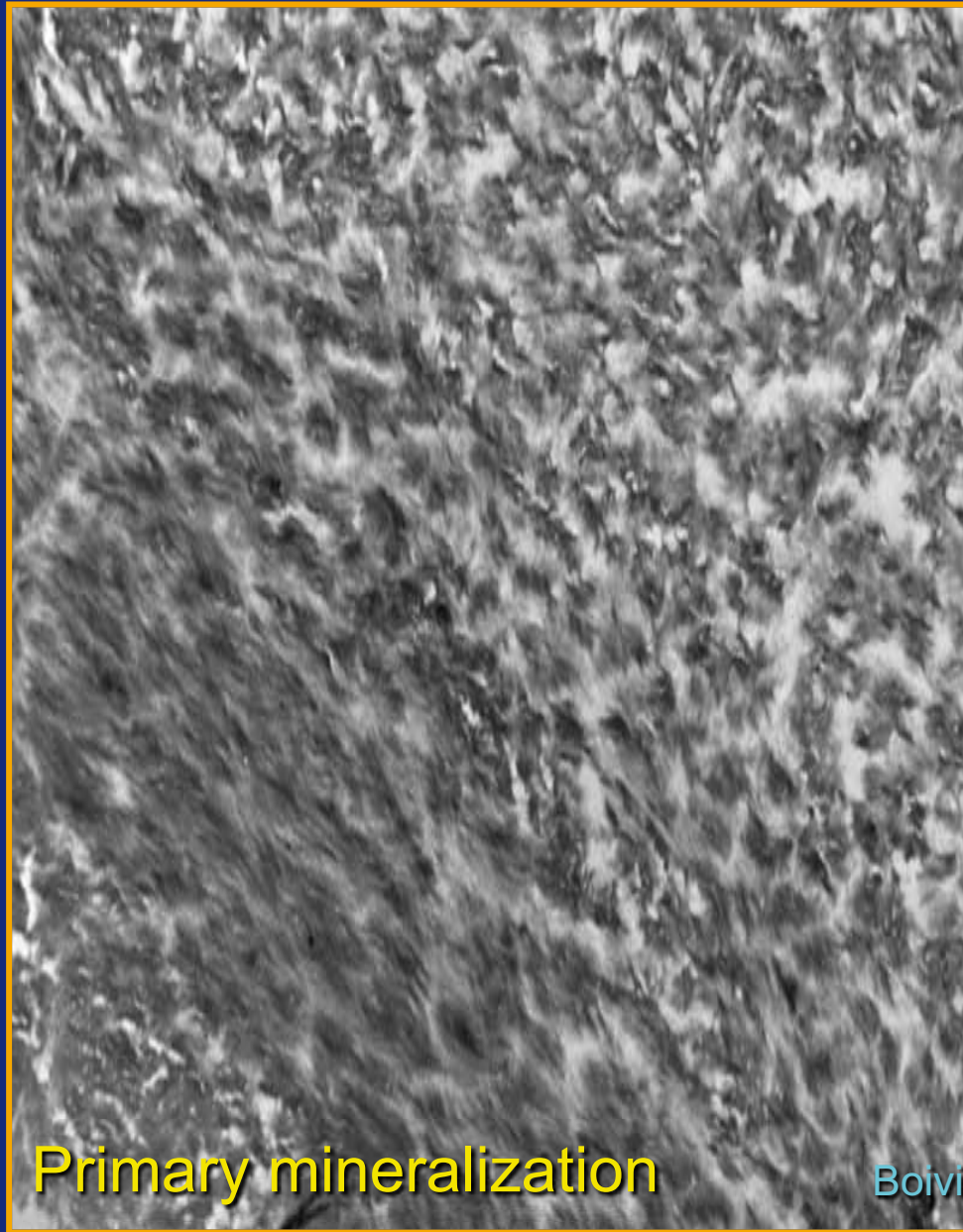
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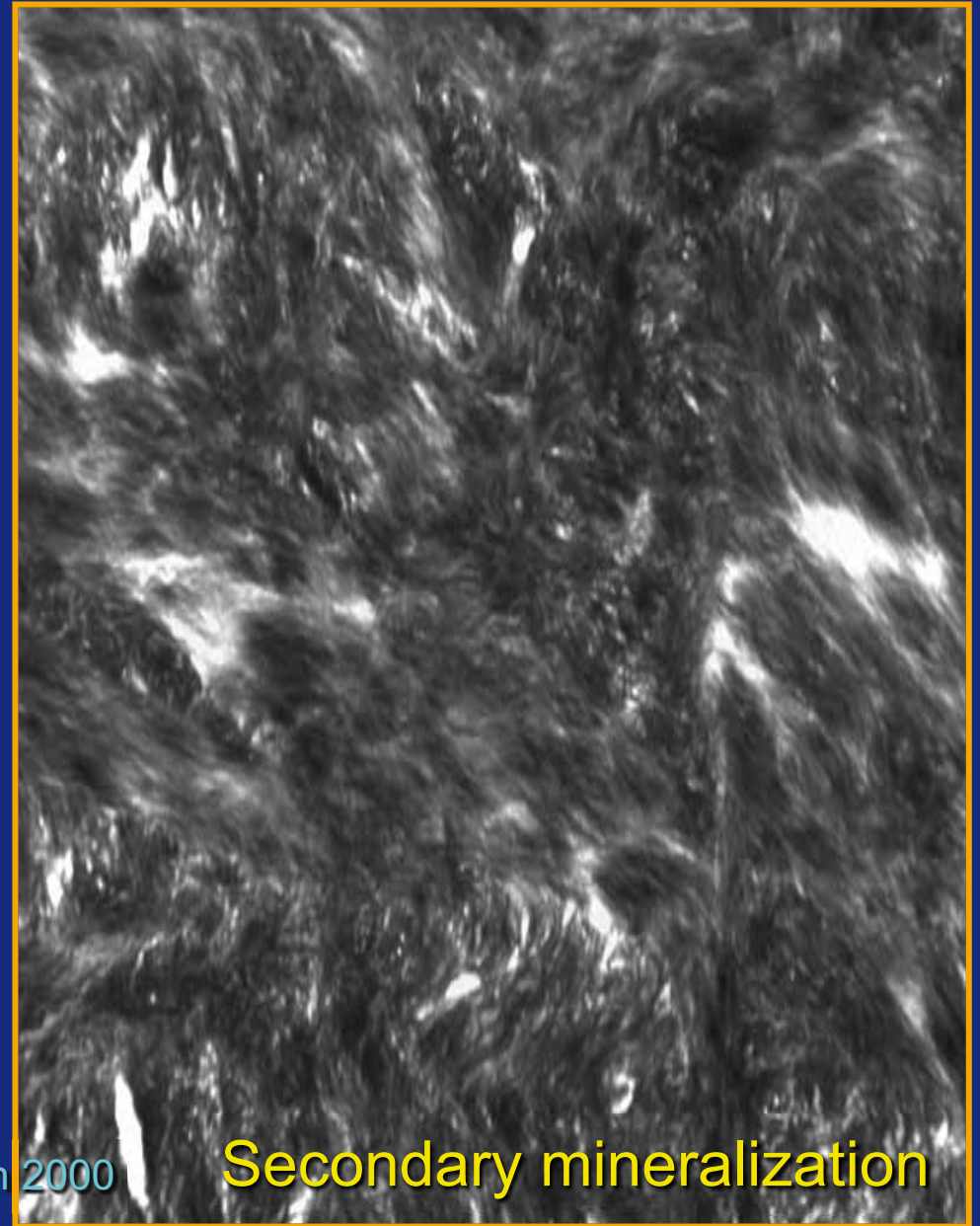
Microradiograph of a human iliac bone section illustrating the heterogeneity of the mineralization



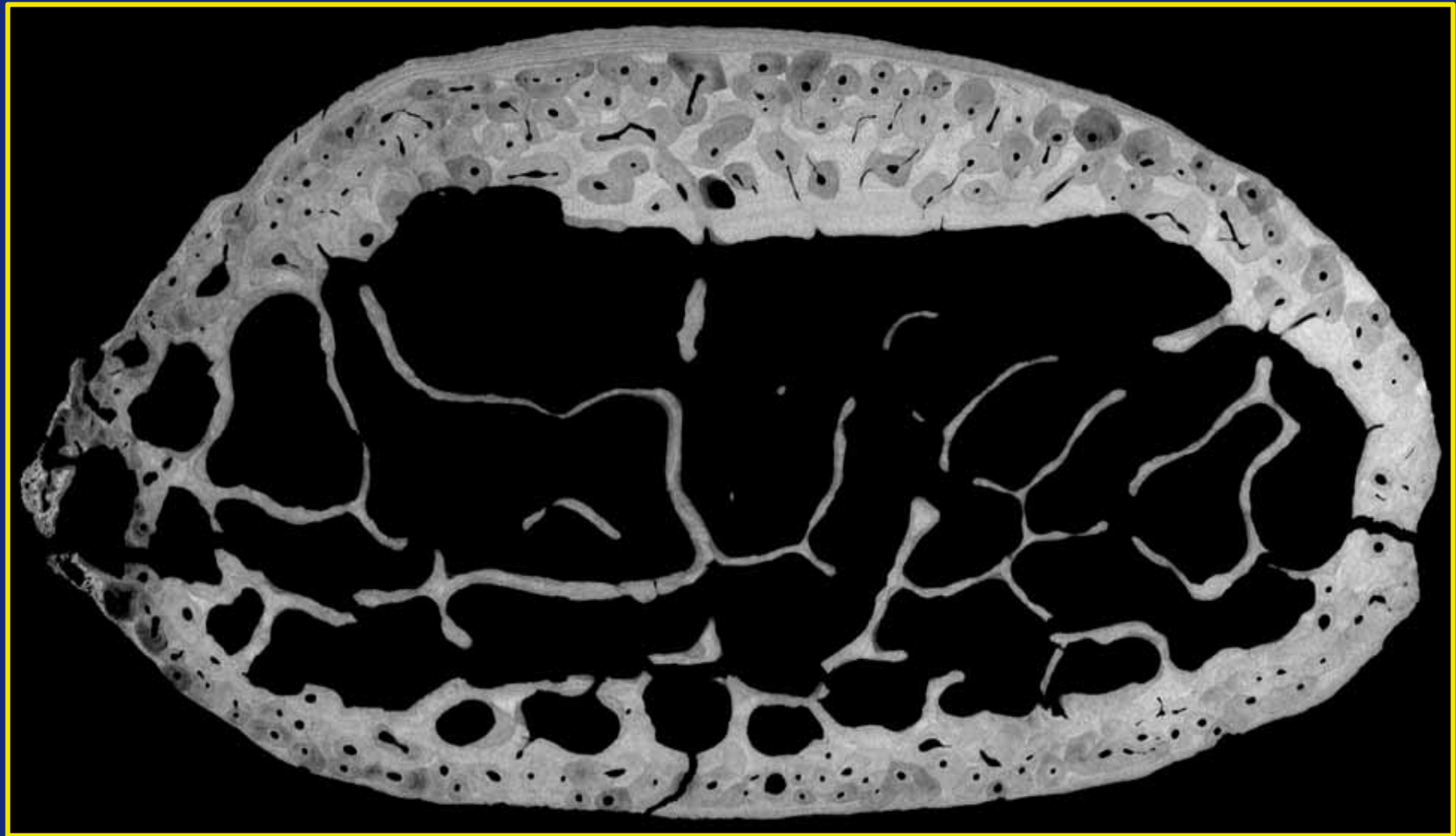
Electron micrographs of human bone tissue (X 10 000)



Boivin 2000



Microradiograph of a section of human rib (men 22 yrs-old with cystic fibrosis) illustrating the heterogeneity of the mineralization all around bone sample and following modeling and remodeling activities.



Mailhot et al. 2016, submitted

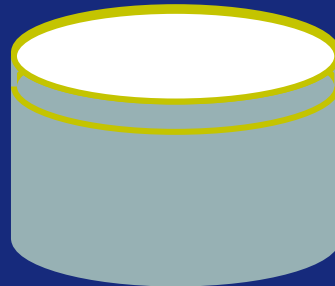
Human iliac bone samples



1cm

Bone sample

fixed > dehydrated > embedded in PMMA



8 μm -thick sections

Histomorphometry

100 \pm 1 μm -thick sections

Microradiography

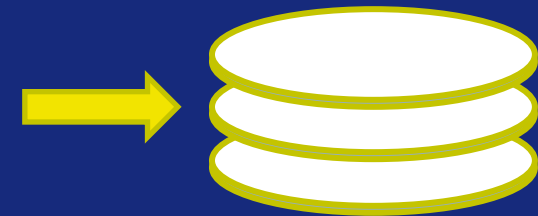
Residual sample

Microindentation

X-ray microanalysis

X-ray diffraction

2-5 μm -thick sections



Fourier Transform
Infra-Red
Microspectroscopy

Pluridisciplinary approach of bone quality

Histomorphometry



In vitro MicroCT



Structure, micro-architecture 2D, and parameters reflecting bone remodeling

Tissue & BSU levels

Microarchitecture 3D

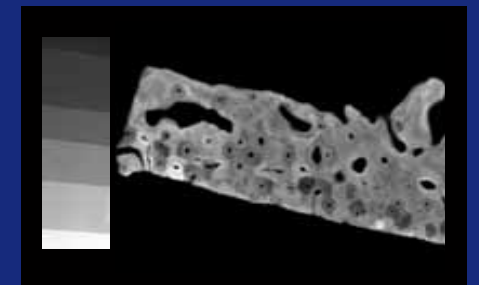
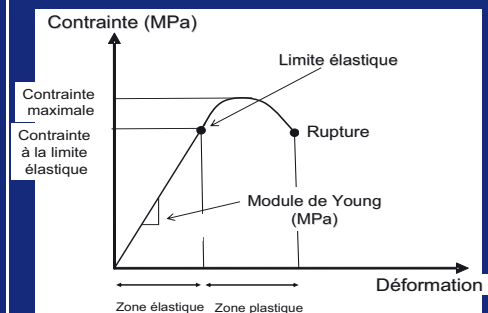
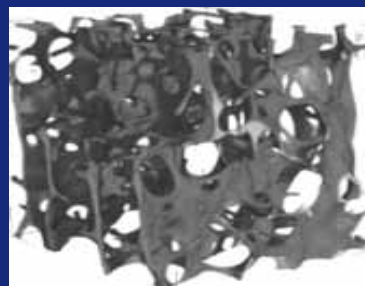
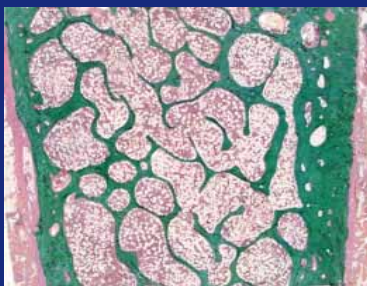
Bone & Tissue levels

Mechanical tests

Bone & Tissue levels

Intrinsic determinants of bone quality

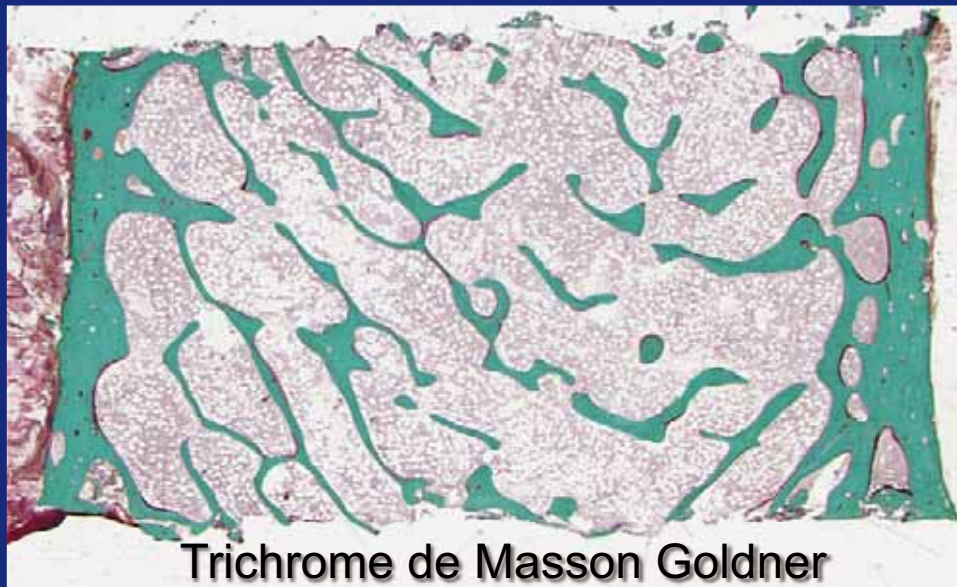
Tissue & BSU levels



Bone Histomorphometry



Microtome



Trichrome de Masson Goldner

Bone structural parameters:

- Cortical thickness (Ct.Th) and porosity (Ct.Po)
- Trabecular Bone Volume (BV/TV), thickness (Tb.Th), number (Tb.N), separation (Tb.Sp), wall thickness (W.Th)

Bone remodeling parameters:

Parameters reflecting the bone resorption

- Eroded surfaces (ES/BS), osteoclast number (Oc.N), erosion depth (E.De)

Parameters reflecting the bone formation

- Osteoid surfaces (OS/BS), osteoid thickness (O.Th)

Coordinated Approach of Bone Quality at Tissue, BSU and Ultrastructural Levels

Quantitative microradiography



Micro-, nanoindentation



Fourier Transform Infra-Red Microspectroscopy



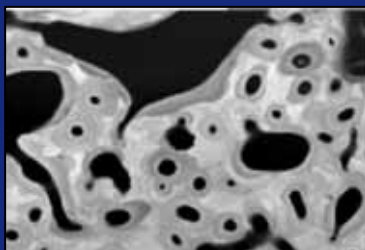
X-ray Microanalysis



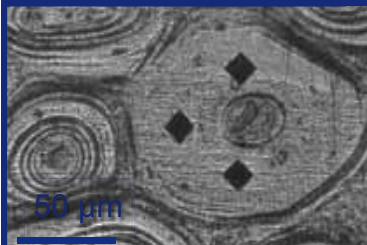
X-ray Diffraction



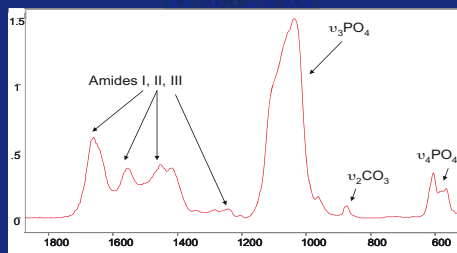
Degree of mineralization



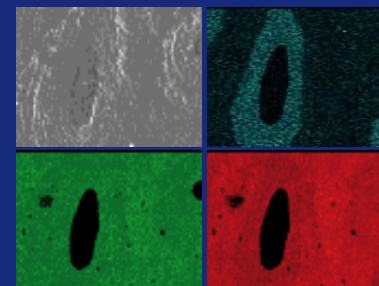
Elastic modulus
Hardness



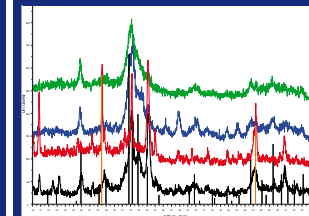
Characteristics of mineral and organic matrix



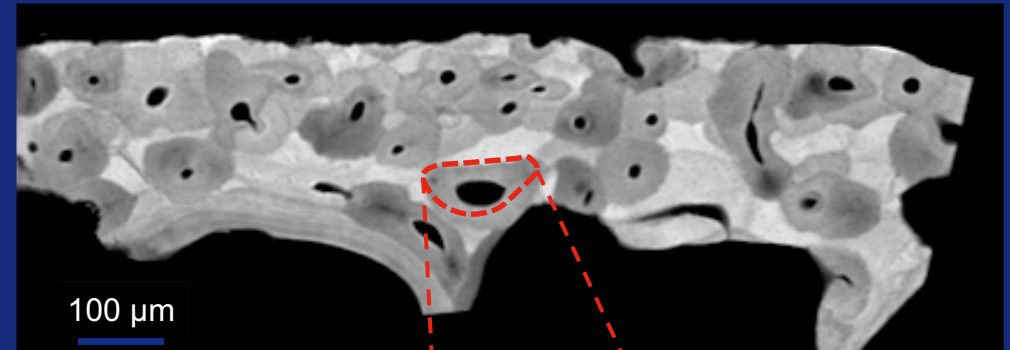
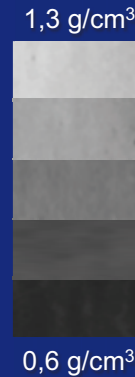
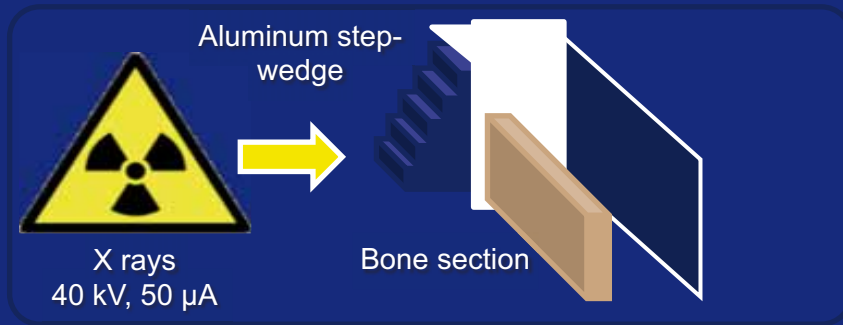
Localization and content of elements



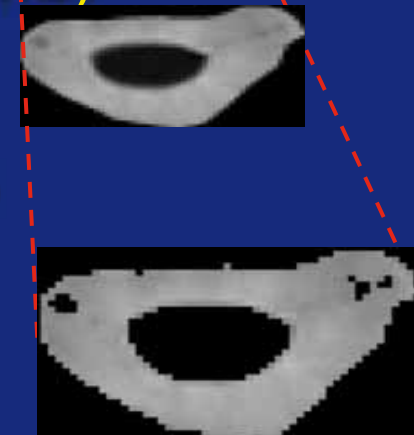
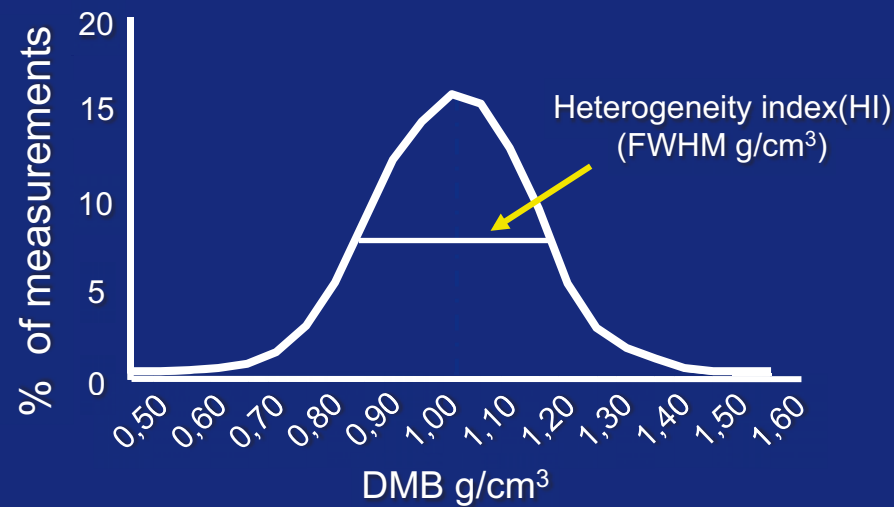
Nature and size of crystals



Quantitative digitized microradiography

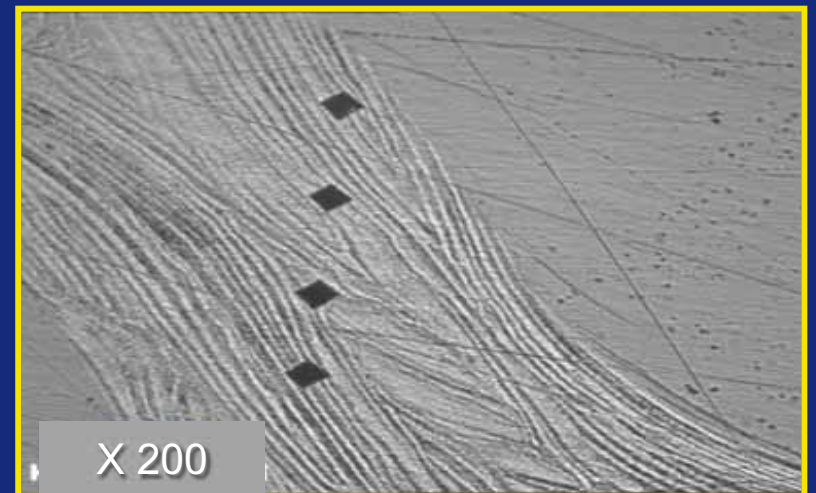
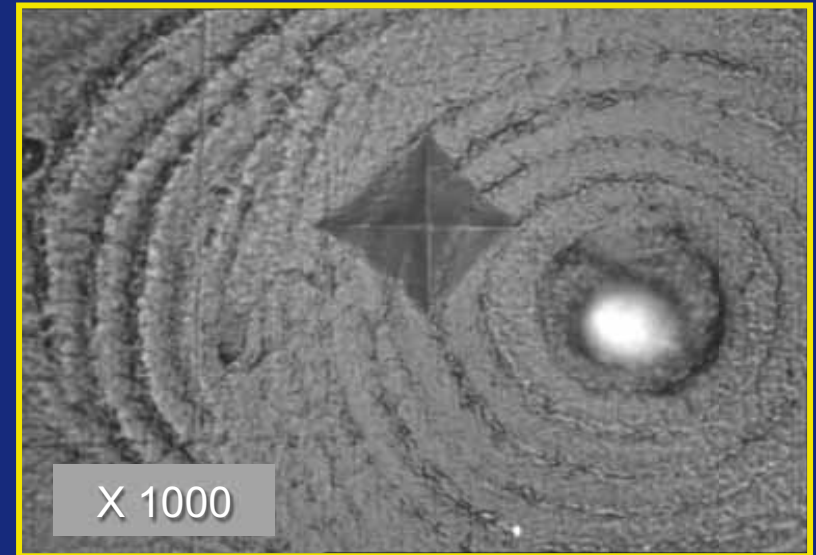


Tissular degree of mineralization (DMB)



**DMB measured
at BSU level**

Global microhardness of bone tissue (Hv, Kg/mm²), measured on surfaced blocks with a Vickers indenter (25g for 10s), corresponded to the mean of 60 indentations performed at random.

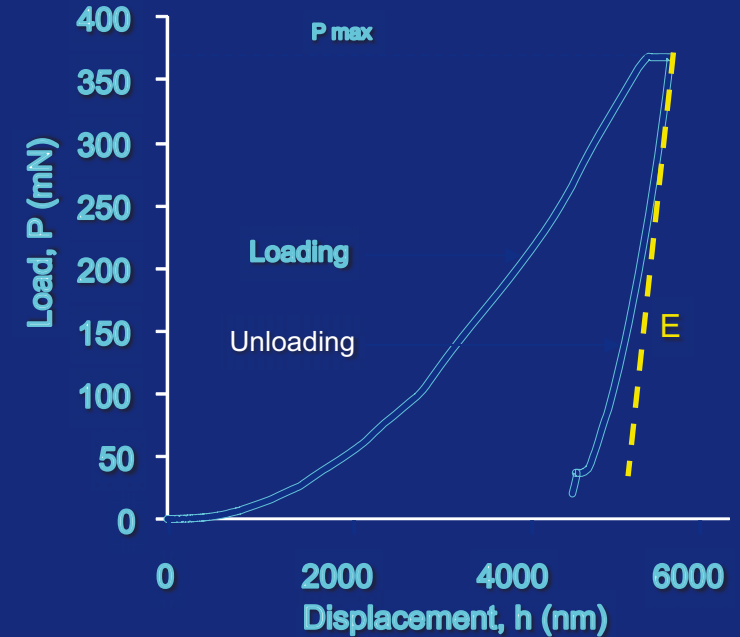
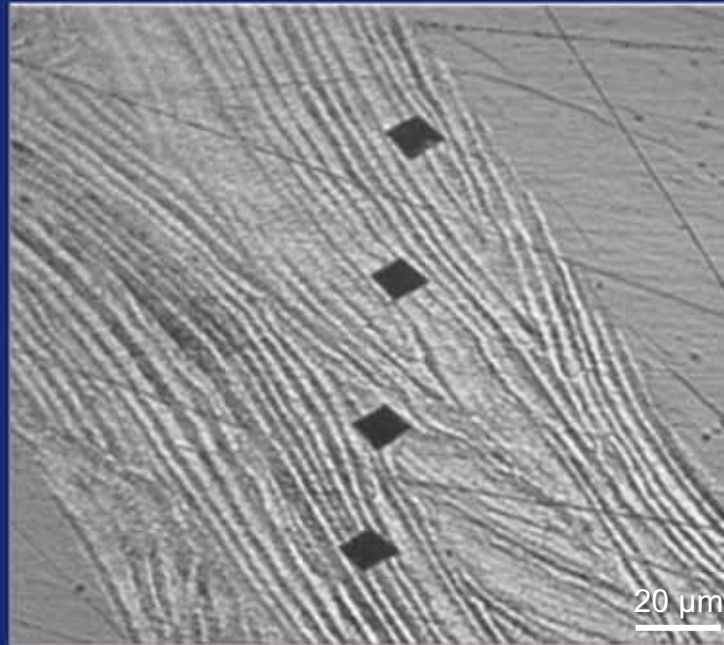
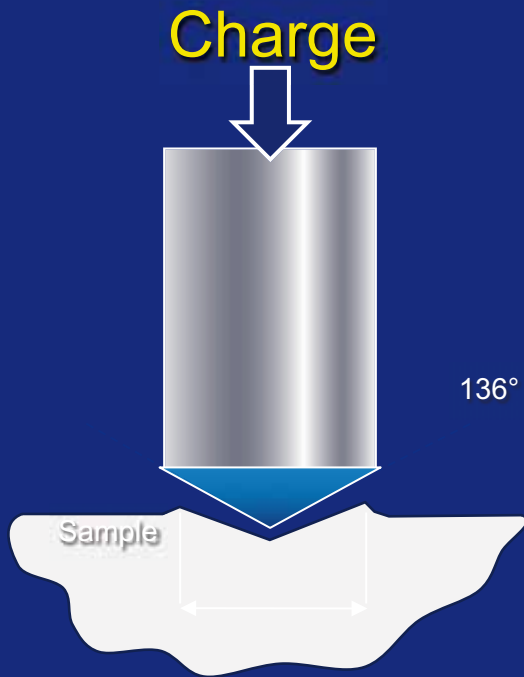


Microhardness of iliac bone tissue in control women

	Total bone	Cortical bone	Cancellous bone
Hv (Kg/mm ²)	49.71 ± 2.01	49.91 ± 2.51	49.33 ± 1.52

Boivin et al. 2008, Bone 43:532-8

Indentation



Propriétés mécaniques locales

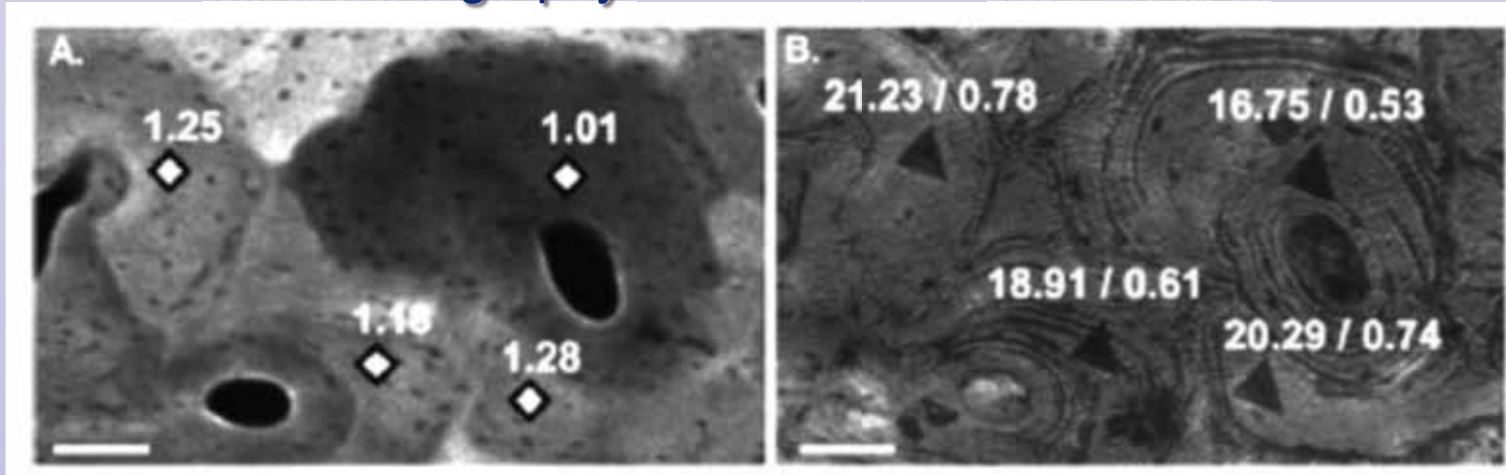
Module élastique (E , Gpa): Propriété intrinsèque décrivant la résistance à la déformation élastique (réversible).

Dureté (Hardness, H , Kg/mm^2 or Gpa): Rapport de la charge sur la surface de contact entre l'indenteur et l'échantillon décrit la résistance à la déformation plastique (irréversible).

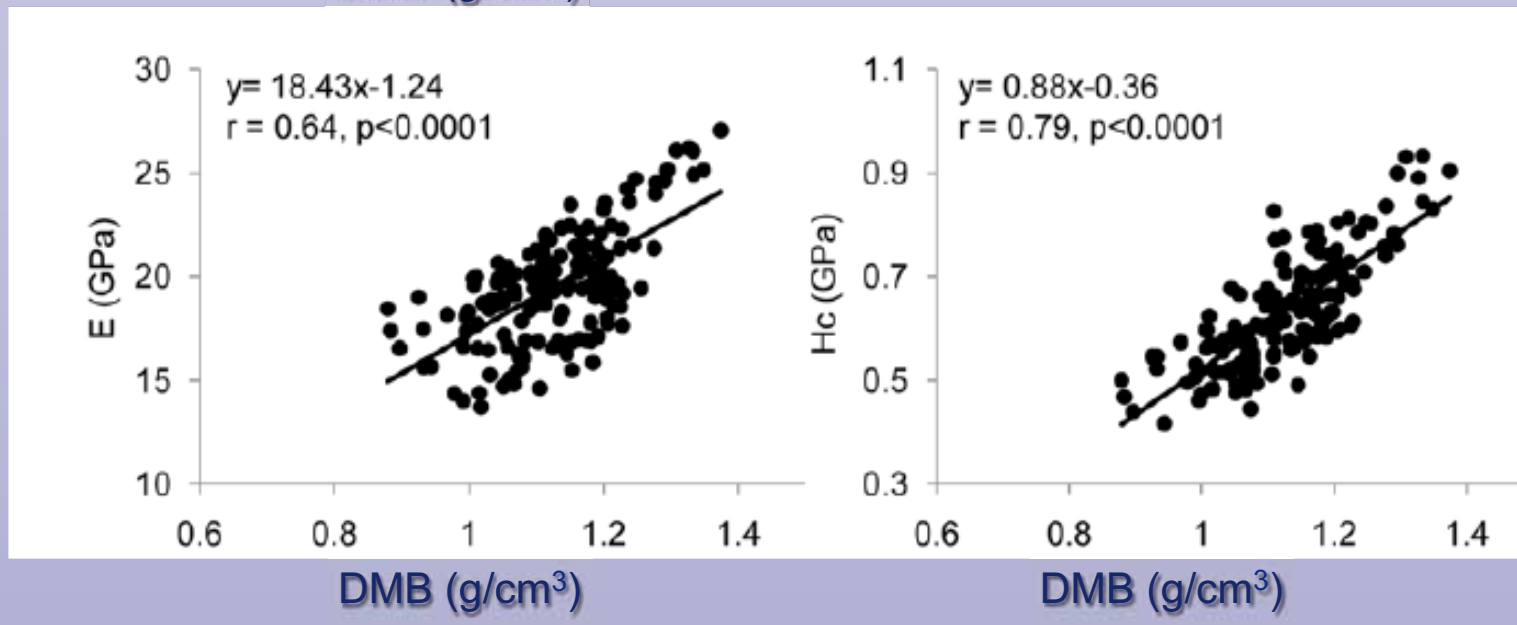
DMB et propriétés mécaniques mesurées par indentation

Microradiography

Indentation



DMB (g/cm³)

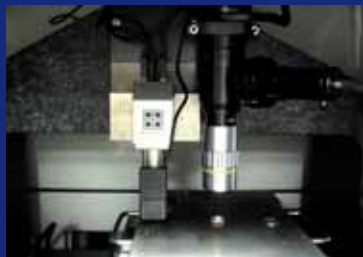


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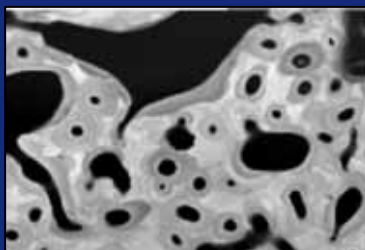
X-ray Microanalysis



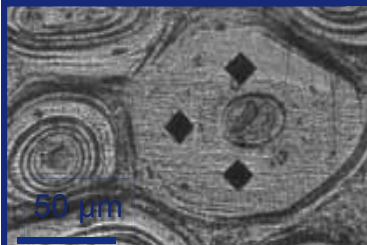
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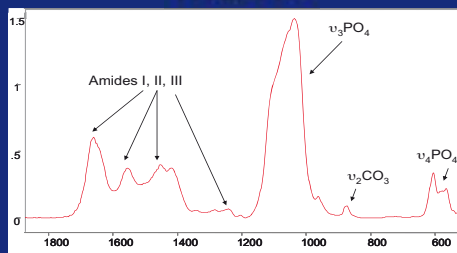
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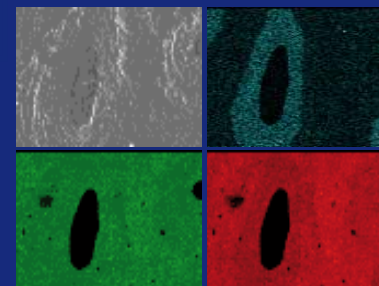
Elastic modulus
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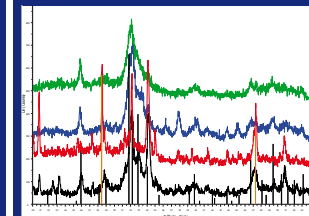
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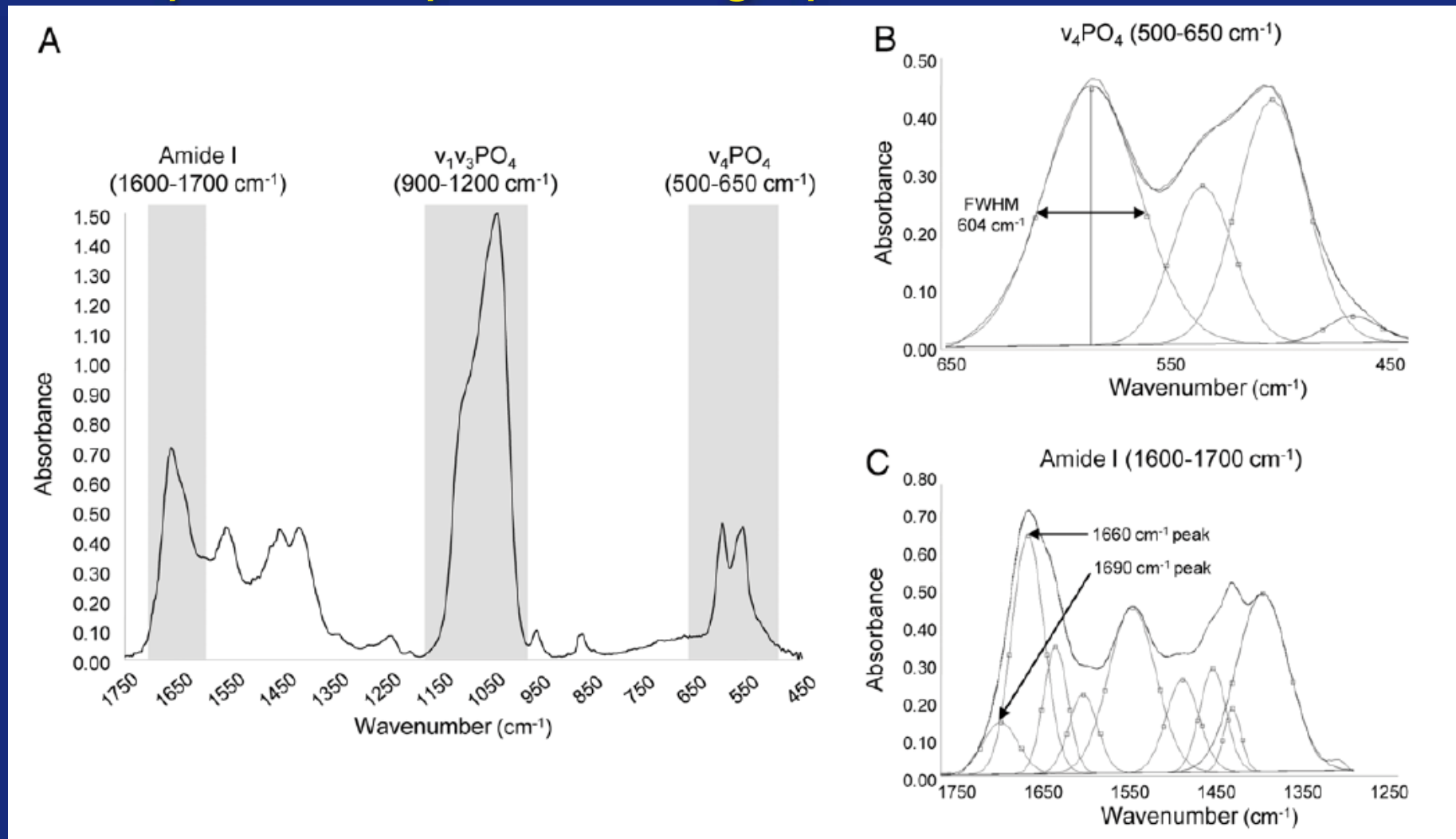
Localization and content of elements



Nature and size of crystals



Microspectroscopie infrarouge par transformée de Fourier



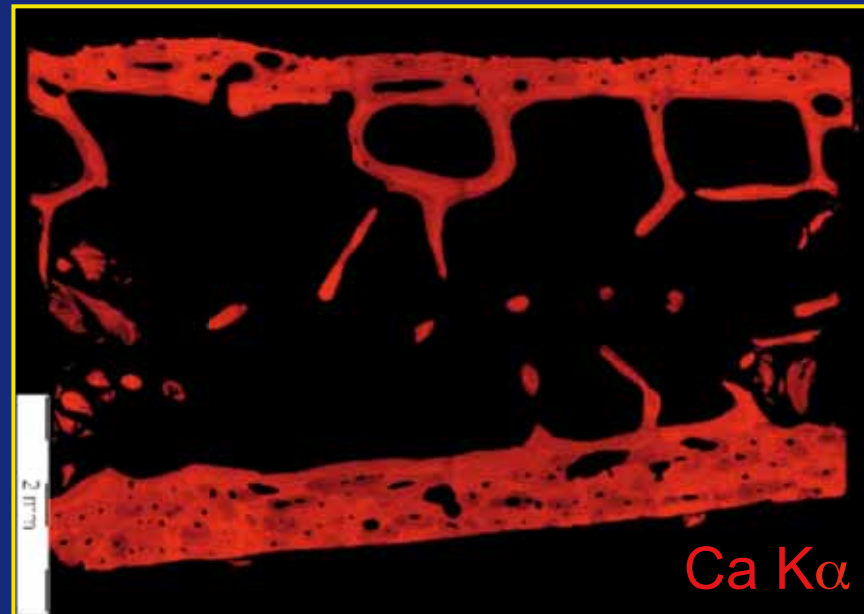
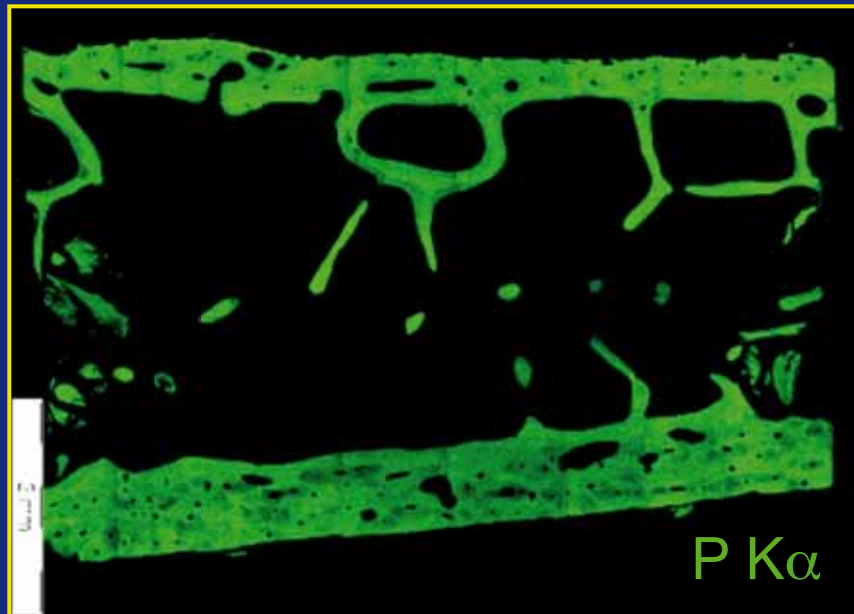
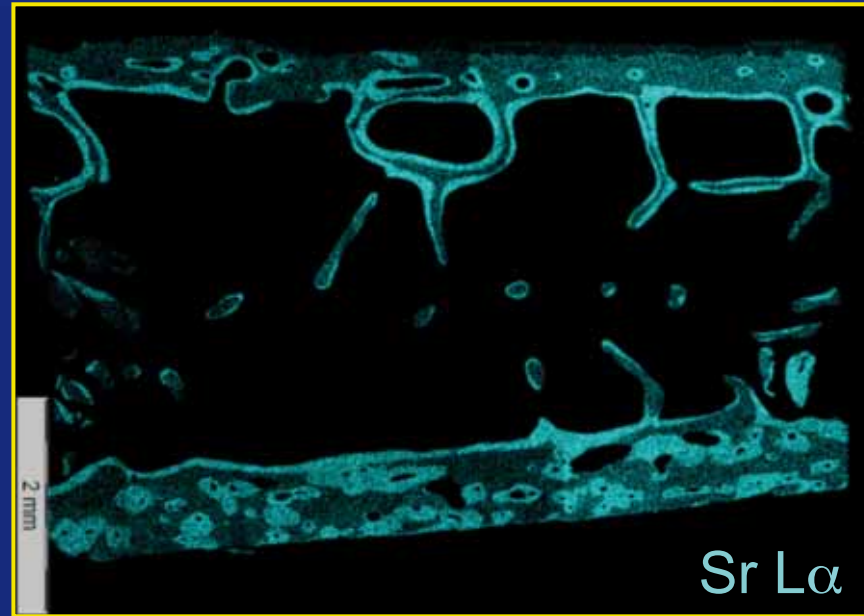
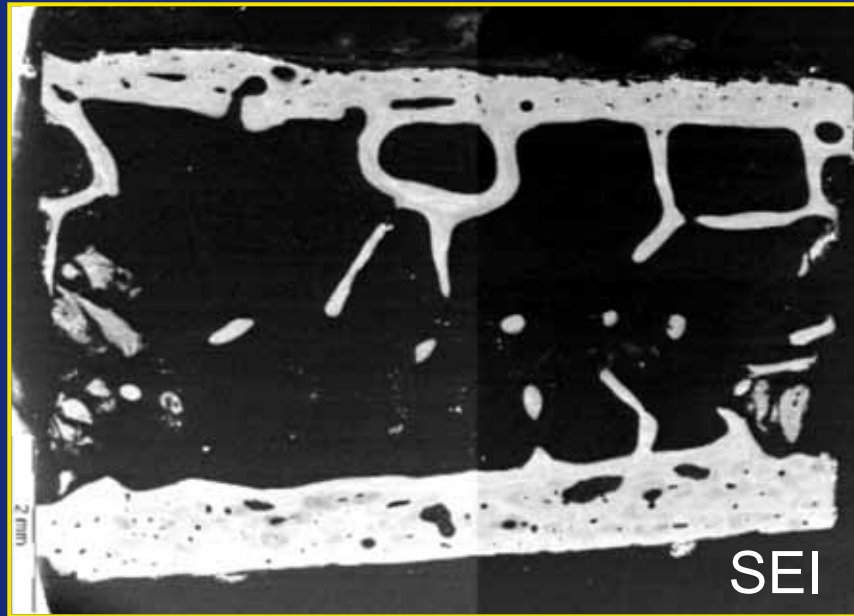
Maturité du collagène 1660 cm^{-1} / 1690 cm^{-1} \longrightarrow Age de la matrice organique

Maturité minérale 1030 cm^{-1} / 1110 cm^{-1} \longrightarrow Age du minéral

Index de cristallinité FWHM of 604 cm^{-1} \longrightarrow Taille / perfection du cristal

Farlay et al. 2010, JBMM 28: 433-45; Farlay et al. 2011, PLoS ONE 6(12): e28736.

Woman treated with strontium ranelate (2g/day) for 36 months
Cartography of Sr, Ca and P on the surface of a bone biopsy



Total area
35%

Cortical 29 %
Cancellous 44%

Boivin et al. 2010,
Osteoporos Int
21: 667-77
Boivin 2010,
IBMS BoneKEy
7:103-7

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Ostéoporose - Cancérologie osseuse - Lyon



Yohann Bala
Delphine Farlay
Sébastien Rizzo
Pascale Chavassieux
Brigitte Burt-Pichat
François Duboeuf

Merci à tous mes collaborateurs de
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