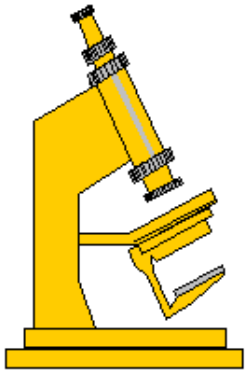


Manchester Microscopical & Natural History Society

Established 1880

www.manchestermicroscopical.org.uk

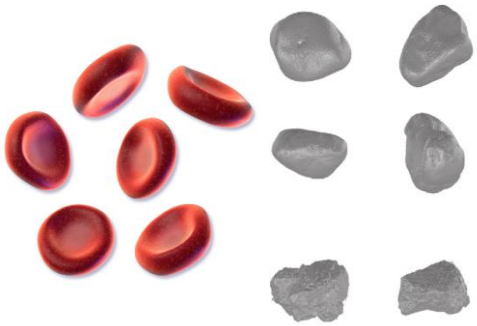
Measurement in Microscopy

Micrometry … a part of the Microscopist's Armamentarium

Guess !

Use experience

Use a ruler/transfer scale



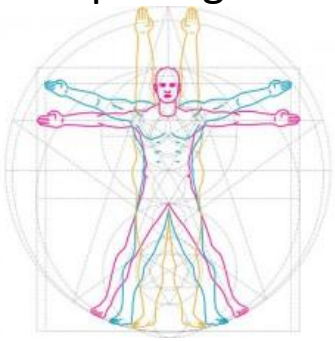
Use a known object

25 years ago! … ***MMS: November 2000***

Measuring Microscopical Objects

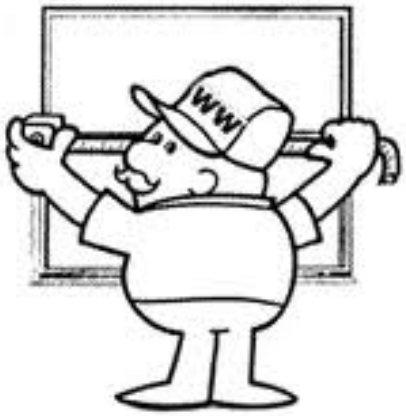
How to measure sizes, surfaces, volumes, lengths, numbers, shapes and patterns of microscopical objects. + Practical

Mike Mahon, *February 22nd 2025*



See MMS Website (Downloads pdf) ....

Quantitation



- Sampling
- Morphometry
- Stereology
- Pattern Analysis
- Image Analysis

ACA4 (2022) - Mike Mahon
School of Medicine, Faculty of Medicine & Health Sciences

Keele University

Questions

- Why measure ?
- What do you want to measure ?
- How do we measure ?
- Are the results unbiased, precise, accurate, valid, meaningful ?

Questions

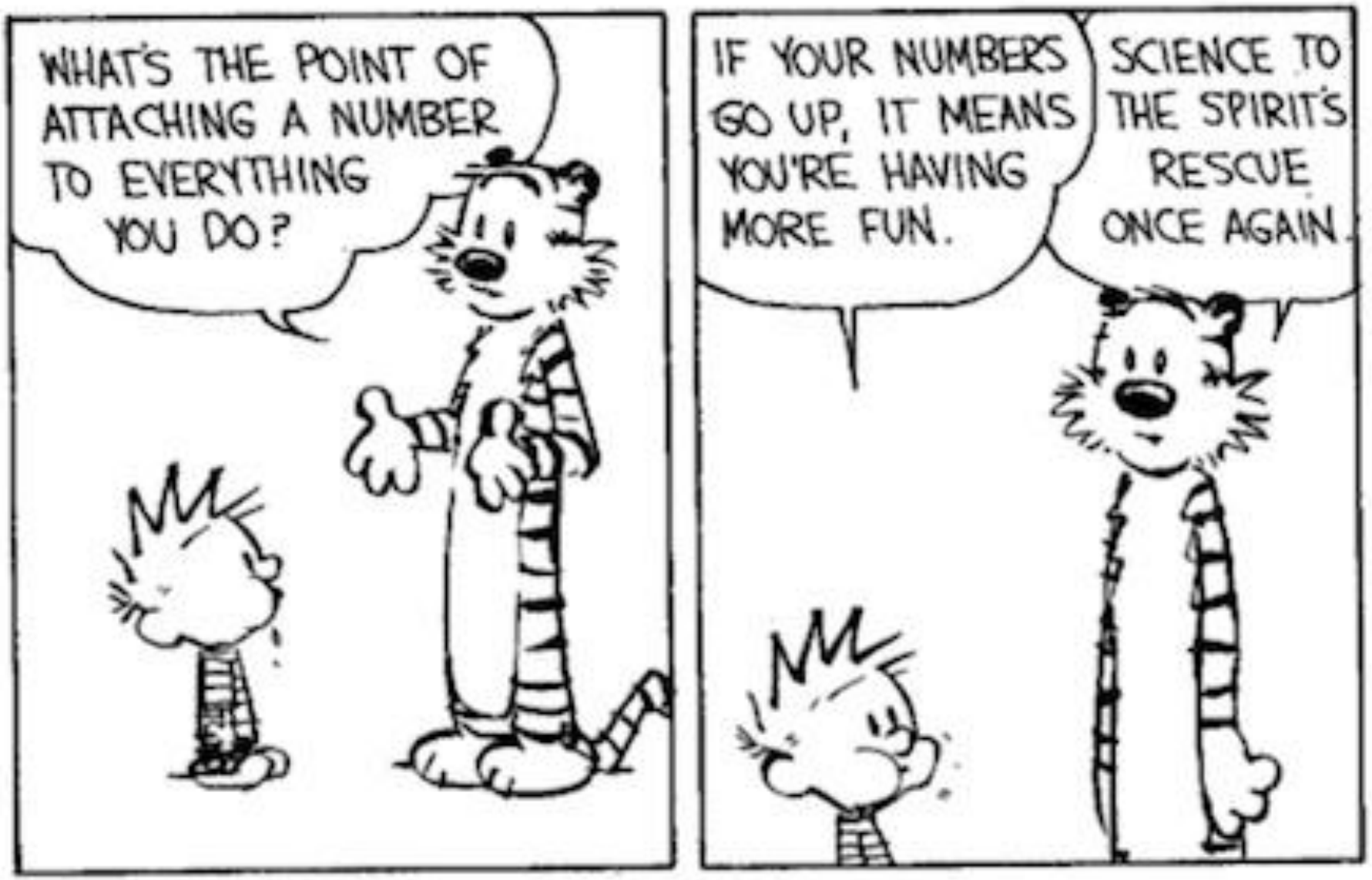
- Why measure ?
- What do you want to measure ?
- How do we measure ?
- Are the results unbiased, precise, accurate, valid, meaningful ?

Why measure ?

" …. When you can **measure** what you are speaking about and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the state of ***Science***, whatever the matter may be."

Lord Kelvin (1883)

MMS …… How big / small is it?


WHAT'S THE POINT OF ATTACHING A NUMBER TO EVERYTHING YOU DO?
IF YOUR NUMBERS GO UP, IT MEANS YOU'RE HAVING MORE FUN.
SCIENCE TO THE SPIRIT'S RESCUE ONCE AGAIN.

Why measure ?

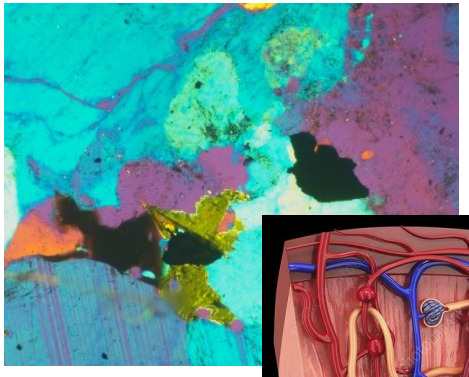
- **Obtain Absolute Size Data**
- **Variability / Constancy**
- **Relative / Comparative Data**
- Experimental v Control Data
- Disease v Healthy Data
- Treatment v Control Data
- Data on Changes / Growth / Ageing / Differences
- Data on Structure / Function Relationships
- Data to Predict / Mathematical Models

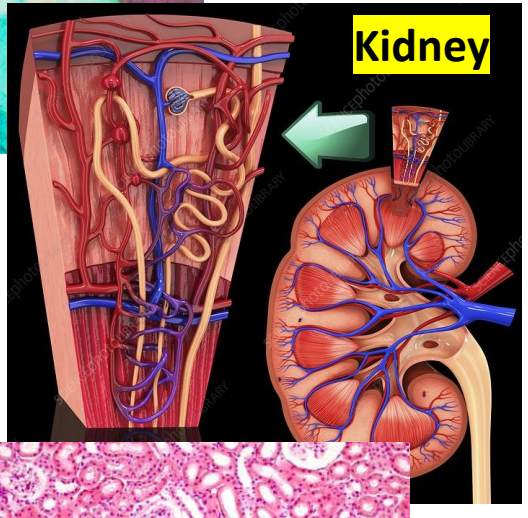
"The plural of anecdote is not data !" … Measure it yourself !

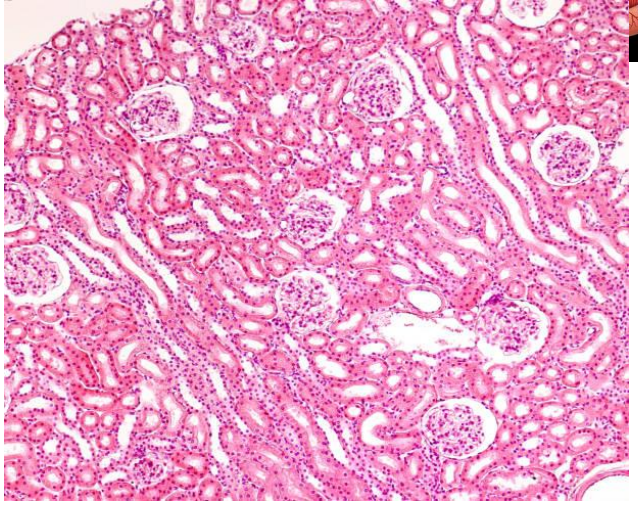
- Why measure ?
- What do you want to measure ?
- How do we measure ?
- Are the results unbiased, precise, accurate, valid, meaningful ?

Individual Objects

Structures in Rock/Tissue Sections







What? Things which may be measured

- **Structures (3D)**
  - **Number, size, surface area, length, volume, shape, …**
- Mass
  - Density/Weights of organelles, cells, tissues, organs, people, …
- Shapes & Arrangement
  - Macromolecules, organelles, cells, tissues, organs, people, …
- Chemical Constituents
  - Storage products, DNA
- Activity – Time (4D)
  - Enzyme activities, intracellular events, cell turnover, movement

What ?

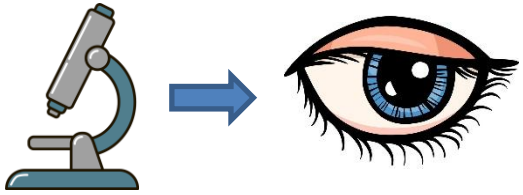
Quantitation

Geometrical Properties

- Sampling
- **Morphometry (… directly)**
- Stereology (… indirectly 2D/3D)
- Pattern Analysis
- Image Analysis

(Units: 1mm = 1000µm)

Minimum size resolved by …



0.25 µm 0.1mm (100 µm)

Microscopists measure sizes about 1µm to 5mm

Optical Properties

- Analytical Microscopy
  - Reflectometry, Phase Contrast/Refractometry, Polarising, Interference, 'Weigh cells', Microdensitometry
- Semi-Quantitative
  - Rating scales, ++++

What ?

What to Measure - Structures

- **Size**
  - **Lengths, Widths, Heights**
- Amount
  - Lengths, Lv; Surface areas, Sv; Volumes, Vv
- Numbers
  - N_A, Nv, N
- Shapes
  - Roundedness, Indentedness, S:V ratios, Form Factors, Tortuosity
- Orientations
  - Angles, Isotropic, Anisotropic, Branching
- Locations & Patterns
  - Random, Clumped, Dispersed, Related/Connectivity

What ?

Examples

- **Size of diatoms, forams, tardigrades, pollen, bugs, hairs, sand grains, blood cells, muscle cells, …**
- Number of neurons in brain samples
- Percentage / Number of dividing cells in sample
- Proportion of cells, nuclei, vessels in liver section
- Length of capillary network in tissue
- Surface area of villi in gut, alveoli in lung
- Orientation/branching of Purkinje fibres in cerebellum
- Relationships of organism type 1 to organism type 2

What ?

Applications

- **Microscopy**, Histology, Pathology
- Botany, Zoology, Anatomy, Embryology
- Radiology
- Food Science
- Metallurgy
- Materials & Computer Sciences
- Geology
- Ecology
- Geography
- Social Sciences
- Astronomy

- Why measure ?
- What do you want to measure ?
- How do we measure ?
- Are the results unbiased, precise, accurate, valid, meaningful ?

How ?

" One ounce of thought is worth one ton of equipment."

Lord Rutherford

How ?

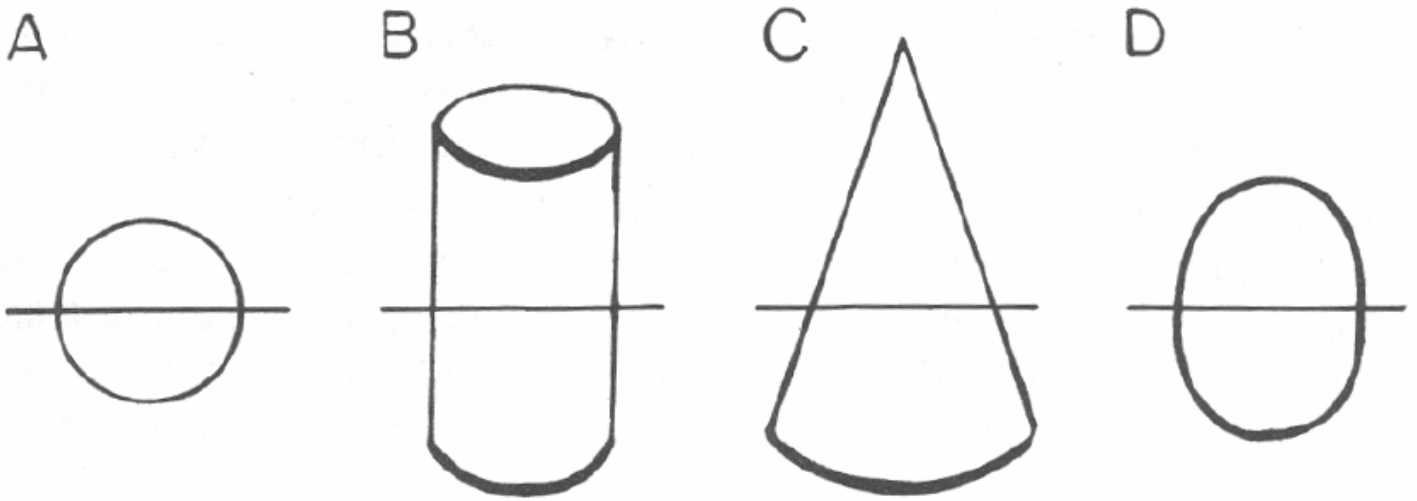
Pre-Quantitation

- Qualitative Analysis
  - Observation / Visualisation / Form / Organisation
  - Recording
  - 2D/3D Interpretation / Serial:Thick Sections / Reconstruction
  - Functional Interpretation
- Subjective Quantification
  - **Guesstimate ??**
    Variability
  - Amount, many, more, larger, 0 to ++++
  - Activity
- Relate to other levels of organisation, up, down
- Relate to other methodologies – Physiol, Biochem, Living
- Artefacts / Misunderstandings

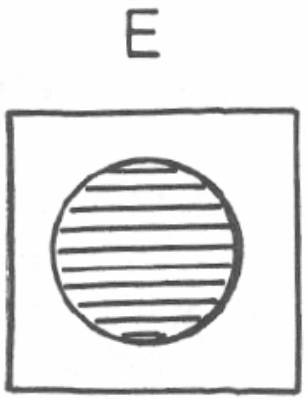
2D < > 3D

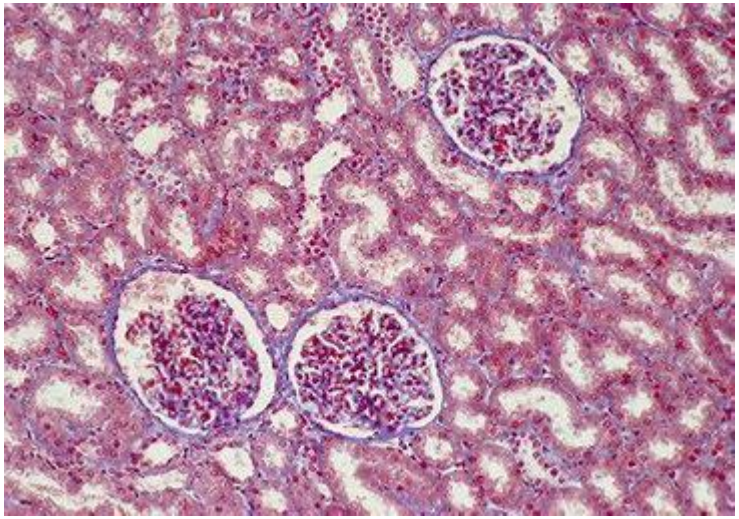
Problems using sectioned tissues !

Objects



Sectioned Profiles of objects





2D < > 3D

Problems using sectioned tissues !

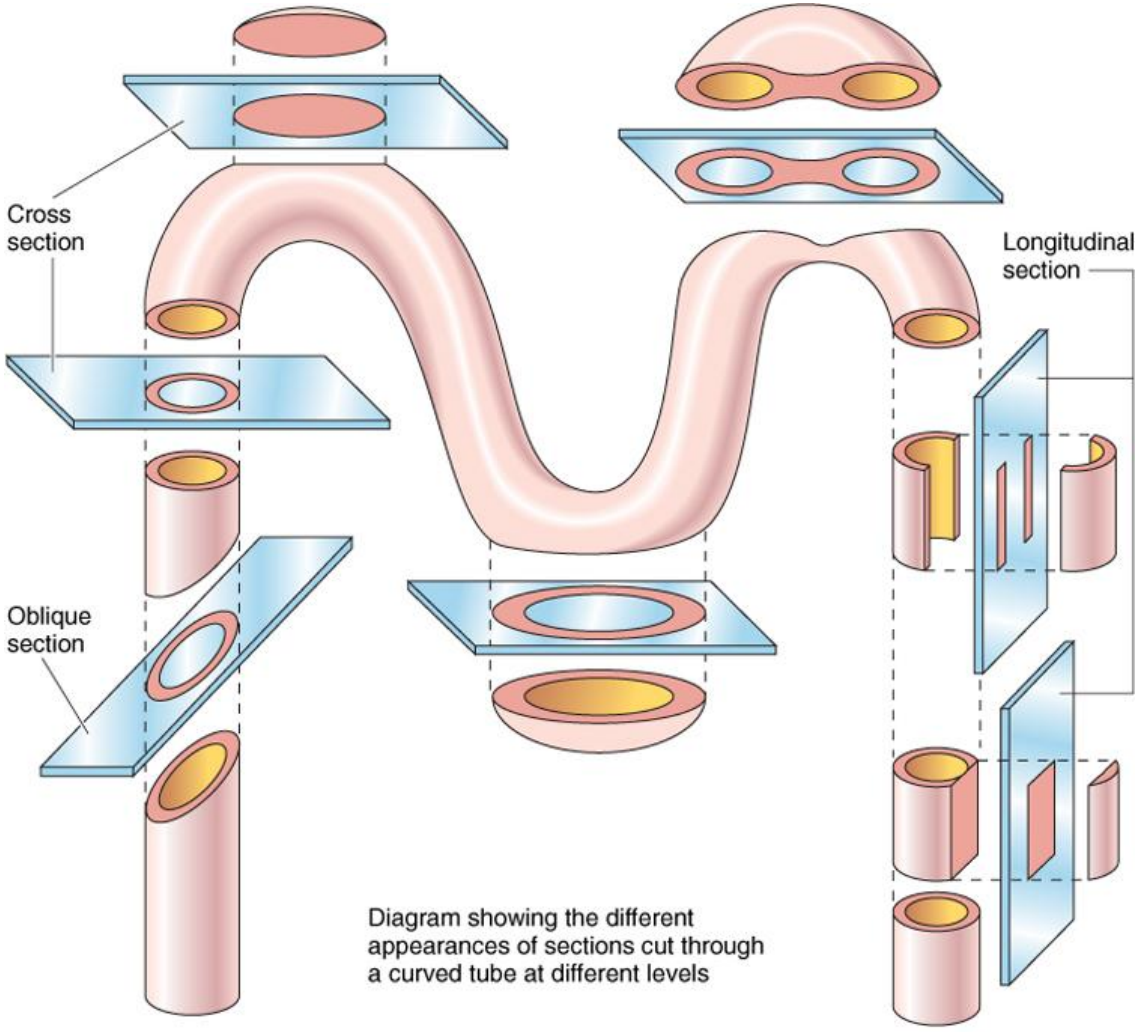
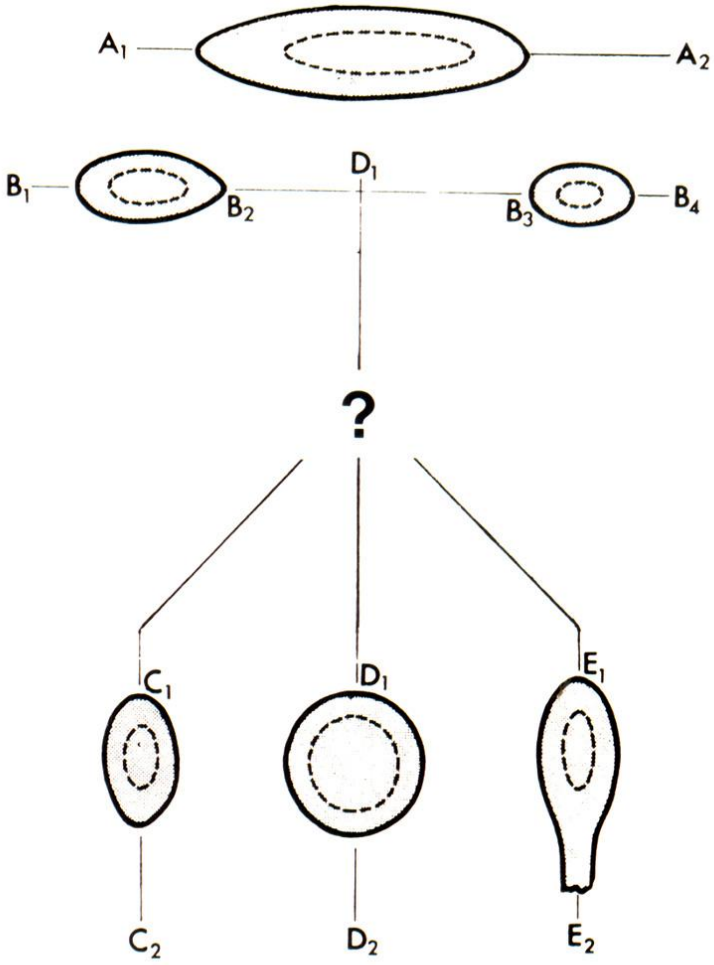
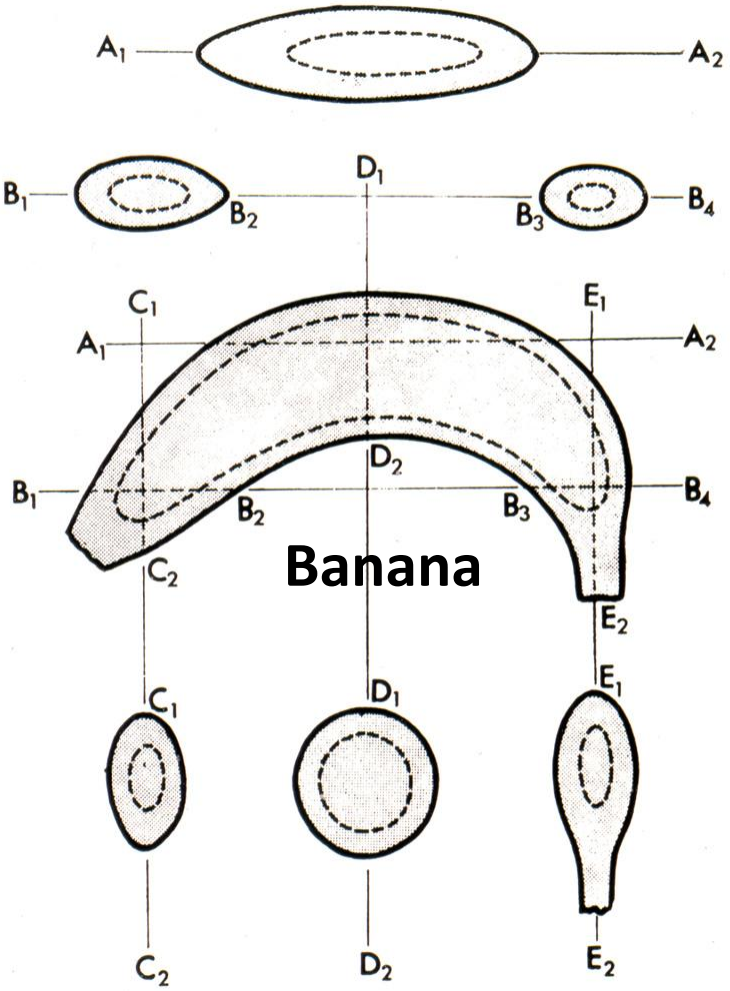


Diagram showing the different appearances of sections cut through a curved tube at different levels

2D < > 3D

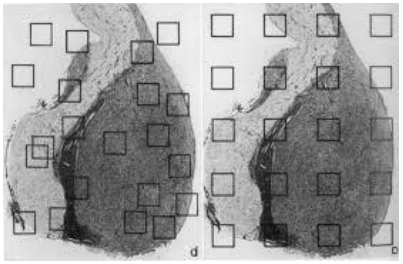

A1
A2
B1
B2
D1
B3
B4
?
C1
D1
E1
C2
D2
E2

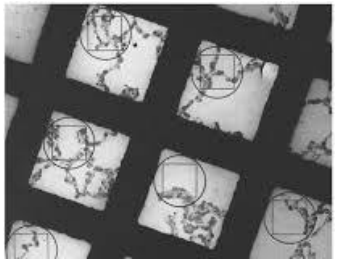

A1
A2
B1
B2
D1
B3
B4
C1
E1
A1
A2
D2
B1
B2
B3
B4
Banana
C2
E2
C1
D1
E1
C2
D2
E2

How ?

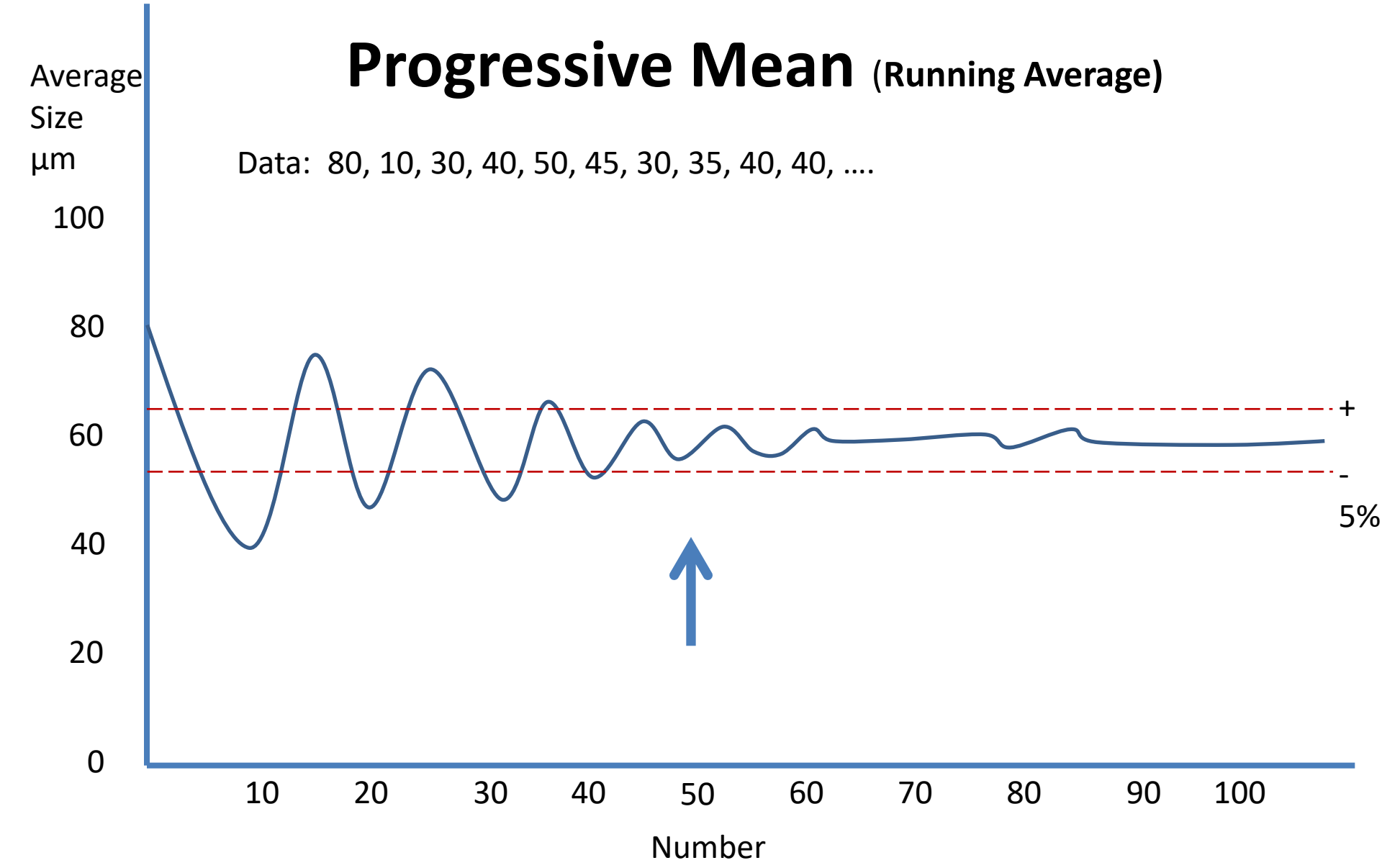
Sampling

- Identify Object
  - Staining
  - Magnification
- Representative
  - Is tissue Homogeneous (Isotropic) / Irregular (Anisotropic)
    Heterogeneous (Anisotropic)
    Gradiential (Anisotropic)
- Random
  - Completely random
  - Systematic stratified random sampling
  - Zonal oblique sector analysis

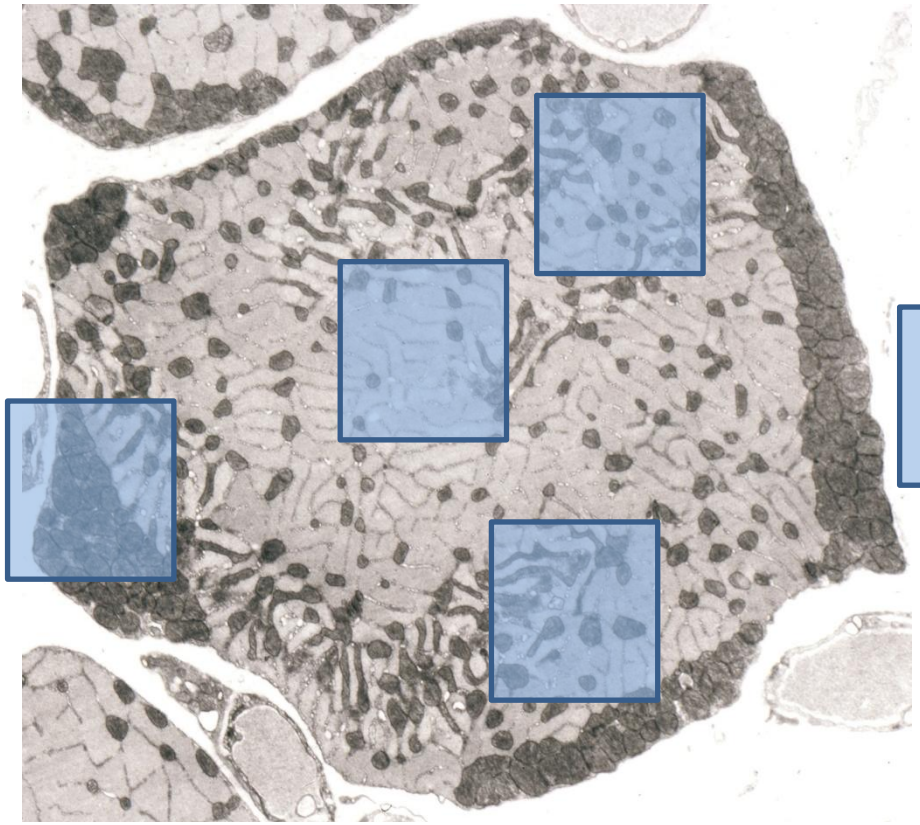




- Manual / Automated
- How many samples? (Experimental Design)
  - Individuals / Organs / Blocks / Sections / Micrographs / Items / Measures
  - Hally Formula RSE= SQRT (1-Vv)/SQRT n
  - Progressive mean, Log Plots
  - **Do More, Less Well !**


Progressive Mean (Running Average)
Data: 80, 10, 30, 40, 50, 45, 30, 35, 40, 40, ….
Average Size µm
100
80
60
40
20
0
+
-
5%
10
20
30
40
50
60
70
80
90
100
Number

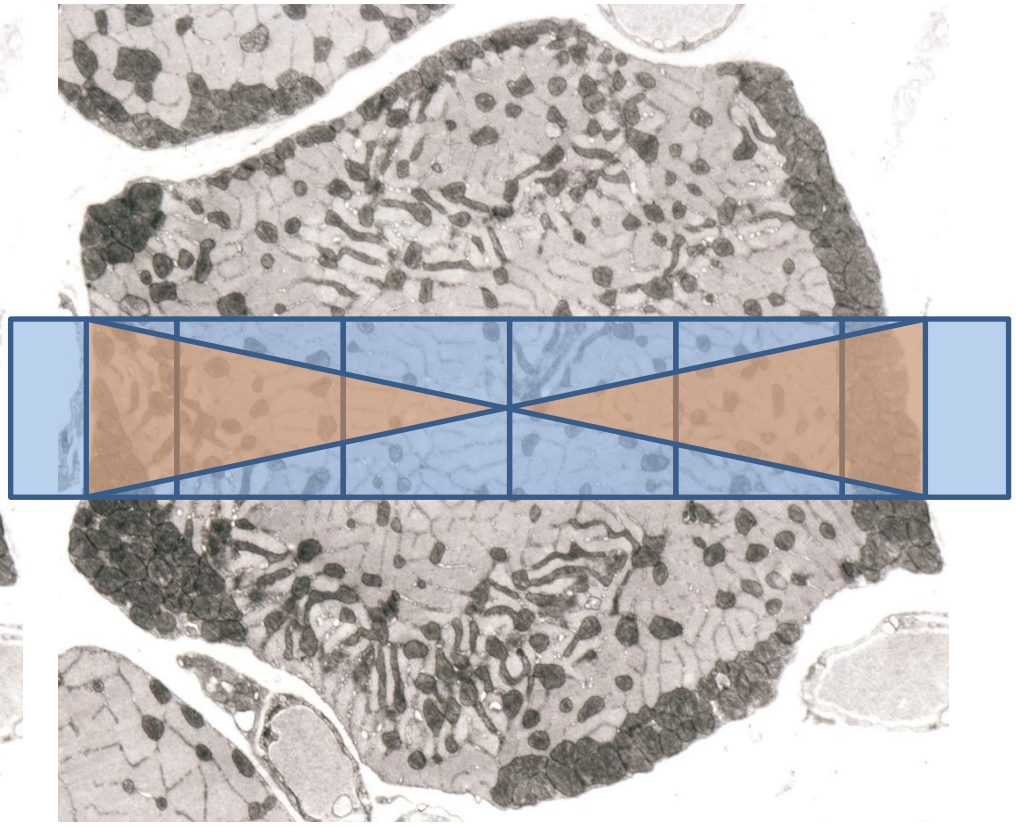
Skeletal Muscle – Random Sampling Micrographs at x18,000



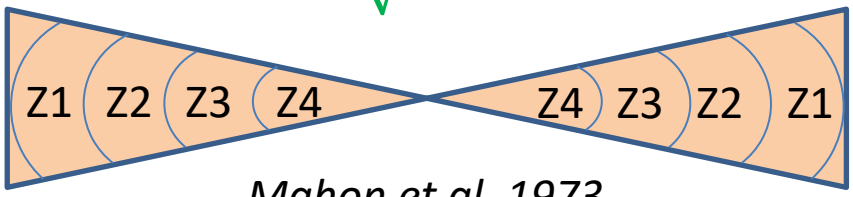
X

Sampling: $\leq 1, RSE$ (, SQRT (1−Vv)/SQRT n) or $Prog.Mean$

Skeletal Muscle – Zonal Oblique Sector Analysis Method (ZOSAM)



√



Mahon et al, 1973

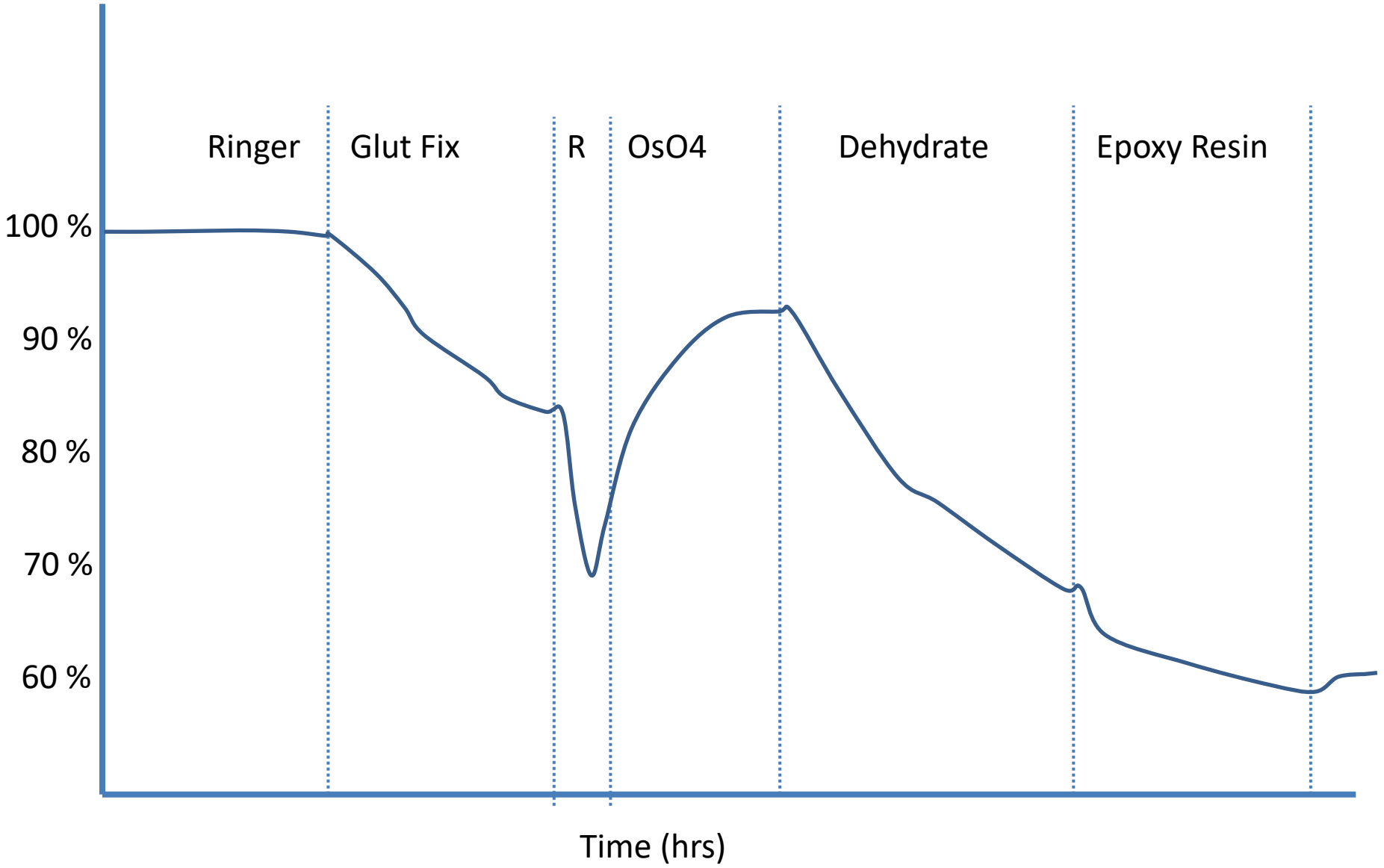
Tissue Preparation Effects

- Fixation Effects (Sissons, Goldspink, Strickland 1960s, 1970s)
  - Flemmings - 3-15%
  - Carnoys -19-36%
  - Bouins -23%
  - Formalin -23-30%
  - Zenker -30-40%

- Length change 10%
- Areal change 21%
- Volume change 33%

- Need a Standard ….. Ringers, Frozen, …

Tissue preparation steps for transmission electron microscopy

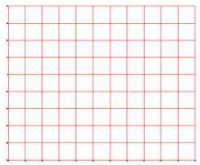


How ?

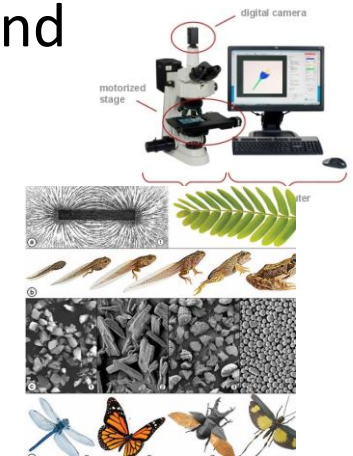
Which Method ?

- **Morphometry**
  - **direct measurement of structures**

- Stereology
  - extrapolation from 2D to 3D using simple counting methods



- Image Analysis
  - combination of above using digital imaging and computers in manual or automatic modes
  - plus data presentation and analysis

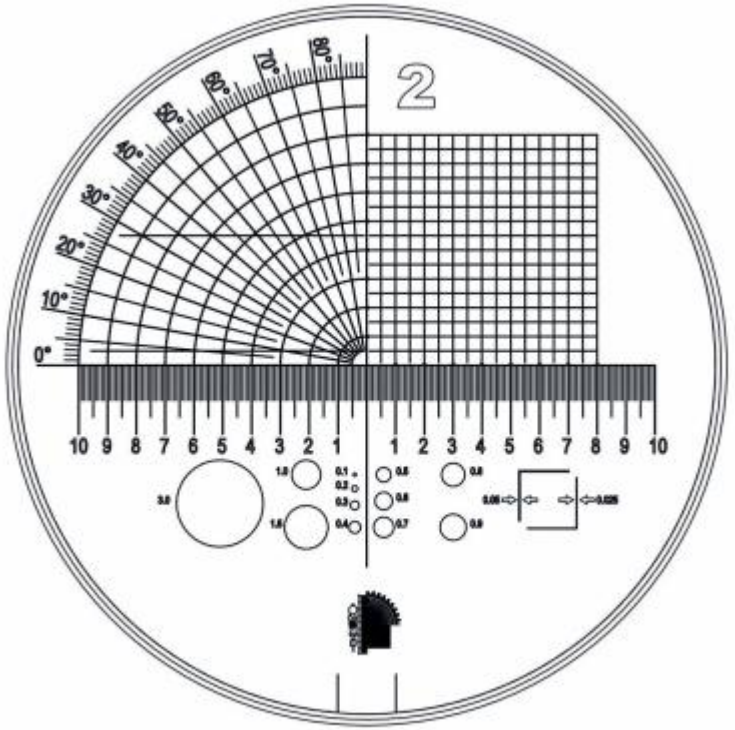


See Russ, 2019 Robust Measurements

If object is large enough ..

Measuring Magnifier (Loupe)

+ Reticle / Graticule



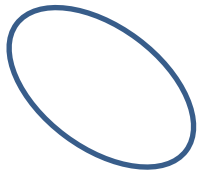
Microscopy Practical

1. **Magnification Calibration**
2. **Morphometry**
3. Stereology
4. (Pattern/Shape analysis)

How ?

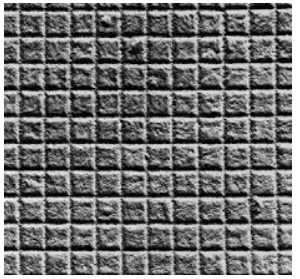
Morphometry

Measurement of Form

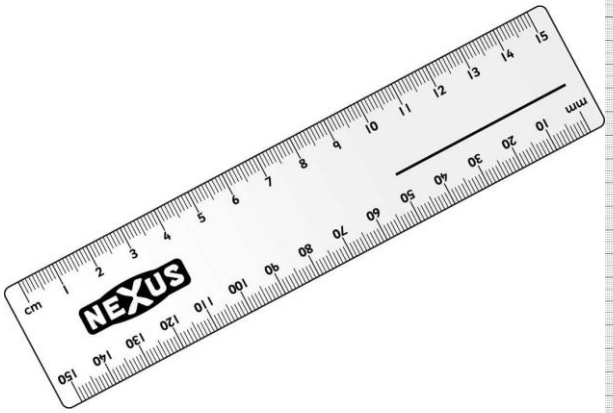


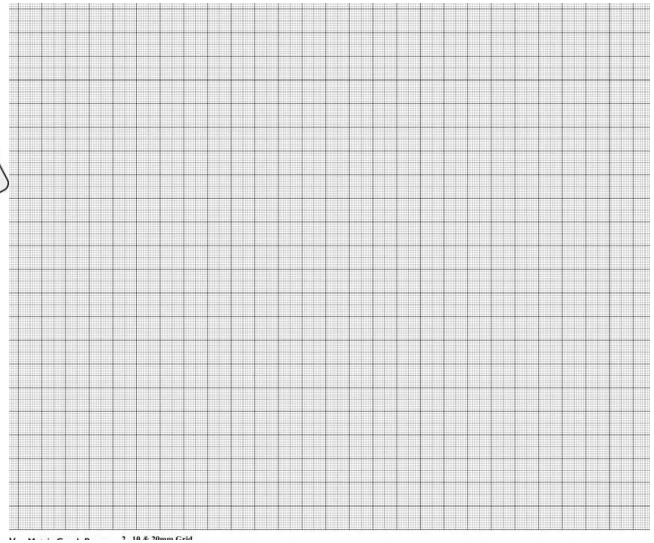
- Diameters & Areas
  - Longest, shortest/narrow, Orthogonal, Feret, Average, Random chord
  - From area (πr^2), Circle of best fit
- Shape
  - Axial ratio, S:V ratios or Form factors(A/P^2, $1=4\ \pi A/P^2$), Angularity (Gulfs & Peaks)
  - Shape of best fit (Identikit), Reconstruction
  - Fourier Analysis, Fractals (Mandelbrot)
- Volumes, Lengths ⚠ , Surfaces ⚠, Numbers ⚠ ⚠ ⚠, Thickness

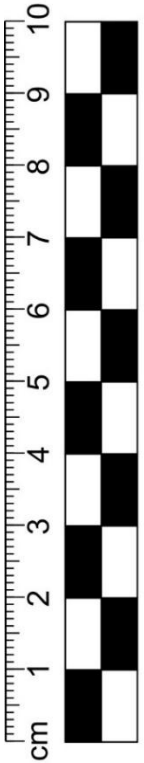
- **Equipment & Magnification Calibration**
  - Rulers, Calipers, Graticules, Stage Micrometer, Cut & Weigh
  - EM: Diffraction Grating, Latex Spheres, Crystals
  - Filar micrometer, Image Shearing micrometer
  - Photographs, Drawings, Projection (Camera Lucida/Drawing Tube)
  - Thread, Map Measurer (Opisometer), Planimeter
  - Stereological lattices
  - Image Analyser

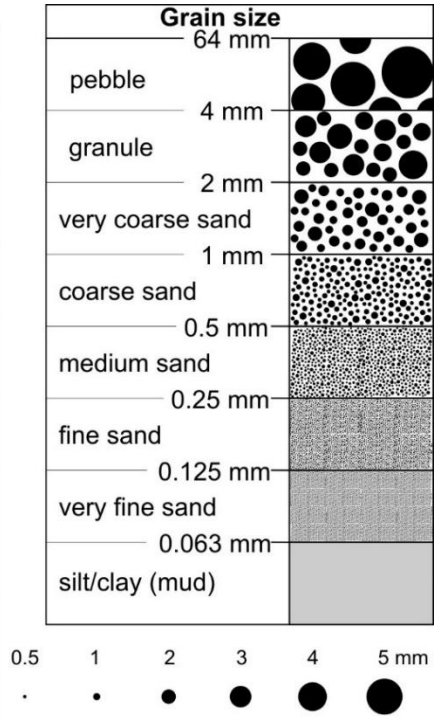


Magnifier or Stereomicroscope

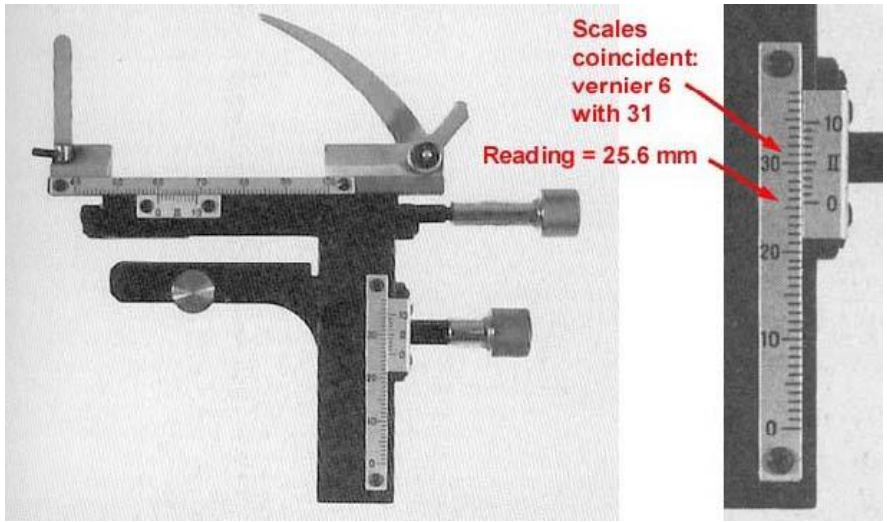


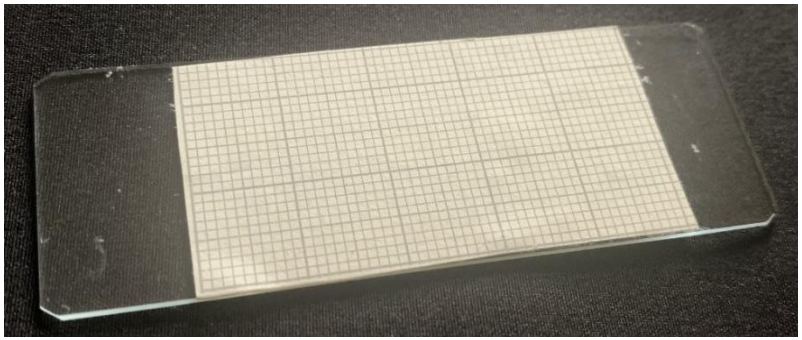






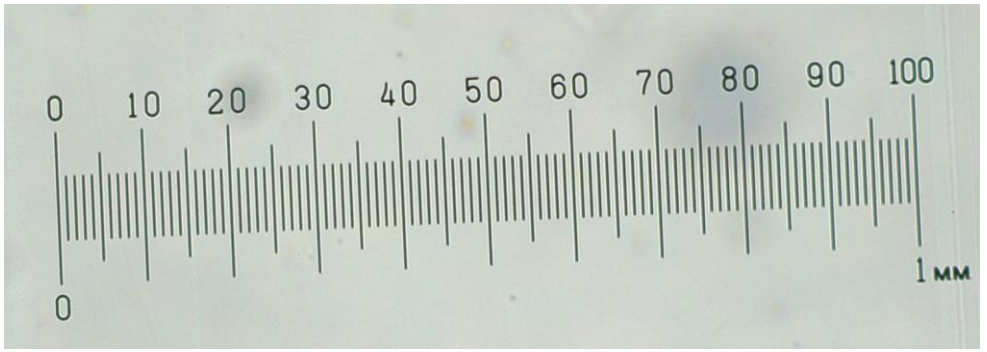
Compound Microscope





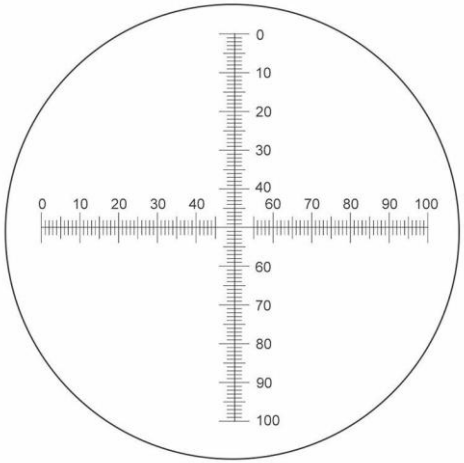
MM scale slide – 1mm squares

Stage Micrometer Slide

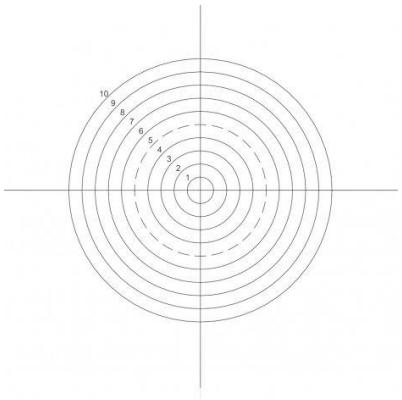


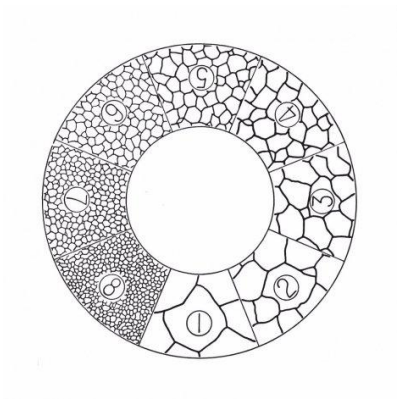
- As a guide / **guestimate**
- Measure width of **field of view** for each objective
- **Magnification / Scale bar**
- **Transfer** scale to **Eyepiece Graticule**

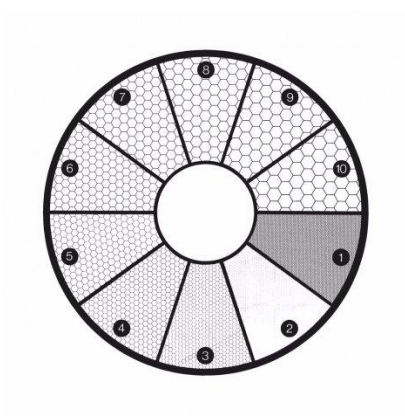
Eyepiece Graticule Scale

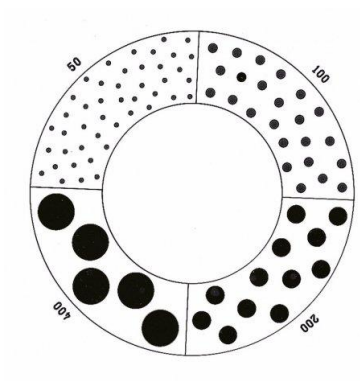


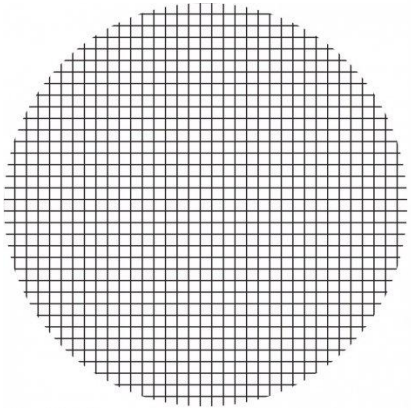
Graticules Optics Limited

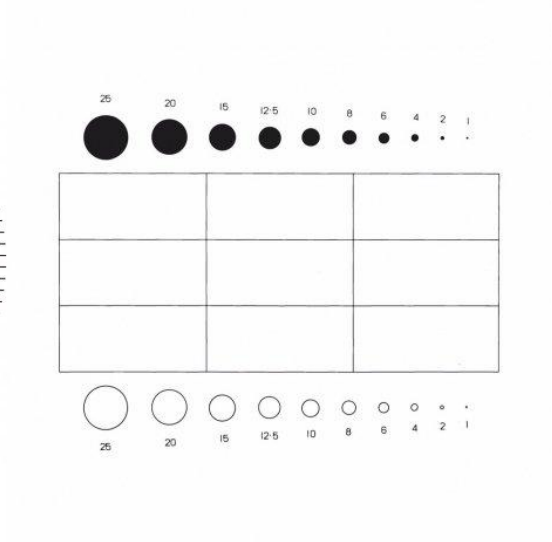


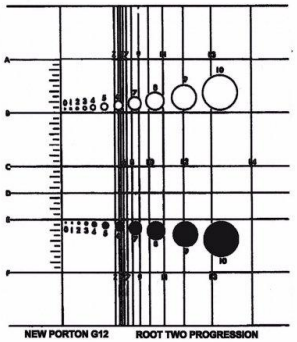


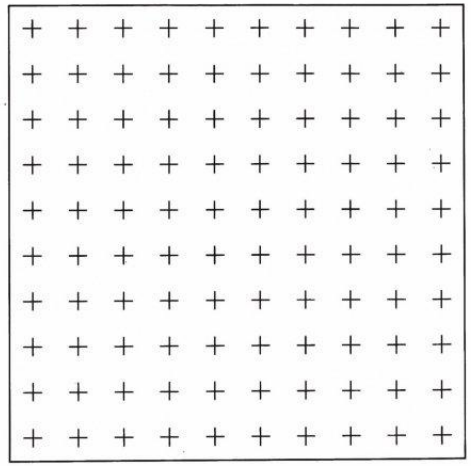








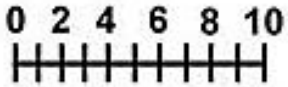




… some of the Eyepiece Graticules made by Graticules Optics Limited

How to calibrate and Eyepiece Graticule against a Stage Micrometer

3(a)

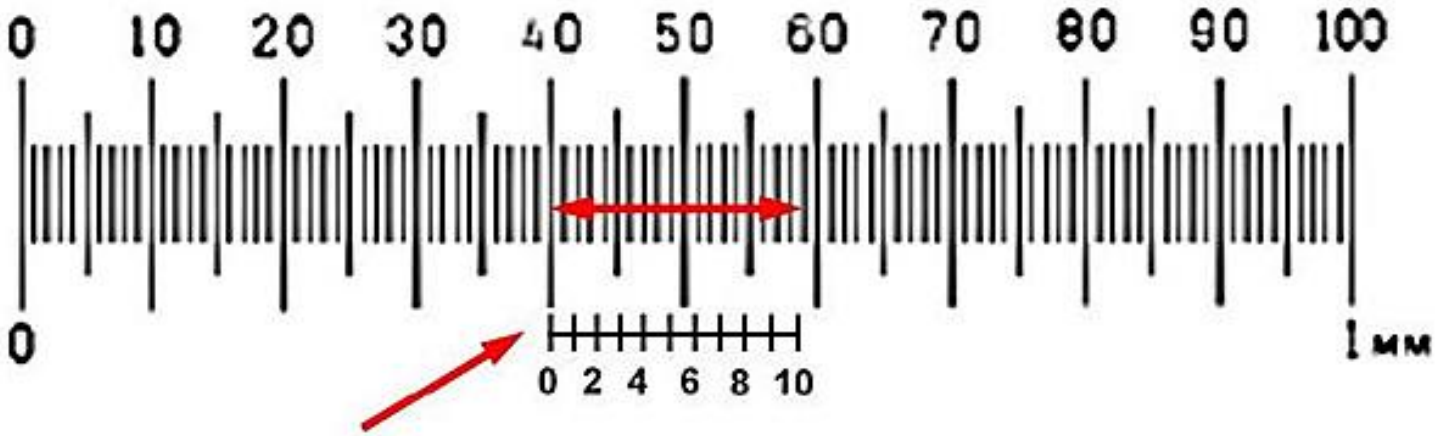


3(b)

3(c)

3(d)

1



Calibration of arbitrary scale: 10 units = 190 μm, at this magnification

Each arbitrary unit = 19μm

2 Then replace Stage Micrometer with your slide and 'measure' objects with Eyepiece scale
e.g. 2 units = 38 um

Transfer Scale …

+ Take a Photo !

Filar Micrometer Eyepiece

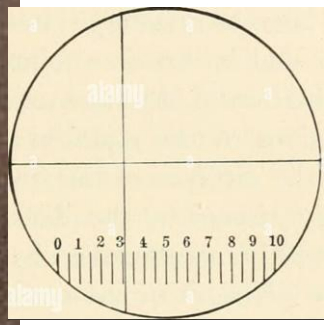


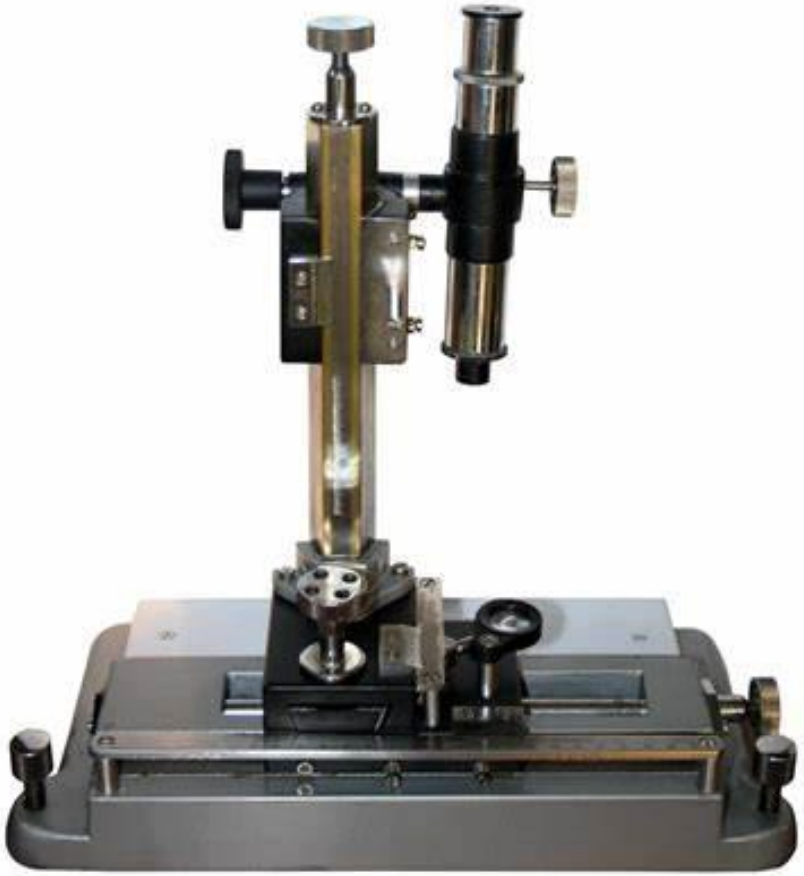
Image Splitting Eyepiece (Watson)

Image Shearing Eyepiece (Vickers)

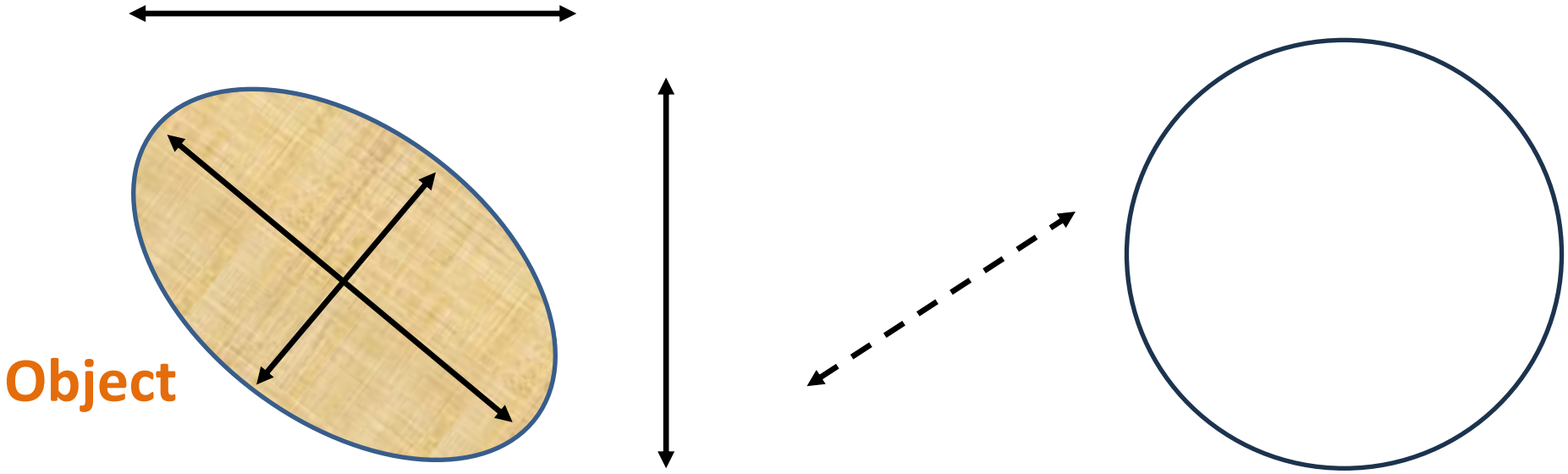
… very precise

Measuring Larger Items precisely …

Travelling Microscope



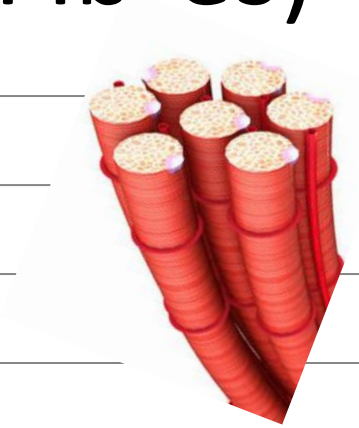
Diameter: Some Problems to consider ….

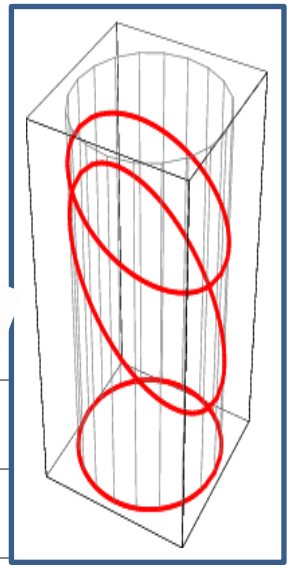


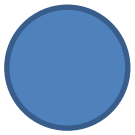
- Narrow diameter
- Maximum diameter
- Average diameter
- Orthoganal diameter
- Feret (Caliper) diameter
- Random chord diameter
- Diameter of Circle of best fit
- Diameter calculated from measured area using πr^2

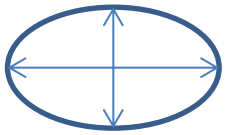
Diameters of Cylinder Profiles

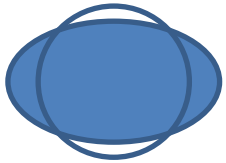
Diameter (Muscle Fibres)





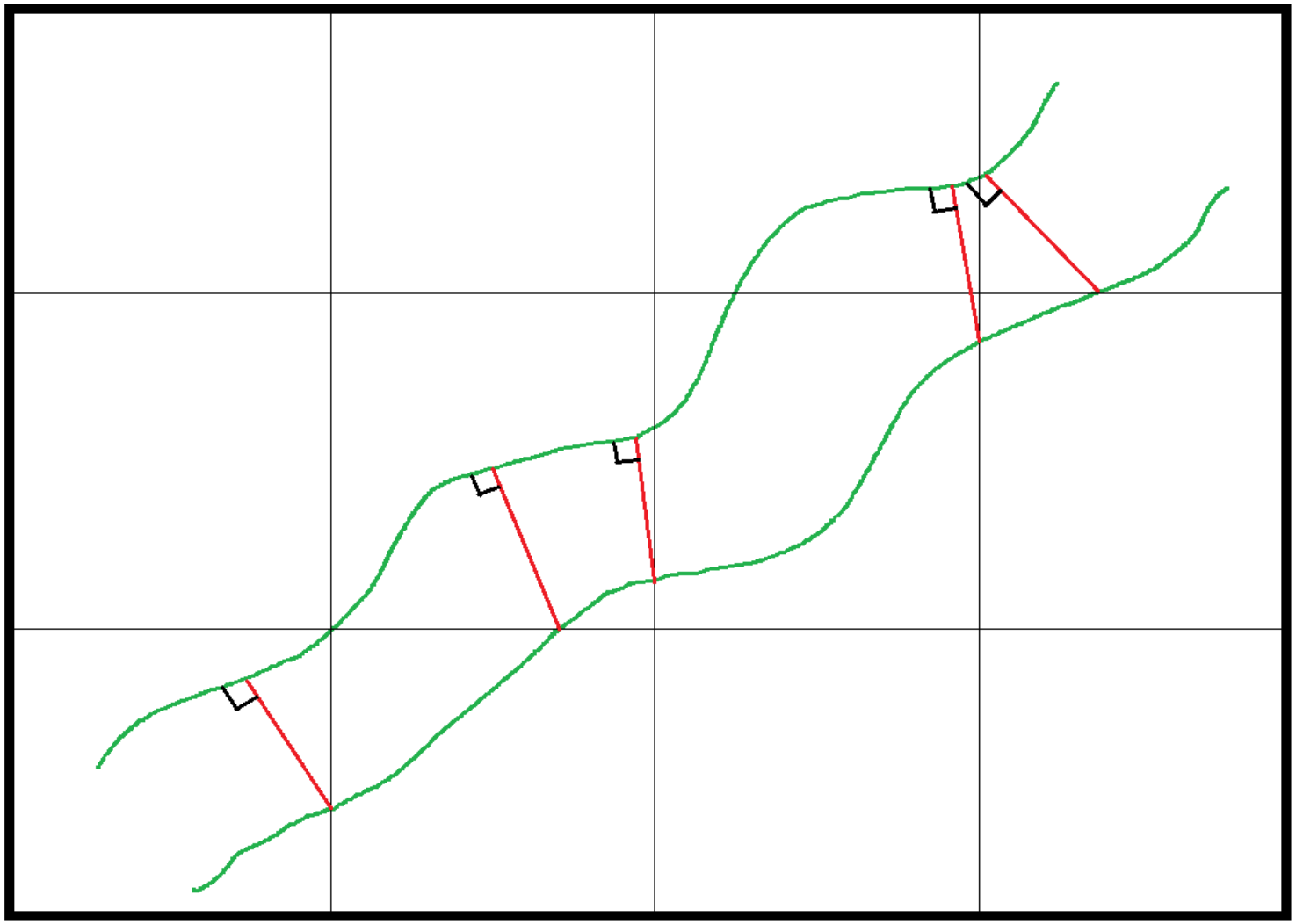






Some problems …

Thickness Measurements

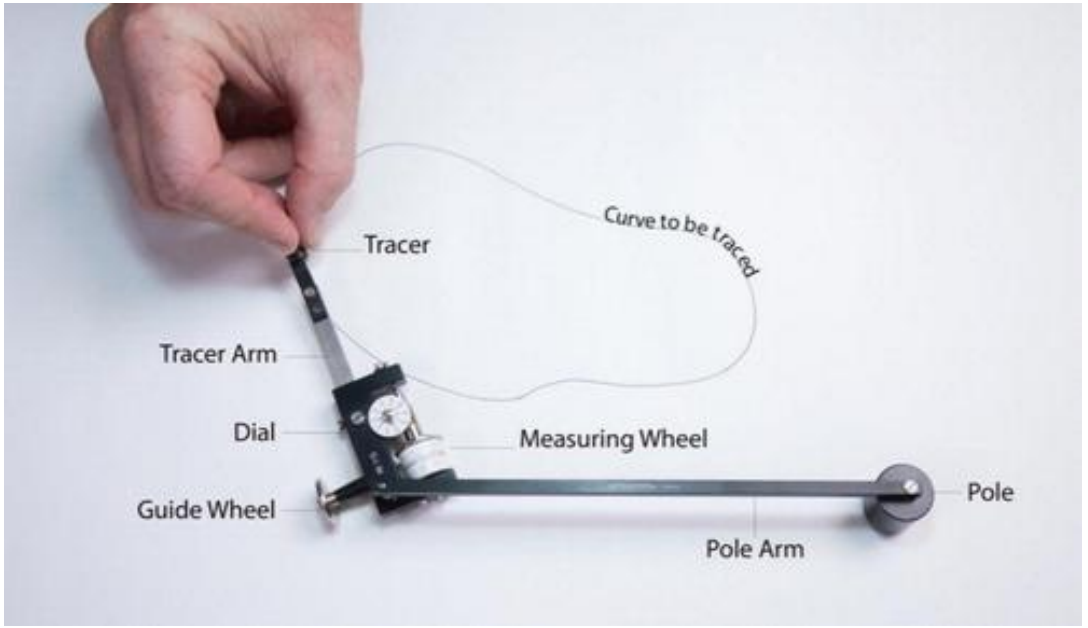


Map Measurer (Opisometer)
for measuring <u>Lengths</u>

… or use a cotton thread !

On projected images, drawings, photographs

Planimeter
For measuring <u>Areas</u>



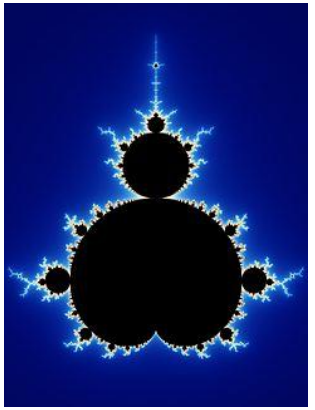
Length

- Dependent on Magnification
- How long is the coastline of Britain? (Mandelbrot (1967) Science)

U 200 km
L 2400 km

U 50 km
L 3400 km

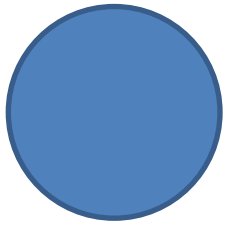
U ?? km
L 12450 km

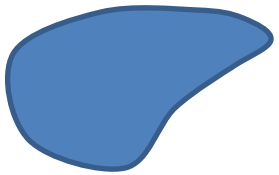


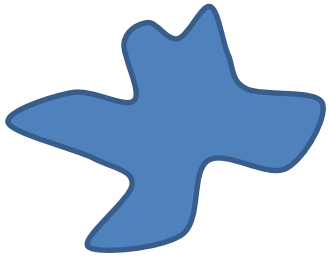
[See also Russ et al 2018 The Problem of Perimeter]

Fractal Dimension of Wigglinesss 1.25 !

Shapes

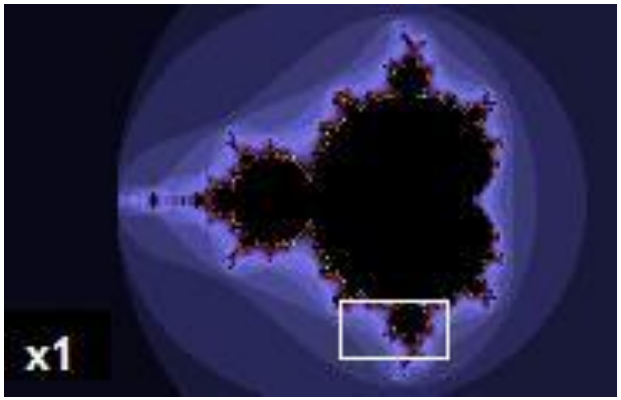


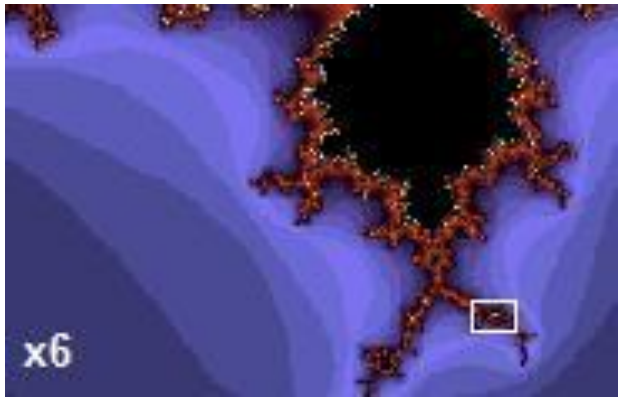


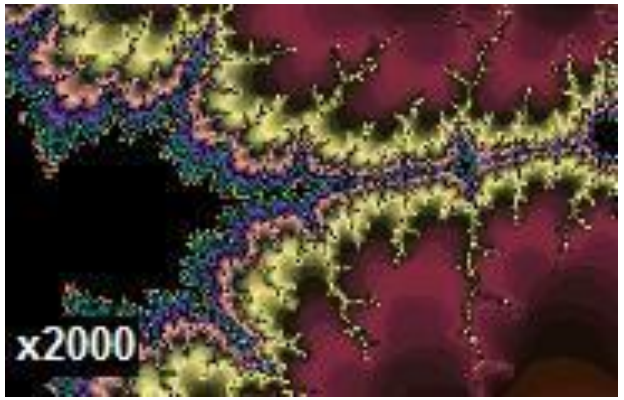


Form Factor	1.0	0.8	0.4
Gulfs	0	1	5

$F=(4 \times \pi \times \text{Area})/\text{Perimeter}^2$



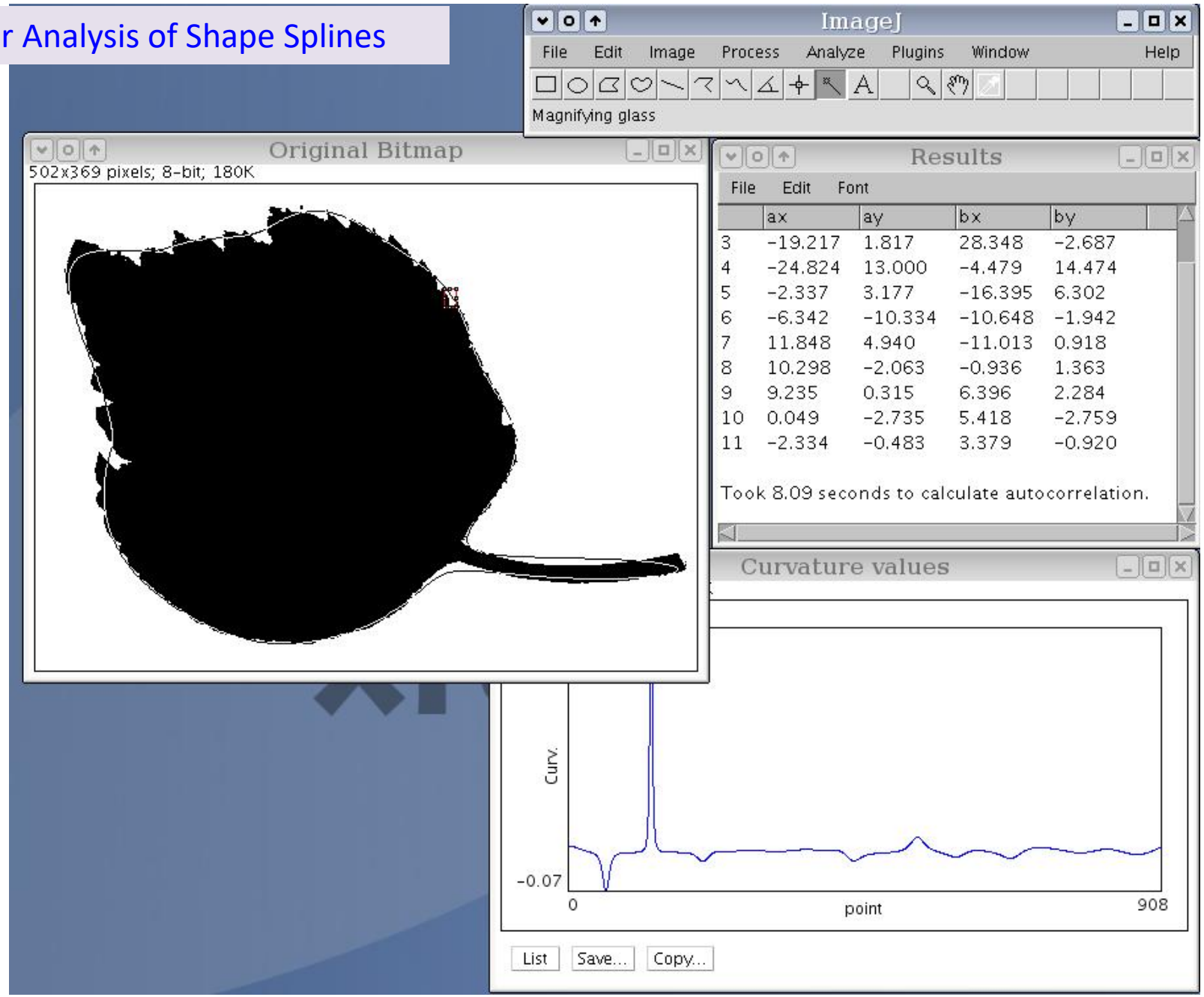


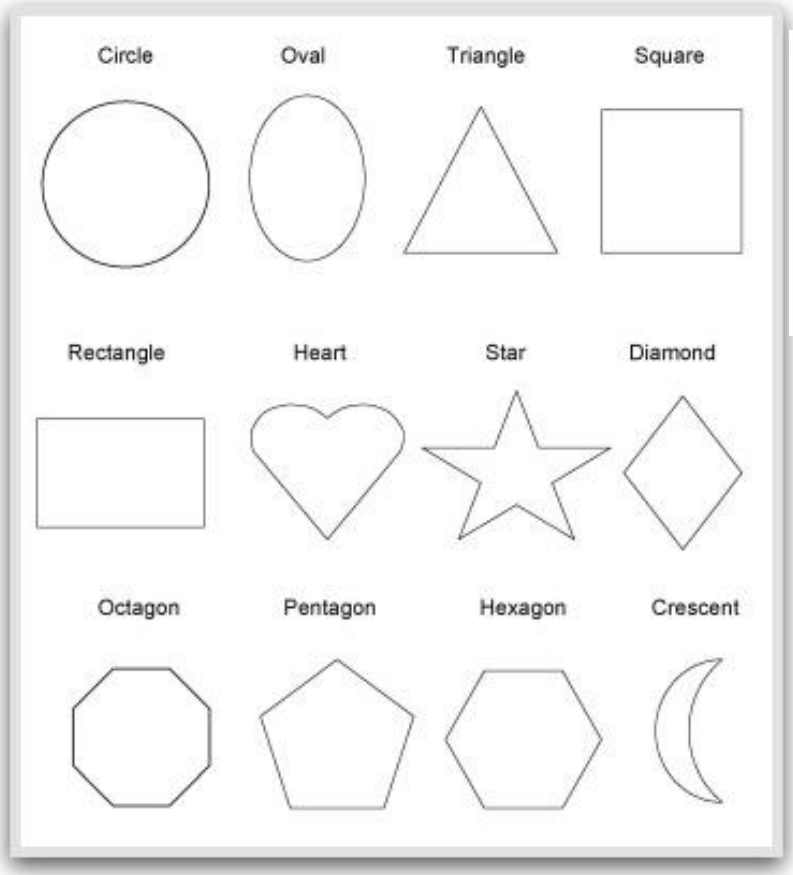


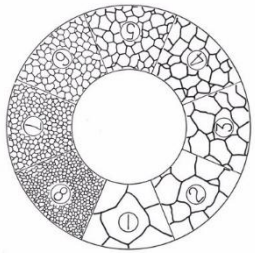
Fractals – Mandelbrot Set

See Mandelbrot, 1982 The Fractal Geometry of Nature

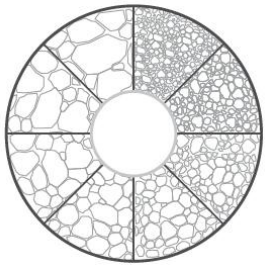
Fourier Analysis of Shape Splines

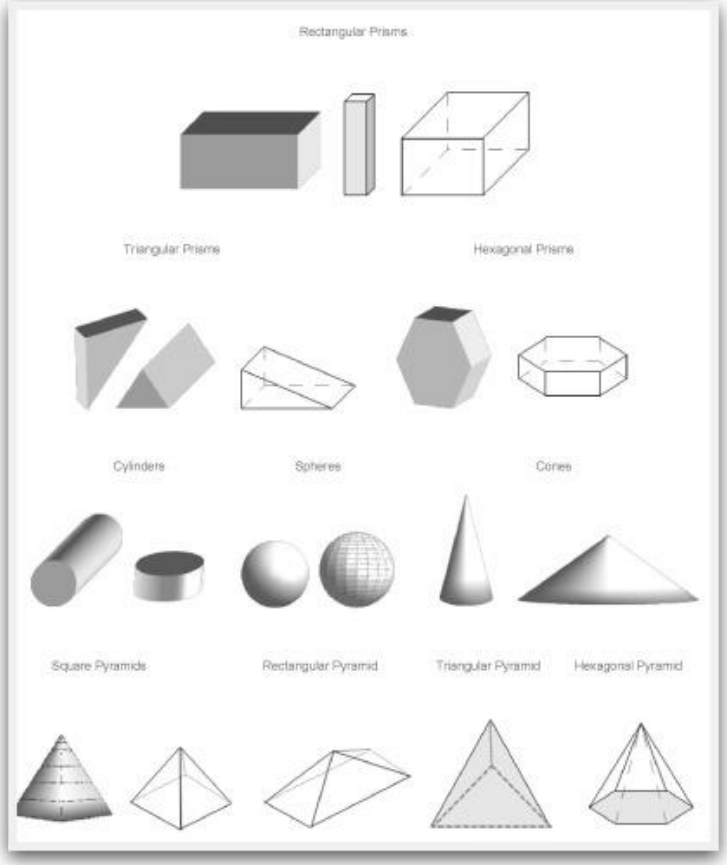




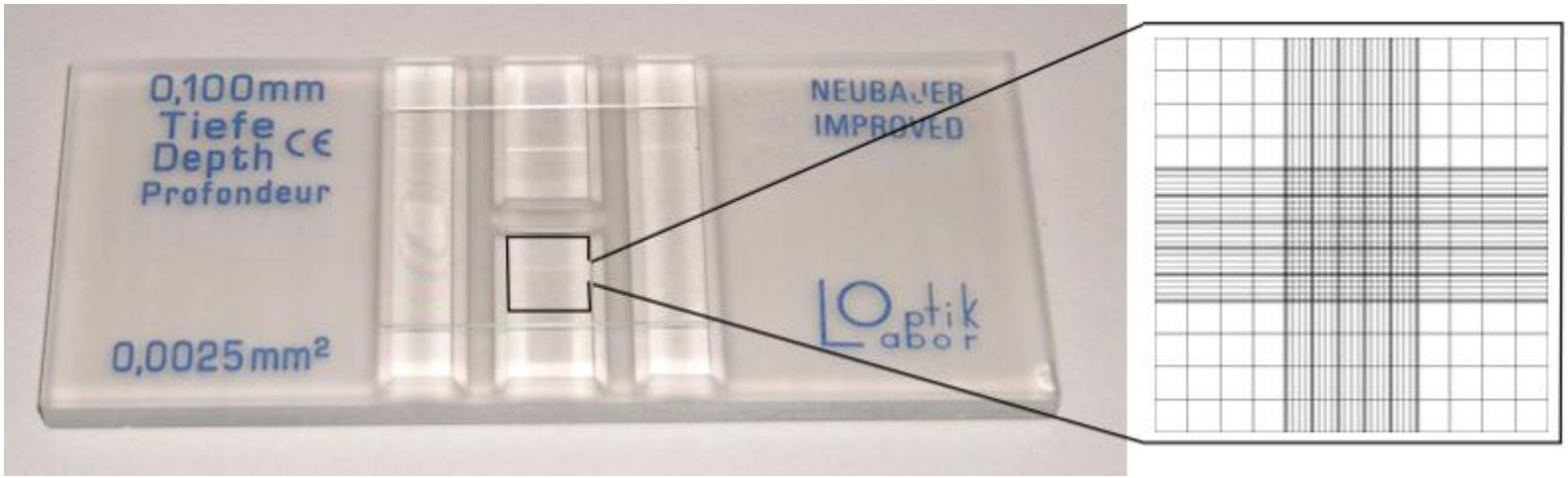


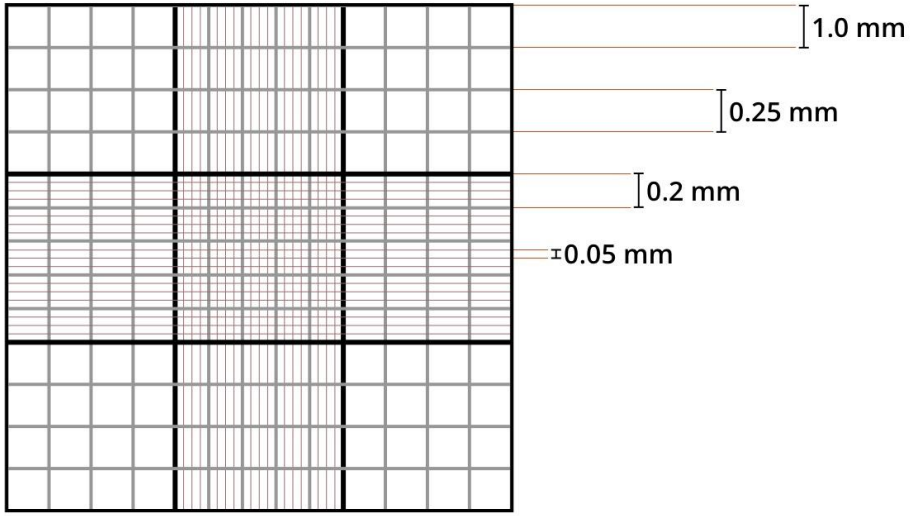
Identikit Match





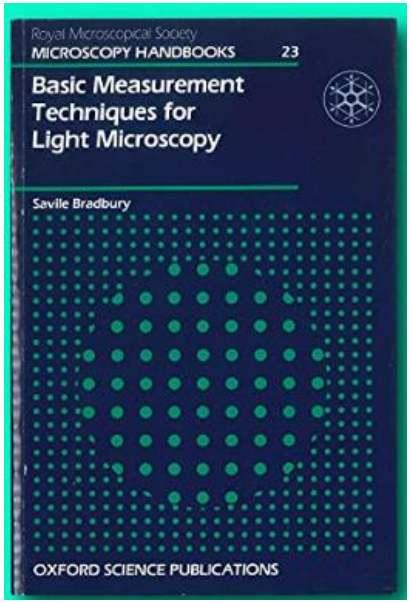
Numbers: Haematocytometer Slide





Number of cells in a $1mm^2$ square x 10^4 = No. cells/ml.

Reading: **Bradbury S 1991 Basic Measurement Techniques for Light Microscopy RMS #23**



Early History of Measurement in Microscopy

- 1665 Hooke - comparisons, scale bars on drawings, counts
- 1673 Leeuwenhoek - compared 'Little animalcules' v sand grains, rbcs
- 1679 " - made a graticule of 600 hairs per inch
- 1716 Hertel – stage micrometer
- 1718 Jurin – hair micrometer
- 1753 Baker – calibrated lattice wire micrometers
- 1771 Adams – screw micrometer
- …….
- 1904 Wright - eikenometer
- 1936 Patterson & Cawood – particle size eyepiece graticules

- Travelling Microscope
- Haemocytometer slides

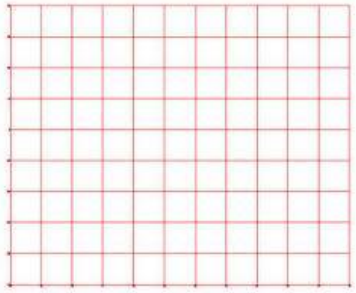
- 1960 Barer /Dyson – image shearing/splitting micrometer
- ……
- **Counts & Proportions** *(Geology, Metallurgy, >Biology)*
- 1847 Delesse – areas ≡ volumes
- 1851 Sorby – tin foil cutouts
- 1916 Shand – mechanical automation
- 1939- 1990s– various electronic 'image analysers'

Henry Sorby – FRS, RMS, MMS

How ?

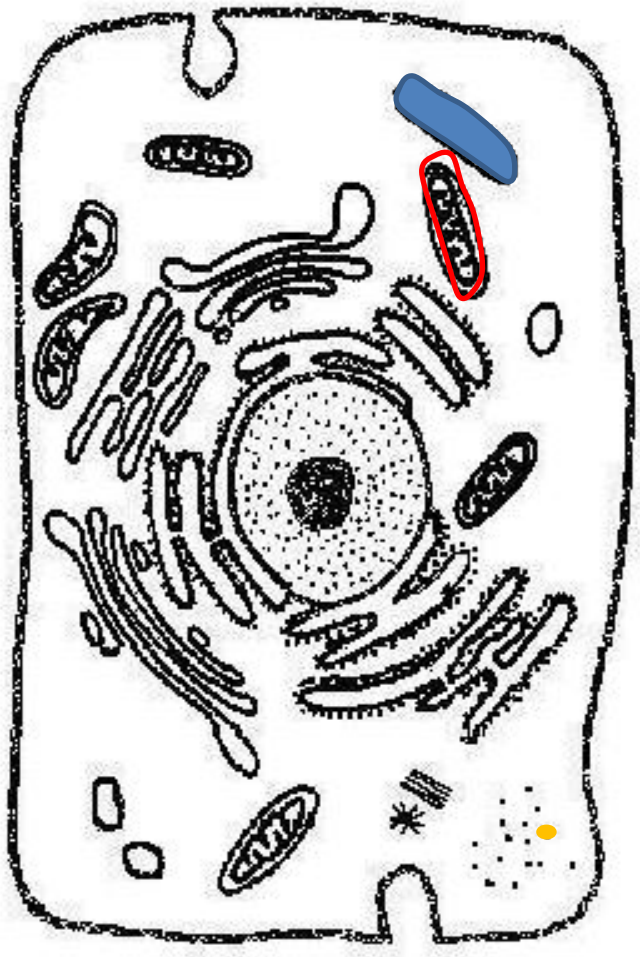
Stereology 1

Extrapolation from 2D to 3D



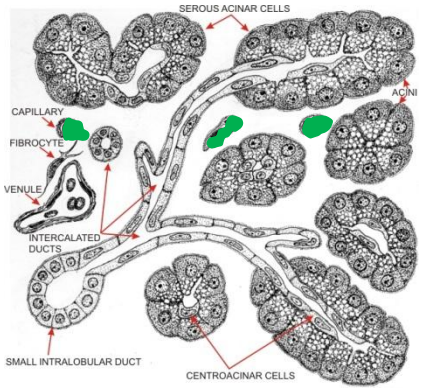
Geology, Metallurgy, Engineering, Astronomy, Biology

- History
  - 1777 Buffon
  - 1850s Delesse, Sorby
  - 1961 ISS, Models; Elias, Weibel, Williams, Mayhew, Cruz-Orive
  - 1983 Unbiased/Designer: Gunderson (Cavalieri, 1635)
- Dimensional Reduction
  - Volumes, Surface Areas, Lengths, Numbers
  - Volume=3D, Area=2D, Length=1D, Point=0D
  - Vv, Sv, Lv, Pp Point Counting, Line Cuts, Counting
- Equipment
  - Probes (Sections, Lattices, Tally Counters, Image Analysers)
  - Isotropic Probes, Merz Lattices, Cycloids

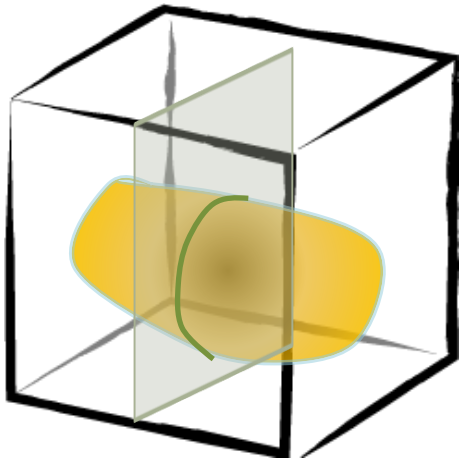


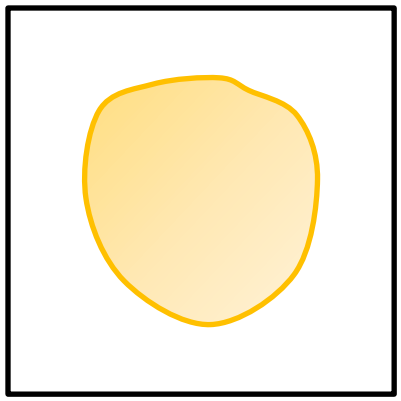
Dimensional Reduction

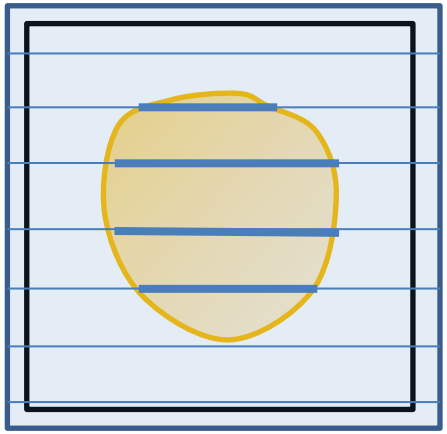
OBJECT		SECTION	
3D VOLUMES	→	AREAS	2D
2D AREAS	→	LENGTHS	1D
1D LENGTHS	→	POINTS	0D
0D POINTS	→	Hit	0D
		Miss	X

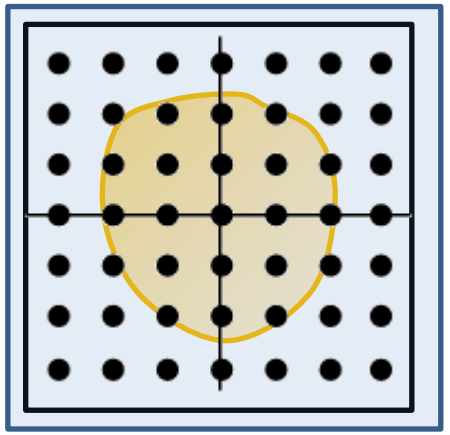


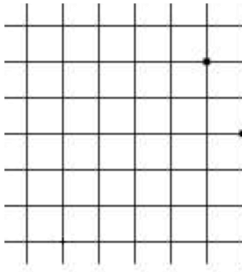
Volume Fraction









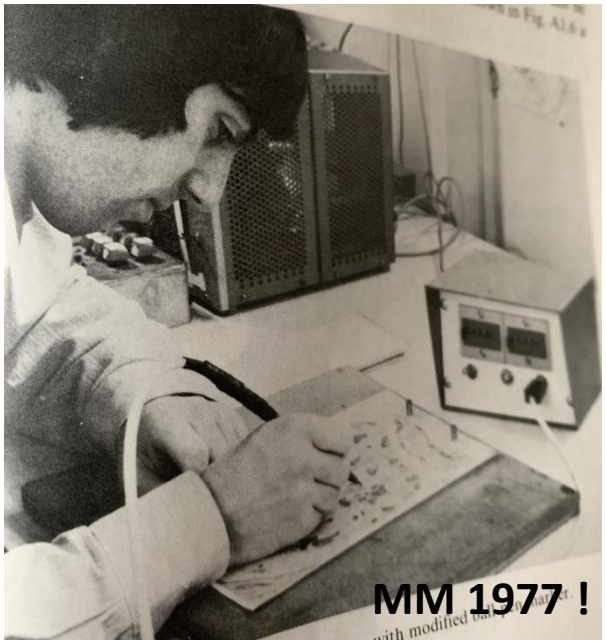


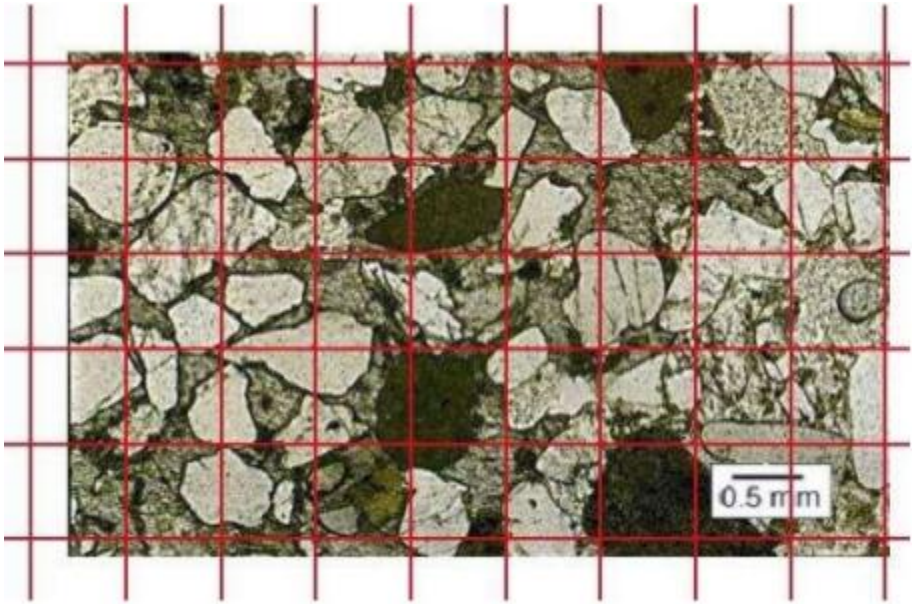
$V_V = A_A = L_L = P_P$

Reconstruct	Planimetry Cut & Weigh Count Squares	Measure Opisometer Computer	Count

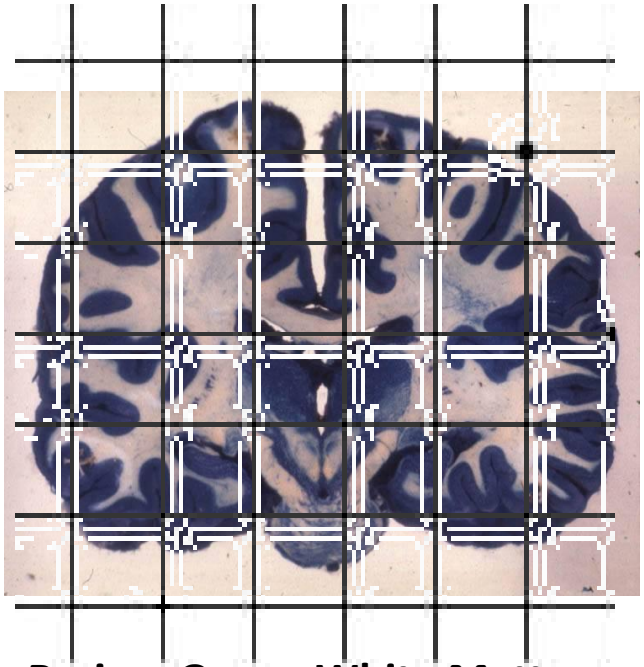
So the relative number of points on object versus background equates to the fractional volume.

Point Counting Lattice Overlay …Cheap!

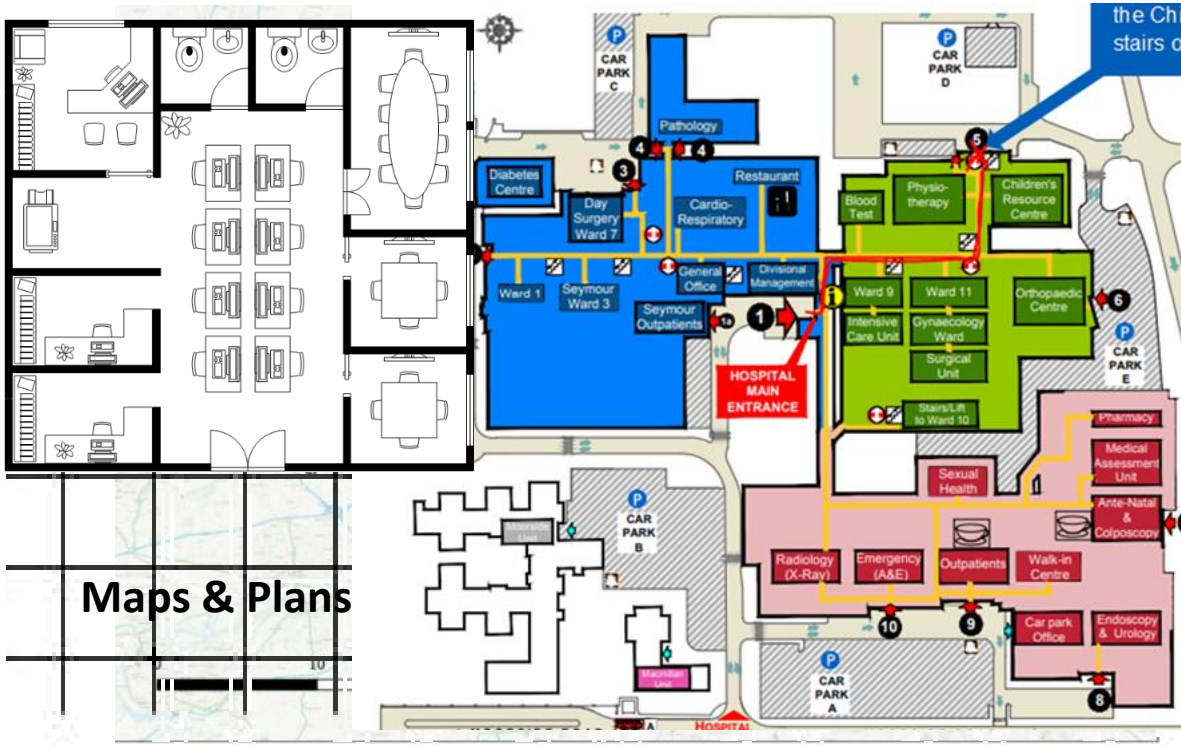




Metallurgy & Geology

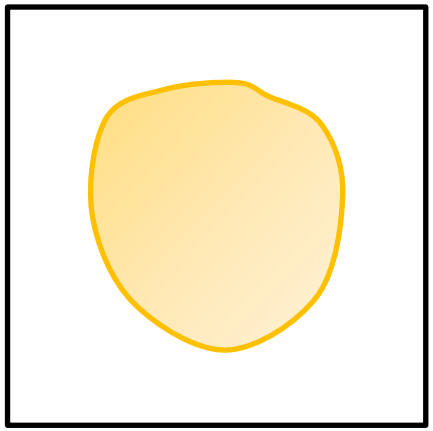


Brain – Grey v White Matter

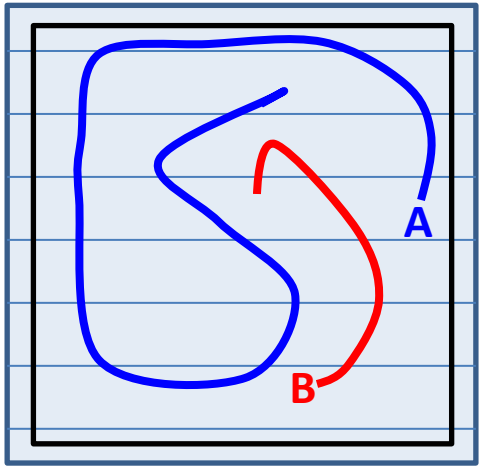


Maps & Plans

Surface Density



$$S_V = L \times 4/\pi$$



A= 14 cuts

B= 5 cuts

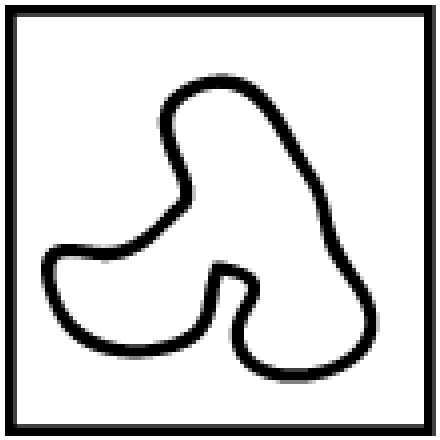
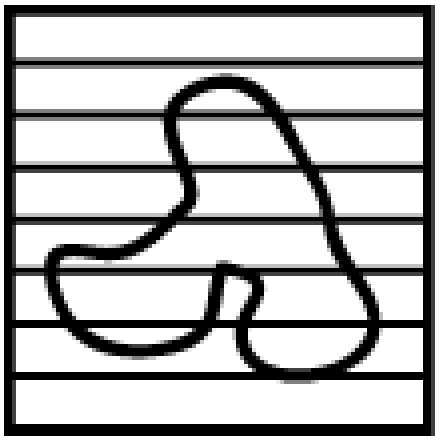
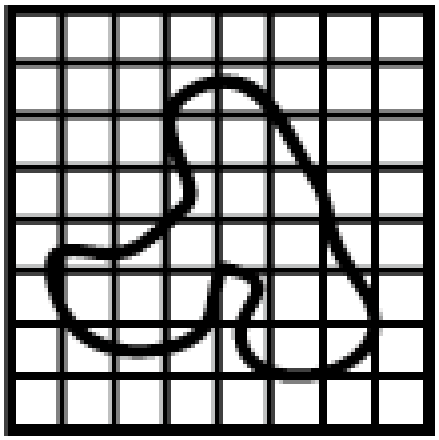
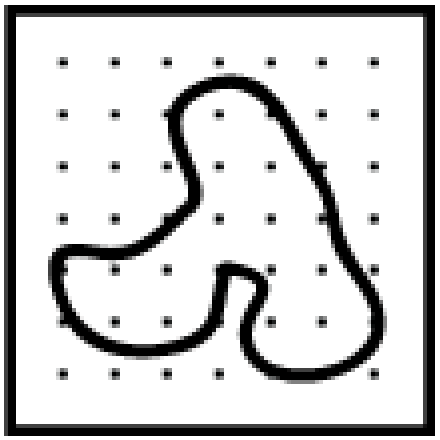
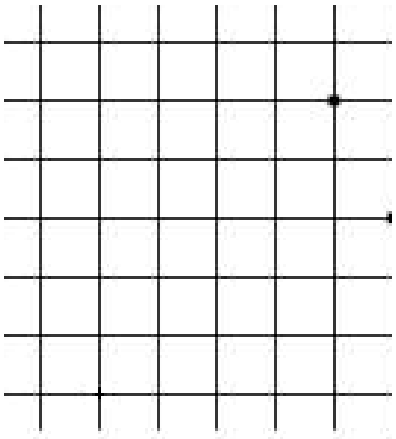
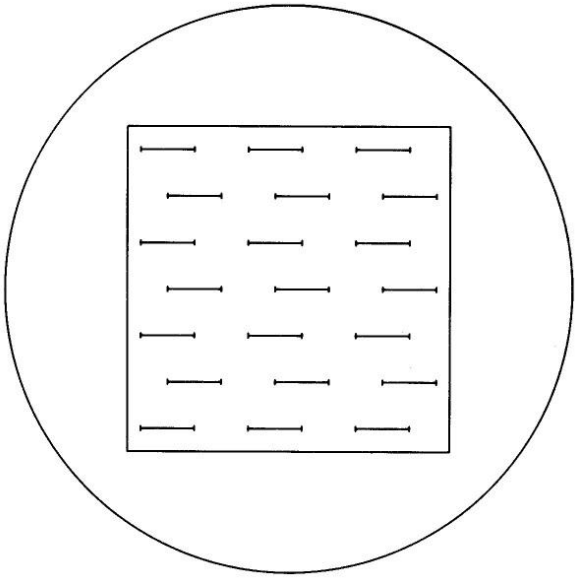
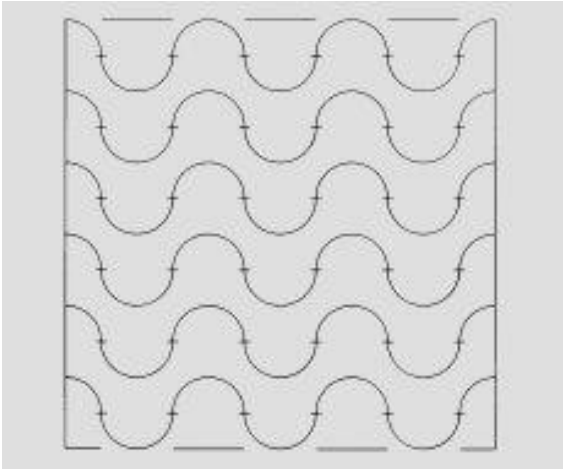
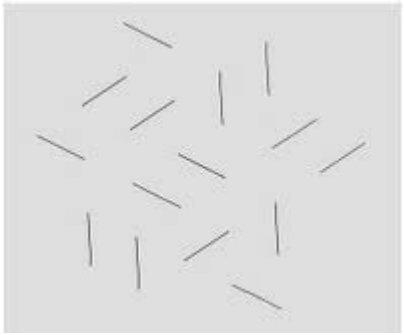
A is about 3 ties longer than B !

$S_V = 2 \times I_L$

$L = \pi / 2 \times I_L$

Line Cut Lattice Overlay …Cheap!

Buffon's Needle (1777)

Stereology Lattices (Probes)

… also available as Eyepiece Graticules

Use Hally formula for number of hits

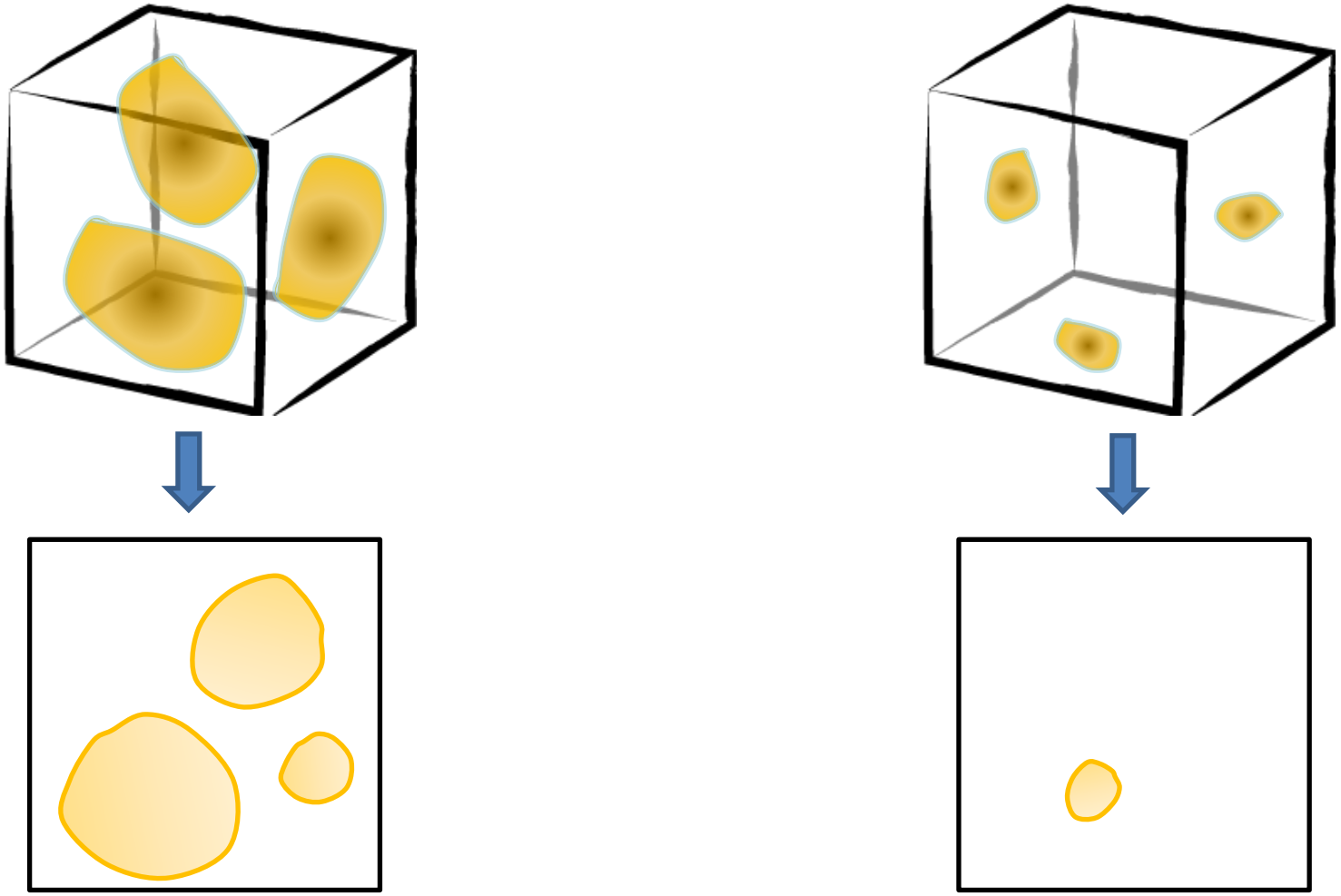
Numbers

… in tissue sections

- N, Nv, N_A
- Counting Objects or Profiles ?
  - Loose cells/smears or sections?
- Depends on size and shape, section thickness
- Reconstruct
- Correction procedures (for Size, Shape, Populations)
  - Abercrombie (1946) $D=dx4/\pi$
  - Schwartz-Saltykov (1958) Unfolding
  - Avoid: use Design Based Stereology

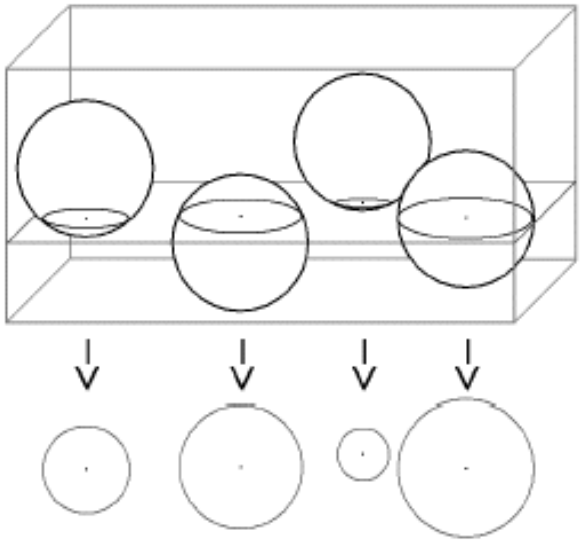
Numerical Density

Although the answer is 3 items .. If they are smaller they are cut less often and apparently seem to be fewer!
So you need to measure their size as well.

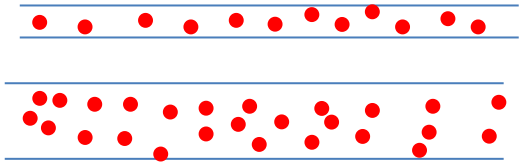


$$N_V = N_A / \bar{D}$$

Size corrections

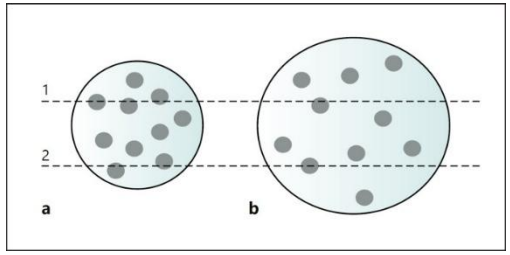


Section Thickness …(Holmes)

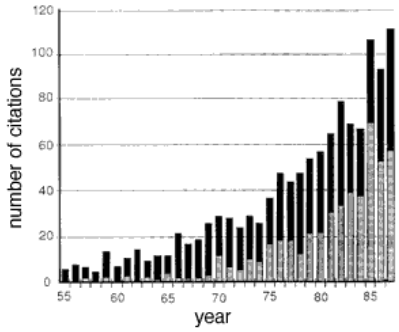


Monodispersed (Same Size)

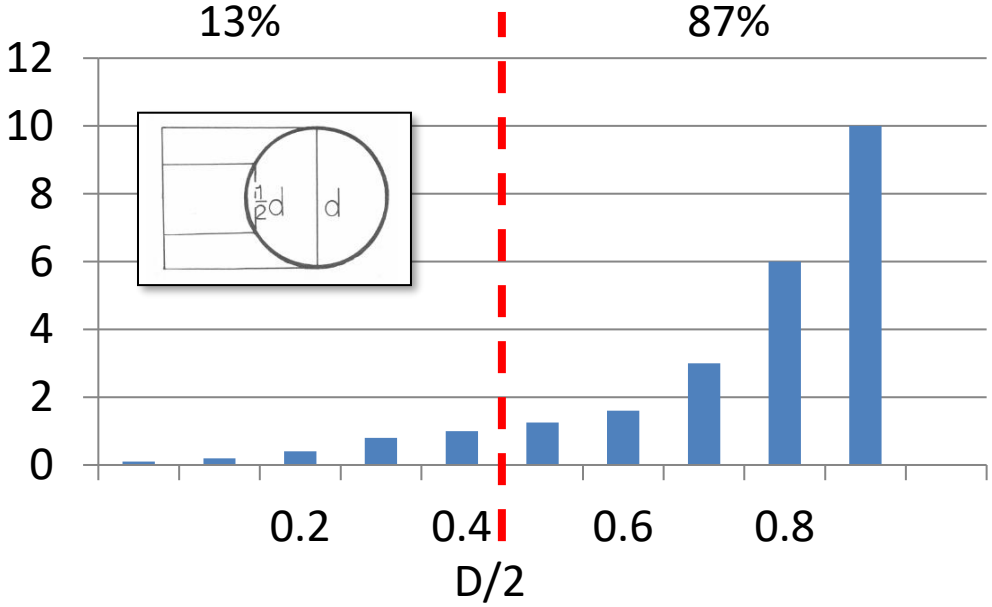
Change in Reference Volume



…aargh!!



Profile Size



Abercrombie (1946) Correction Factor …

$D = d \times 4/\pi$ (ie x1.273)

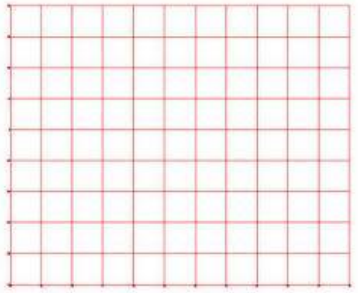
Polydispersed (Different Sizes)

Schwartz / Saltykov Unfolding

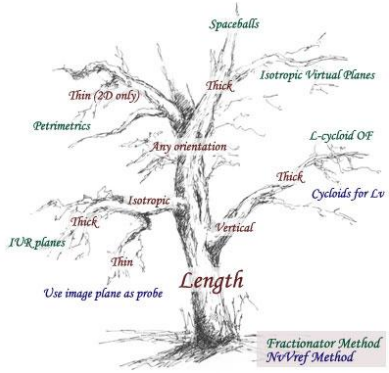
….. **Shape** (Ellipsoids, Cylinders, …) !!

How ?

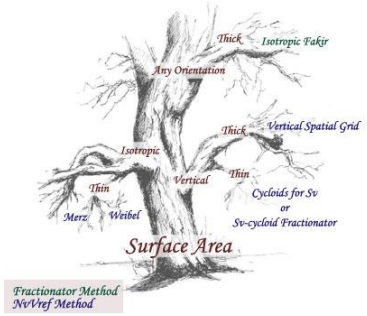
Stereology 2



- New Design Based Stereology
  - Fractionator, Disector, Nucleator, Surfactor, Proportinator, Selector, Rotator, Cycloids
  - Surface Weighted Star Volume
  - Unbiased Brick, Isotropic Fakir
  - Spaceballs, Petrimetrics

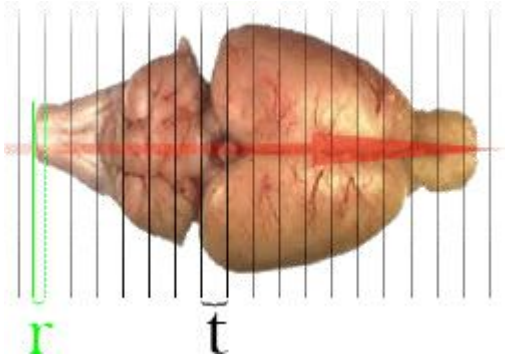


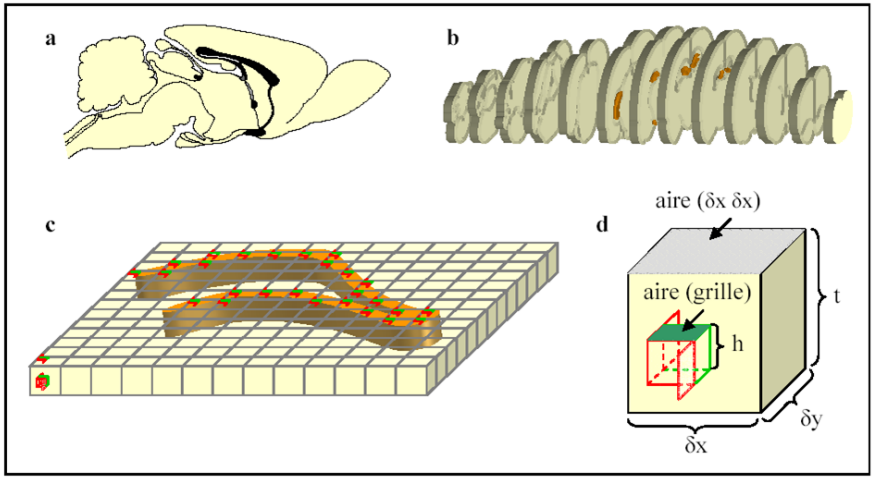
- Equipment / Design
  - Specialised Sampling; IUR, VUR, SRS sections
  - Thick sections, Optical sections
  - Unbiased Counting Frames

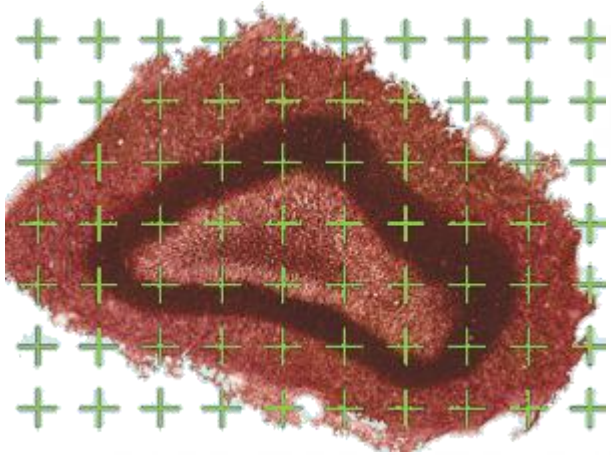


Gunderson, Sterio 1980s *See Howard & Reed, 1998, 2010* www.stereology.info

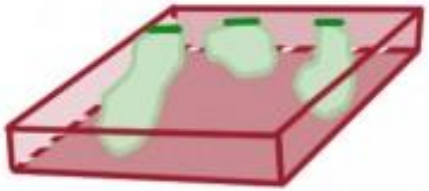
Cavalieri Method (1635)

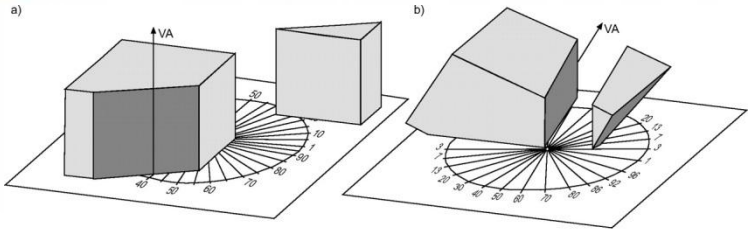


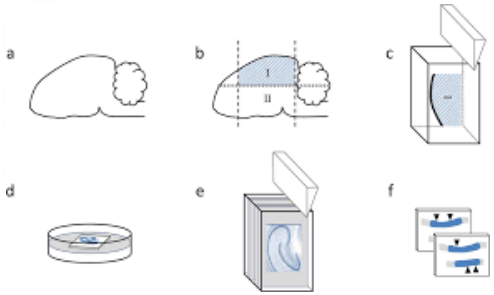


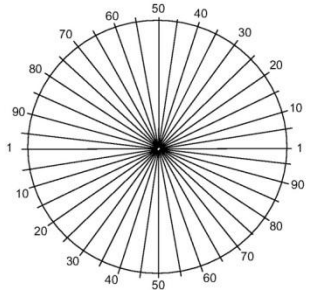


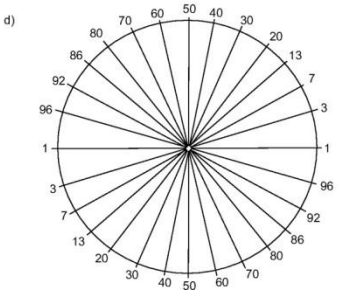
Random start then serial sections, random slices, randomised probes …



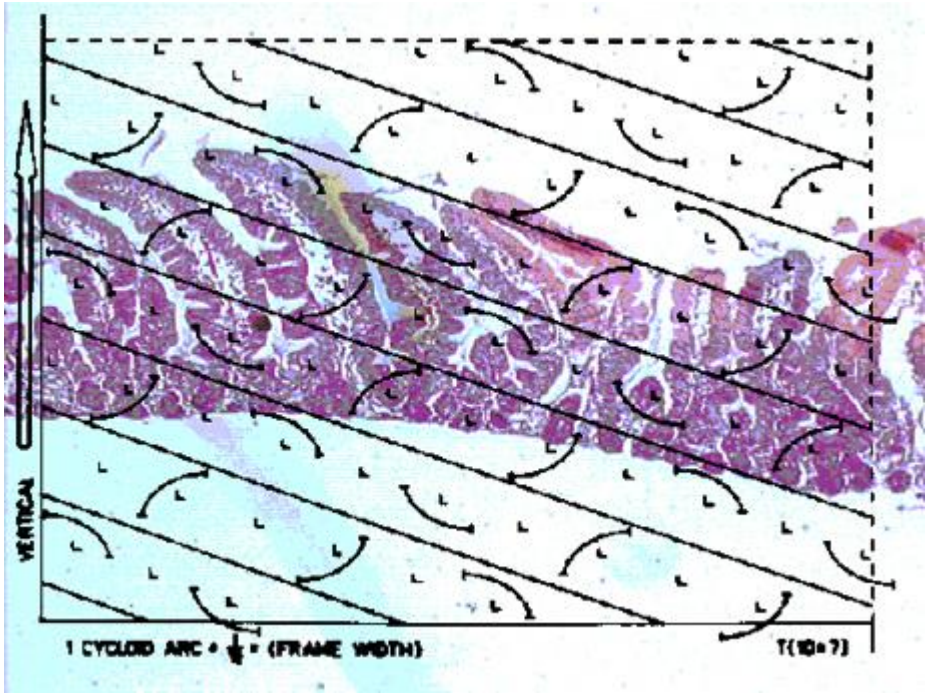


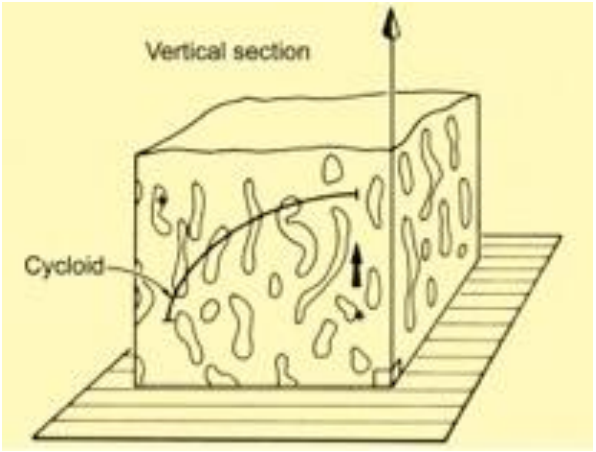


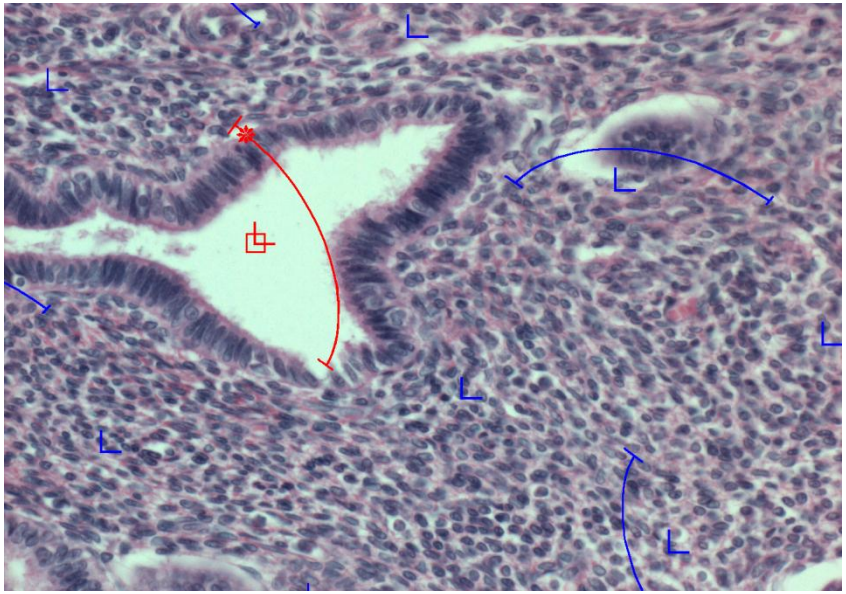


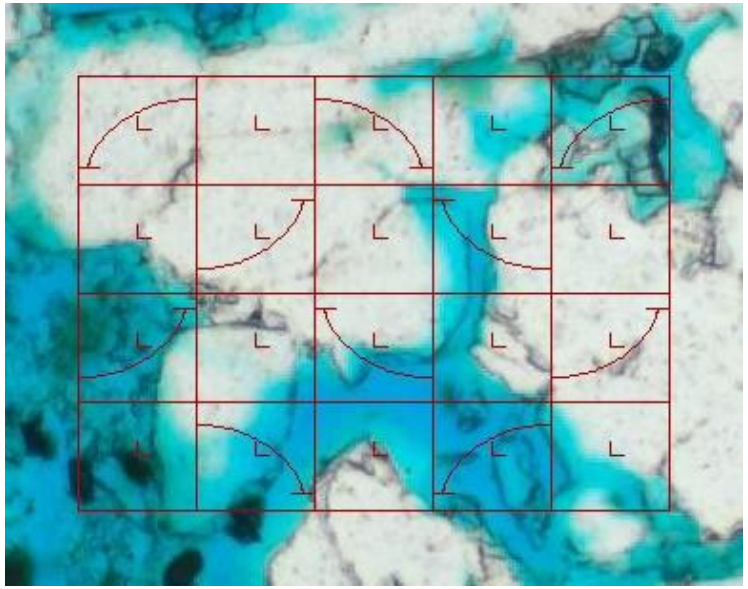


Cycloids for estimating Surface Area in a Volume

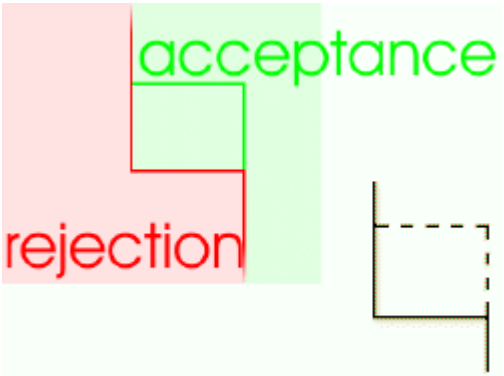




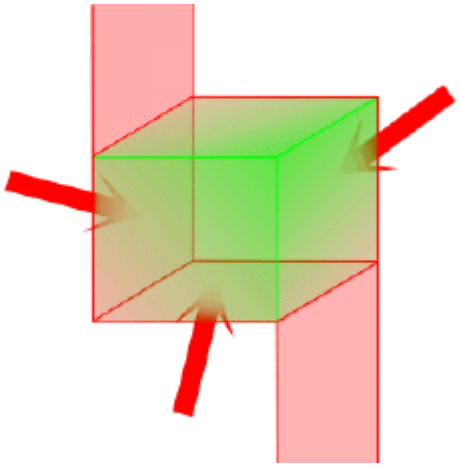




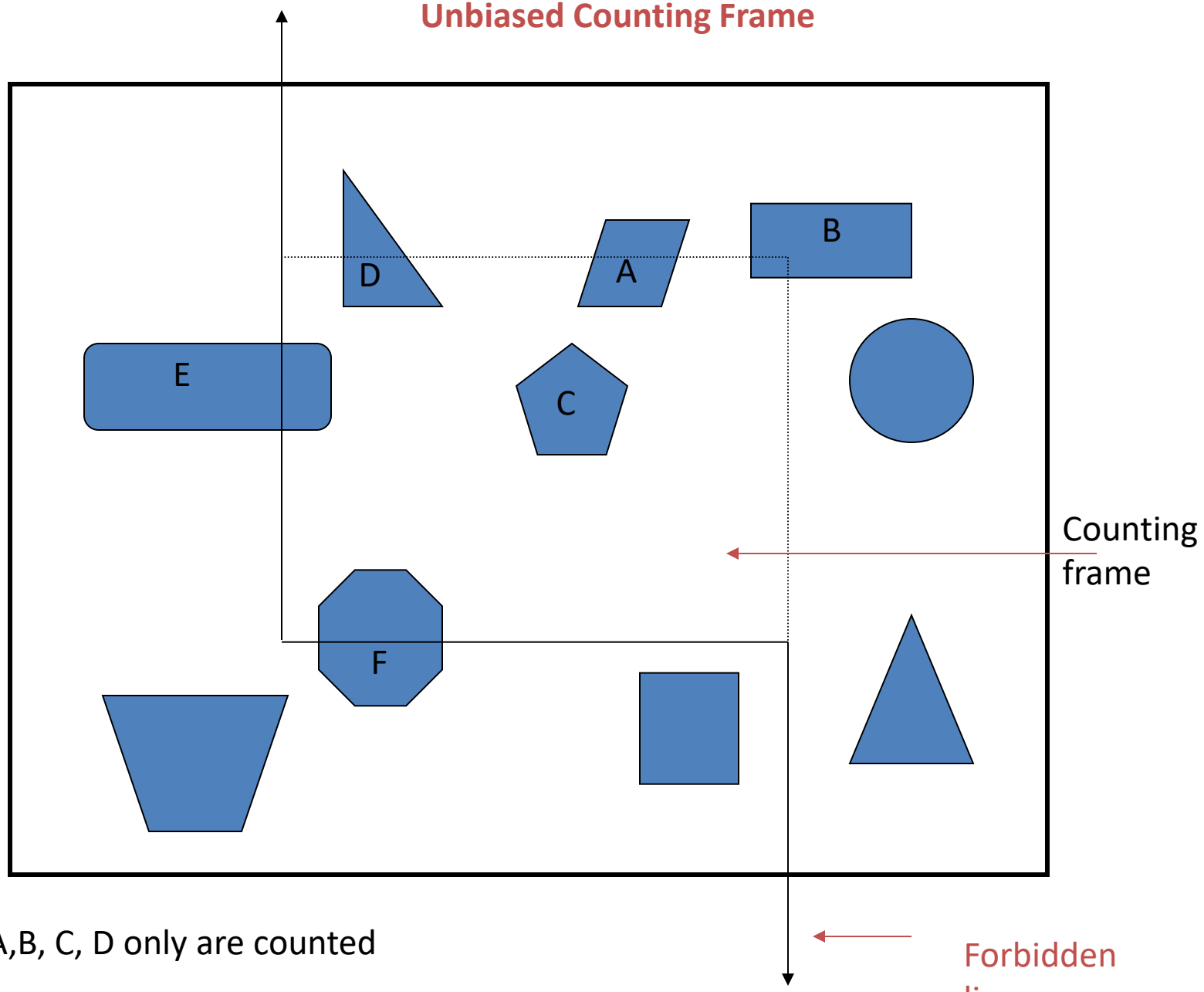
Unbiased Stereology for numbers Gunderson



- Measuring (see Picture 1)
  Since small objects are more likely to fit as complete objects within a measuring field it is best to remove this bias by measuring ALL objects within the frame and ALL objects hitting the dashed (allowed) lines but NOT those hitting the solid (forbidden) lines.
- Counting (see Pictures 1 & 2)
  Objects allowed in the reference section (Picture 1 - A,B,C,D) are then checked in the next (look-up section (Picture 2)). If they DO NOT APPEAR in the look-up section these are the objects which are counted - ie object A ONLY.
  Therefore one object occupies a volume equal to the frame area times the section spacing.
  This is called the Disector Method.
- See: Howard CV, Reed MG (1998) Unbiased Stereology. RMs Handbook 41; Bios Scientific.



2D

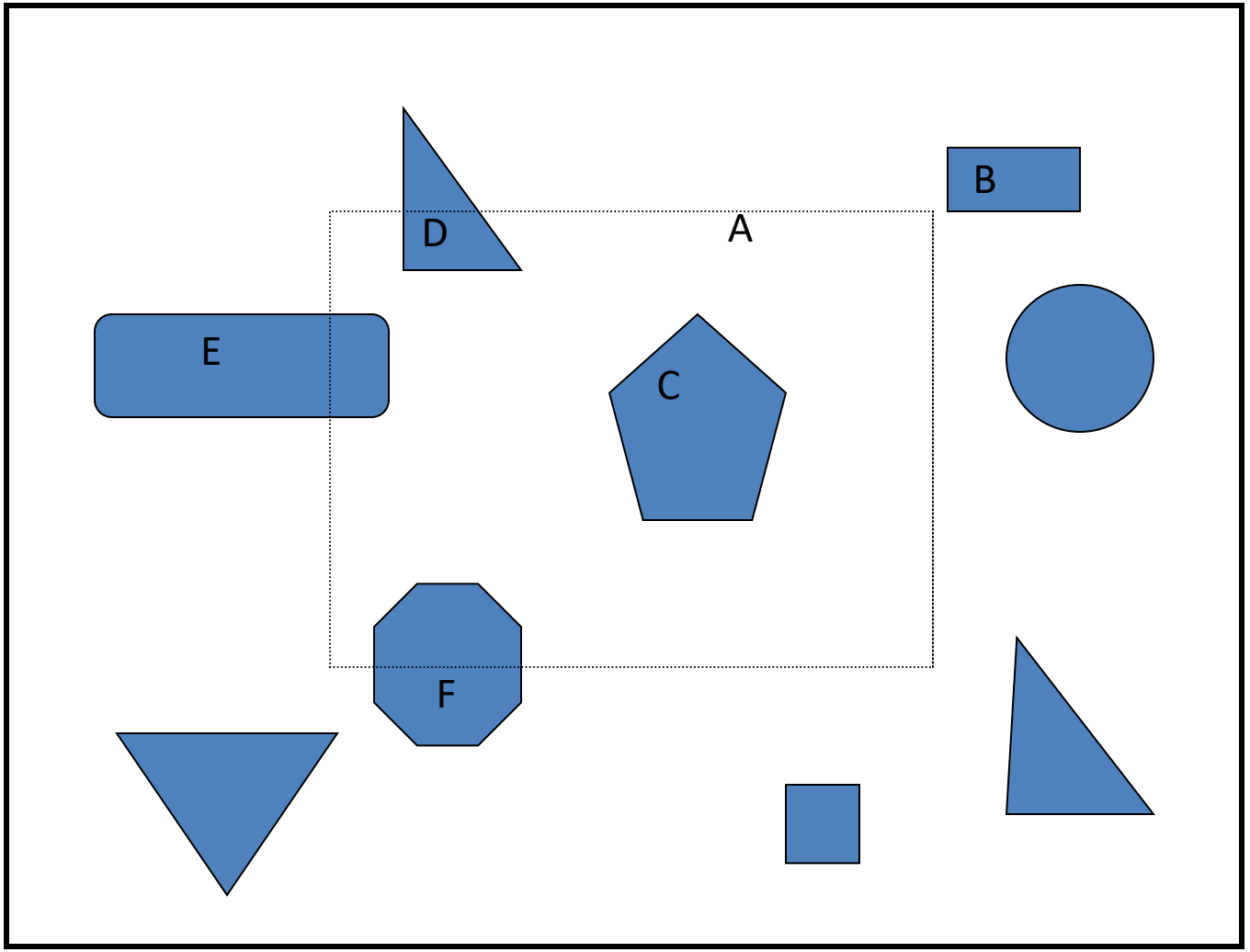


Objects A,B, C, D only are counted

1) Reference Section

2D

Disector method



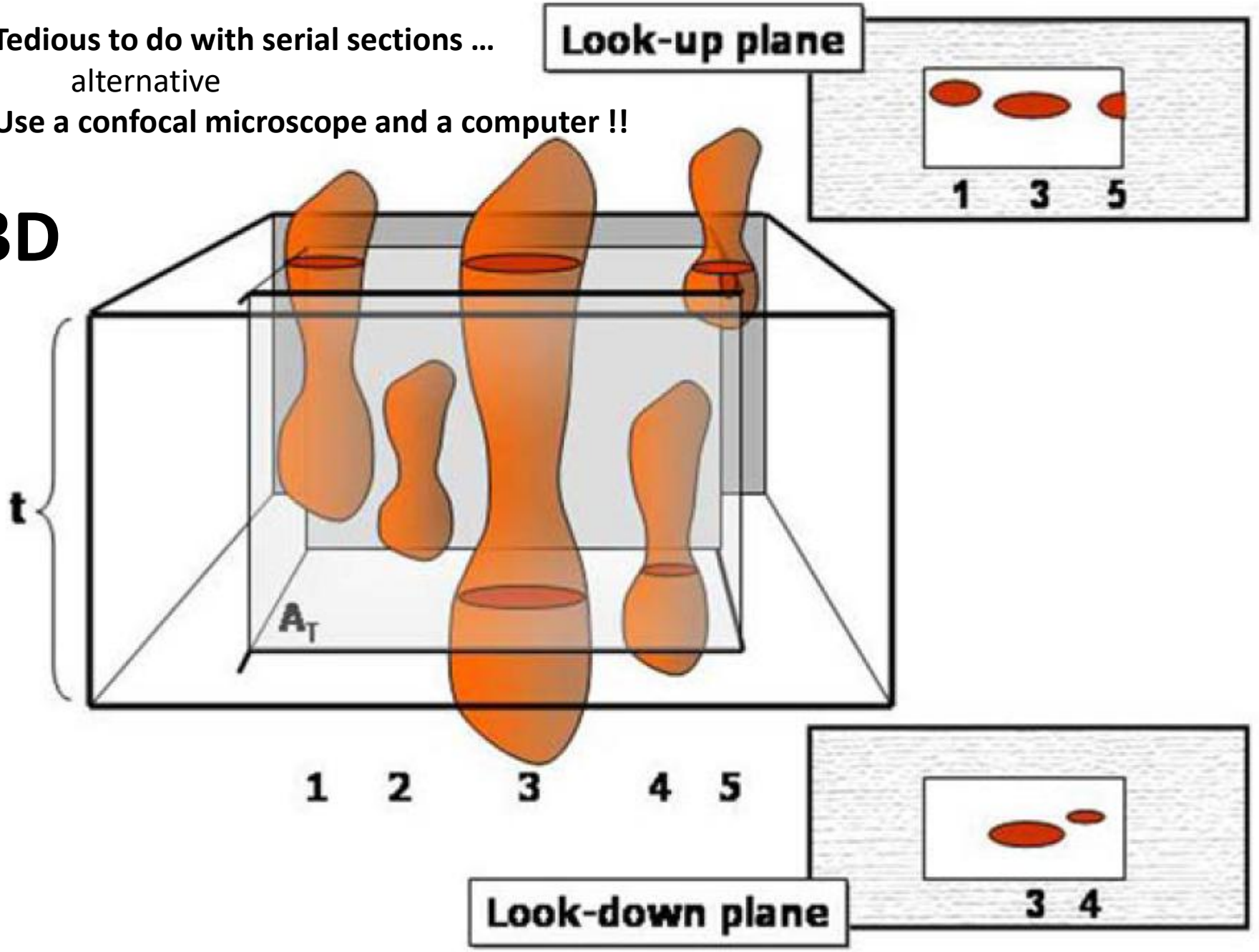
2) Look-Up Section

Of the objects counted in the reference section (A,B,C,D) only the objects NOT present in the look-up section are counted, ie only object A is counted.

Tedious to do with serial sections …

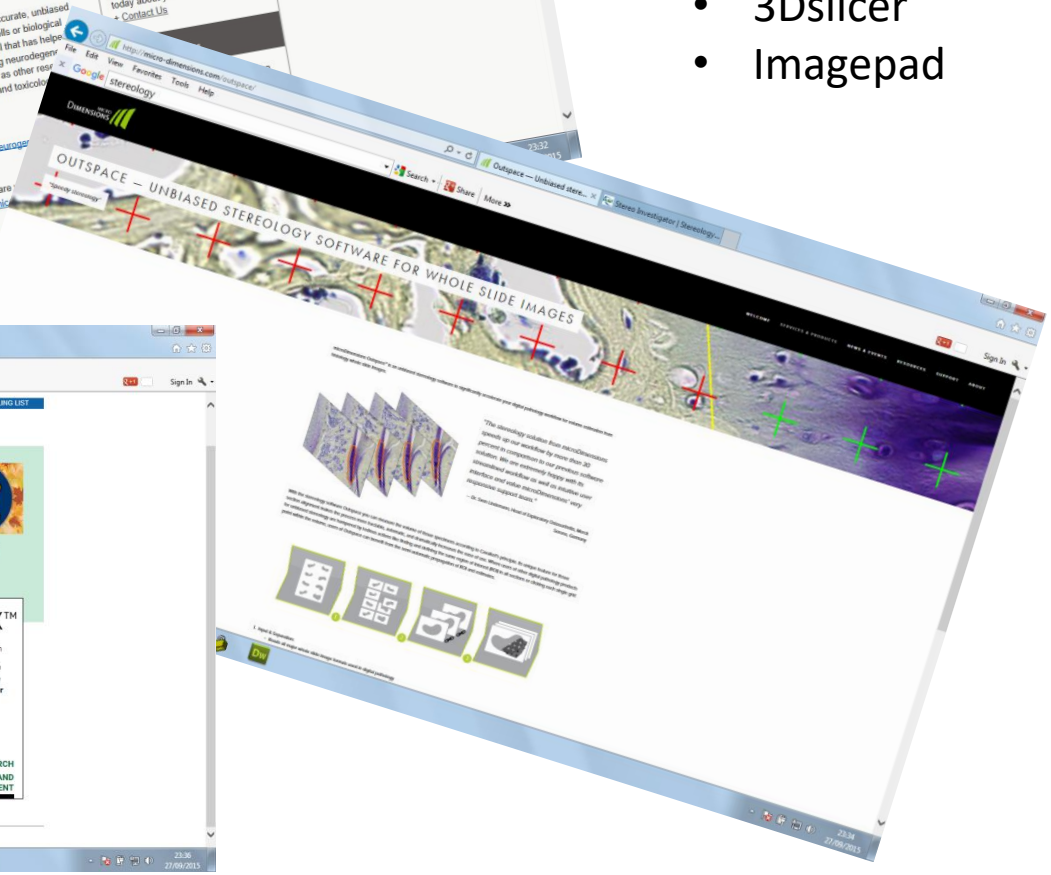
alternative

Use a confocal microscope and a computer !!



Mandarim-De-LaCerda (2003)

Commercial Stereology Software Packages



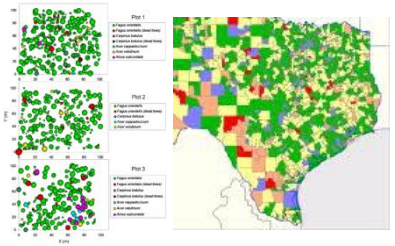
Free

- Stepanizer
- MorhoJ
- 3Dslicer
- Imagepad

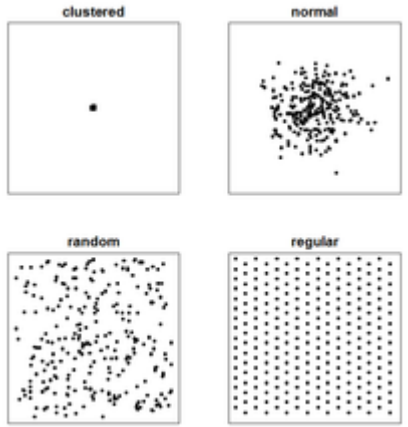
How?

Pattern Analysis

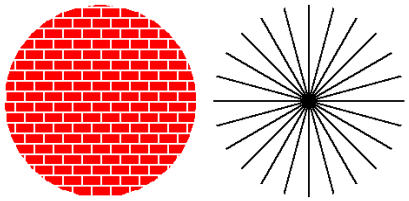
Measurements of 'Organisation'

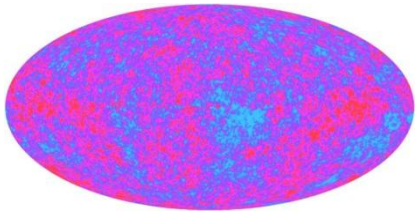


- Location / Distribution / Spatial Arrangement / Association / Connectivity / Interaction
  - ???: Random, Regular, Clumped, Dispersed, Associated/Related
  - Distance: Nearest Neighbour, Mean free path
  - Grouping: Enclosed, Contiguity, Runs Test, SPAM
  - Autocorrelation
  - Tesselation / Joins / Overlay methods
  - Regional Density, Point Swarms
- Orientation / Branching
  - Dendritic methods (fields, segments, nodes)
  - Isotropic, Anisotropic



See: *Uylings, Berry, Aherne, Underwood, Johnson, Sokal & Rohlf, James, Mahon, Cruz-Orive, Diggle, Unwin*

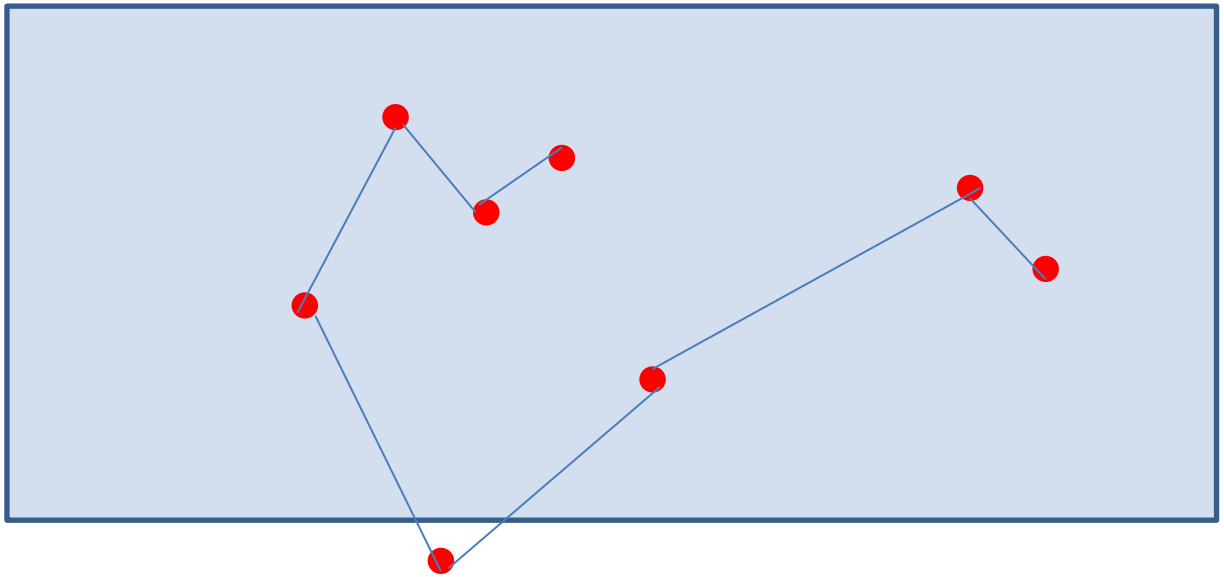




Nearest Neighbour Analysis

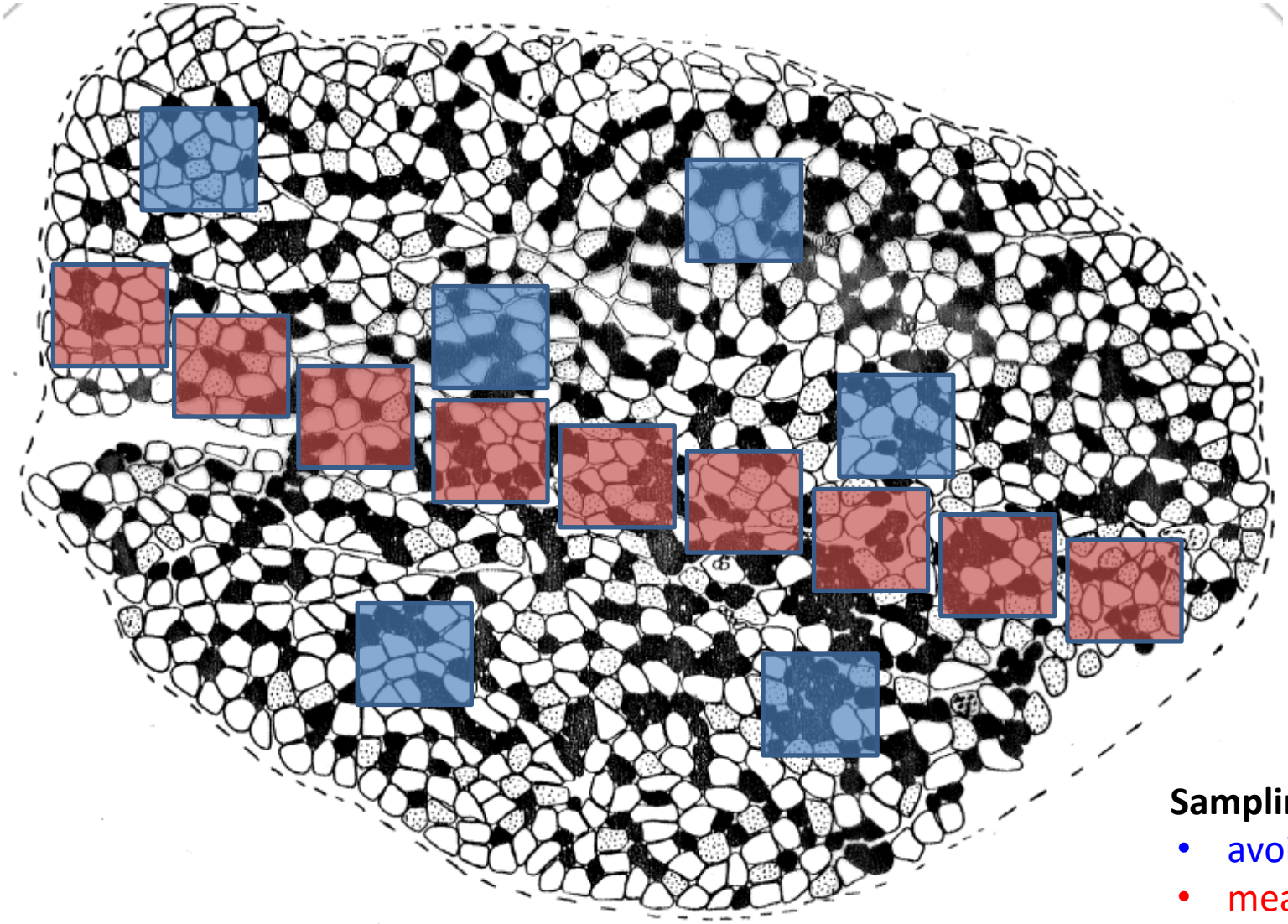
- Developed from Ecology studies (Poisson distribution) *Clark & Evans, 1954, Kendal & Moran, 1963*

- Average distance between objects or Mean area / cell



- Begin at random cell / point *Problems:* reflexive pairs / high densities

Homogeneity / Heterogeneity

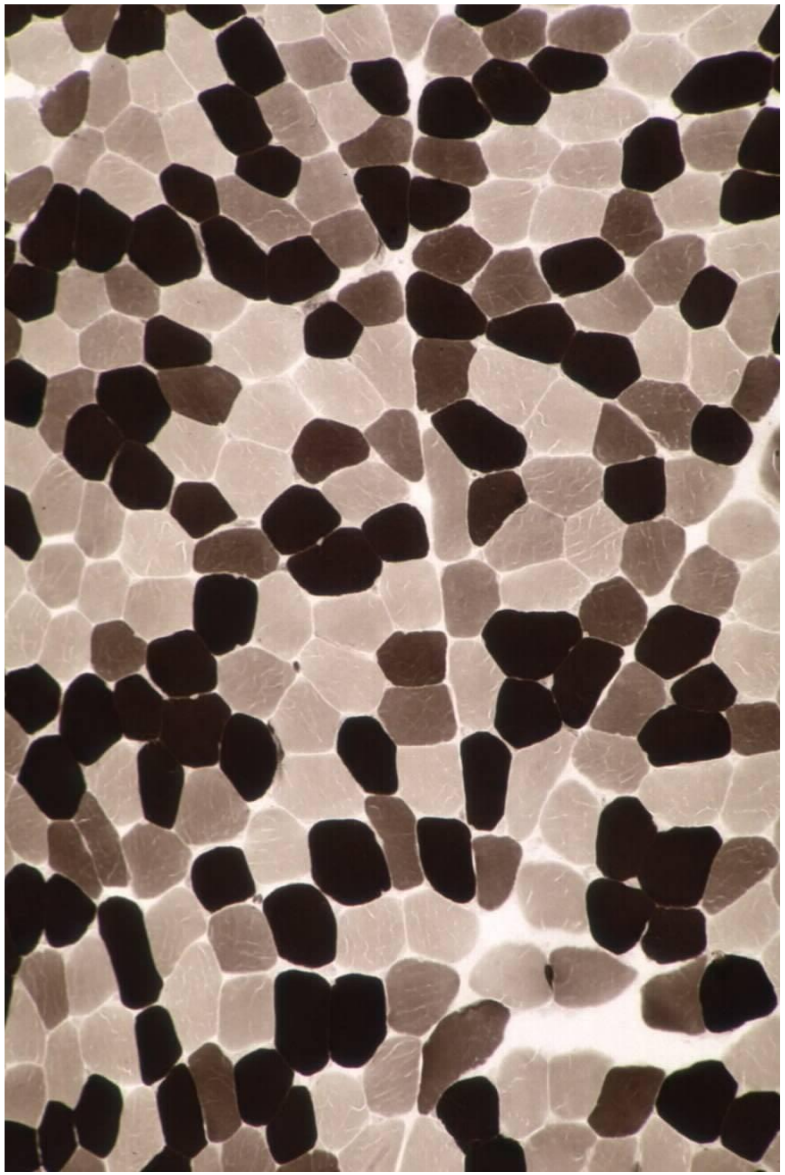


Sampling :

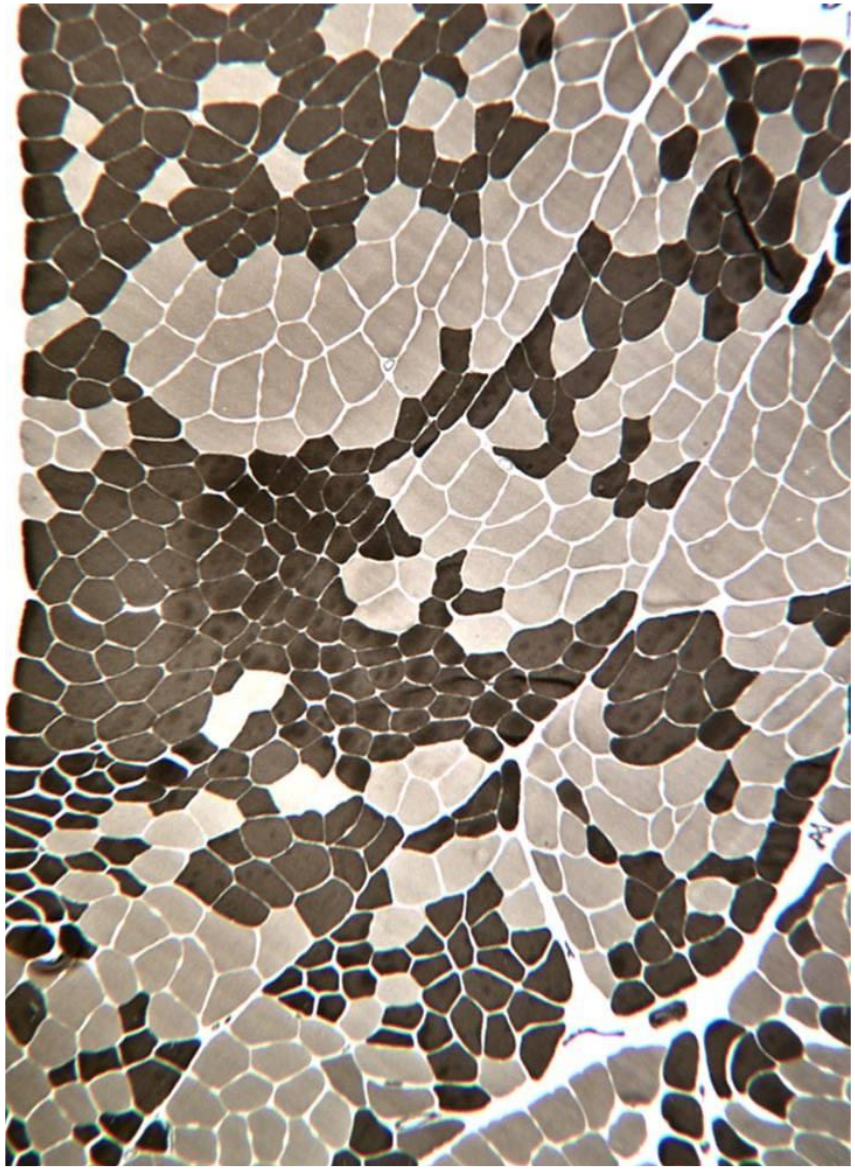
- avoid it
- measure it

Randomness ?

Healthy Muscle



Diseased Muscle

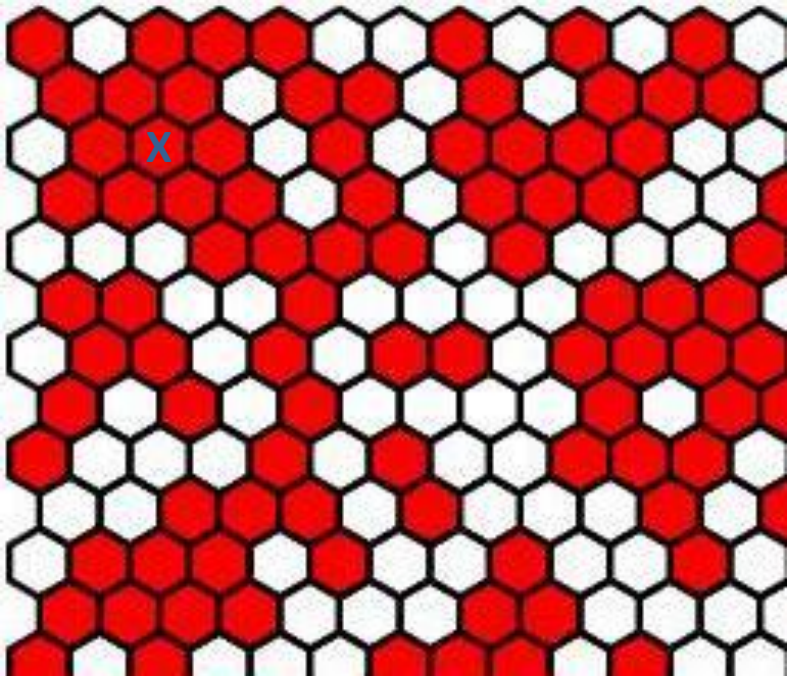


DISTRIBUTION

Enclosed “cell” method

- Observed versus Expected
- Predicted E= Np^7 +/_ SD
- Depends on percentage occurrence
  - 30% R = 0 enclosed
  - 50% R = 1
  - 70% R = 8
  - 90% R = 50

Johnson, 1973



DISTRIBUTION

Runs Test

- RRRR W RRR WWWW R WW r = 6
- R W R W R W R W R W R W R W R r = 15

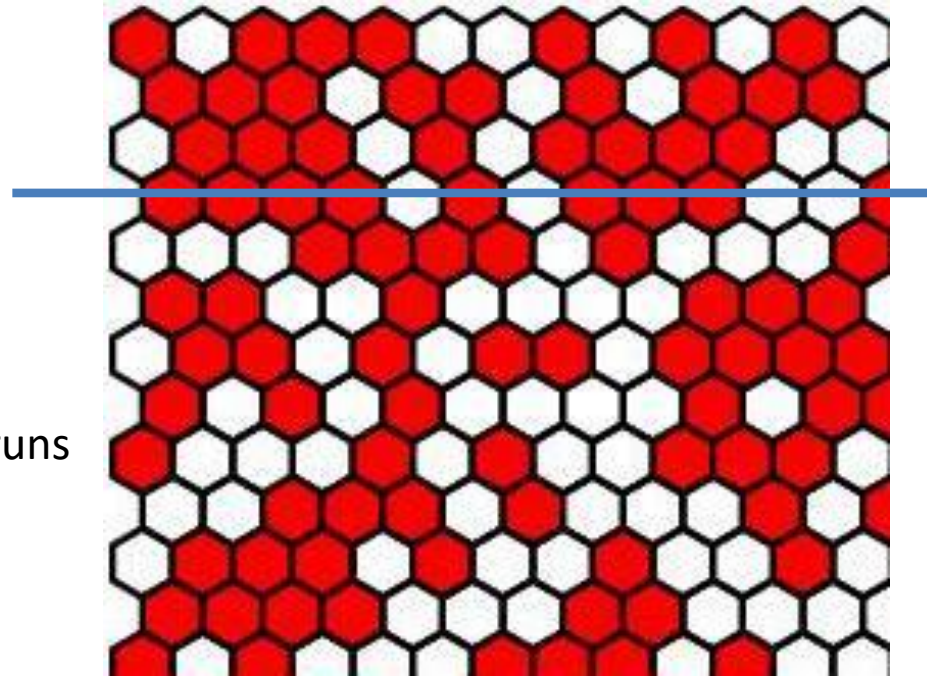
- N = 15, n1 R = 8, n2 W = 7

- Exp F = [2 x (n1 x n2 / n1 + n2)] -1 = 6.5

- T = (F - Exp F) / SD

- Distribution IS Random
- Distribution IS NOT Random

- Predict Runs eg 100 cells 60% R = 47 +/-5 runs



Sokal & Rohlf, 1973

DISTRIBUTION

Run Lengths (Clumps)

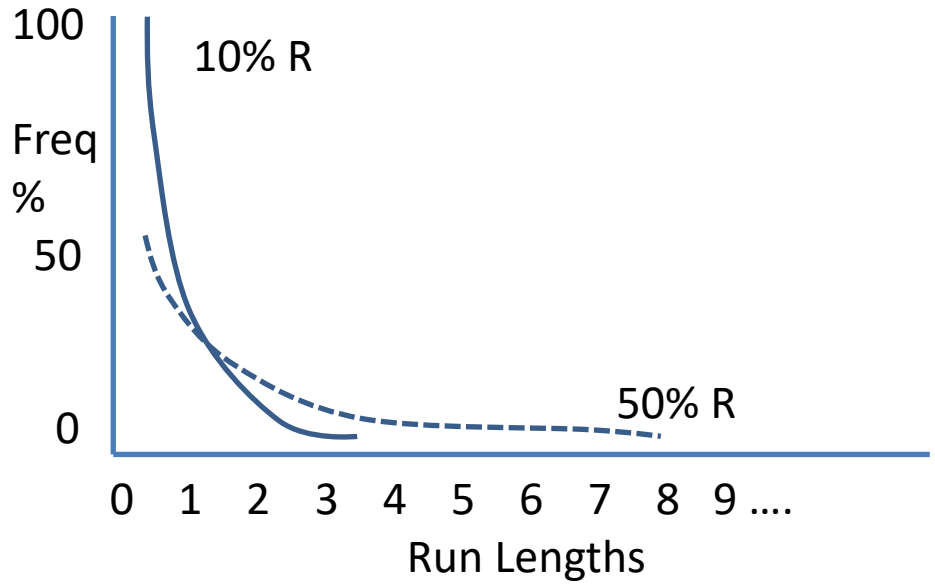
Roach, 1968

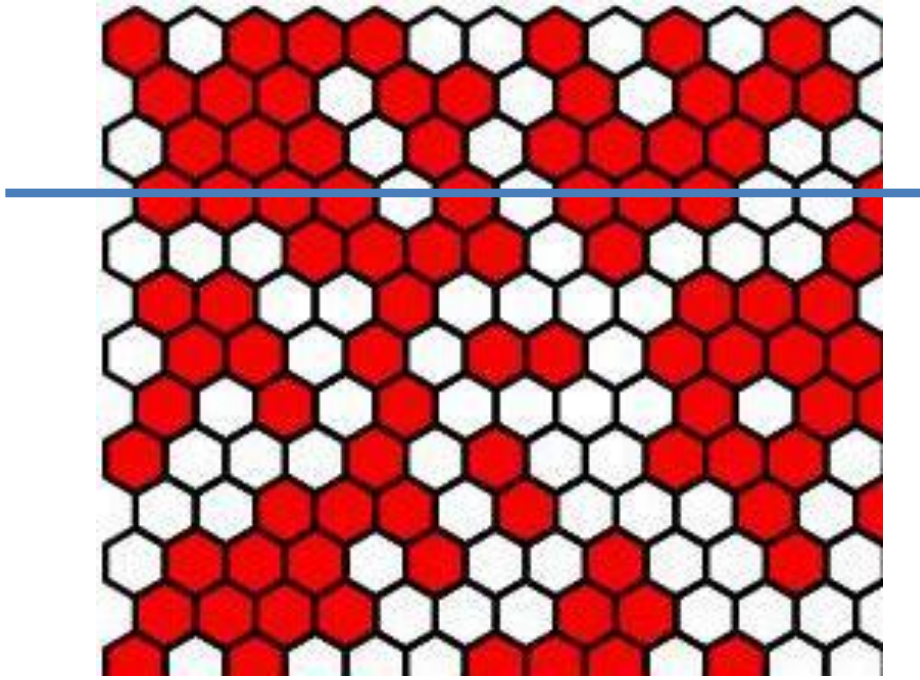
- RRRR W RRR WWWW R WW — Avlength R = 2.66
- R W R W R W R W R W R W R W R — Avlength R = 1.00

- Predict expected run lengths for a random distribution

$Cl_R = N_R + P_{RL^{R-1}} \times (1-P_R)^2$

$Cl_W = N_R \times P_R \times (1=P_R)^{LW}$





DISTRIBUTION

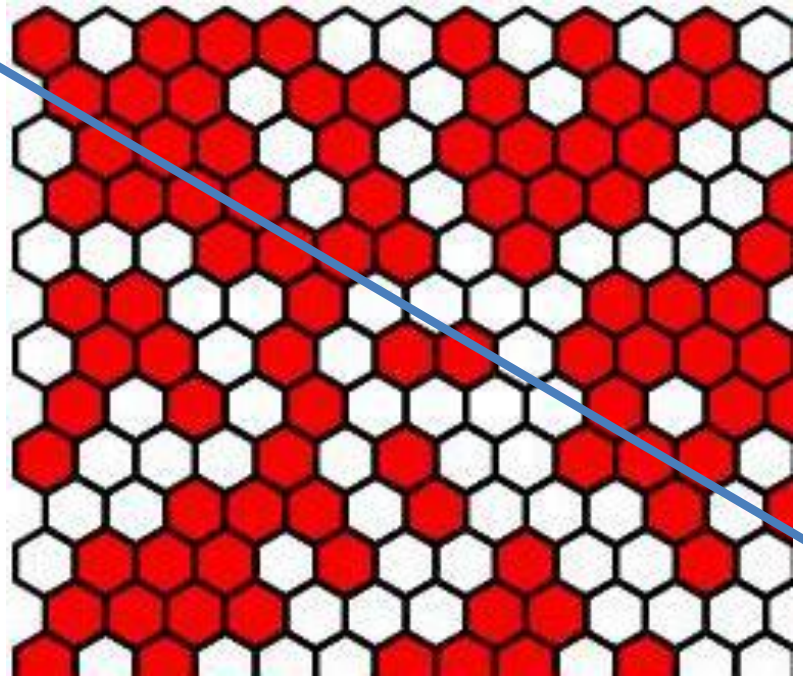
Contiguity

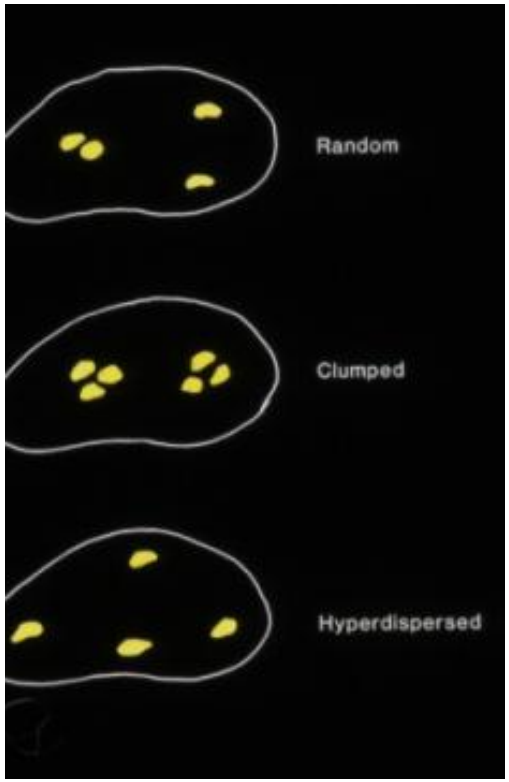
Underwood, 1970 *Gurland, 1975* *James, 1980*

- Apply test line and look at intersections with boundaries
- Need to know length of test line and

N_{RR} = R_R
N_{WW} = W_W
N_{WR} = W_R_W

- Use stereological SV formulae modified for 2D
- $L_A = \pi/2 \times P_L$
- Estimate Interface lengths for LA_{RR}, LA_{WW}, LA_{WR}
- **Index of Contiguity** $C_{RR} = LA_{RR}/(LA_{RR} + LA_{WR})$

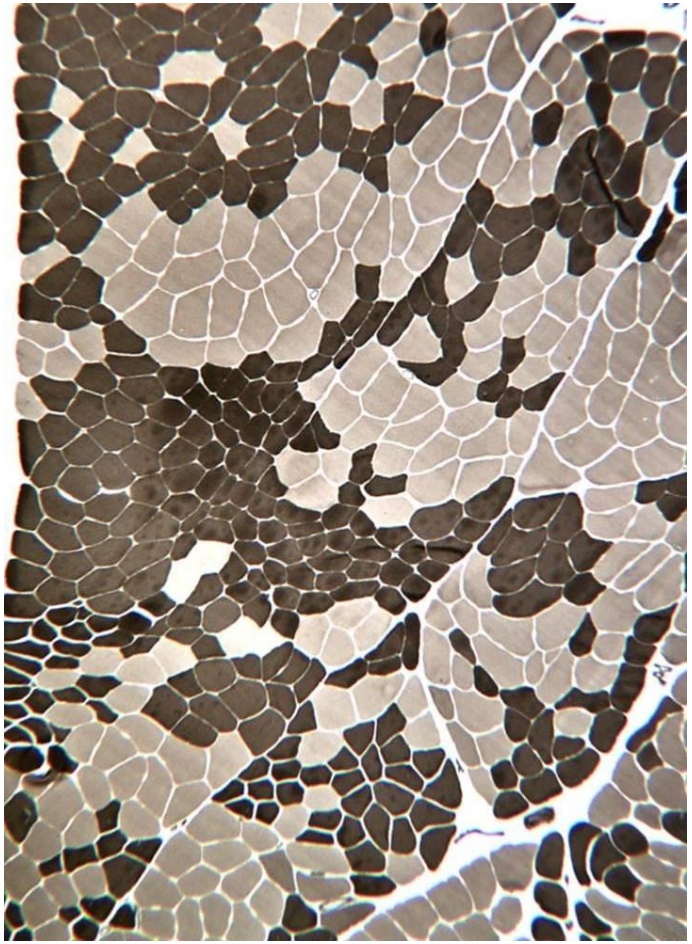


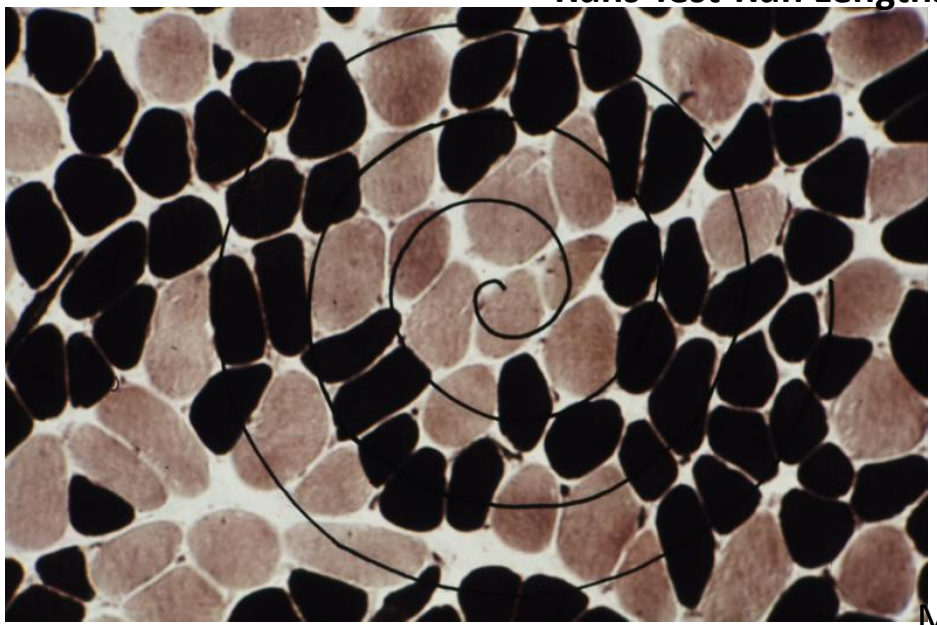


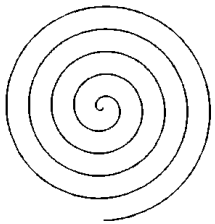
DISTRIBUTION

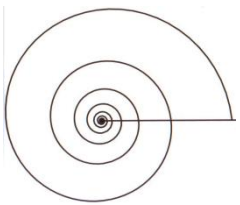
Spatial/Spiral Pattern Analysis of Muscle

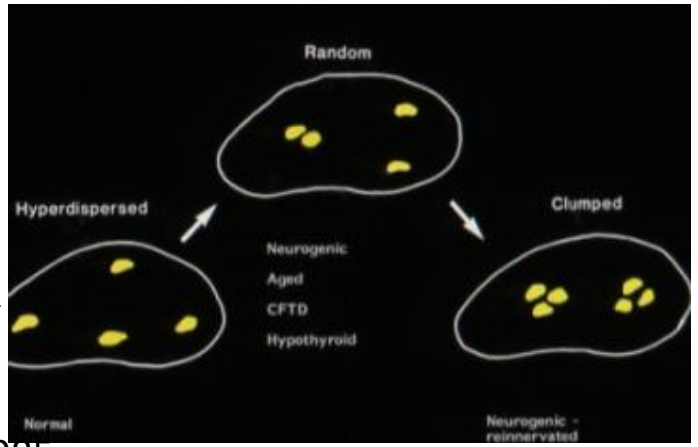
Runs Test-Run Lengths-Contiguity









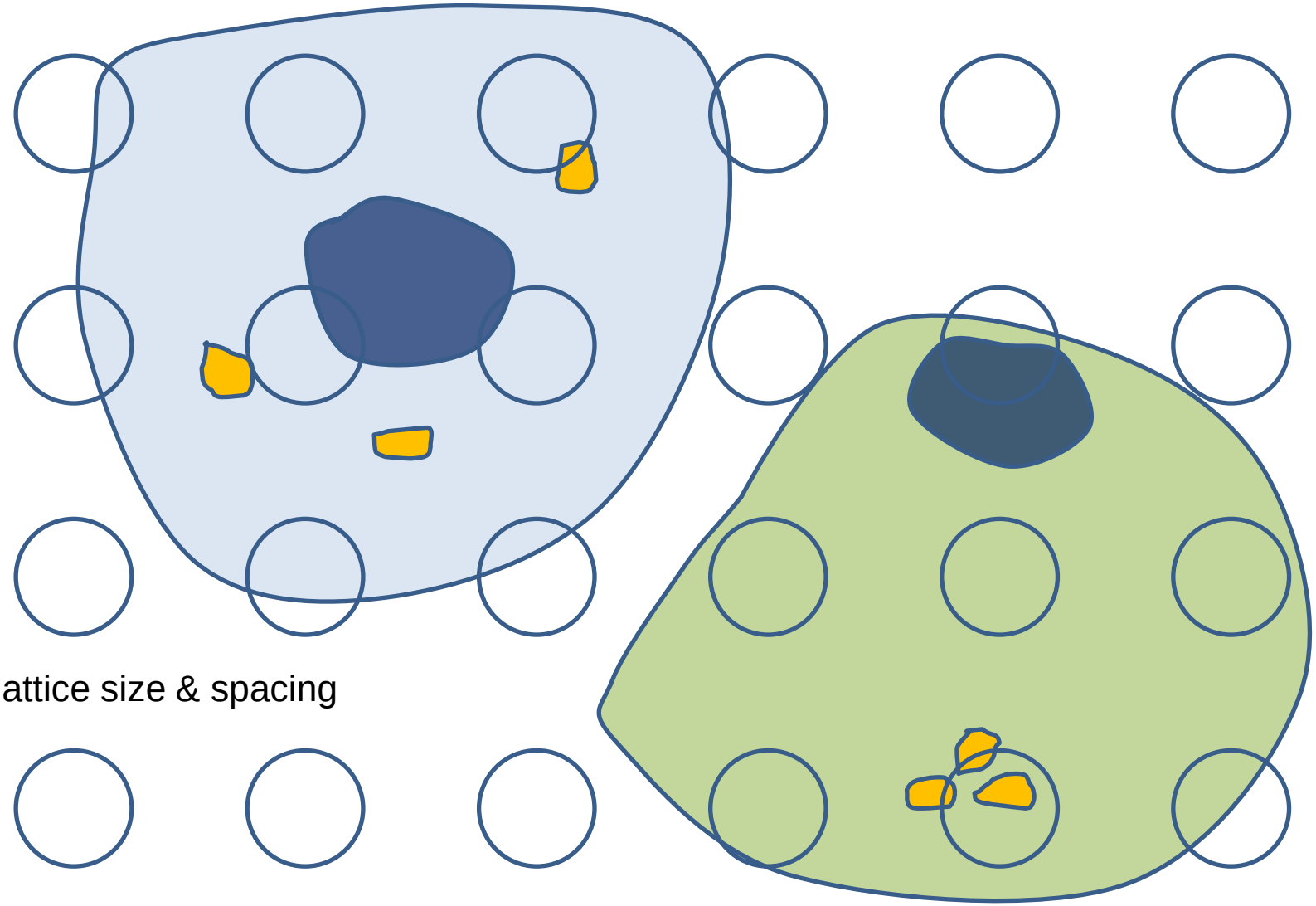


Mahon, 1977,1985

Measure of **Association** – Circle Overlay Method

Cruz-Orive, 1976

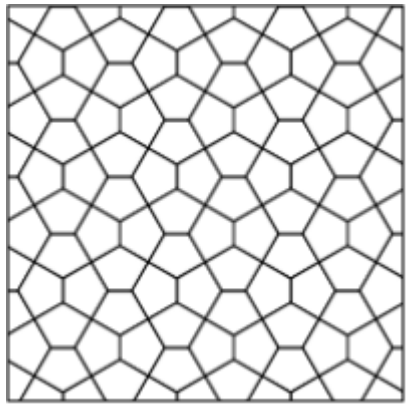
eg: Eccentric nuclei, organelle clumping or autoradiography *(Williams, 1977)*

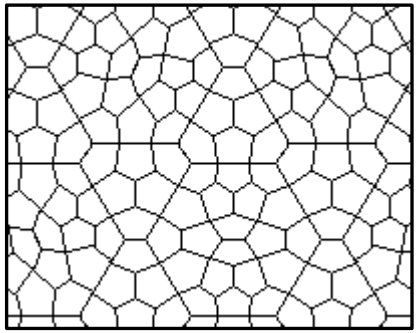


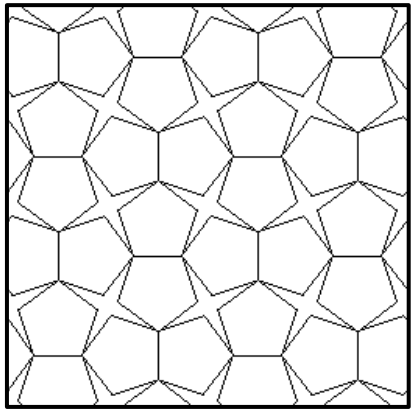
Problems: lattice size & spacing

Shows that in the pathology example to the right, more likely to get circles overlapping groups of orange objects, normal (left) orange associated with nucleus.

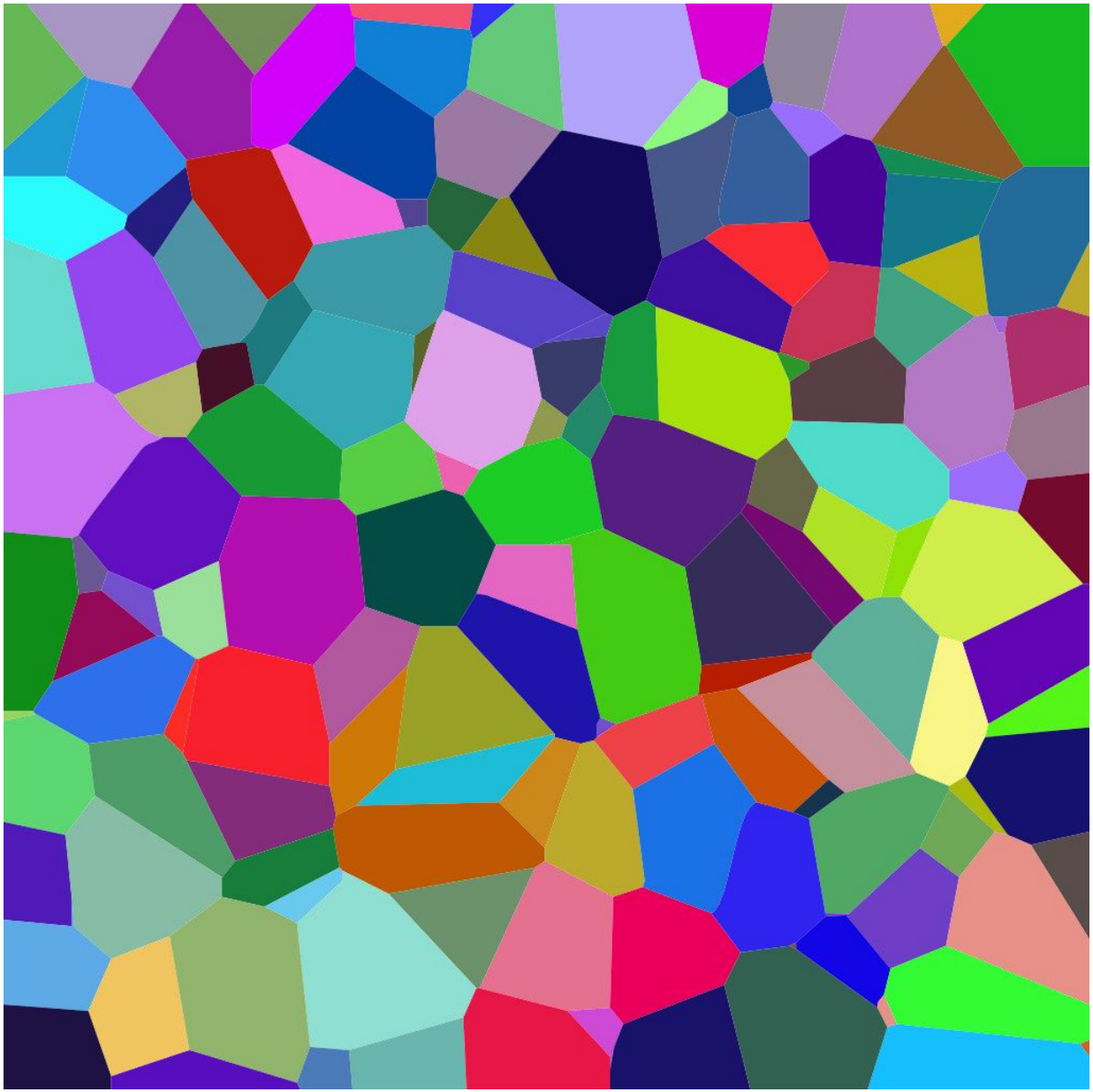
Tesselation



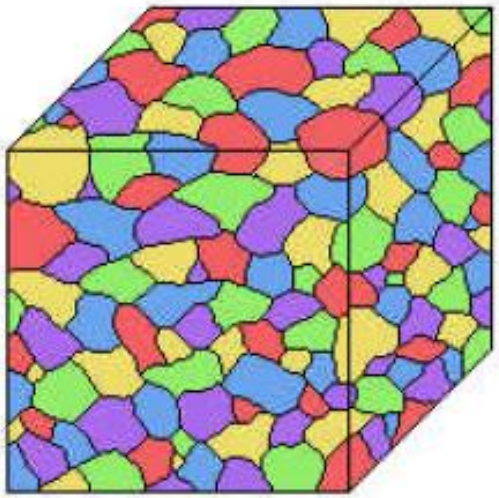




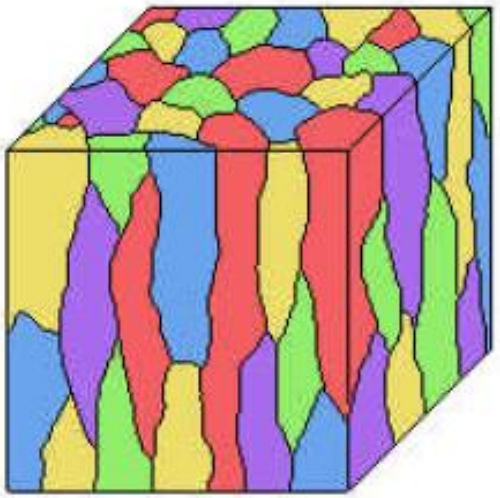
Apply lattices and use mathematical concepts of
“Lattice tesellation of congruent domains”



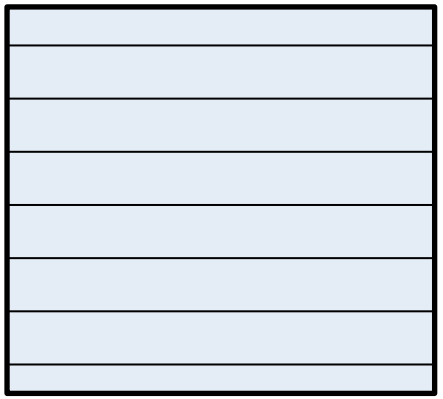
ORIENTATION

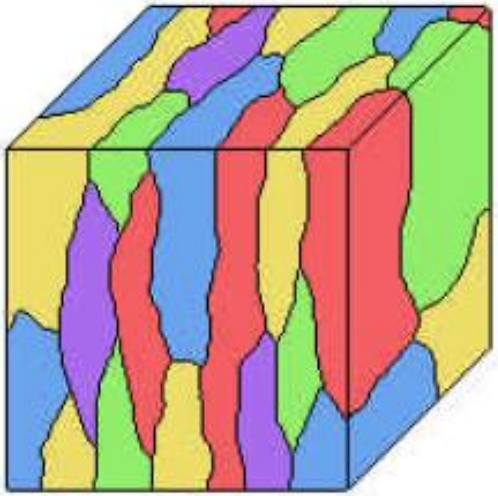


a



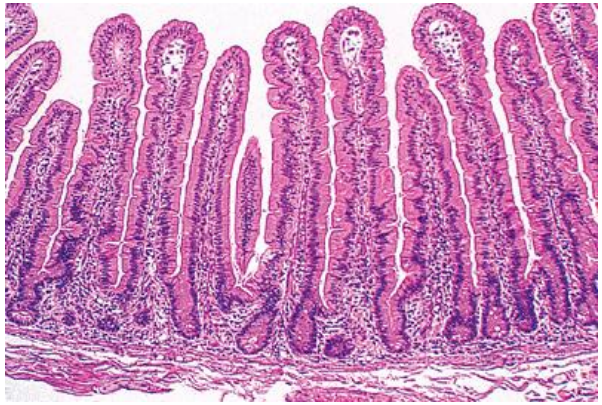
b

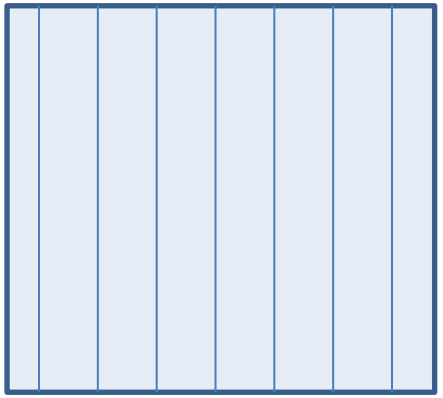




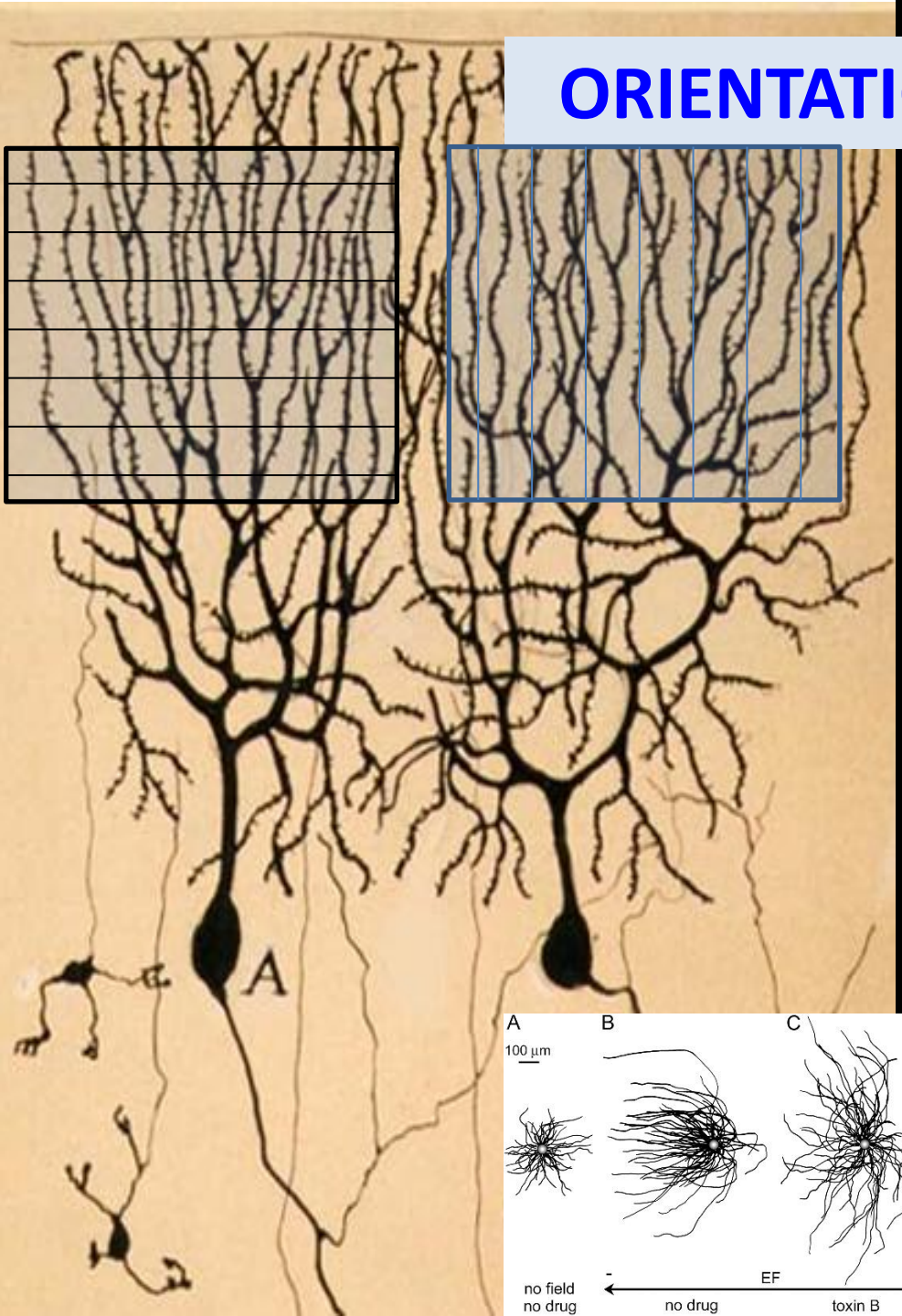
c

Apply line lattice





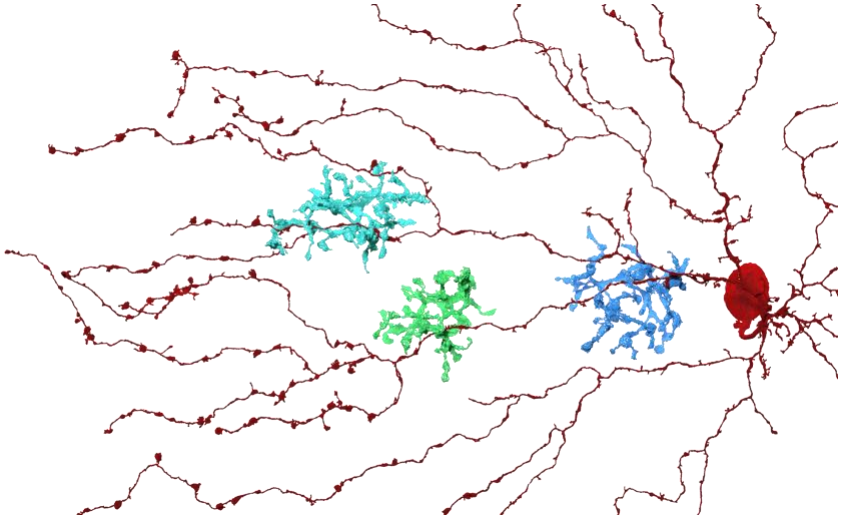
ORIENTATION

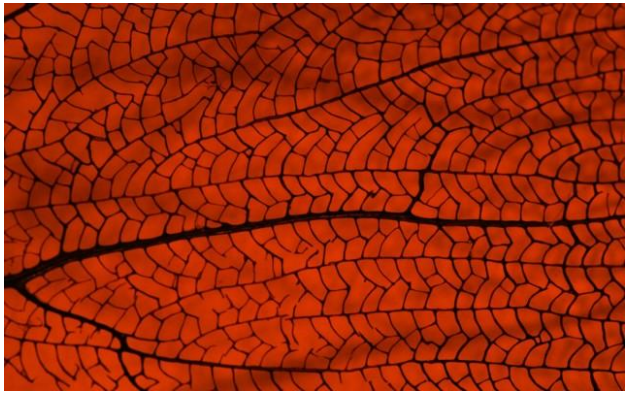


BRANCHING

Sparse growth of dendrites
in an aging, inactive brain

Typical dendritic growth in an active brain





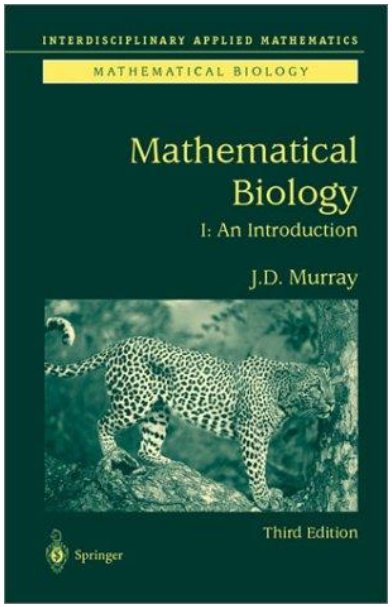
Topology

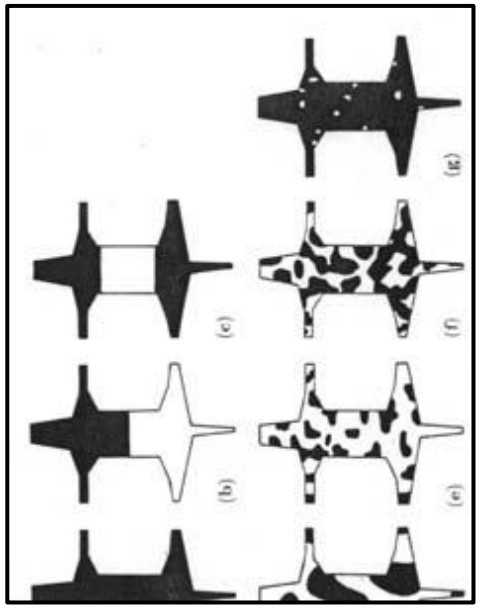
- Arbor or Tree analysis
- Vertex / Branch / Segment
- Bifurcations / tri .. / multi ..
- Angles

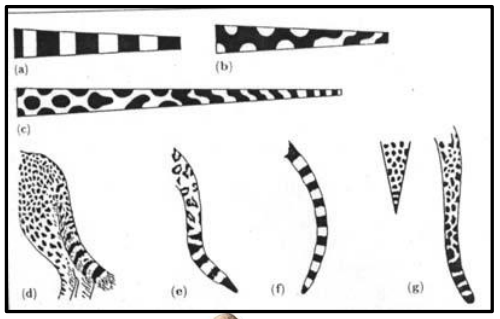
Berry, 1980s

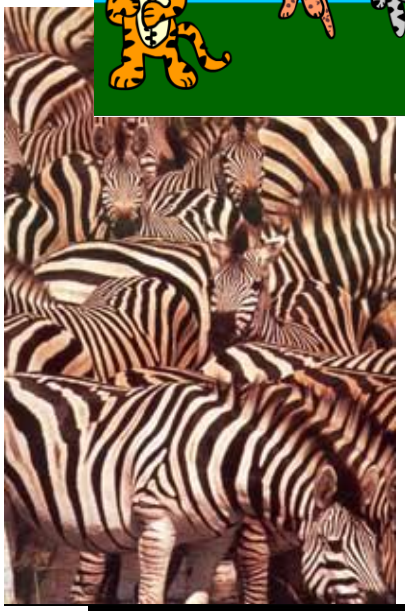
Mathematical Modelling of Topography & Development

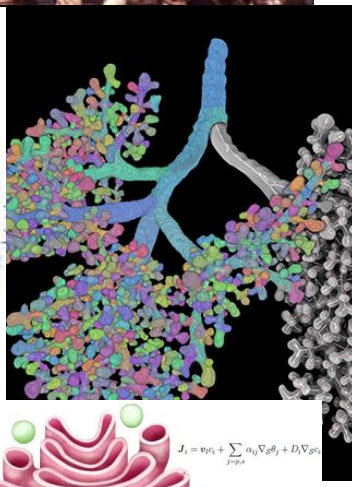
1952 Alan Turing , 1980 JD Murray

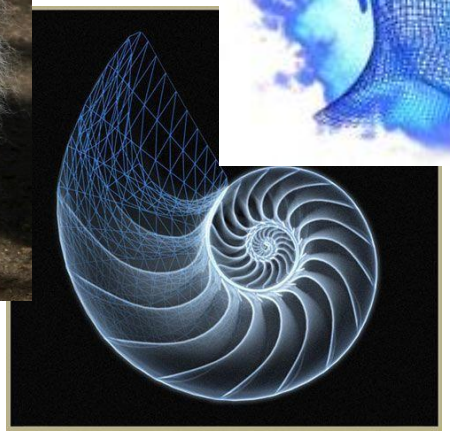


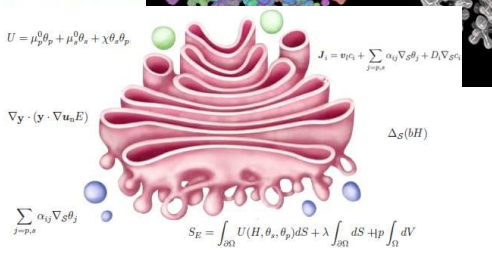






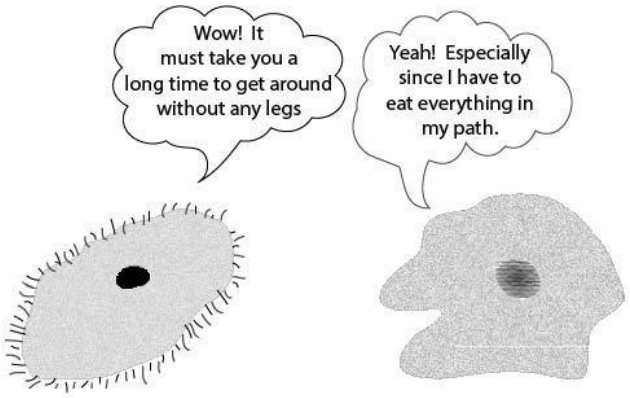






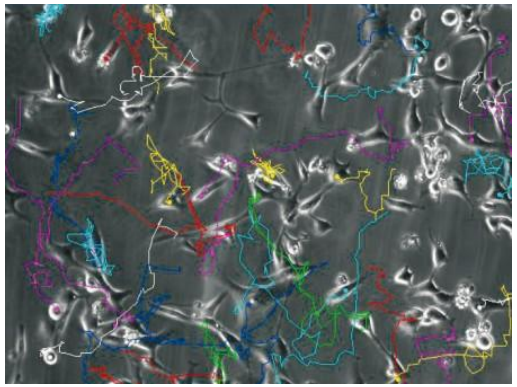
Movement

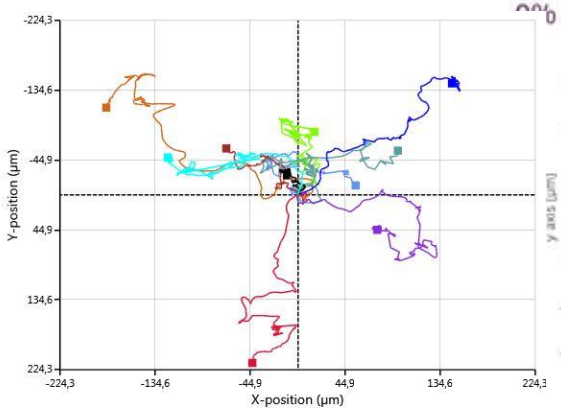
How ?

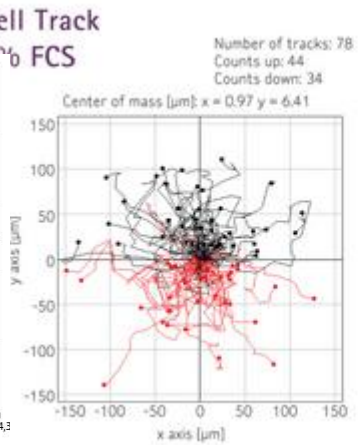


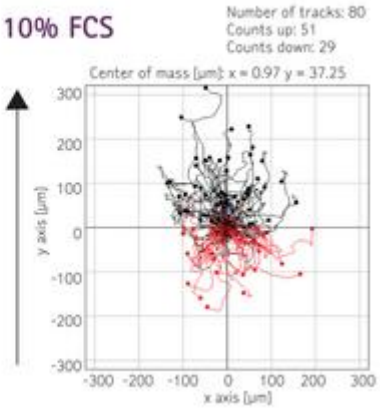
- Organisms, cells, organelles
- Subjective recording

- Tracing
- Direction
- Velocity
- Diffusivity
- Association





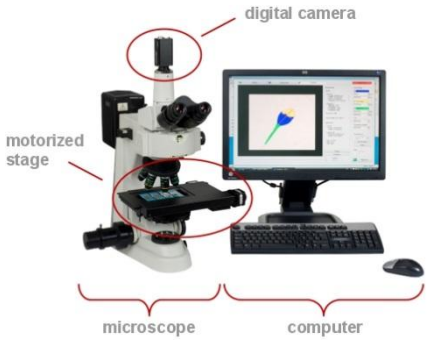




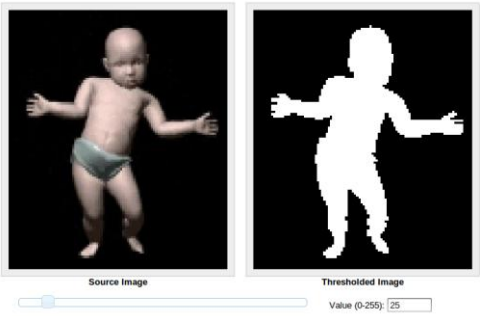
How ?

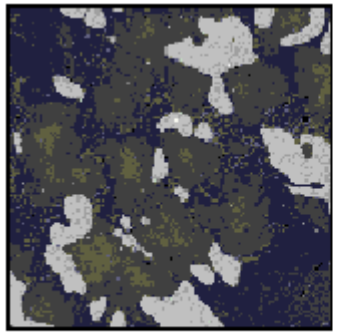
Image Analysis

- Automated/Computerised Measurement vs Eye/Brain
- Early: 1969-1990 … then software based
  - MOP, Quantimet, Magiscan, Imagan, …. **NIH Image-J**, Matlab, LAS, Image Pro, i-Solution, QuPath
    Recent (2010-now): Machine Learning, AI
- Procedure
  - Sampling
  - Calibration
  - Image capture
  - **Segmentation**
    - Thresholding, Edge Detection, Erosion/Dilation
    - Object detection
  - Measurement
    - Size, shape, number, density (IOD?), arrangement, …
  - Data analysis and display
- + / -
  - Speed & Measurement / Identification, User, Cost, GIGO

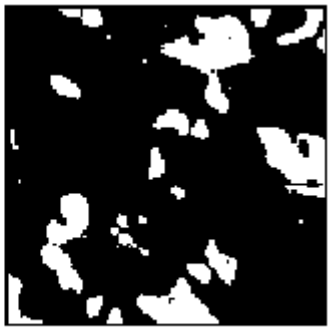


Thresholding

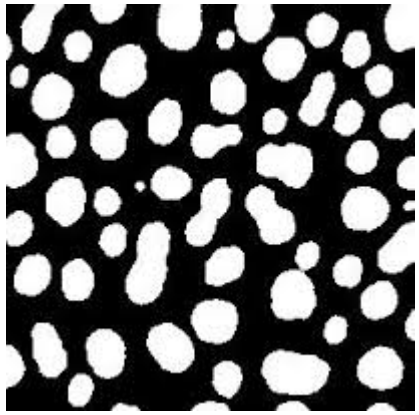




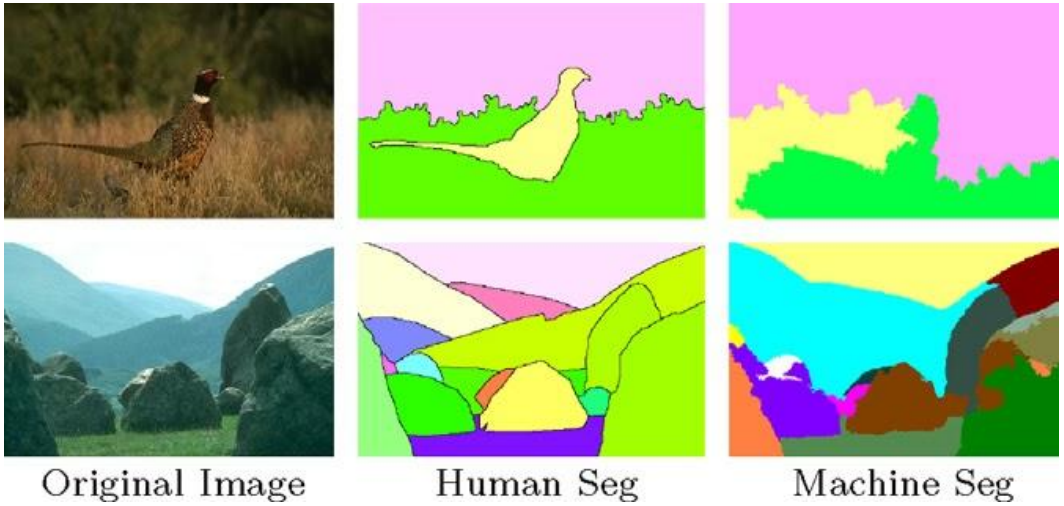
Original image



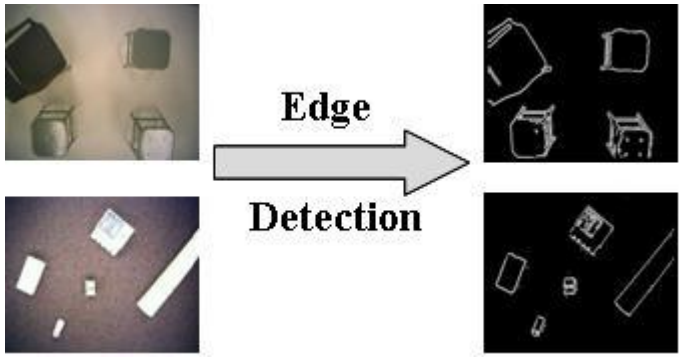
Thresholded binary image



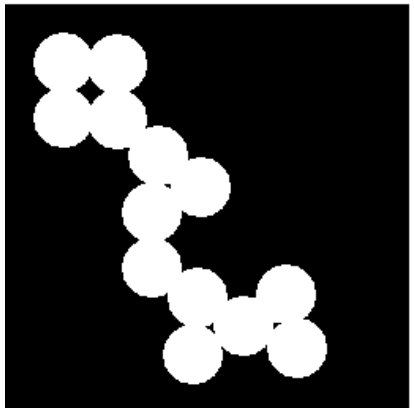
Segmentation

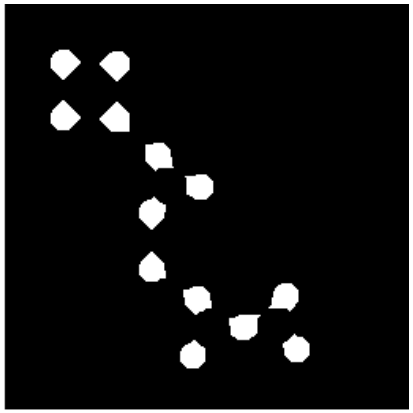


Edge Detection

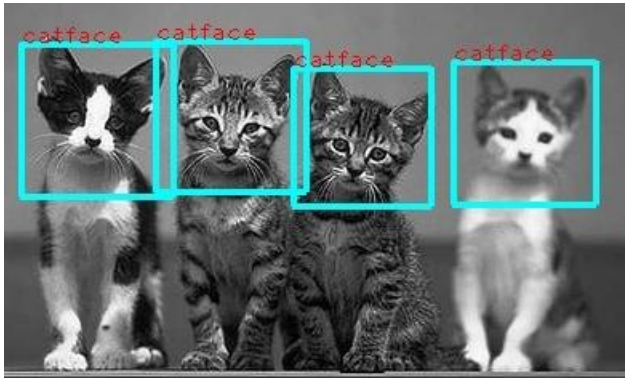


Erosion / Dilation

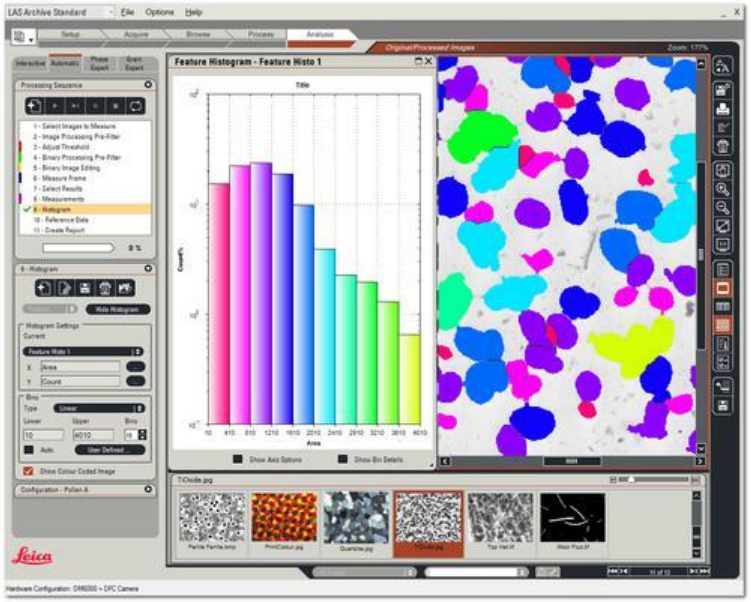


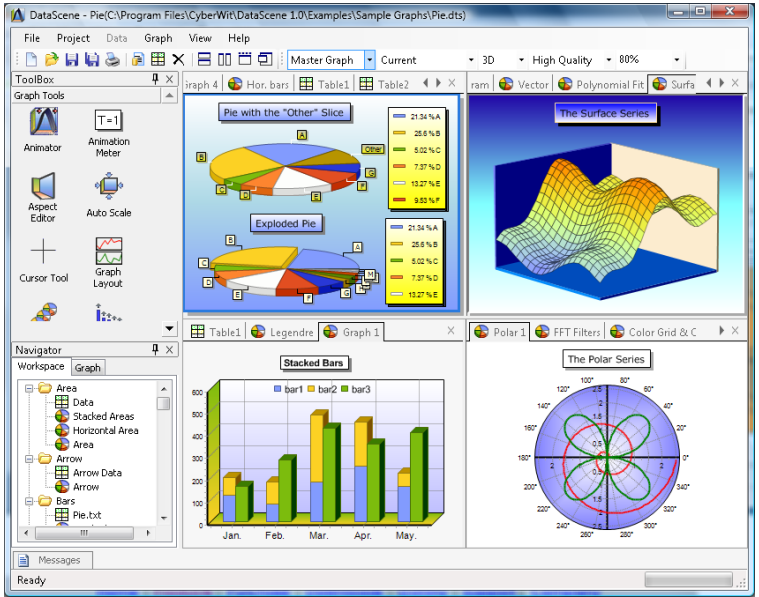


Object Detection



Problems – brilliant results output **But** does the user know what the machine is doing and have they considered bias, caveats etc. ?? !!





1980s advertisement …

THANKS TO MAGISCAN – IMAGE ANALYSIS IS NO LONGER THE PRESERVE OF THE SPECIALIST

Results ?

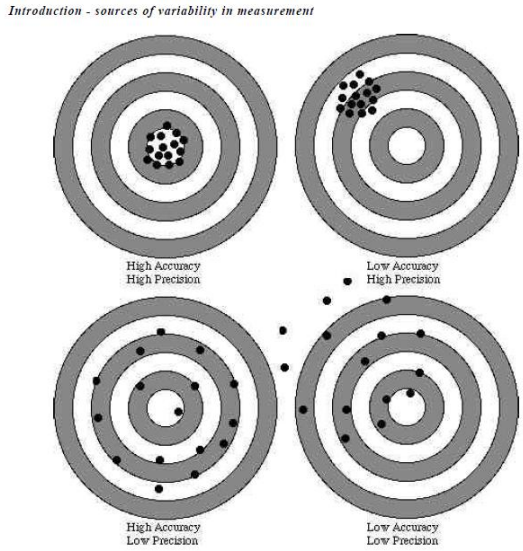
GIGO

Garbage In – Garbage Out !

"Garbage to ten decimal places is still garbage !"

Are the results unbiased, precise, accurate, valid, meaningful ?

Statistics !!!



- Accuracy
  - Degree of closeness to true value
- Precision
  - Related to reproducibility and repeatability
  - Improve by increasing sample size
- Bias
  - Random or Systematic error
- Valid
  - Measurement system which is ACCURATE and PRECISE and UNBIASED

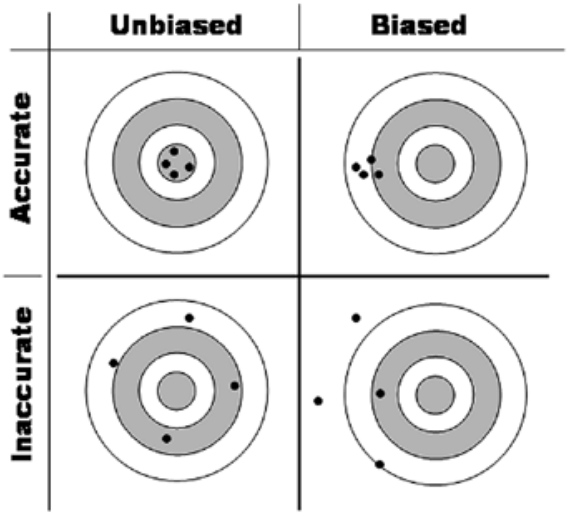


Fig. 2 Schematic illustration of the difference between accuracy and bias. The top row of targets shows accuracy that is the hits are closely clustered together. The bottom row shows inaccuracy and there is a marked scatter of hits. In the left hand column the average of the cluster of hits tends towards the bull s-eye, which means that they are unbiased. The right hand column shows the converse case, these hits are biased (based on Howard and Reed 1998).

How to conduct a measurement project

But 1st …Guess !!

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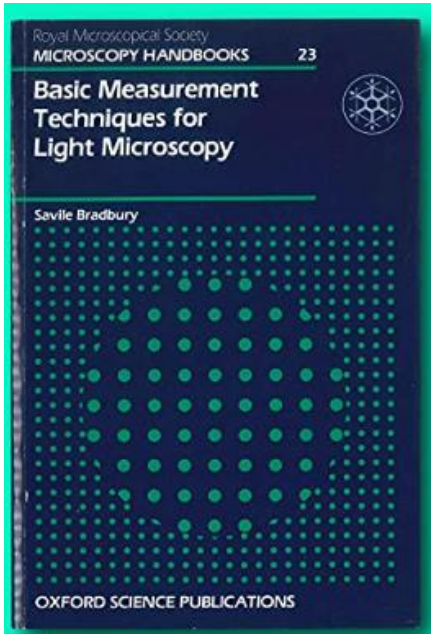
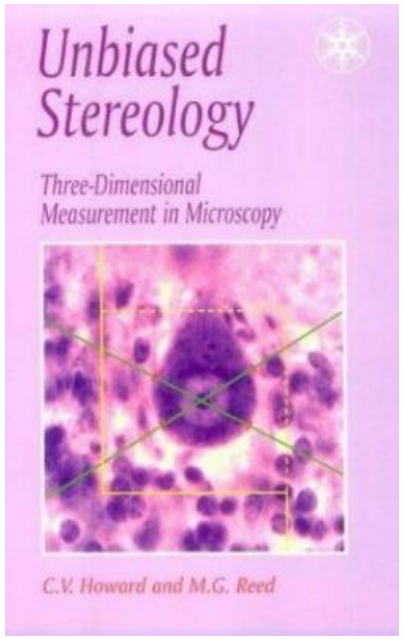
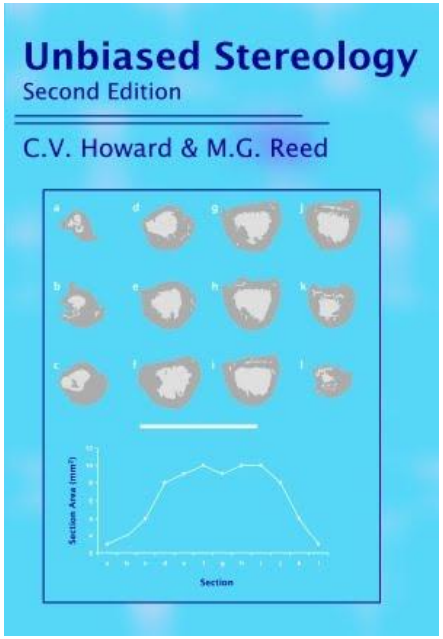
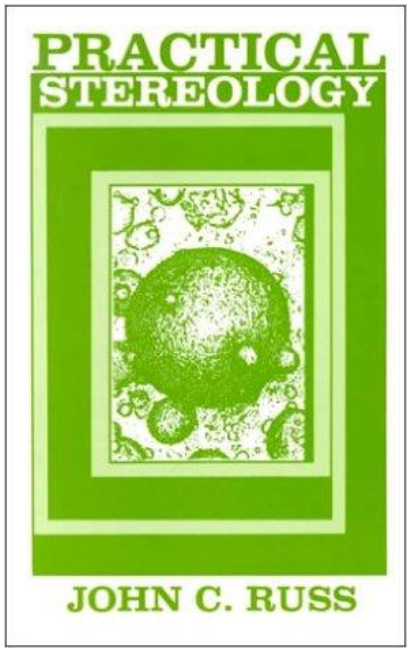
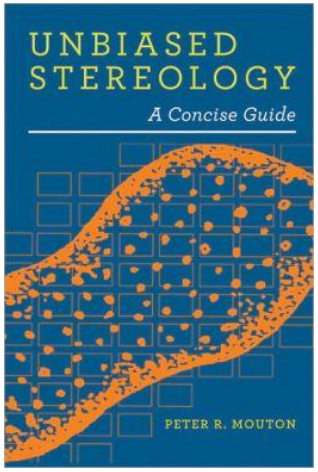
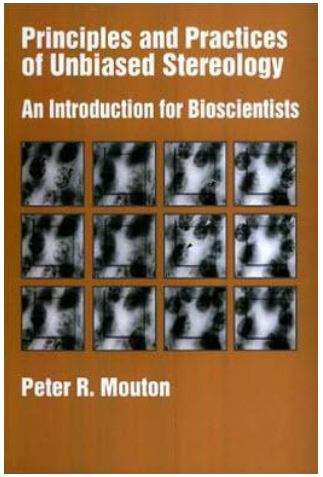


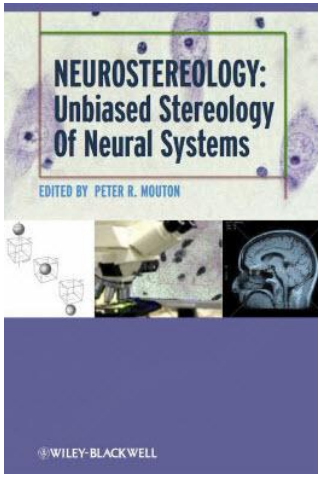


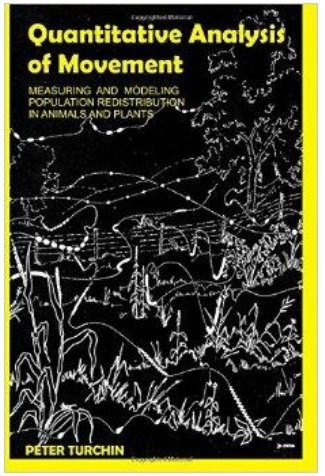












http://www.lab.anhb.uwa.edu.au/mb140/scope/stereology/stereology.htm

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652003000400006

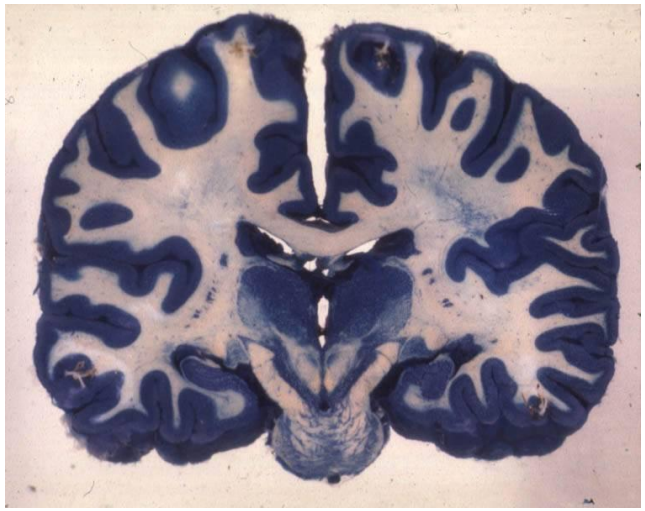
Question

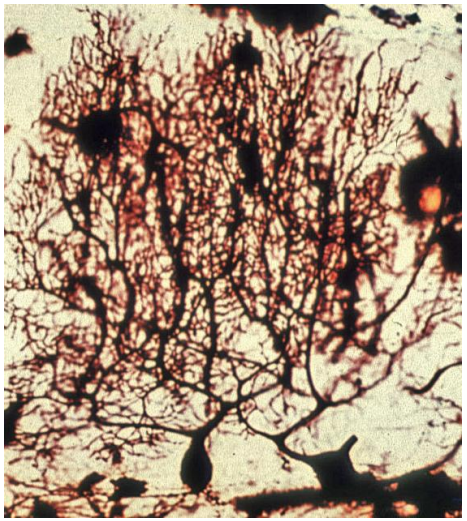
What is the Answer to the Ultimate Question of Life, the Universe & Everything ?

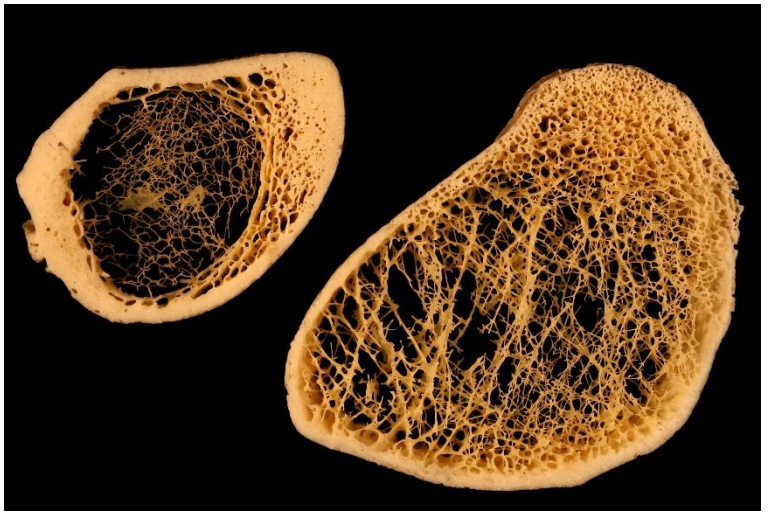
= ??

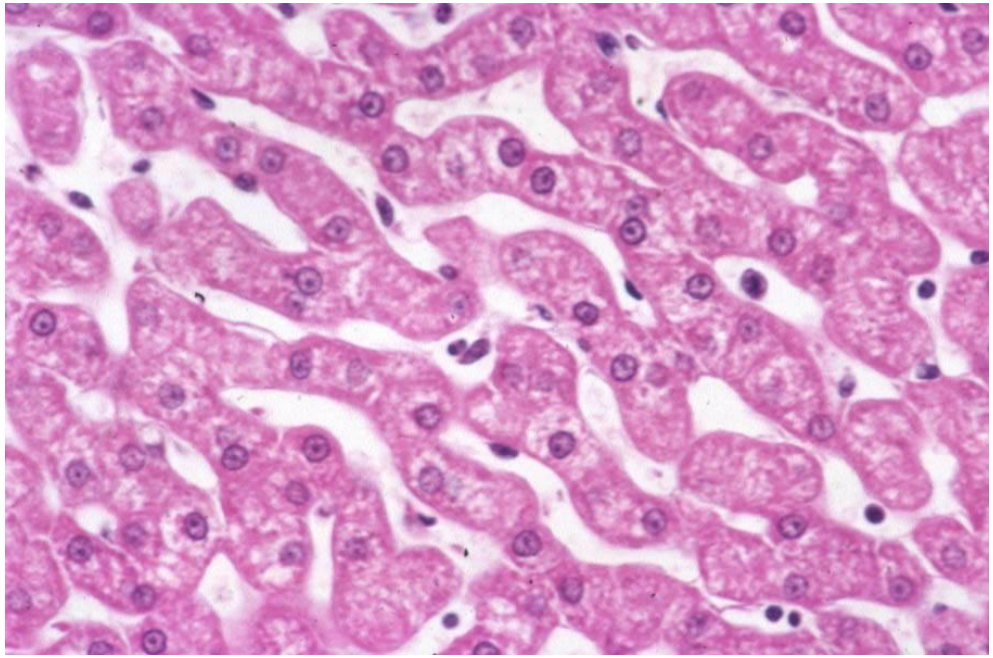
Key Reference: Douglas Adams “Hitchhikers Guide to the Galaxy”

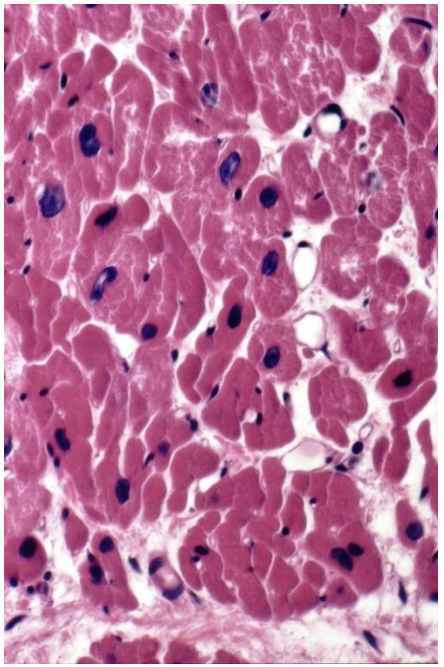
Volumes – Surface Areas – Lengths – Numbers - Branching ???

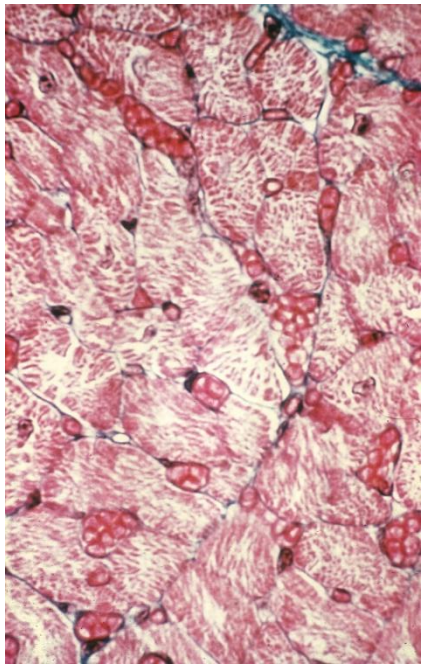


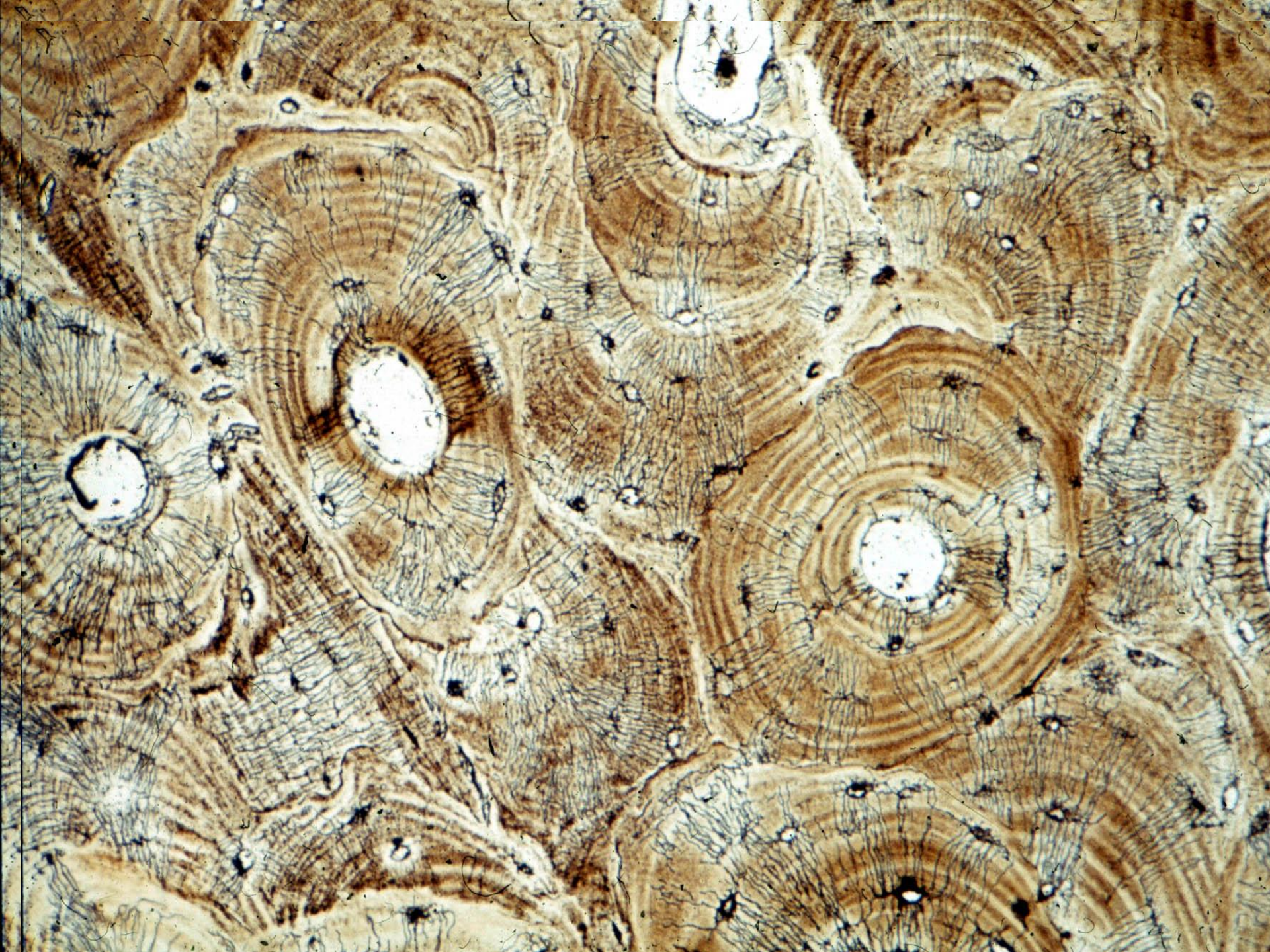












Practical

1. **Magnification Calibration**
2. **Morphometry**
3. Stereology
4. (Pattern/Shape analysis)