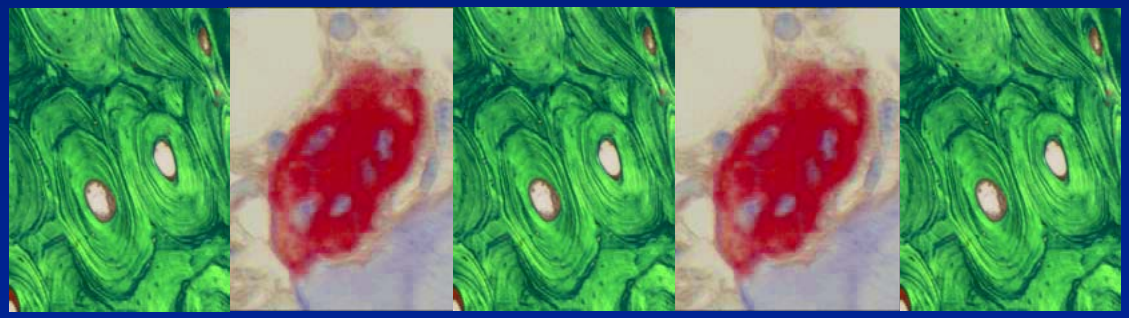
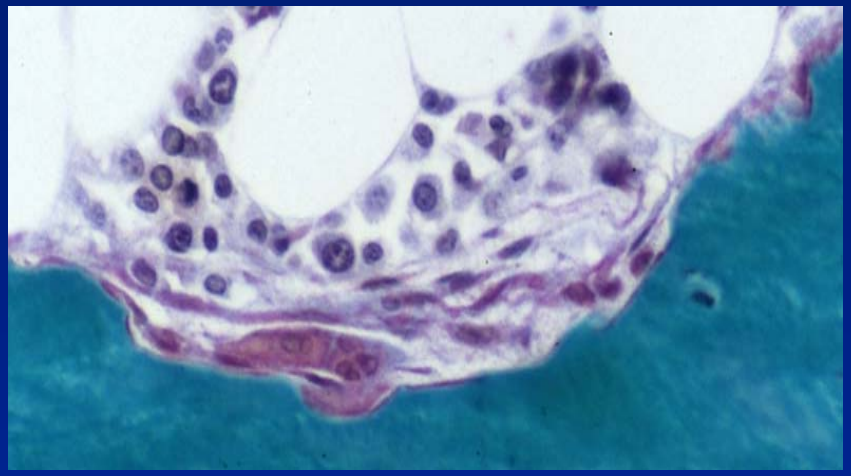
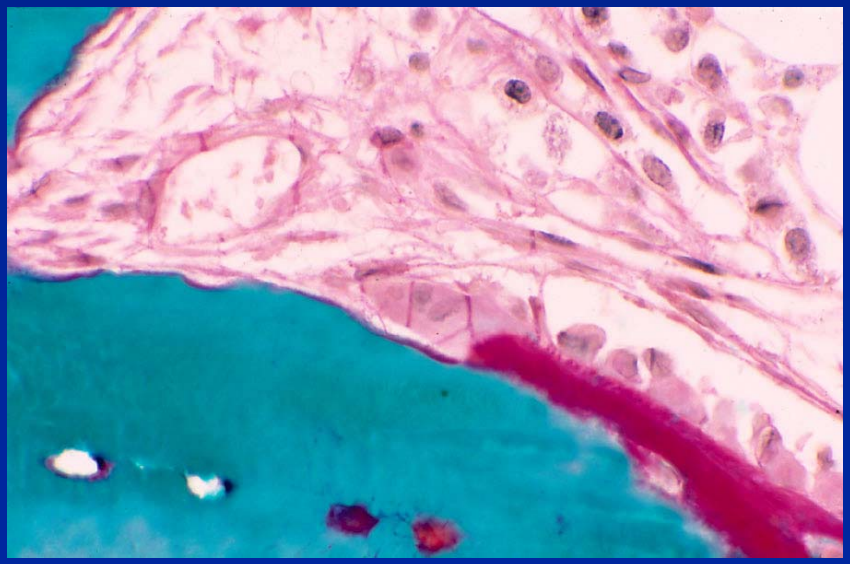
Histomorphometry



Ellen M. Hauge, MD, ph.d.
Dept. Of Rheumatology
Aarhus University Hospital

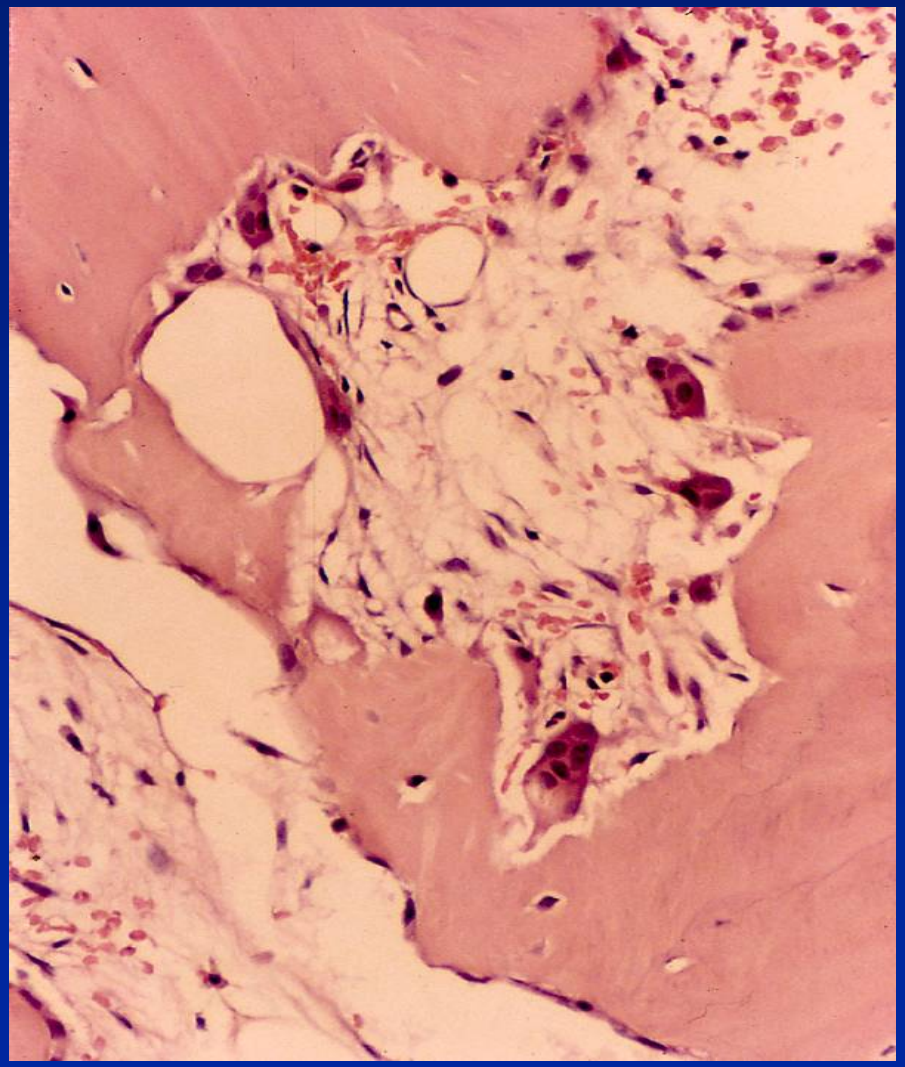
Bone histomorphometry – the only way to measure cellular remodeling activity

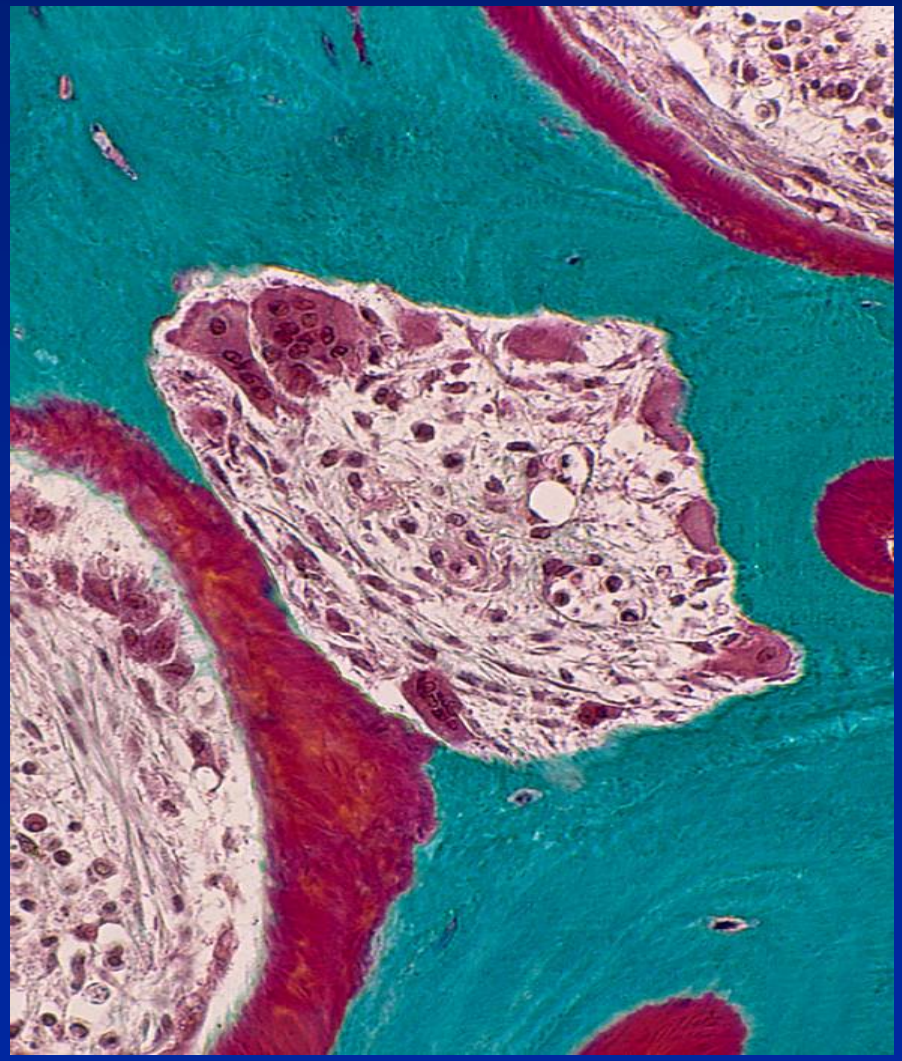




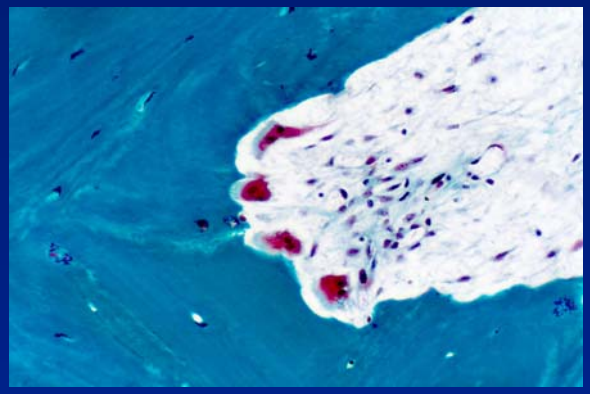
- Micro-organisation
- Remodeling surfaces
- Bone cells

Undecalcified plastic embedding and trichrome staining identifies osteoid

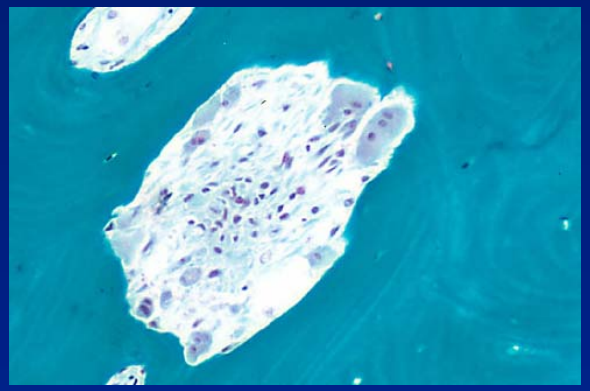




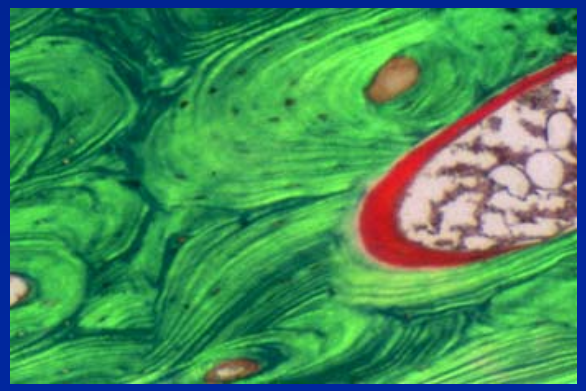
Cortical bone remodeling cycle



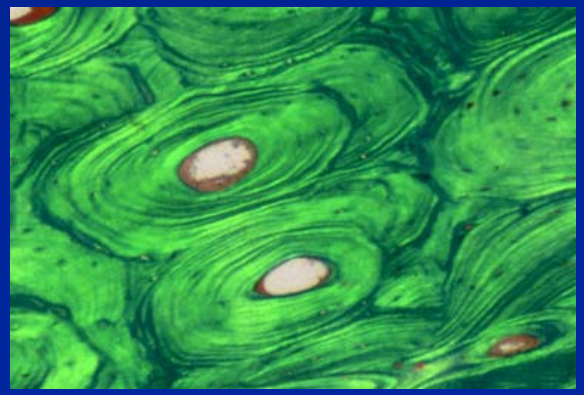
Activation



Resorption

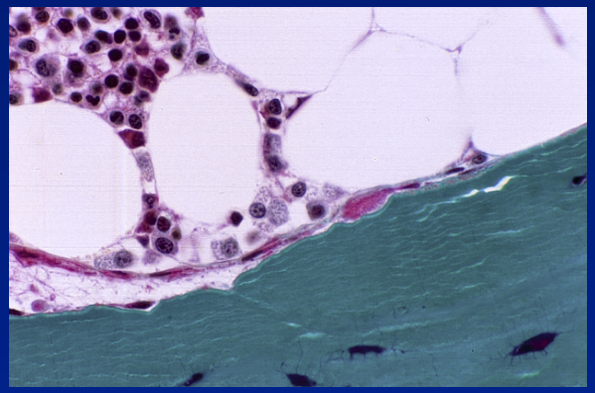


Formation

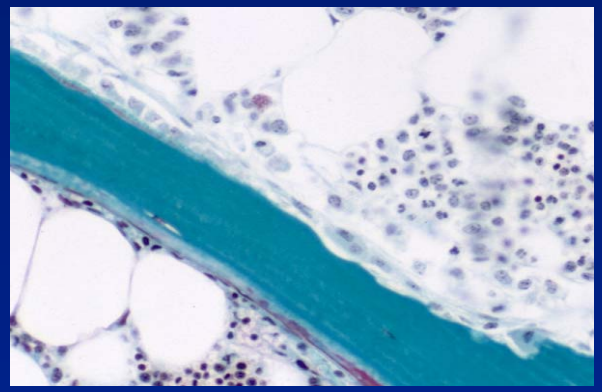


Quiescent

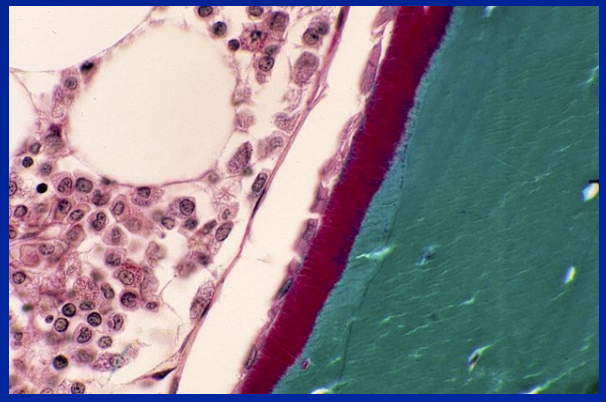
Cancellous bone remodeling cycle



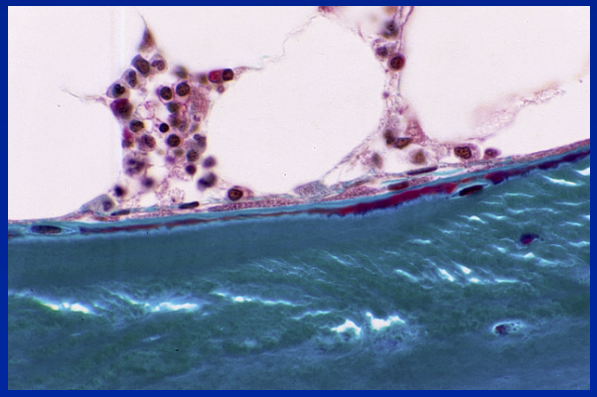
Activation



Resorption

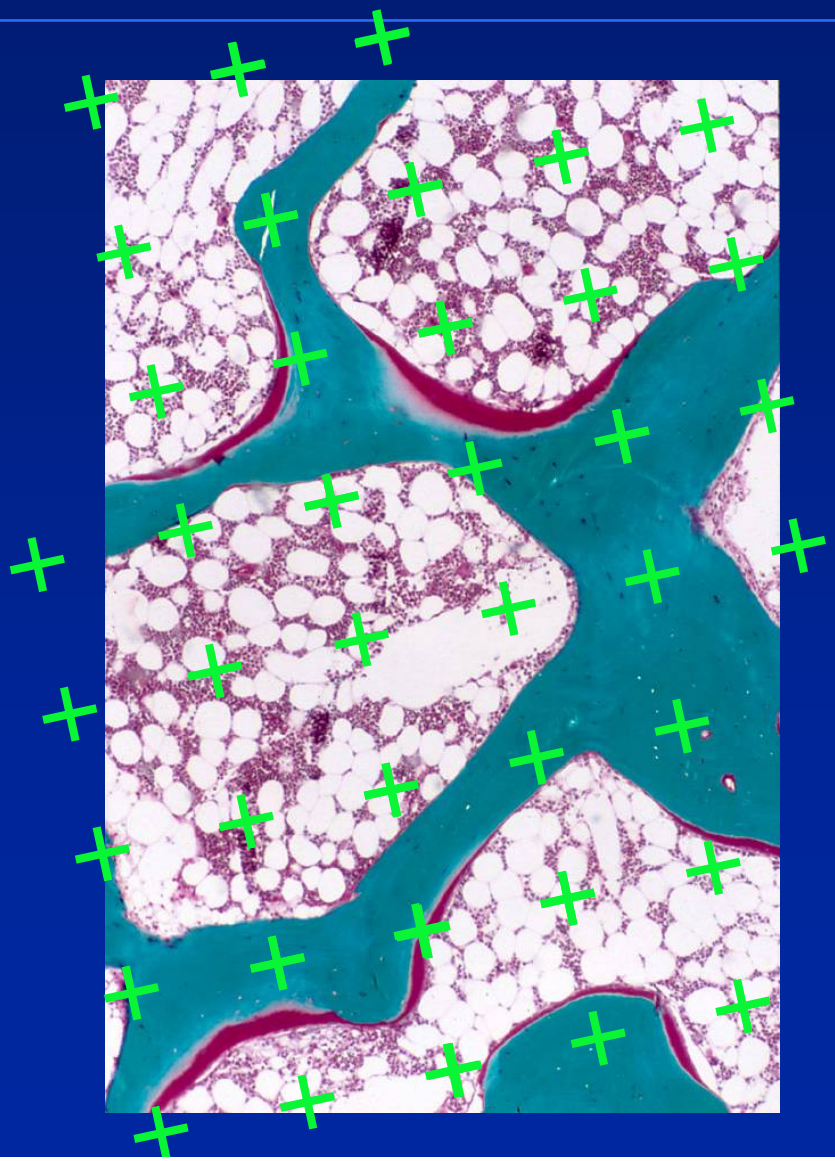


Formation



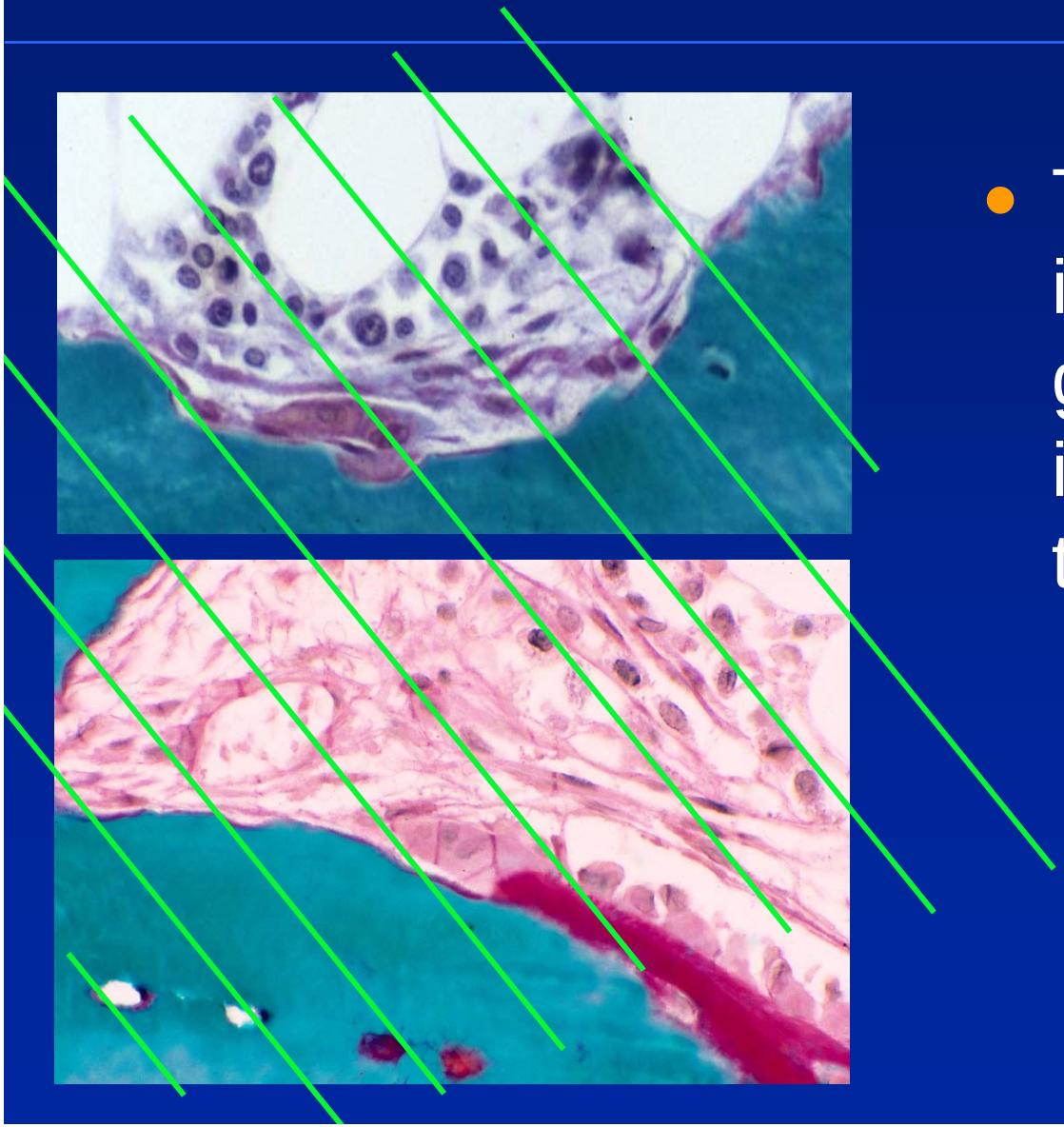
Quiescent

Measuring bone volume



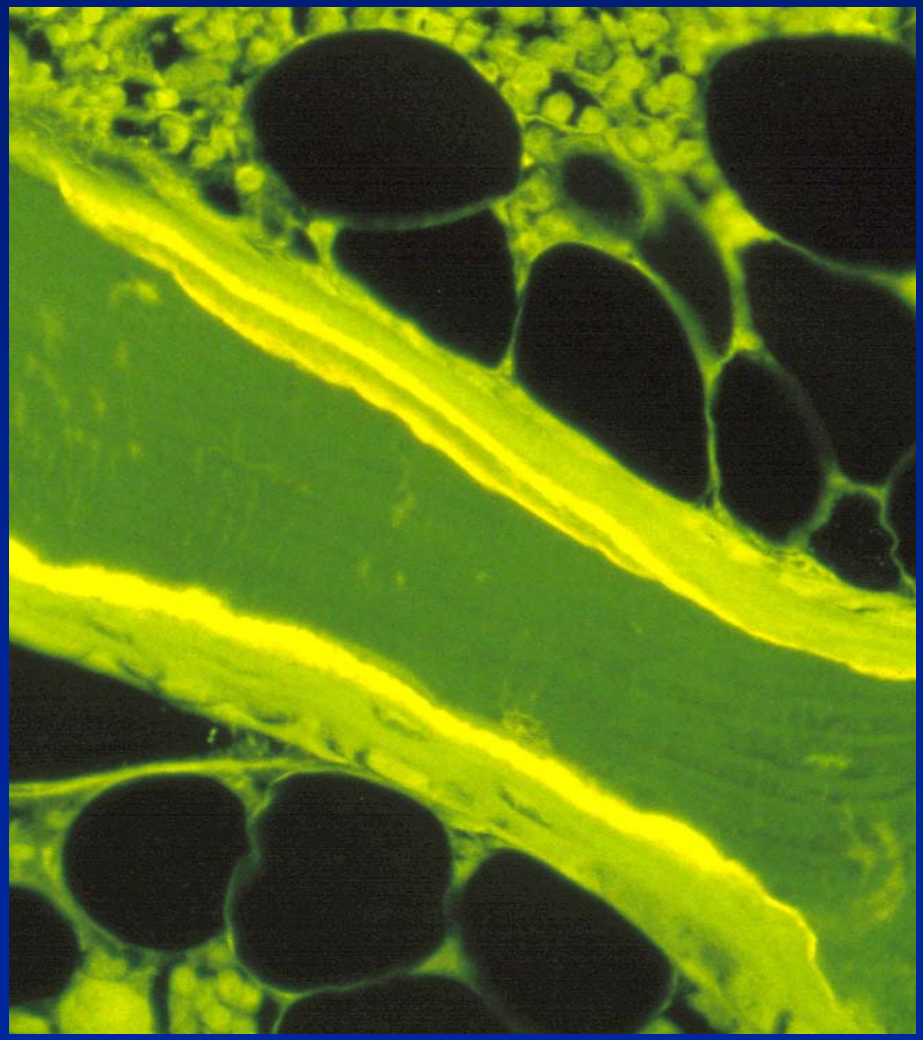
- The number of gridpoints on bone tissue is correlated to the volume of bone in 3D.
- Not very sensitive.

Measuring eroded and osteoid surfaces



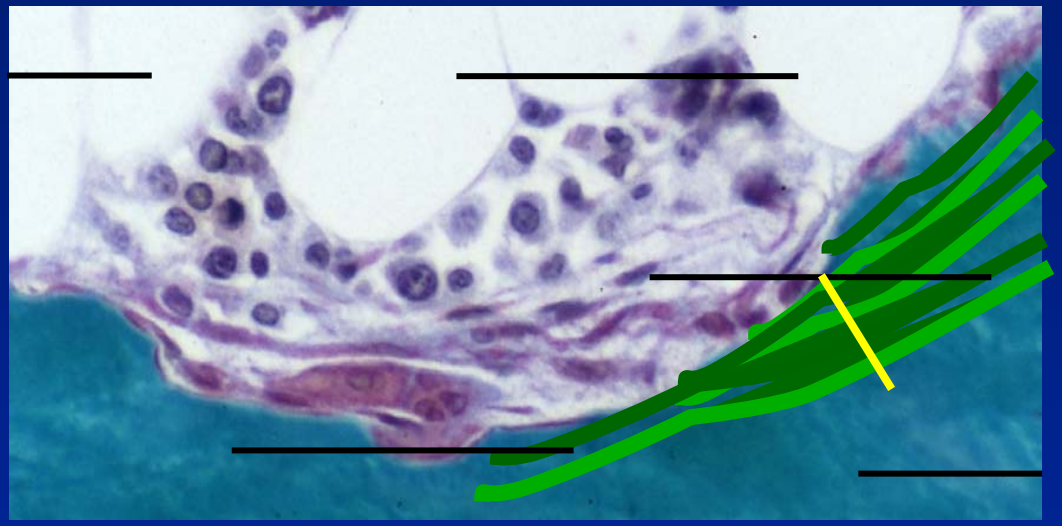
- The number of intersections between gridlines and bone surface is correlated to the area of the surface in 3D.

Measuring tetracycline-labelled surface and the mineral apposition rate

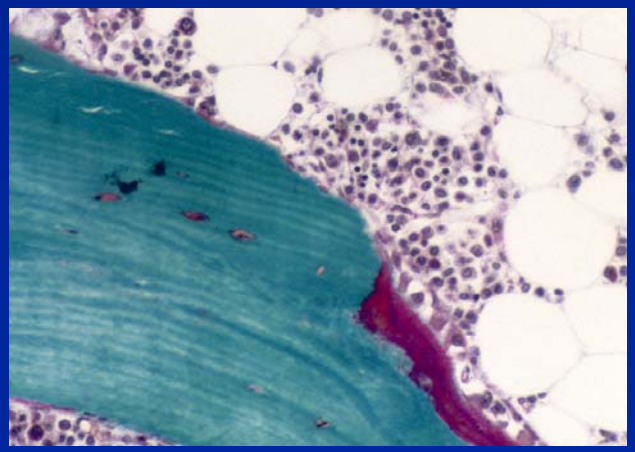


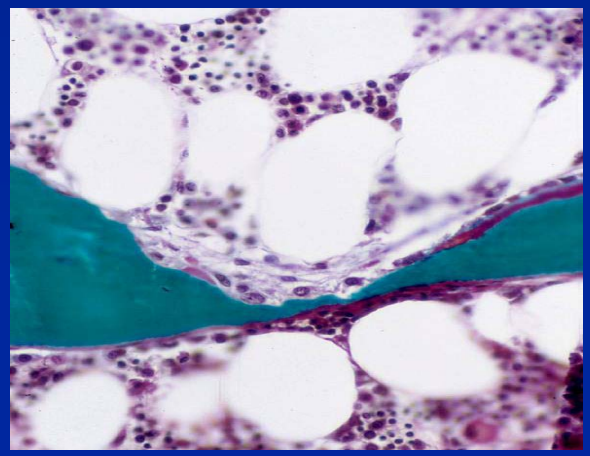
- Two tetracycline doses with an interval. Distance between labels gives an apposition rate.
- Active mineralizing surface.
- Lamellar bone.

Measuring erosion depth - Distance along orthogonal intercepts



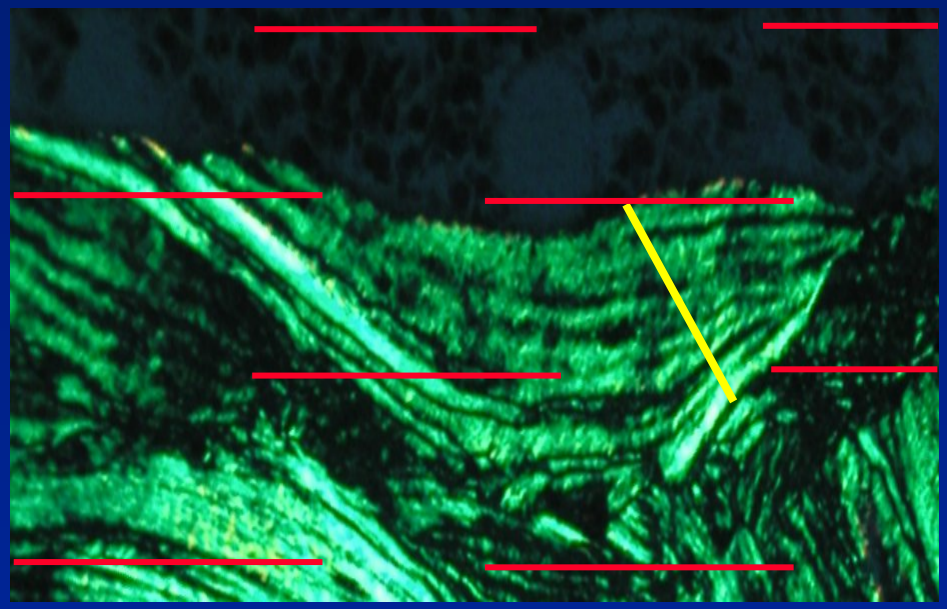
- Sampled by gridlines
- Number of lamellae.
- The ”untouched” surface as the reference.
- Final resorption depth ?



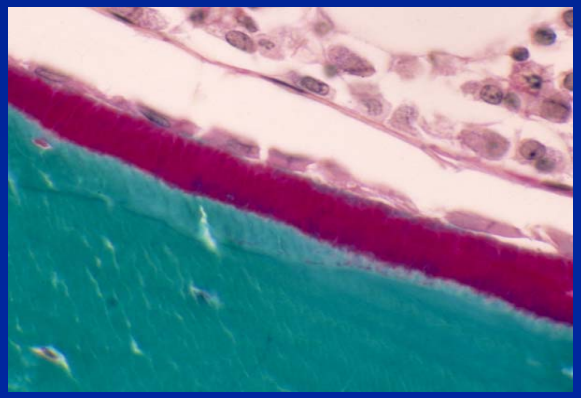


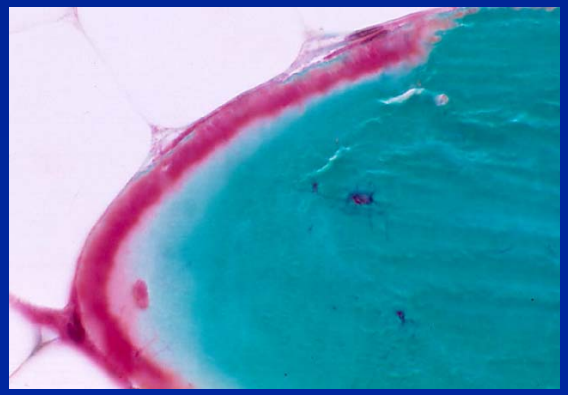
Eriksen et al, 1993

Measuring wall thickness - Distance along orthogonal intercepts



- Sampled by gridlines
- Number of lamellae
- Length of intercept
- Old or new walls ?





Eriksen et al, 1993

Calculating the derived parameters of bone remodeling

- Formation period
- Erosion period
- Activation frequency
- Remodeling balance
- Bone formation rate

Parfitt et al, JBMR 1987

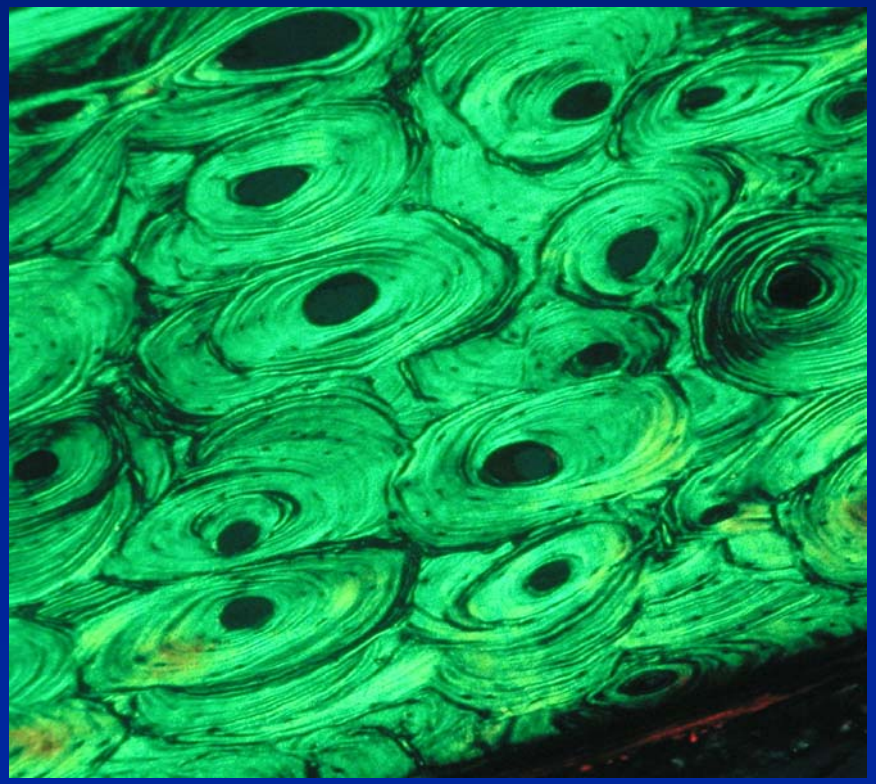
Back to Basics

Again ask the questions, that were answered 20-30 years ago.

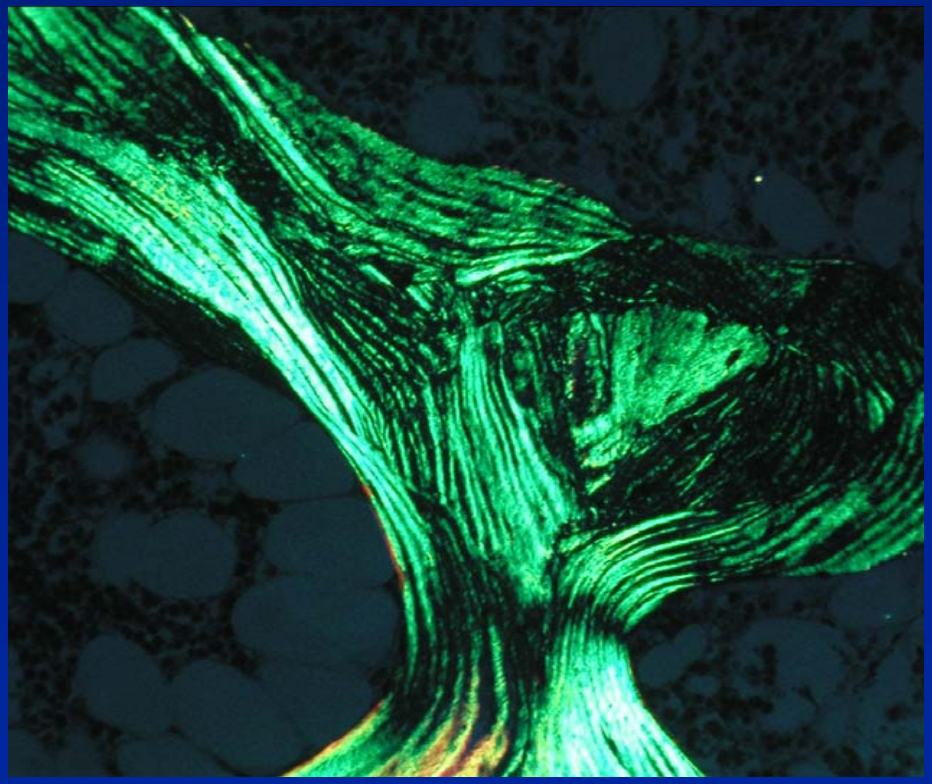
- What are the premises for interpreting bone histomorphometry ?
- Is unbaised cell number out of reach ?
- Is tissue orientation to be ignored ?

Micro-anatomical structure of bone

Cortical bone

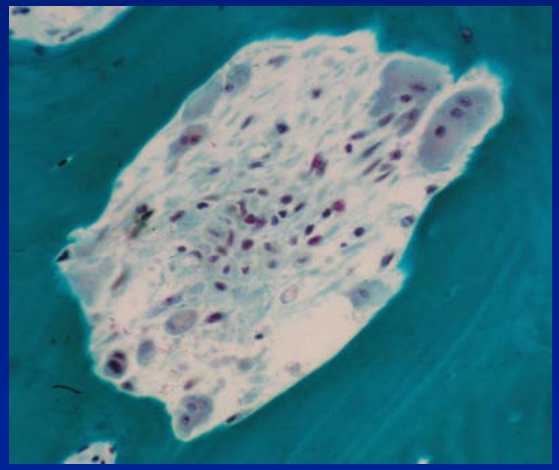


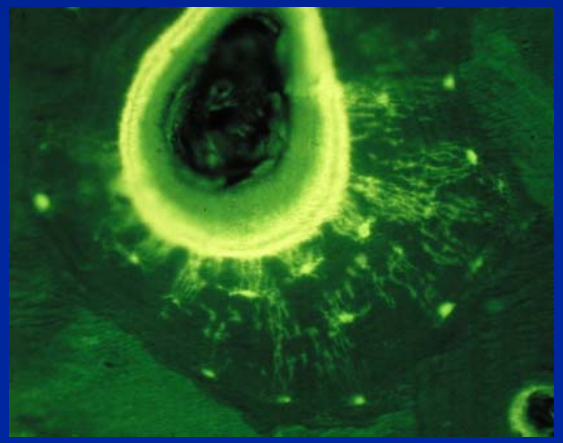
Cancellous bone



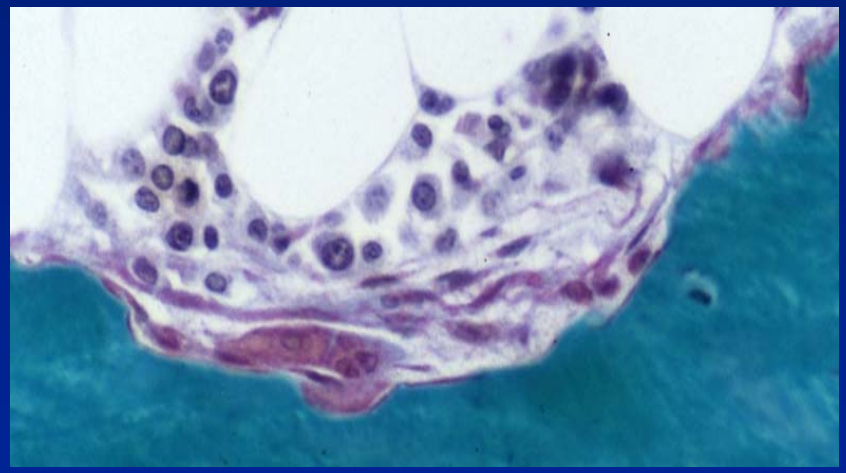
The anatomical unit is also a functional unit

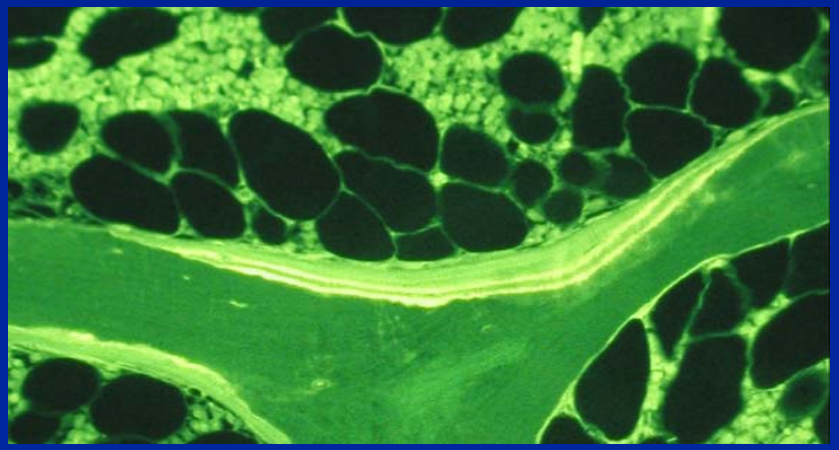
Compact bone



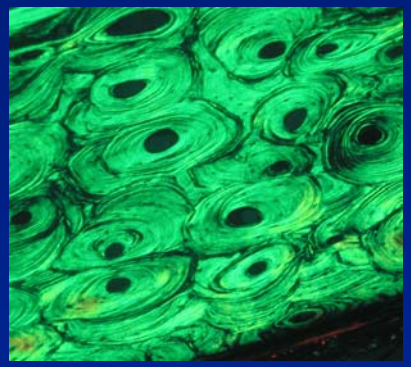


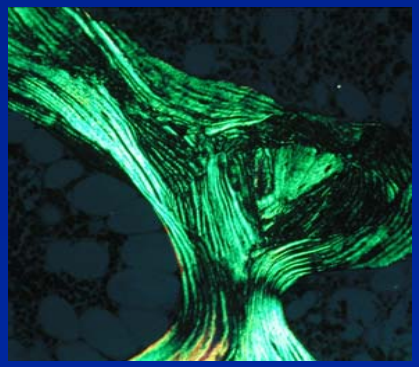
Cancellous bone





Frostian theories are based on ...





- Turnover of bone in discrete geometric units
- Investigations in humans
- Steady state

Steady state is present when …

Incidence of resorption = Incidence of formation

In steady state bone remodeling …

- The surface extent of resorption and formation are determined by ...

$$\text{prevalence} = \text{incidence} \cdot \text{duration}$$

- Since incidences of resorption and formation are equal, then …

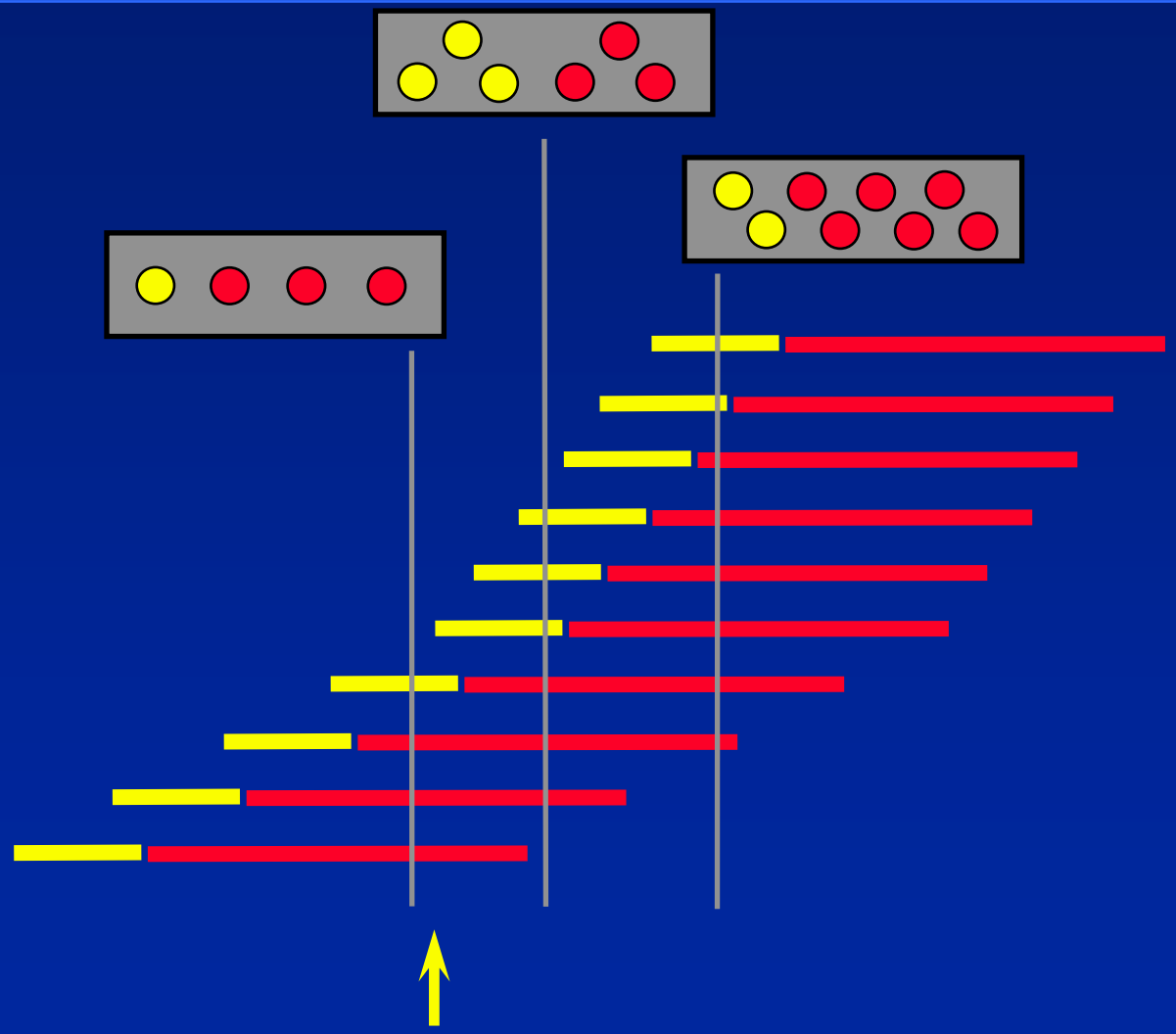
$$\frac{ES/BS}{OS/BS} = \frac{EP}{FP}$$

Why bother about treatment and transients ?

- Diseases and interventions often induce sudden changes in turnover that have profound effects on remodeling surfaces.
- Steady state is shifted into a transient state.

Transient state

Resorption
Formation



Turnover

Avoid transient states in bone remodeling studies

- At least 1 remodeling period must pass after the intervention.
- The remodeling period varies with location, type of bone, and species.

Measuring transients or modeling

- Interpret direct measurements in terms of uncoupling and imbalance.
- Avoid using the derived estimates that assume coupled remodeling and steady state.

The standardization paper from 1987

JOURNAL OF BONE AND MINERAL RESEARCH
Volume 2, Number 6, 1987
Mary Ann Liebert, Inc., Publishers

Times cited: 2,447

Bone Histomorphometry: Standardization of Nomenclature, Symbols, and Units

REPORT OF THE ASBMR HISTOMORPHOMETRY NOMENCLATURE COMMITTEE

A. MICHAEL PARFITT (CHAIRMAN),[1] MARC K. DREZNER,[2] FRANCIS H. GLORIEUX,[3] JOHN A. KANIS,[4] HARTMUT MALLUCHE,[5] PIERRE J. MEUNIER,[6] SUSAN M. OTT,[7] and ROBERT R. RECKER[8]

PRACTITIONERS OF BONE HISTOMORPHOMETRY communicate with each other in a variety of arcane languages, which in general are unintelligible to those outside the field. Many in phometry, not to help bone histomorphometrists understand each other. Second, names should be self-explanatory and descriptive, without implicit assumptions. Third, symbols should

A standardized nomenclature – and method

Bone histomorphometric methods have been largely unchanged

for two decades.

Important statements on stereology

- Bone cannot be fully understood unless conceived in 3D terms.
- The ultimate goal is understand 3D reality by the application of stereology.
- But as a practical matter, it is premature to insist on universal adoptation of a 3D format.

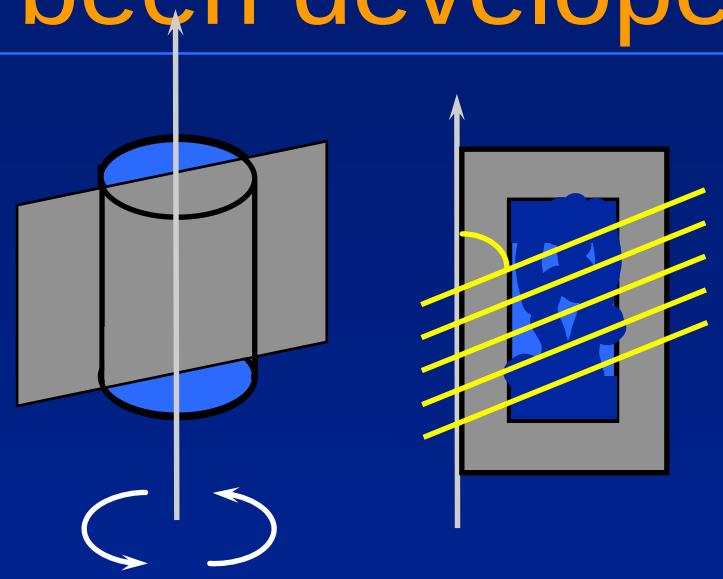
Parfitt et al, JBMR 1987

Several stereological limitations of bone histomorphometry were mentioned

- Random and unbiased sampling is only rarely fulfilled
- The anisotropy of bone is an acceptable error.
- There is no convenient way of extrapolating number to three dimensions.
- Investigation of the correct stereological approach to iliac cortical bone has only just begun.

Parfitt et al, JBMR 1987

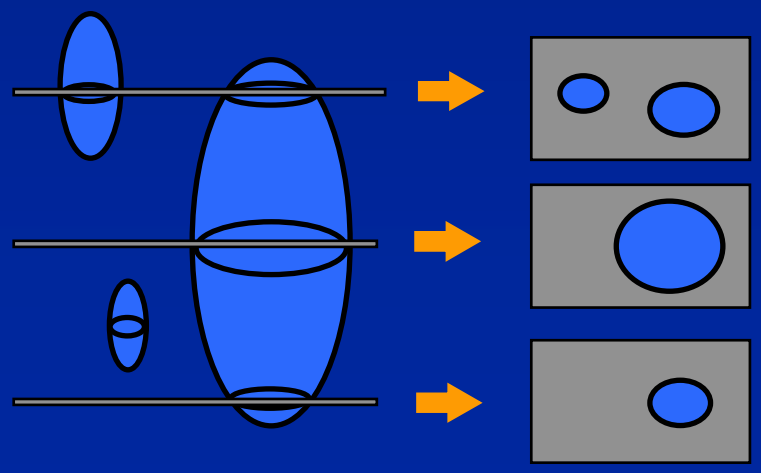
The necessary stereological tools have been developed and proved their value



Anisotropic orientation

- Vertical sections design

Baddeley, J.Microsc. 1986



Biased cell number count

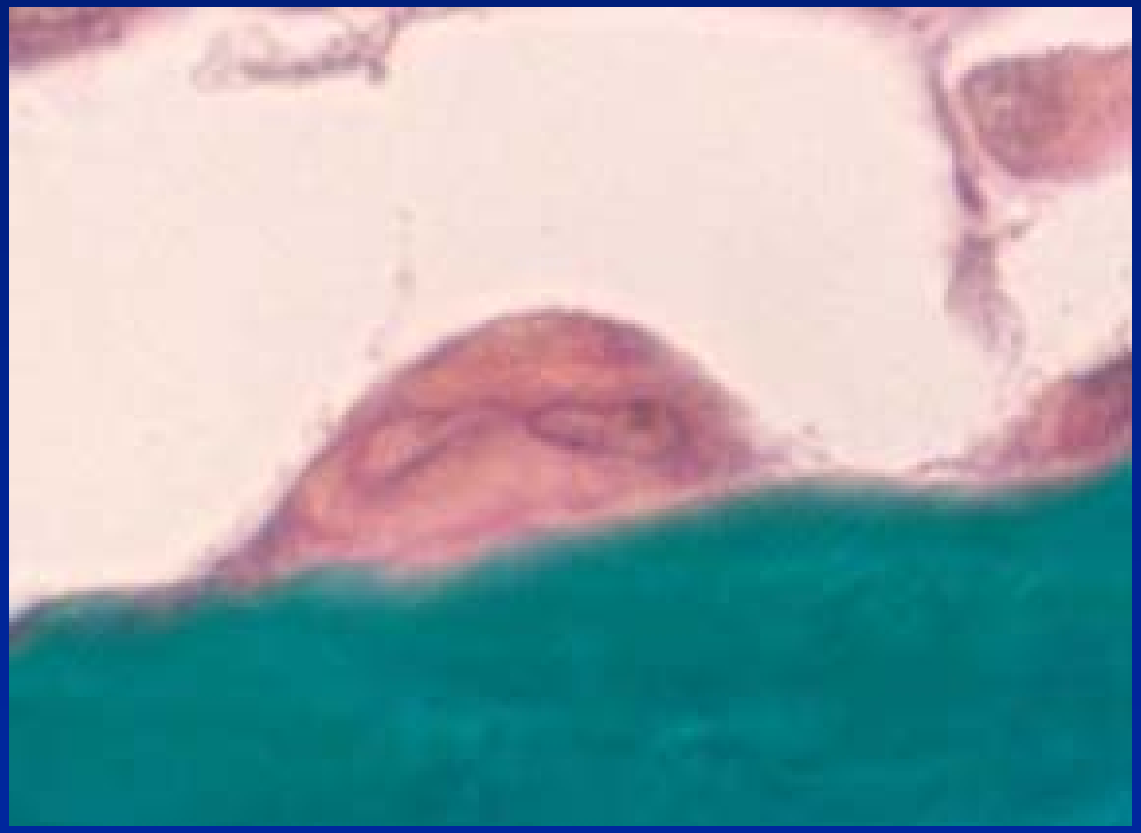
- Disector design

Sterio, J.Microsc. 1983

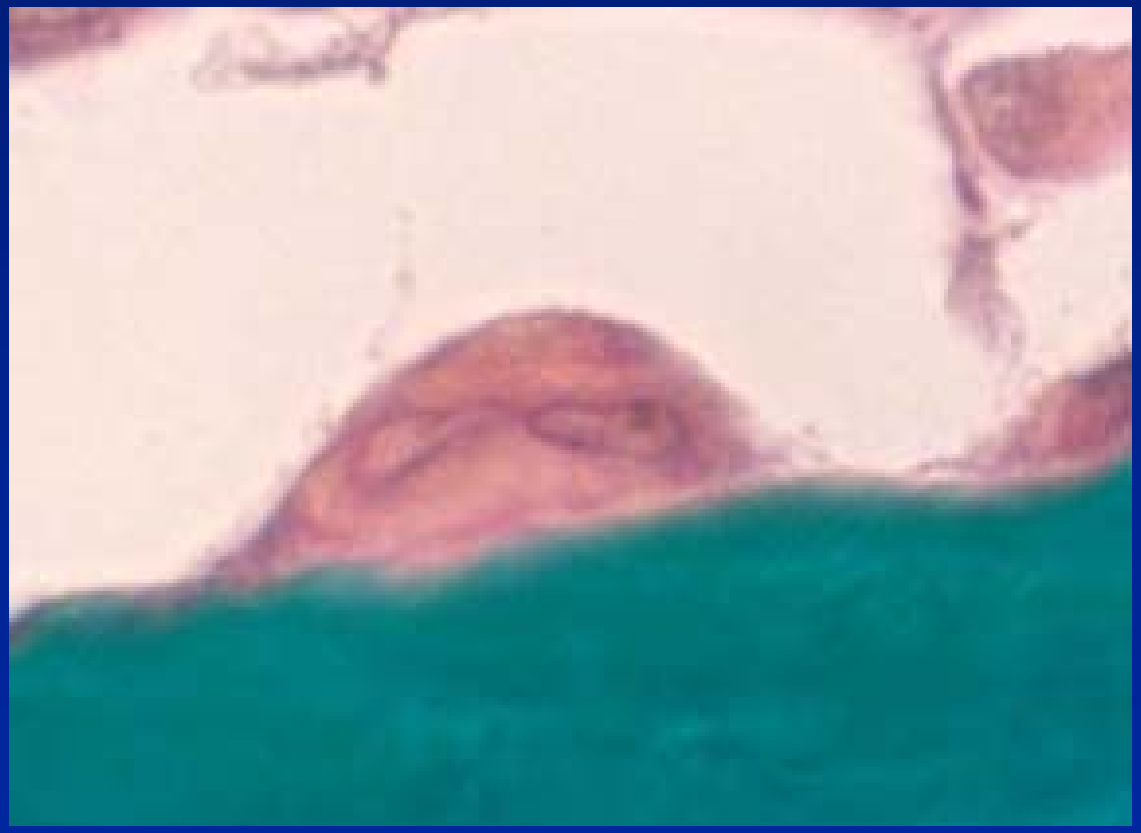
Fair sampling needs to be…

Random	Without preferences
Uniform	The same for all the tissue
Isotropic	The same for all directions

Is this a cell ?

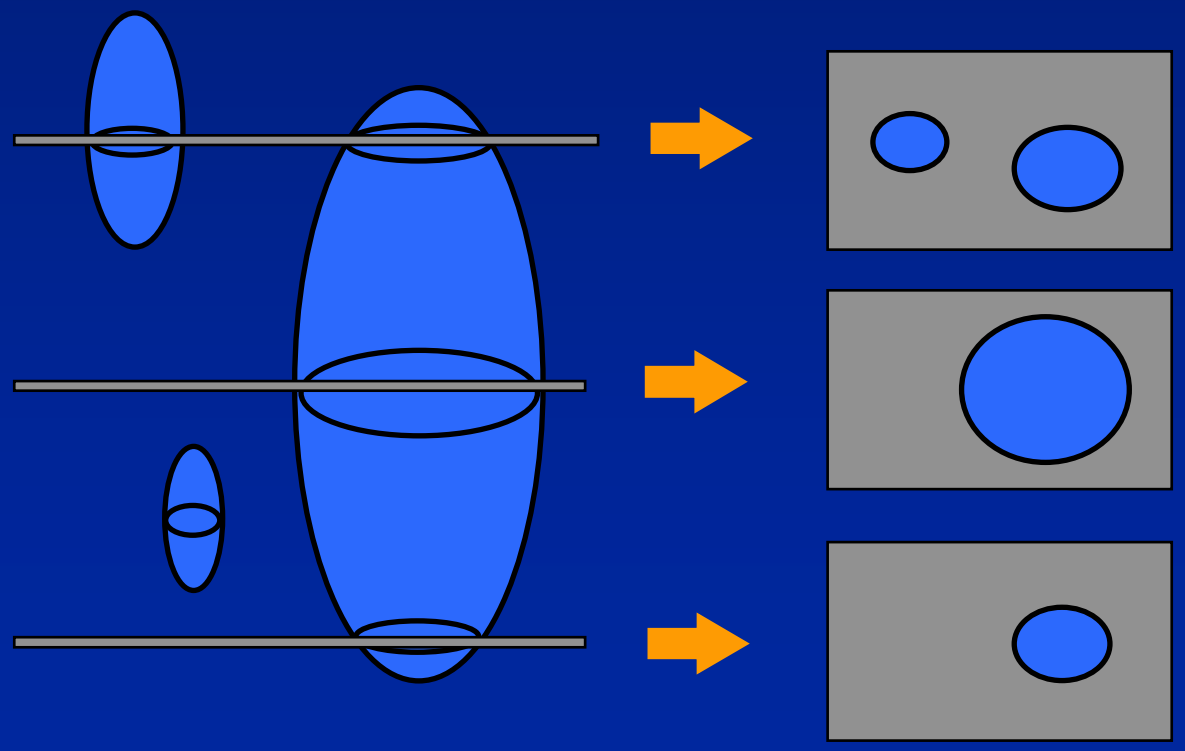


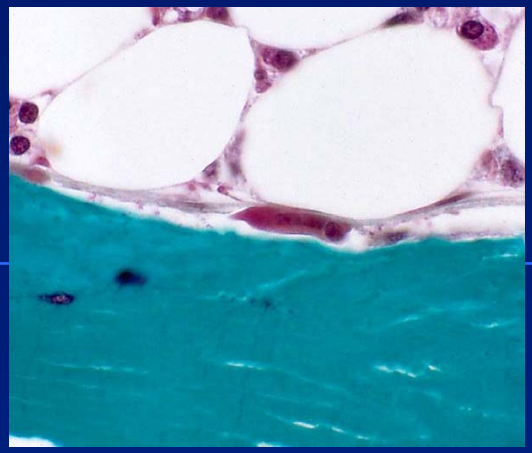
No, it is a section of a cell !

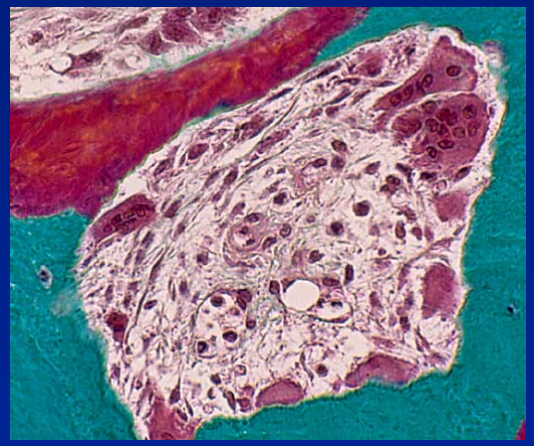


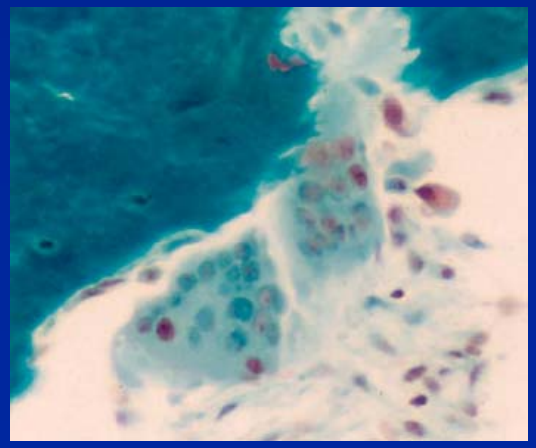
Cells cannot be identified in one section – and therefore not counted

Large particles have a higher probability of getting sampled - just because they are larger.







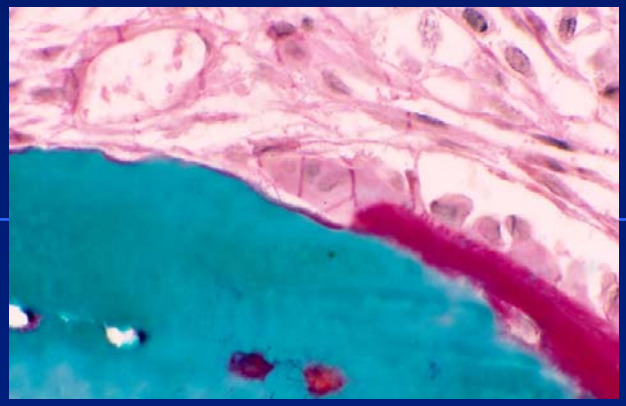


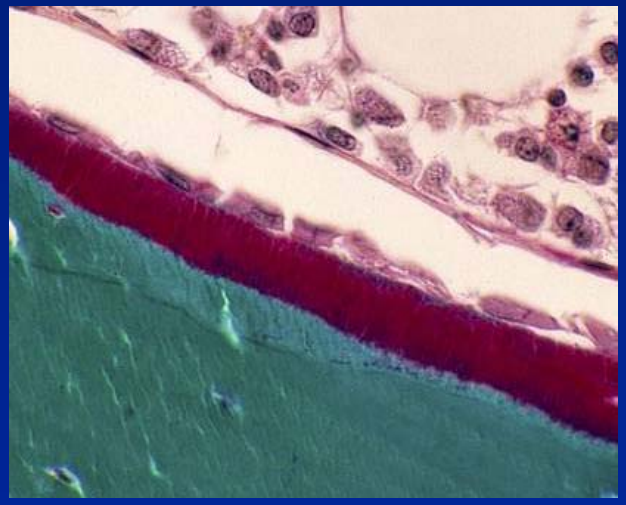
Osteoclasts increase in size when active or pathological

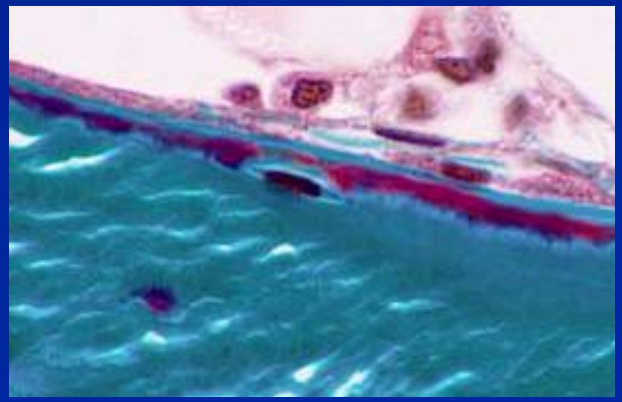
Such as in…

- Secondary hyperparathyroidism
- Paget's disease
- Osteopetrosis
- Pycnodysostosis

Osteoblasts decrease in size when inactive



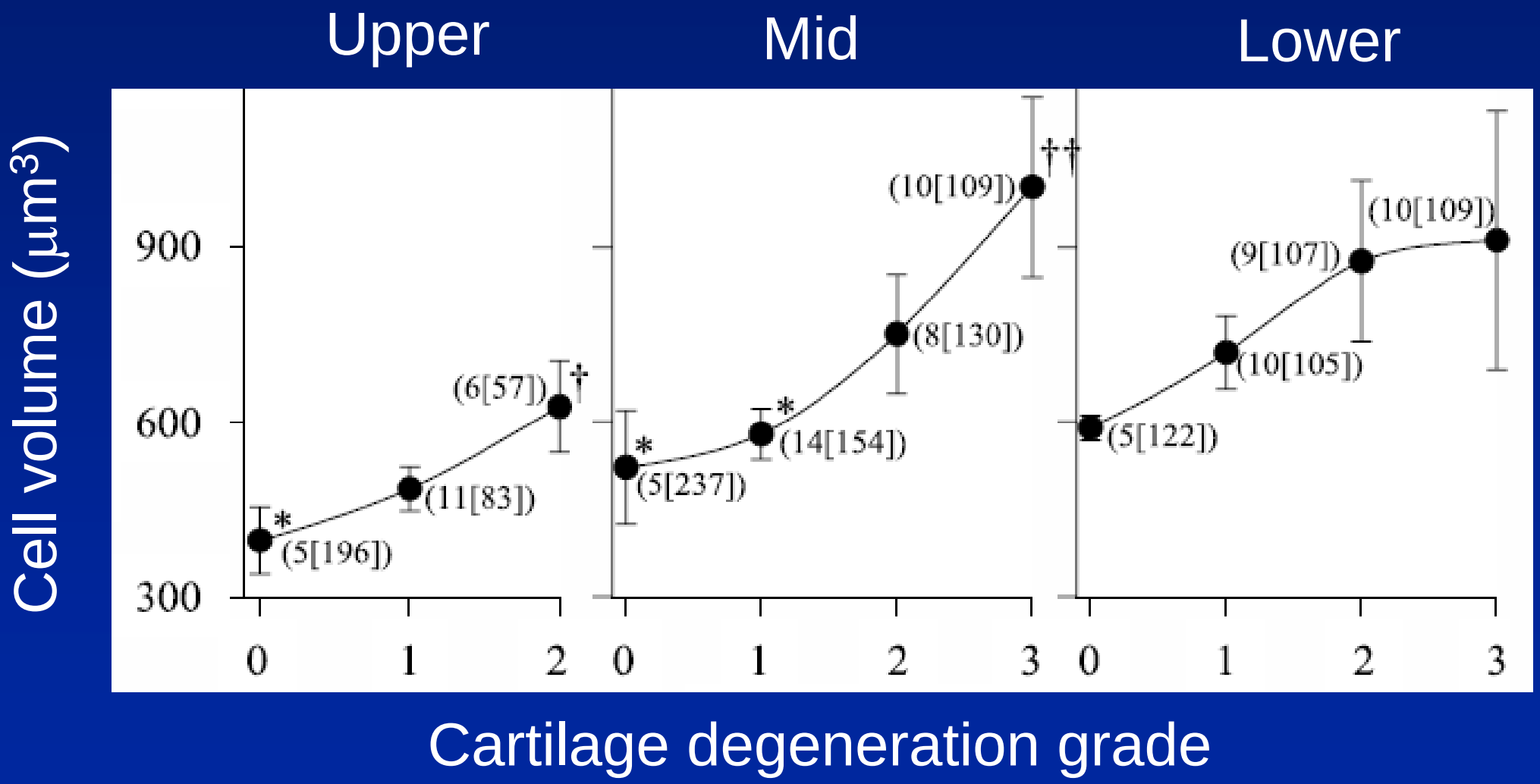




Such as in…

- Normal remodeling

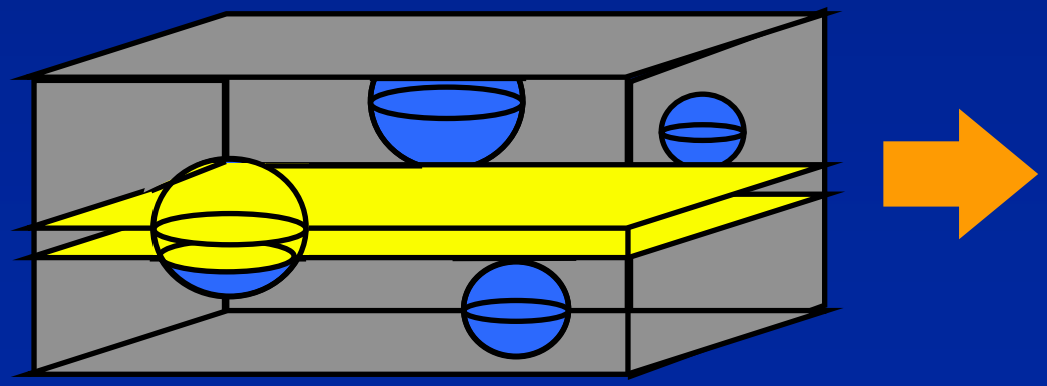
Chondrocytes increase in size in OA

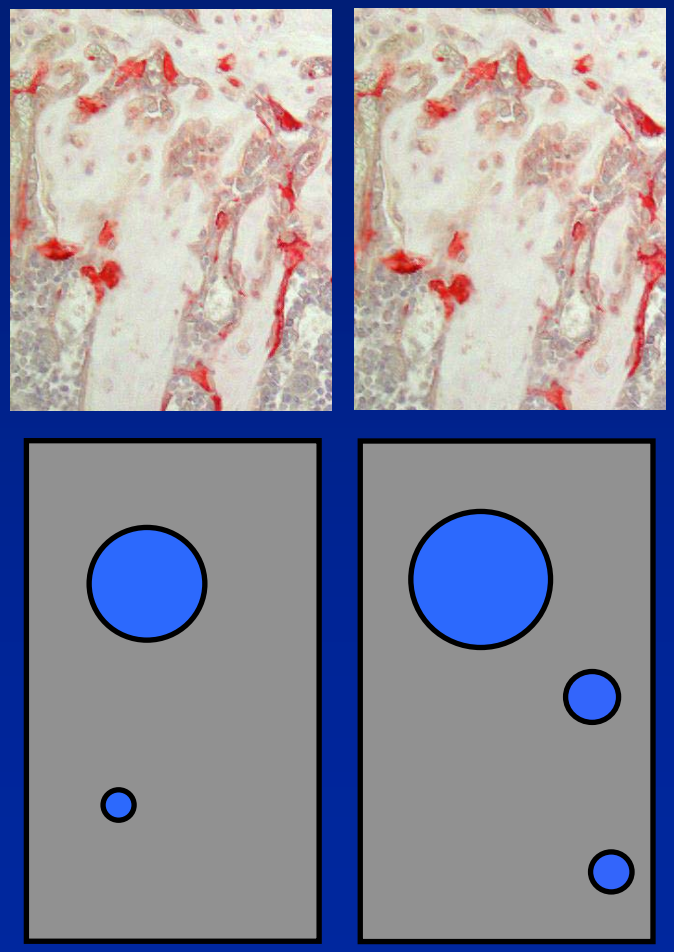


Bush, O&C 2003

Using pairs of two sections, we can identify, count and measure cells unbiased

The disector samples particles with uniform probability.





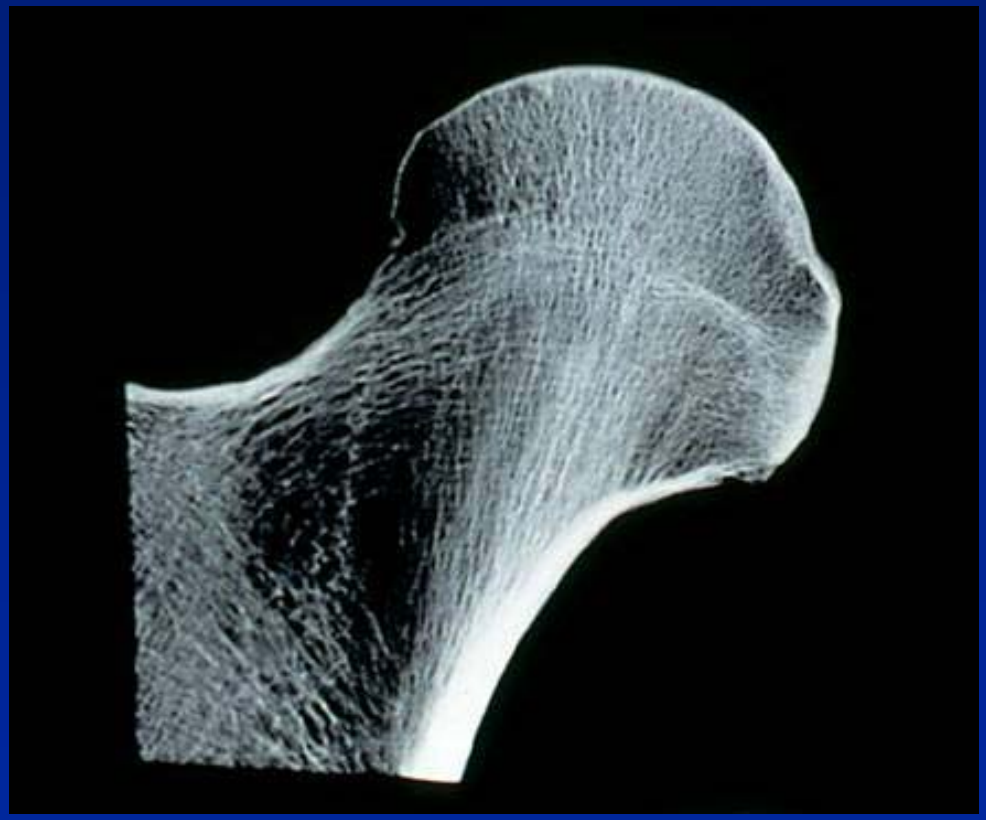
Sterio, J.Microsc. 1983

Stereology has provided unbiased tools to count cells

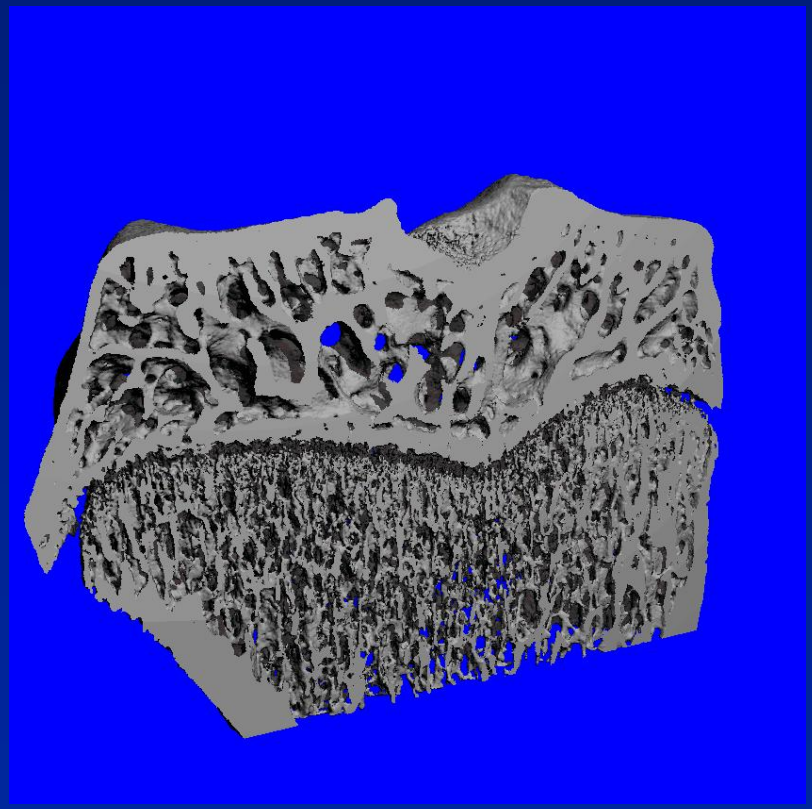
- There is a convenient way to count number of cells in three dimensions.

Sterio, J.Microsc. 1983

Bone tissue has a preferred orientation - it is anisotropic

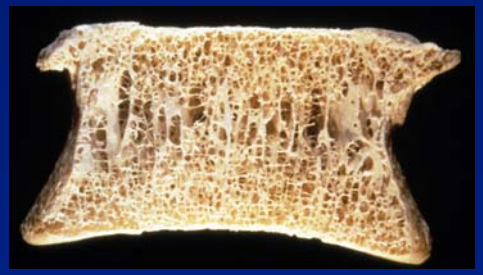


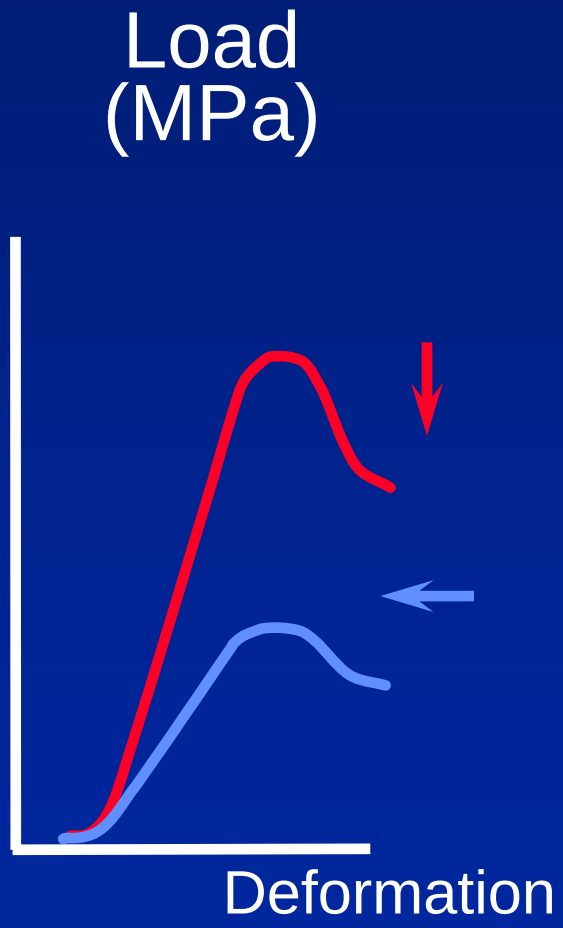
Human proximal femur

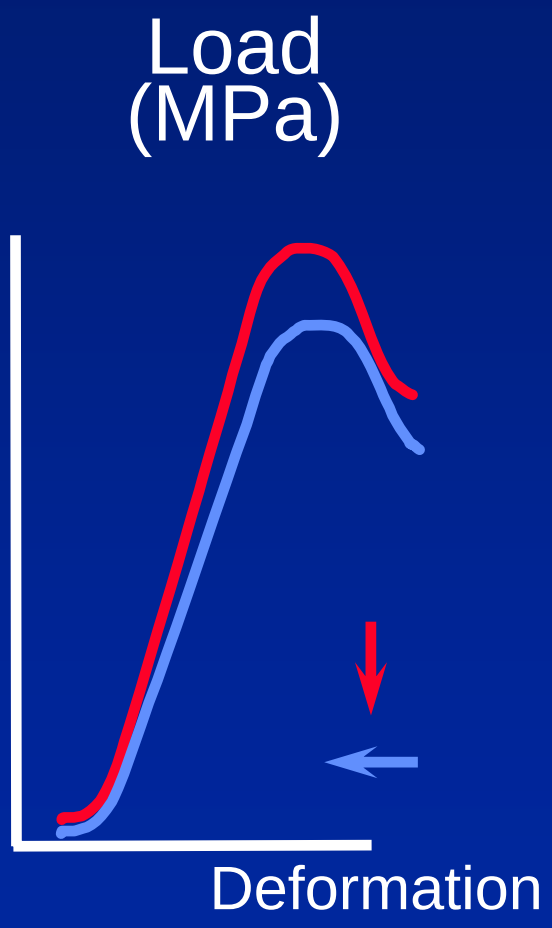


Rat proximal tibia

Bone tissue is anisotropic in most locations - iliac crest seems to be an exception

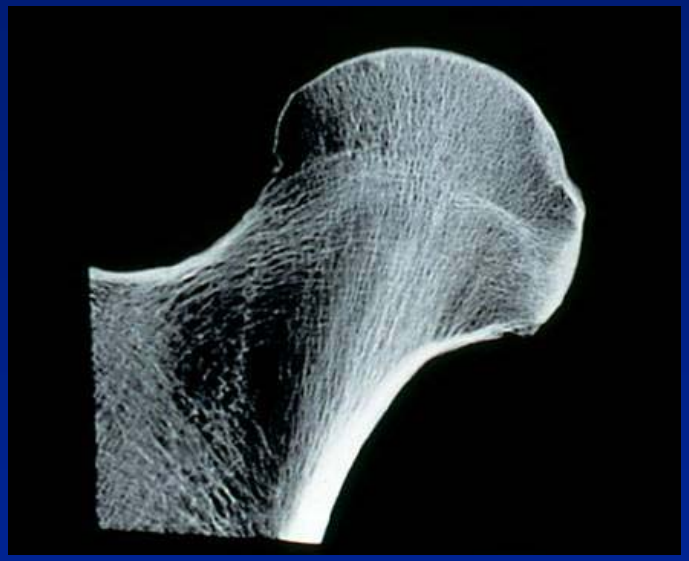


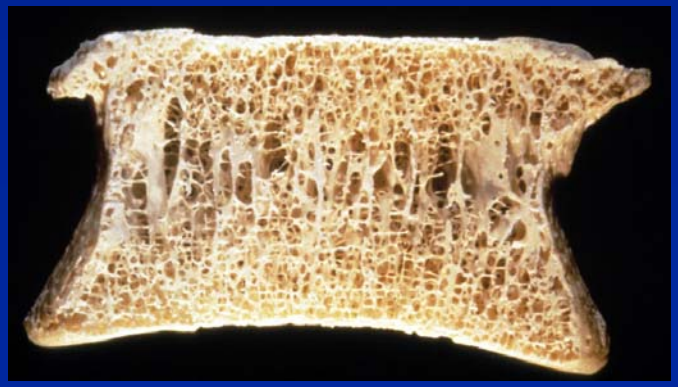




Mosekilde, Bone 1985

Bone tissue is anisotropic …





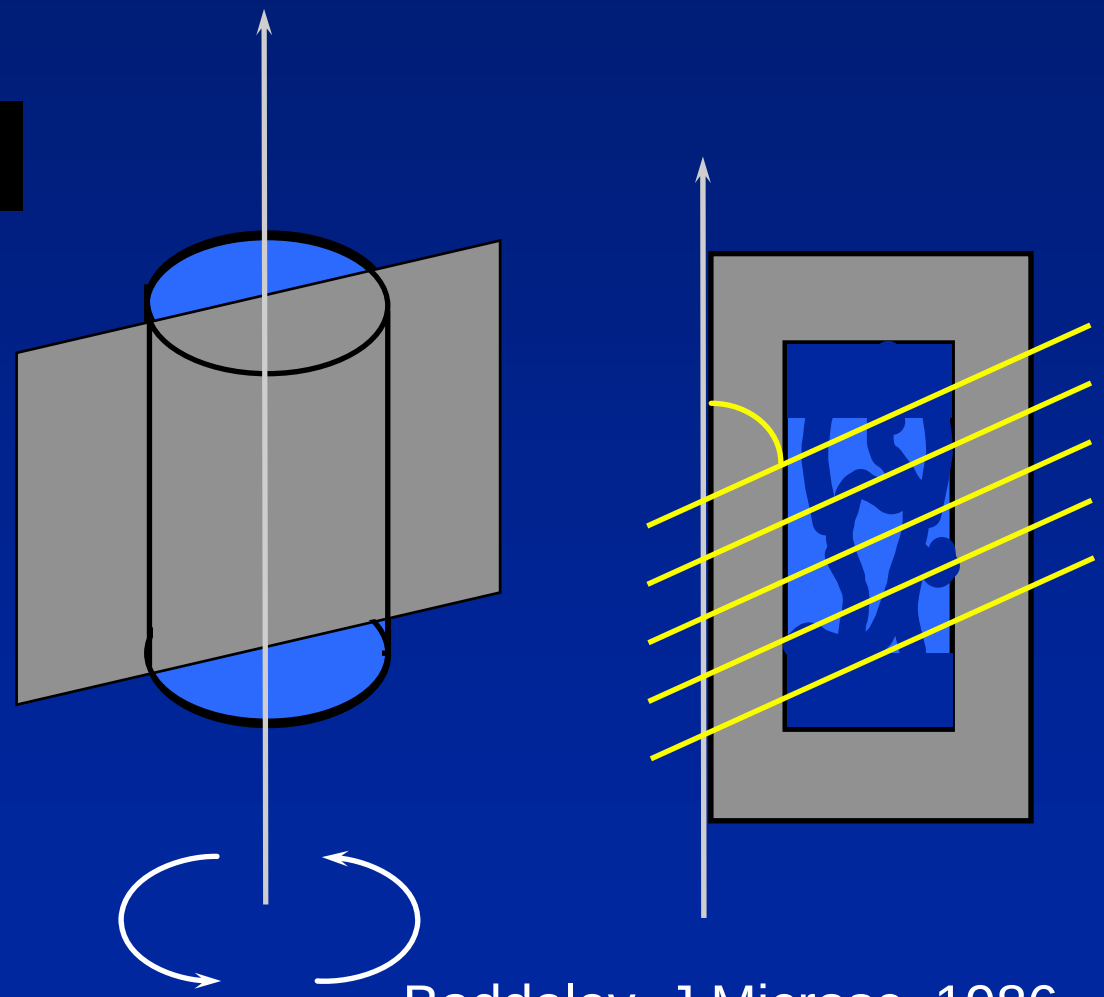
… therefore we must do a trick to fulfill the requirements of fair sampling, i.e. IUR.

- isotropic
- uniform
- random

Using vertical sections design, we can estimate surface without bias

The simple requirements are

- Random rotation of the sample around a vertical axis
- Sine-weighted rotation of the grid lines.

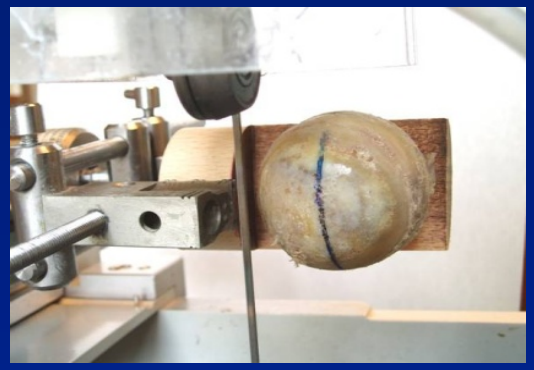


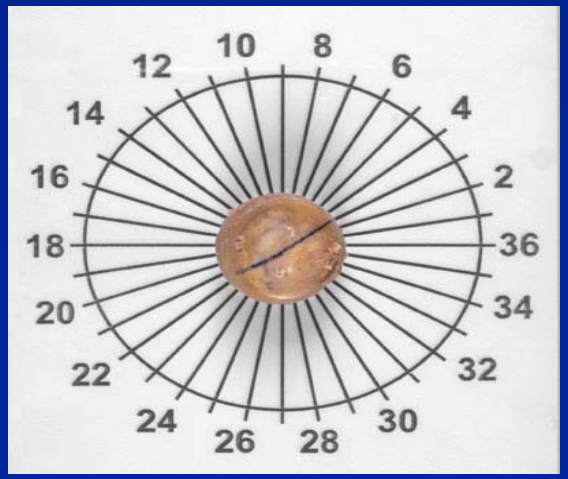
Baddeley, J.Microsc. 1986

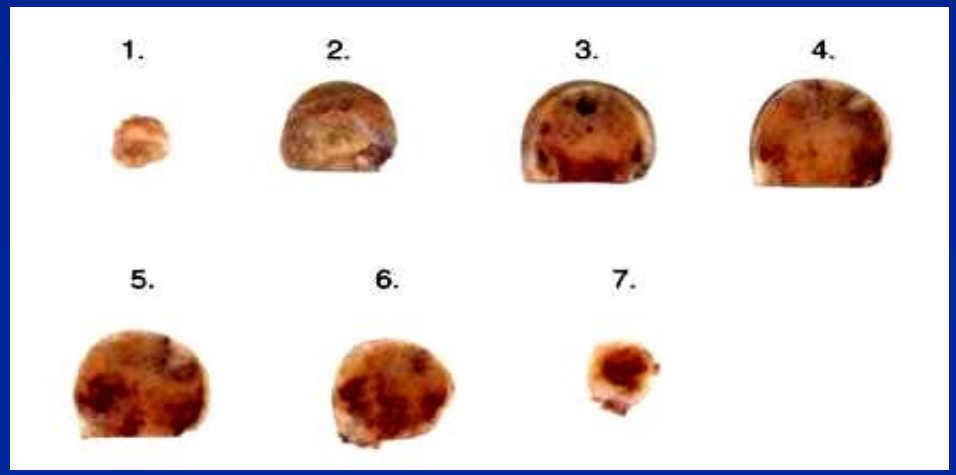
Inhomogenous involvement of the OA joint imply IUR sampling of surface

- OARSI recomends
  - One coronal section through worst affected area
- But we can do
  - Random sampling of the *entire* femoral head
  - Global mean surface (mm^2)

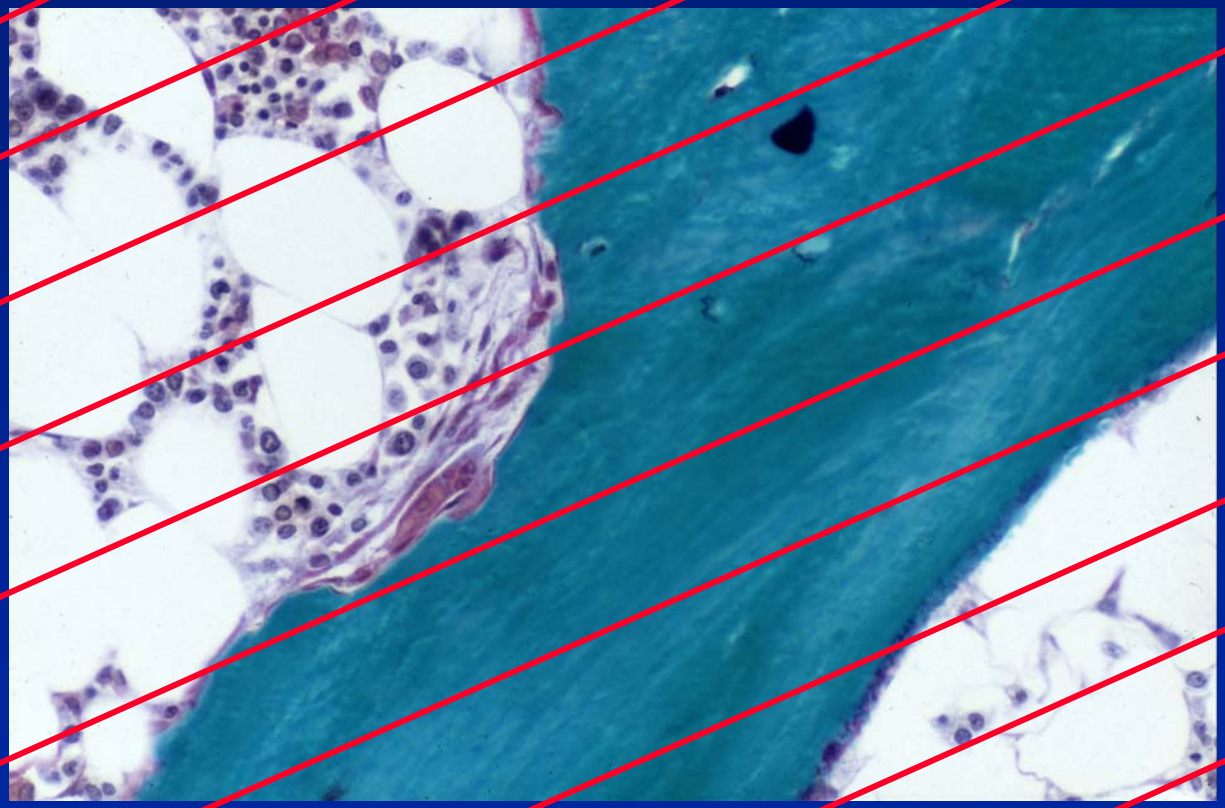
The vertical sections design used on entire femoral heads to sample surface IUR

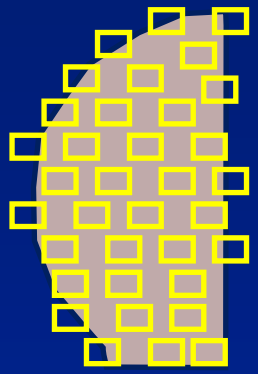






IUR sampling of bone resorptive surface





Grid lines are rotated between each field of view.

Rotation is sine-weighted

Stereology has provided unbiased tools to estimate surface unbiased

- Random and unbiased sampling of bone can be fulfilled.
- The anisotropy of bone is not an acceptable error since little effort is needed to avoid it.

It may be time for applying new stereological tools in quantitative bone histology

- In most bone tissues we need to care about orientation.
- A meaningfull number estimate must be unbiased in 3D.
- The vertical sections design and the disector allows unbiased estimation of surface and number.
- A unique tool to study bone cell activities.