ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

III. Morphometry: from features to statistics

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Topics

1. Morphometry
   1. Individual Features
   2. Collective Features
2. Examples

Literature

John C. Russ, “The Image Processing Handbook”,
Boca Raton, CRC Press
Available online within domain ethz.ch (or proxy.ethz.ch / public VPN)
http://dx.doi.org/10.1201/9780203881095

1. Motivation

Why do we do experiments?

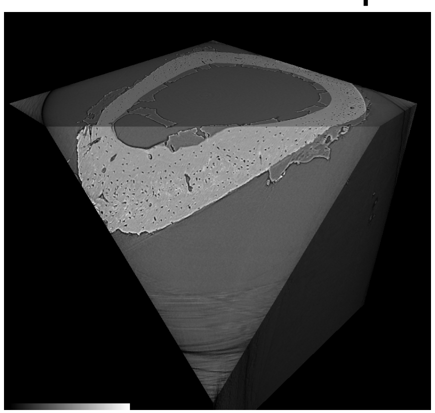
1. To get an idea of what is going on
2. To test a hypothesis
   1. Does temperature affect bubble size?
   2. Is this gene important for cell shape and thus mechanosensation in bone?
   3. Does higher canal volume make bones weaker?
   4. Does the granule shape affect battery life expectancy?

1. Motivation

Why do we do experiments?

1. To test a hypothesis

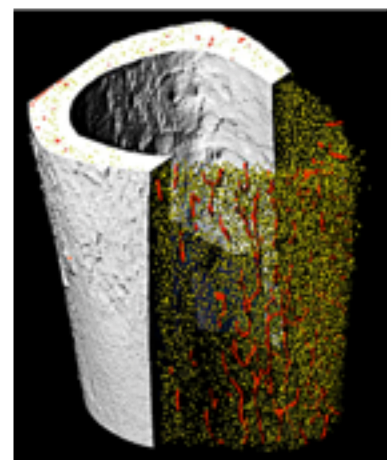
2560 (pixels in x)
x 2560 (pixels in y)
x 2160 (pixels in z)
x 32 bit
= 56GB / sample



Highly Absorbing Regions — Weakly Absorbing Regions

Segmentation

…
x 1-2 bit
~ 2GB / sample



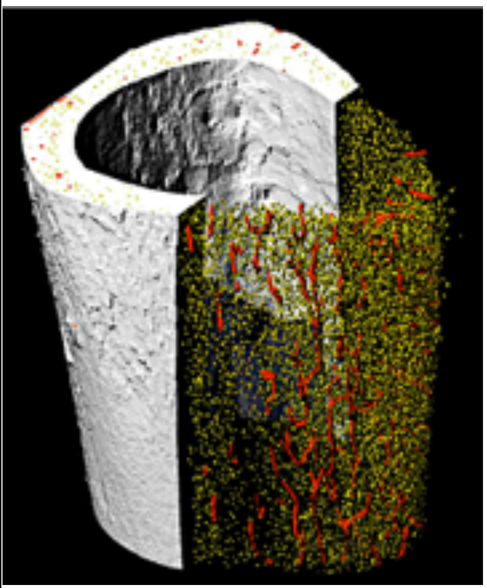
still a long way to go
….

1. Motivation

Why do we do experiments?

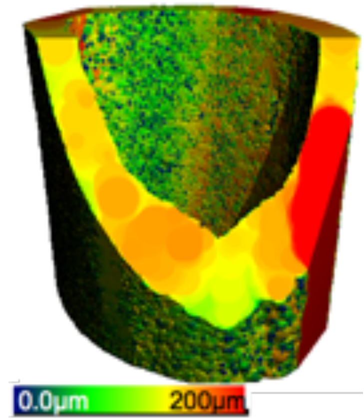
1. To test a hypothesis

Segmented Image



Morphology

Cortical Bone



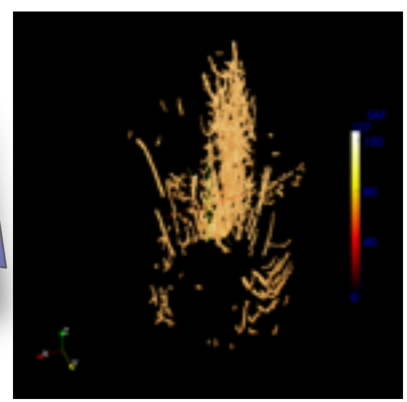
Mean Thickness

= 122.5μm

Std Thickness

= 20μm

Canal Network



Component

Labeling

Canal Count = 30

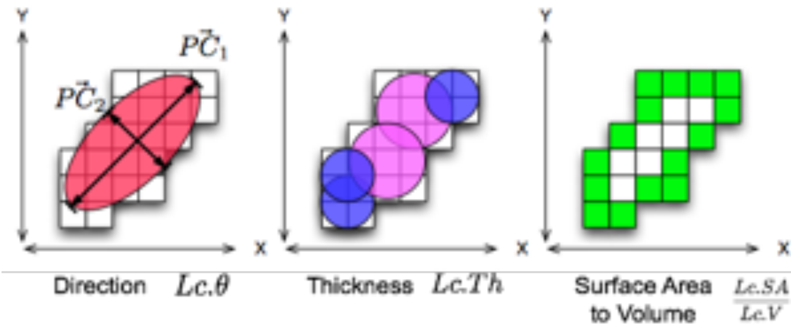
Mean Canal Volume = 40,000μm^3

Canal Volume Fraction = 3%

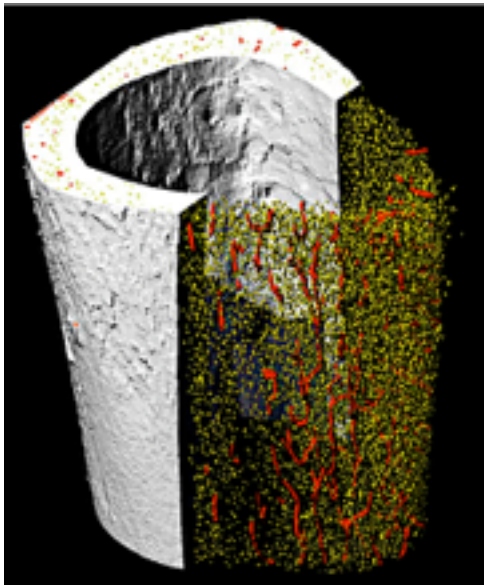
1. Morphometry

Global and feature measurements

Classification of image measurements



Segmented Image



Ensemble, summarizing, entire structure analysis

Component Level Analysis looking at individual features

Location, Shape, Thickness, Correlation, Wavelet/Fourier Representation, …

1. Morphometry

Determining location

Bounding box

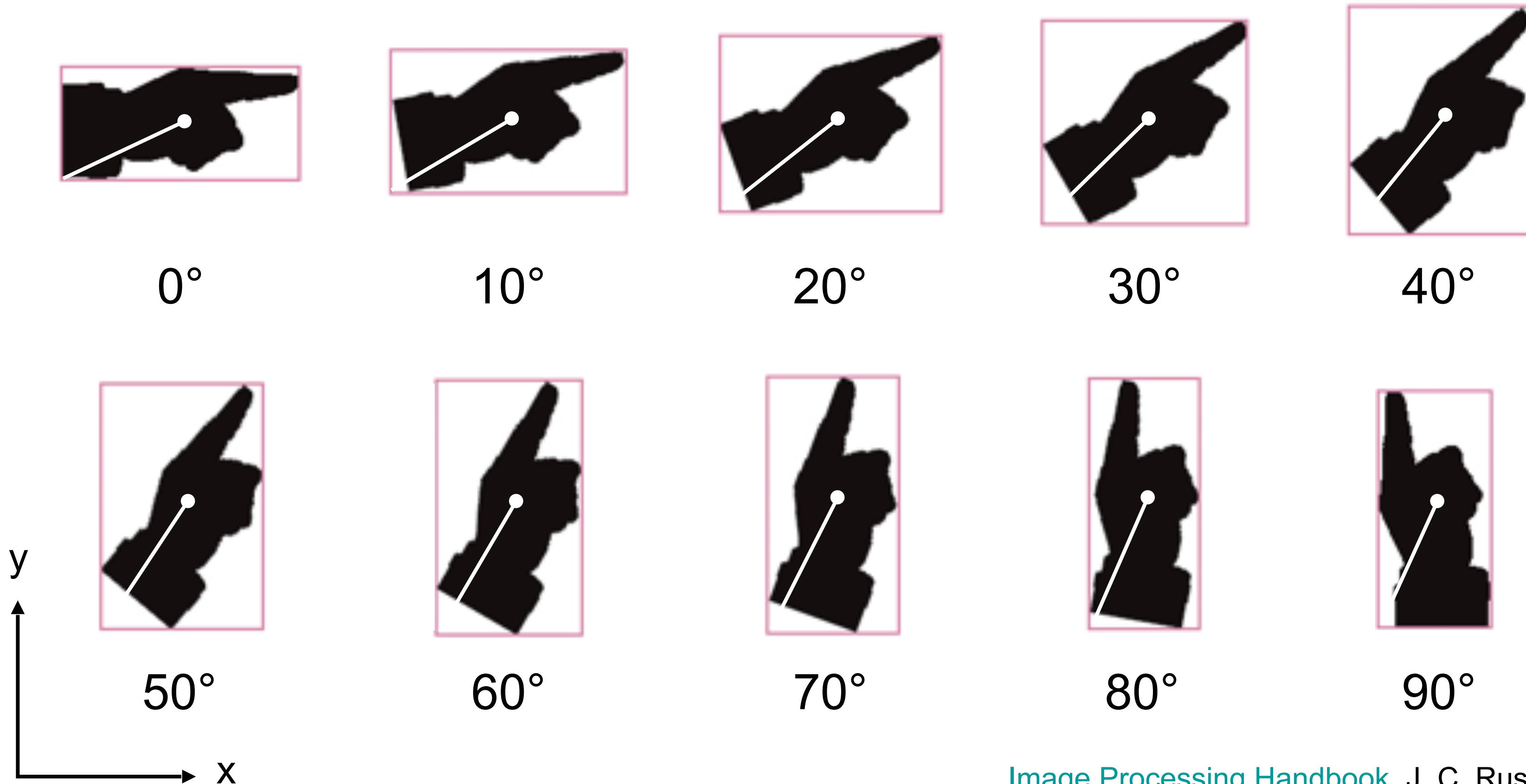
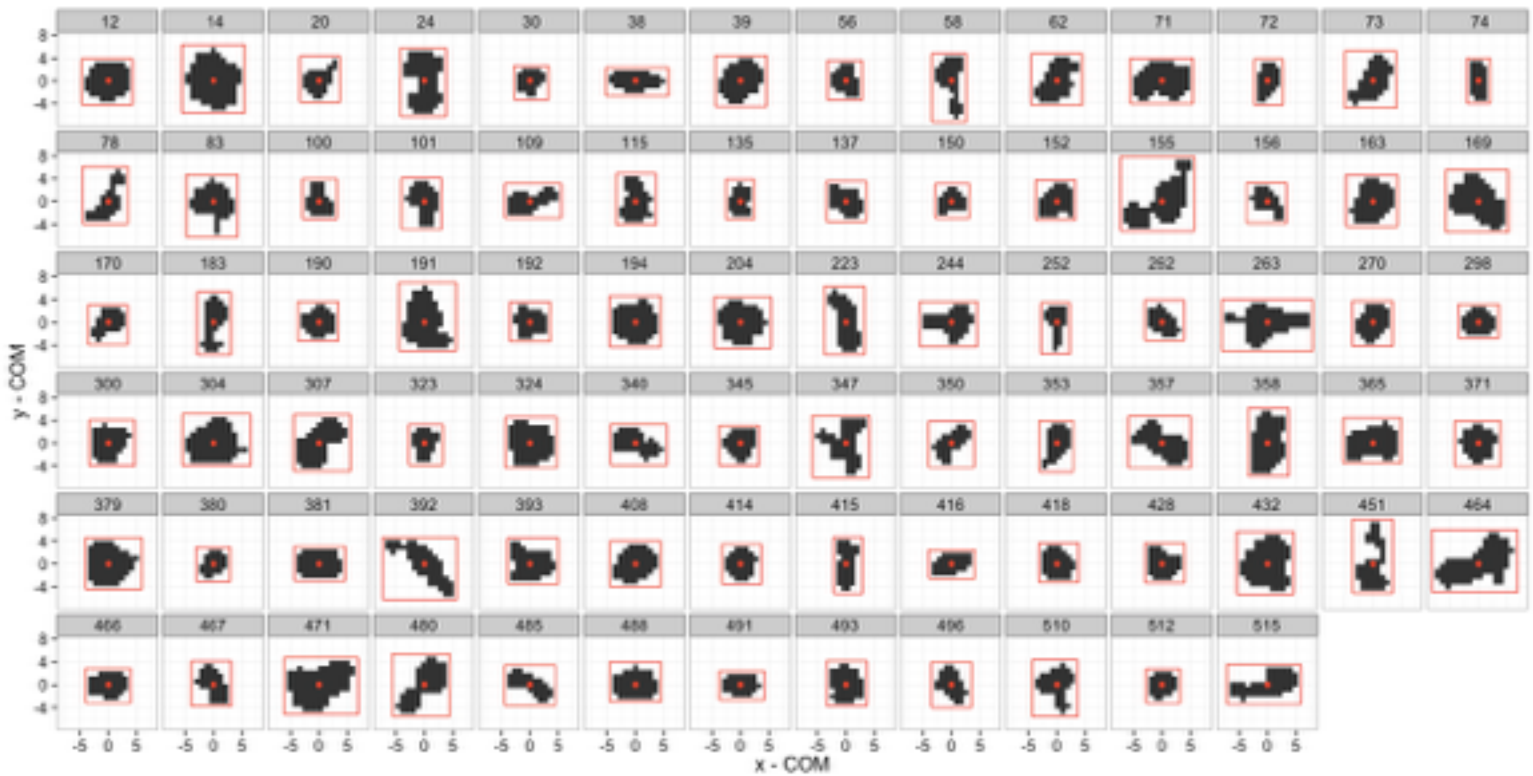


Image Processing Handbook, J. C. Russ

1. Morphometry

Determining location

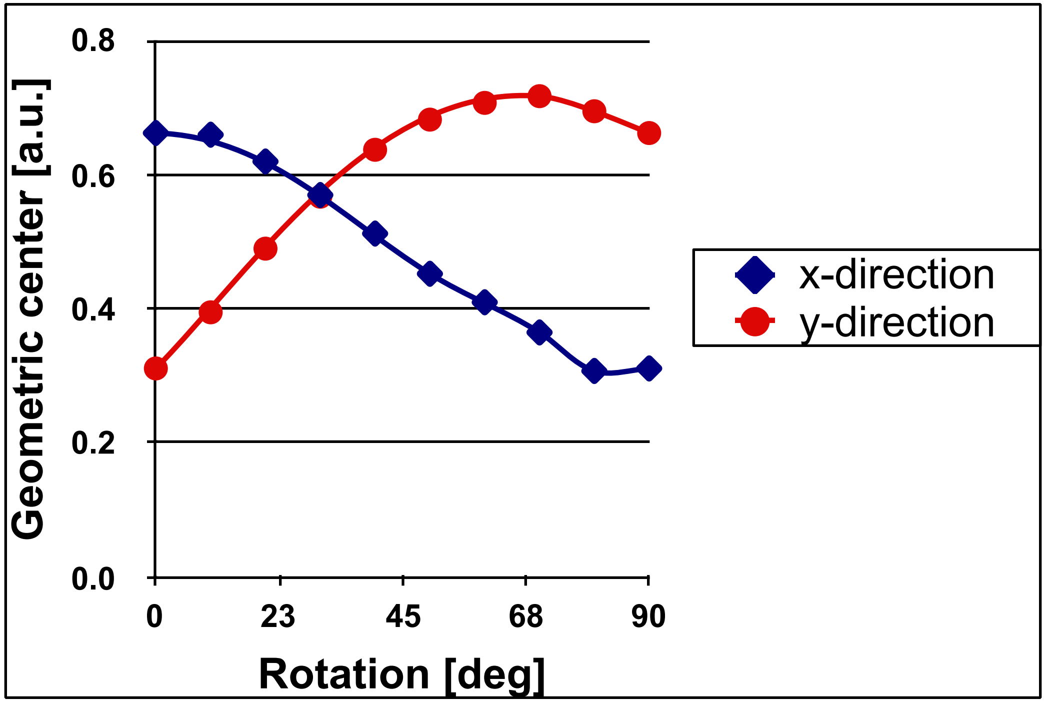
Bounding box of many different cells



1. Morphometry

Determining location

Bounding box



=> Sensitive to object orientation

1. Morphometry

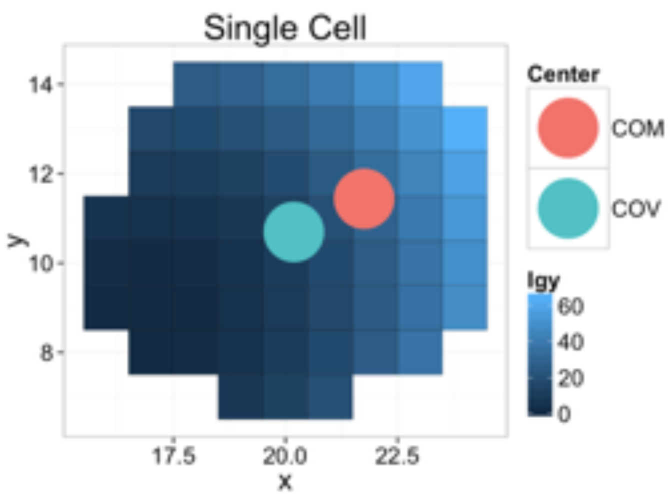
Determining location

Center of Volume

$$\bar{x} = \frac{1}{N} \sum_{\vec{v} \in I_{id}} \vec{v} \cdot \vec{i}$$

$$\bar{y} = \frac{1}{N} \sum_{\vec{v} \in I_{id}} \vec{v} \cdot \vec{j}$$

$$\bar{z} = \frac{1}{N} \sum_{\vec{v} \in I_{id}} \vec{v} \cdot \vec{k}$$



Center of Mass

$$\Sigma I_{gy} = \frac{1}{N} \sum_{\vec{v} \in I_{id}} I_{gy}(\vec{v})$$

$$\bar{x} = \frac{1}{\Sigma I_{gy}} \sum_{\vec{v} \in I_{id}} (\vec{v} \cdot \vec{i}) I_{gy}(\vec{v})$$

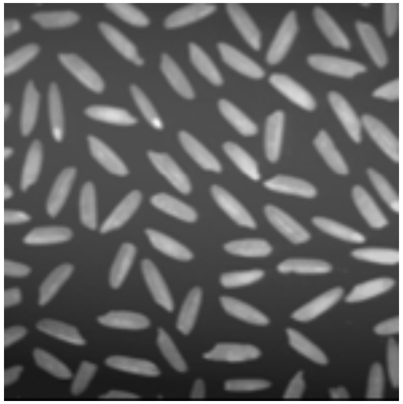
$$\bar{y} = \frac{1}{\Sigma I_{gy}} \sum_{\vec{v} \in I_{id}} (\vec{v} \cdot \vec{j}) I_{gy}(\vec{v})$$

$$\bar{z} = \frac{1}{\Sigma I_{gy}} \sum_{\vec{v} \in I_{id}} (\vec{v} \cdot \vec{k}) I_{gy}(\vec{v})$$

1. Morphometry

Orientation

Principal axis

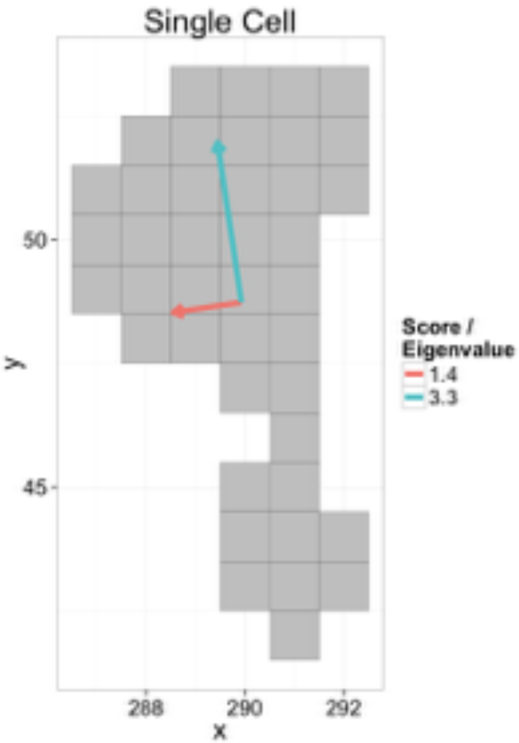


Original

Threshold →

Thresholded

Eigen-transform →

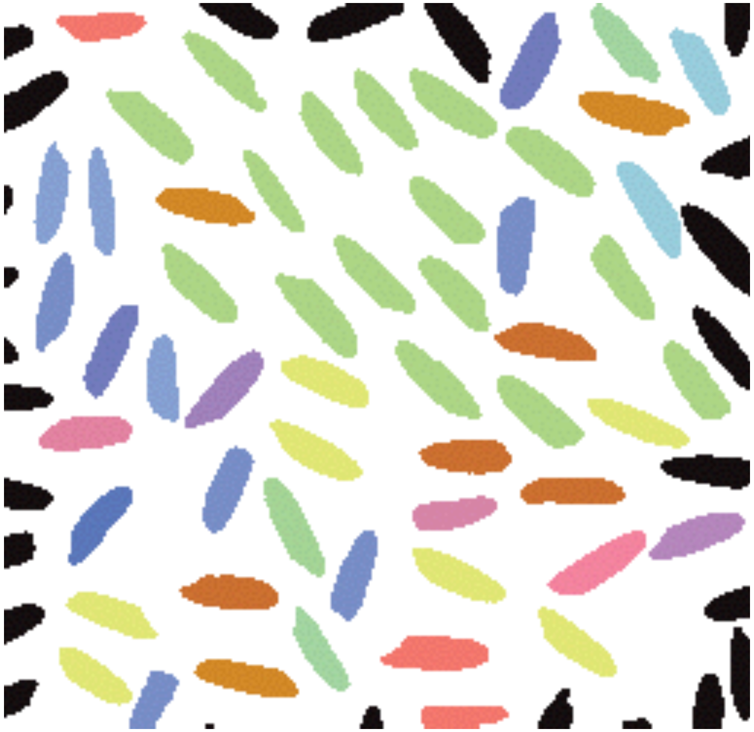


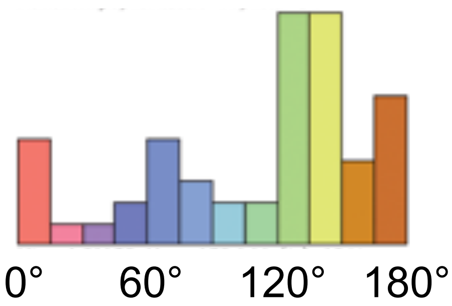
1. Morphometry

Orientation

Principal axis

Orientation





1. Morphometry

In 3D

Centroid and Position Independence

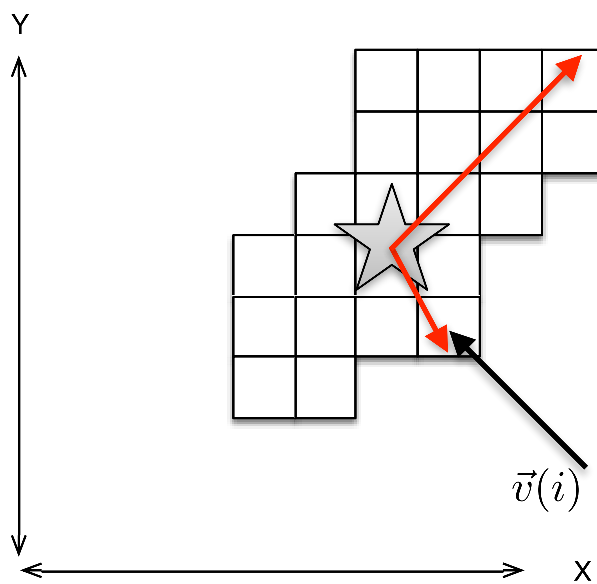
Original Voxel Positions

$$\vec{v}(i) = [v_x(i), v_y(i), v_z(i)]$$

Calculate Centroid

Subtract centroid

$$\vec{p}(i) = \vec{v}(i) - \frac{1}{m}\sum_{j=0}^{m}\vec{v}(j)$$



$$\boxed{\vec{p}(i) = \vec{v}(i) - \frac{1}{m}\sum_{j=0}^{m}\vec{v}(j)}$$

$$\vec{v}(i) = [v_x(i), v_y(i), v_z(i)]$$

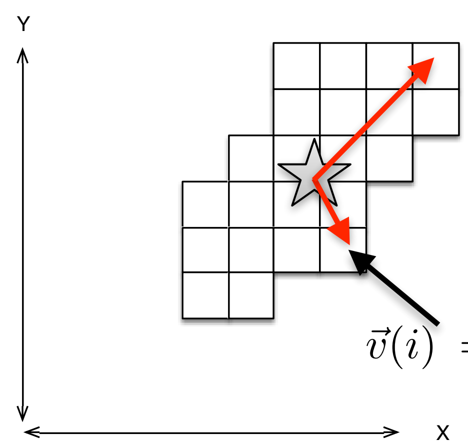
1. Morphometry

In 3D

Principal axis

Calculate covariance matrix -> shape tensor

$$\mathbf{S} = \frac{1}{m}\sum_{i=0}^{m}\begin{bmatrix} p_x(i)p_x(i) & p_x(i)p_y(i) & p_x(i)p_z(i) \\ p_y(i)p_x(i) & p_y(i)p_y(i) & p_y(i)p_z(i) \\ p_z(i)p_x(i) & p_z(i)p_y(i) & p_z(i)p_z(i) \end{bmatrix}$$

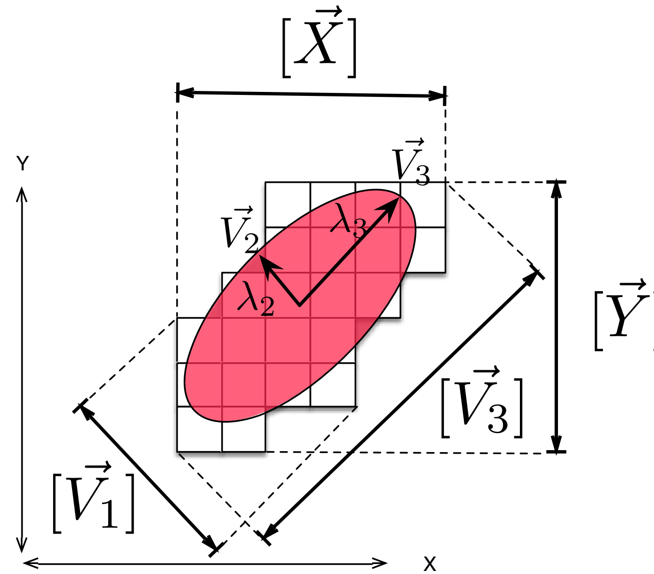


$$\boxed{\vec{p}(i) = \vec{v}(i) - \frac{1}{m}\sum_{j=0}^{m}\vec{v}(j)}$$

$$\vec{v}(i) = [v_x(i), v_y(i), v_z(i)]$$

Eigentransform

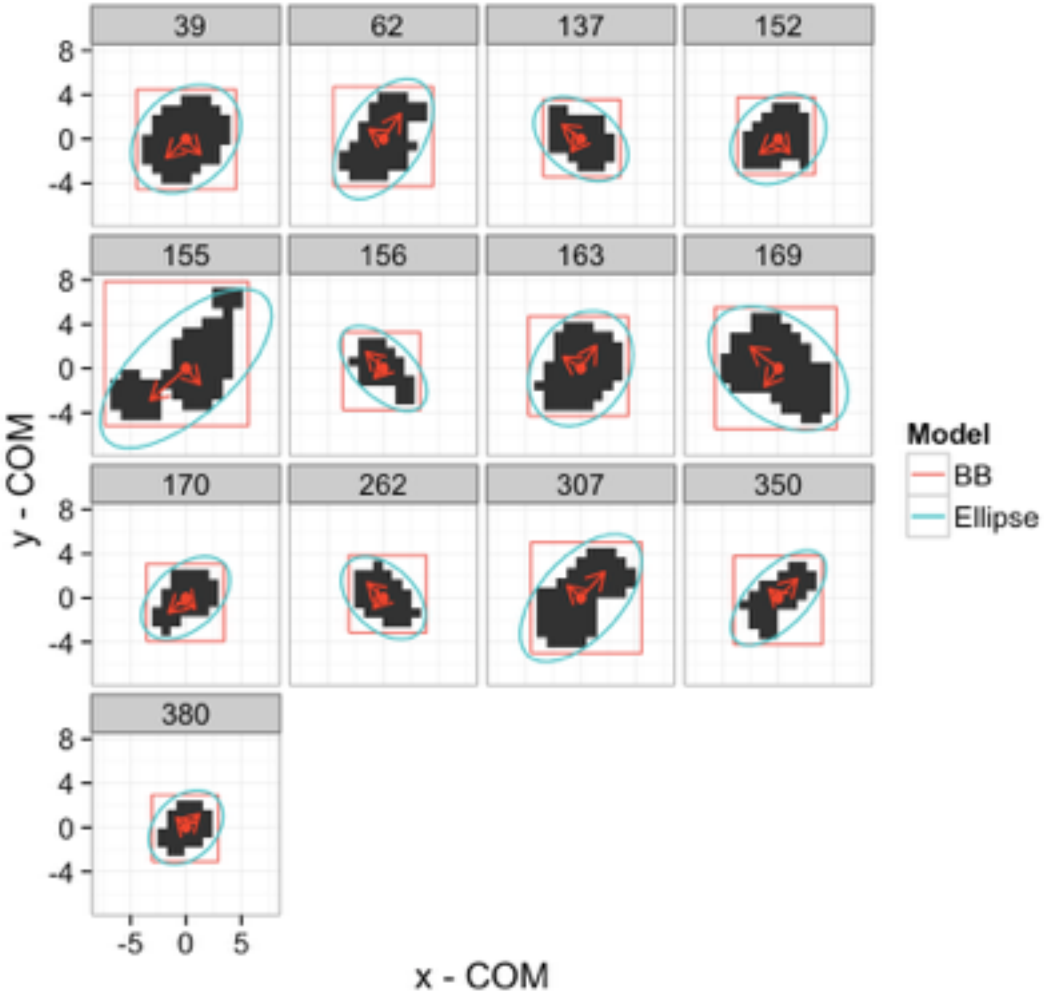
Eigenvectors are primary orientation (V1,V2, V3)
Eigenvalue are ‘lengths’
ƛ1, ƛ2, ƛ3



1. Morphometry

In 3D

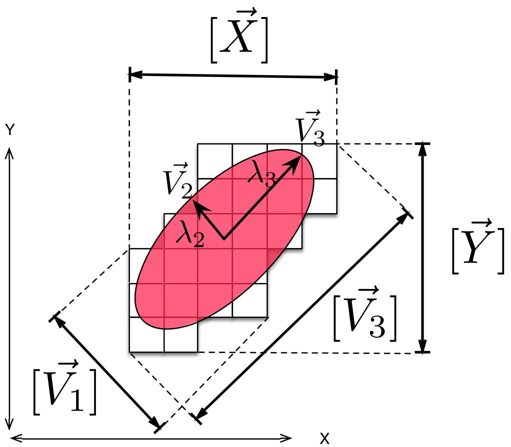
Why Eigentransform?



1. Morphometry

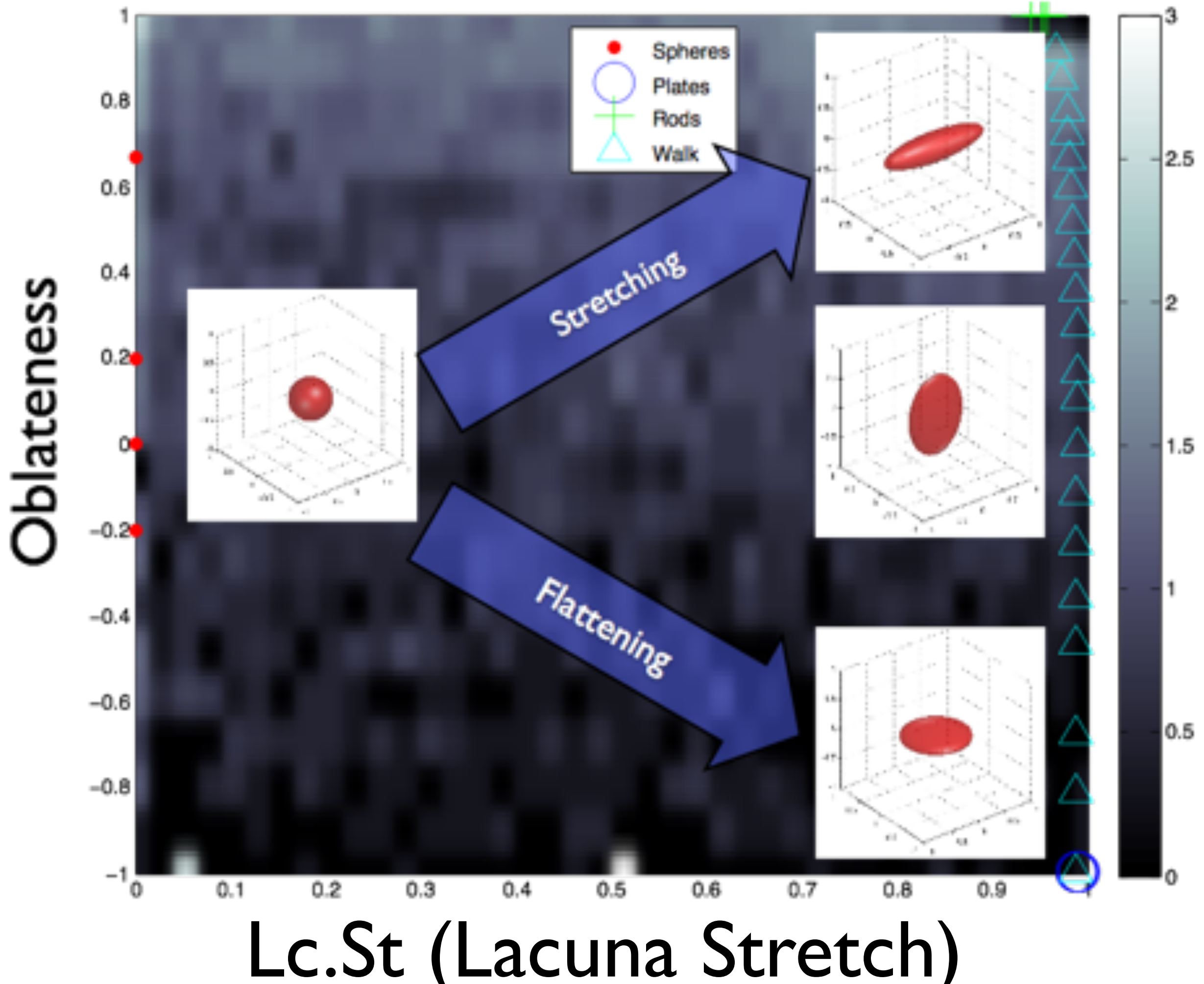
Shape

Anisotropy



$$Lc.St = \frac{\lambda_{S,3} - \lambda_{S,1}}{\lambda_{S,3}}$$

$$Lc.Ob = 2\frac{\lambda_{S,2} - \lambda_{S,1}}{\lambda_{S,3} - \lambda_{S,1}} - 1$$

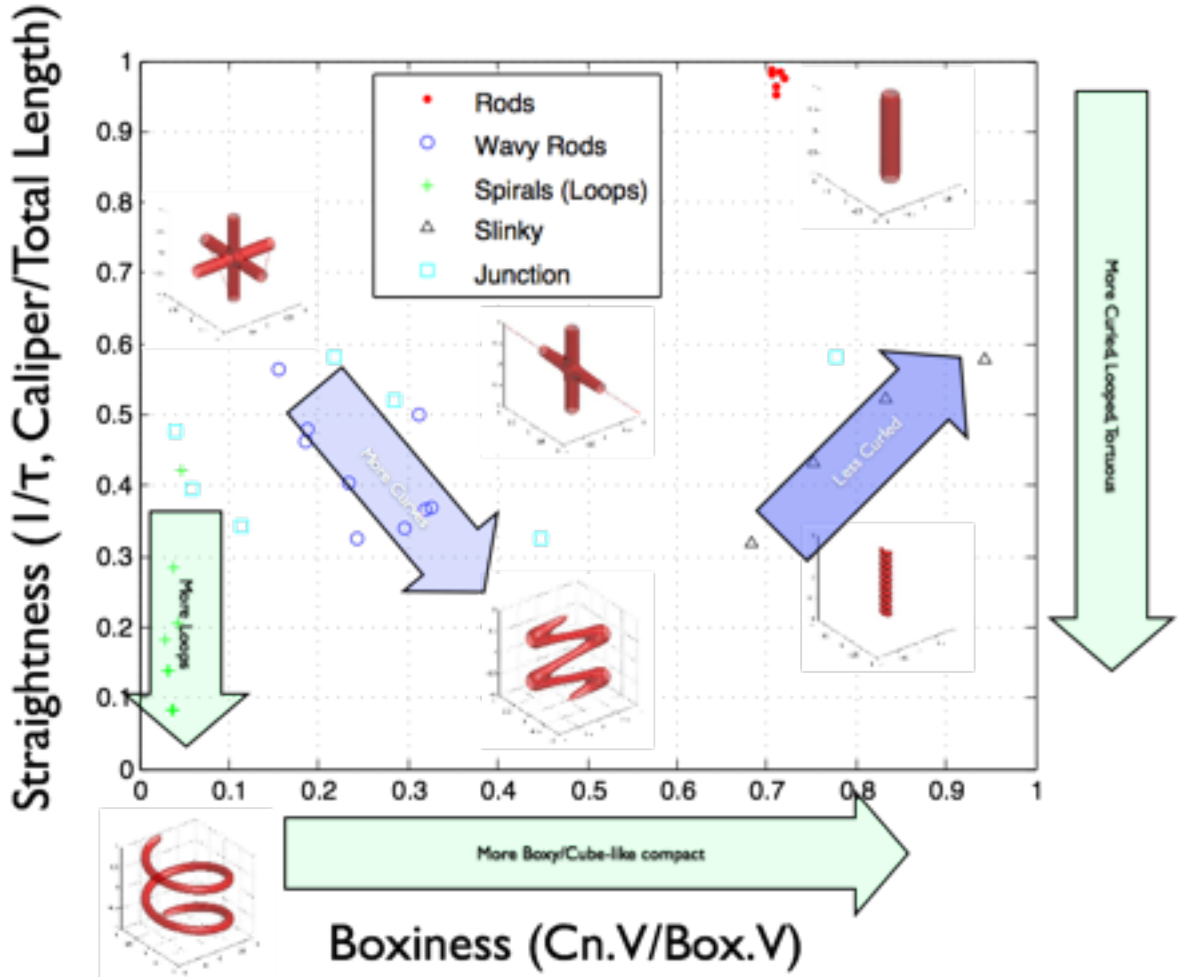


Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

1. Morphometry

Shape

Other metrics

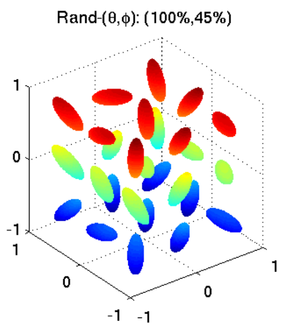


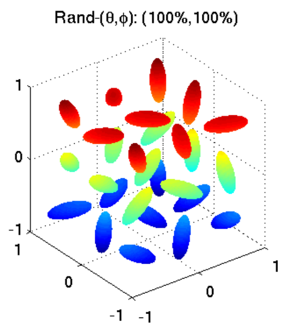
1. Morphometry

Orientation

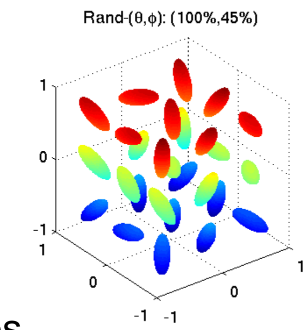
Alignment

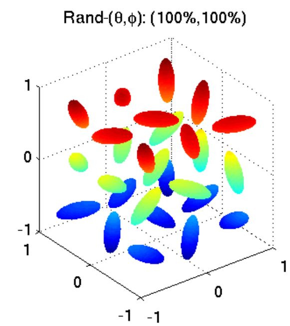
Features



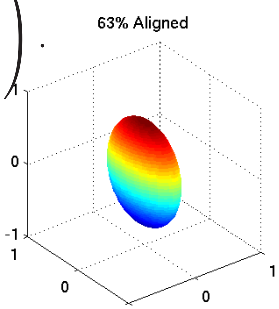


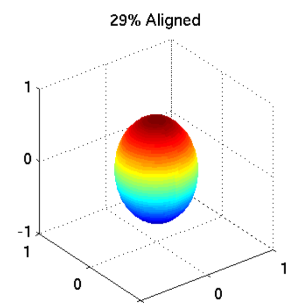
Make a shape tensor from orientations -> shape of shapes



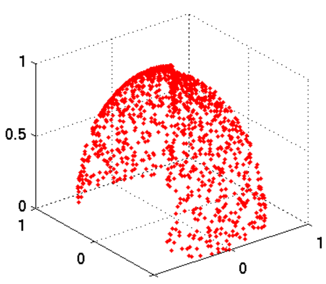


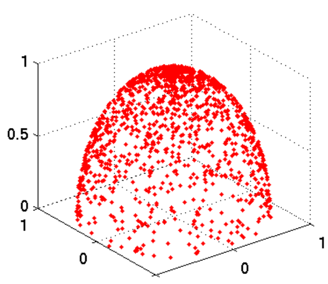
$$\mathbf{M} = \langle \mathbf{m} \rangle = \begin{pmatrix} \langle X^2 \rangle & \langle XY \rangle & \langle XZ \rangle \\ \langle YX \rangle & \langle Y^2 \rangle & \langle YZ \rangle \\ \langle ZX \rangle & \langle ZY \rangle & \langle Z^2 \rangle \end{pmatrix}.$$





Primary Orientation Vector





Take anisotropy of this tensor

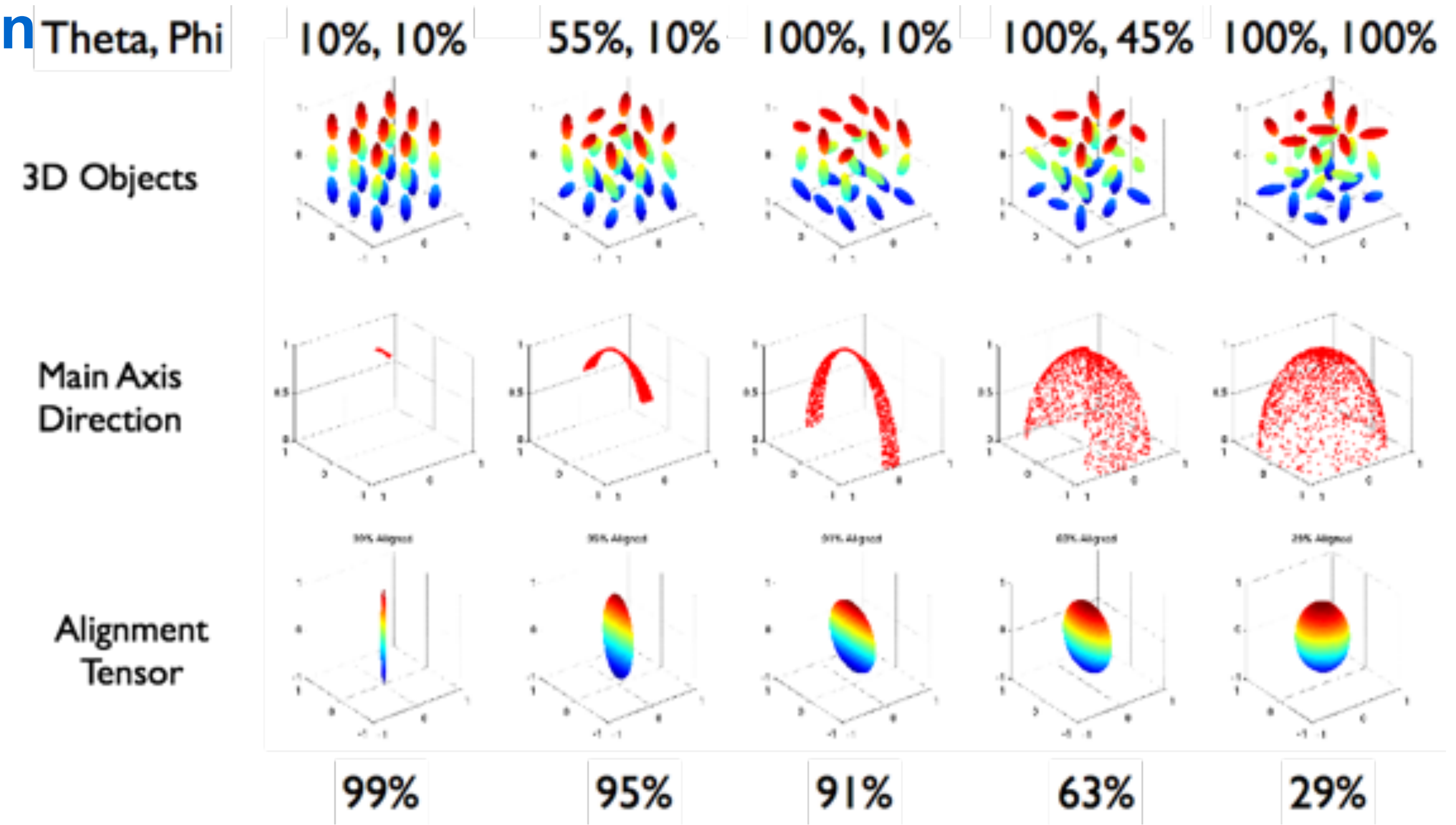
63% **29%**

[1] C. Raufaste, P. Marmottant, and F. Graner, "Discrete rearranging disordered patterns: Prediction of elastic and plastic behavior, and application to two-dimensional foams," *Physical Review E*, vol. 81, Mar. 2010.
[2] Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

1. Morphometry

Orientation

Alignment



[1] C. Raufaste, P. Marmottant, and F. Graner, "Discrete rearranging disordered patterns: Prediction of elastic and plastic behavior, and application to two-dimensional foams," *Physical Review E*, vol. 81, Mar. 2010.
[2] Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

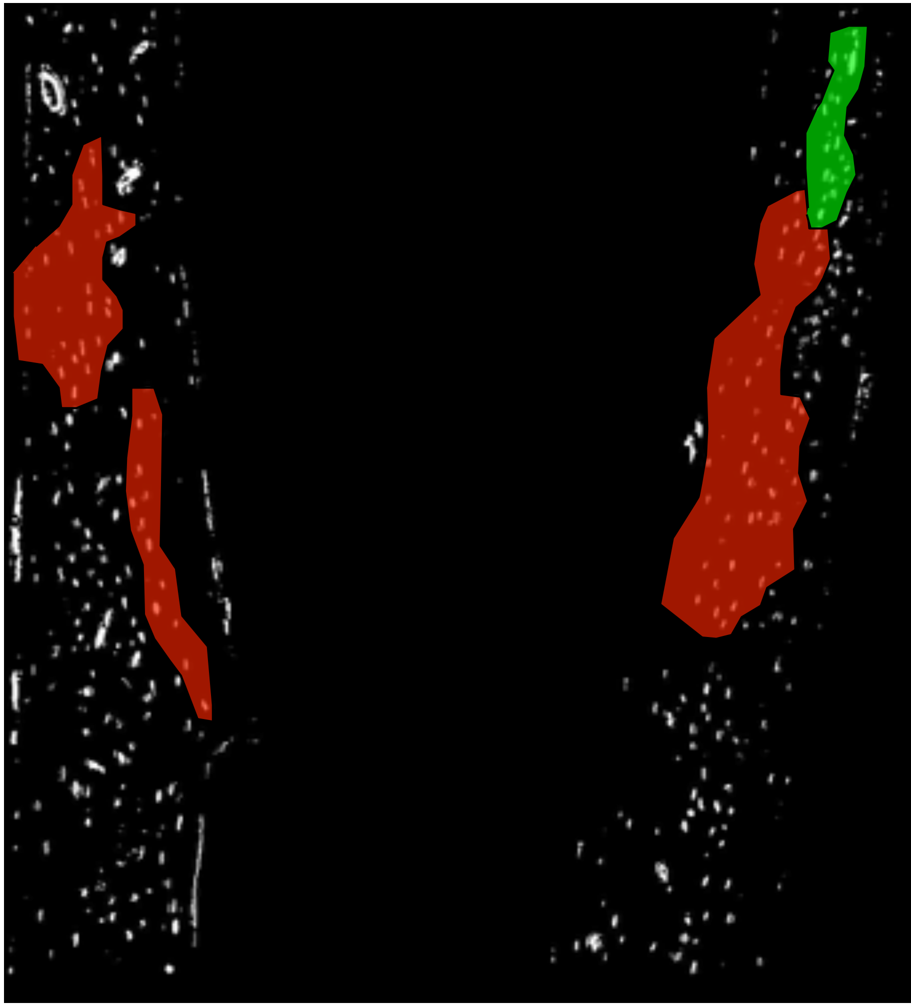
1. Morphometry

Orientation

Alignment

Alignment patterns have been observed, but NOT quantified in 2D slices [1] and small-scale 3D studies

Alignment plays important role in identification of collagen in various bone types [3]



[1] G. Marotti, "Osteocyte orientation in human lamellar bone and its relevance to the morphometry of periosteocytic lacunae," *Metabolic Bone Disease and Related Research*, vol. 1, 1979, pp. 325-333.

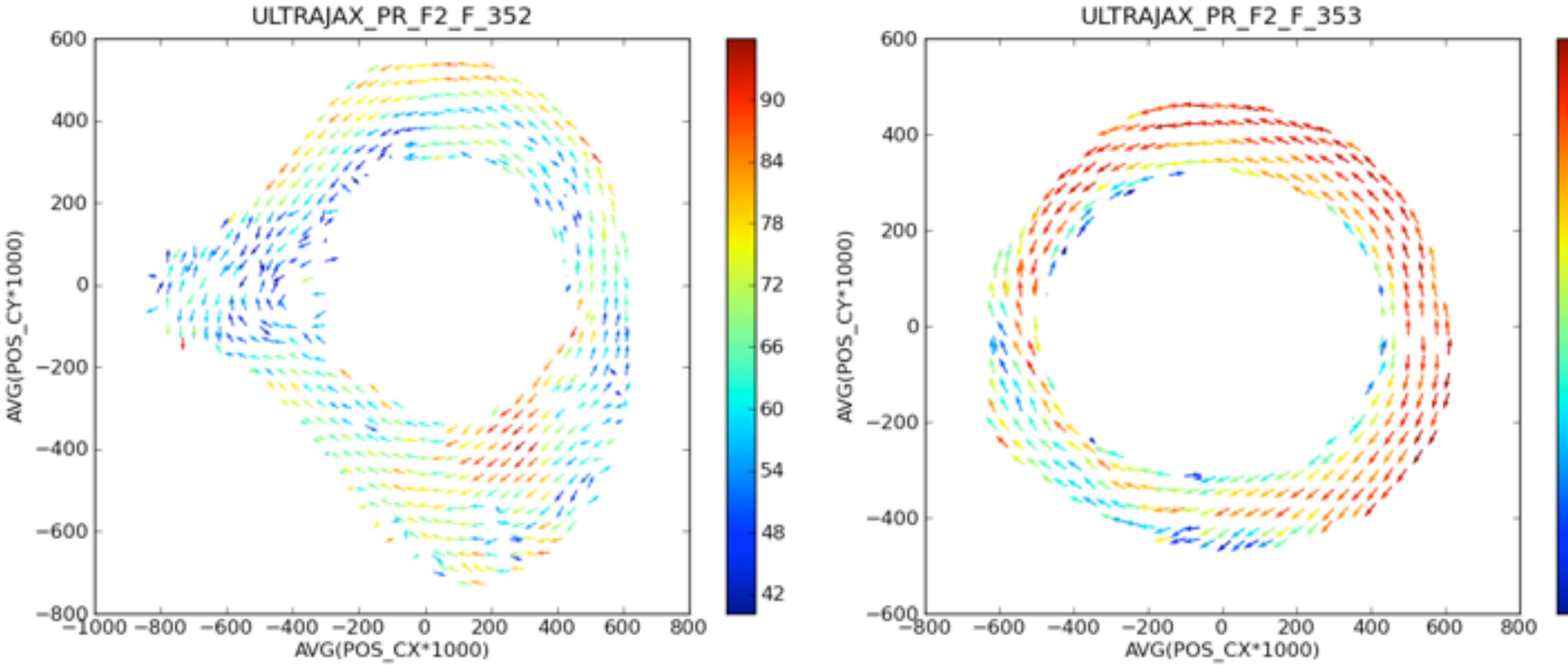
[2] A. Vatsa, R.G. Breuls, C.M. Semeins, P.L. Salmon, T.H. Smit, and J. Klein-Nulend, "Osteocyte morphology in fibula and calvaria --- is there a role for mechanosensing?," *Bone*, vol. 43, Sep. 2008, pp. 452-8.

[3] M.-grazia A. Ã, J. Gill, and A. Lomovtsev, "Orientation of collagen at the osteocyte lacunae in human secondary osteons," *Journal of Biomechanics*, vol. 41, 2008, pp. 3426-3435.

1. Morphometry

Orientation

Alignment

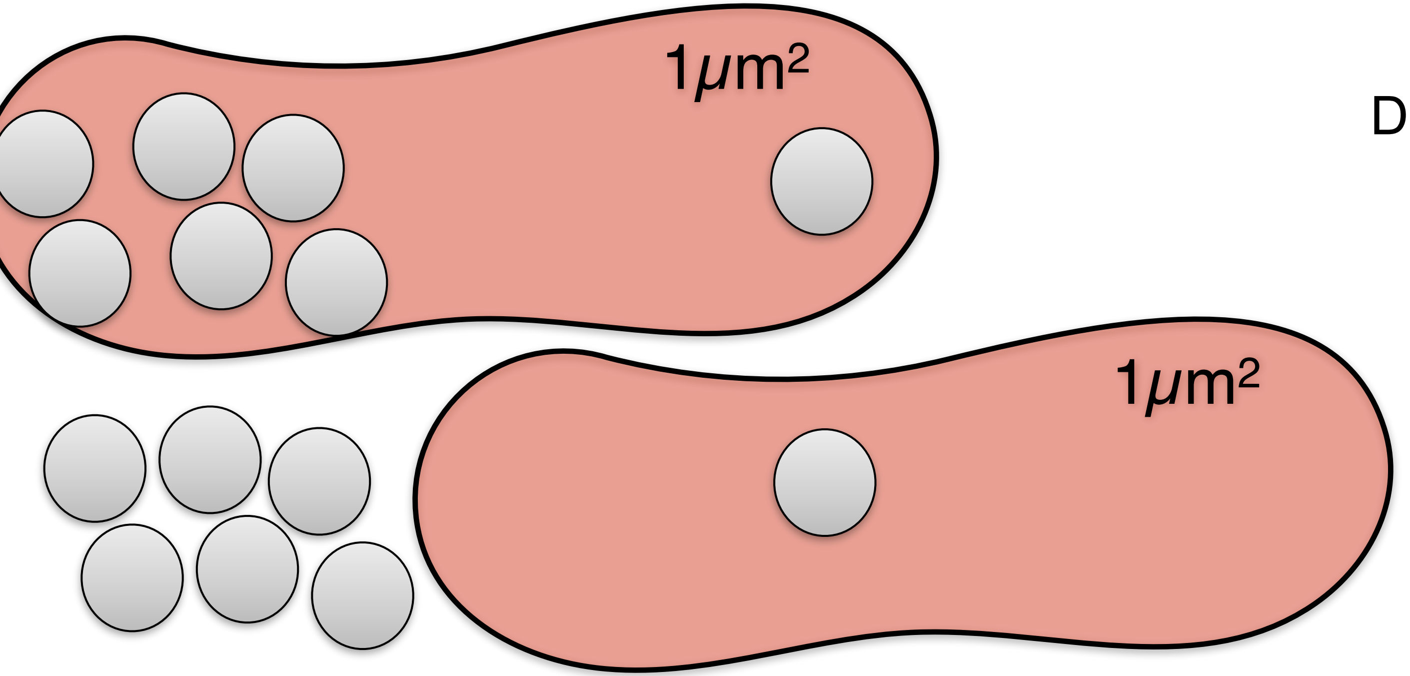


1. Morphometry

Spatial Density

Number of features per unit volume

Standard approach is to count the number of features and divide by the volume of the region



Density is 7 / μm^2

Density is 1 / μm^2

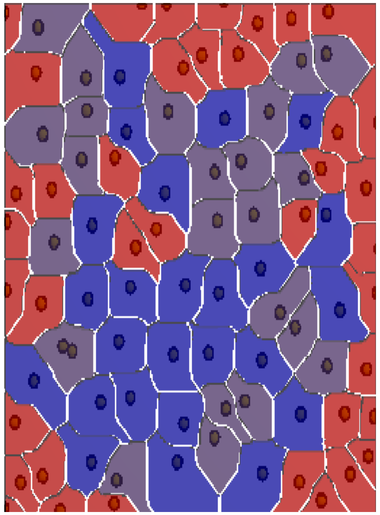
Very sensitive to region selection

1. Morphometry

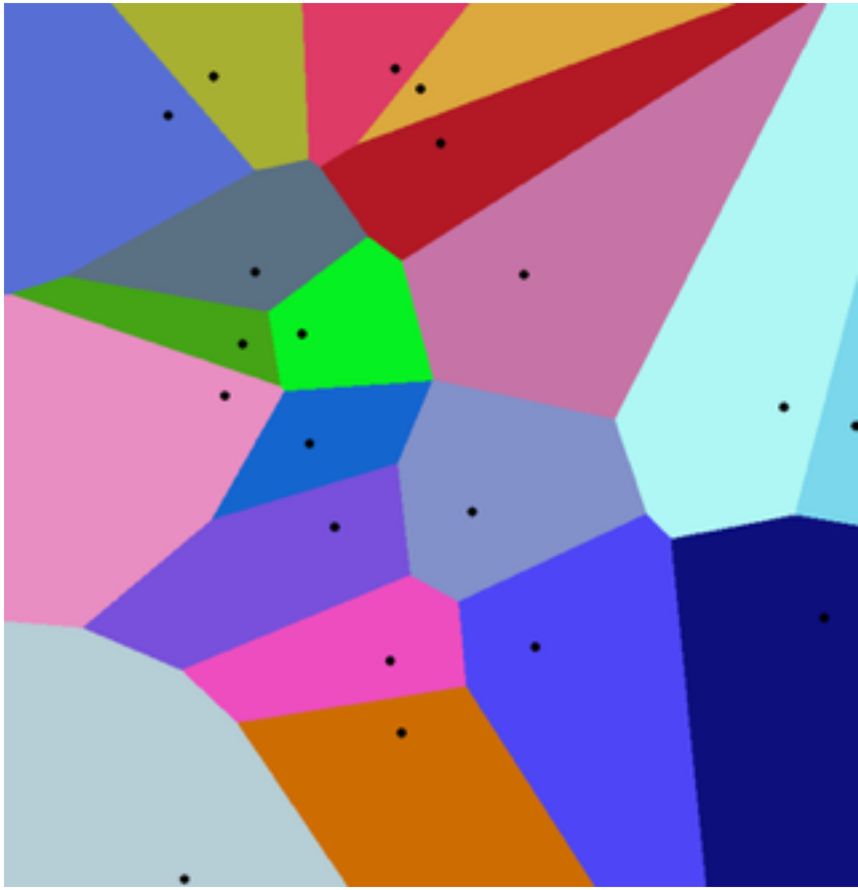
Spatial Density

Number of features per unit volume

A much better approach is to calculate the volume for each feature and define density individually (then average for larger regions



Features are shown as yellow dots, volume (territory) is shown as filled in regions



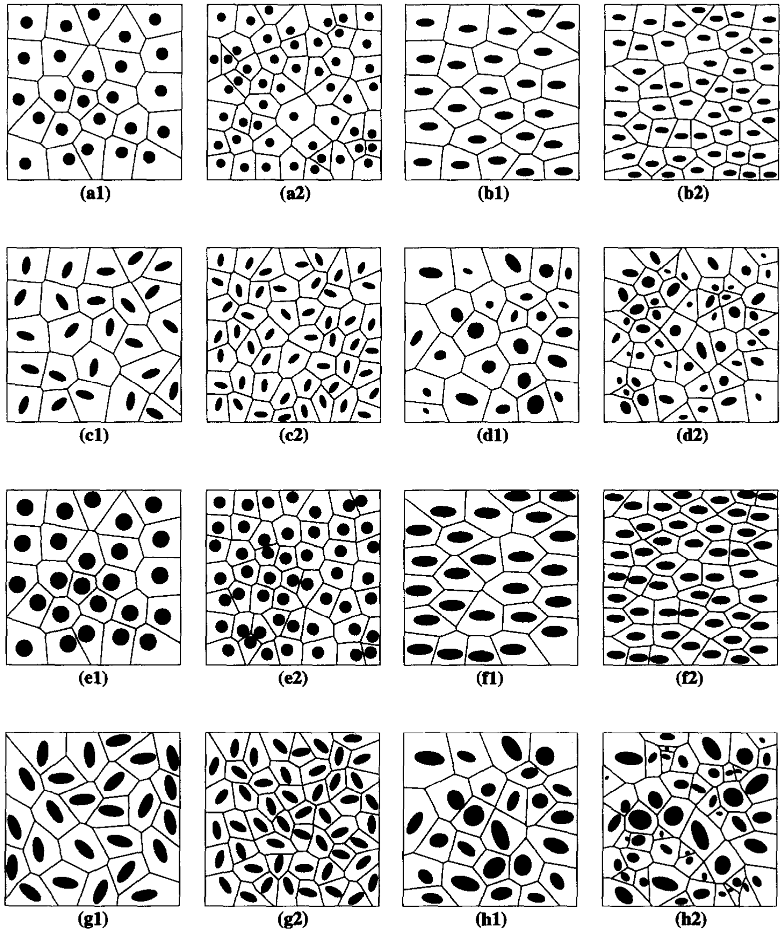
http://en.wikipedia.org/wiki/Voronoi_diagram

1. Morphometry

Spatial Density

Number of features per unit volume

Can also be used for identifying states of materials: crystalline structures have a narrow distributions of density, random structures are much higher



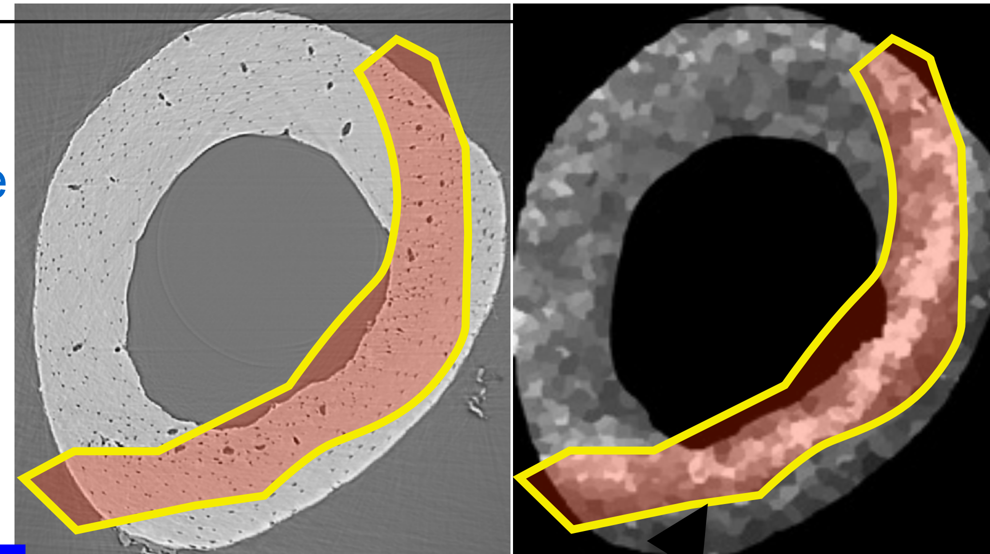
Ghosh, S. (1997). Tessellation-based computational methods for the characterization and analysis of heterogeneous microstructures. Composites Science and Technology, 57(9-10), 1187–1210. doi:10.1016/S0266-3538(97)00042-0

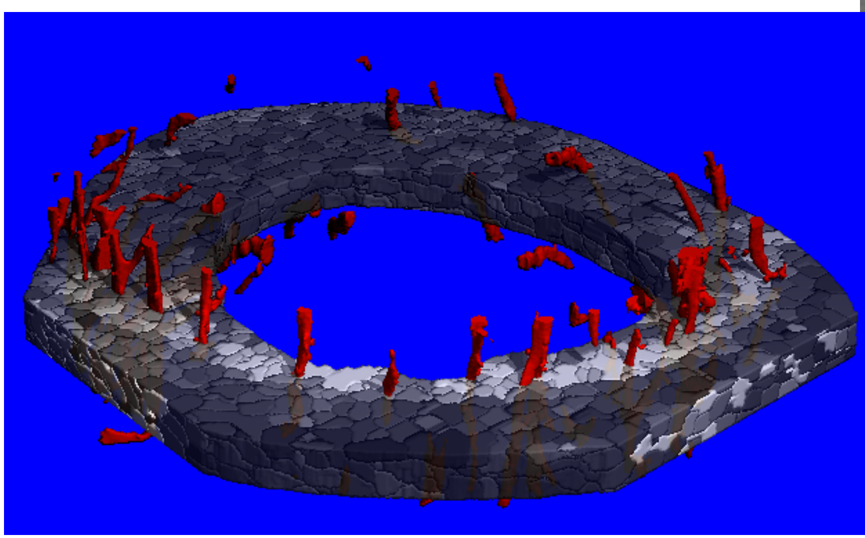
1. Morphometry

Spatial Density

Number of features per unit volume

Provides a visual indicator for density and arrangement -> also useful for qualitative interpretation of complex images





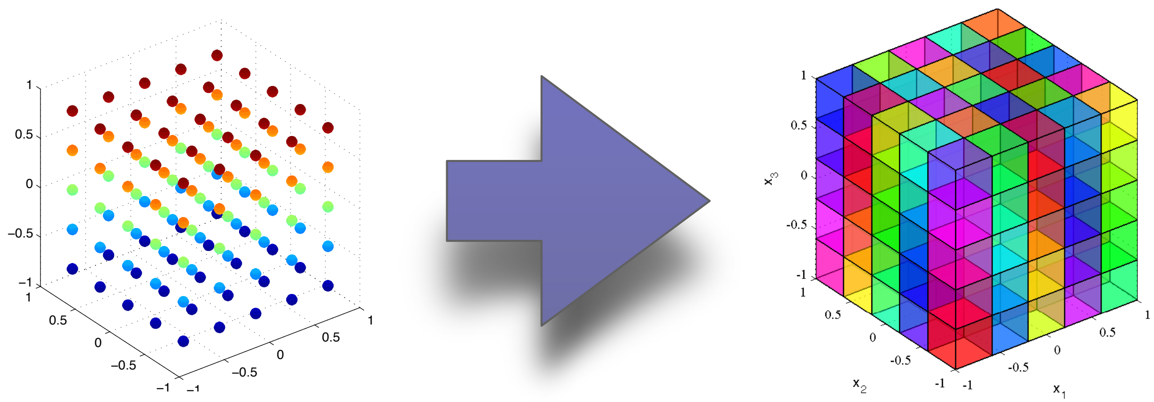
Growth direction?
High turnover volume?

1. Morphometry

Neighbor relationships

Number of adjacent neighbors

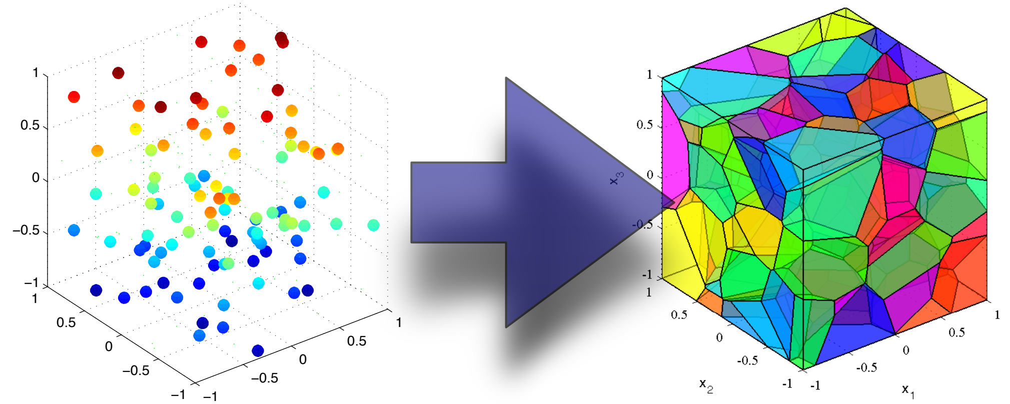
Different features are defined by component labeling
Therefore they have by definition no neighbors
We can use tessellation, specifically Voronoi tessellation to grow the features until they they touch and then call the ones in contact 'neighbors'

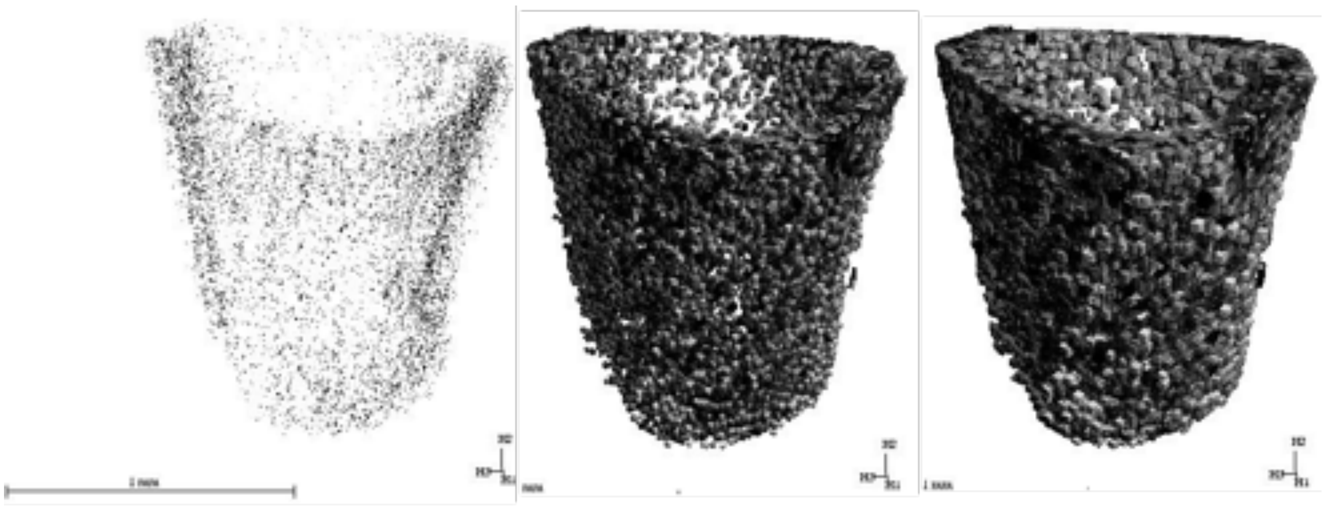


1. Morphometry

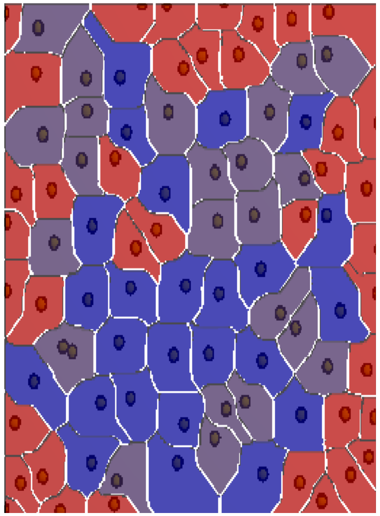
Neighbor relationships

Number of adjacent neighbors





Starting Image   4 iterations   10 iterations

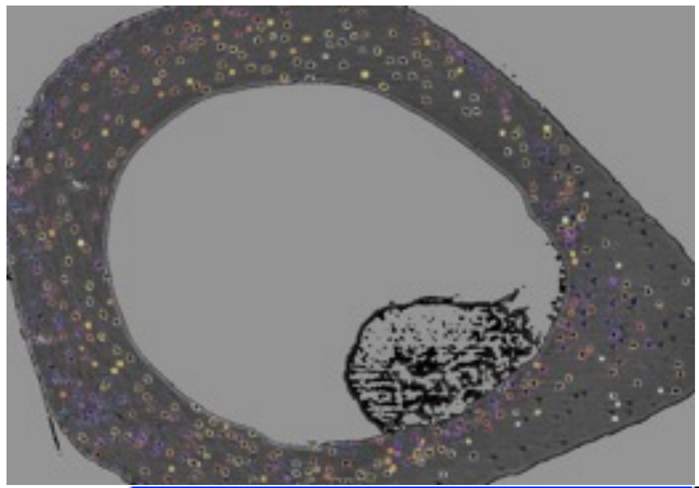


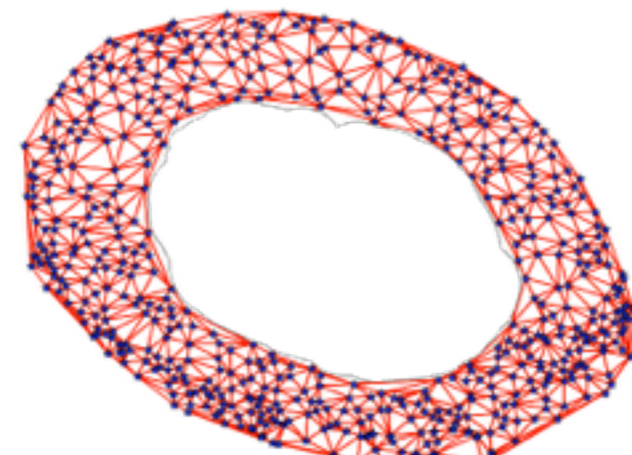
1. Morphometry

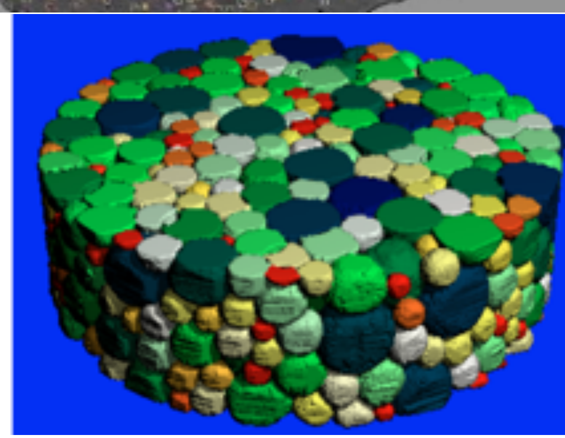
Neighbor relationships

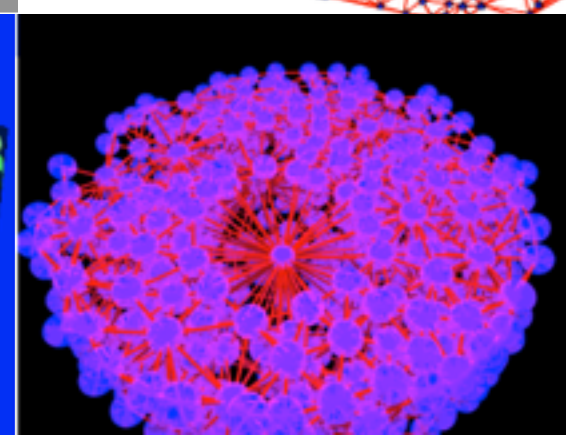
Number of adjacent neighbors

Creates a network inside of the sample which can be analyzed and studied
Are the connections evenly distributed or is there a hub and spoke arrangement?





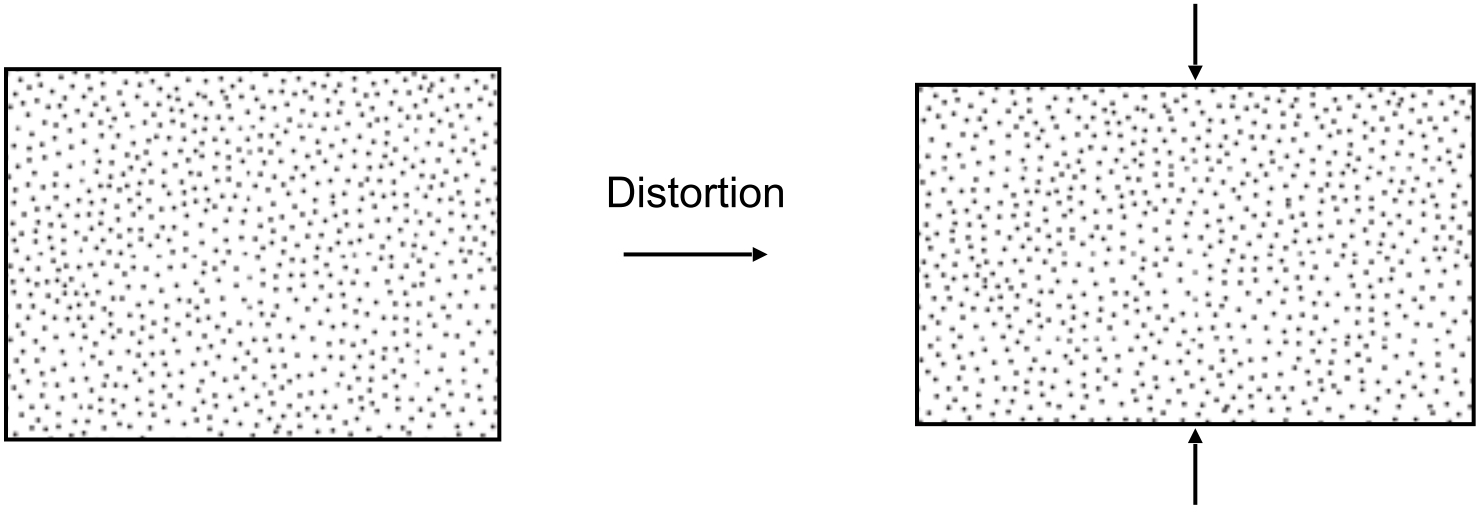




1. Morphometry

Neighbor relationships

Separation: anisotropy



Original

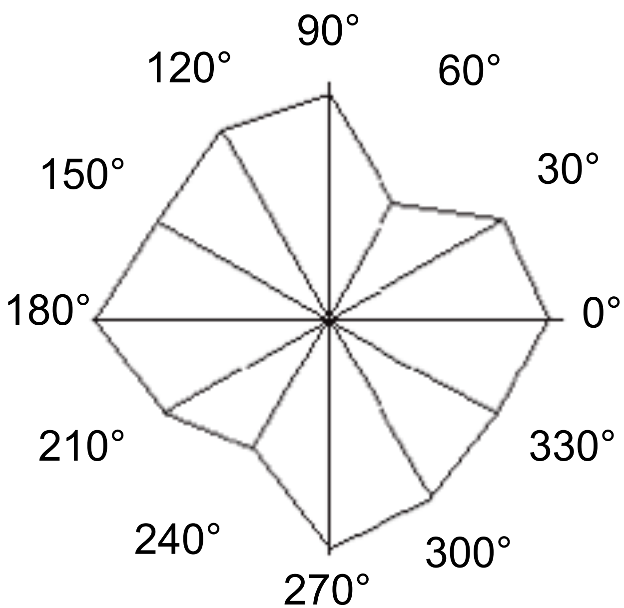
Distorted
= compressed in 1 direction

Image Processing Handbook, J. C. Russ

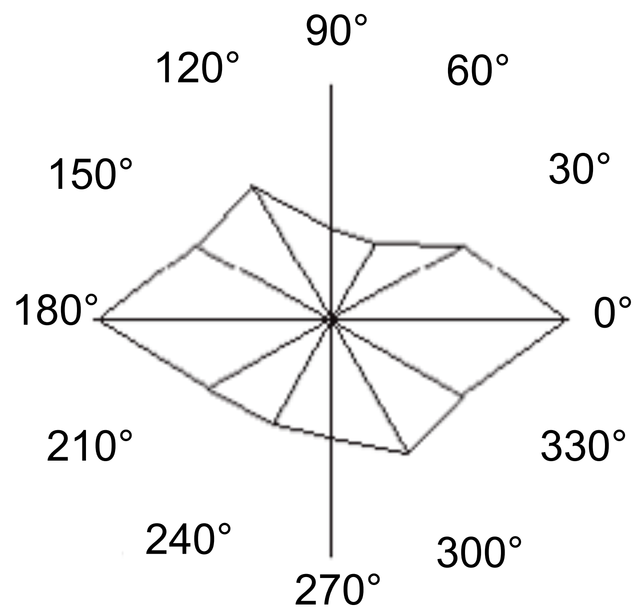
1. Morphometry

Neighbor relationships

Separation: rose plot



Original



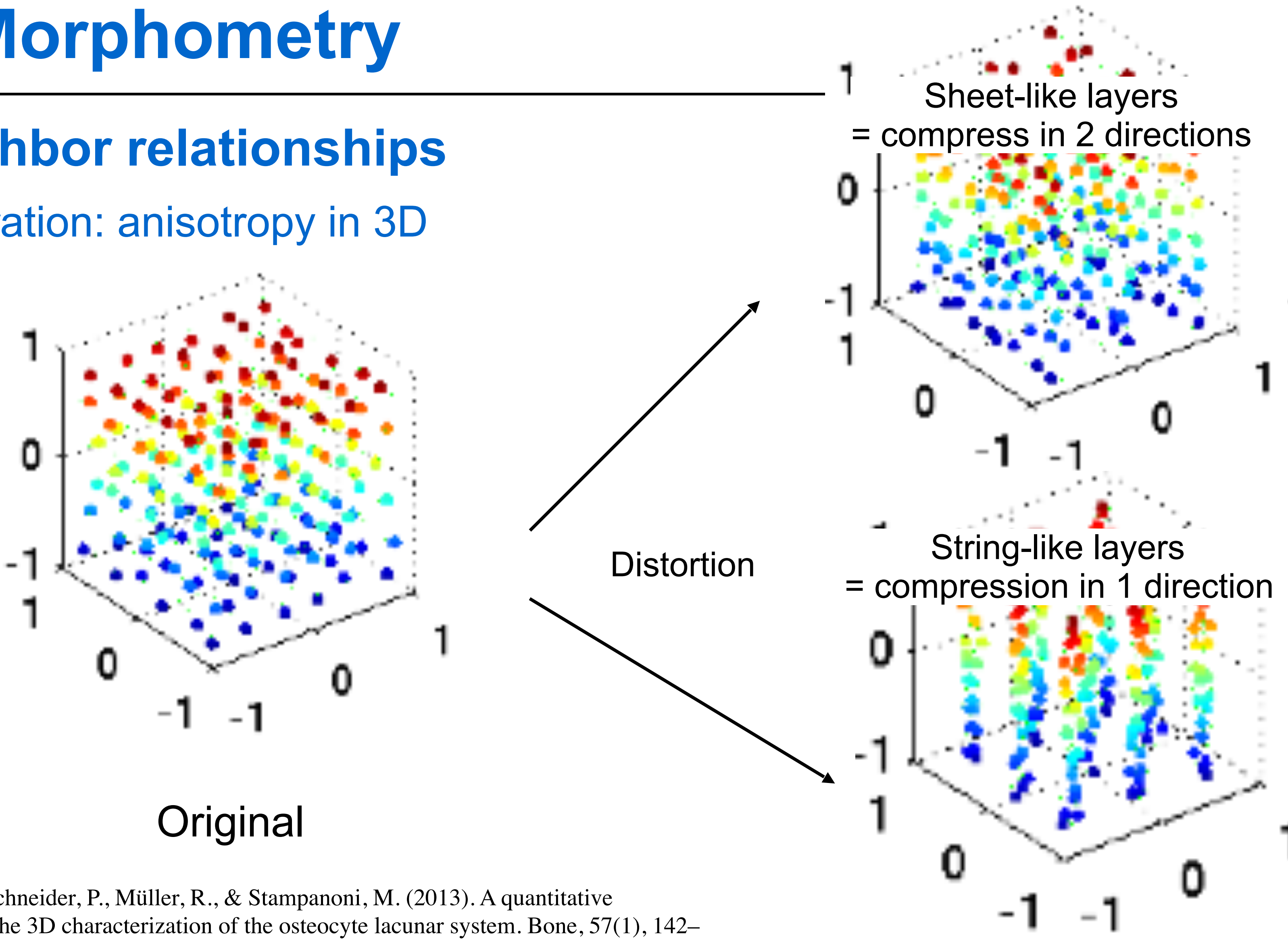
Distorted

Image Processing Handbook, J. C. Russ

1. Morphometry

Neighbor relationships

Separation: anisotropy in 3D



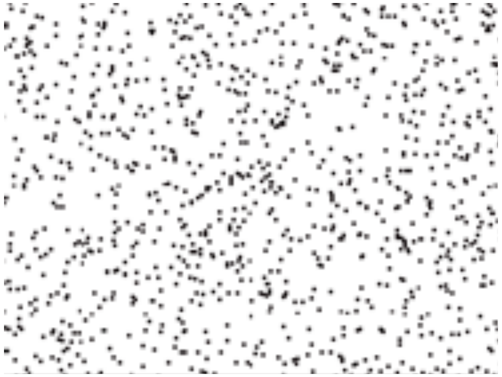
Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

1. Morphometry

Neighbor relationships

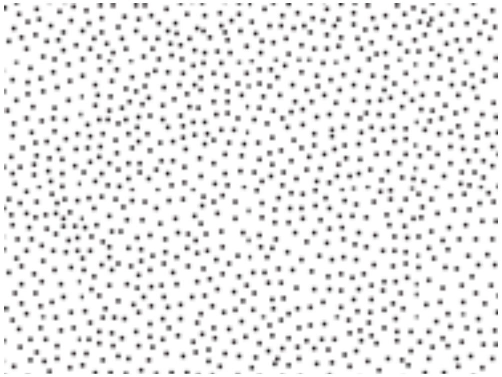
Separation: distribution

Feature distribution



Random
(independent)

Clustered



Regular/uniform
(self-avoiding)

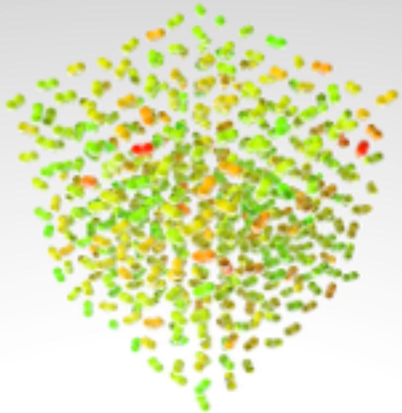
Image Processing Handbook, J. C. Russ

1. Morphometry

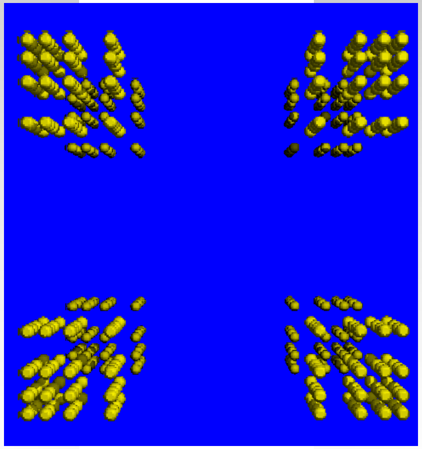
Neighbor relationships

Separation: distribution

Feature distribution



Random
(independent)



Clustered

Regular/uniform
(self-avoiding)

Image Processing Handbook, J. C. Russ

1. Morphometry

Neighbor relationships

Separation: distribution

Distance between nearest neighbors

$$Mean_{Poisson} = \frac{1}{2 \cdot \sqrt{\frac{N}{Area}}}$$

$$Std\ dev_P = \sqrt{Mean_P}$$

N: number of points

Random
(independent)

Most points have at least one close neighbor

$$Mean_{Cluster} < Mean_{Poisson}$$

$$Std\ dev_C < Std\ dev_P$$

Clustered

$$Mean_{Regular} > Mean_{Poisson}$$

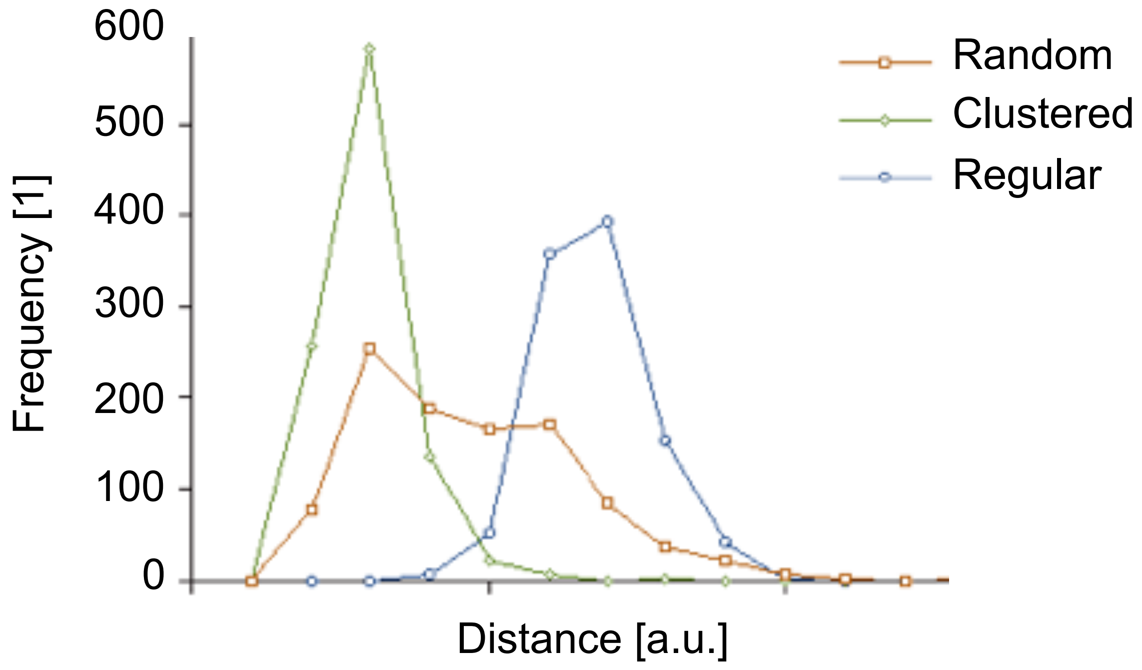
$$Std\ dev_R < Std\ dev_P$$

Regular/uniform
(self-avoiding)

1. Morphometry

Neighbor relationships

Separation: distribution



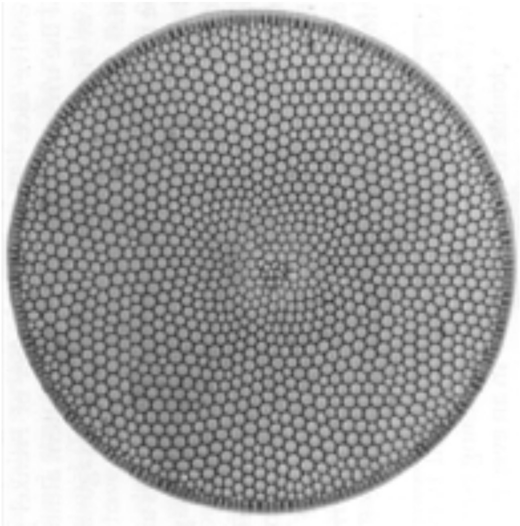
Histogram

Image Processing Handbook, J. C. Russ

1. Morphometry

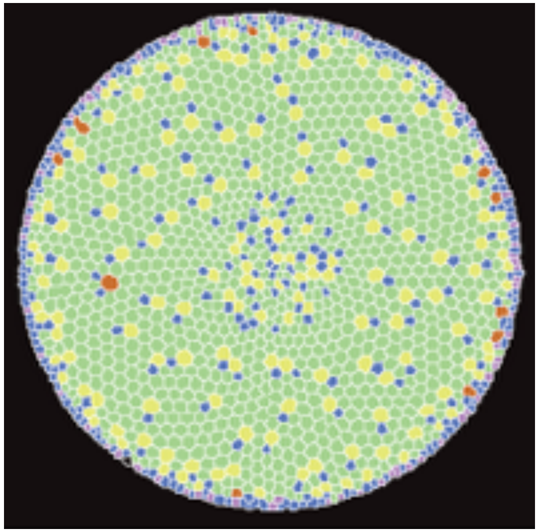
Neighbor relationships

Number of adjacent neighbors



Skeletonization and component labeling

→



Original

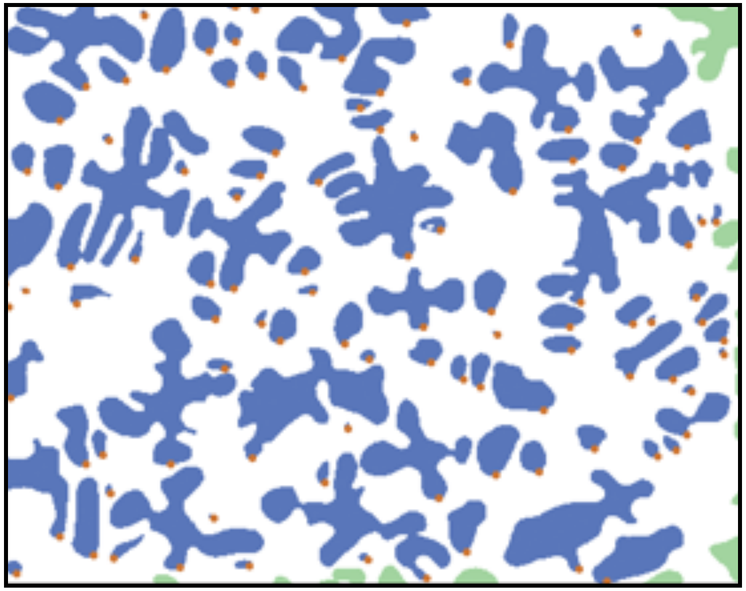
Number of neighbors

Light micrograph: diatom (phytoplankton type)
Image Processing Handbook, J. C. Russ

1. Morphometry

Counting

Unbiased counting



Do not count features touching two adjacent edges (e.g. right & bottom)

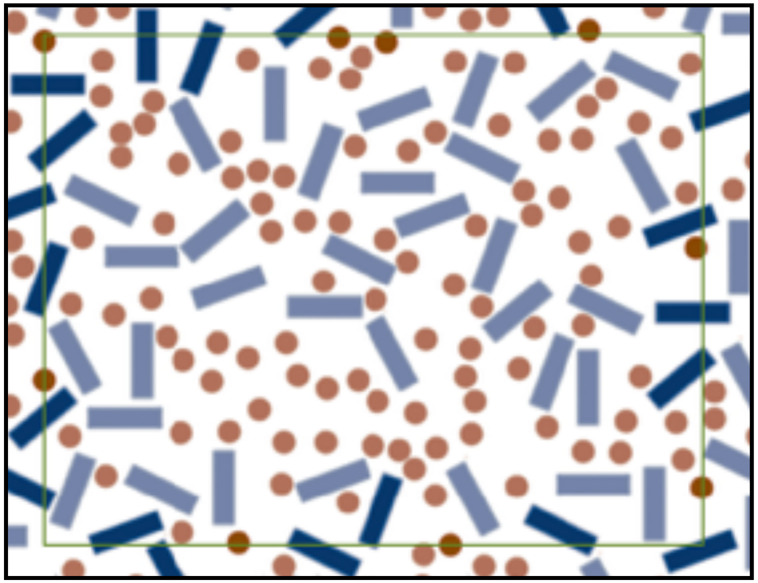
=> Not implemented very often

Image Processing Handbook, J. C. Russ

1. Morphometry

Counting

Unbiased counting



Do not count features touching any edge

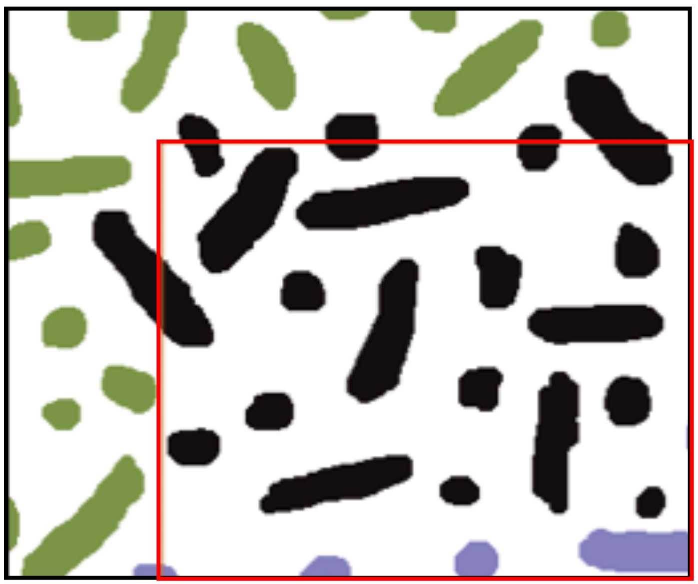
=> Proportions between small and big features wrong

[Image Processing Handbook](), J. C. Russ

1. Morphometry

Counting

Unbiased counting



Do not count features touching any outer edge (blue and green) or lying outside of the guard frame (green)

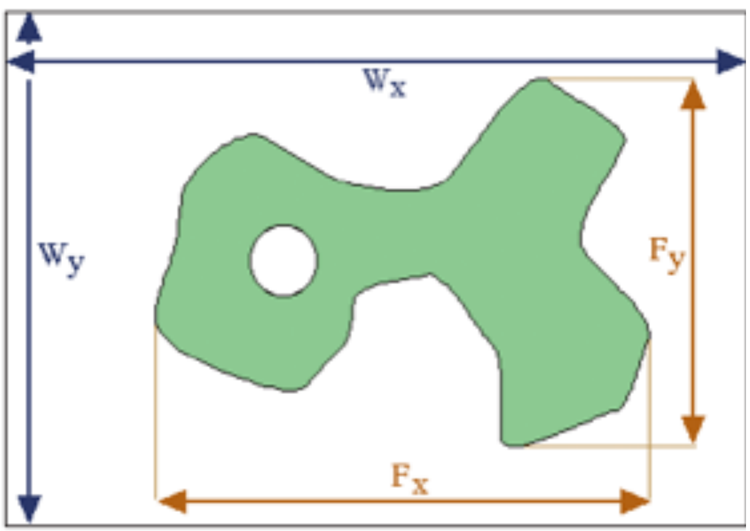
=> Guard frame (red) can become (very) small for big features

Image Processing Handbook, J. C. Russ

1. Morphometry

Counting

Unbiased counting



Do not count features touching any edge, but adjust counts:

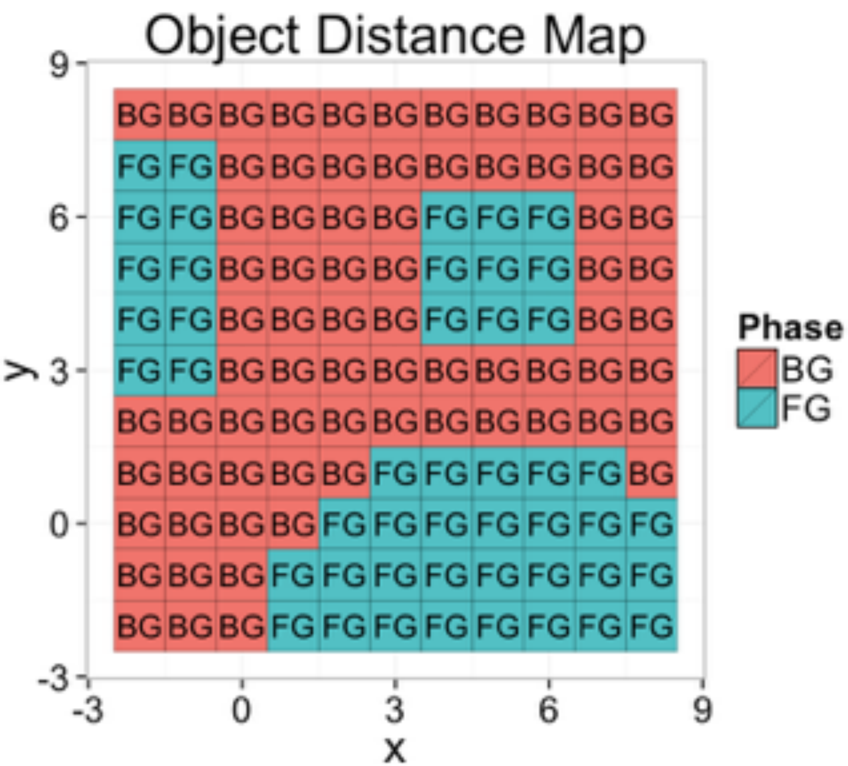
$$Count = \frac{W_x \cdot W_y}{(W_x - F_x) \cdot (W_y - F_y)}$$

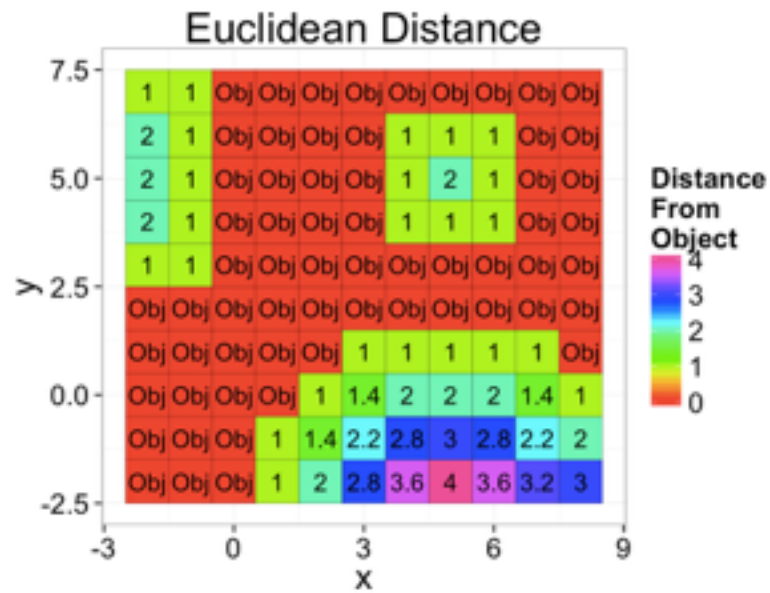
=> Proportions between small and big features correct

Image Processing Handbook, J. C. Russ

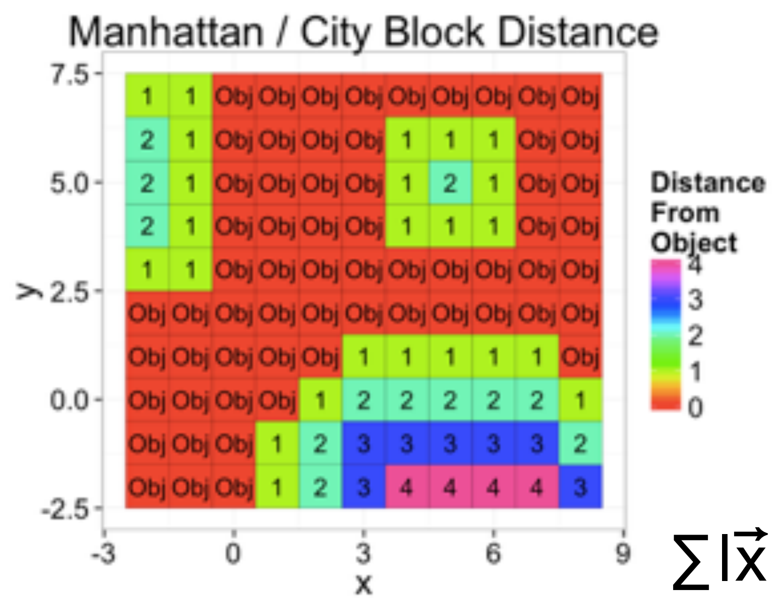
1. Morphometry

Distance Maps





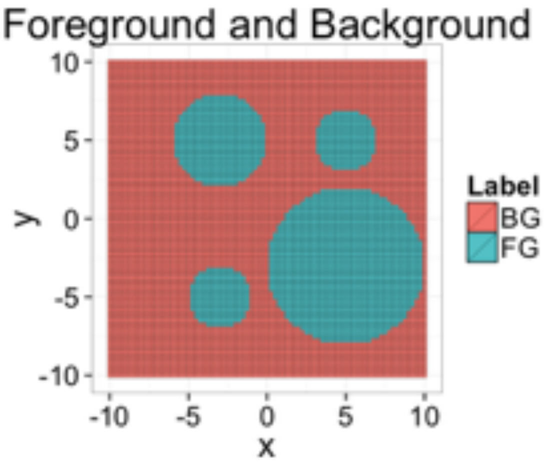
$$\mathrm{dist}(\vec{x})=\min(\|\vec{x}-\vec{y}\| \, \forall \vec{y} \in \mathrm{Background})$$

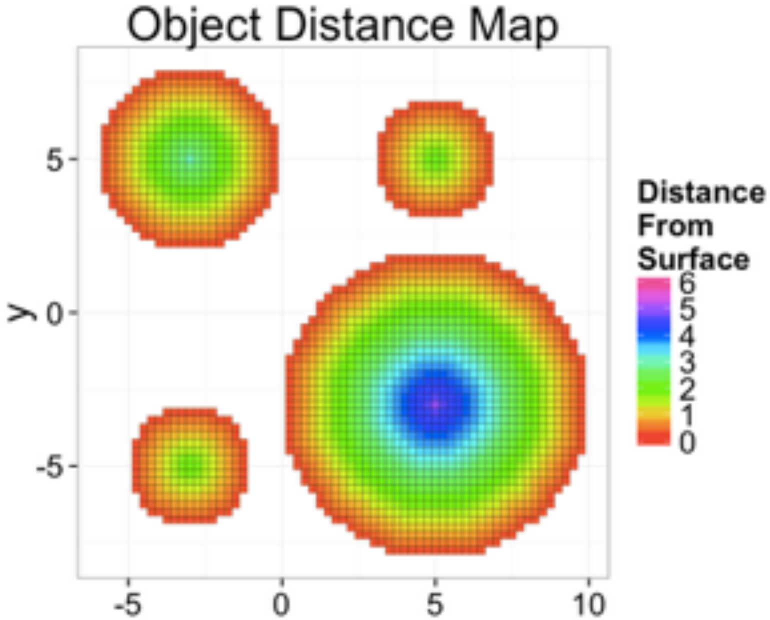


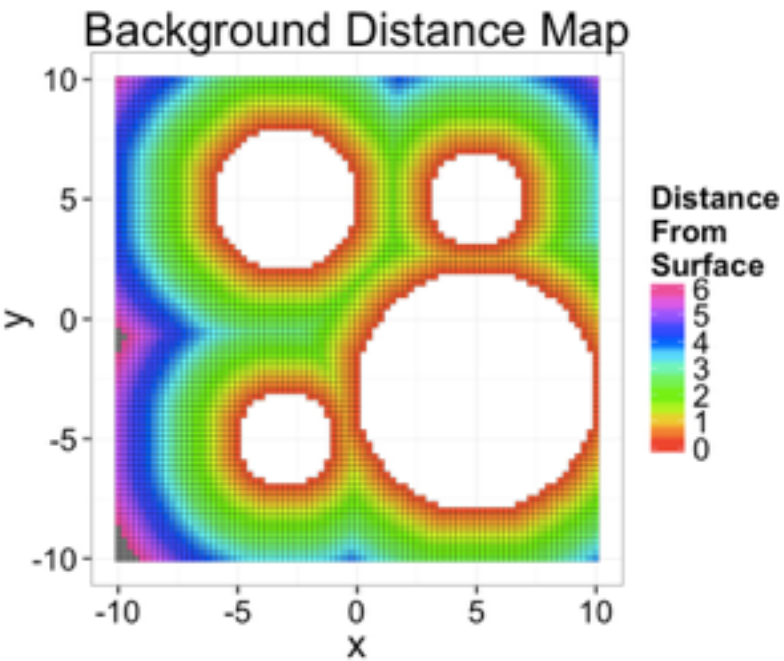
$$\sum |\vec{x}-\vec{y}|$$

1. Morphometry

Distance Maps



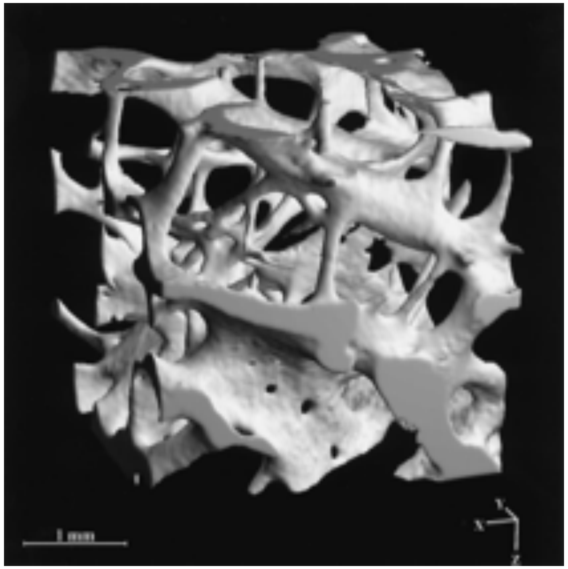




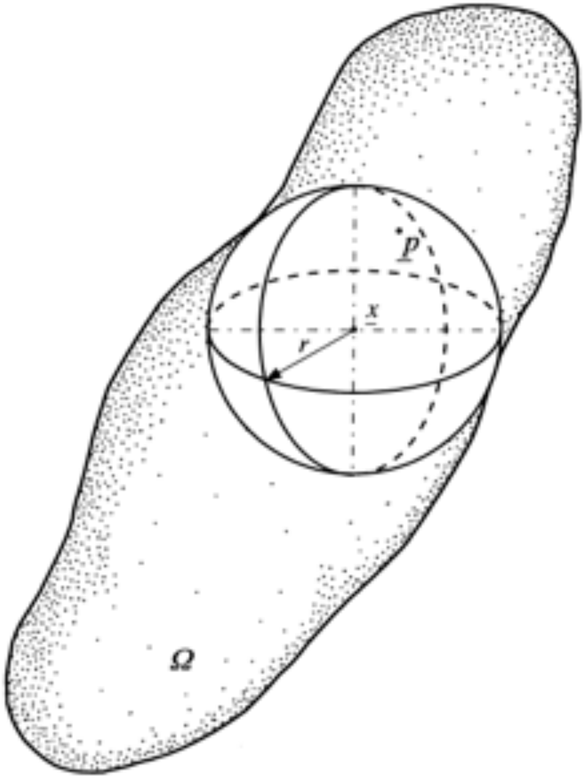
1. Morphometry

Dimensions

Dimensions in 3D



Trabecular bone
(human iliac crest)



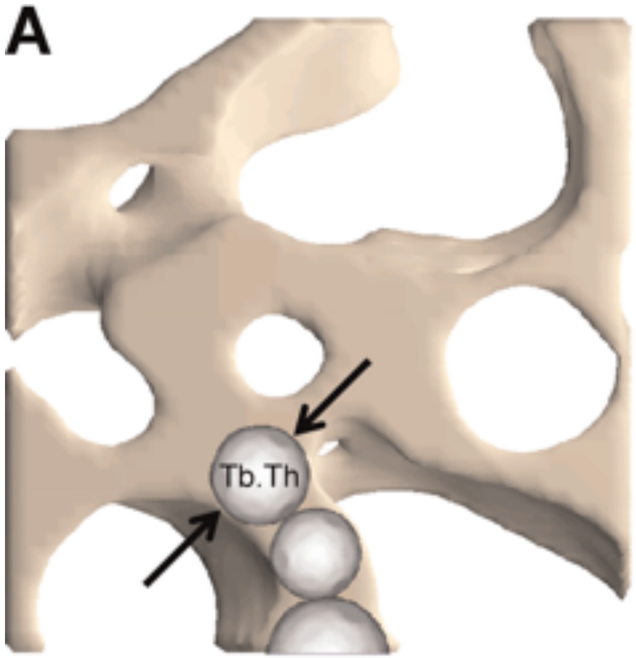
Thickness
assessment

Hildebrand T and Rüegsegger P, J Microsc-Oxford 185:67-75 (1997)

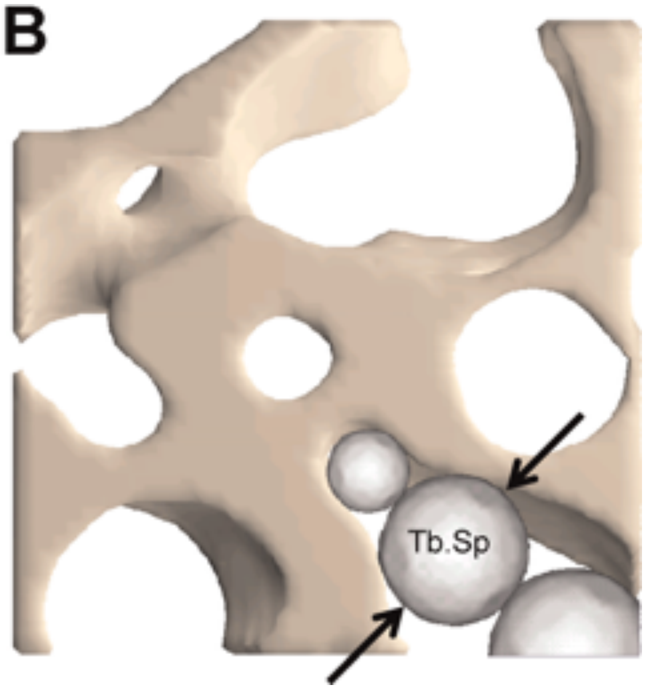
1. Morphometry

Dimensions

Dimensions in 3D



Trabecular thickness



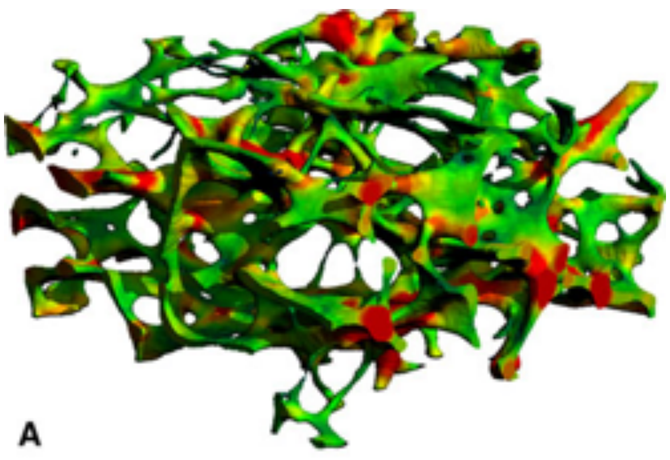
Trabecular spacing

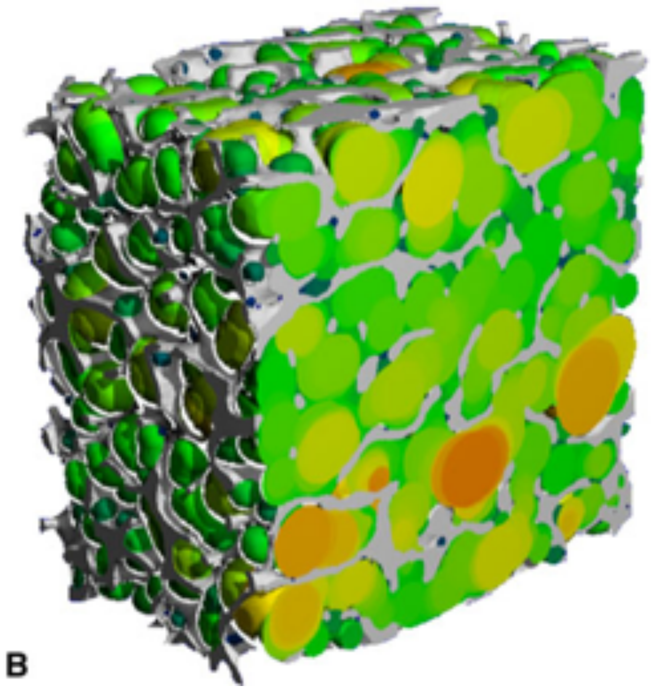
Bouxsein ML *et al*, [J Bone Miner Res 25:1468-86 (2010)]

1. Morphometry

Dimensions

Dimensions in 3D





Trabecular thickness

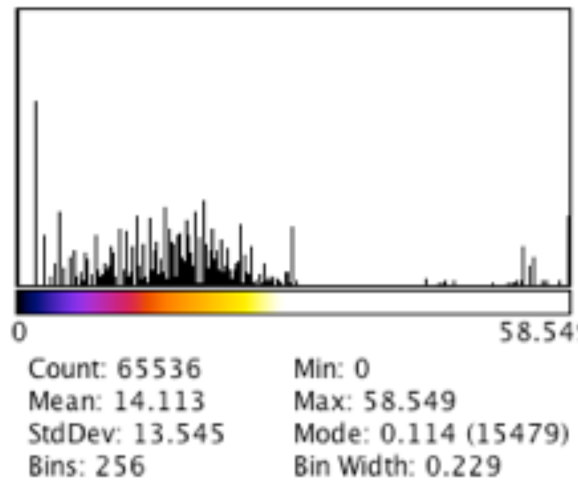
Trabecular spacing

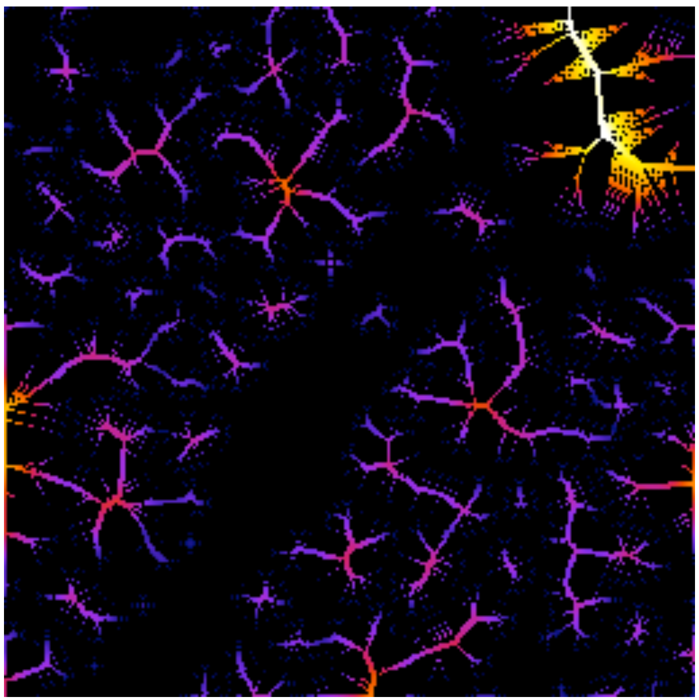
Burghardt AJ *et al*, Clin Orthop Relat Res 469:2179-93 (2011)

3. Demonstration

Baby Rat Lung Structure

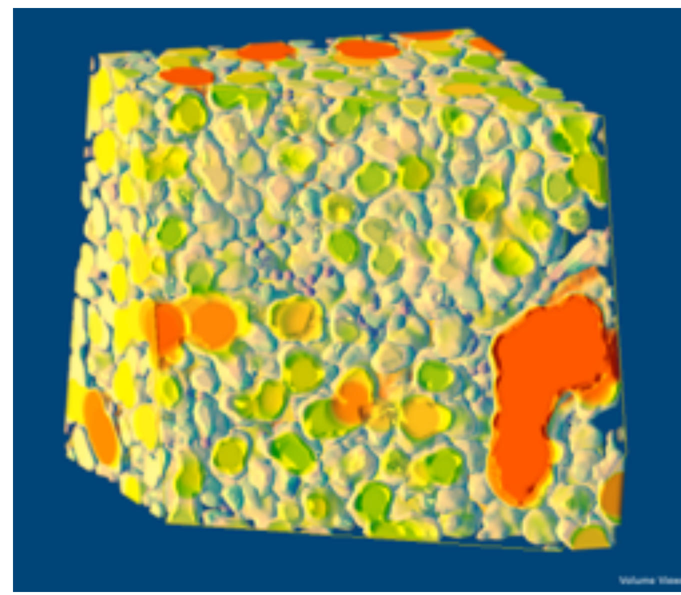
Micron-scale aveolar structure





Ridge Map

Thickness Filling

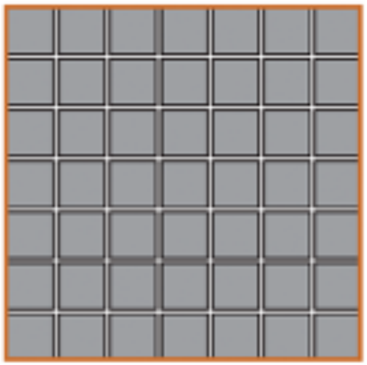


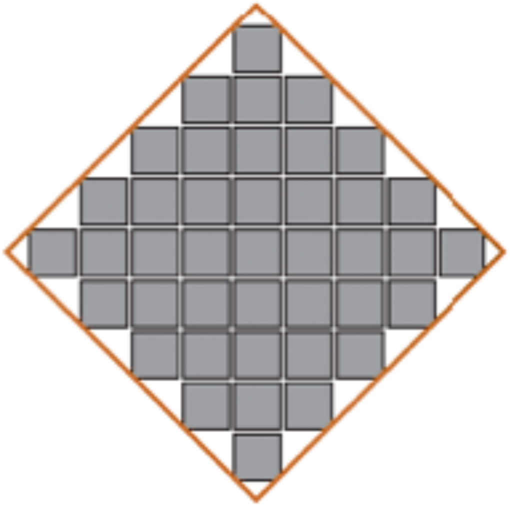
Thickness map of alveoli

1. Morphometry

Perimeter

Orientation





Edge sum: 28
Interpolated perimeter: 28

Edge sum: 36
Interpolated perimeter: 28

=> Perimeter dependent on rotation
=> Perimeter (2D)/surface (3D) must be interpolated

Image Processing Handbook, J. C. Russ

1. Morphometry

Perimeter

Interpolation

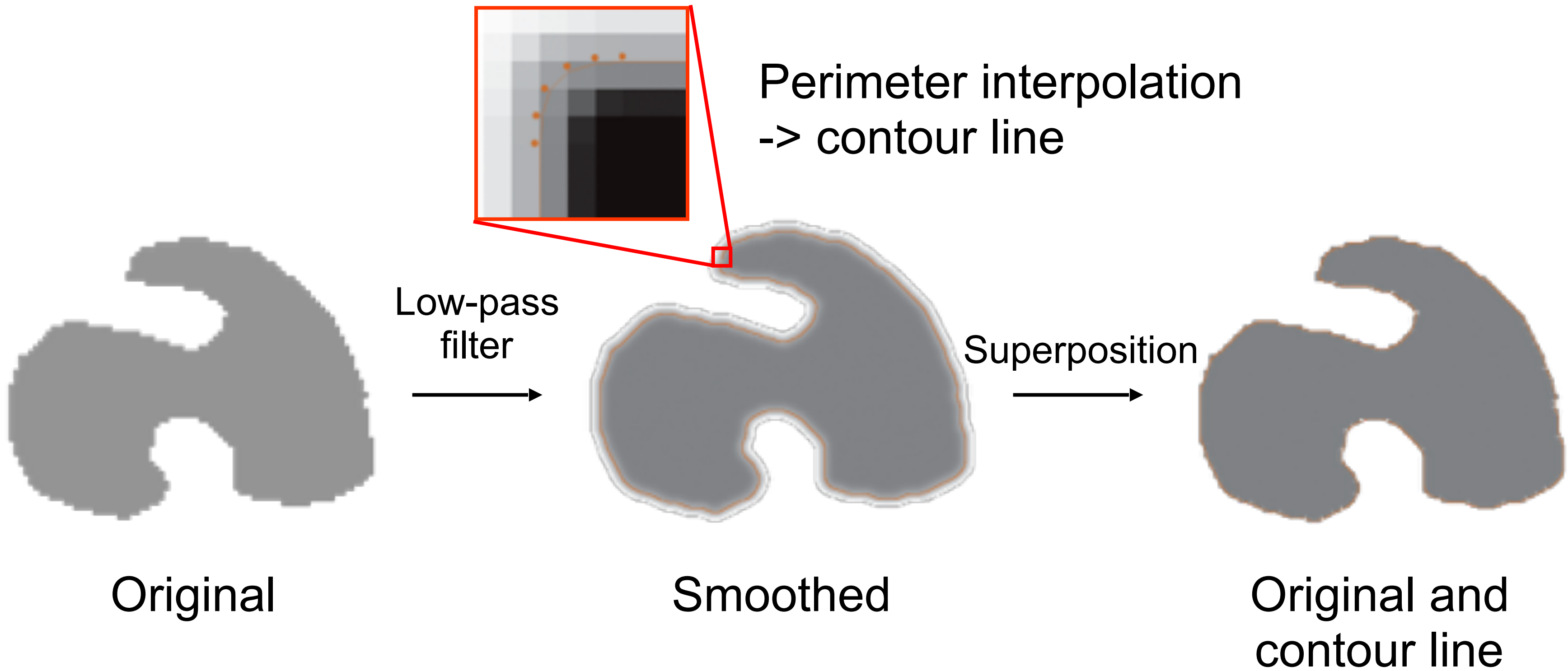
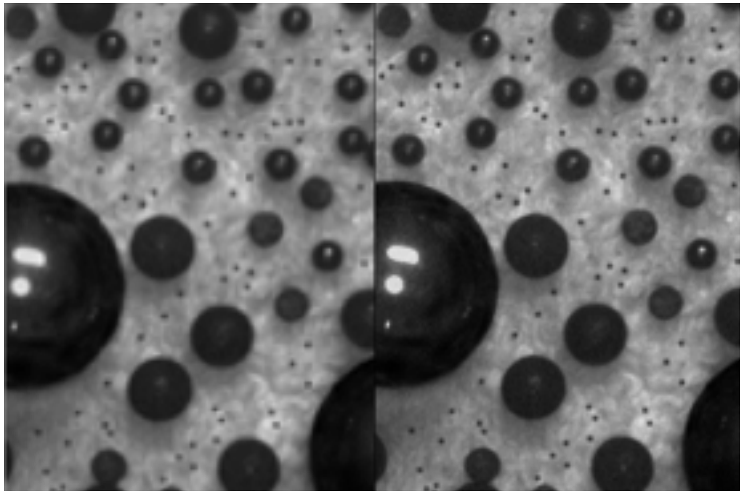


Image Processing Handbook, J. C. Russ

1. Morphometry

Perimeter

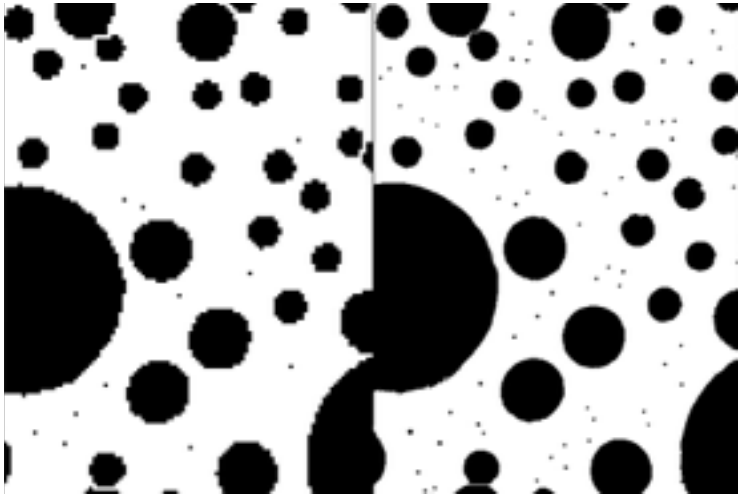
Resolution



Low resolution High resolution

Original

Segmentation →



Low resolution High resolution

Segmented

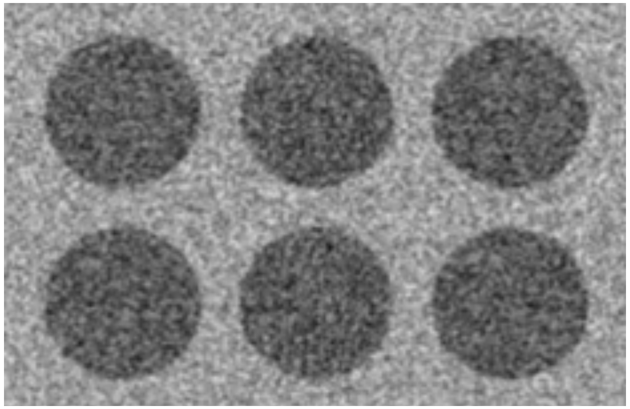
Photo: spherical particles
Image Processing Handbook, J. C. Russ

=> Perimeter depends on resolution

1. Morphometry

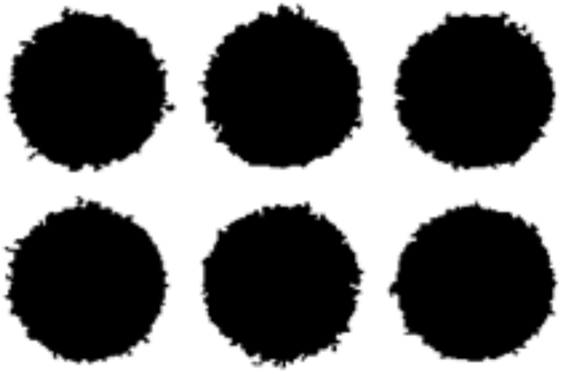
Perimeter

Noise



Noisy original

Segmentation →



Segmented

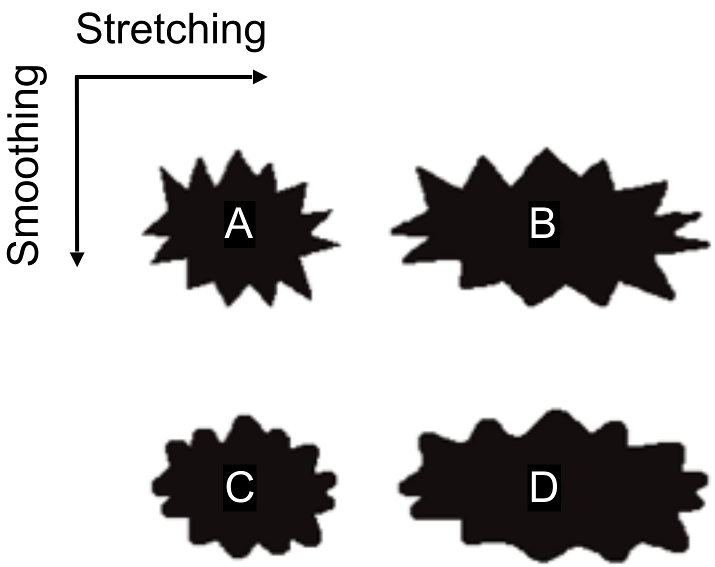
=> Perimeter is prone to noise

Image Processing Handbook, J. C. Russ

1. Morphometry

Shape

Shape descriptors



Shape variations

$$\textit{Aspect ratio} = \frac{\textit{Max diameter}}{\textit{Min diameter}} \qquad \textit{Form factor} = \frac{4\pi \cdot \textit{area}}{\textit{perimeter}^2}$$

	Aspect ratio	Form factor
A	1.339	0.257
B	2.005	0.256
C	1.294	0.459
D	2.017	0.457

Shape descriptors

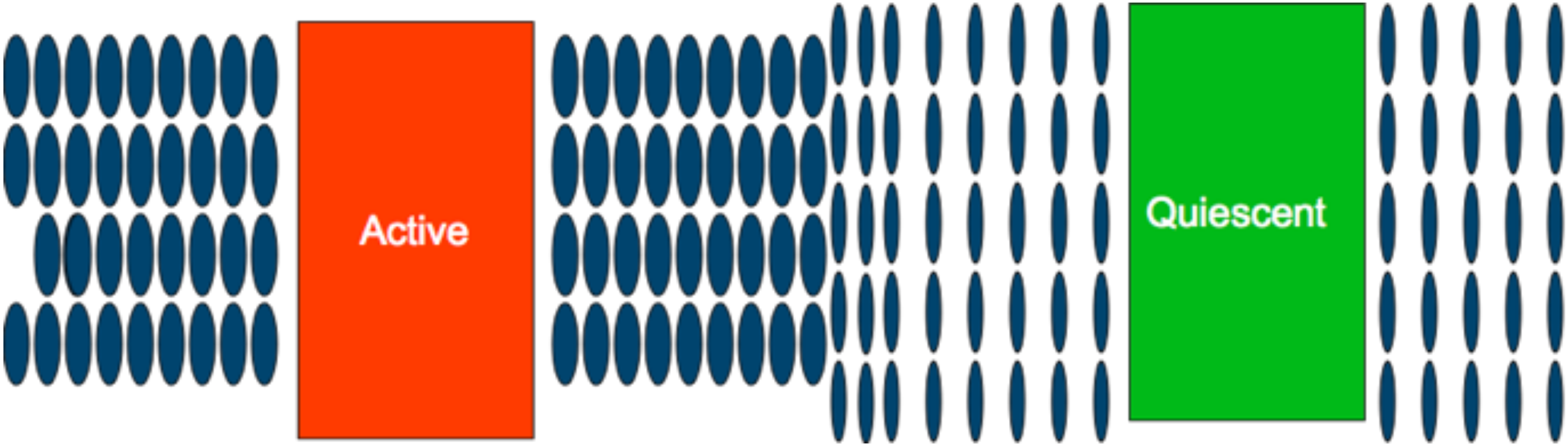
Stretching Smoothing

Image Processing Handbook, J. C. Russ

2. Examples

Activity of Canals in Bone

- The lacunae surrounding a canal show the viability of the canal
  - Histologically observable lacuna density differences between active and quiescent canals [1]
- Identify function through shape and relational analysis of structure

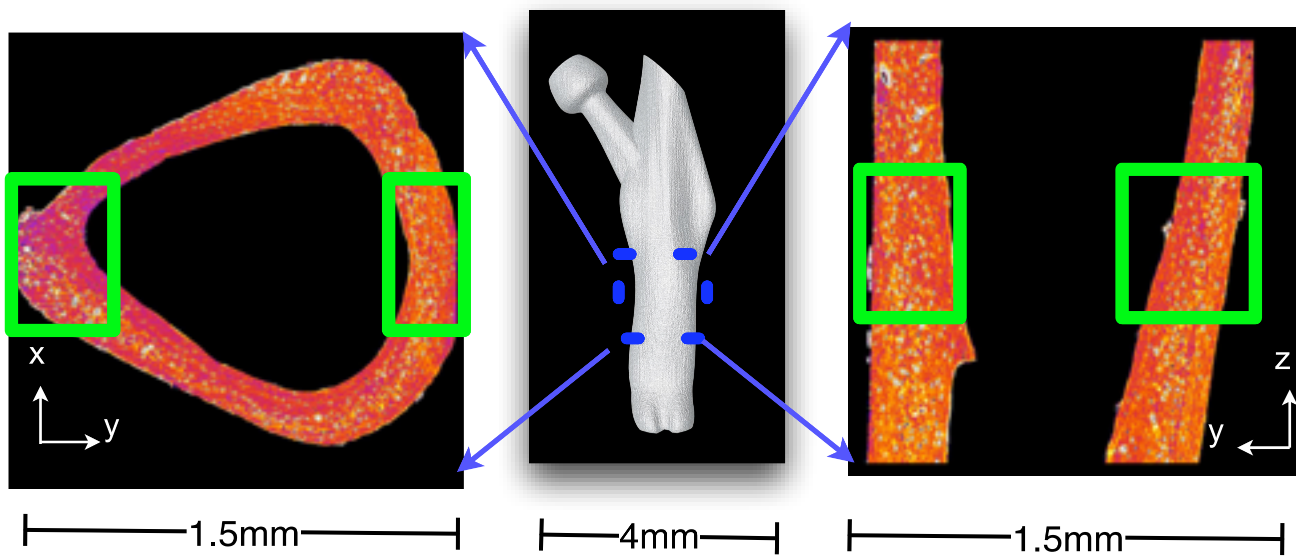


- [1] J. Power et al. / Bone, 859-865 (2002)

2. Examples

Activity of Blood Vessels

- 7 femurs from healthy 4 month old female mice
- Measured in 2 ROIs
- 740nm voxel size, 750um field of view



uzh | eth | zürich

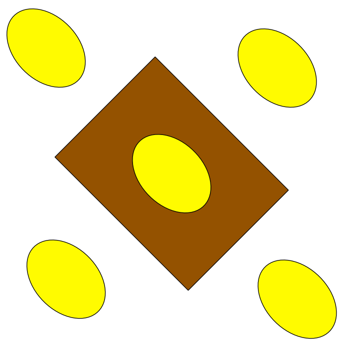
Lacuna Territory

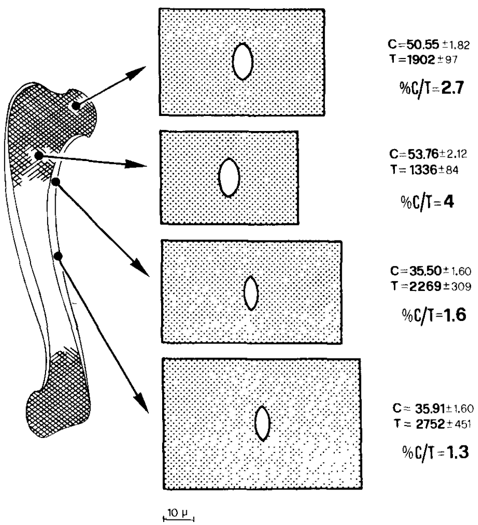
2D

$$< Lc.Ter >= \frac{Ct.A}{Lc.N}$$

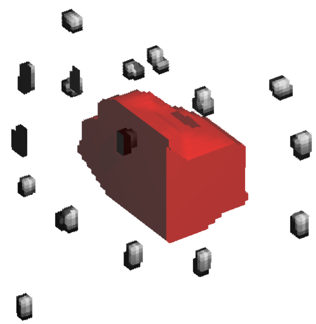
3D

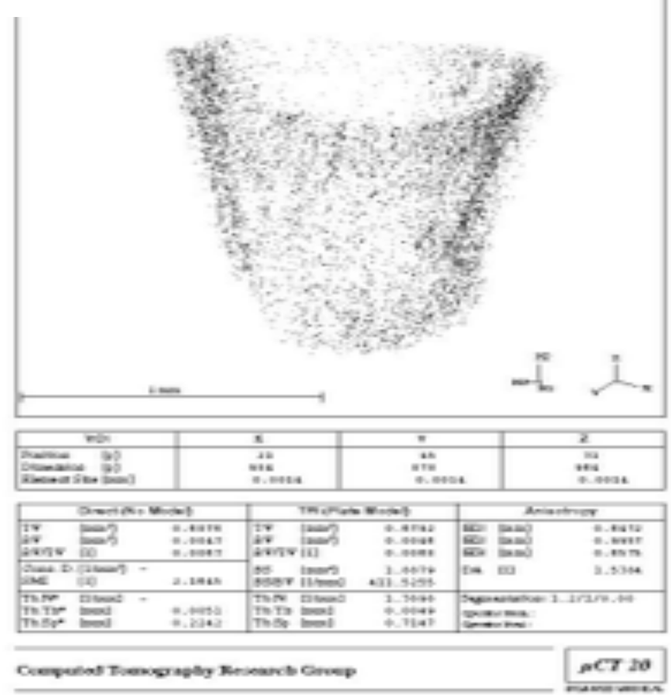
$$< Lc.TerV >= \frac{Ct.V}{Lc.N}$$





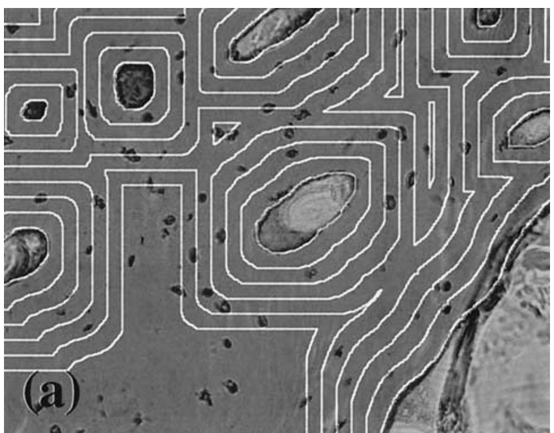
- V. Canè et al. / CTI 558-563 (1982)



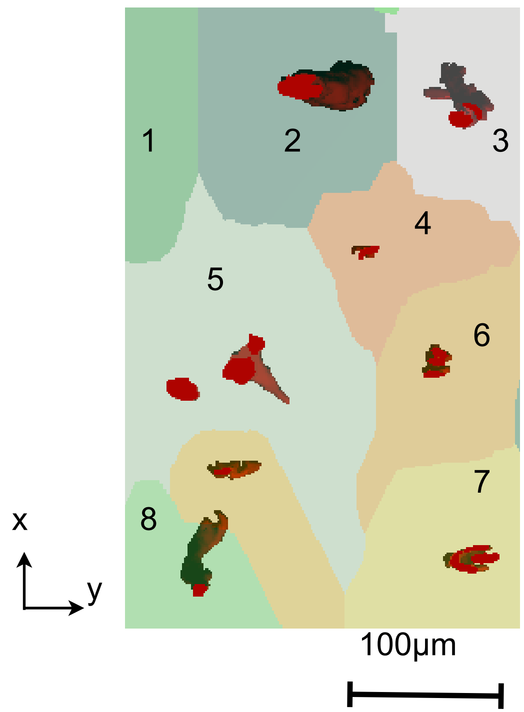


Canal Support Region

- Bone Volume divided into canal support regions by expanding canals until bone volume is filled
- The canals are then compared by examining at the lacunae within their support regions

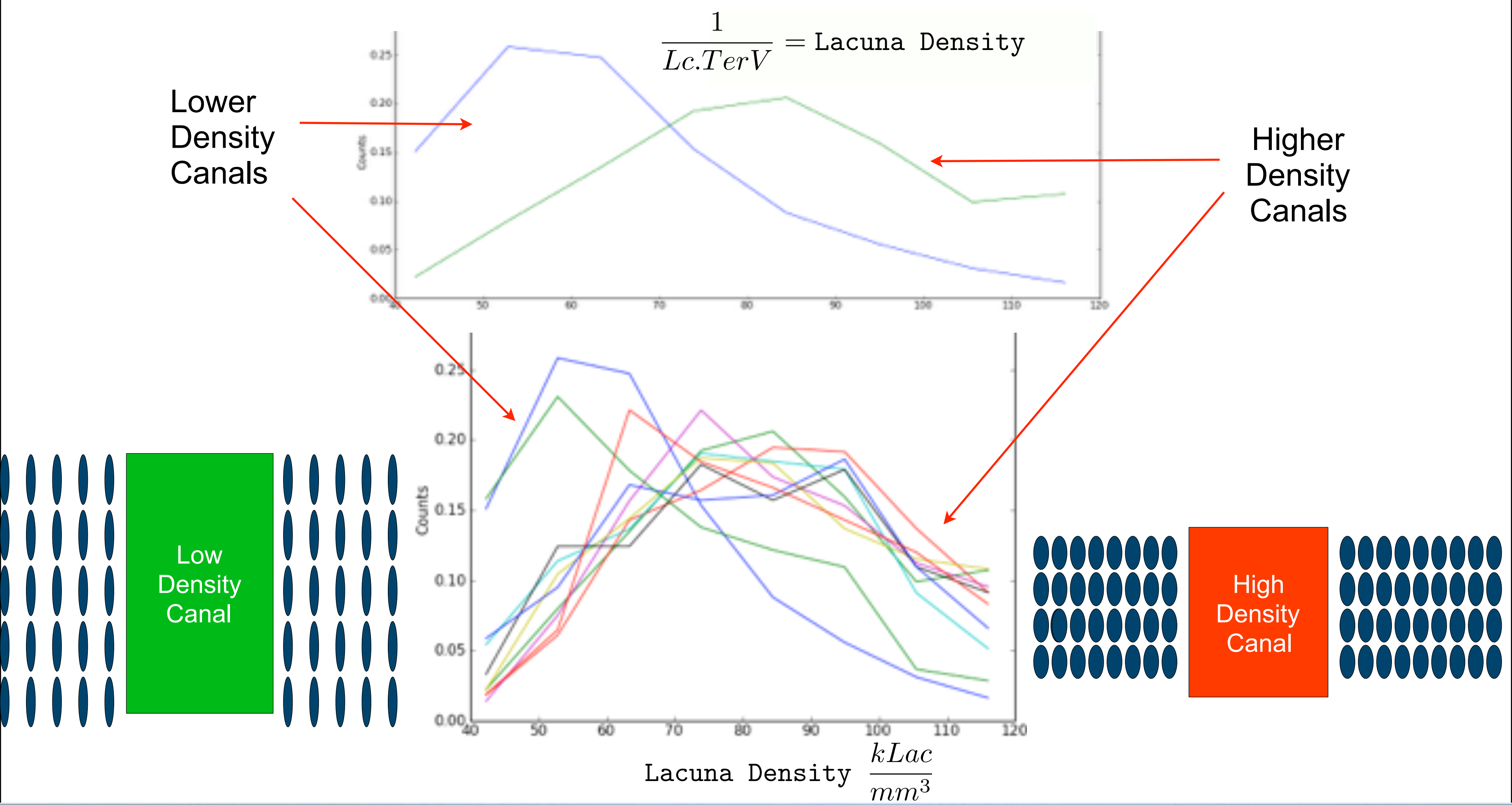


- J. Power et al. / Bone, 859-865 (2002)



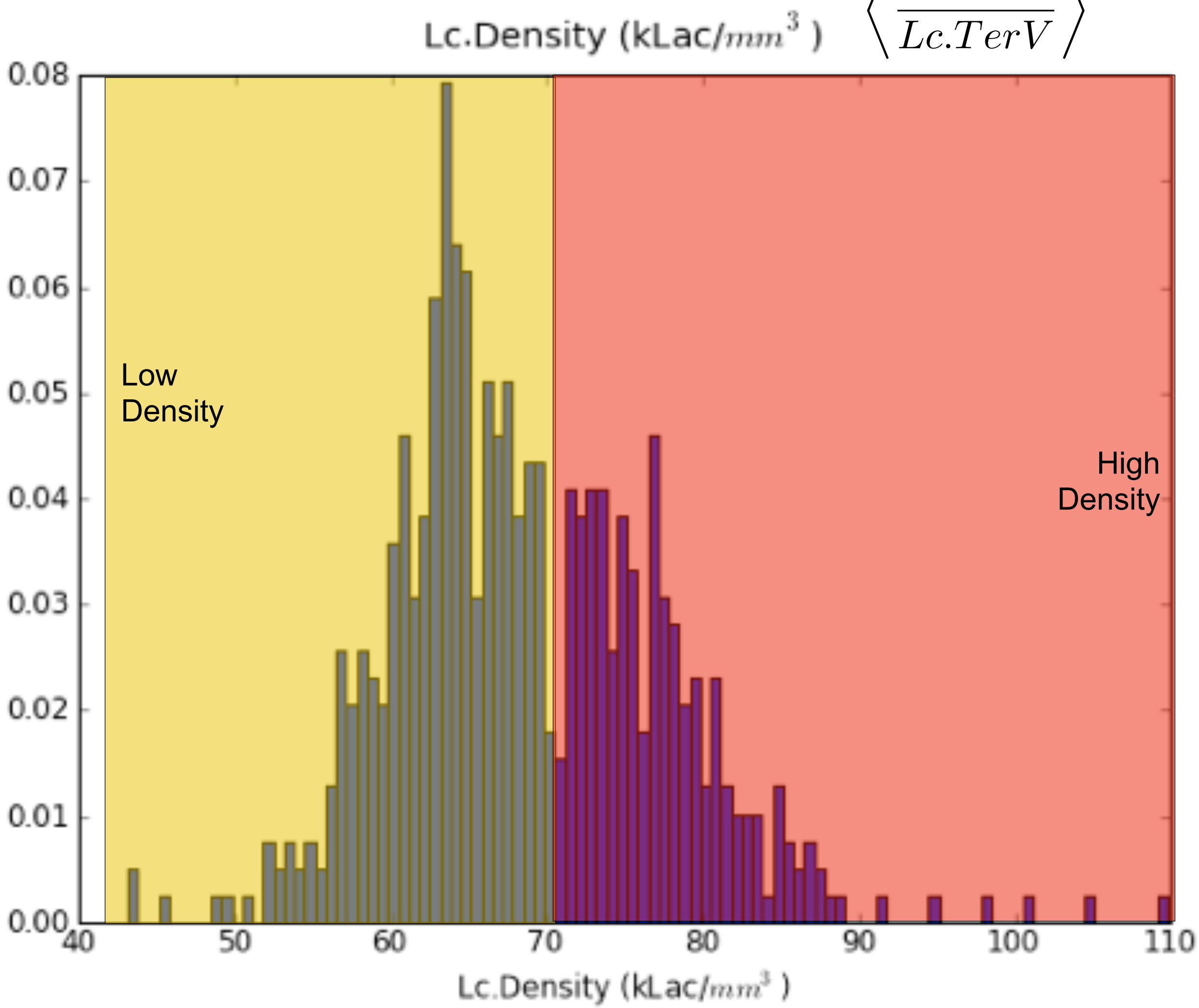
Two Classes of Canals

- For each canal the surrounding lacunae were analyzed and the histogram of the densities was made

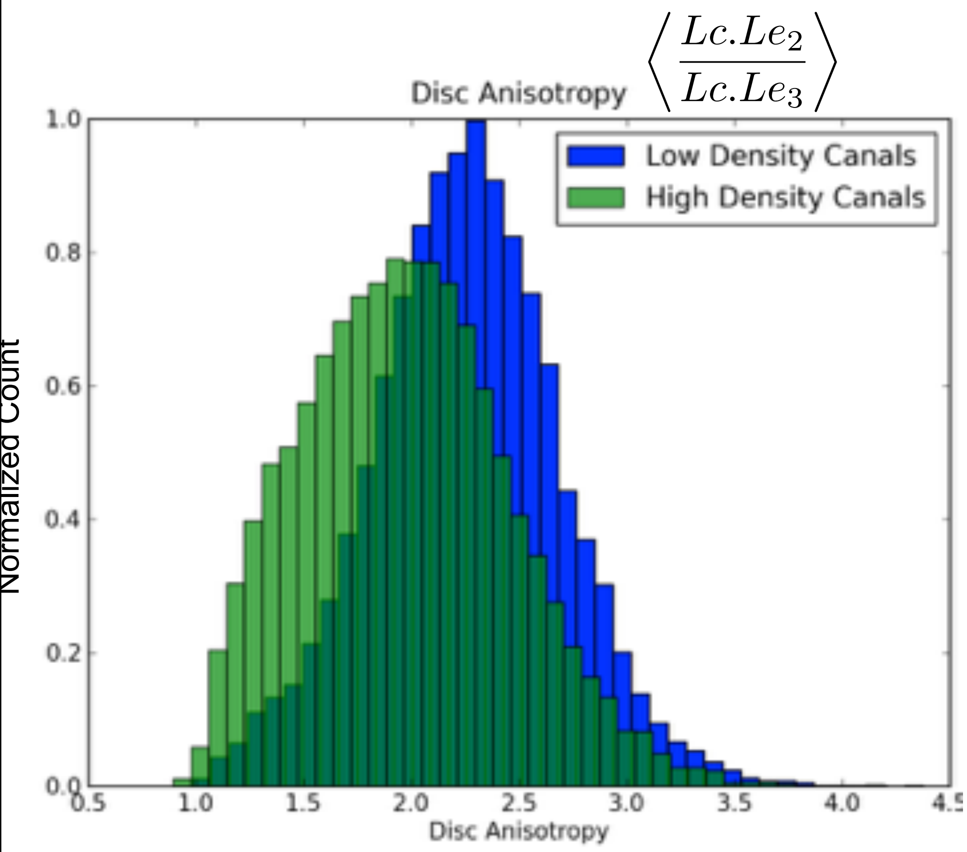


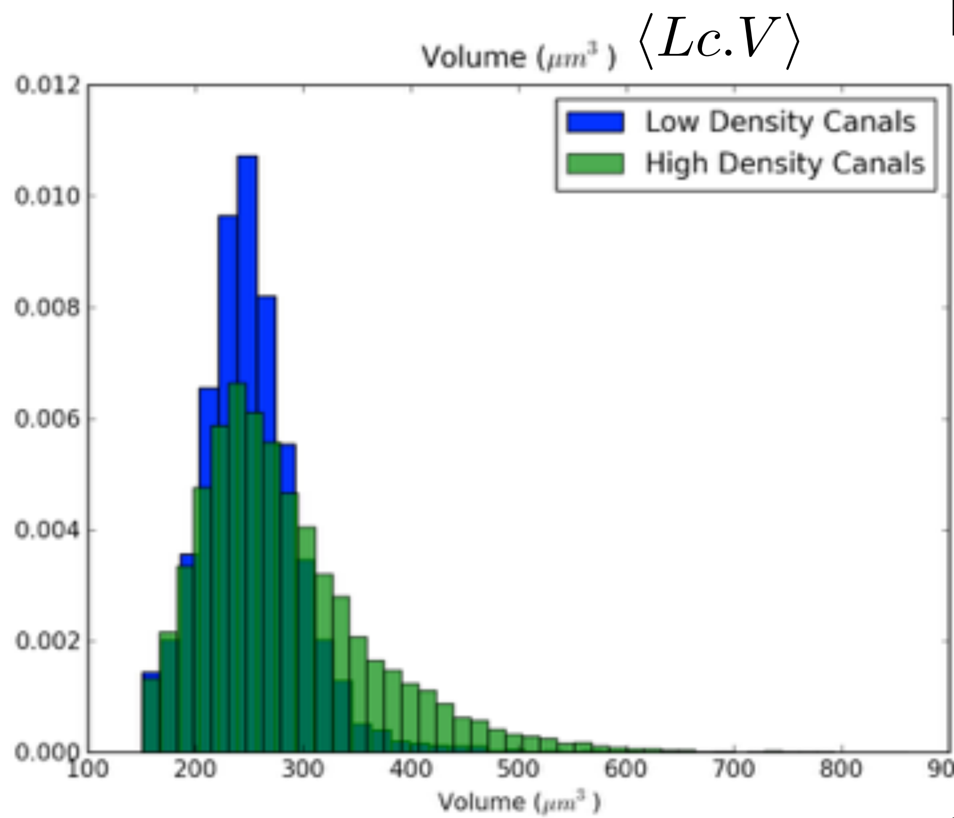
Density Distribution of Canals

$$\left\langle \frac{1}{Lc.TerV} \right\rangle$$



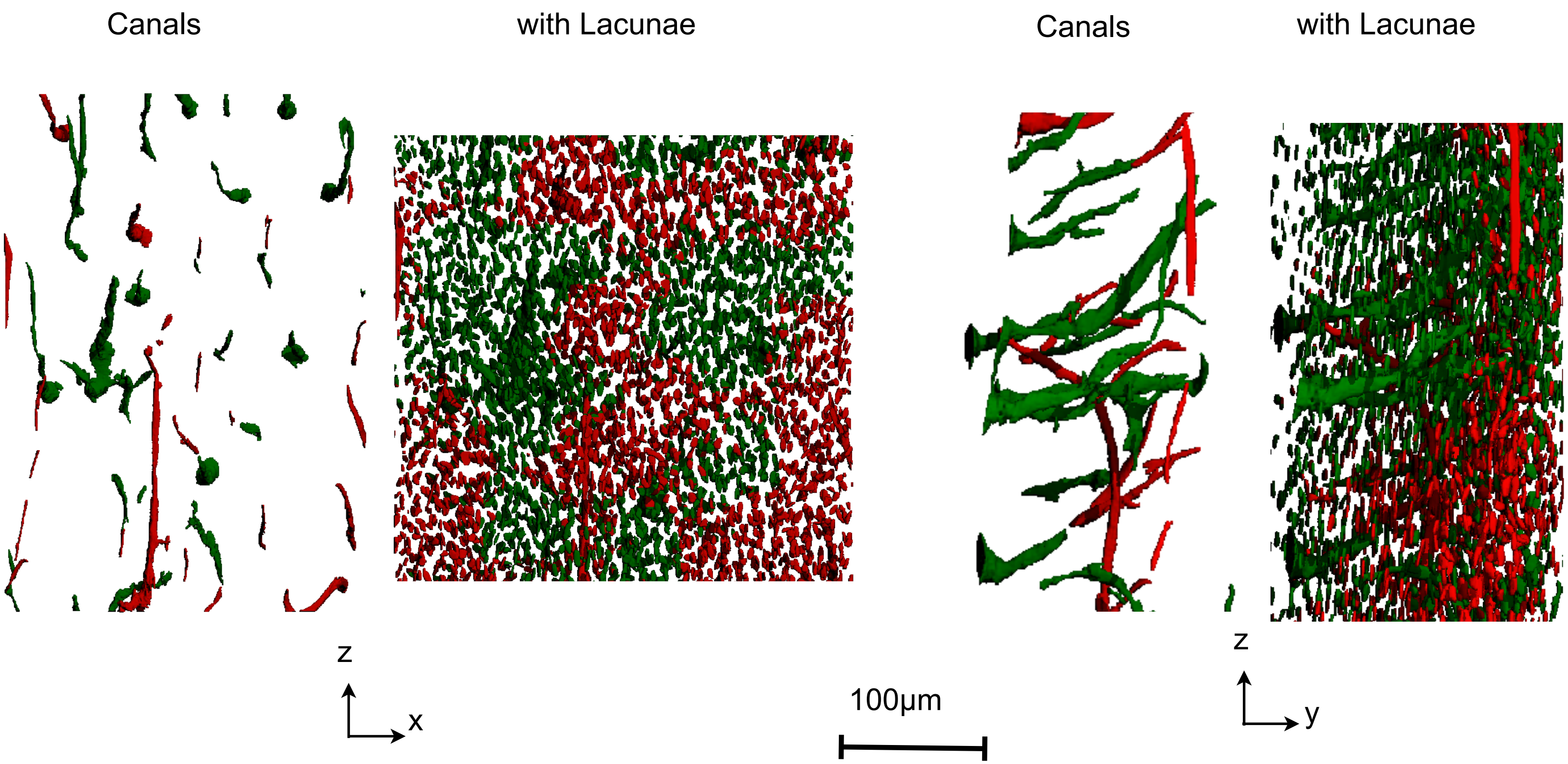
Shape Differences





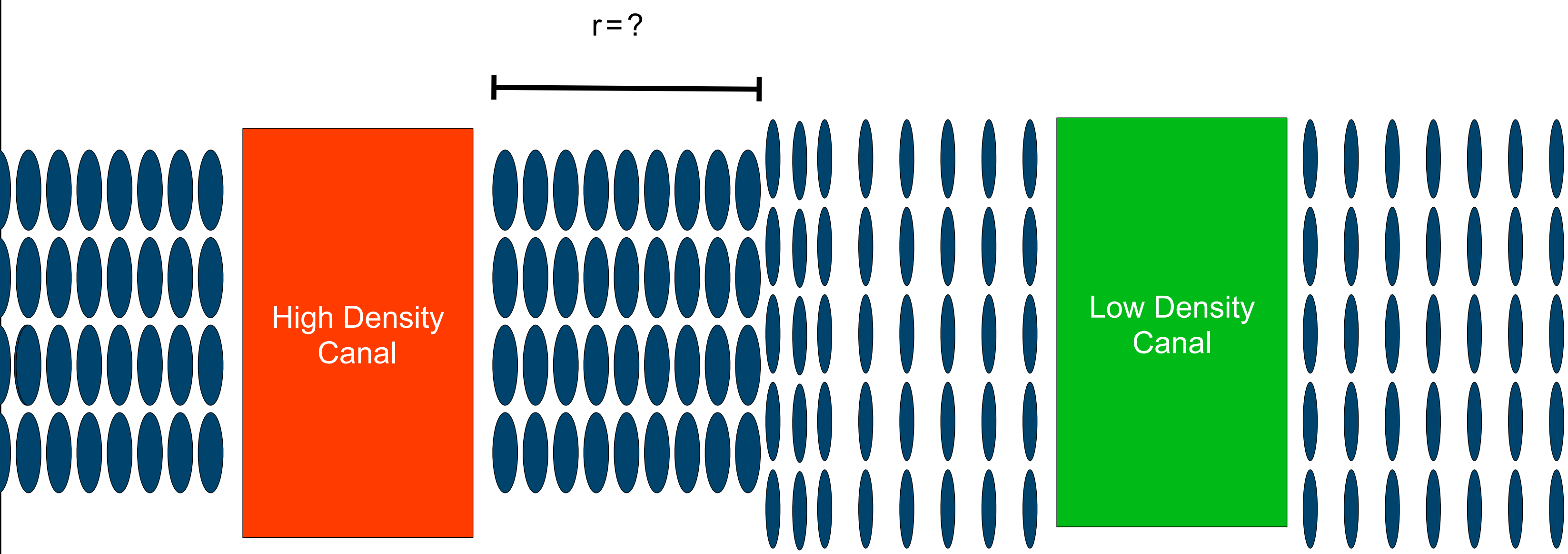
Visualization

- **Red** is higher lacuna density
- **Green** is lower lacuna density



Range of Influence

- The greater the distance from the surface of the canal the less the effect the canal should have on the lacunae and thus the smaller the observable differences between lacunae belonging to the classes of canals



Range of Influence Canals

Lacuna Density $\left\langle \frac{1}{Lc.TerV} \right\rangle$

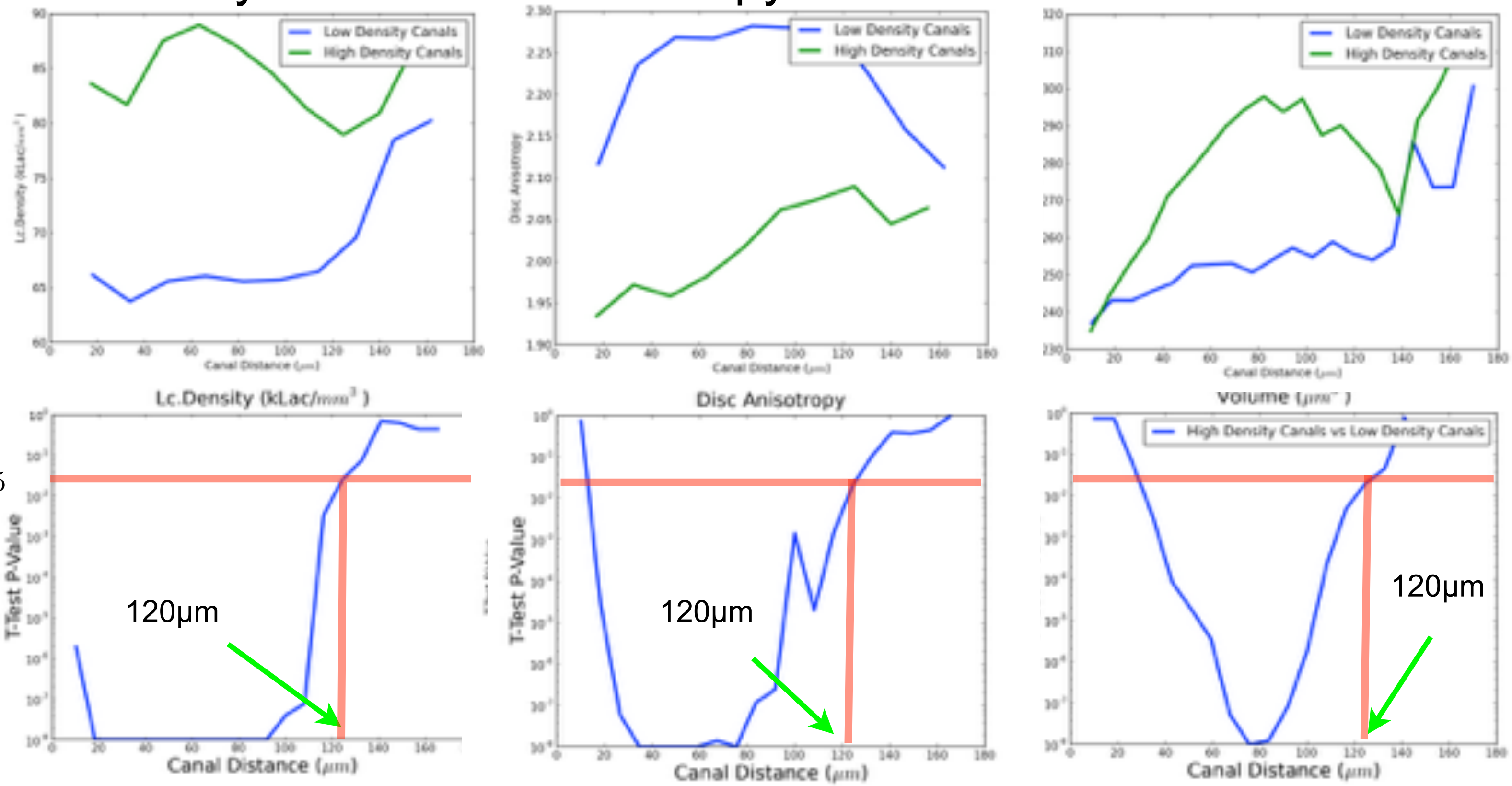
Disc Anisotropy $\left\langle \frac{Lc.Le_2}{Lc.Le_3} \right\rangle$

Lacuna Volume $\langle Lc.V \rangle$

Index vs Distance

$p = 0.05$

T-Test p-value vs Distance



Discussion

- Distinguish between two classes of canal based on lacunae distribution and shape
- Estimate the range of influence for canals where the differences in surrounding lacuna are maintained ~ 120μm

- **J. Power et al. / Bone, 859-865 (2002)**

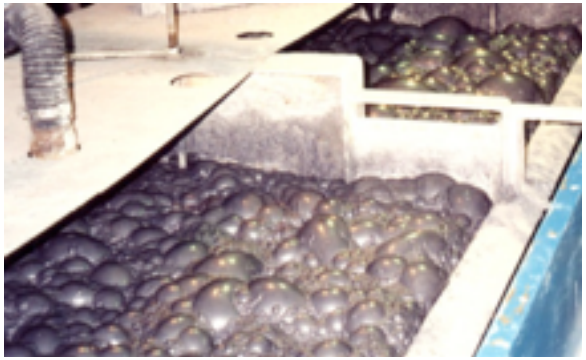
Our Results

Lacuna	$\left\langle \frac{Lc.N}{Ct.V} \right\rangle$	Density (%)
Forming	$932 \frac{Lac}{mm^2}$	125%
Quiescent	$750 \frac{Lac}{mm^2}$	100%
High Density	$85,300 \frac{Lac}{mm^3}$	129%
Low Density	$65,800 \frac{Lac}{mm^3}$	100%

2. Examples

Evolution of Liquid Foam



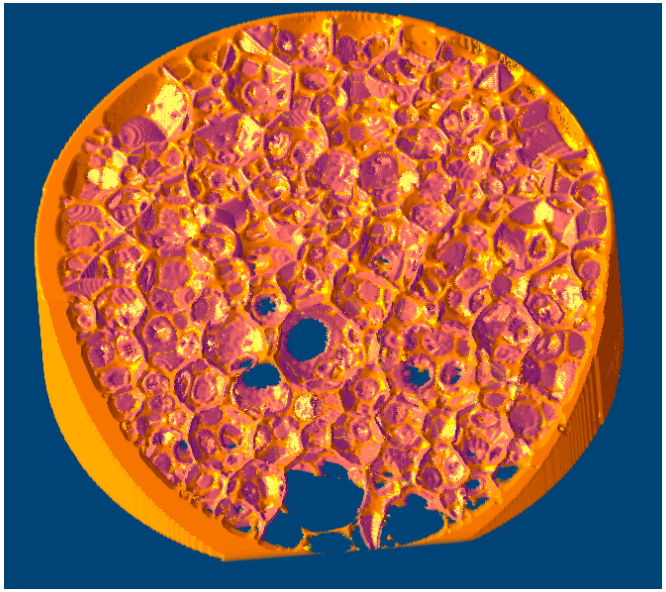



- Foam and cellular materials in general allow tuning of mechanical properties by changing topology and organization, applications in food, biology, firefighting, ore extraction, etc.
- Model system for elastic, plastic, and viscous behavior
- Understanding basic physics and rheology of liquid foam provide a simple experimental model to validate theory

2. Examples

Evolution of Liquid Foam

- Liquid foam evolves (also called coarsening) over time and it is important to understand the rate at which bubble change volume, which bubbles grow and how the material moves

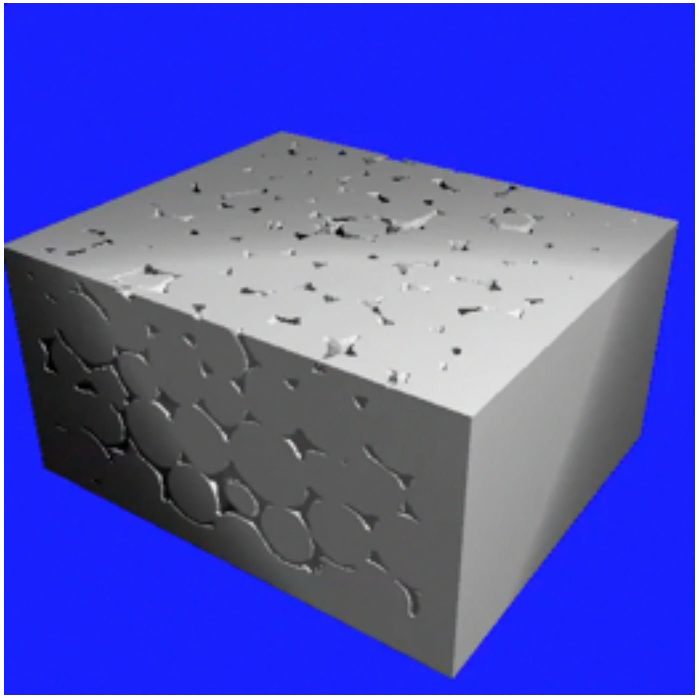


- hypothesis the biggest bubbles get bigger and the smallest get smaller / disappear

2. Examples

Evolution of Liquid Foam

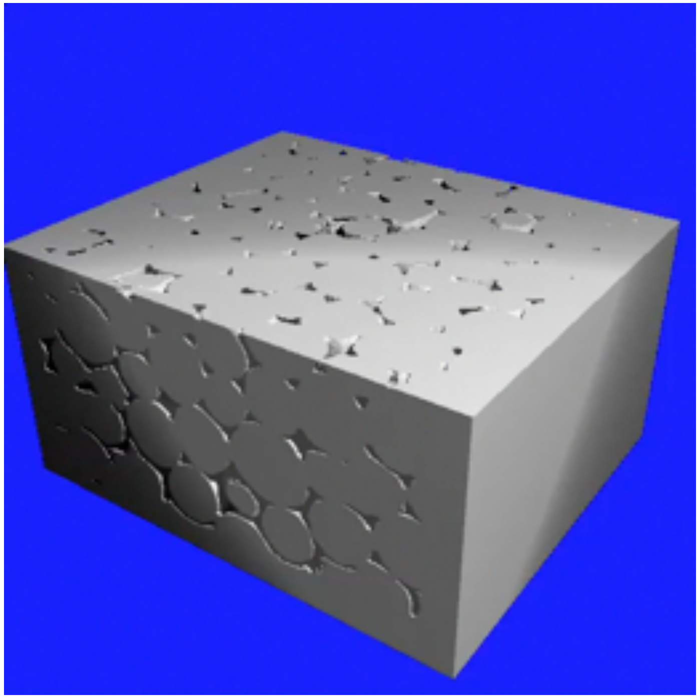
- Measurement just shows liquid and all of the bubbles are connected together



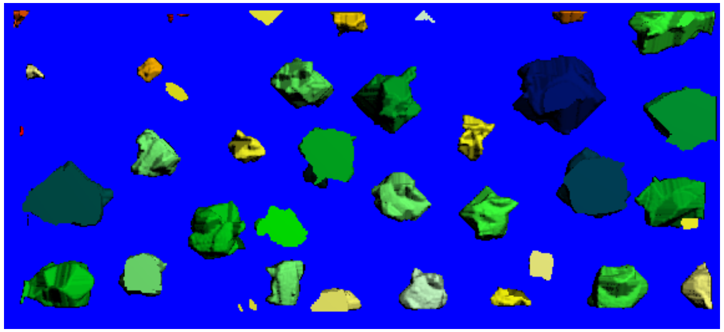
2. Examples

Evolution of Liquid Foam

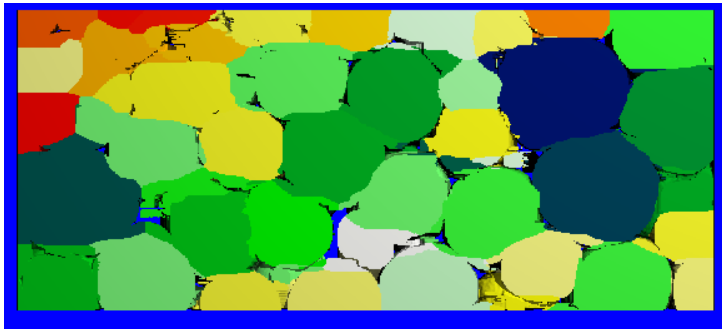
- Measurement just shows liquid and all of the bubbles are connected together



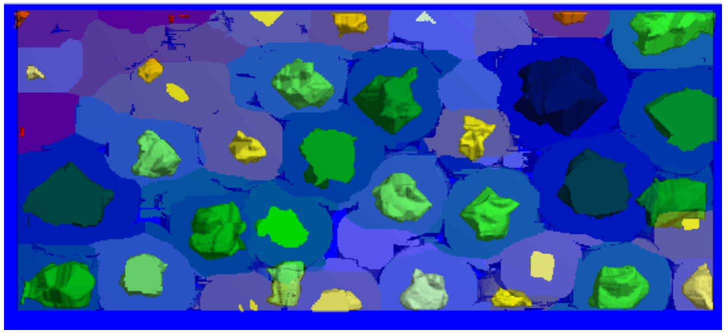
Labeled “cores”



Regrown bubbles



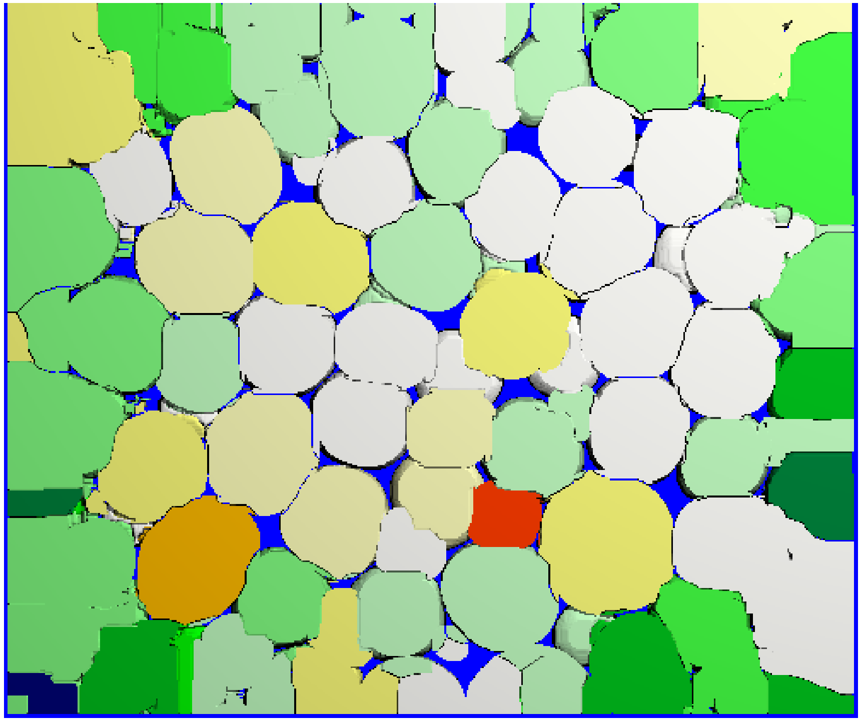
Overlap



2. Examples

Evolution of Liquid Foam

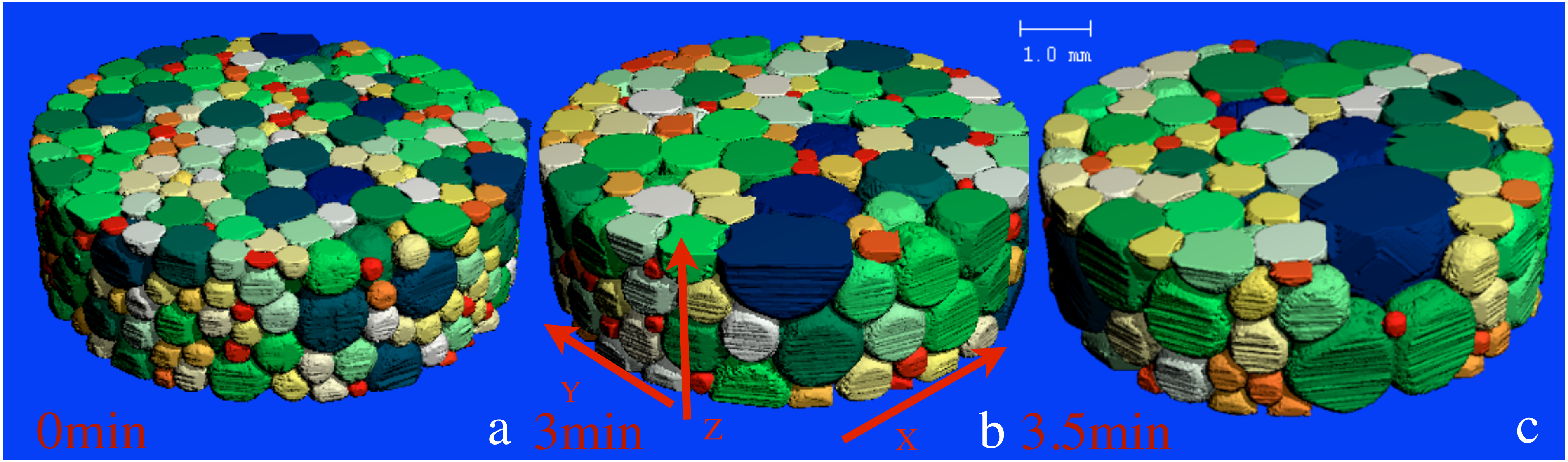
- Bubbles can now be colored by volume, neighbor count, etc
- Here neighbor count is shown



2. Examples

Evolution of Liquid Foam

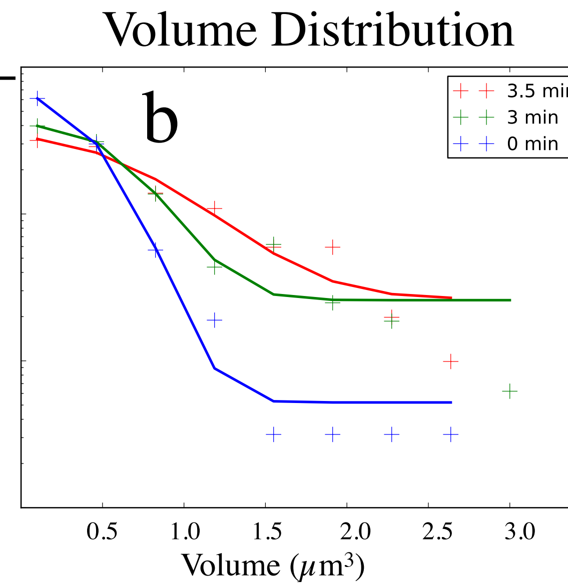
- The exact procedure can then be applied to 3 different measurements at 0, 3, and 3.5 minutes

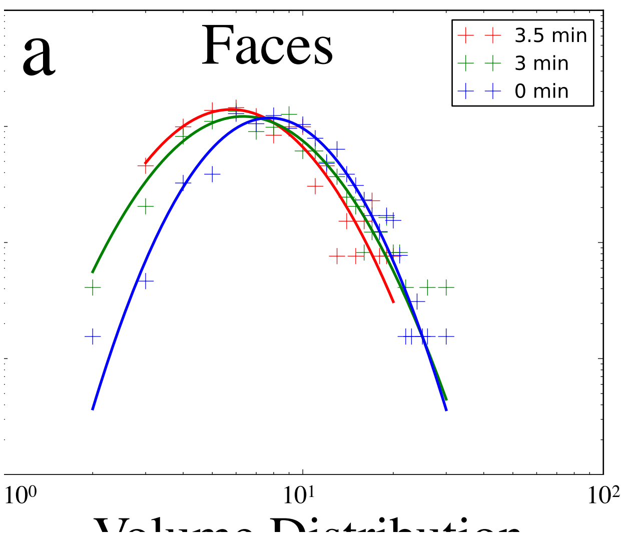


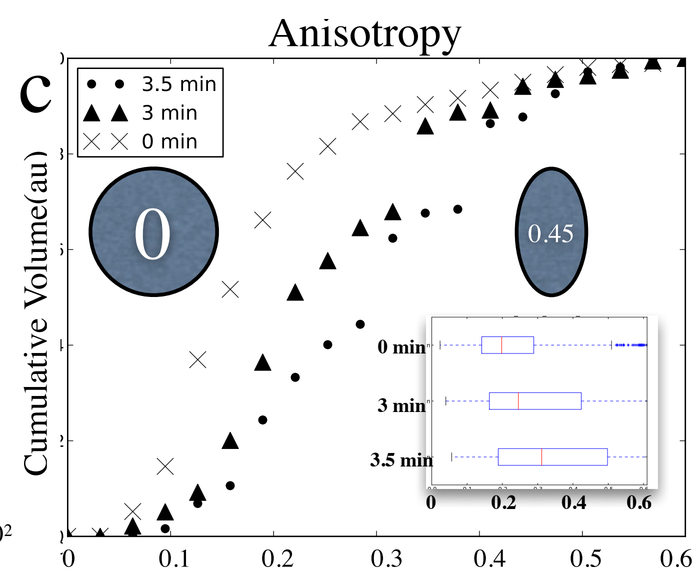
2. Examples

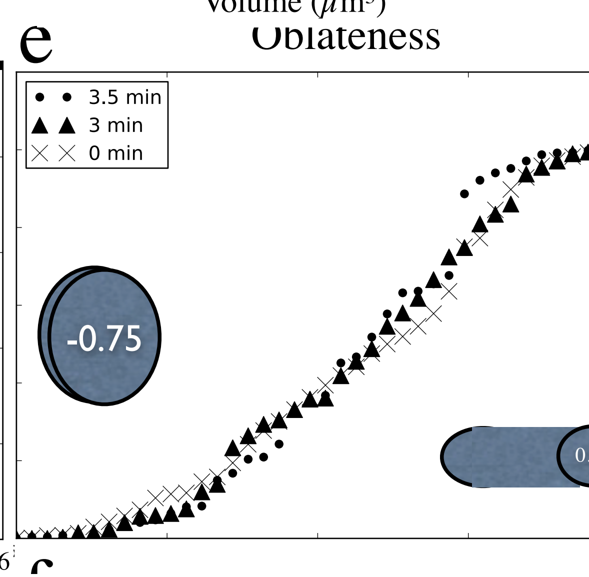
Evolution of Liquid Foam

- Volume, Number of faces and bubble shape can be compared
- Number and fraction of larger bubbles increases
- Faces steadily decreases, shape shifts towards more distorted bubbles but oblateness does not change







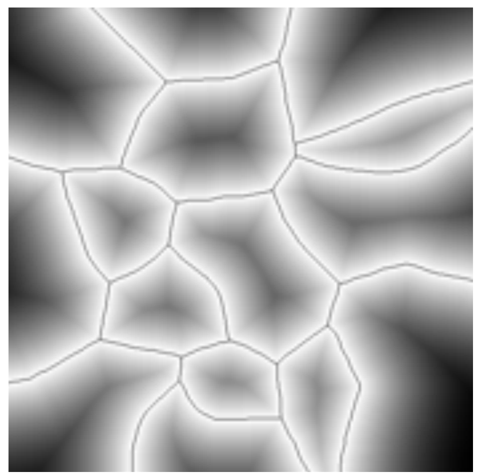


1. Morphometry

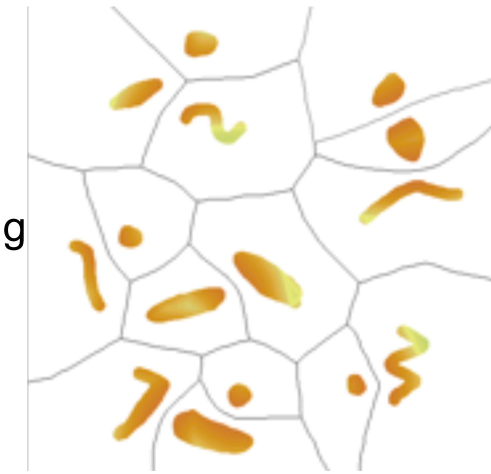
Neighbor relationships

Separation: edge-to-edge distance

Skeleton and EDM →



Masking →



Original

Skiz (skeleton of the background) and EDM

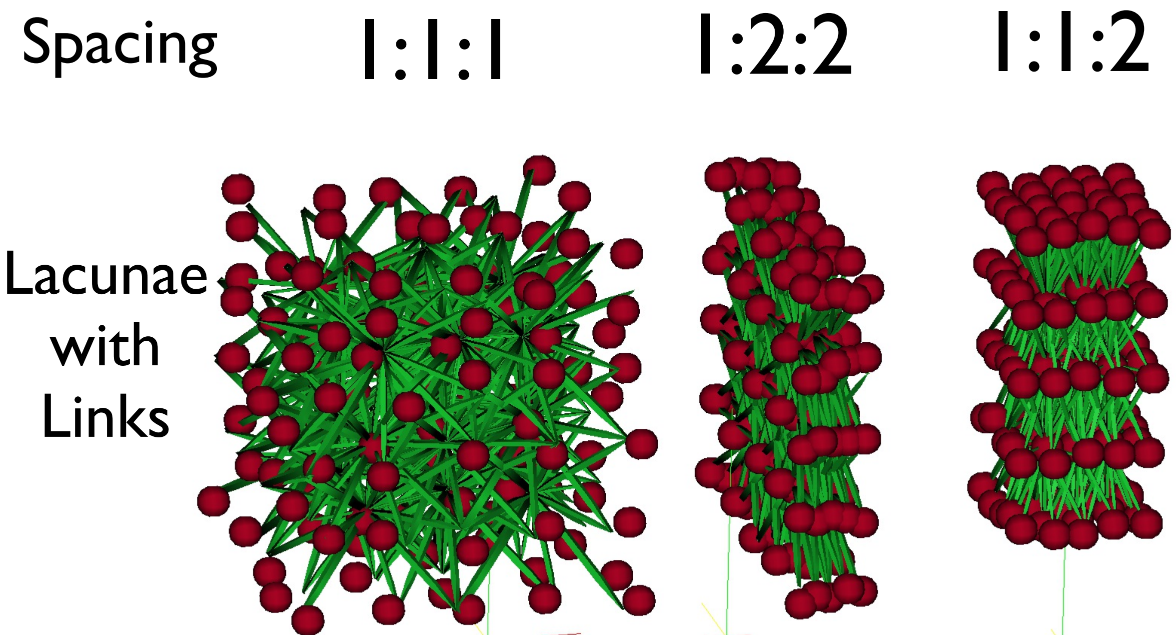
Image Processing Handbook, J. C. Russ

1. Morphometry

Neighbor relationships

Number of adjacent neighbors

1) Perform Voronoi tesselation
2) Draw edges (links) between touching features



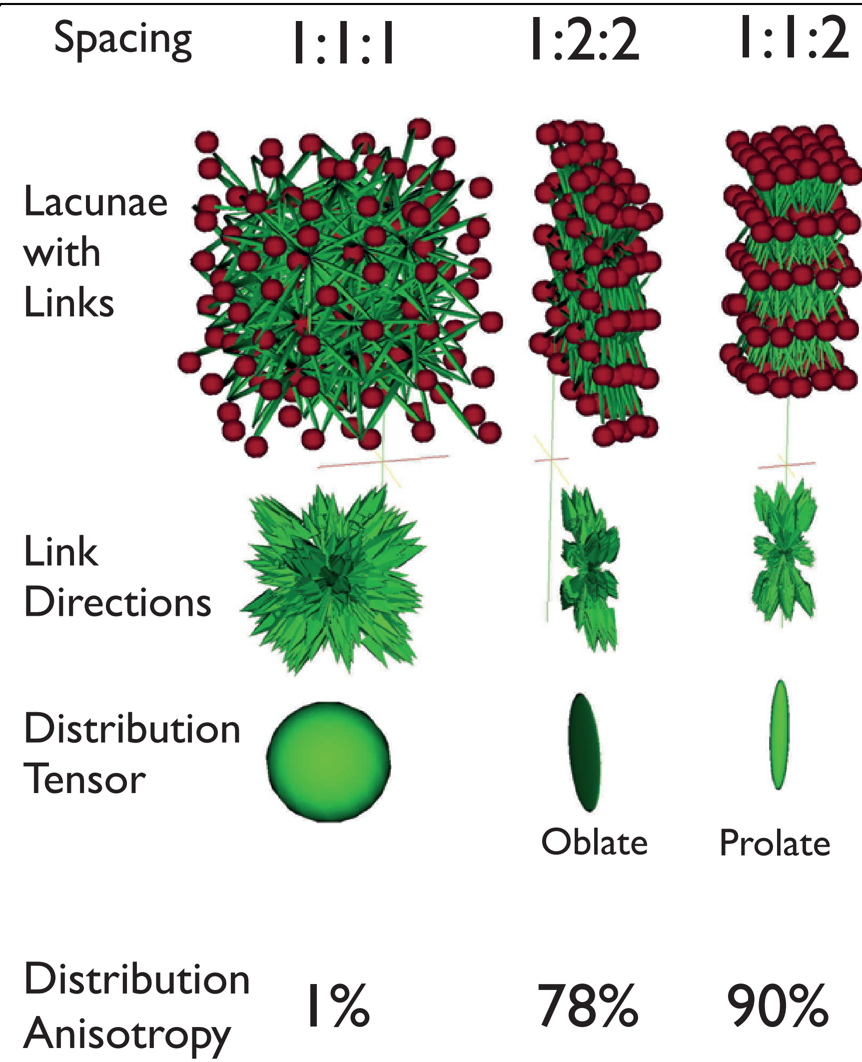
Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

1. Morphometry

Neighbor relationships

Number of adjacent neighbors

1) Calculate the covariance of links and create a distribution tensor
2) Anisotropy shows absolute degree of compression
3) Oblateness shows mode



Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026