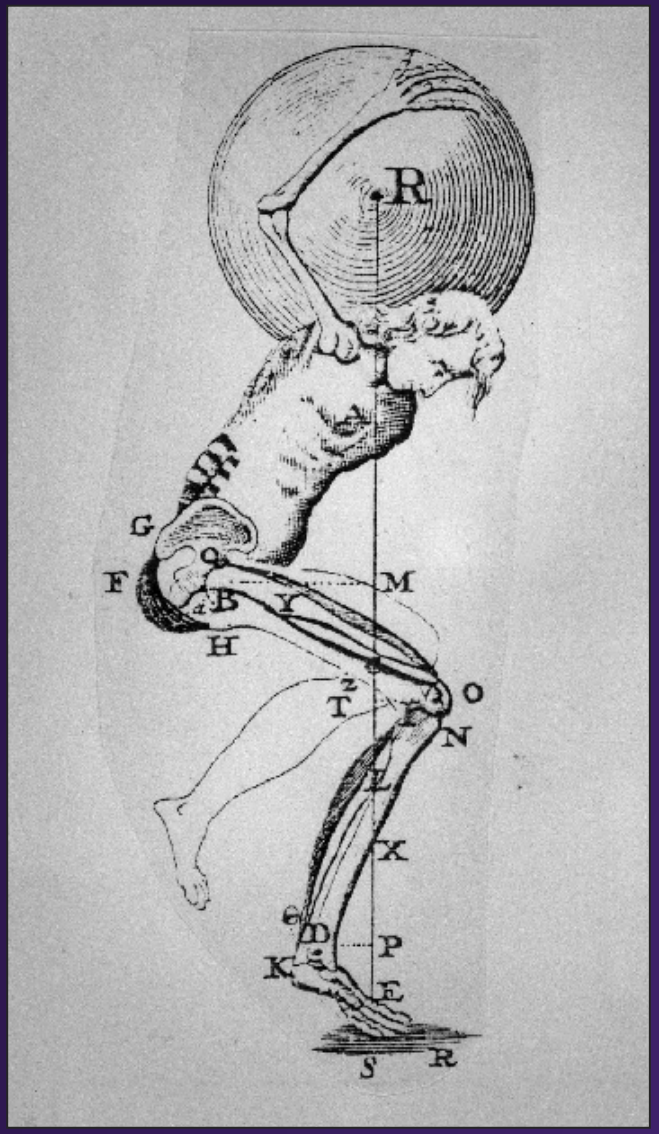


Musculoskeletal Biomechanics

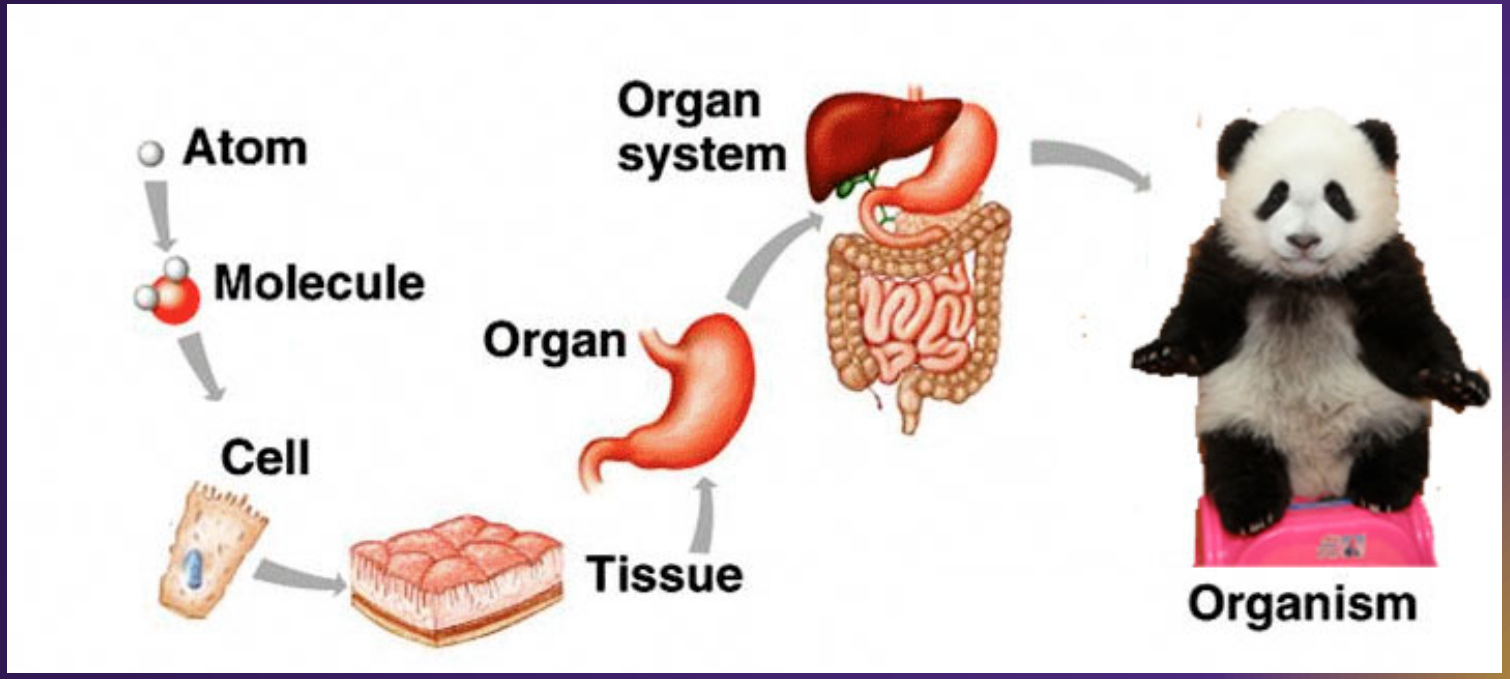
BIOEN 520 | ME 527

Session 5A

Histology and Biochemistry

What is Tissue?

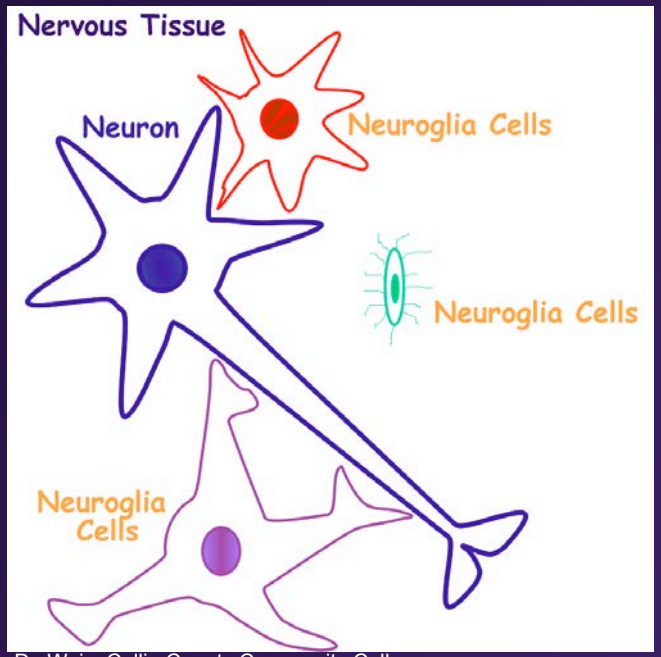
OED: Any of the distinct types of material of which animals or plants are made, consisting of specialized cells and their products.



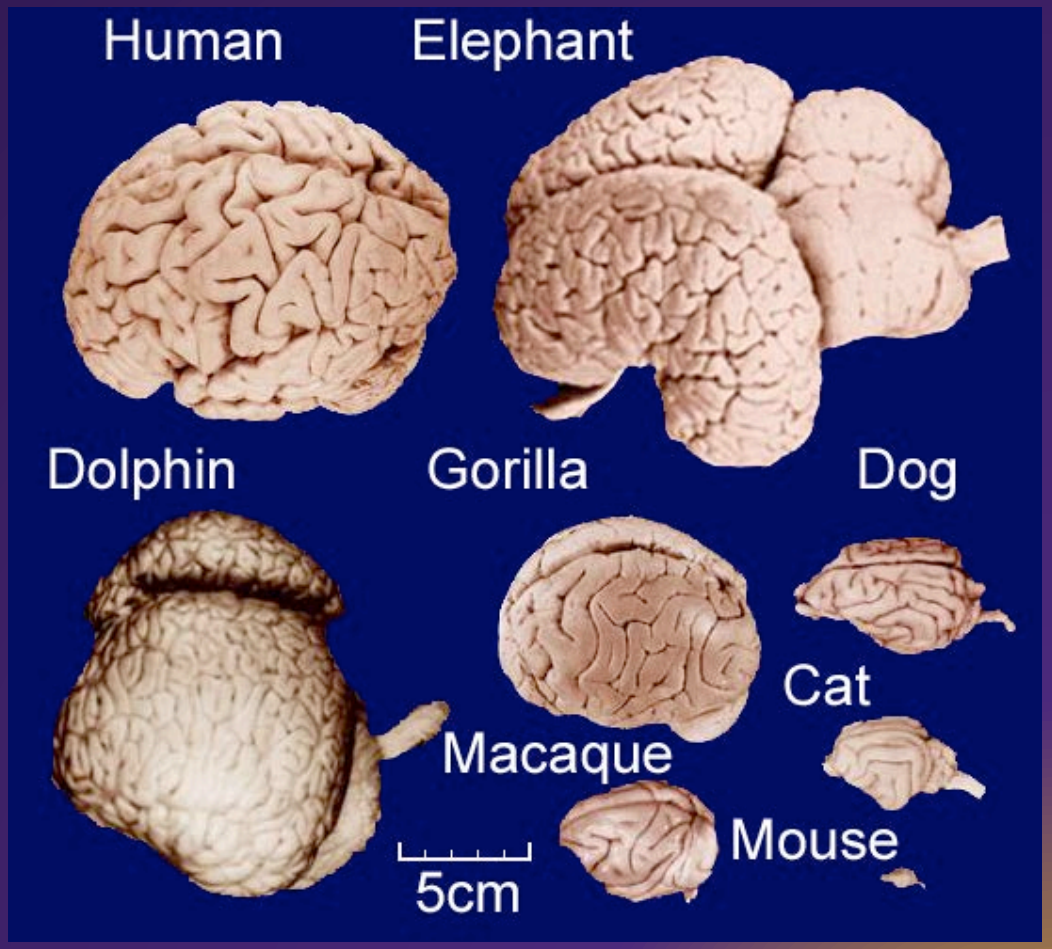
How many tissue types are there?

- Nerve
- Epithelial
- Connective Tissue
- Muscle

Nerve



Dr. Weis, Collin County Community College.

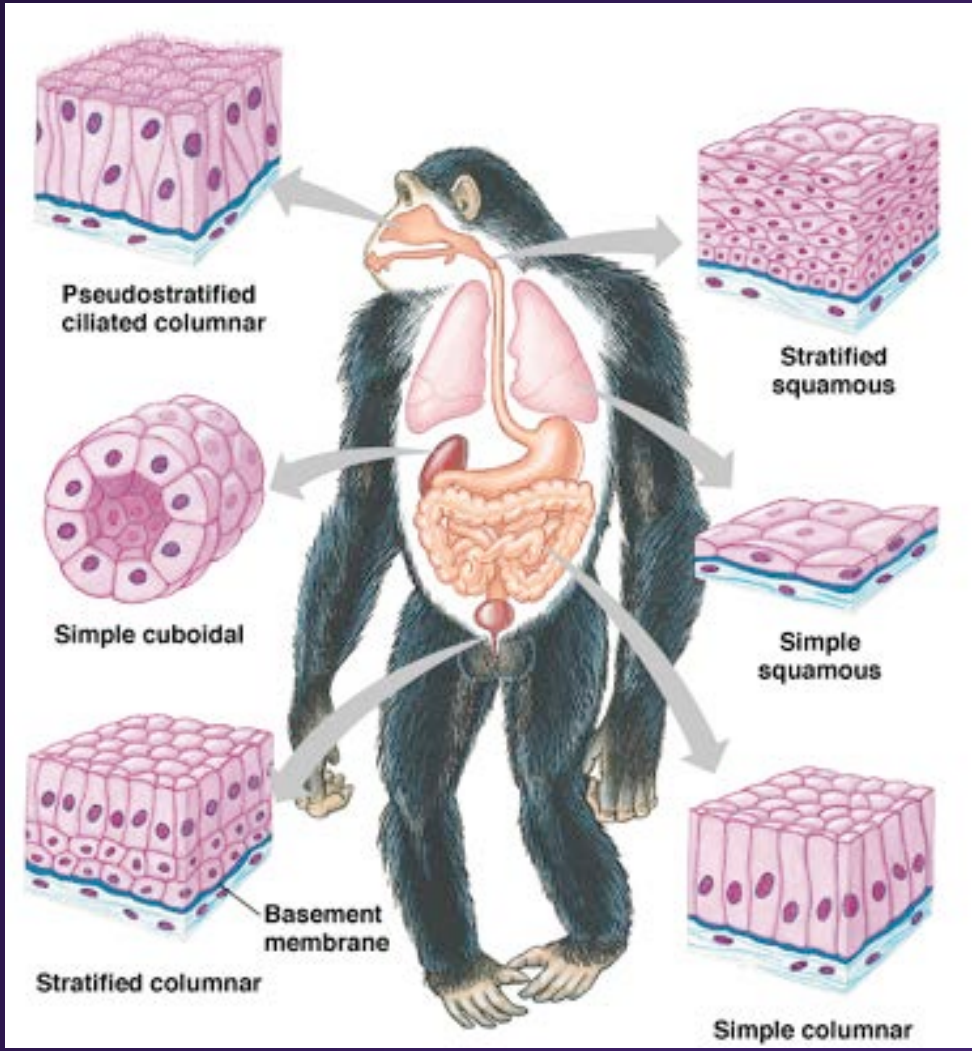


http://www.brainmuseum.org/

Function:

- Sensing stimuli
- Transmitting signals

Epithelial

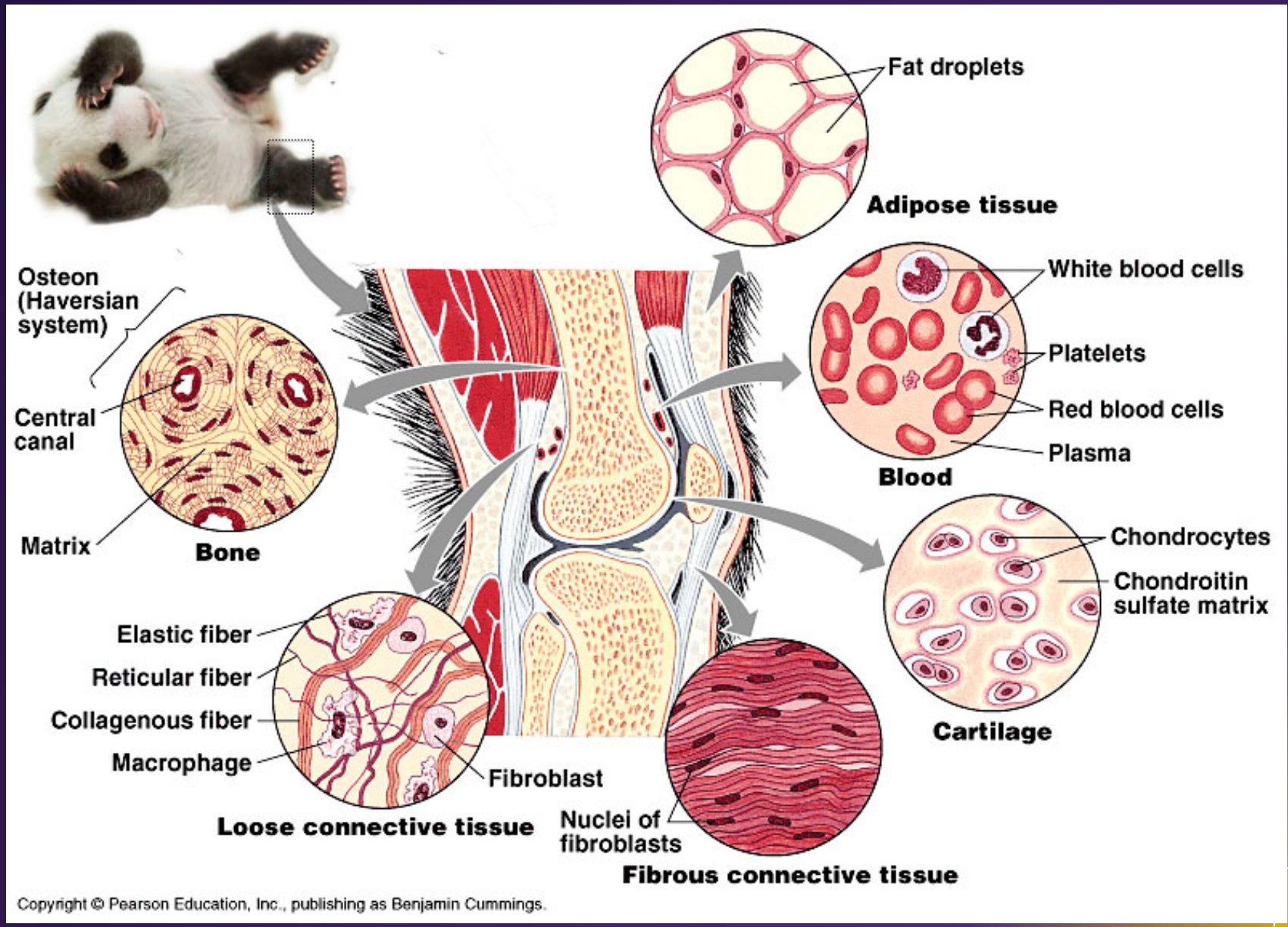


http://anatomycorner.com/histology/

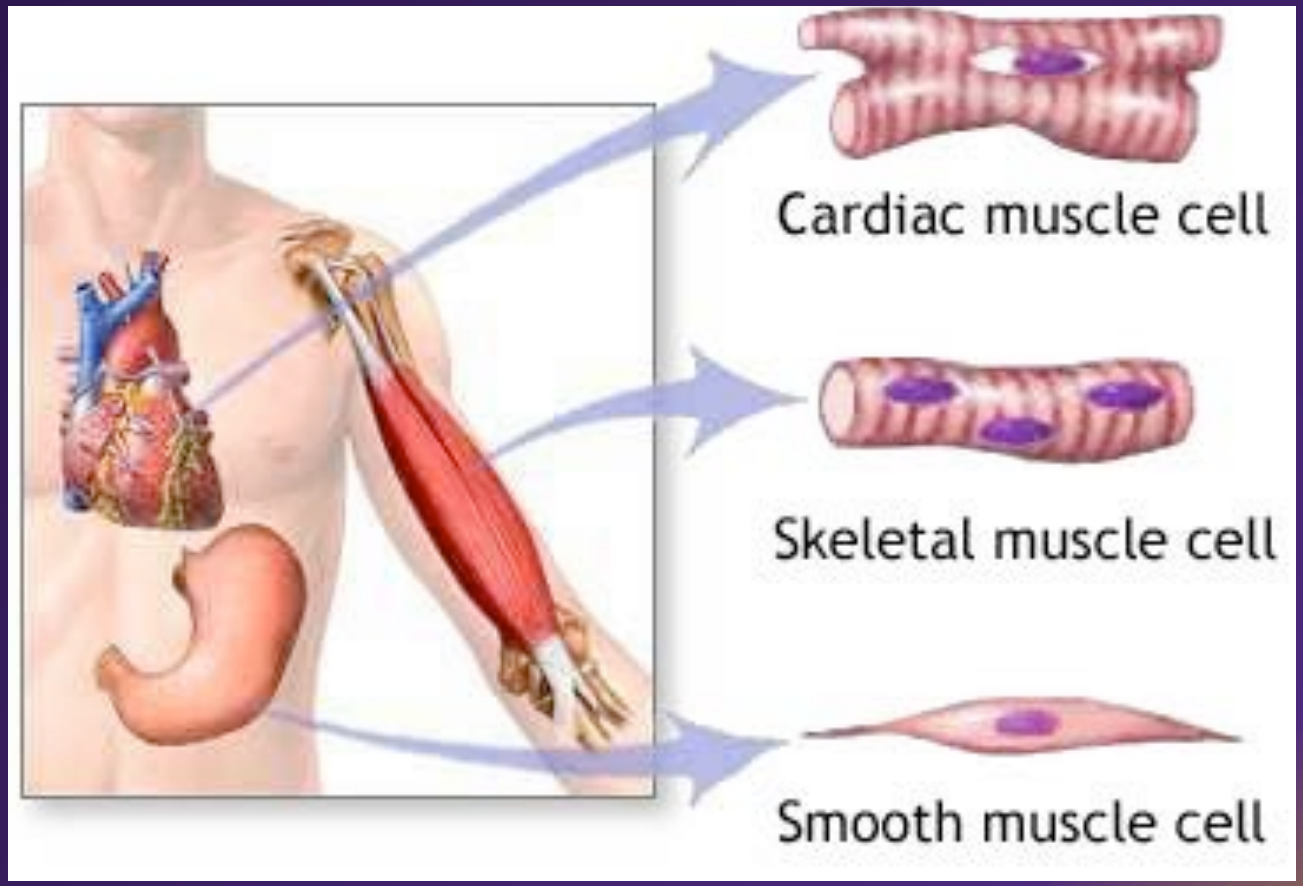
Function:

- Protection
- Secretion
- Absorption
- Excretion
- Sensory perception
- Diffusion
- Cleaning
- Reduces friction

Connective Tissue



Muscle



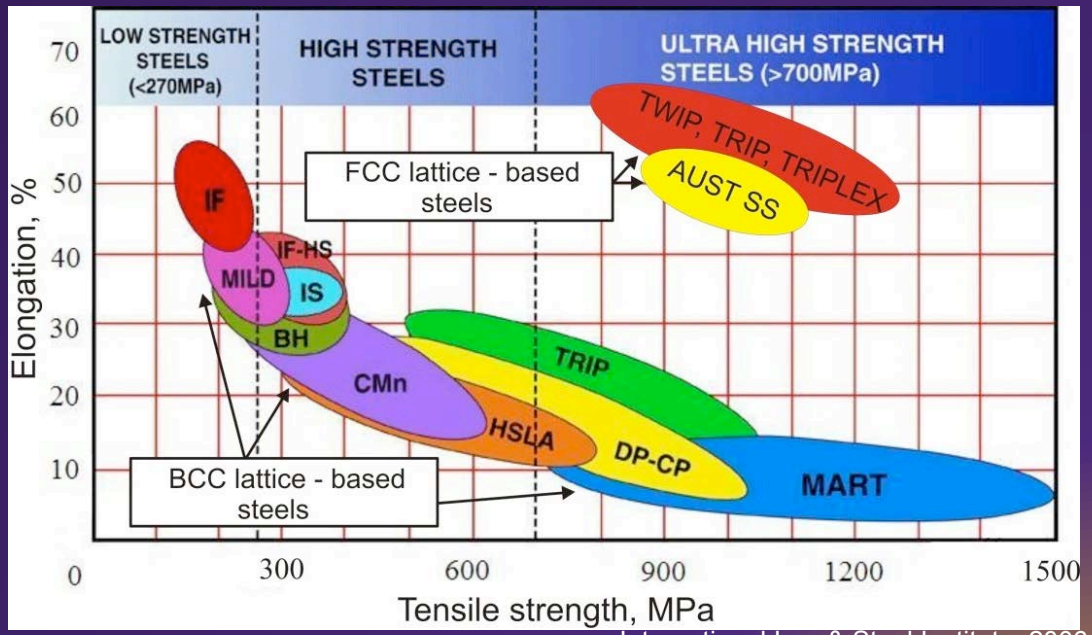
Function:

- pumps blood
- Contracts and moves part of the skeleton
- Contracts to help exude contents

How does this relate to mechanics?

What is mechanical behaviour dependent on?

- Constituents
- Structure



International Iron & Steel Institute, 2006

Traditional Engineering → Biology

Constituents

How do we quantify the constituents of a tissue?

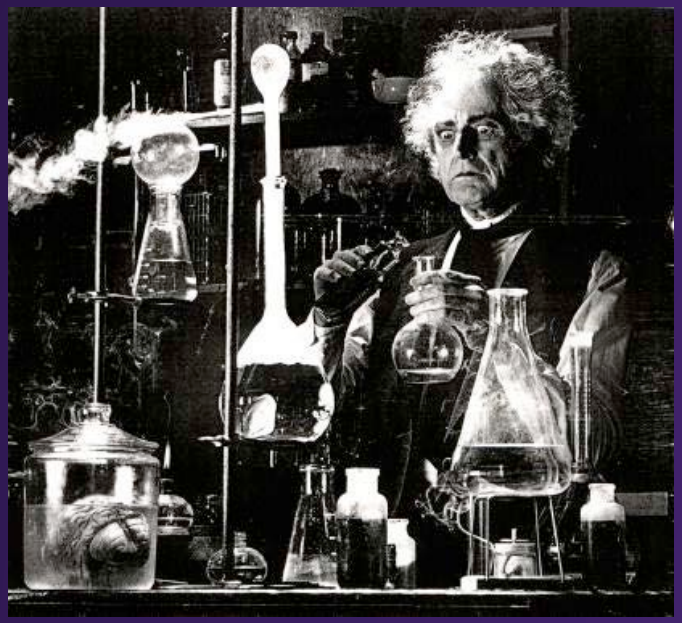
What do tissues consist of?

- Water
- Proteins (extracellular matrix: collagen, elastin)
- Carbohydrates (ground substance)
- Lipids
- Nucleic acids

Biochemical Analysis

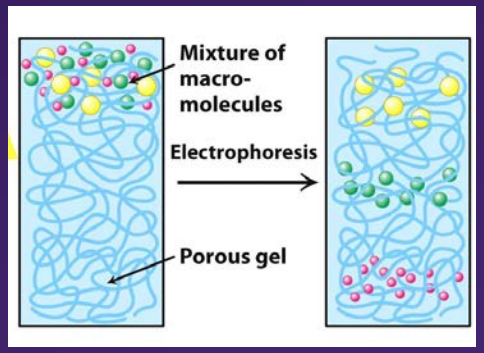
Used to evaluate the composition and function of tissue

Many methods to evaluate the same component

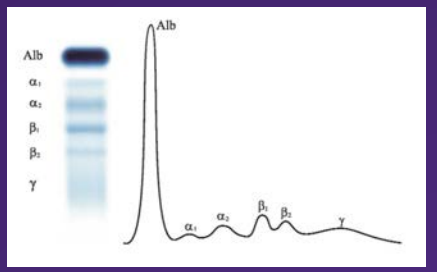


Techniques

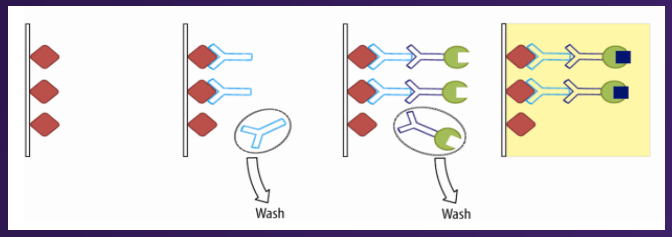
- Physical characteristics
  - Differences in charge and/or weight
  - Centrifugation
  - Electrophoresis
  - Chromatography
- Chemical or enzymatic reactions
  - biological activity
  - UV absorption or colour change

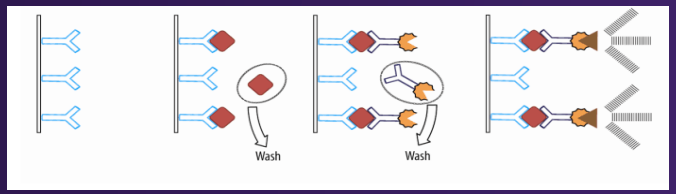


Freeman *et al.* Biochemistry

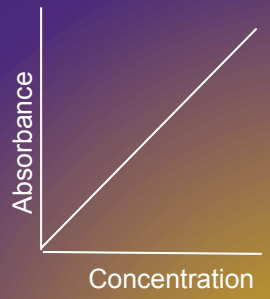


Interlab.com





Rockland Inc.

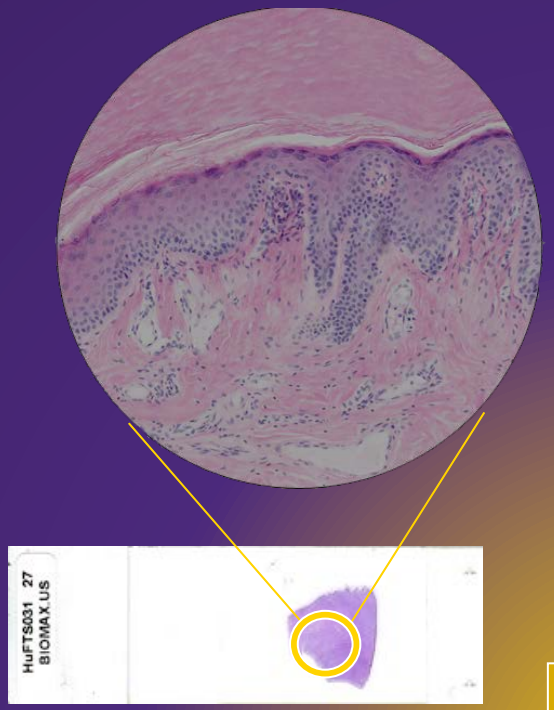


Structure

How do we examine the structure of tissue?

Histology: The study of the microscopic anatomy of cells and tissues

?



Tissue Processing

What do we need to consider?

- How fresh is tissue?
- What has been done to the tissue before?
- What do we want to look at?
  - How big is the structure you want to look at?
  - What stain, label or enzyme reaction
- What do we want to do with the images?
  - Just take pictures
  - Make measurements

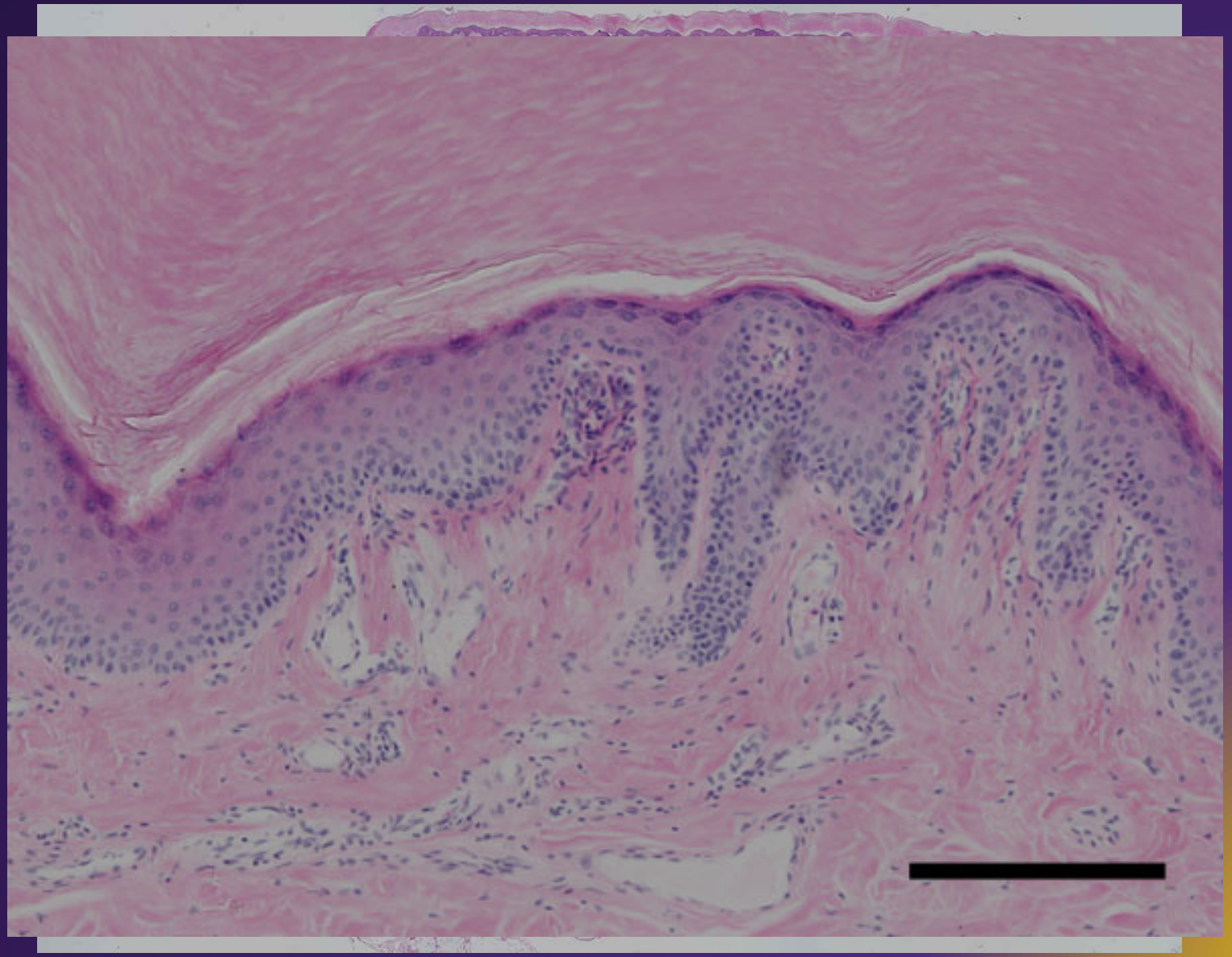
Tissue Processing

Stains, labels and enzyme reactions
General structure

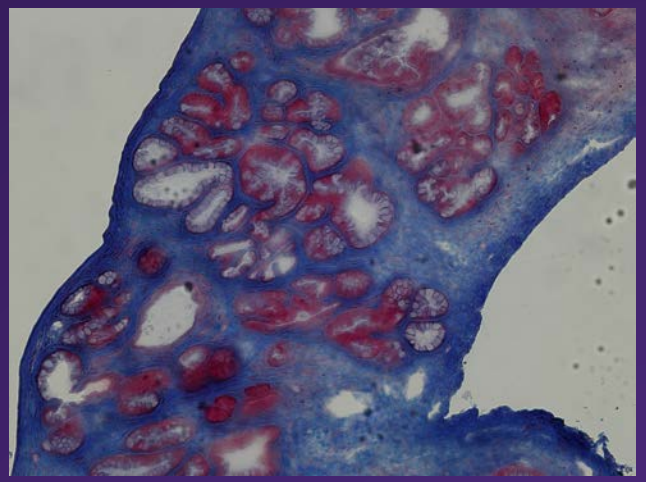
Haematoxylin and Eosin (H&E)

- Haematoxylin
  - Haematin is the active ingredient of haematoxylin
  - Haematin demonstrates cell nuclei
- Eosin
  - Counterstain
  - Mechanism of action not known but believed to be electrostatic in nature

General Structure: Plantar Tissue



Collagen



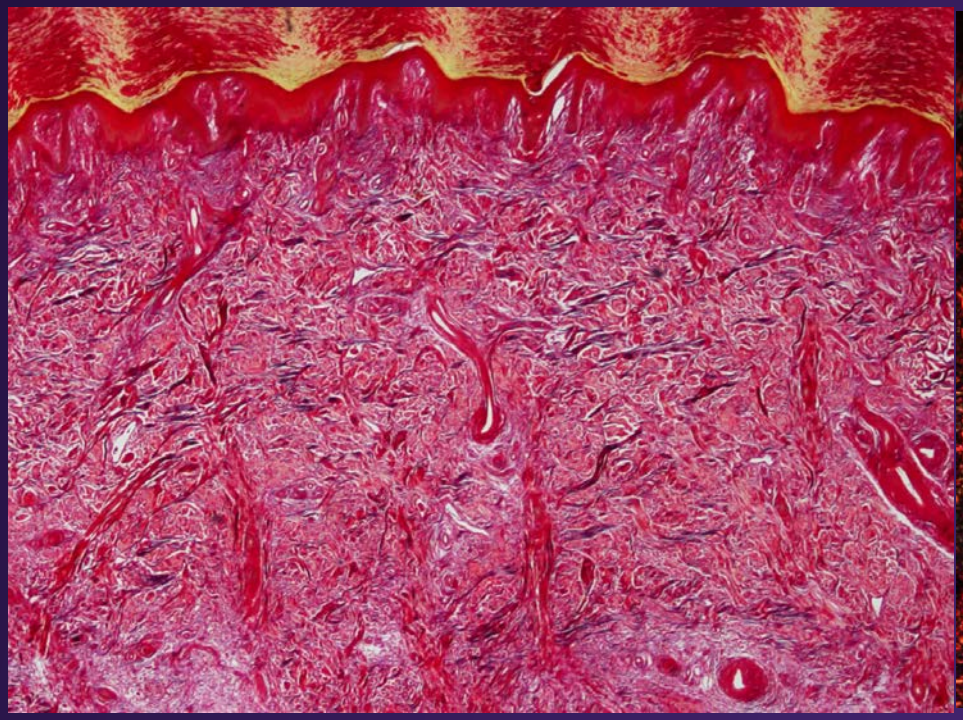
Massons trichrome

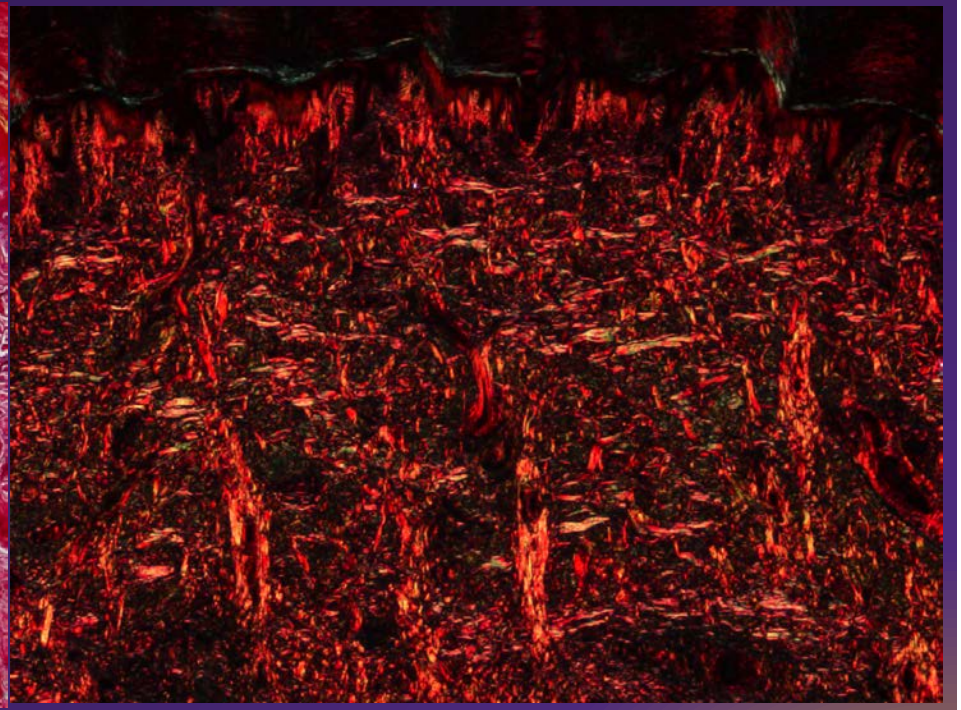
- Many different formes
- Collagen is blue

Picro Sirius Red

- Enhances the birefringence of collagen fibres, which is largely due to co-alinged molecules of Type I collagen

Collagen: Skin

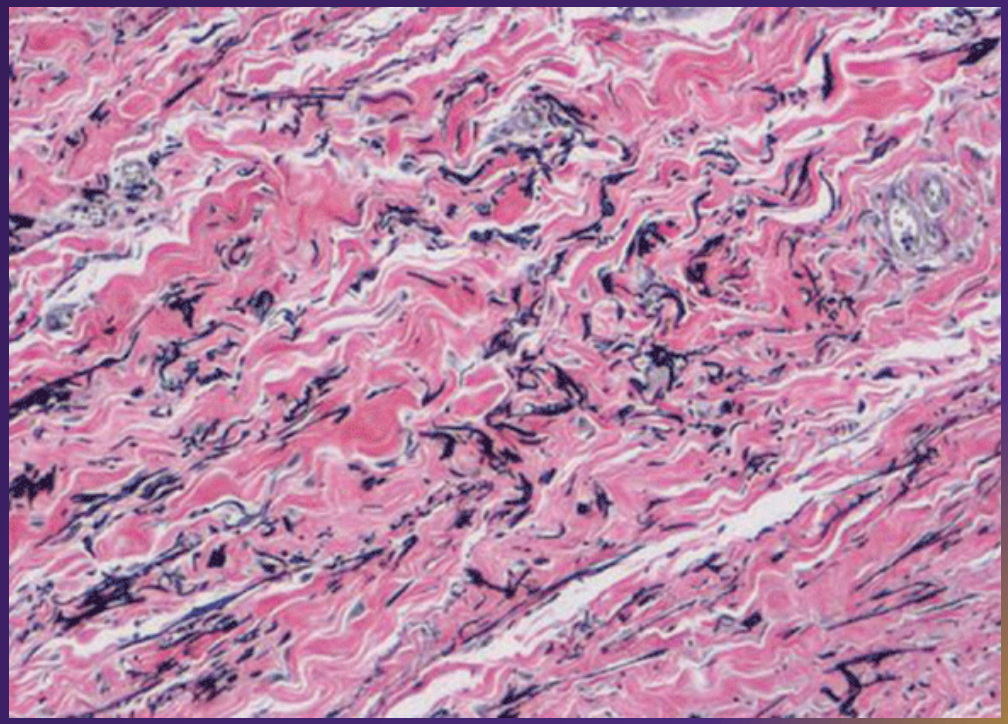




Elastin

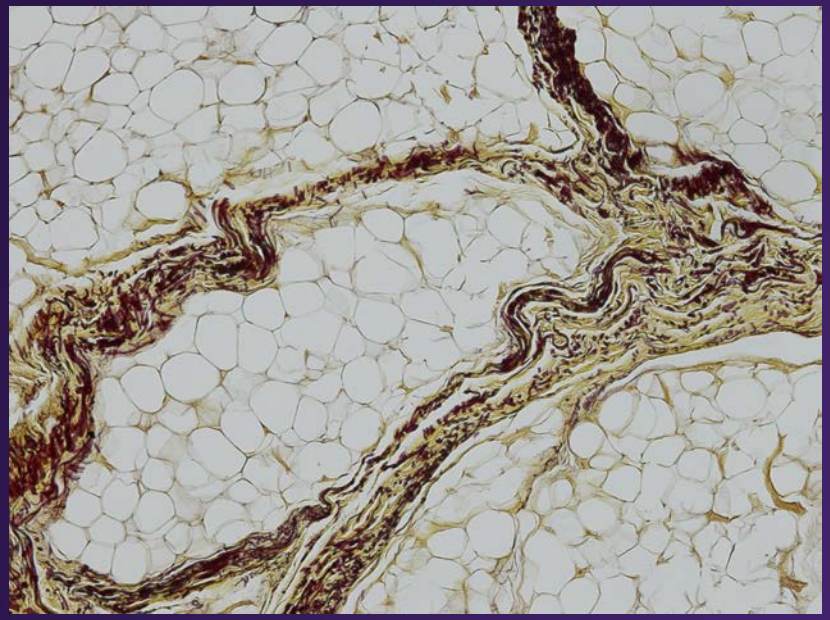
Modified Hart's

Verhoffs



Breuing 2009

Elastin: Plantar Tissue

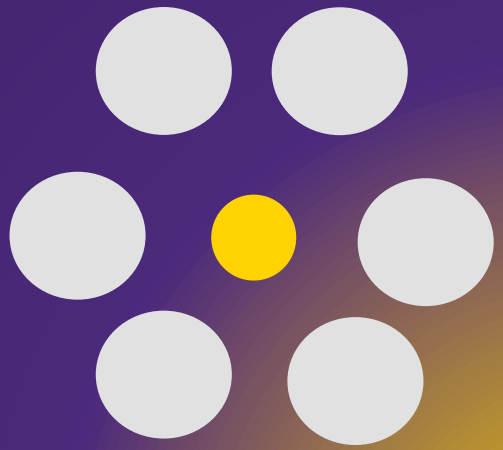


Pictures are great to have but……

How do we define differences in tissue features?

Qualitative description

- Pattern recognition
- Changes may be too small
- Subjective estimations are remarkably inaccurate



Objective measurements needed
Methods?

Quantitative Evaluation

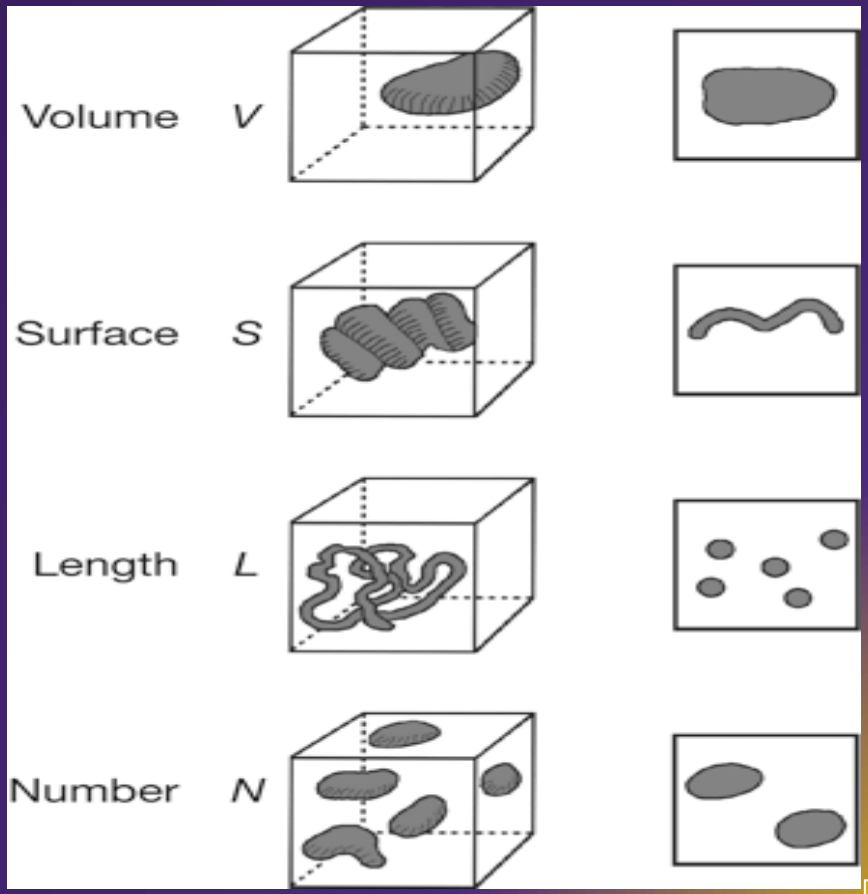
- Densitometry (or histophotometry)
  - Measurement of the absorption
- Morphometry
  - Quantitative description of a structure
- Stereology
  - Defined protocol that allow direct derivation of quantitative features of structures from two dimensional sections on the basis of geometrico-statistical reasoning

Stereology

Defined protocol that allow direct derivation of quantitative features of structures from two dimensional sections on the basis of geometrico-statistical reasoning

Structural information is lost when embedded structures are sectioned

The science of estimating geometrical quantities



Stereology

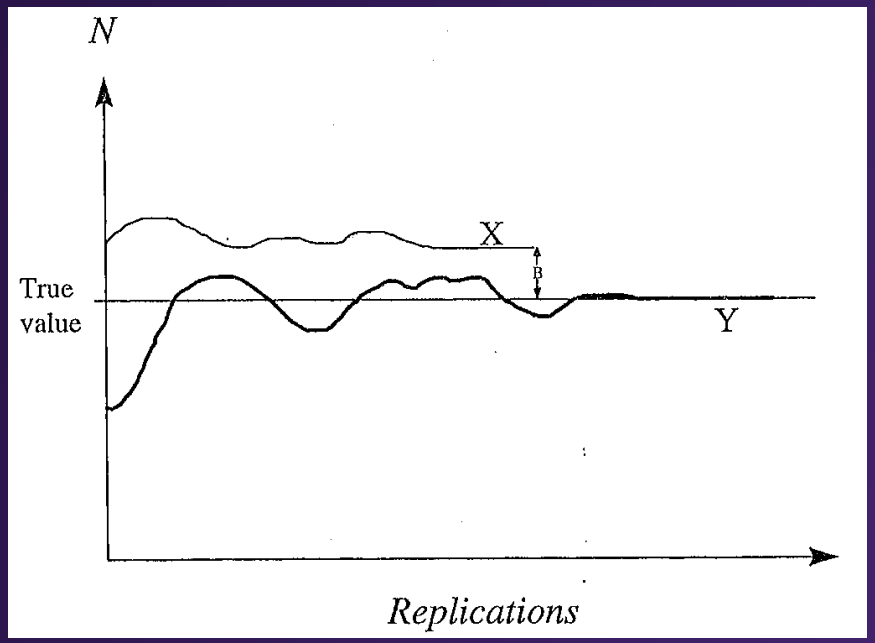
Foundation of the study is important

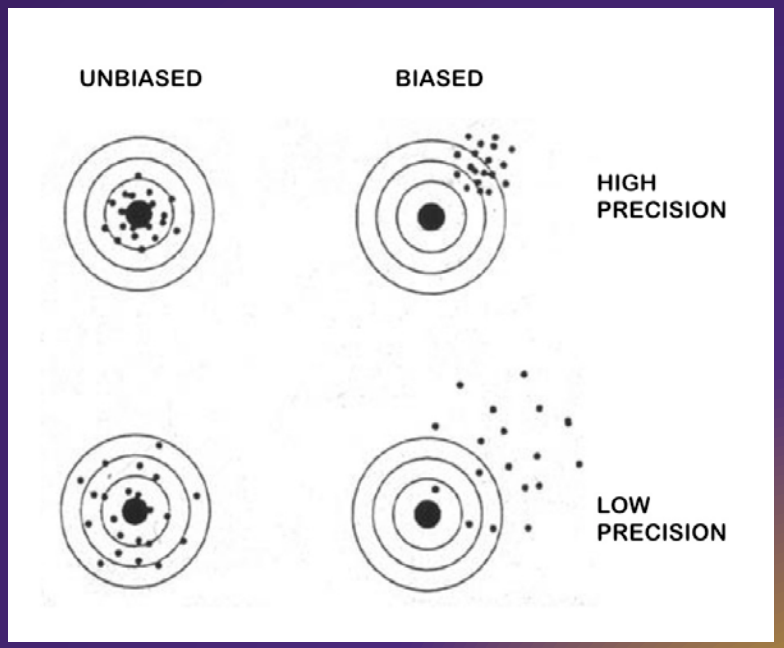
What do we need to consider?

- Sampling
  - Sampling should be uniformly random; every part of the sample needs to have an equal chance of being selected for measurement (sampling bias)
- Measurements
  - Sample must be interrogated in the same way (systematic bias)

Bias

Just because you have a small sample deviation, it doesn't mean your value is accurate!





Accuracy comes before precision

Howard and Reed 1998
Moutron 2013

Process

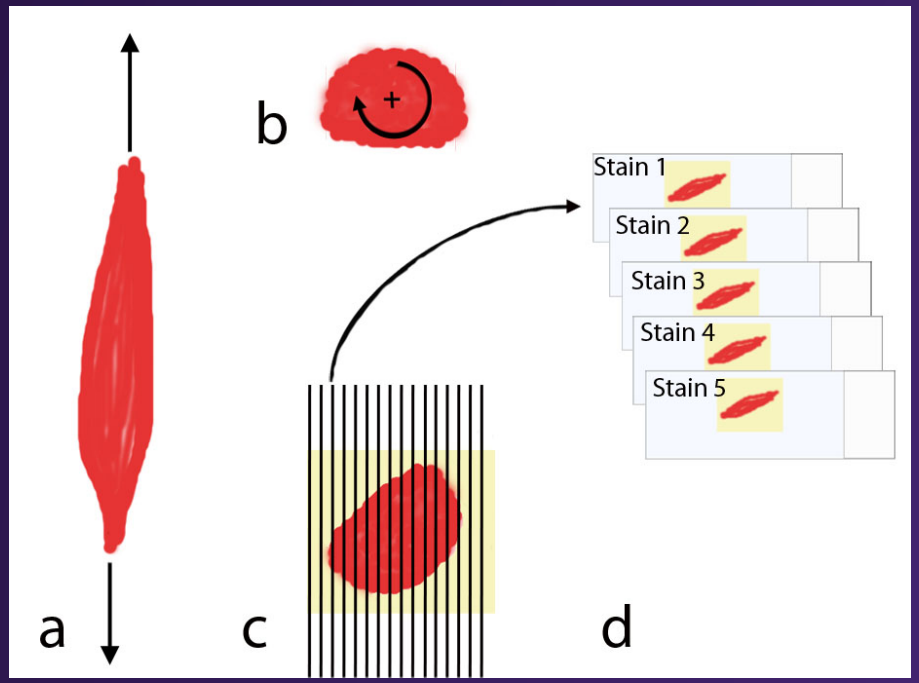
- Plan the investigation
  - Feature of interest
  - Properties that can be measured
  - Orientation
- Perform the investigation
  - Sample processing
  - Make unbiased selection from which measurements are made
  - Analysis

What are some measurement?

- Area or Volume
- Length
- Surface
- Thickness
- Number

How does isotropy change the orientation of sample?

- Particular orientation
- Well defined orientations (vertical sections)
- Isotropic orientation (not common)



Remove sample bias

Unbiased selection

Geometric probes

- Point grids
- Sampling frames (optical dissectors)
- Line grids
- Sweeping planes

Unbiased selection: Point grid

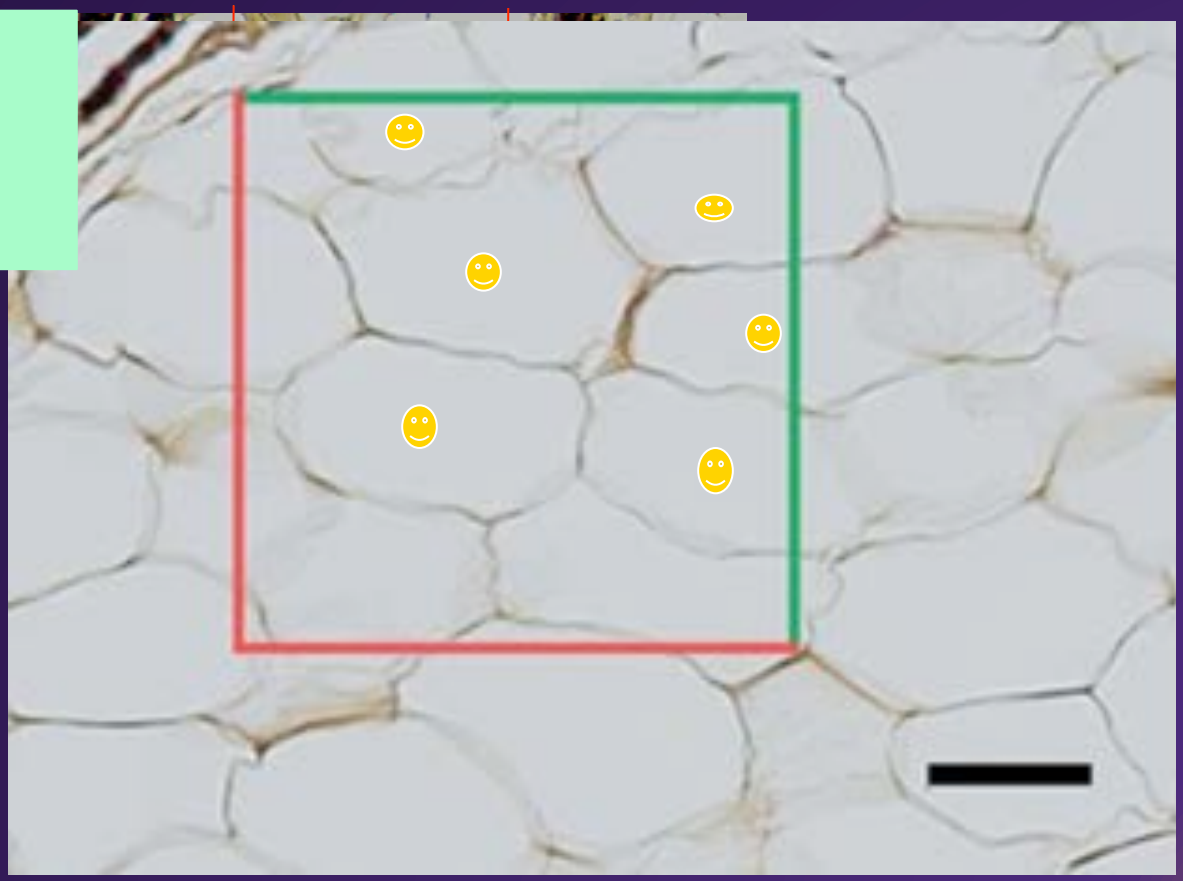
Area:
$A = \Delta x . \Delta y . P$

Volume:
$V = S . t . \Delta x . \Delta y . \Sigma P$

Things to keep in mind:

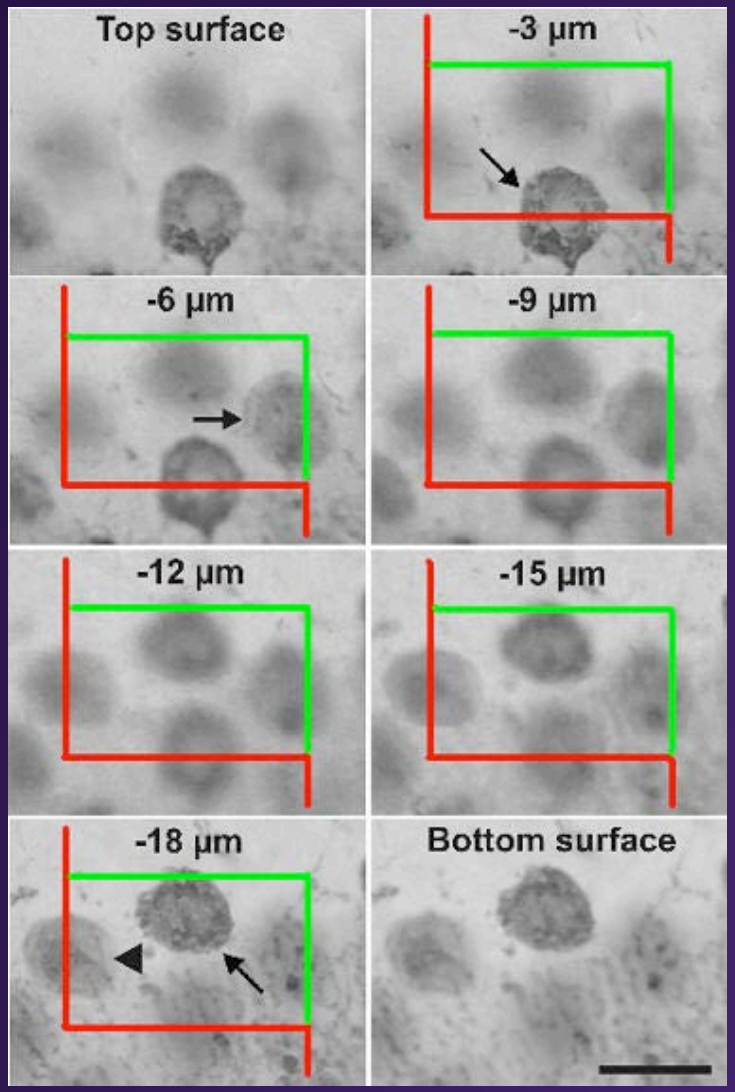
- Section thickness (t)
- Number of sections (S)

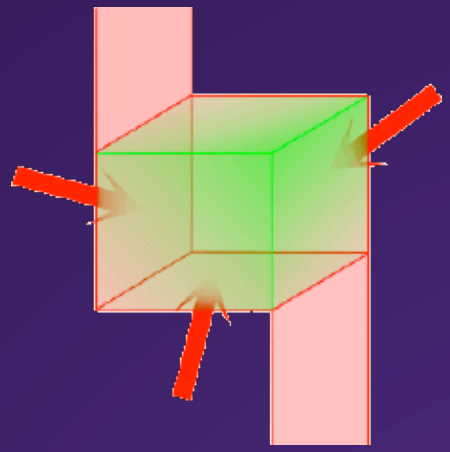
Unbiased selection: Optical Dissector



What is wrong with this method if you are using it to determine numerical density?

Unbiased selection: Number estimations

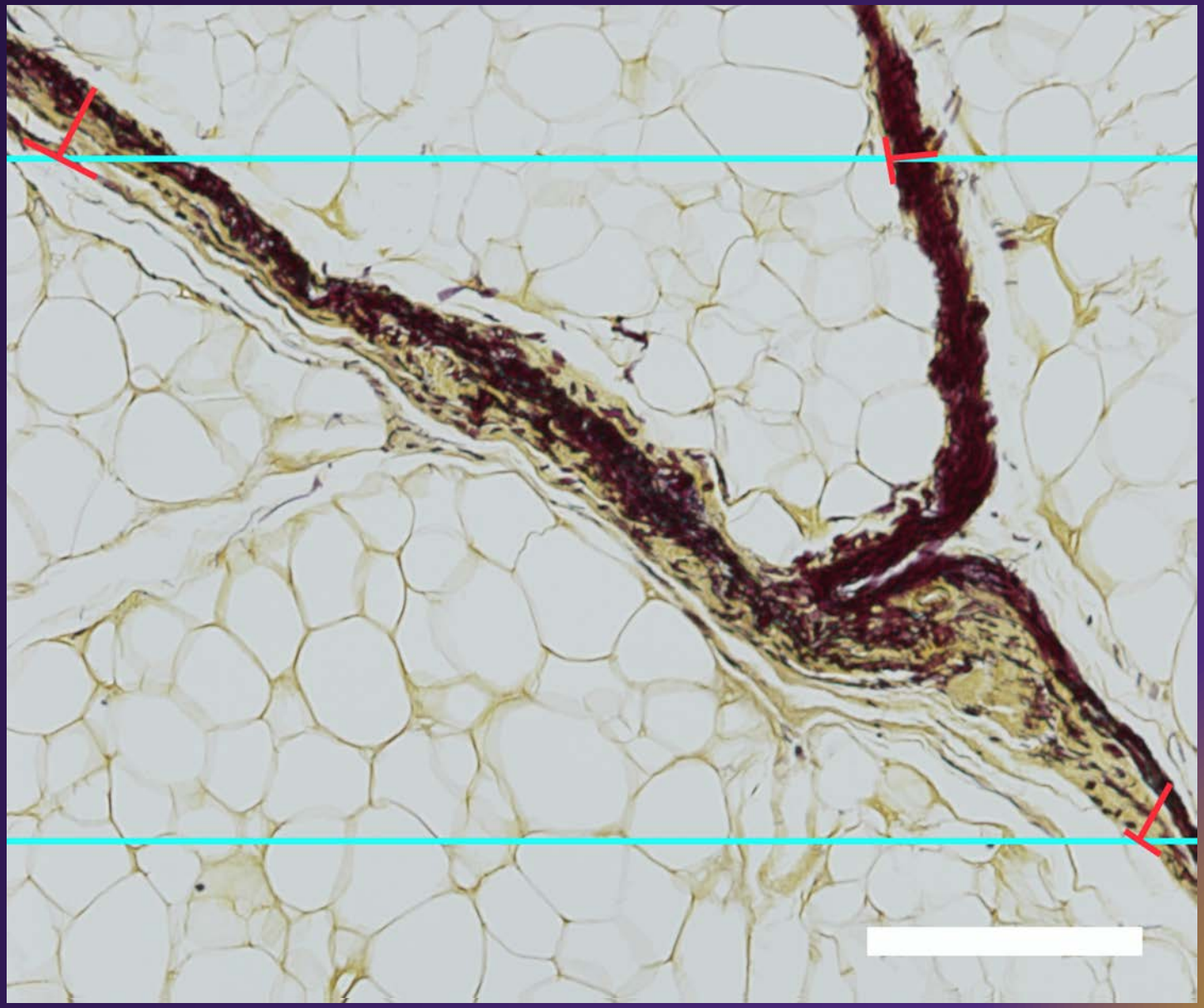




Things to keep in mind:

- Sections need to be thick
- Decide on a unique/ characteristic point to count

Unbiased selection: Line probes



Analysis

Defined protocol that allow direct derivation of quantitative features of structures from two dimensional sections on the basis of geometrico-statistical reasoning

⟶ Statistics are important for stereological analysis

Good statistical practice is based upon good experimental practice

Stereology is a science in and of itself!

Things to remember:

- Remove bias
- Perform a smaller scale pilot to determine probe features, sampling design
- Always be alert (reference trap)

Efficient stereological design avoids all known sources of experimental bias, and allows for data collection for maximum efficiency

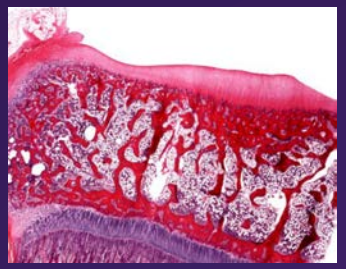
Experimental Design

- All aspects of experimental design must be planned carefully through selection of features to be evaluated through to the analysis
- Even small details count for a lot

Summary

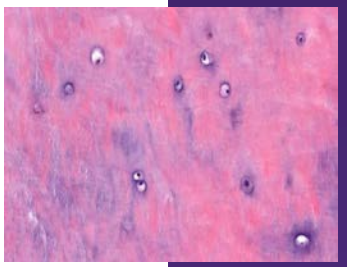
- Constituents : Biochemistry
- Structure : Histology

Welcome Library

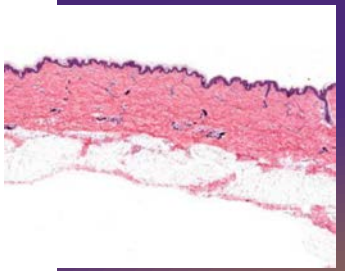


bone

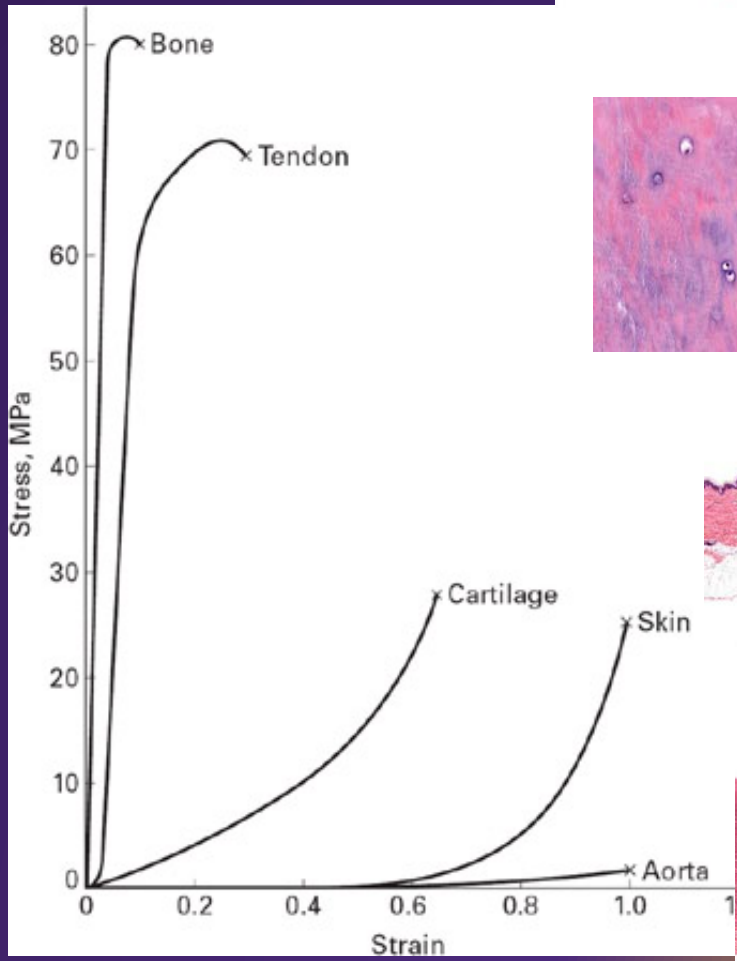
tendon



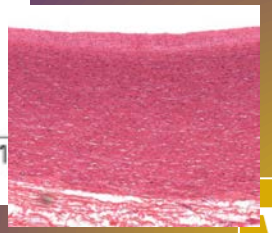
cartilage



skin



Chawla K, Mechanical behavior of materials



aorta

Histologyguide.org