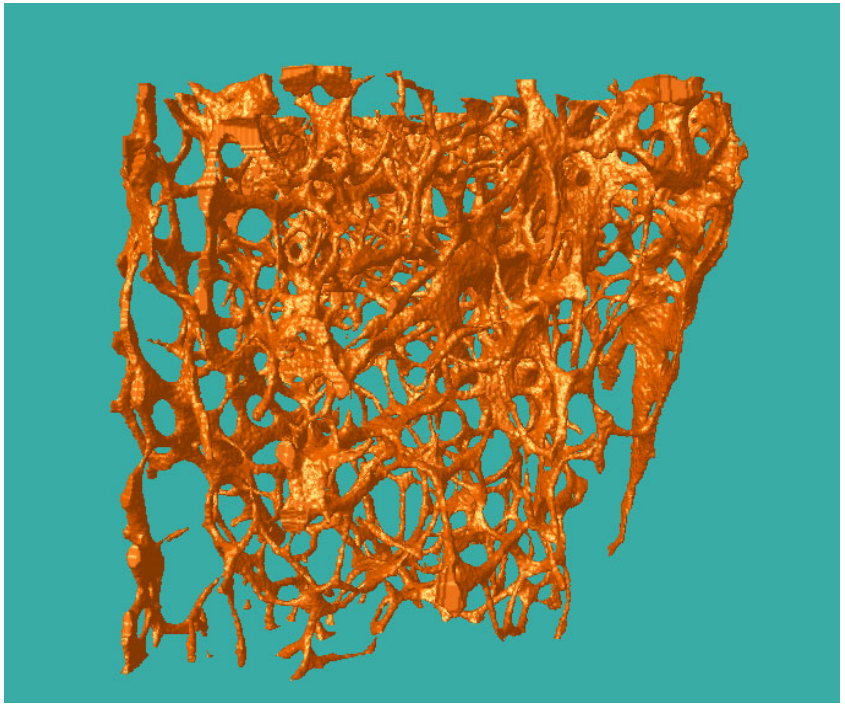
Practical bone histomorphometry in mice

Reinhold G. Erben, M.D. D.V.M.

Department of Biomedical Sciences
University of Veterinary Medicine Vienna

Purpose of histomorphometry

Histomorphometry is always related to mechanistic questions or safety issues



- ***Bone structure***
- ***Bone formation***
- ***Bone resorption***
- ***Bone mineralization***
- ***Bone modeling and remodeling***
- ***Osteocyte lacunae***

Age-related changes in the femur

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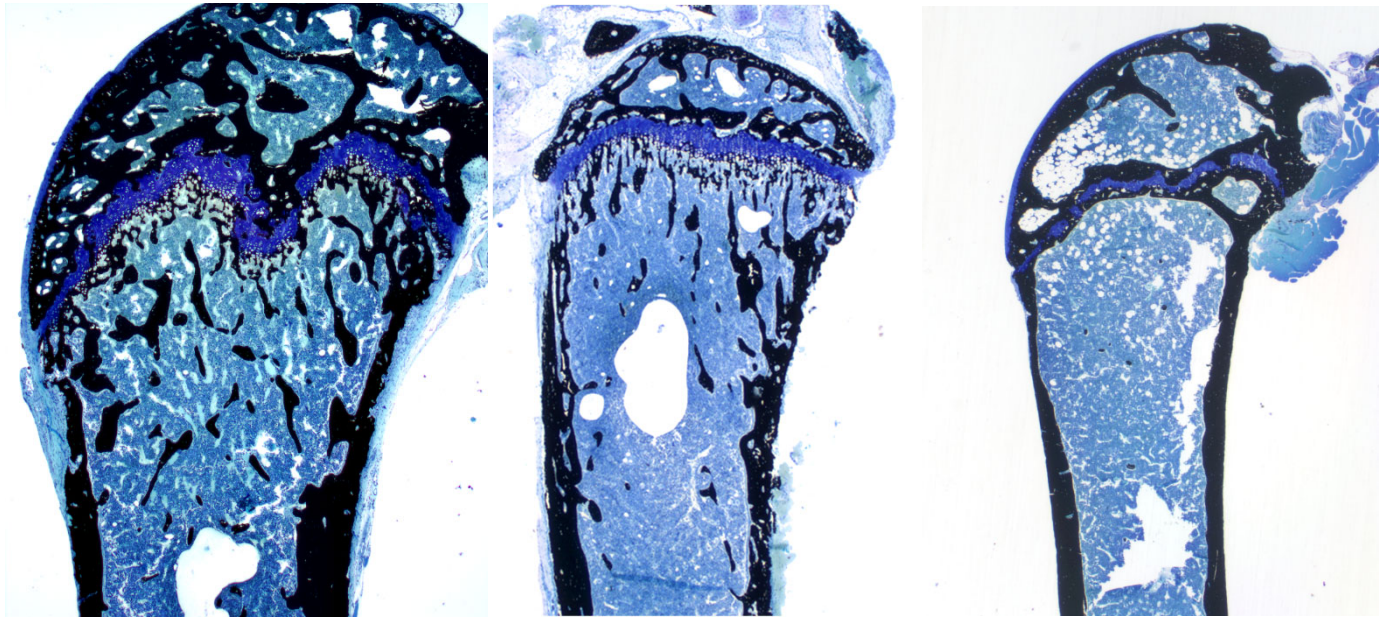
3 mo, femur | 3 mo, tibia | 1 y, femur

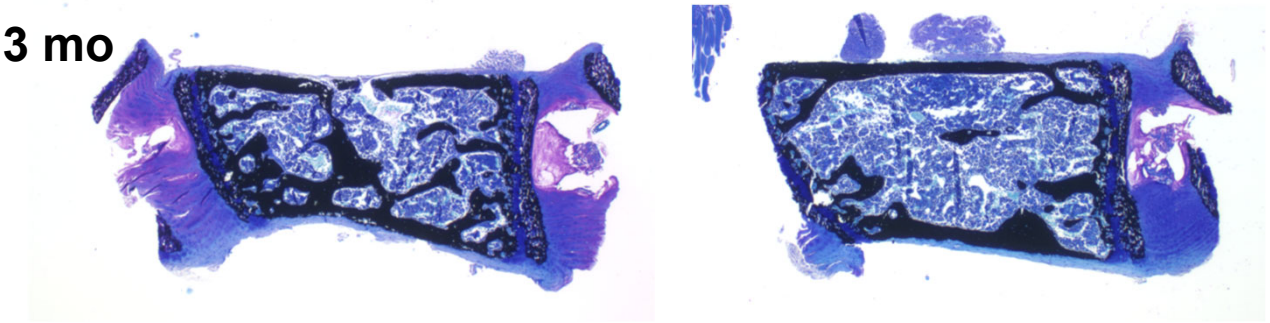


⇒ **The distal femur is more suitable for histomorphometry than the proximal tibia in mice.**

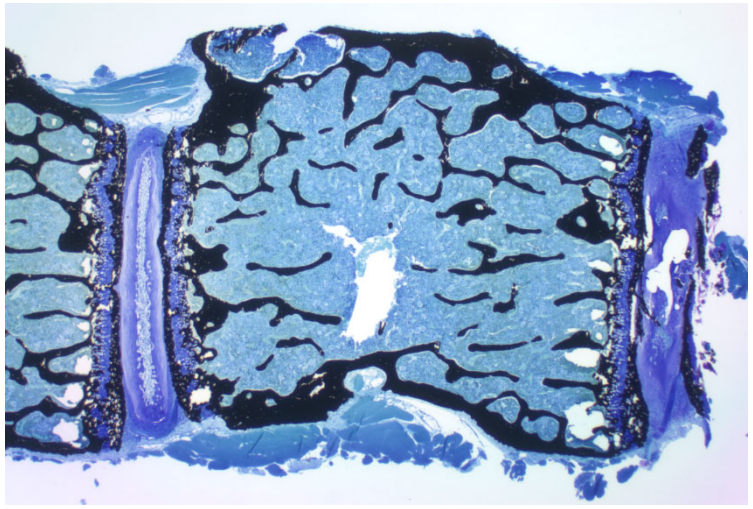
⇒ **Age-related osteopenia can make a sound analysis of cancellous bone impossible in the distal femoral metaphysis of aged mice.**

Age-related changes in vertebrae

3 mo



10 mo



⇒ **Always take out the verts in aged mice! Use frontal sections.**

Gender-related differences in mice

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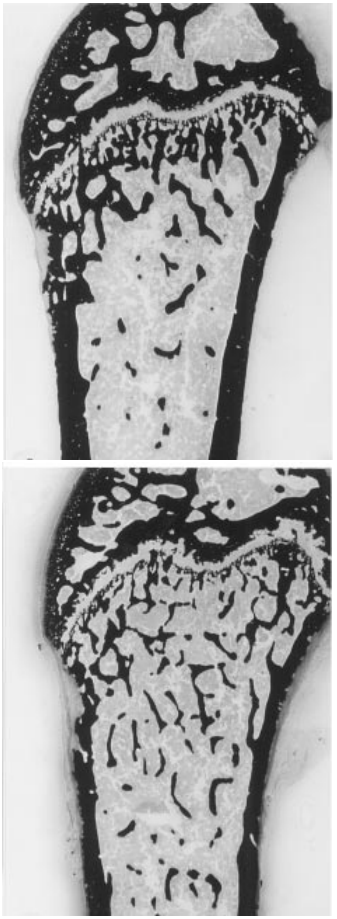
Mice



Female

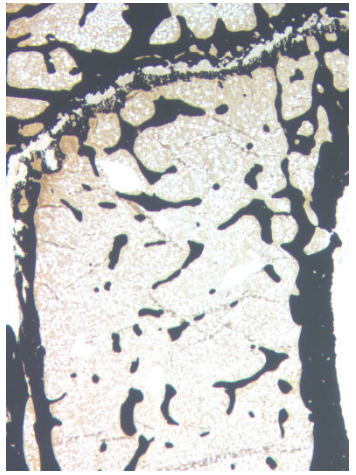
Male

→ Estrogen is a bone anabolic hormone in mice, unlike other mammalian species.

Rats

Female

Male



Schmidt et al. Am J Pathol 155:557, 1999

Embedding methods

Distal femur and vertebrae

- ***MMA mixture suitable for histochemistry (Erben, J Histochem Cytochem 45:307,1997)***

Cortical cross-sections & implants

- ***Conventional MMA embedding (80% MMA, 20% dibutylphthalate, 3% benzoylperoxide)***

More information: Erben & Glösmann (2019) Histomorphometry in Rodents. Methods Mol Biol 1914:411-435

Routine stains

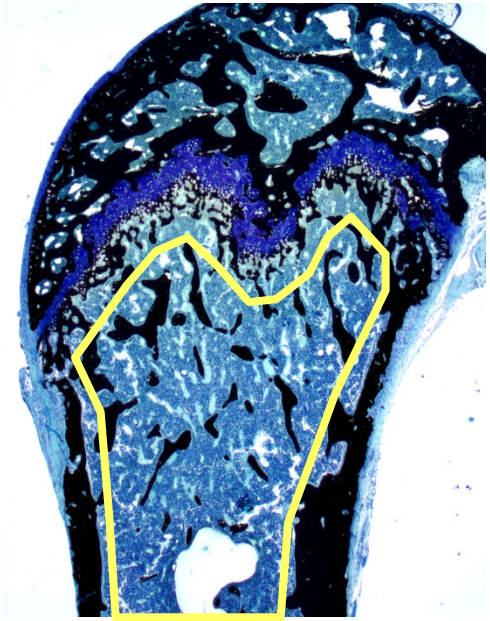
Distal femur and verts

- *Von Kossa/McNeal's tetrachrome*
- *TRAP staining*
- *(Cement line stain)*

Microground cortical bone cross-sections&implants

- *Toluidine blue*

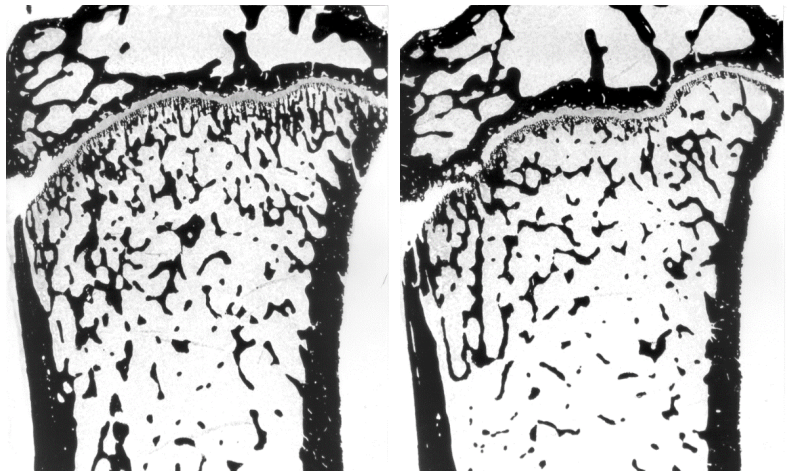
How to measure structural parameters



Primary measurements

- **Tissue area**
- **Bone area**
- **Bone perimeter**
- **Number of structural elements**

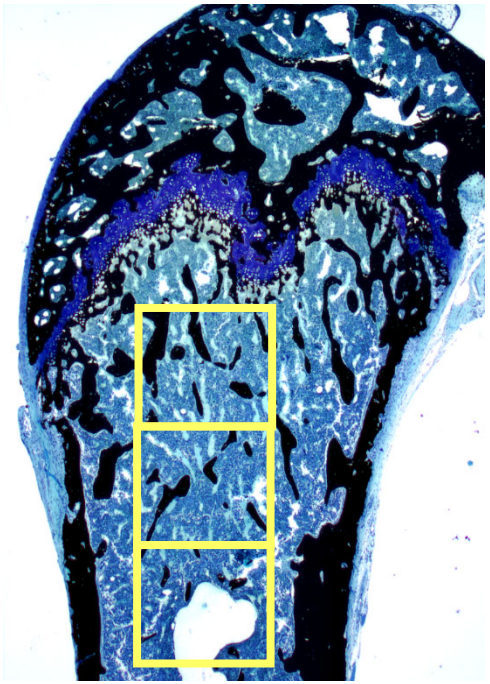
Control **4 weeks of immobilization**

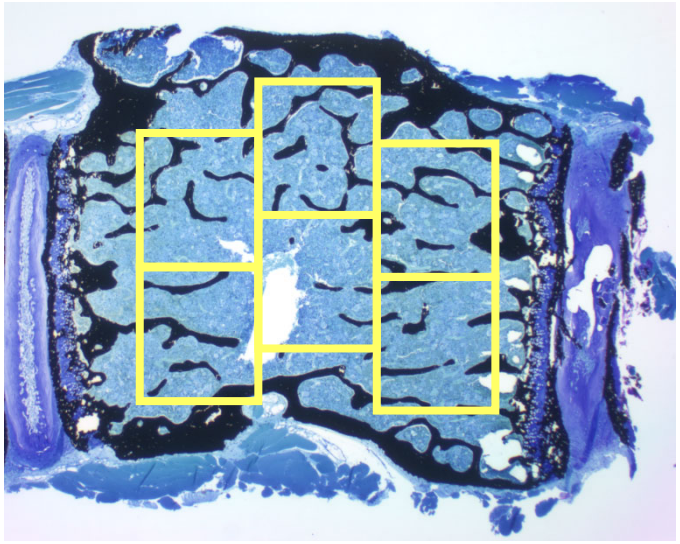


Distance from growth plate:
Femur: 500 µm in 3 - 4-week-old mice, 250 µm in mice ≥ 2 mo of age
Vertebrae: 250 µm

→ Histomorphometry expresses in numbers that what you see

How to measure turnover parameters





Distance from growth plate: 250 µm typically
500 µm in young, fast-growing mice

Measurement at x200 or x400

Bone turnover. Bone formation

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Primary measurements

- ***Mineral apposition rate***
- ***Mineralizing perimeter (double labeled perimeter or D.L.Pm + 0.5 * S.L.Pm)***

↓

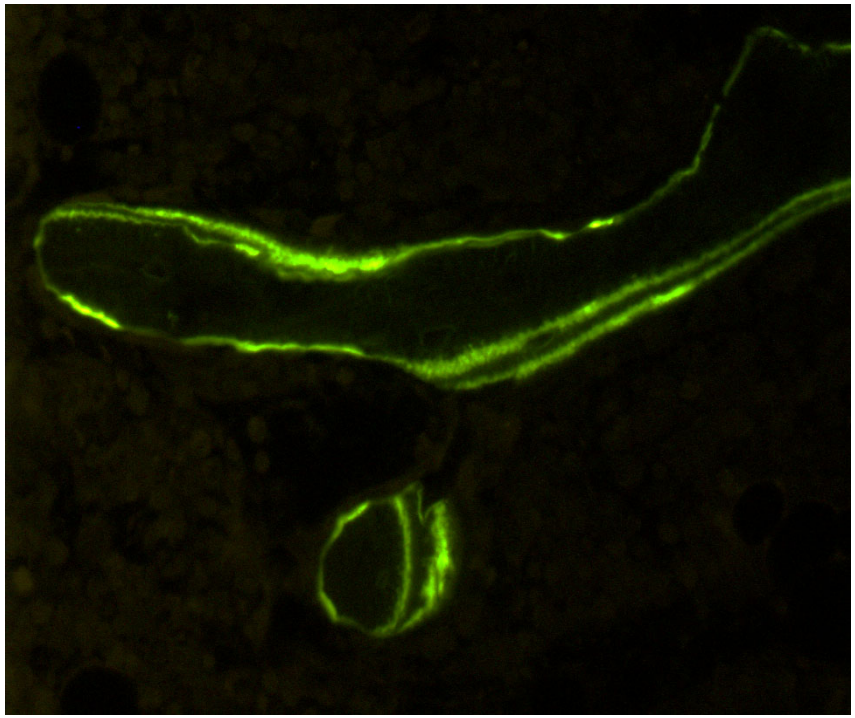
Bone formation rate (BFR/B.Pm = BFR/BS)

Marker interval (cancellous bone formation!)

24 h in 3 – 4-wk-old mice

48 h in 8 – 12-wk-old mice

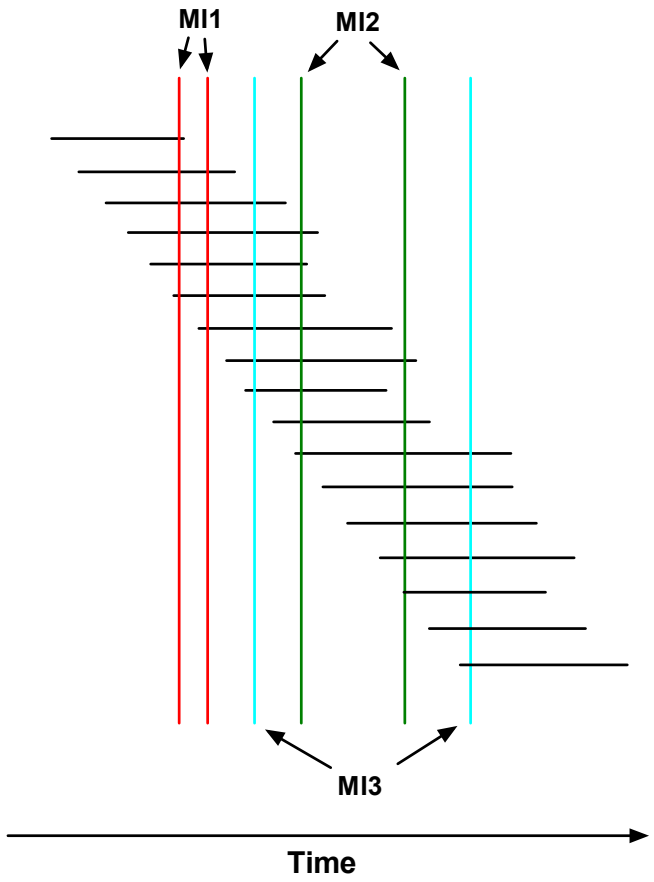
2 - 3 days in aged mice >5 mo



→ Allow for enough time (1 day) between last label and sampling!

→ More information: Erben RG (2003) Bone Labeling Techniques. In: Handbook of Histology Methods for Bone and Cartilage. An YH, Martin KL (eds) Humana Press Inc., Totowa, NJ, USA, pp 99 – 117

Labeling escape error

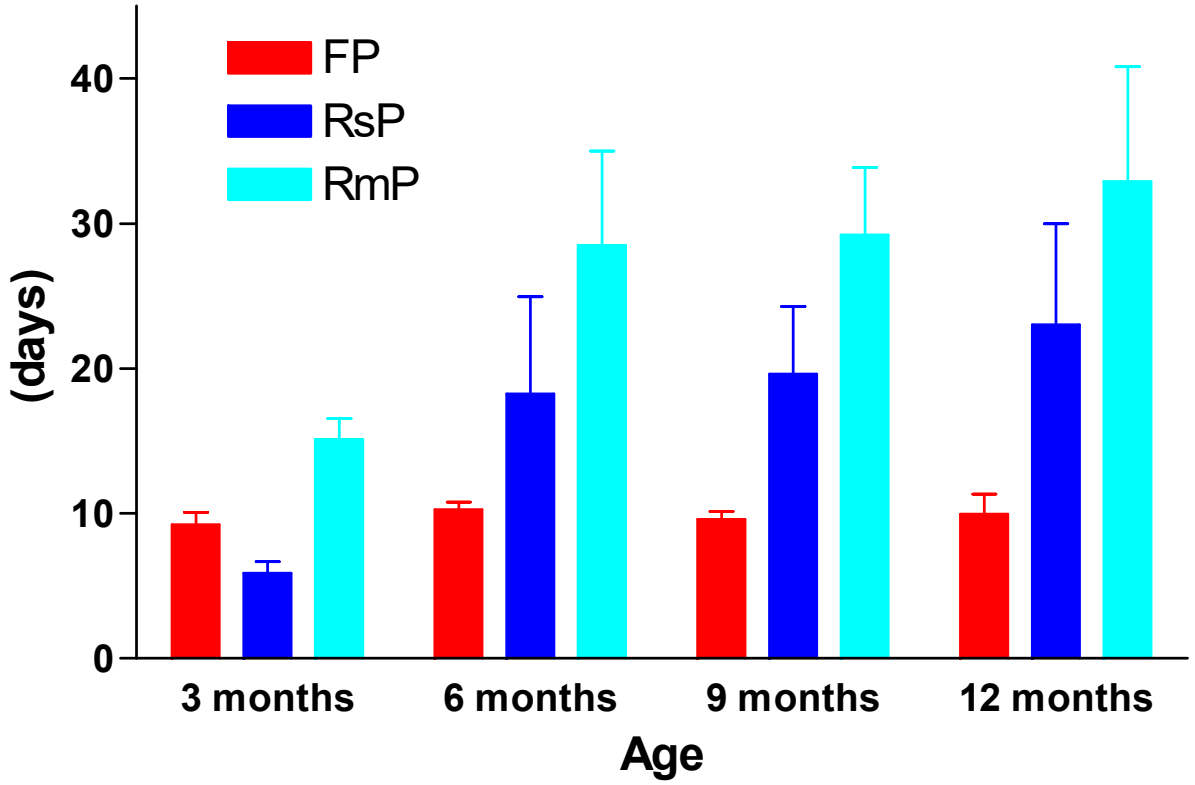


⇒ To minimize the labeling escape error, the marker interval should be less than about 1/5 of the formation period.

From: Erben RG (2003) Bone Labeling Techniques. In: Handbook of Histology Methods for Bone and Cartilage. An YH, Martin KL (eds) Humana Press Inc., Totowa, NJ, USA, pp 99 – 117

Bone remodeling in mice

Murine vertebral cancellous bone



Bone turnover. Bone formation

Primary measurements

- *Mineral apposition rate*
- *Mineralizing perimeter (double labeled perimeter)*

↓

Bone formation rate (BFR/B.Pm = BFR/BS)

Marker interval (cancellous bone formation!)

24 h in 3 – 4-wk-old mice

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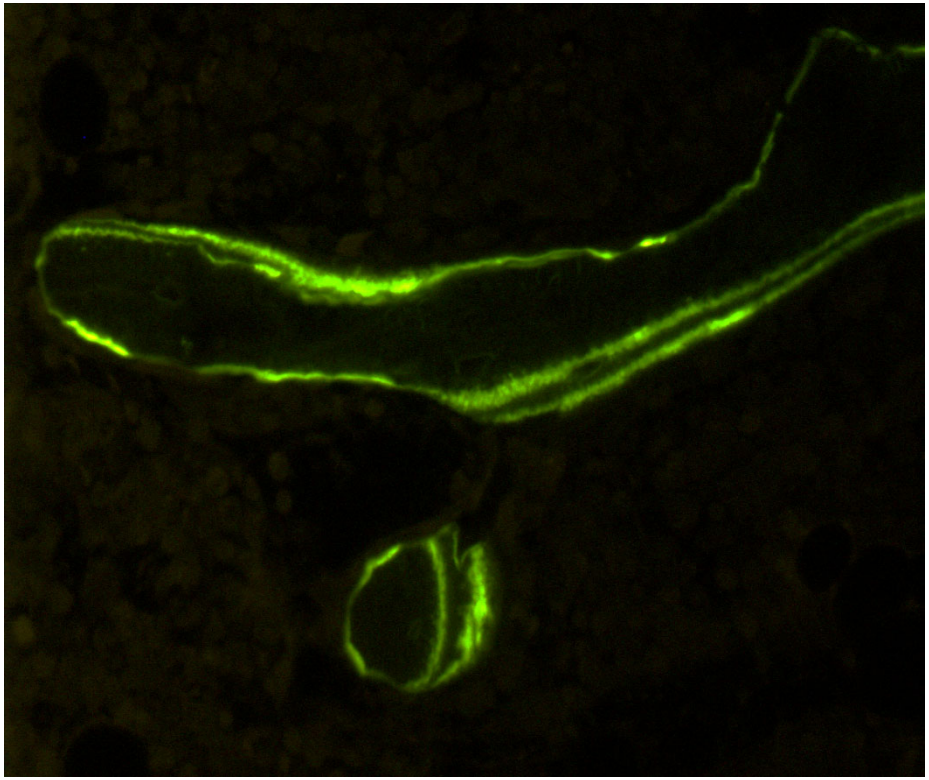
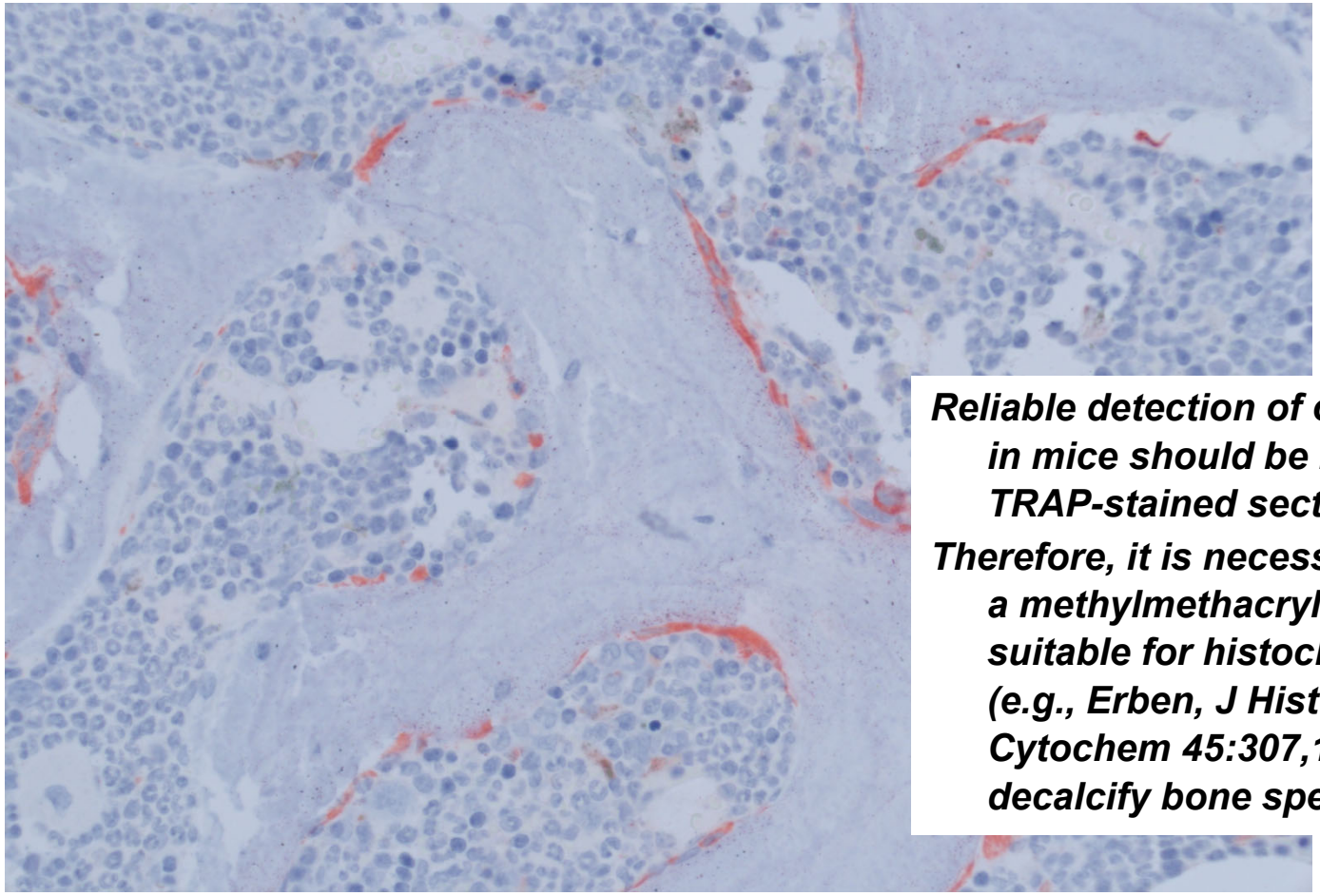


Bone resorption. TRAP staining



Reliable detection of osteoclasts in mice should be based on TRAP-stained sections!

Therefore, it is necessary to use a methylmethacrylate mixture suitable for histochemistry (e.g., Erben, J Histochem Cytochem 45:307,1997), or to decalcify bone specimens.

Bone turnover. Bone resorption

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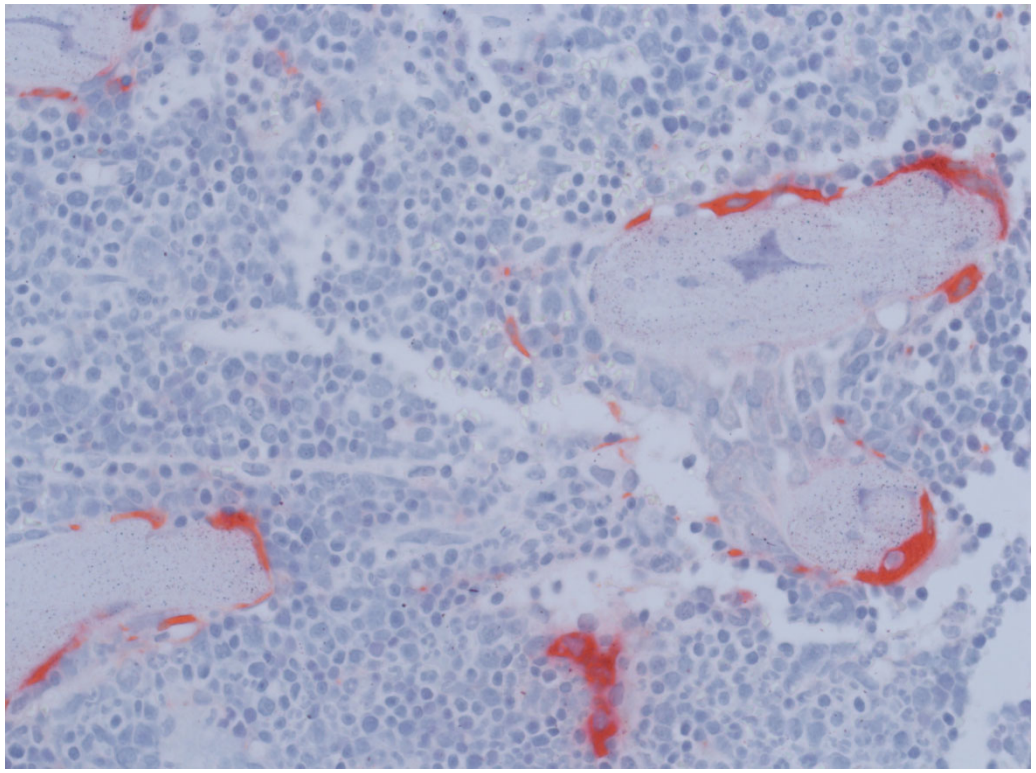
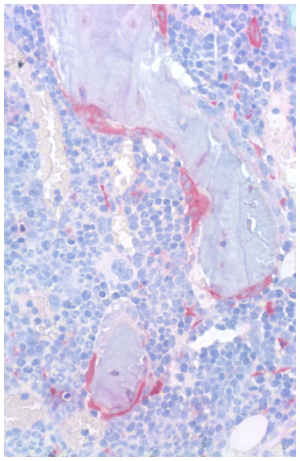
Primary measurements (only nucleated cells in contact with bone are counted!)

- ***Osteoclast number (no./mm or no./mm²)***
- ***Osteoclast perimeter (Oc.Pm/B.Pm, %)***

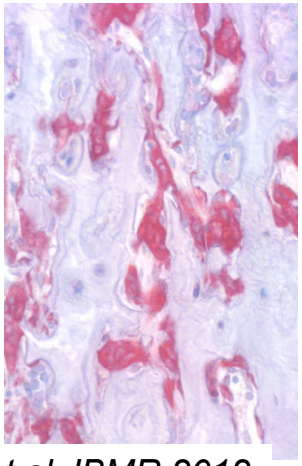


Bone turnover. Bone resorption

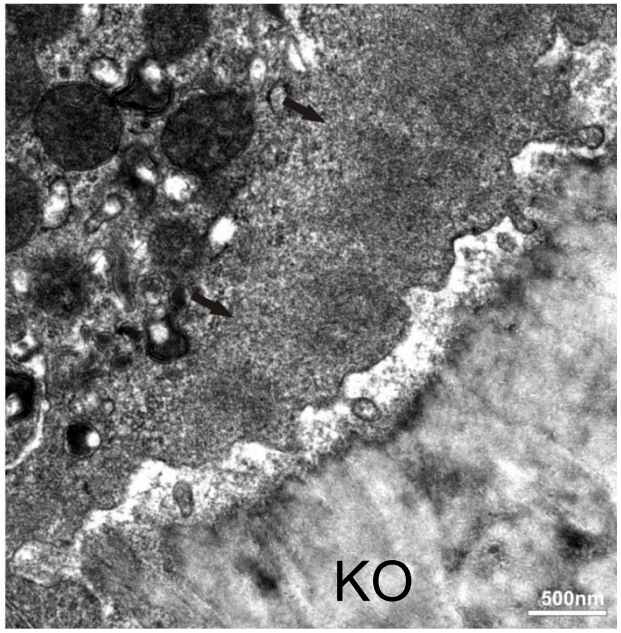
WT



KO



Garbe et al JBMR 2012



→ Osteoclast numbers are not a functional read-out!

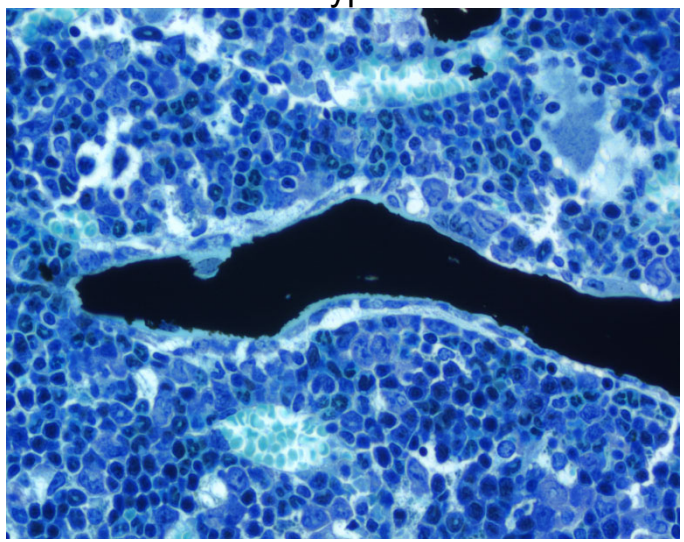
→ As a functional endpoint for bone resorption urinary collagen crosslinks are the best parameter!

Bone mineralization/formation
Osteoid & Osteoblasts

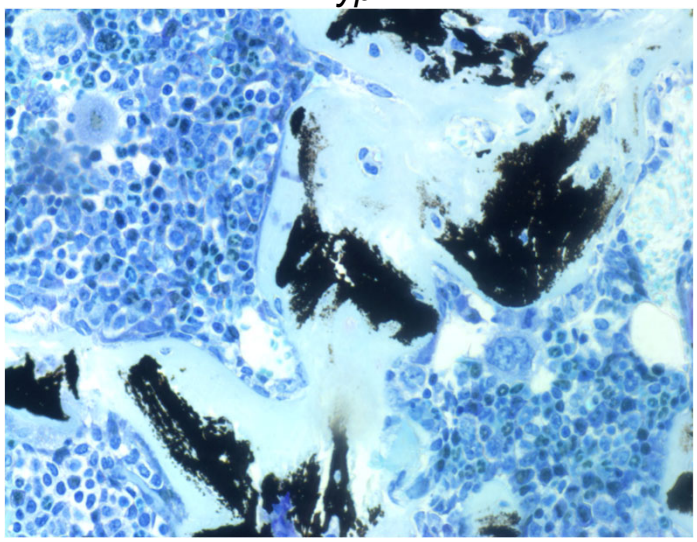
Primary measurements in von Kossa/McNeal-stained sections

- ***Osteoid width (O.Wi, µm)***
- ***Osteoid perimeter (O.Pm/B.Pm, %)***
- ***Osteoid area (O.Ar/B.Ar, %)***
- ***Osteoblast perimeter (Ob.Pm/B.Pm, %)***

Wildtype



Hyp



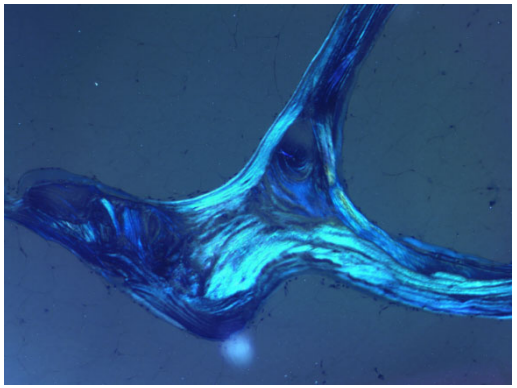
Modeling and Remodeling

Modeling

- Activation ⇨ Resorption, Activation ⇨ Formation
- Continuous process
- Induction of resorption and formation drifts in trabecular (mini-modeling) or cortical bone (macro-modeling): ⇒ Always goes along with changes in shape!
- Fast: adapts a structure within days
- **Function: dynamic adaptation mechanism to changes in biomechanical strain**

Remodeling

- Activation ⇨ Resorption ⇨ Formation
- Cyclical process
- *Cortical bone:* leaves behind osteons. *Trabecular bone:* reconstitutes bone surface more or less in its original shape.
- Slow: takes weeks to months to complete
- **Function: renewal mechanism in biomechanical steady state**



Cancellous bone remodeling in mice?

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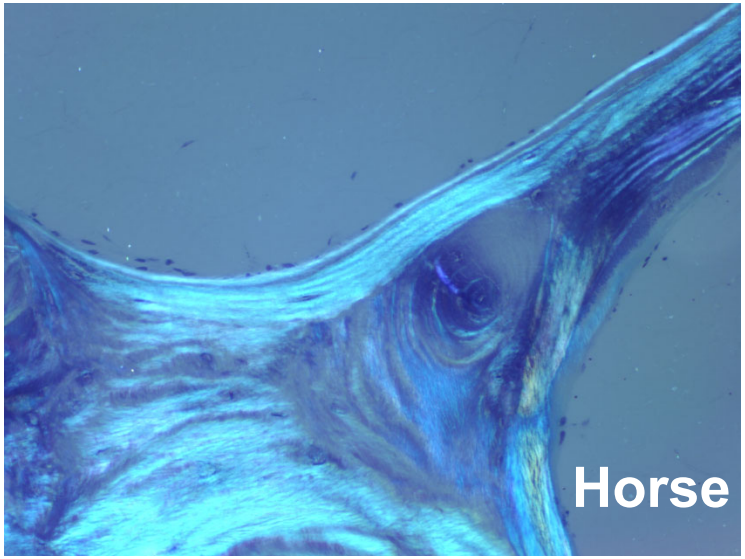
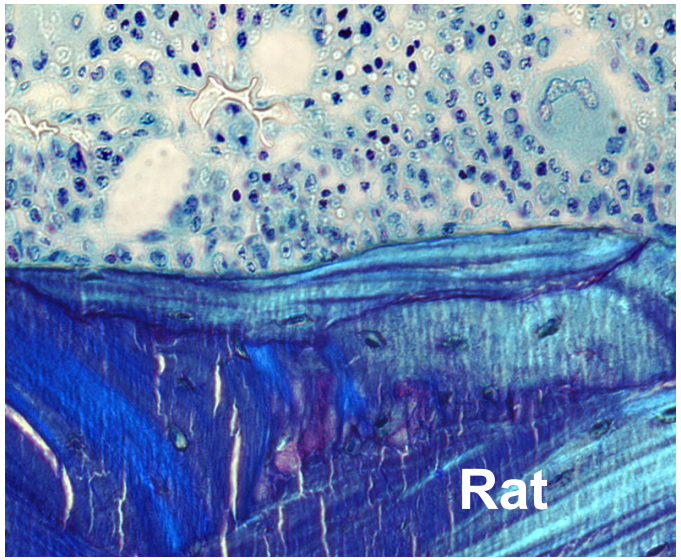
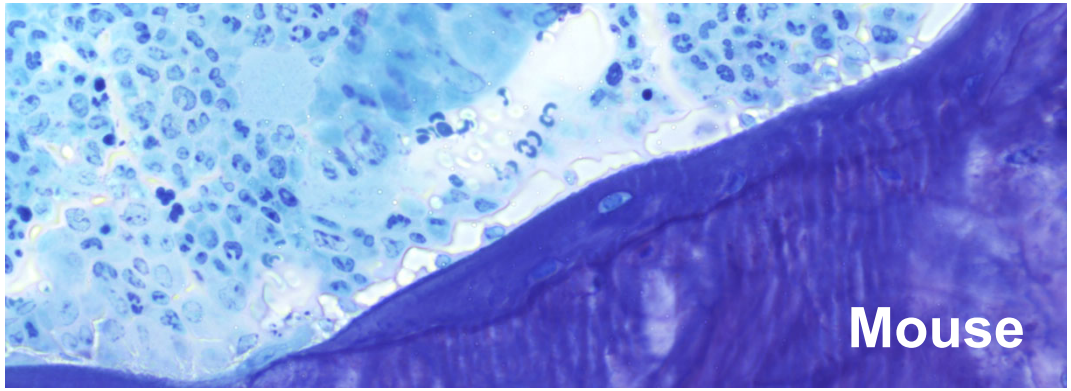


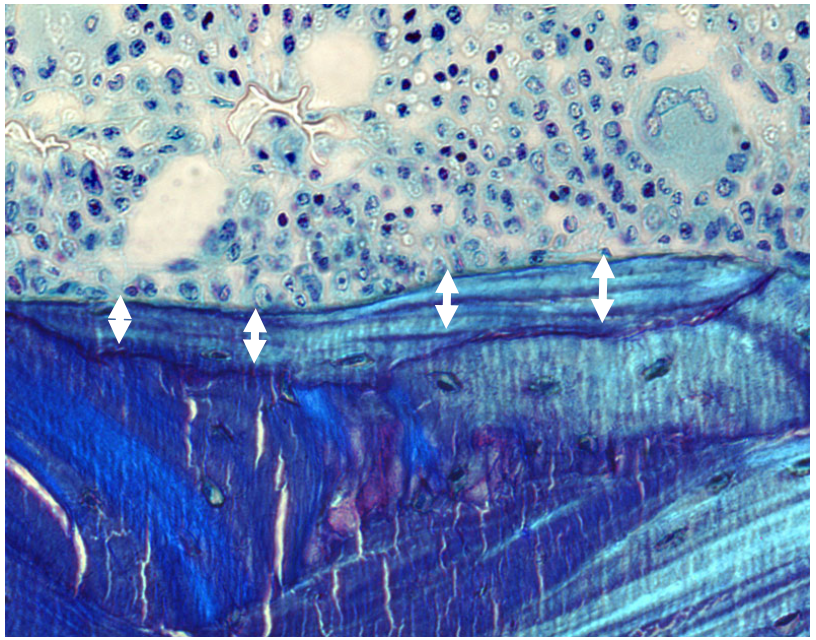




Remodeling-based parameters

Primary measurements

- ***Bone perimeter***
- ***Osteoid perimeter***
- ***Eroded perimeter***
- ***Wall width***



- **Wall width (W.Wi, µm)**
- **Resorption period (Rs.P, d)**
- **Formation period (FP, d)**
- **Remodeling period (Rm.P, d)**
- **Activation frequency (Ac.F, 1/y)**

W.Wi ≥ 15 sites, 4 measurements per site

Active FP = W.Wi/MAR
All other periods are calculated based on the length of the FP:
(“fractions of space are equivalent to fractions of time”, e.g., Rs.P = ES/OS * FP
Rm.P = Rs.P + FP
Ac.F = 1/Tt.P = 1/(FP * BS/OS)

Cortical bone remodeling?

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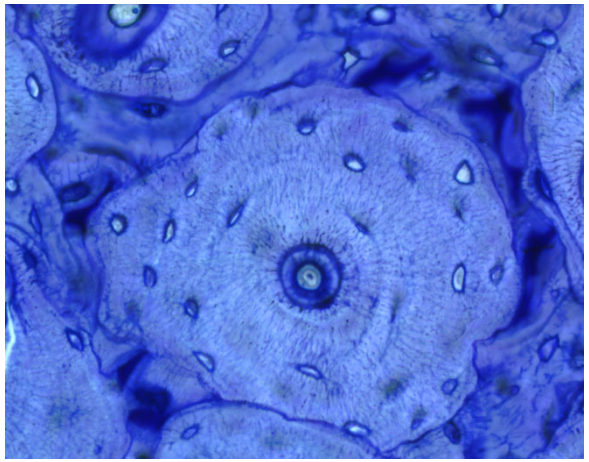
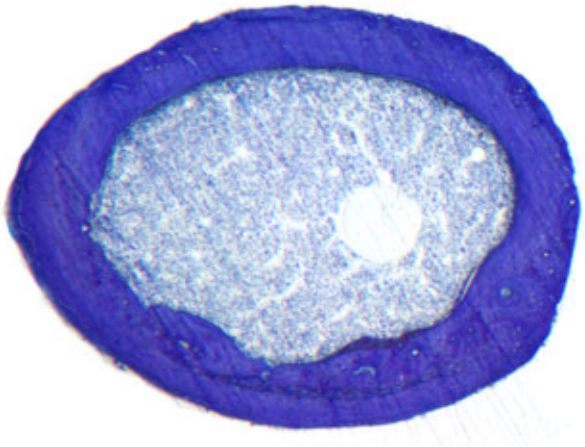
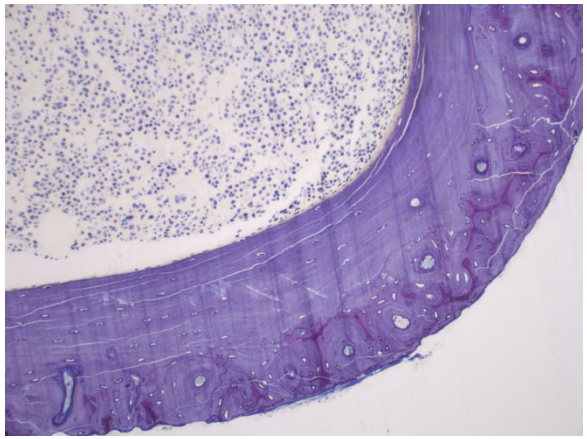
Rabbit



Mouse



Toluidine blue surface stain



Analysis of cortical bone cross-sections

Primary measurements

- **Cortical bone area**
- **Cross-sectional area**
- **Marrow area**
- **Periosteal perimeter**
- **Endocortical perimeter**
- **Cortical thickness**
- **Ps. and Ec. MAR + M.Pm**

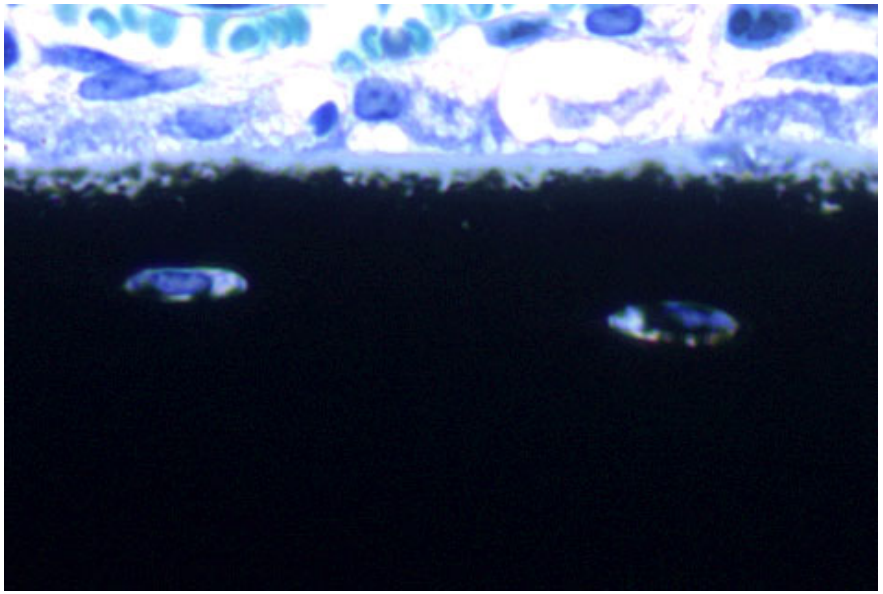
⇒ The femoral midshaft is usually used for cortical bone analysis in mice.

Analysis of osteocytes

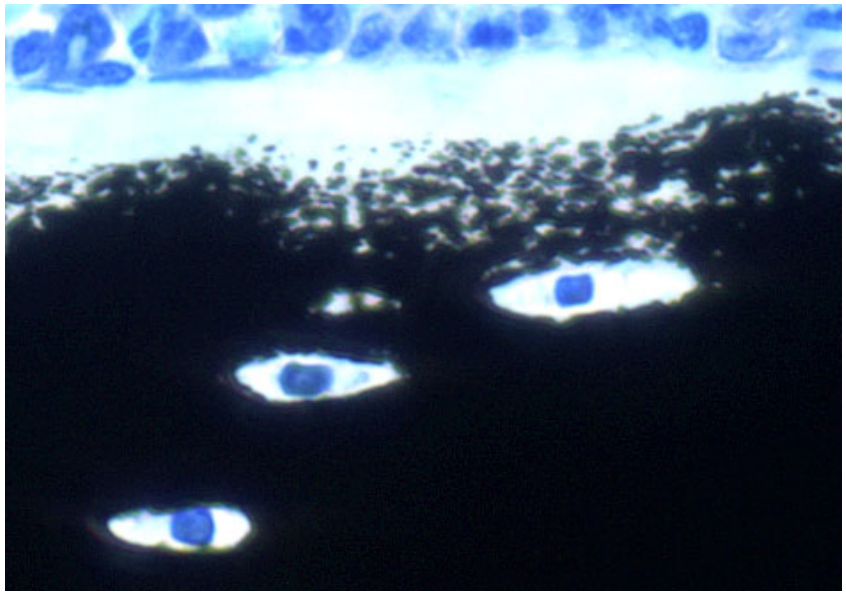
Primary measurements

- **Osteocyte lacunar area (Ot.Lc.Ar, µm²)**
- **Osteocyte number (Ot.N, no./mm²)**

Wildtype



Hyp



Take home messages

- ***Always take out vertebrae in aged mice.***
- ***Consider gender differences in mouse experiments.***
- ***For the quantification of osteoclasts always use TRAP staining.***
- ***For the assessment of bone formation fluoro-chrome labeling with an appropriate marker interval is essential. Consider different marker intervals for cancellous and cortical bone.***
- ***Mice lack true Haversian cortical bone remodeling, but not cancellous bone remodeling activity.***

More information

Erben & Glösmann (2019) Histomorphometry in Rodents. Methods Mol Biol 1914:411-435

Ma, Burr & Erben (2019) Bone histomorphometry in Rodents. In: Principles in Bone Biology. Bilezikean et al (eds), Elsevier, pp 1899-1922

www.bonemorphometry.org (members-only section)

Dempster DW et al. 2013 Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 28:2-17