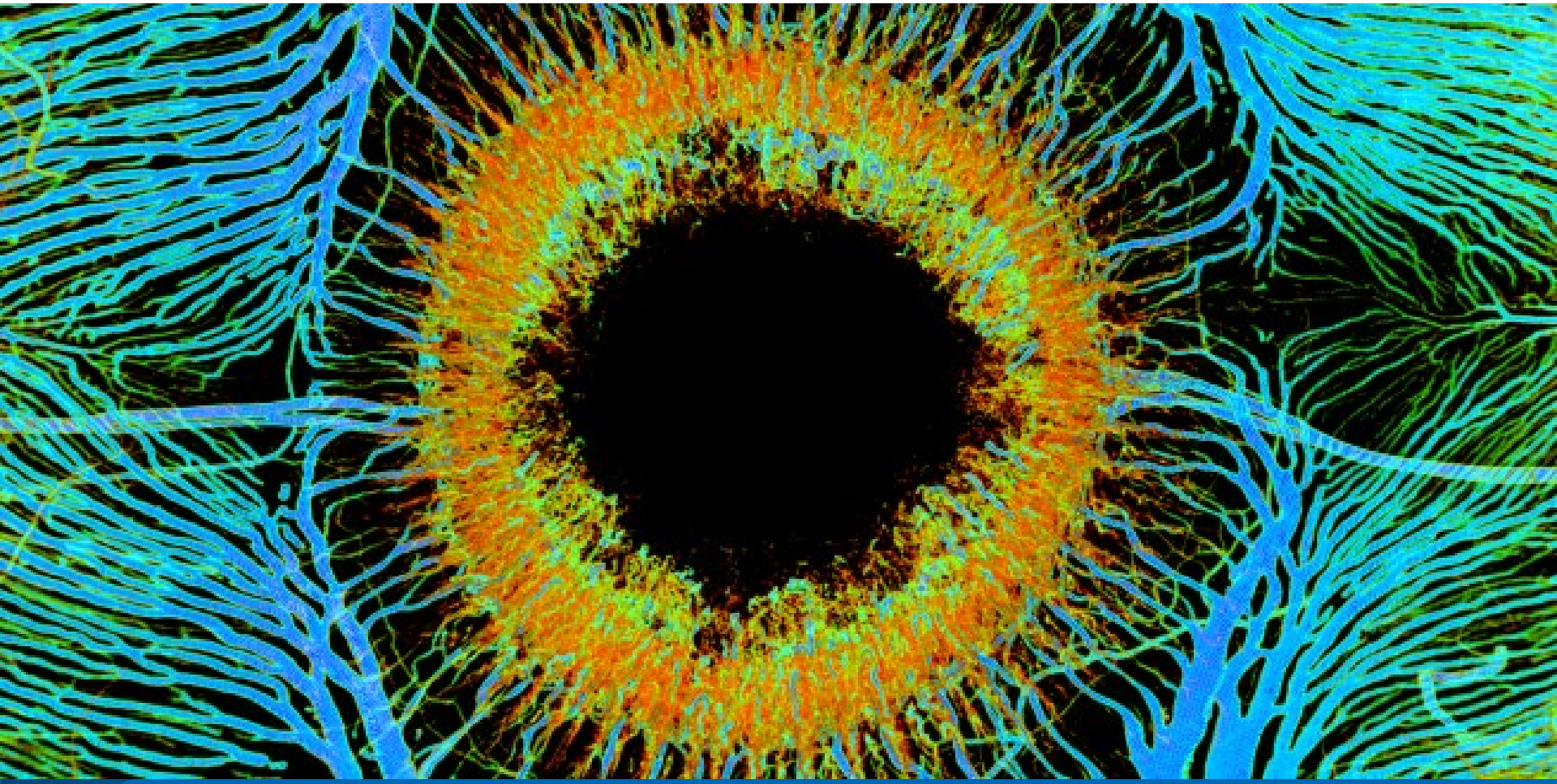
Growth plate and metaphysis: Fractality and gradient

Phil Salmon, Behzad Javaheri, Line Oste. (Bruker microCT, Kontich, Belgium; Royal Vet College, London, UK; Galapagos Ltd., Paris, France.

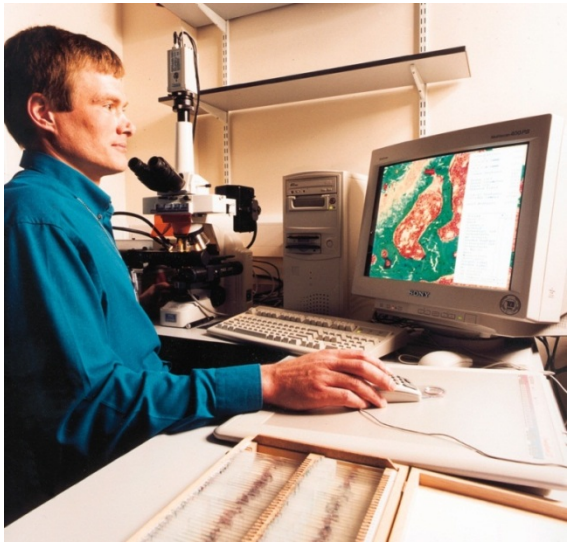
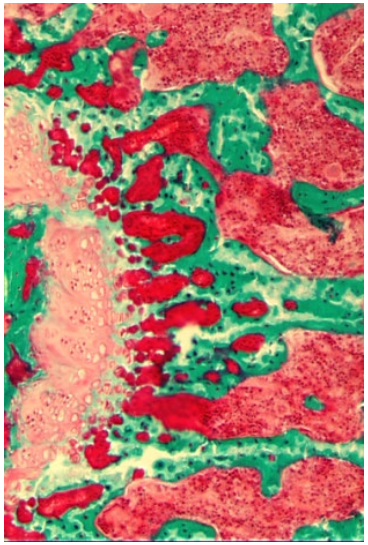
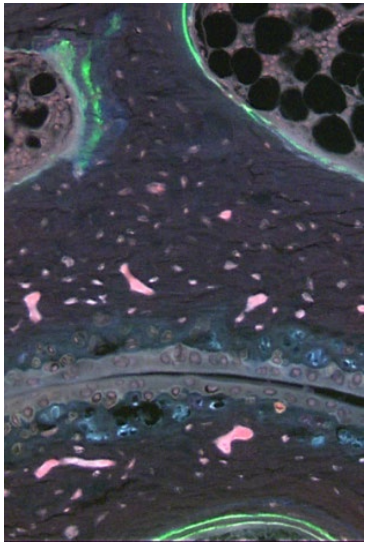


What is the purpose of this study?

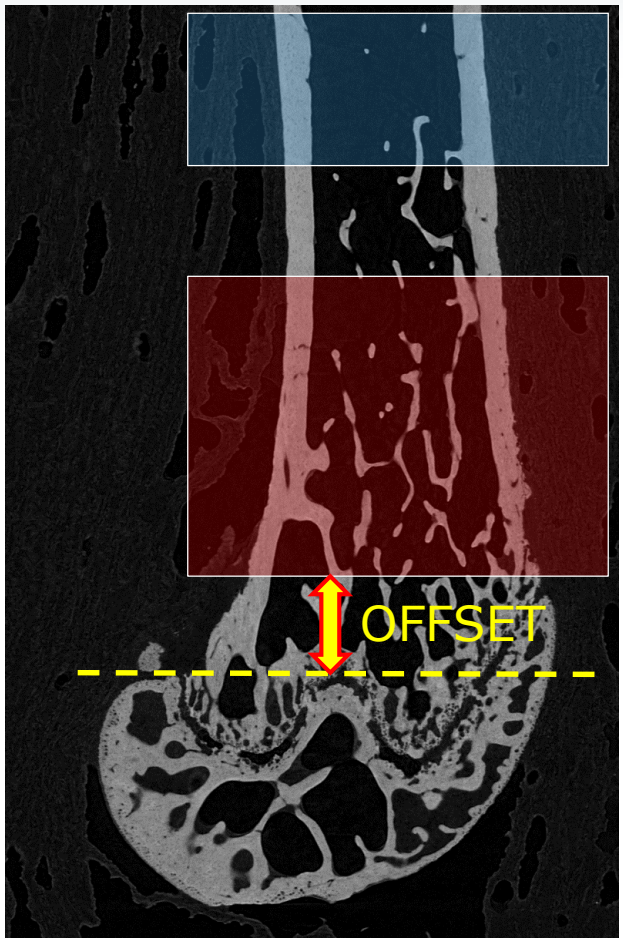
First – a reminder of the principles guiding trabecular bone morphometry of the rodent tibia or femur

Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro–computed tomography. Journal of bone and mineral research. 2010 Jul 1;25(7):1468-86.

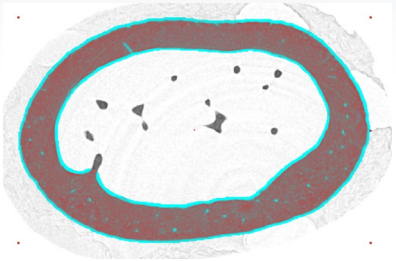
In histomorphometry the growth plate is the reference structure, and trabecular bone selected for analysis starting one or two microscope fields away from the growth plate

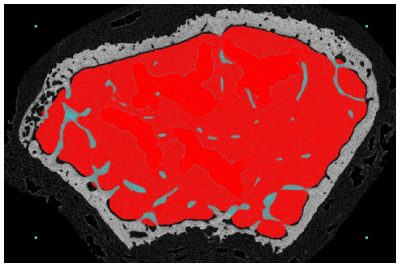
In uCT, we also setan **"offset"** distance between the GP and the region for analysis of trabecular bone



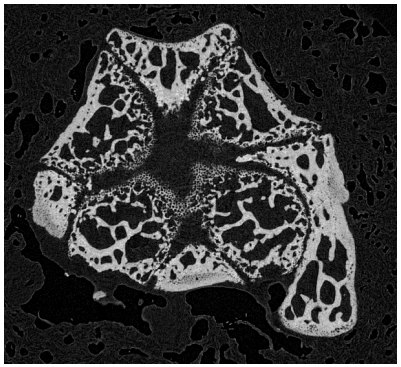
Cortical VOI



Trabecular VOI



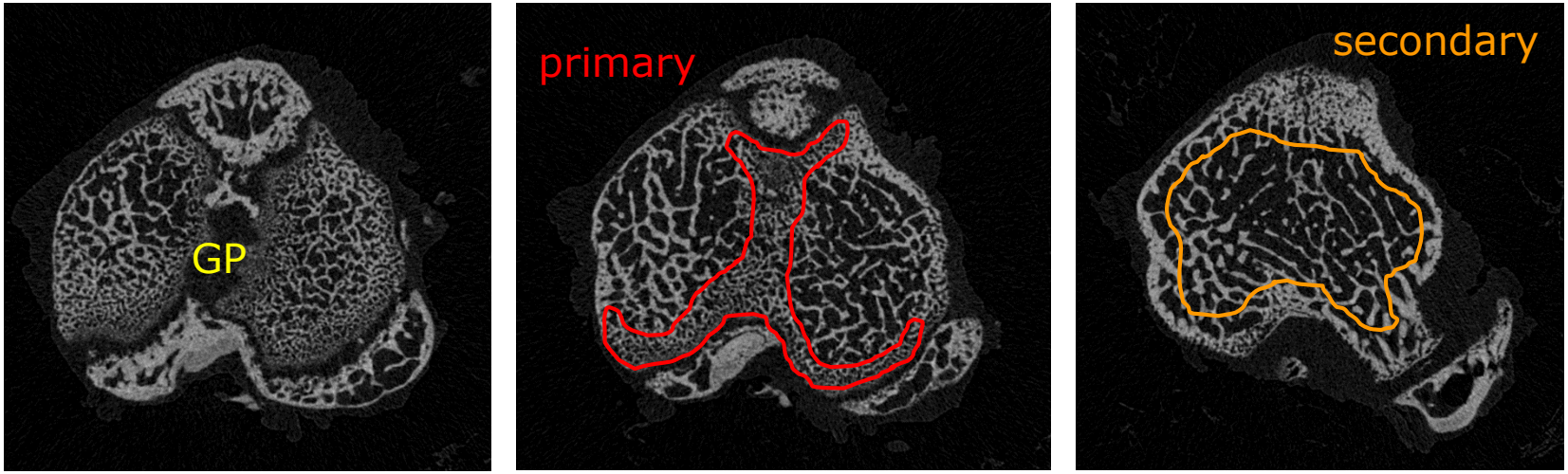
Growth plate reference



Then select appropriate ranges for trabecular (and cortical) analysis

Why the offset?

The rationale from histomorphometry has been that trabecular morphometry should be done on the mature remodeled secondary spongiosa, not the immature fine-textured “primary spongiosa”.



This implies a distinct transition between primary and secondary spongiosal trabecular bone

This sometimes causes a problem with old mice

beyond the GP there is little or no trabecular bone

Questions

- Is there a real transition between primary and secondary spongiosa?
- Is it correct to assume that the analysed femur / tibia metaphysis is a homogeneous volume of bone?
- What would happen if we include rather than exclude the growth plate – primary spongiosa region on trabecular morphometry?

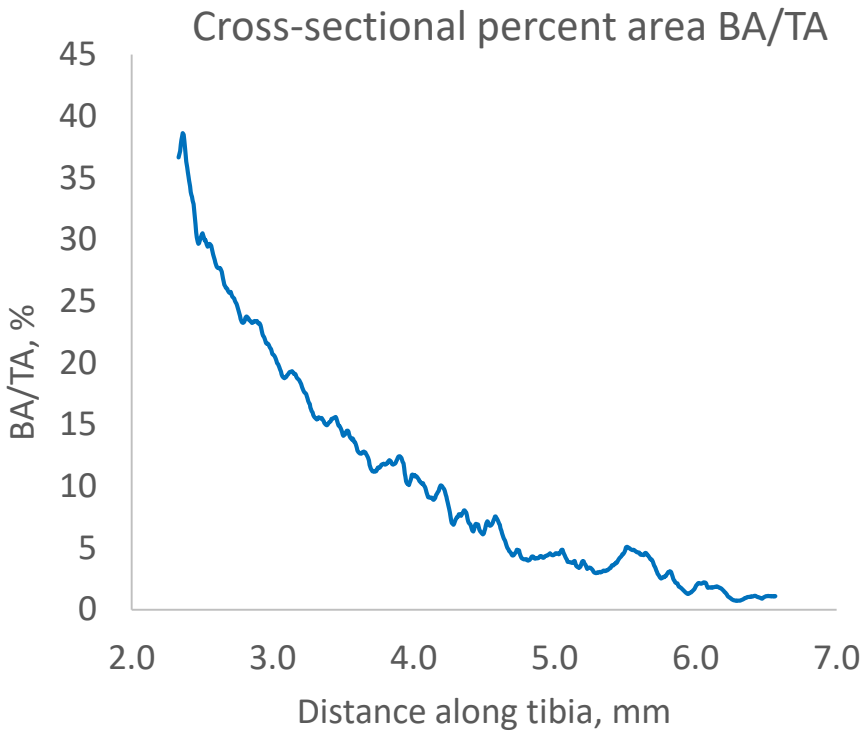
What we did

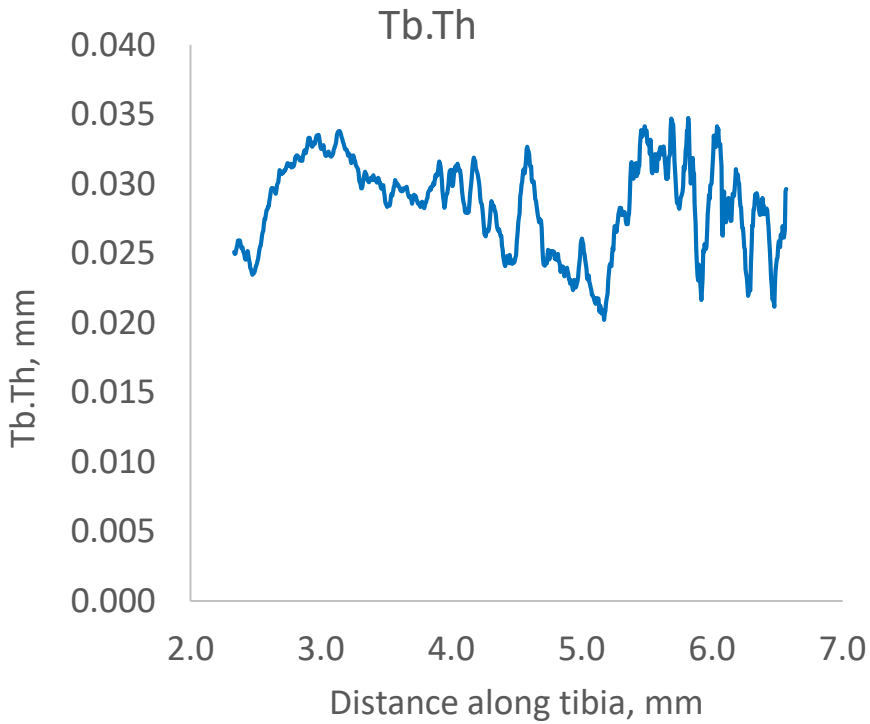
- We also re-analysed an OVX study with alendronate and estradiol, including or excluding the growth plate region
- We measured 2D cross-sectional profiles of bone parameters from the growth plate well into the metaphysis/diaphysis
- We derived some new parameters related to the metaphyseal gradient

The study

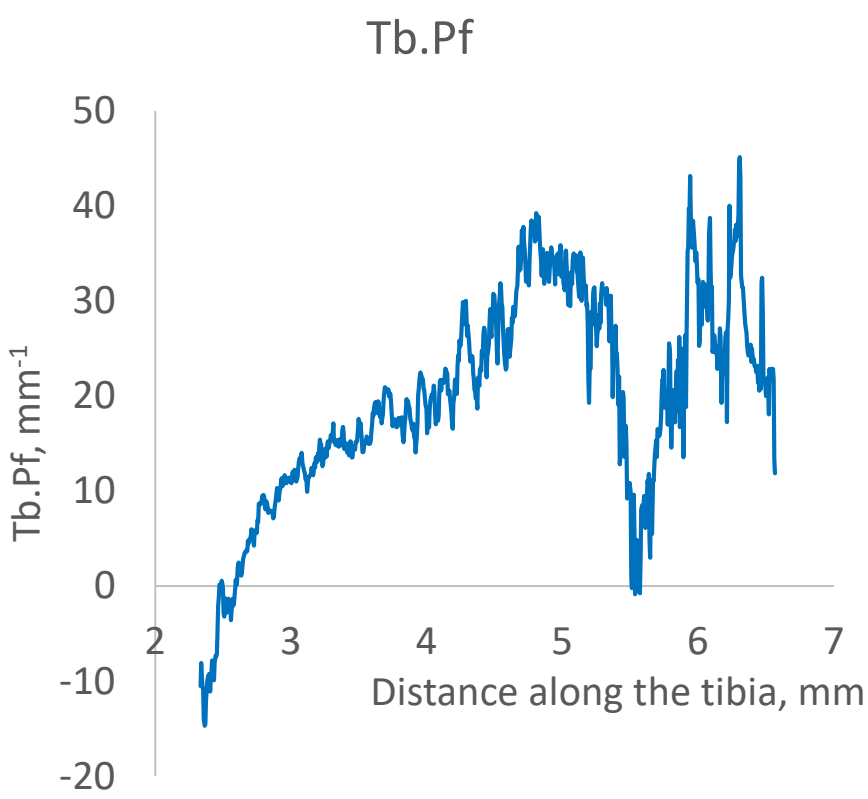
- Mice aged 10 weeks at commencement
- Sham, OVX, alendronate treated and estradiol treated by injection
- Six weeks study duration after OVX
- Mice euthanized, distal femurs analysed by MicroCT (4.4 micron pixel)

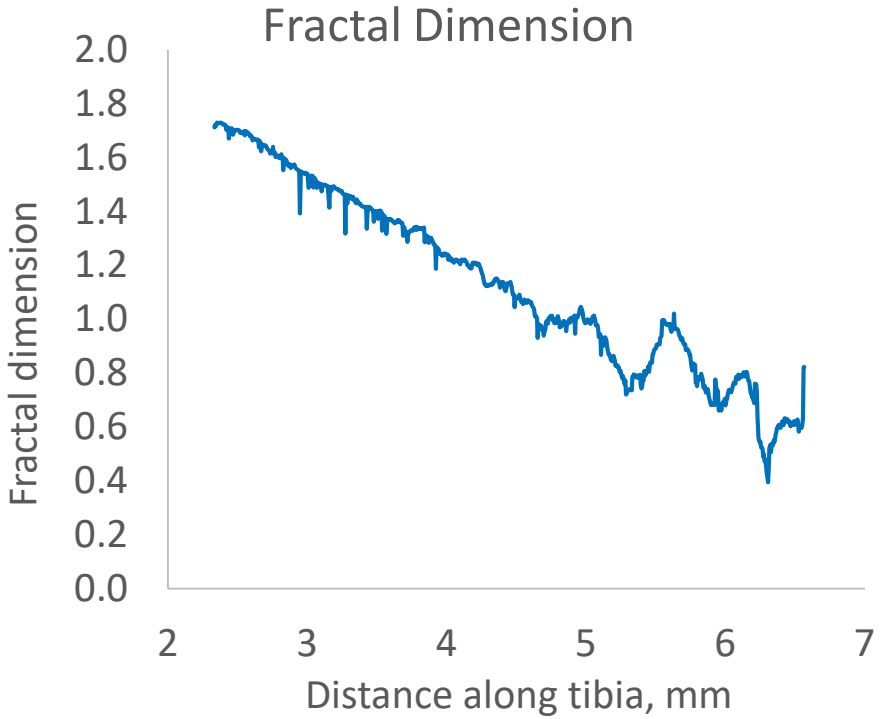
Longitudinal profiles from the growth plate



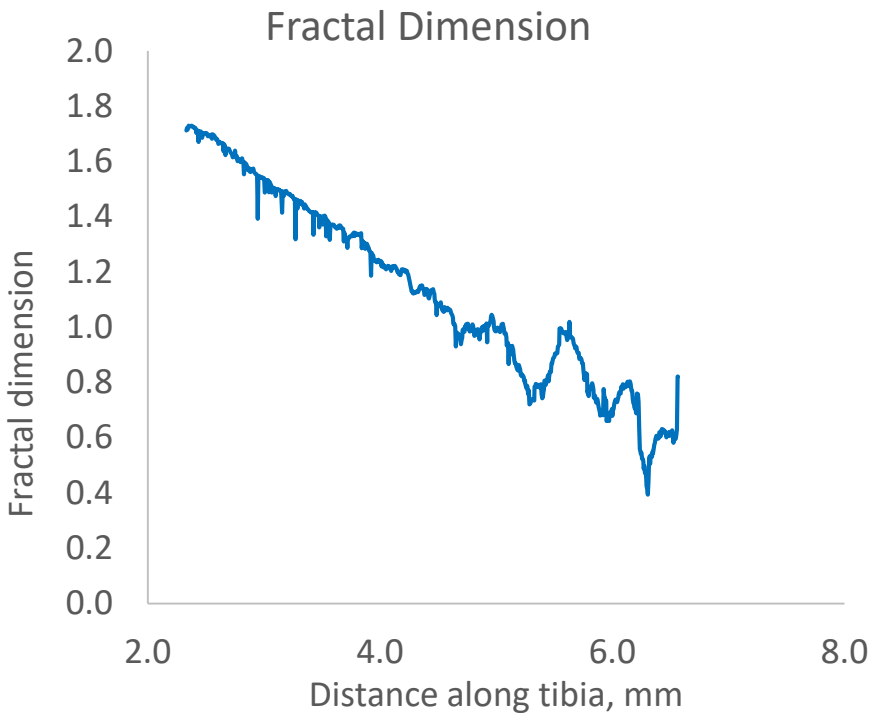


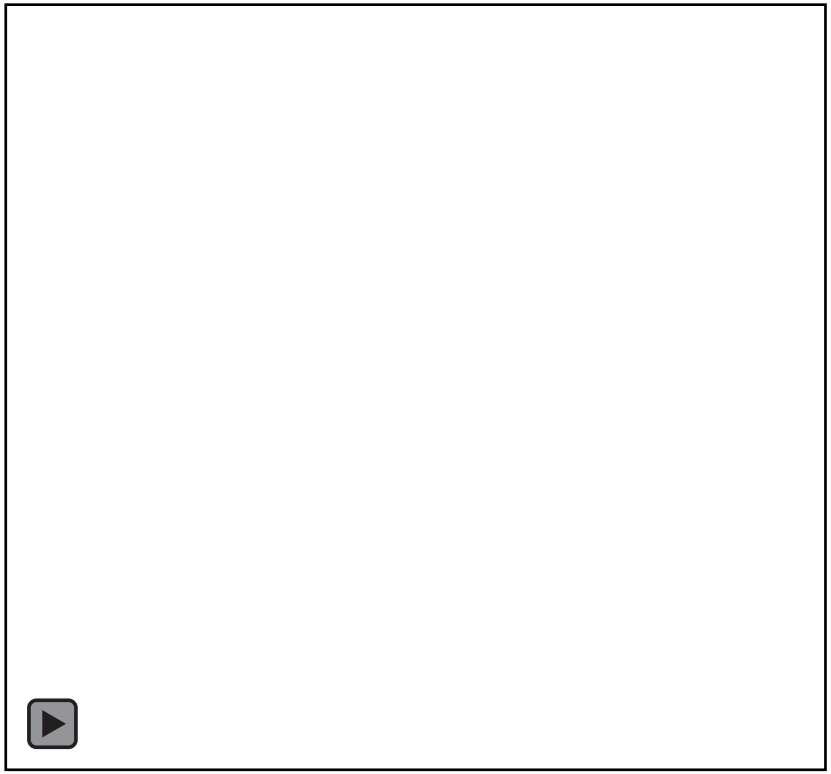
Sham animal


Tb.Pf
Tb.Pf, mm-1
50
40
30
20
10
0
-10
-20
2
3
4
5
6
7
Distance along the tibia, mm


Fractal Dimension
Fractal dimension
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2
3
4
5
6
7
Distance along tibia, mm

A linear gradient of FD occurs over most of the metaphysis accompanying big apparent changes in geometry





Fractal pattern is generated by the bone modeling cycle

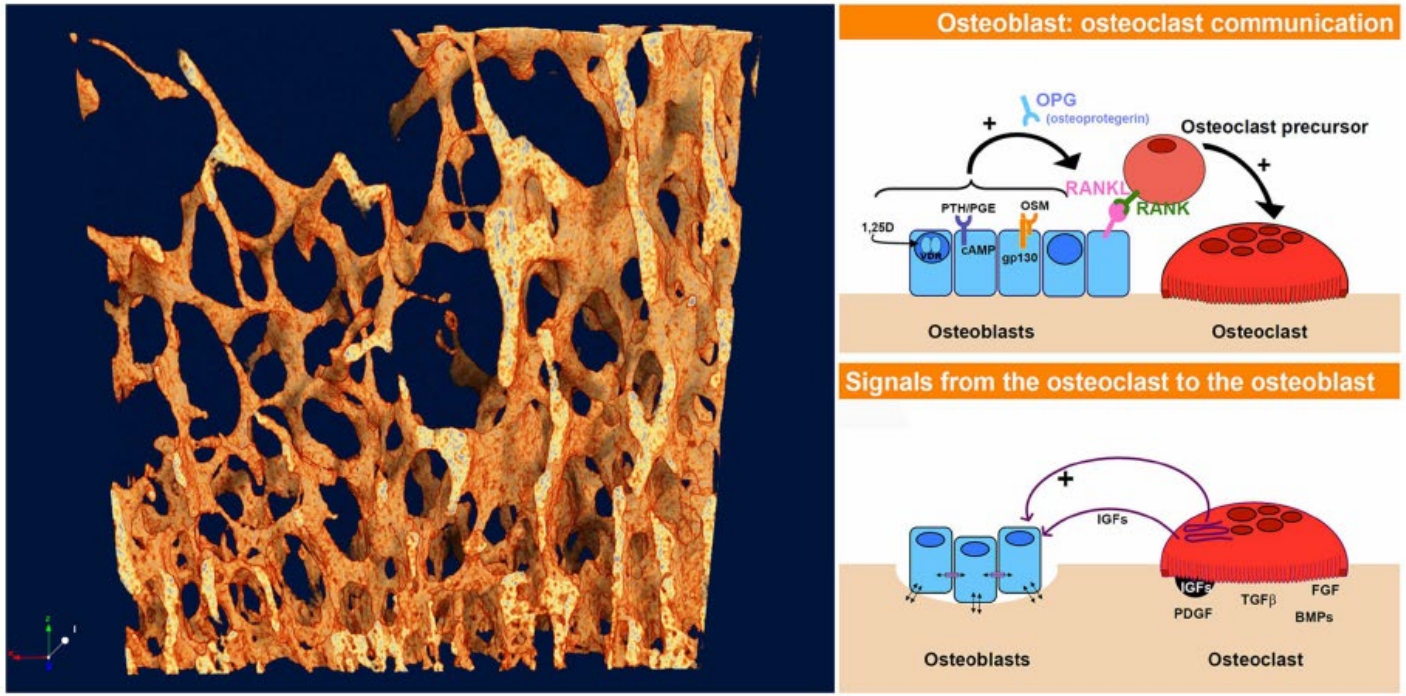
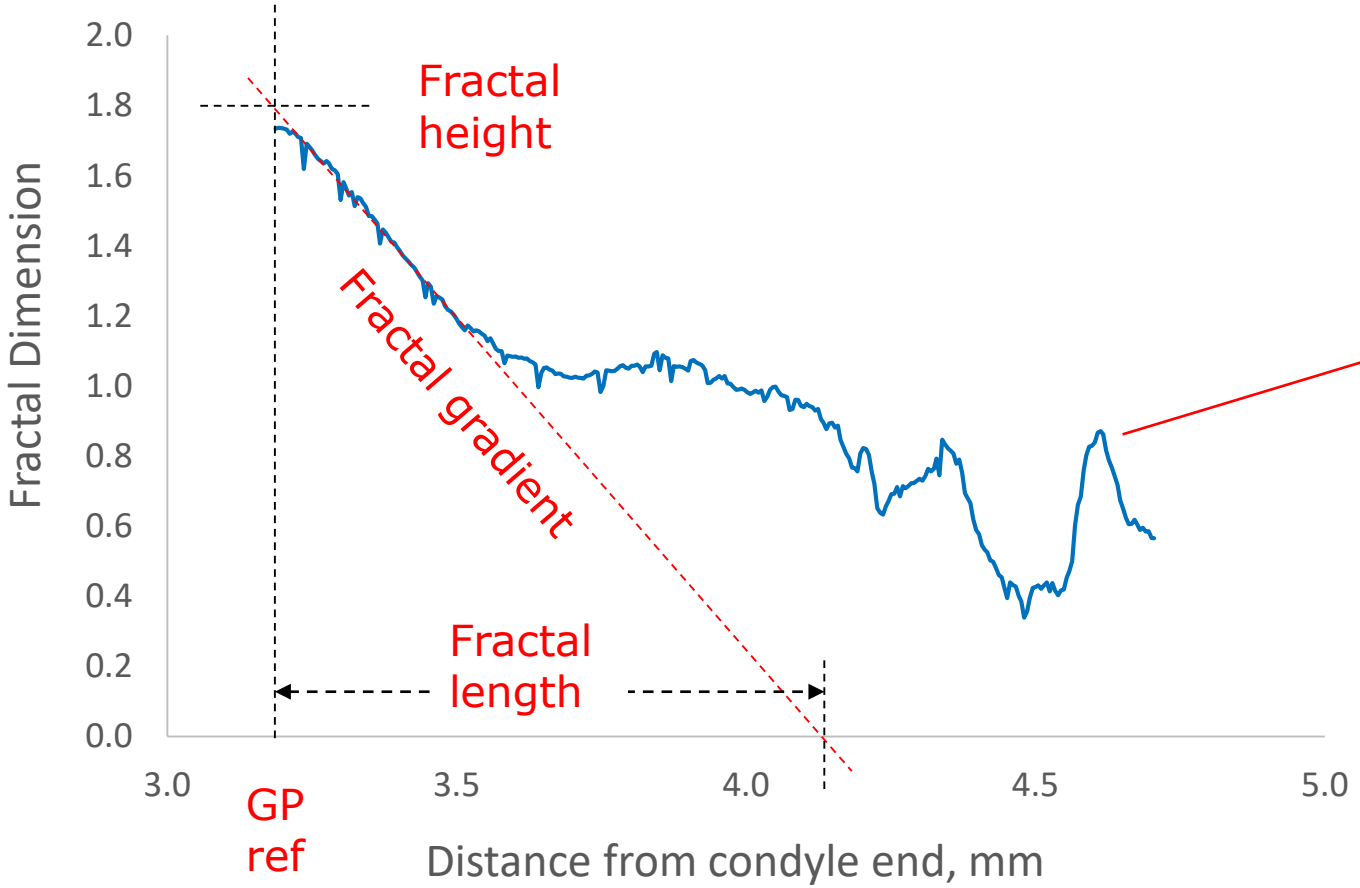


FIGURE 2 | The dynamics of interaction and coupling between osteoblasts and osteoclasts give rise to the complex evolving 4-D pattern of trabecular bone, just as the responses between starlings give rise to the highly patterned swarming murmurations.

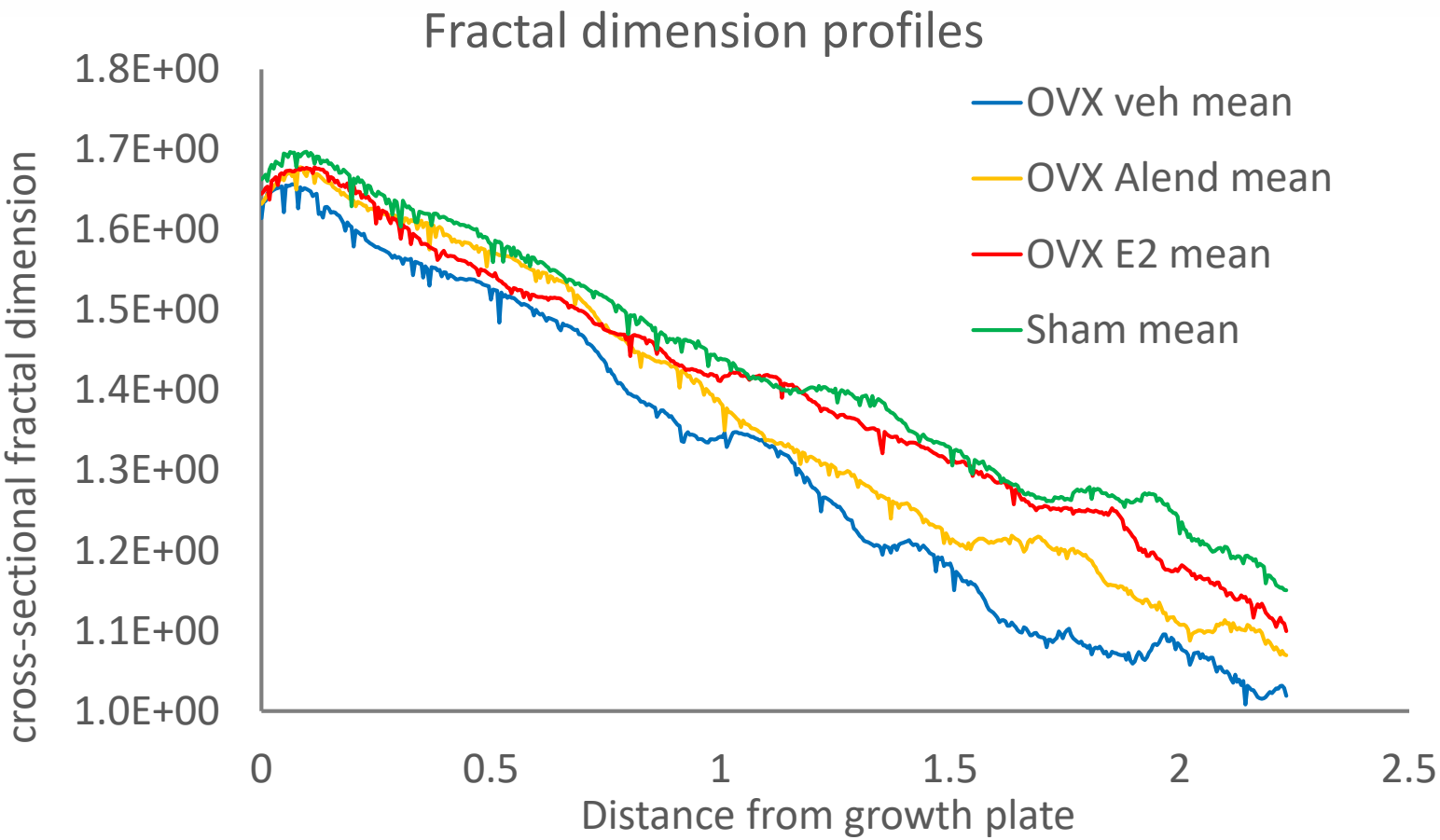
The osteoblast–osteoclast diagrams (right) were taken from an online slideshow provided by the group of Natalie Sims and Ron Martin at the Saint Vincent's Institute, Melbourne, VIC, Australia [Ref. (4), http://www.ectsoc.org/c020709/sims.pdf].

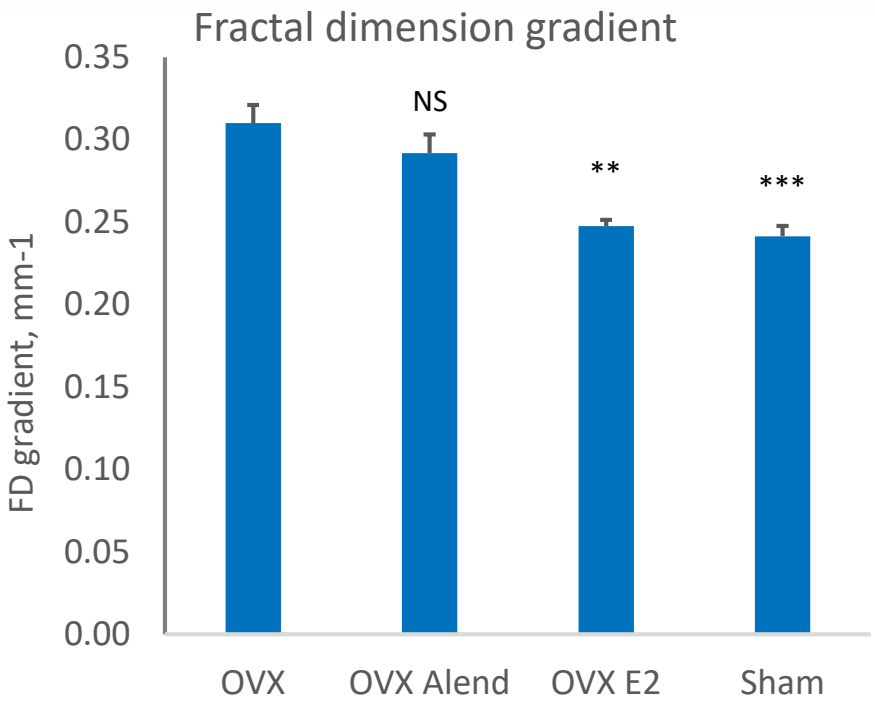
We can derive new morphometric parameters for the metaphysis from this FD profile, reflecting underlying pattern formation

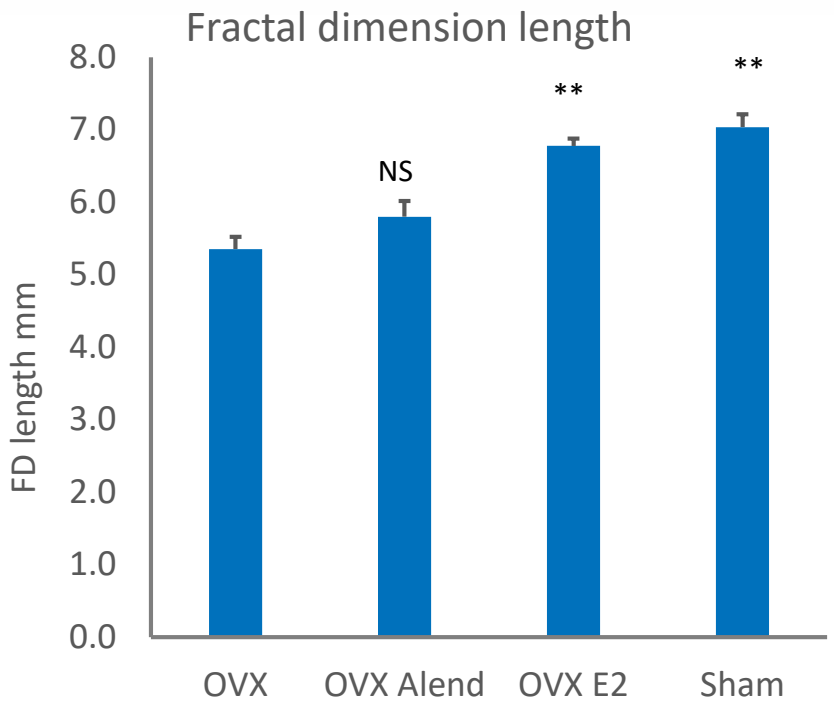


FD profile of a 20 month old BL-6 mouse

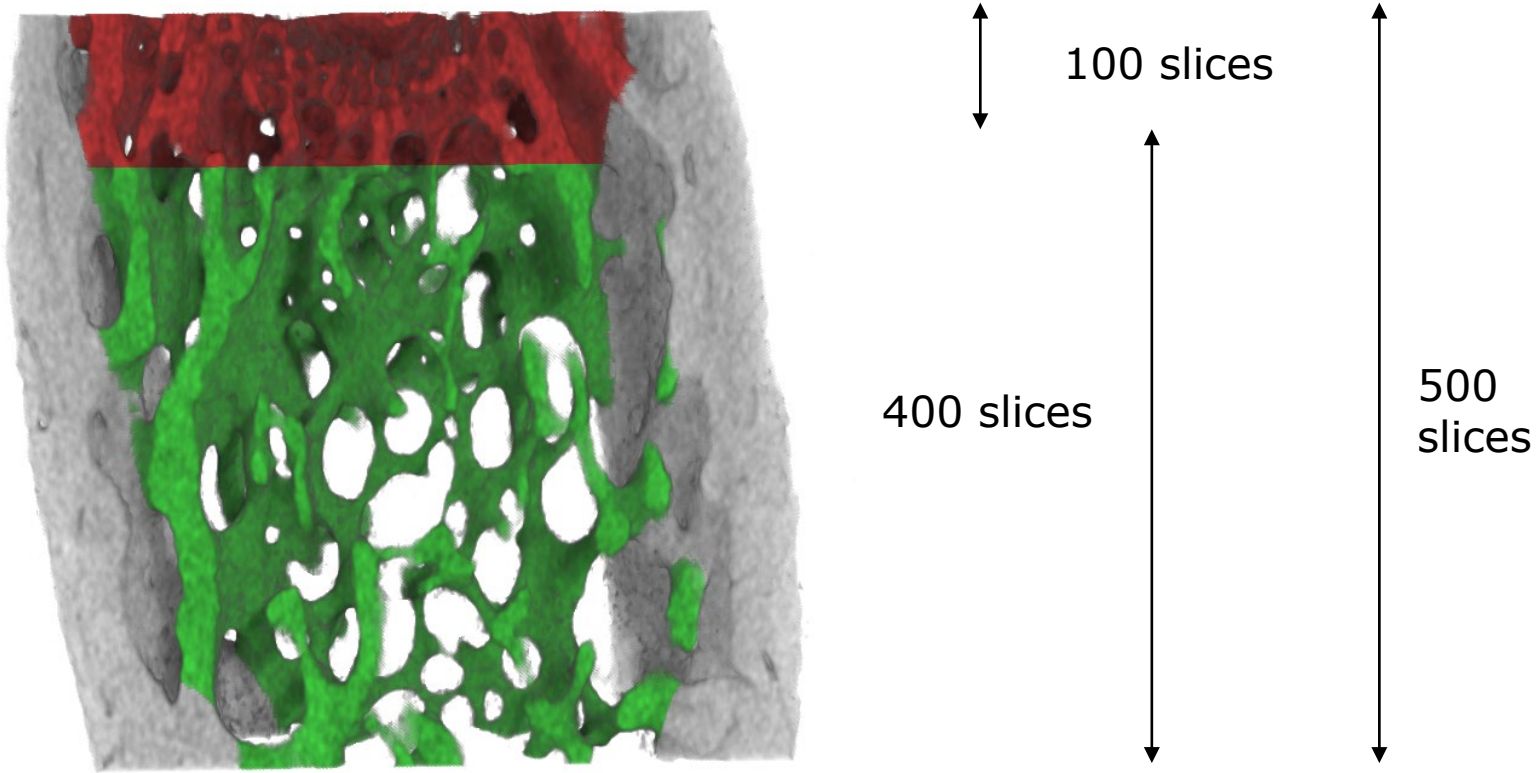
Fractal gradient in our OVX study



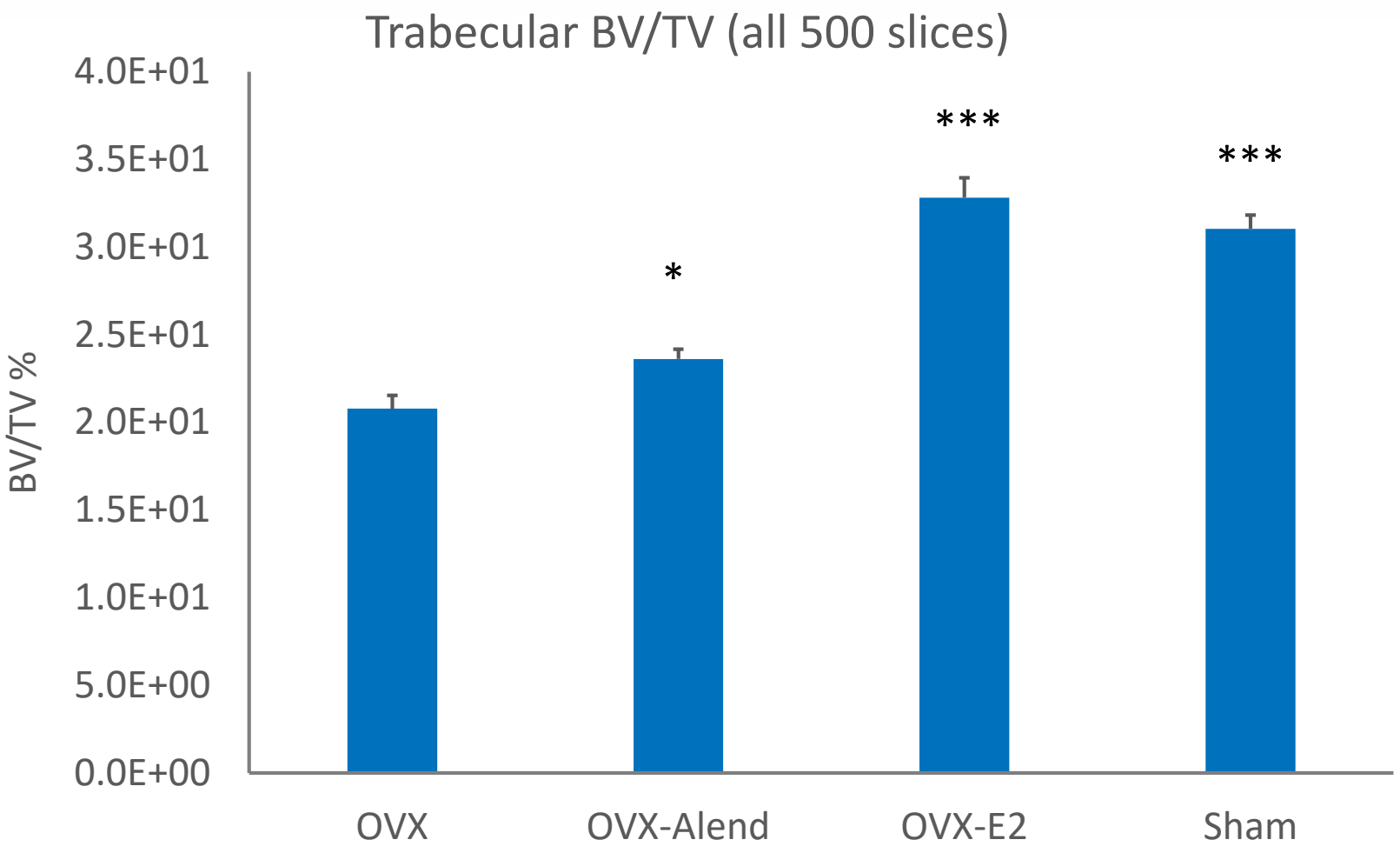

Fractal dimension gradient
FD gradient, mm-1
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
NS
**
***
OVX
OVX Alend
OVX E2
Sham


Fractal dimension length
FD length mm
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
NS
**
**
OVX
OVX Alend
OVX E2
Sham

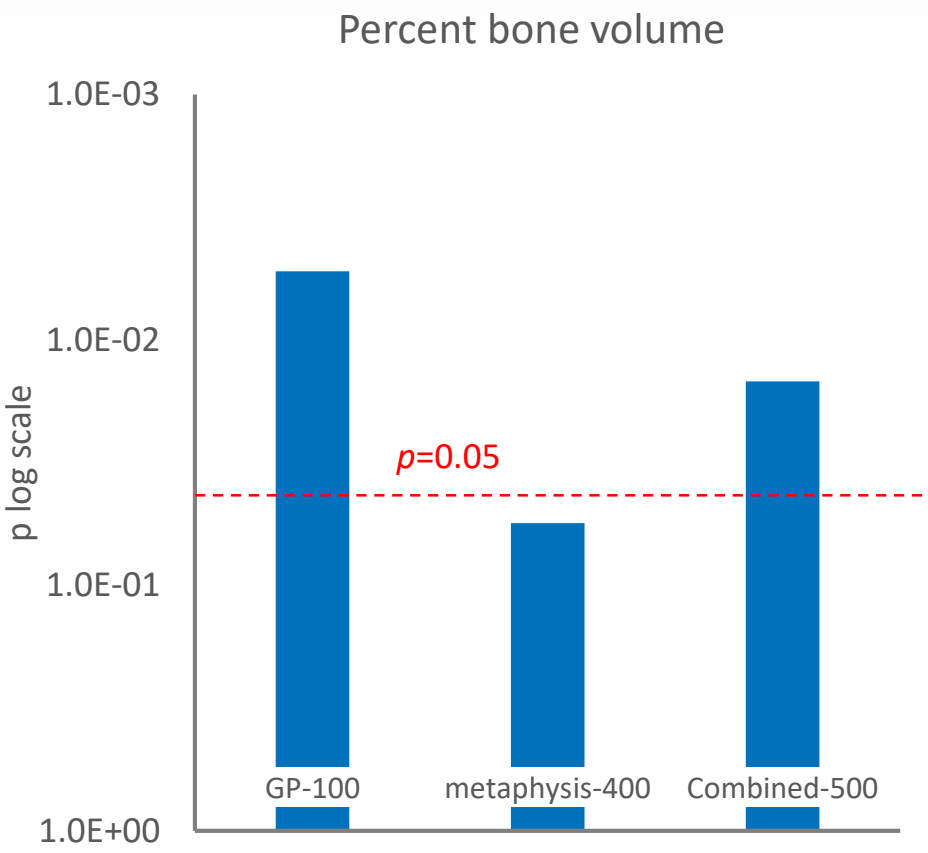
3D morphometry with and without the growth plate region

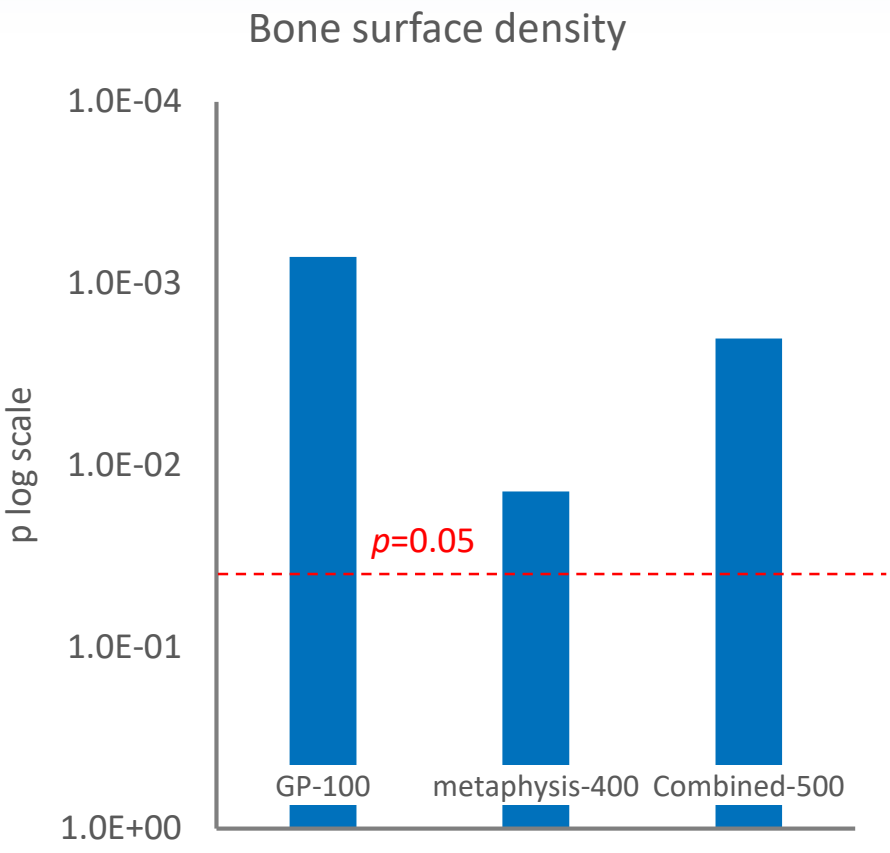


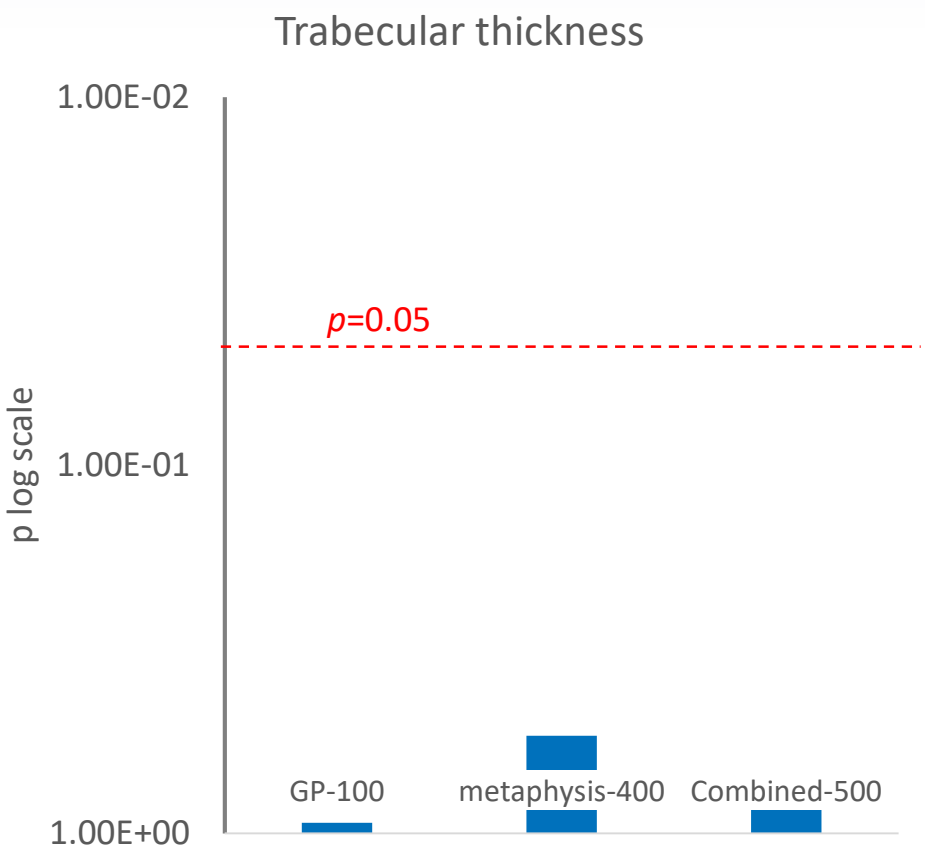
Overall result: strong preventive effect of estradiol, weak effect of alendronate (dose issue?)

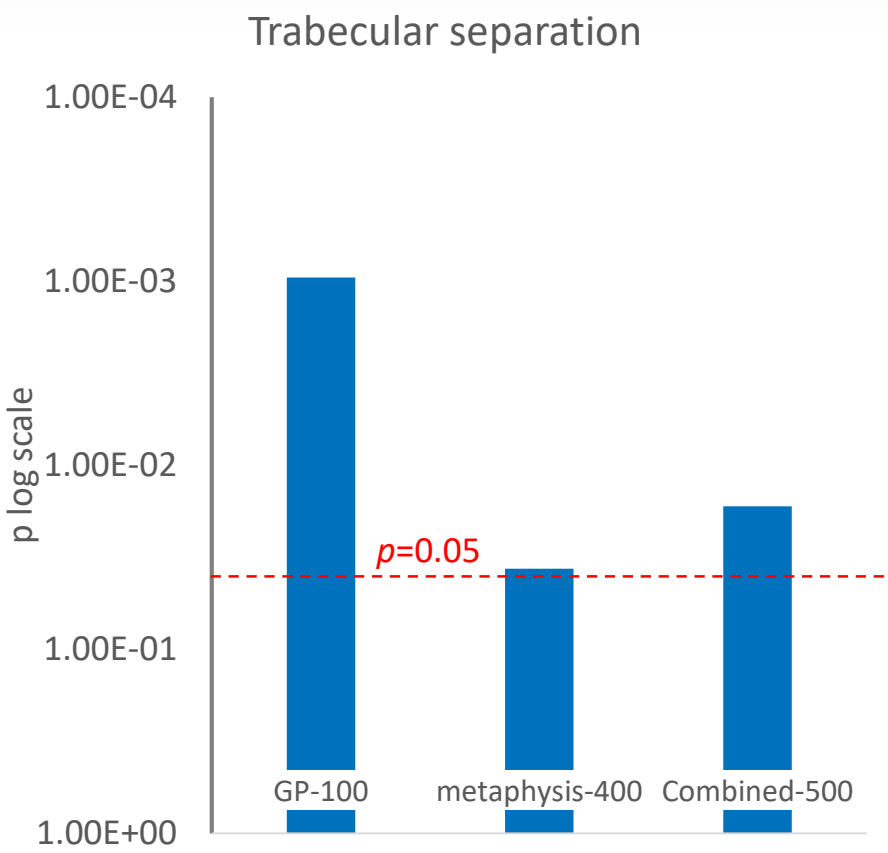


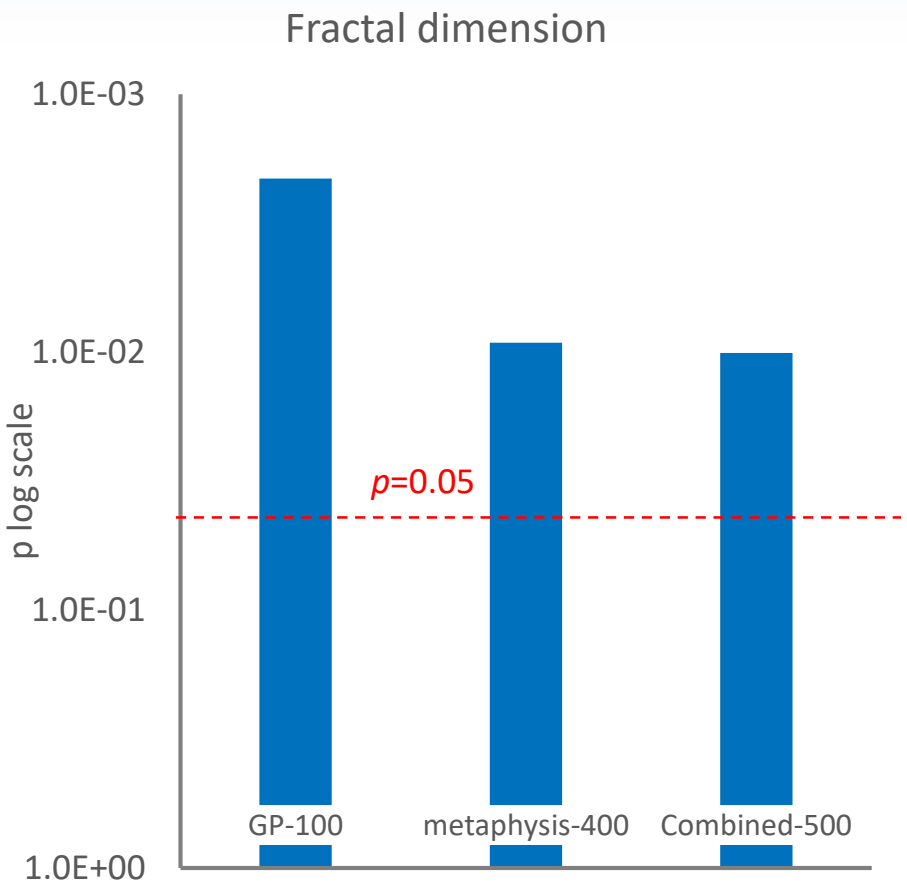
What effect did including or excluding the growth plate region have on the p values of inter-group differences?

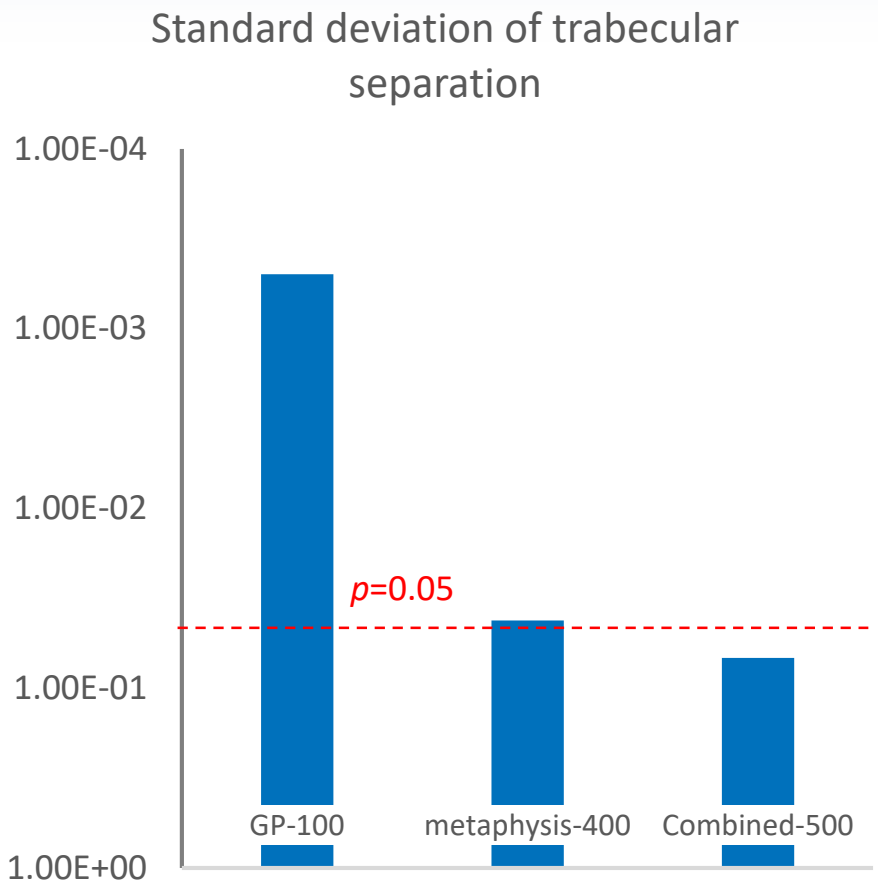

Percent bone volume
1.0E-03
1.0E-02
1.0E-01
1.0E+00
p log scale
p=0.05
GP-100
metaphysis-400
Combined-500


Bone surface density
1.0E-04
1.0E-03
1.0E-02
1.0E-01
1.0E+00
p log scale
p=0.05
GP-100
metaphysis-400
Combined-500


Trabecular thickness
1.00E-02
1.00E-01
1.00E+00
p log scale
p=0.05
GP-100
metaphysis-400
Combined-500


Trabecular separation
1.00E-04
1.00E-03
1.00E-02
1.00E-01
1.00E+00
p log scale
p=0.05
GP-100
metaphysis-400
Combined-500

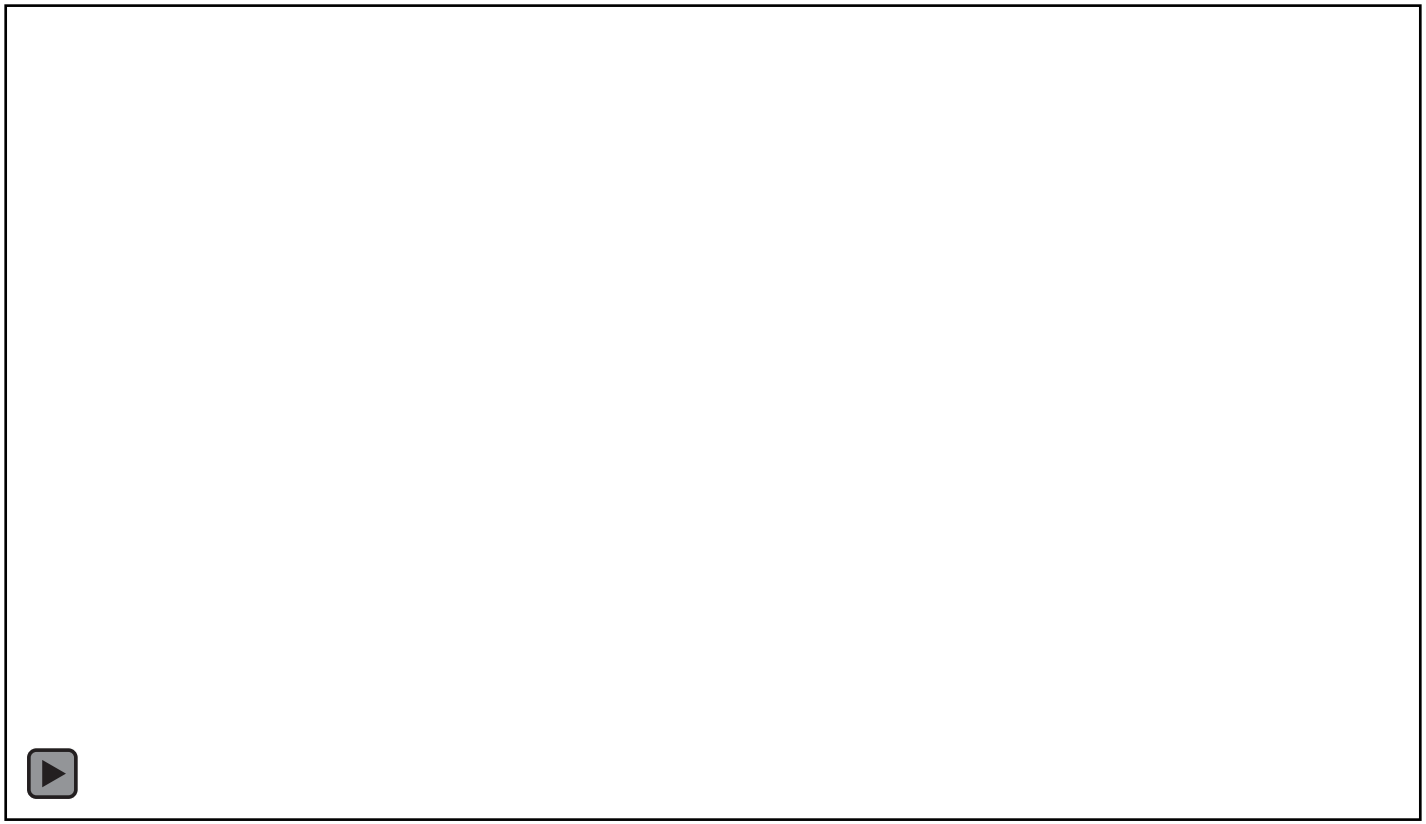

Fractal dimension
1.0E-03
1.0E-02
1.0E-01
1.0E+00
p log scale
p=0.05
GP-100
metaphysis-400
Combined-500

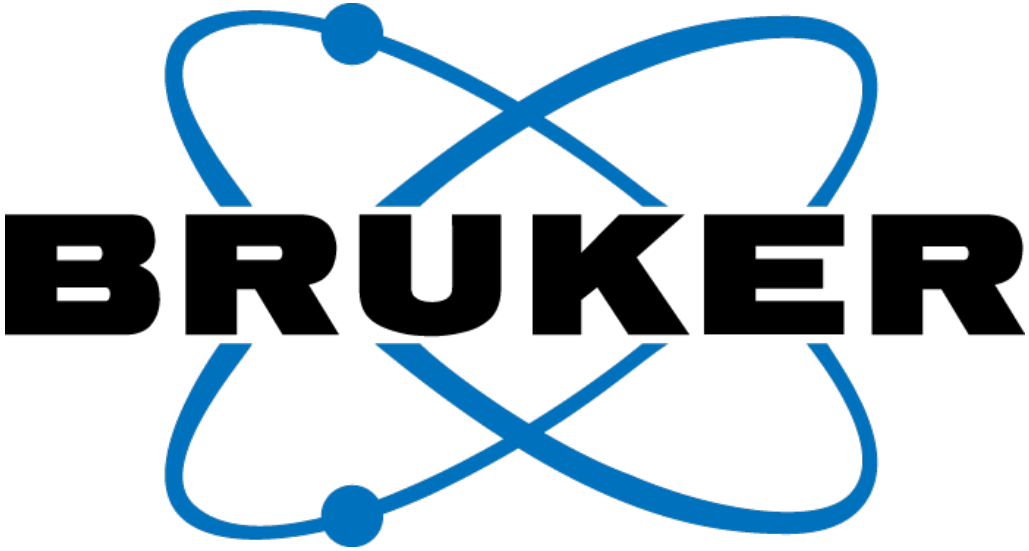

Standard deviation of trabecular separation
1.00E-04
1.00E-03
1.00E-02
1.00E-01
1.00E+00
p=0.05
GP-100
metaphysis-400
Combined-500

Conclusions

- There is no real transition between primary and secondary spongiosal trabecular but a uniform linear gradient
- This is shown most clearly by the linear gradient of cross-sectional fractal dimension
- So the metaphysis all the way to the growth plate can be considered a unitary modelling volume
- This means it might be OK to include the growth plate and primary spongiosal region in trabecular morphometry
- This could help us in morphometry of very old mice with little or no bone beyond the growth plate

Thank-you for your attention





Innovation with Integrity

© Copyright Bruker Corporation. All rights reserved.