ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

II. Segmentation: from shades of gray to black and white

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Topics

1. Segmentation and thresholding
2. Processing binary images
3. Demonstration

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Literature

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

Machine Learning Stanford: http://cs231n.github.io/

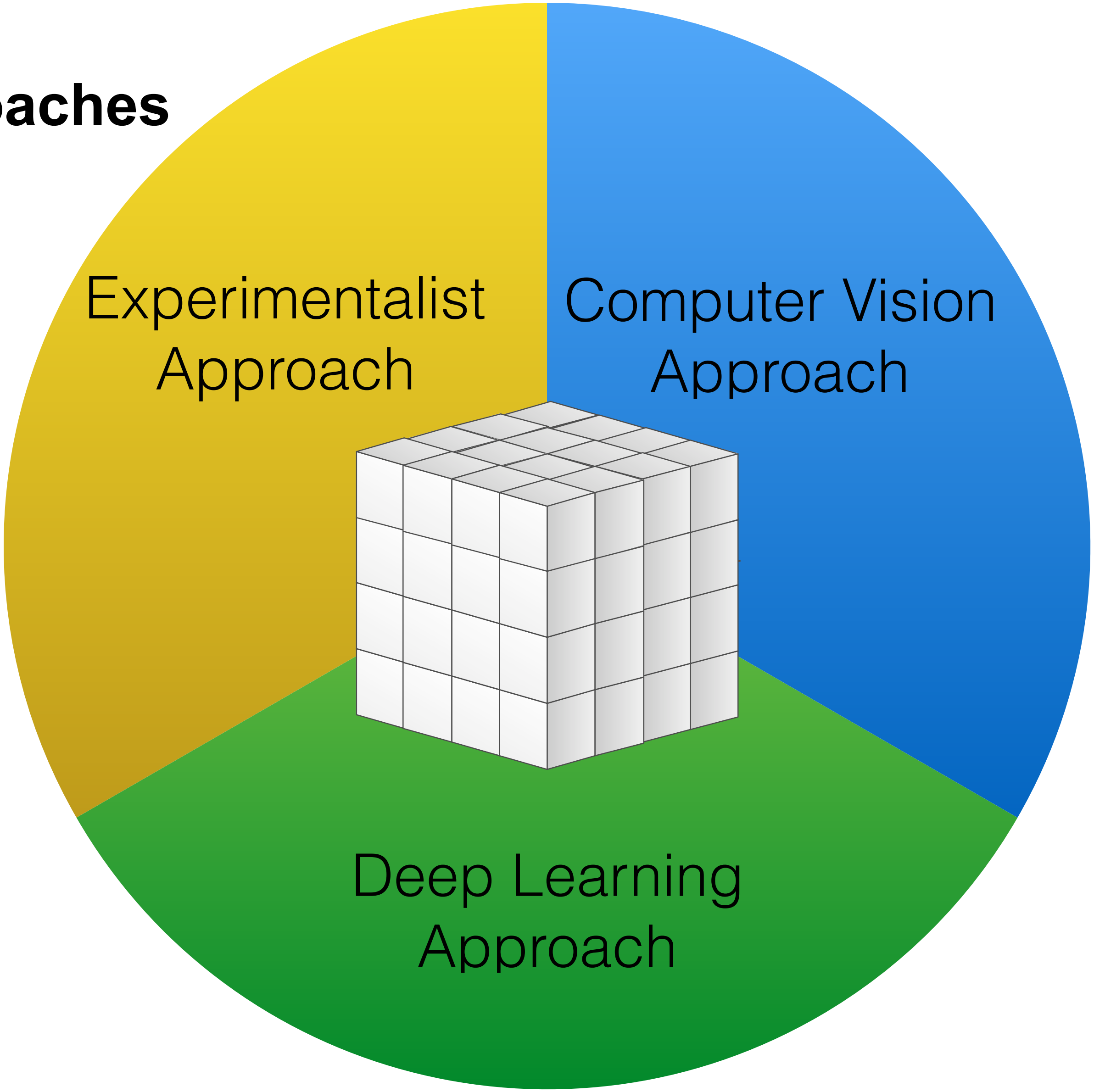
More detailed Slides: Quantitative Big Imaging Course

kmader.github.io/Quantitative-Big-Imaging-2015/

- Basic Segmentation, Discrete Binary Structures
- Advanced Segmentation
- Analyzing Complex Objects

Image Analyst Approaches

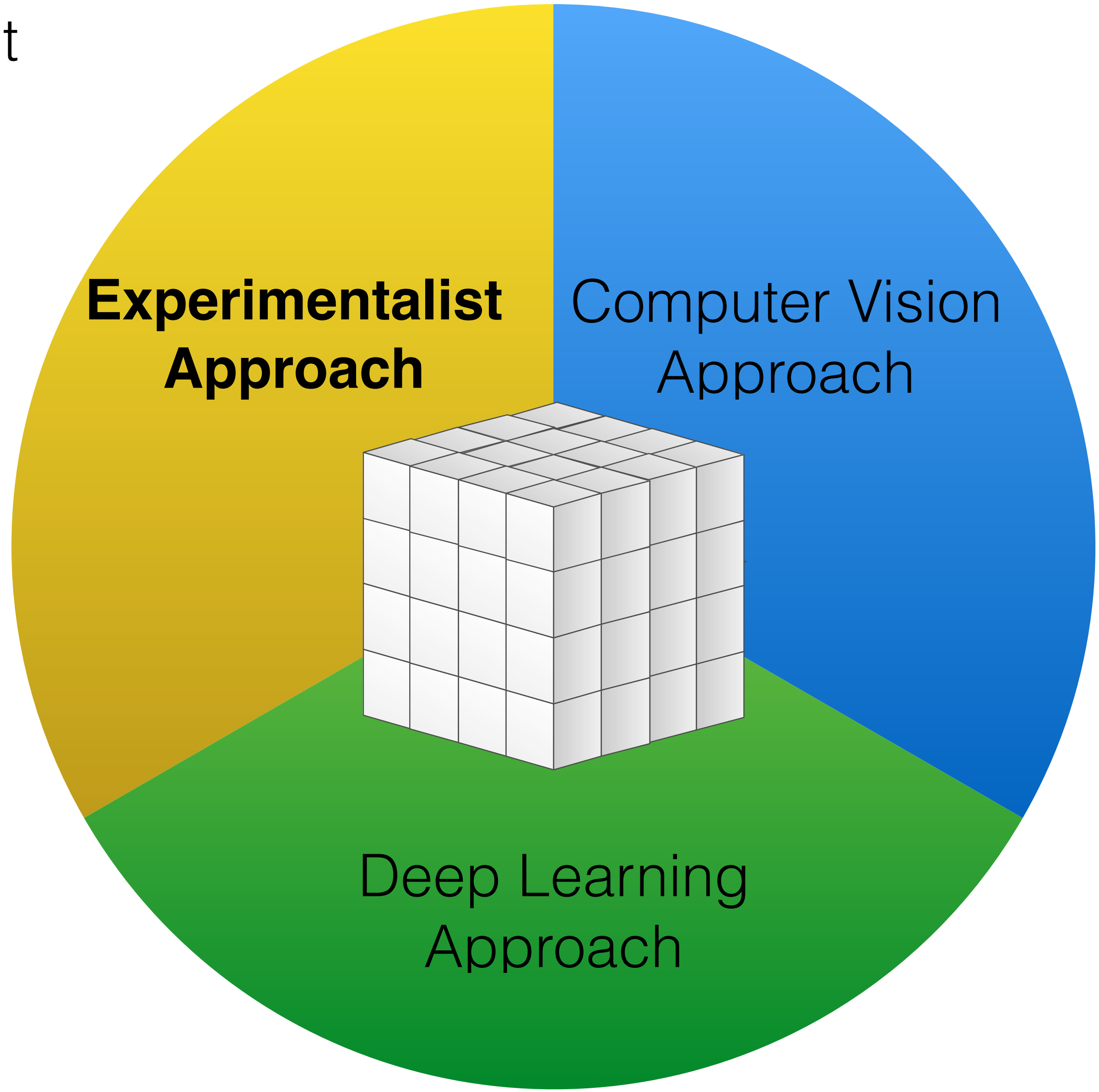
- An image is a bucket of pixels.
- How you choose to turn it into useful information is strongly dependent on your background



Experimentalist Approach

- Problem-driven
  - Top-down
  - ‘Reality’ Model-based

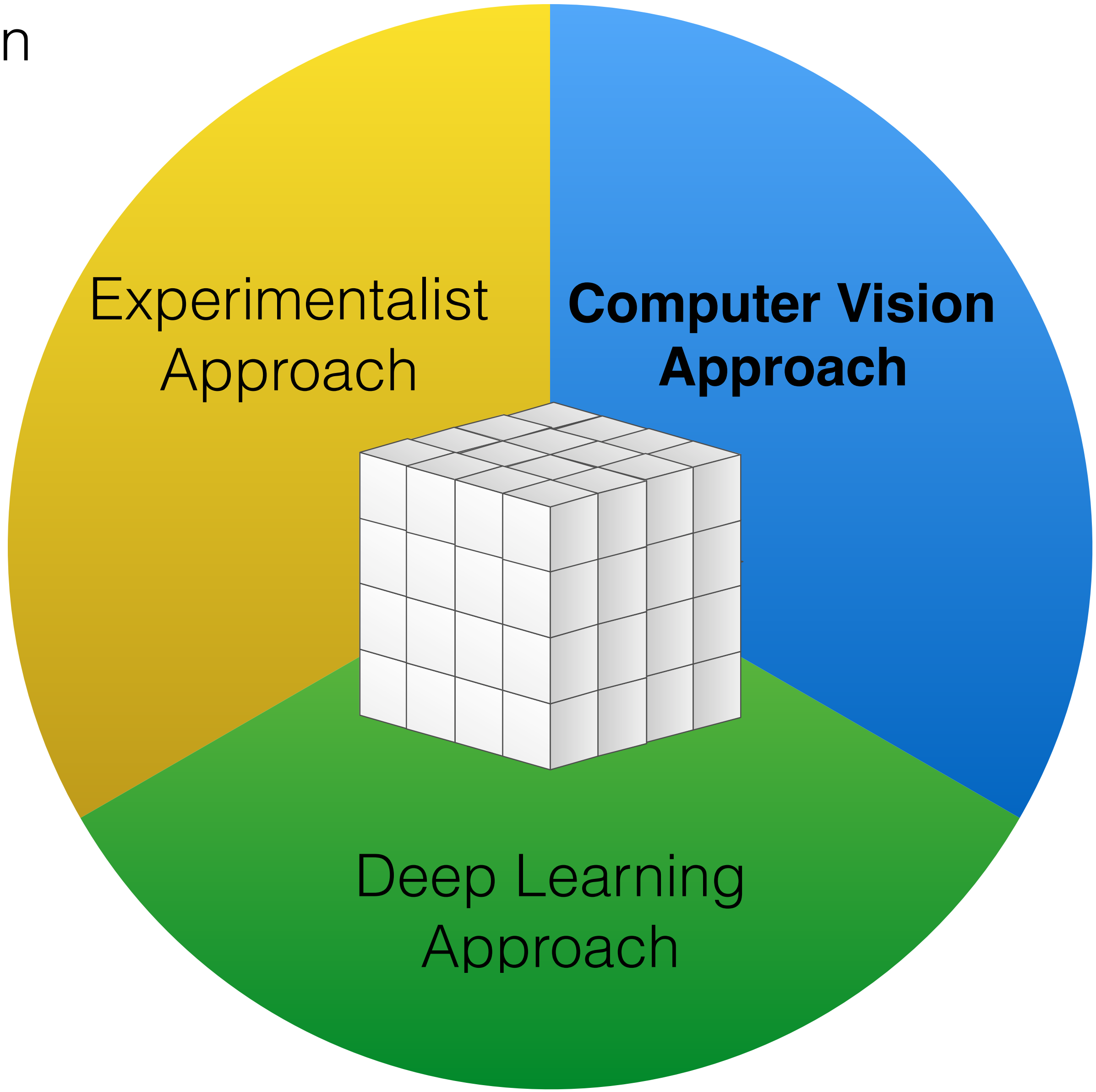
- cell counting
- porosity



Computer Vision Approach

- Method-driven
  - Feature-based
  - ‘Image’ Model-based
- Engineer features for solving problems

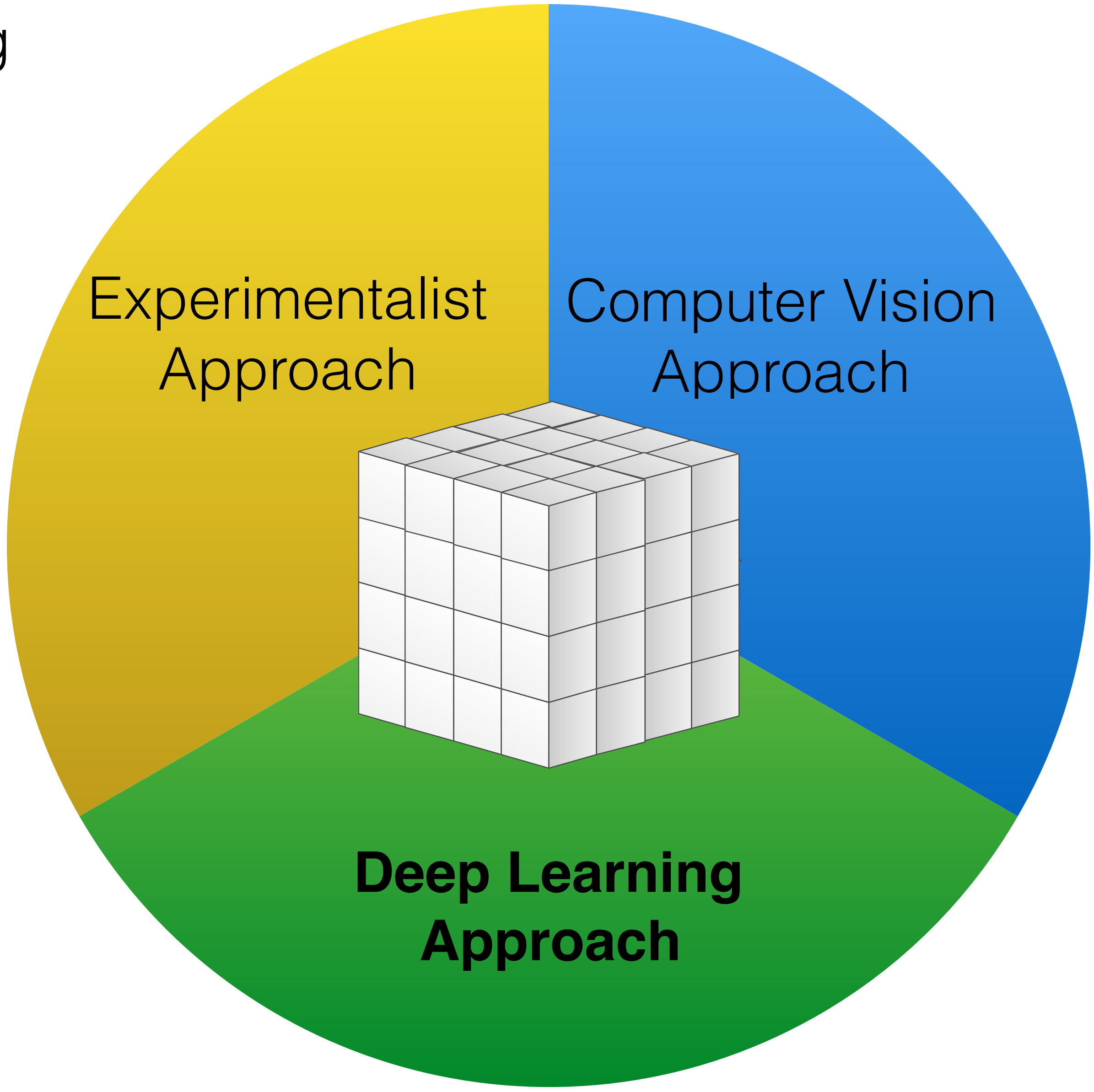
- edge detection
- face detection



Deep Learning Approach

- Results-driven
  - Biology 'inspired'
- Build both image processing and analysis from scratch

- Captioning images
- Identifying unusual events

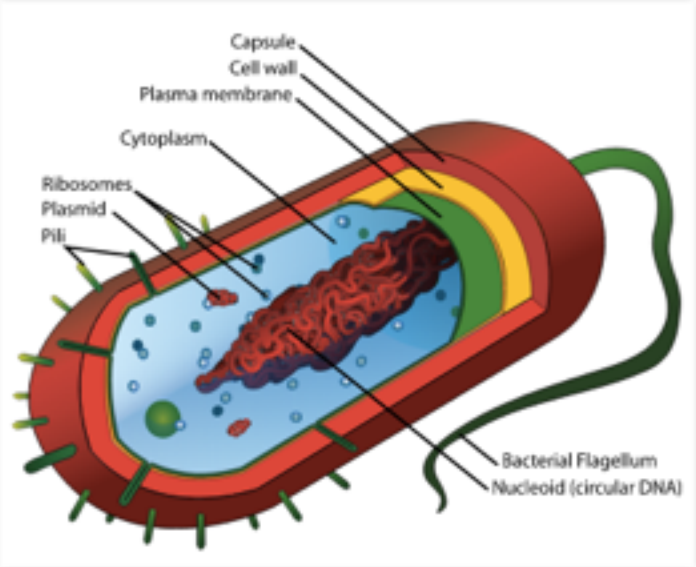


1. Motivation

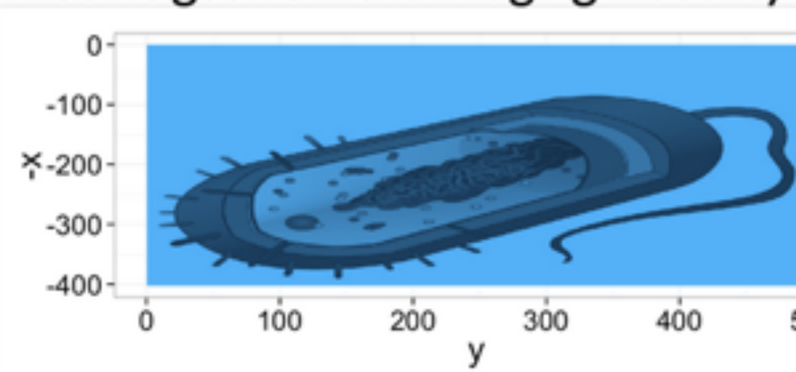
Why do we do experiments?

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?

- What we are looking at



- What we get from the imaging modality

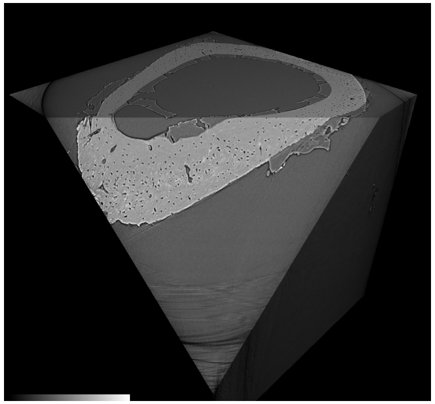


1. Motivation

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

Filtering / Preprocessing

Same exact size :(

....

Way too much, need to reduce

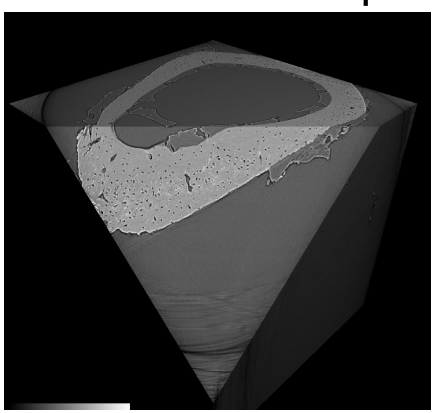
Single number: volume fraction, cell count, average cell stretch, cell volume variability

1. Motivation

Why do we do experiments?

1. To test a hypothesis

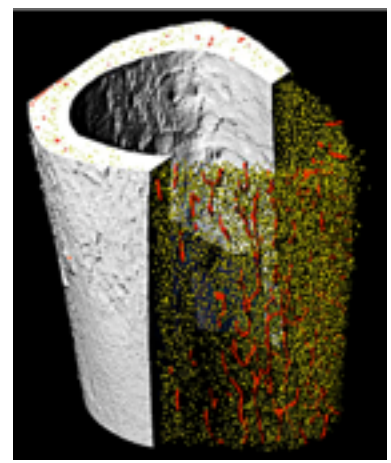
2560 (pixels in x)
x 2560 (pixels in y)
x 2160 (pixels in z)
x 32 bit
= 56GB / sample



Highly Absorbing Regions — Weakly Absorbing Regions

Segmentation

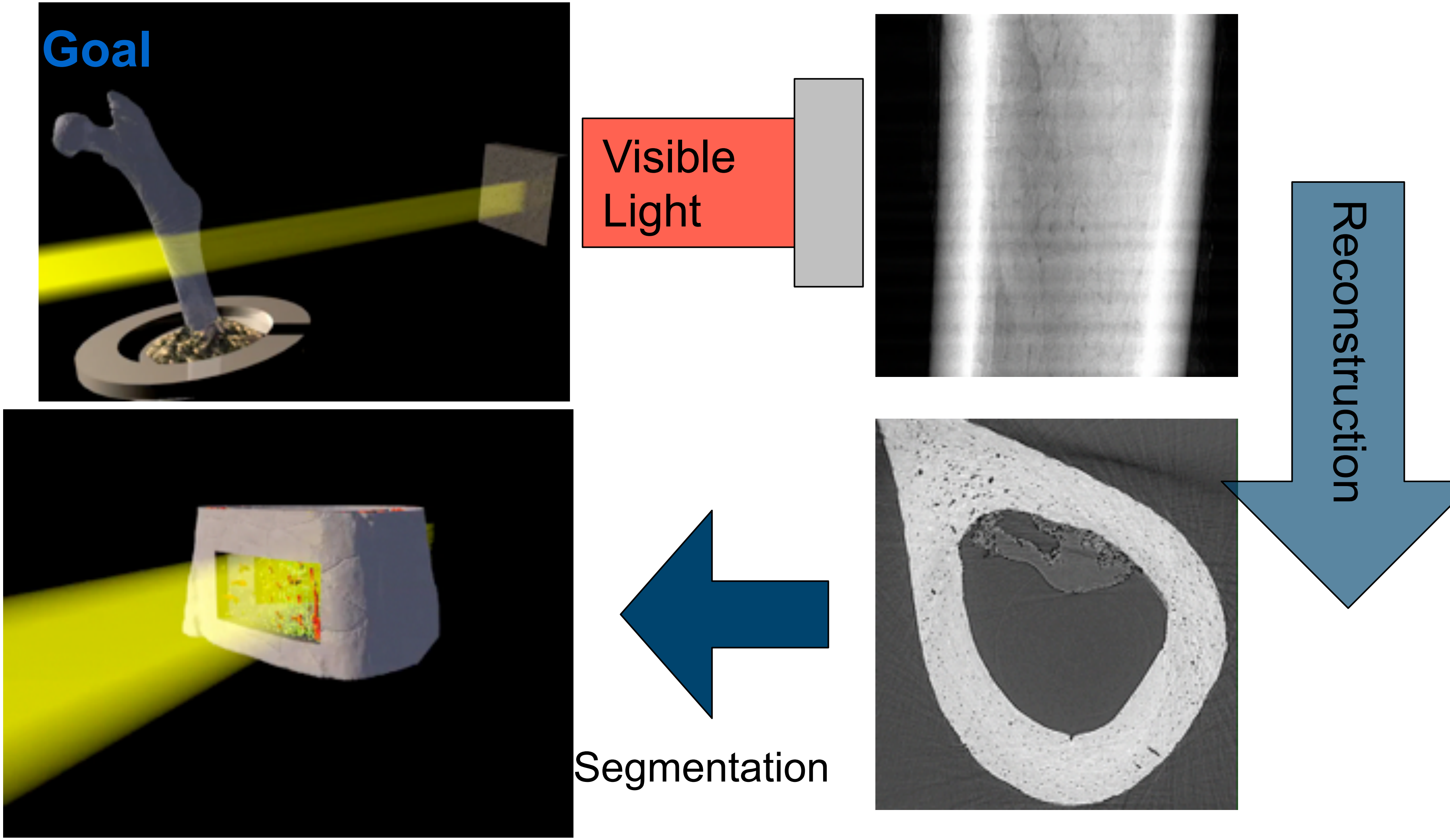
…
x 1-2 bit
~ 2GB / sample



still a long way to go
….

CCD

1. Segmentation and thresholding



1. Motivation

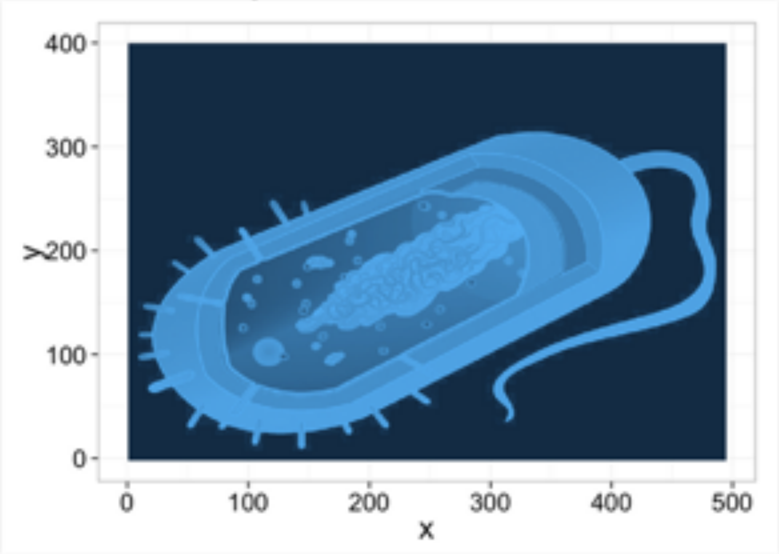
Review: Filtering and Image Enhancement

- This was a noise process which was added to otherwise clean imaging data
- $$I_{measured}(x,y) = I_{sample}(x,y) + \mathrm{Noise}(x,y)$$
- What would the perfect filter be
  - $$Filter * I_{sample}(x,y) = I_{sample}(x,y)$$
  - $$Filter * \mathrm{Noise}(x,y) = 0$$
  - $Filter * I_{measured}(x,y) = Filter * I_{real}(x,y) + Filter * \mathrm{Noise}(x,y) \rightarrow \mathbf{I_{sample}(x,y)}$
- What most filters end up doing
$$Filter * I_{measured}(x,y) = 90\% I_{real}(x,y) + 10\% \mathrm{Noise}(x,y)$$
- What bad filters do
$$Filter * I_{measured}(x,y) = 10\% I_{real}(x,y) + 90\% \mathrm{Noise}(x,y)$$

1. Motivation

What did people used to do?

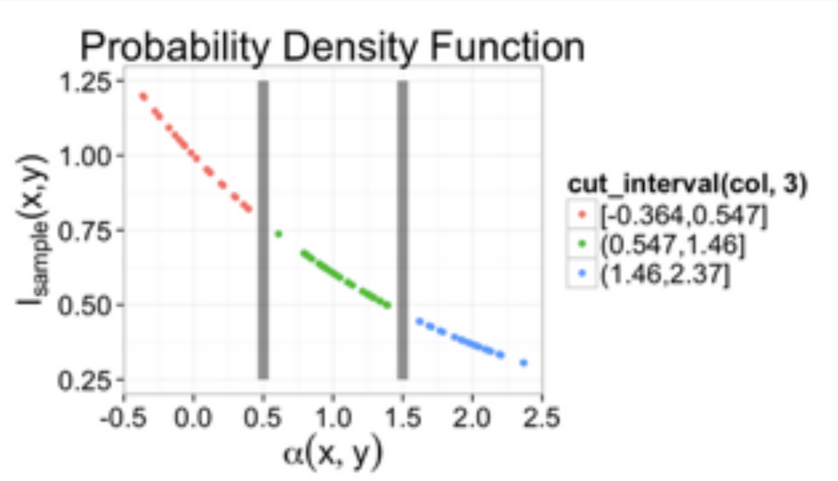
- What comes out of our detector / enhancement process

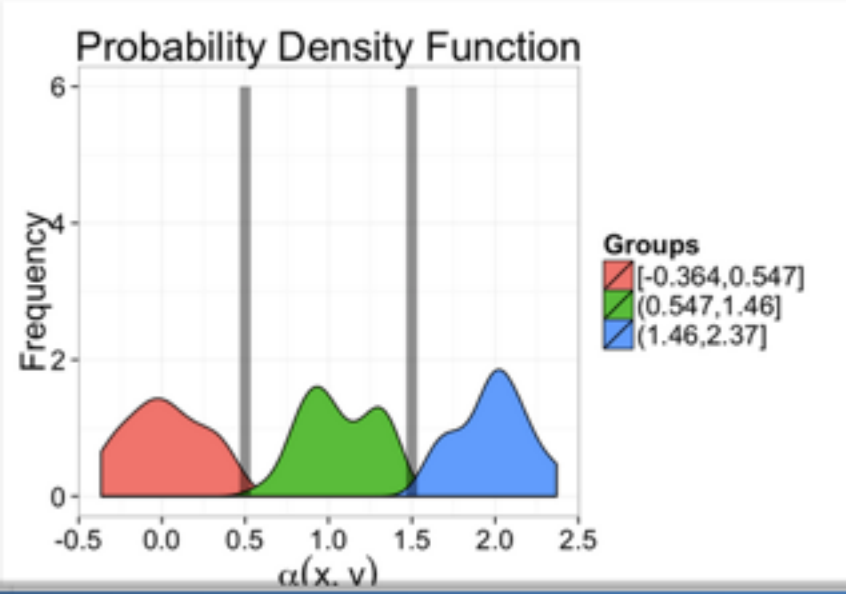


- Identify objects by eye
  - Count, describe qualitatively: "many little cilia on surface", "long curly flaggelum", "elongated nuclear structure"
- Morphometrics
  - Trace the outline of the object (or sub-structures)
  - Can calculate the area by using equal-weight-paper and employing the "cut-and-weigh" method

Quantitative Analysis

- For segmentation this model is:
  - there are 2 (or more) distinct components that make up the image
  - these components are distinguishable by their values (or vectors, colors, tensors, ...)
  - For absorption/attenuation microscopy, Beer-Lambert Law
  $$I_{detector} = I_{source} \exp(-\alpha d)$$
  - Different components have a different α based on the strength of the interaction between the light and the chemical / nuclear structure of the material
  $$I_{sample}(x, y) = I_{source} \exp(-\alpha(x, y) d)$$
  $$\alpha = f(N, Z, \sigma, \cdots)$$

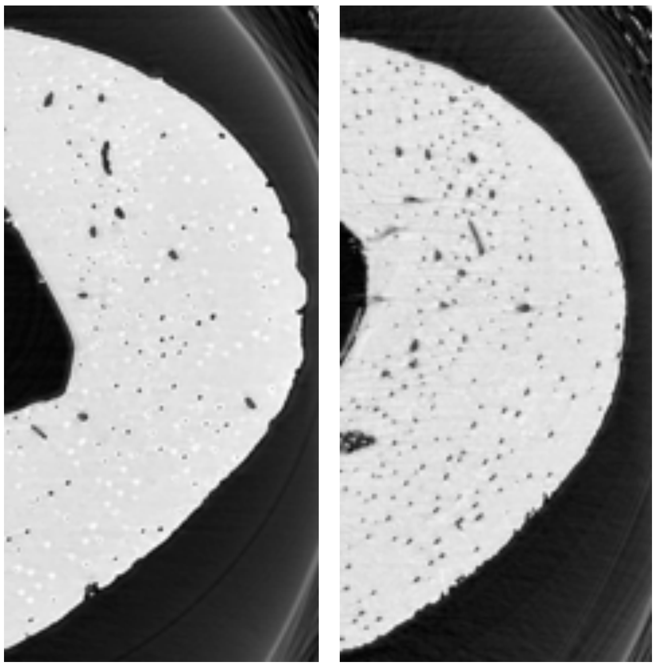




1. Segmentation and thresholding

Thresholding

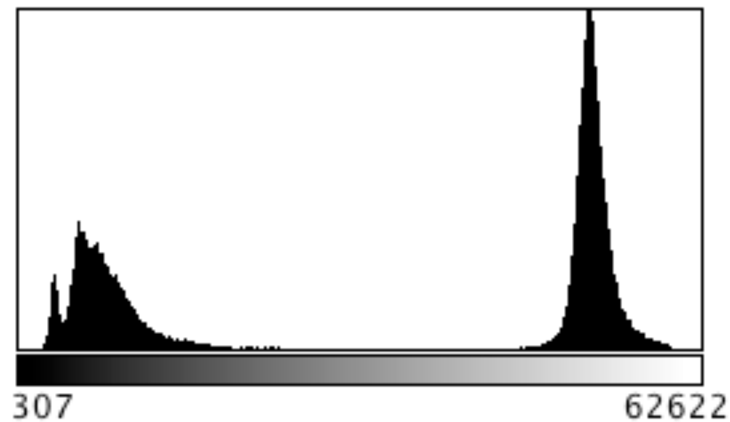
Histogram



Slices of Cortical Bone Tissue from a mouse

100µm

Original



Count: 70089 Min: 307
Mean: 34408.424 Max: 62622
StdDev: 21994.130 Mode: 52654 (3220)
Bins: 256 Bin Width: 243.418

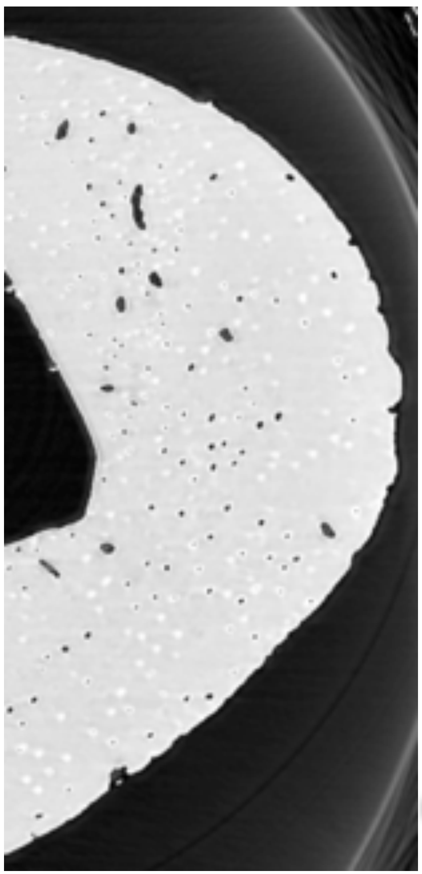
Histogram

1. Segmentation and thresholding

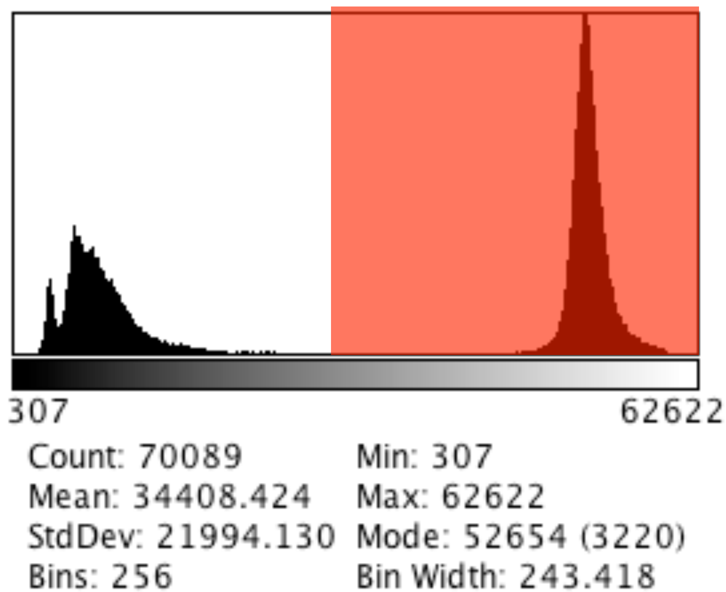
Thresholding

=> Brightness peak correspond to a bone

Histogram



100µm



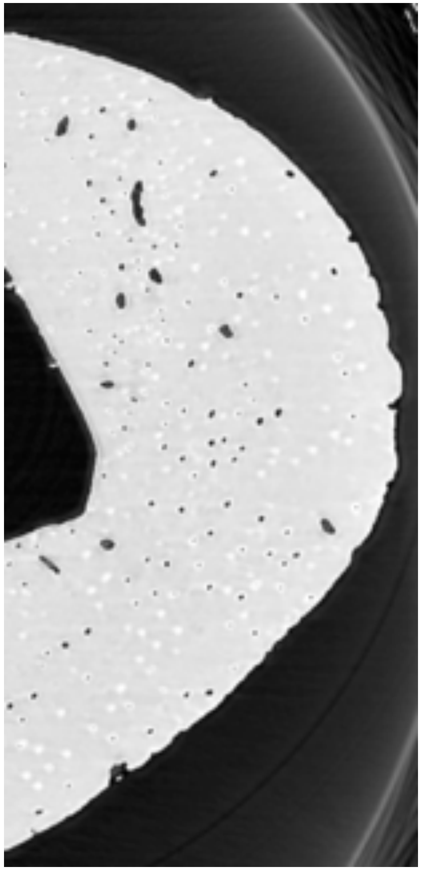
Threshold

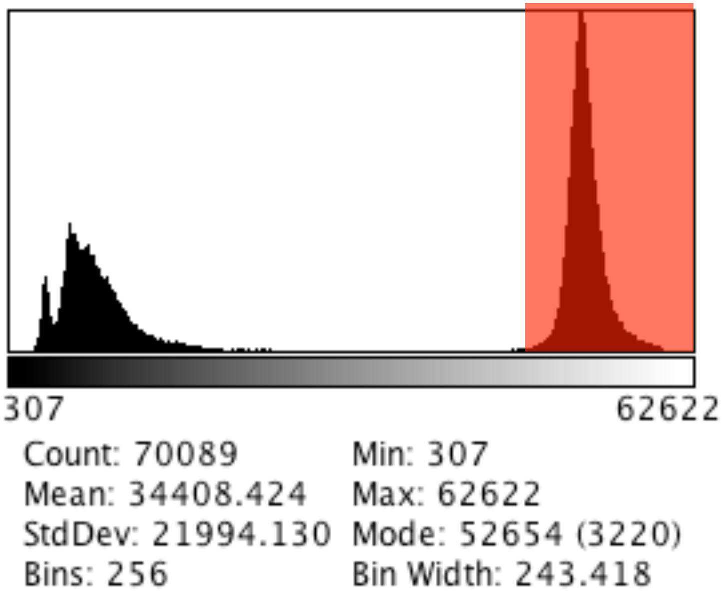
1. Segmentation and thresholding

Thresholding

Histogram

Highly mineralized bone only





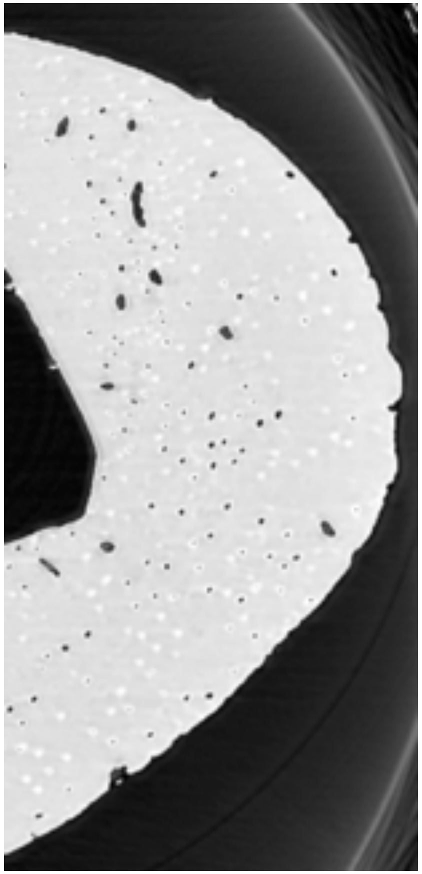
Threshold

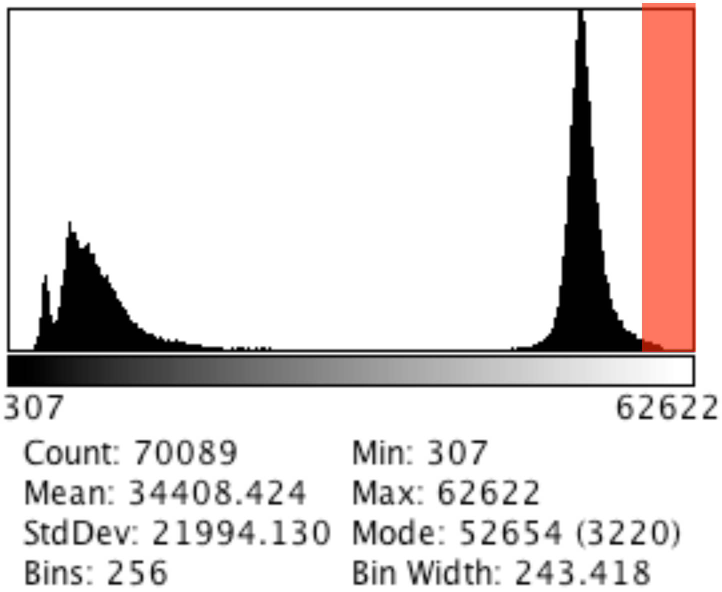
1. Segmentation and thresholding

Thresholding

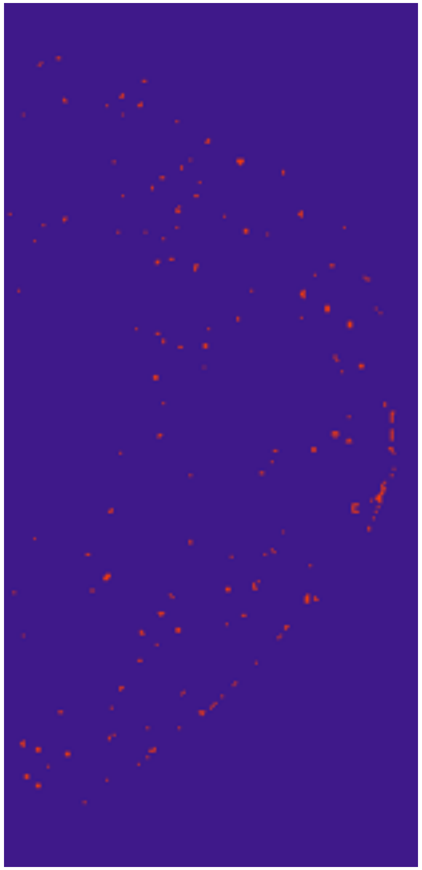
Histogram

Very Highly mineralized spots only





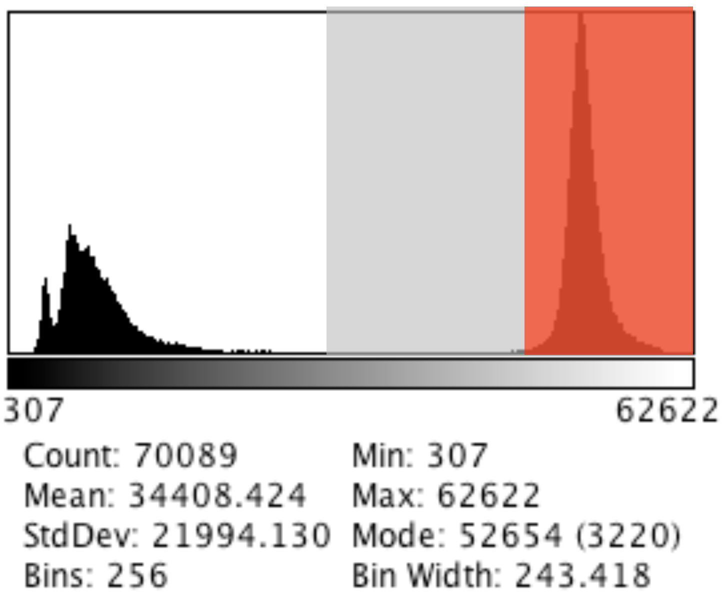
Threshold



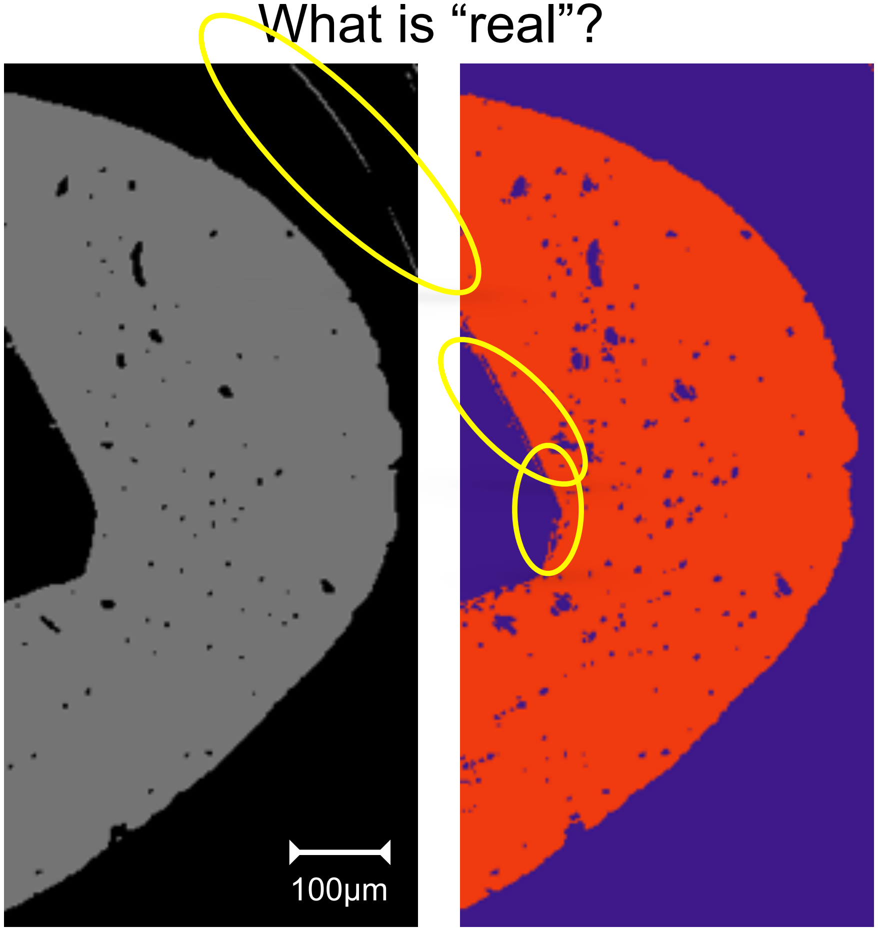
1. Segmentation and thresholding

Selecting a Threshold

Histogram



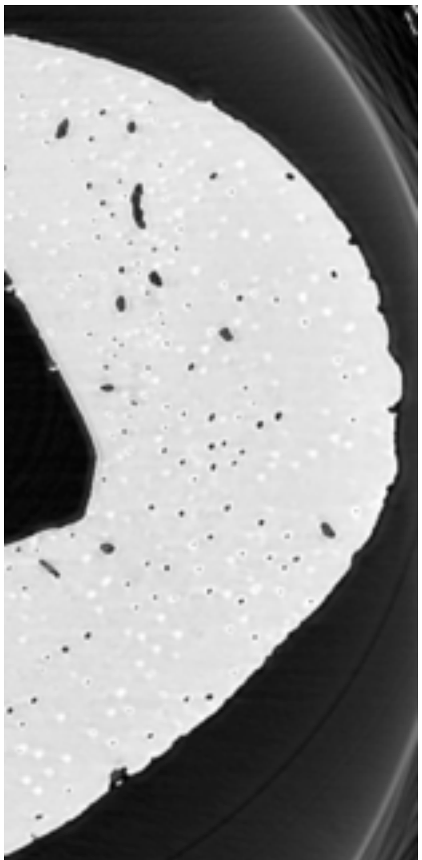
How do you pick a threshold?
What is “real”?

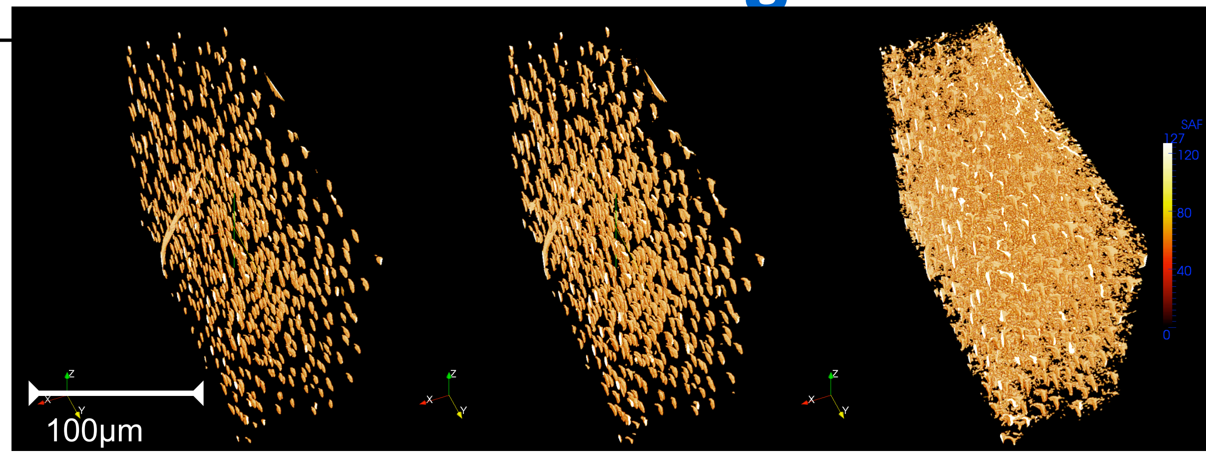


1. Segmentation and thresholding

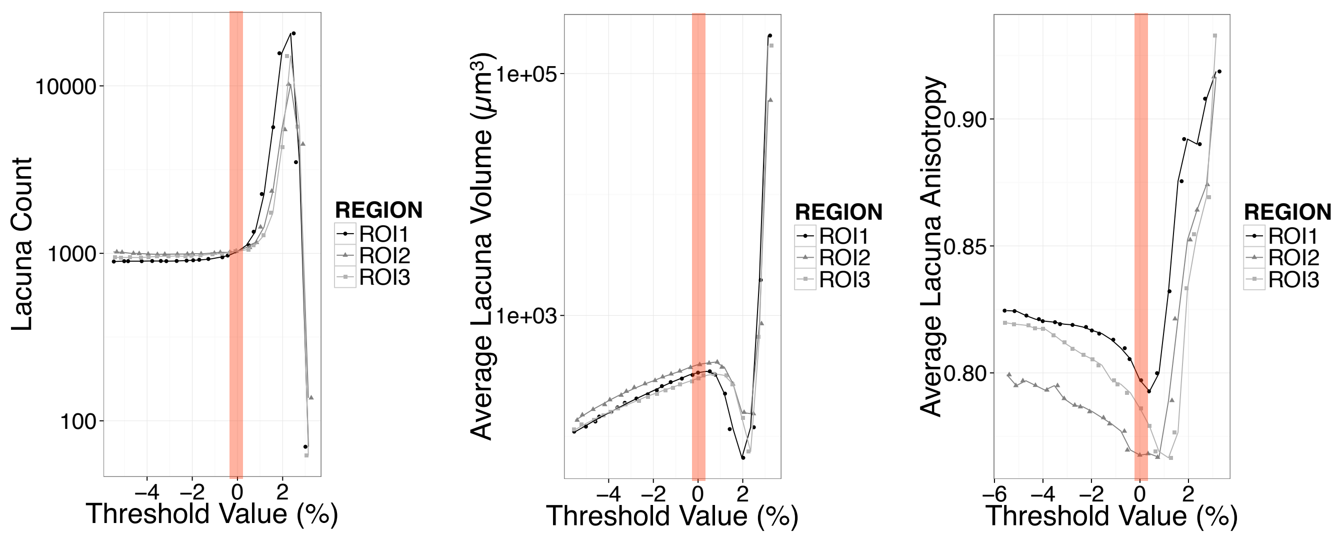
Thresholding

Picking a threshold





-4% 0% +4%



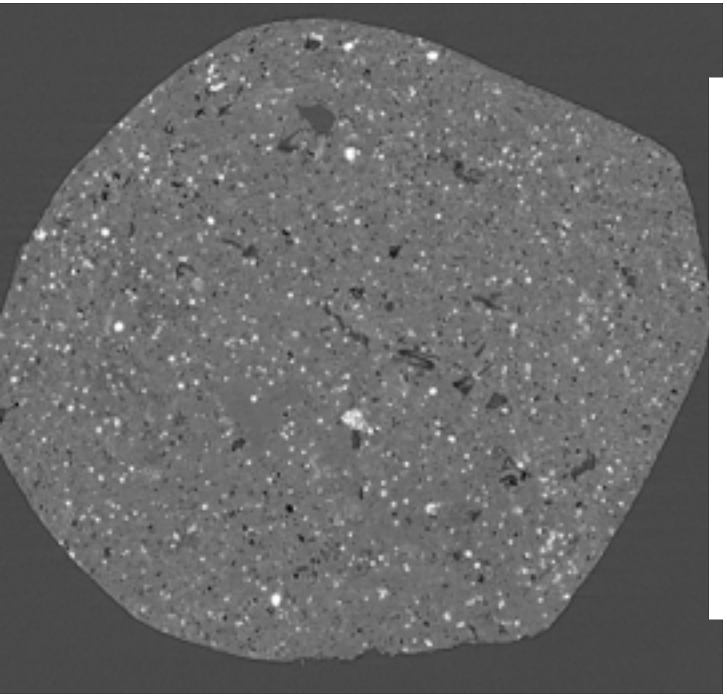
Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1), 142–154. doi:10.1016/j.bone.2013.06.026

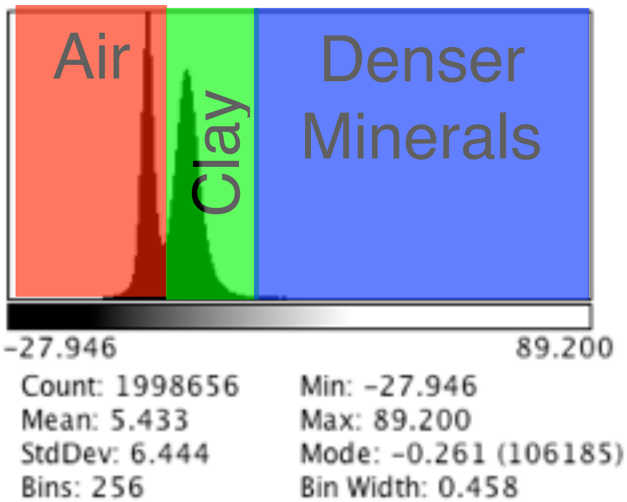
1. Segmentation and thresholding

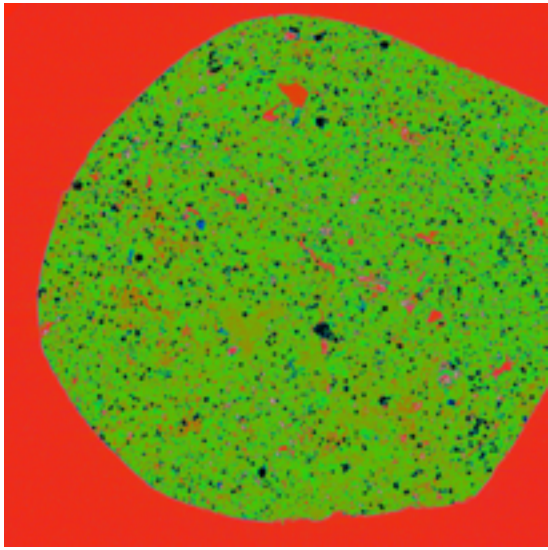
Thresholding

Histogram

All three materials identified







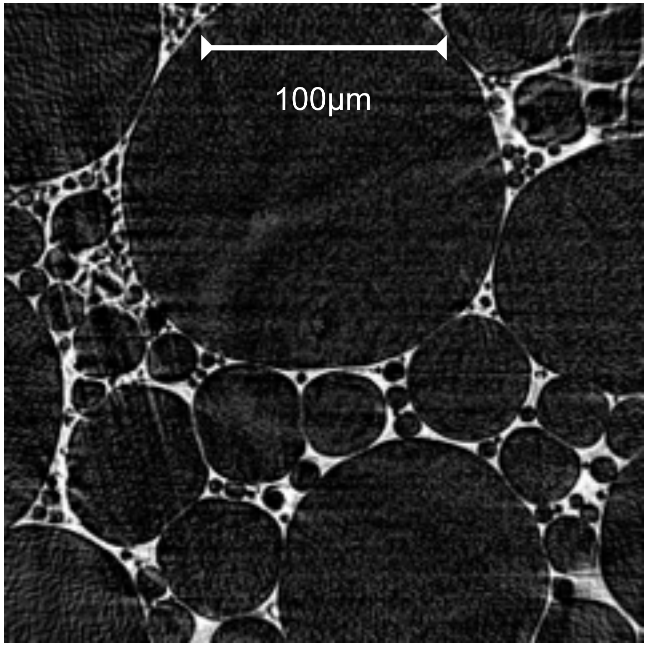
Apply Each Threshold

Shale provided from Kanitpanyacharoen, W. (2012). Synchrotron X-ray Applications Toward an Understanding of Elastic Anisotropy.

1. Segmentation and thresholding

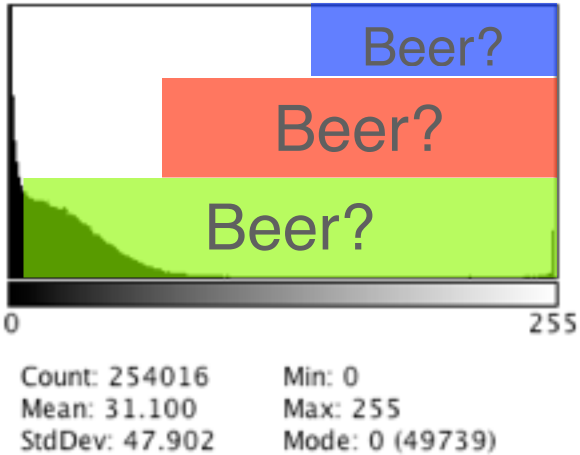
Thresholding

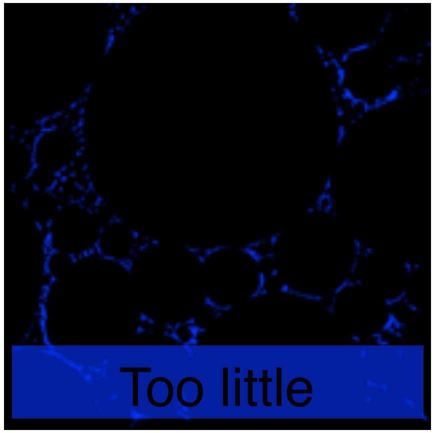
Histogram

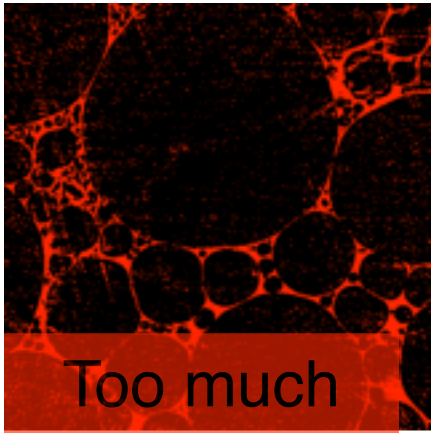


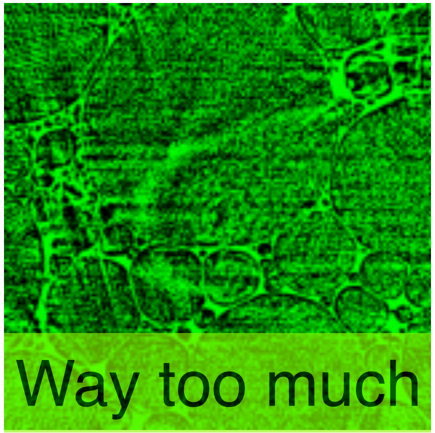
Beer foam
Courtesy of R. Mokso

More difficult histogram to interpret









1. Segmentation and thresholding

Thresholding

Texture

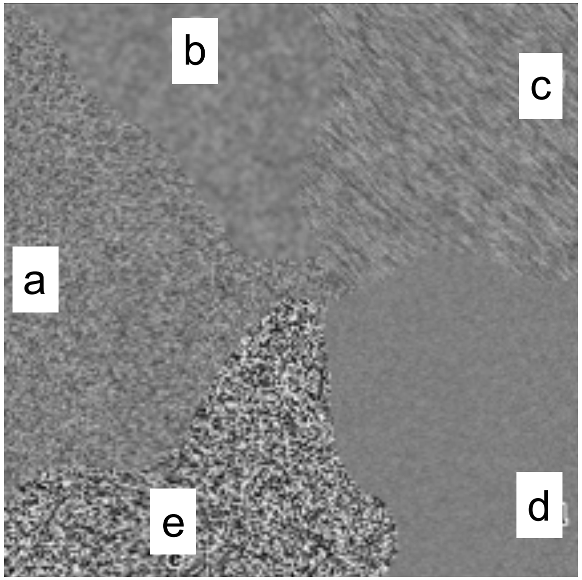


Image Processing Handbook, J. C. Russ

Different textures

a) Gaussian brightness variation
b) Gaussian filtered a)
c) Directionally averaged a)
d) Gaussian brightness variation
e) Uniformly random brightness distribution

1. Segmentation and thresholding

Thresholding

Texture

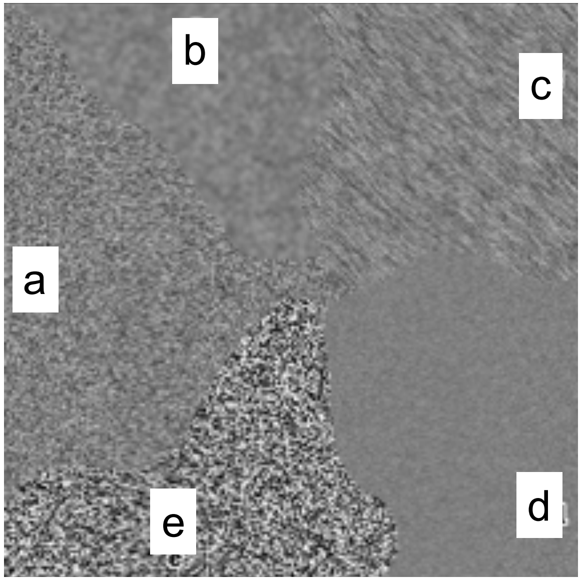
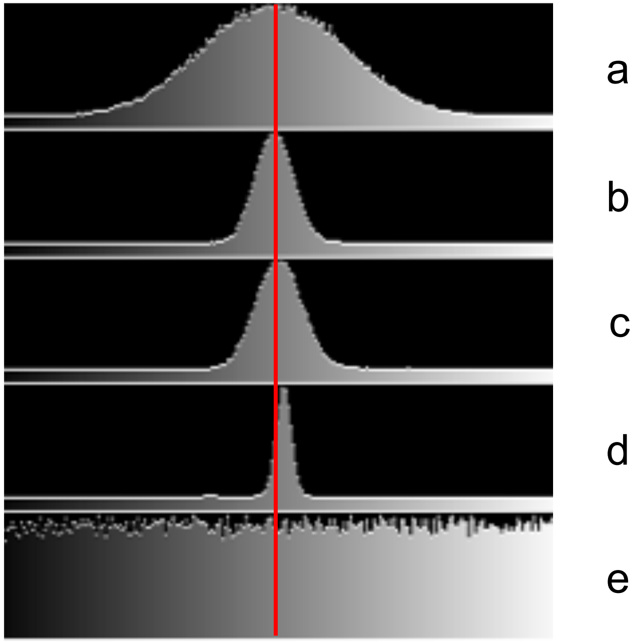


Image Processing Handbook, J. C. Russ

Different textures

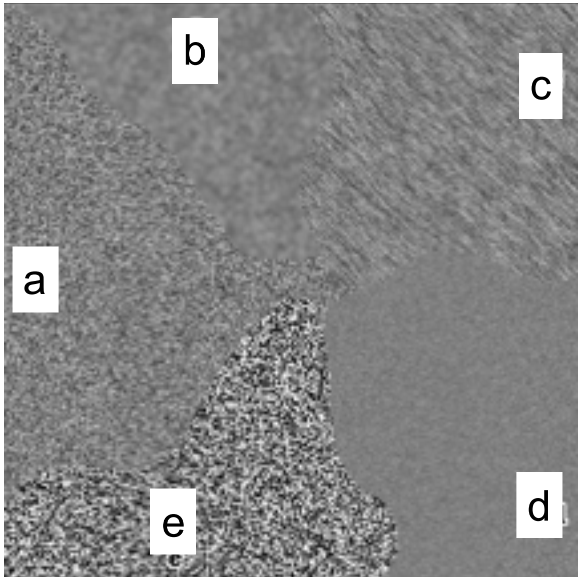


Histograms

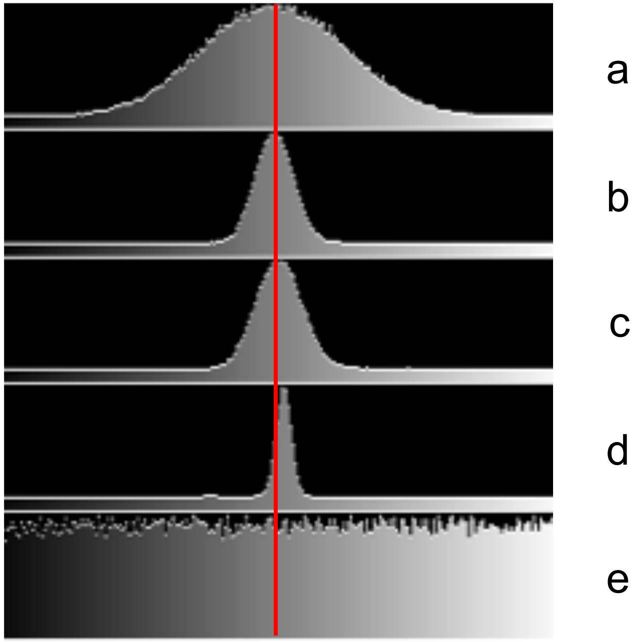
1. Segmentation and thresholding

Thresholding

Texture



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



Different textures

=> Average brightnesses identical
=> Histogram-based thresholding unfeasible

1. Segmentation and thresholding

Thresholding

Texture-sensitive operators

- Laplacian
- Frei and Chen
- Variance
- Haralick
- Hurst
- Range
- …

Already discussed for sharpening and finding edges (within block I. Preprocessing)

1. Segmentation and thresholding

Thresholding

Texture-sensitive operators

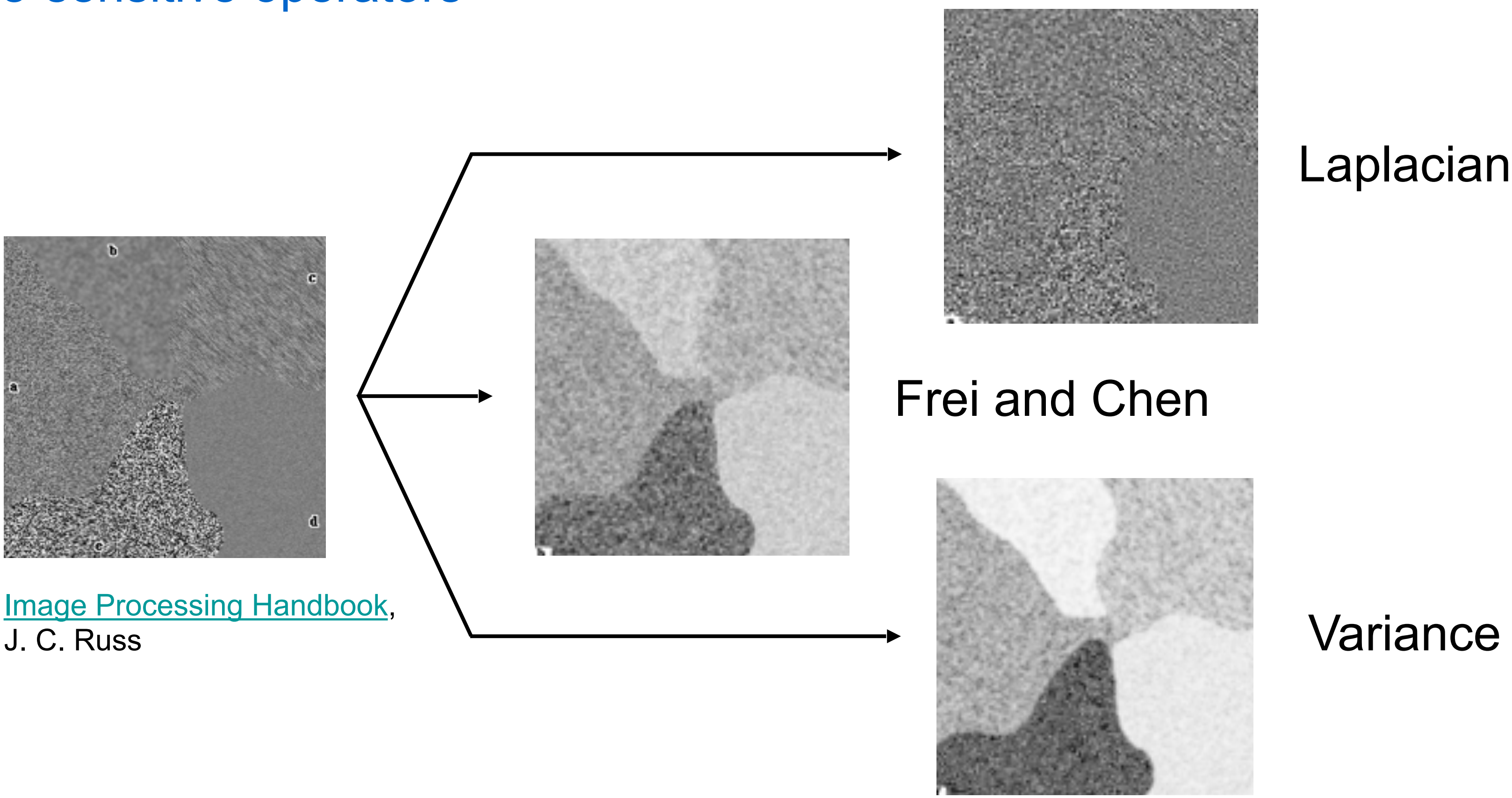


Image Processing Handbook, J. C. Russ

1. Segmentation and thresholding

Thresholding

Thresholding from texture

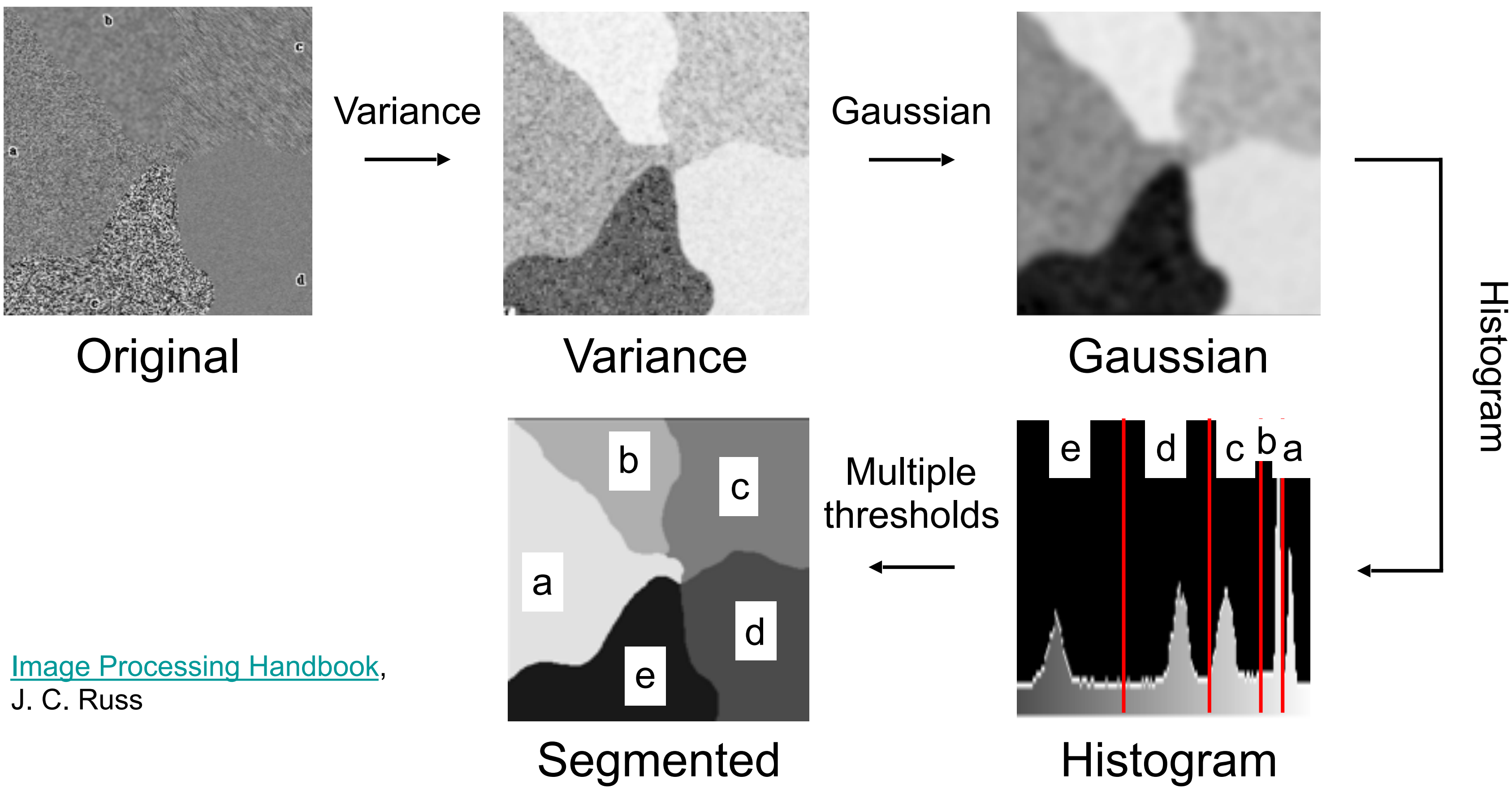


Image Processing Handbook,
J. C. Russ

1. Segmentation and thresholding

Thresholding

Multiple thresholding criteria

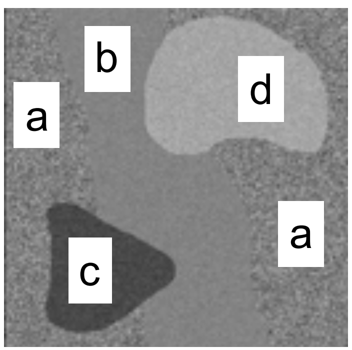
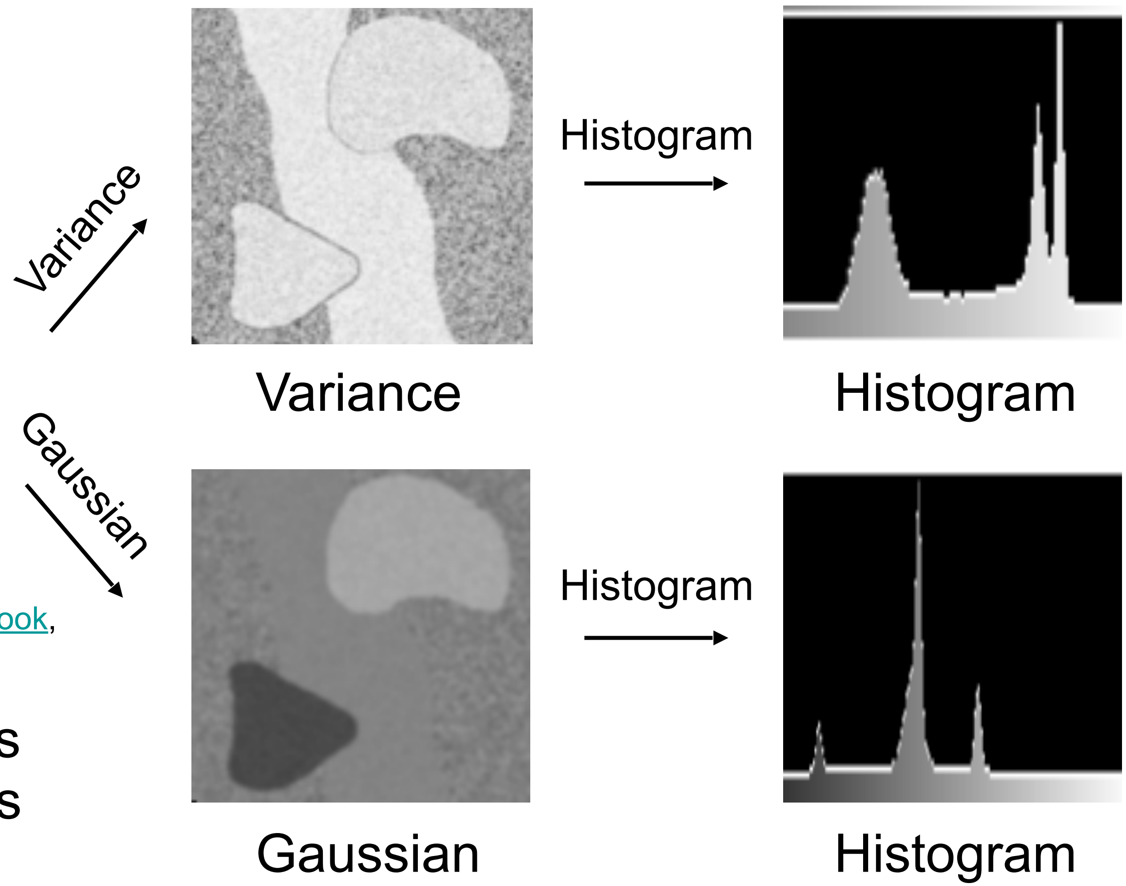


Image Processing Handbook, J. C. Russ

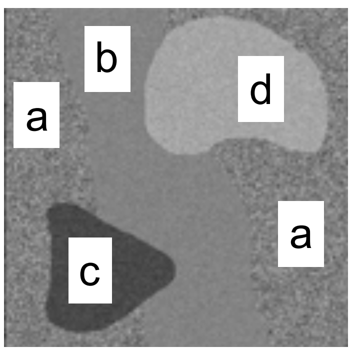
Different textures and brightnesses



1. Segmentation and thresholding

Thresholding

Multiple thresholding criteria



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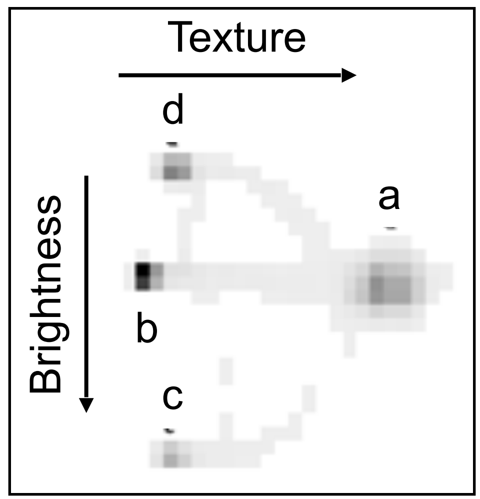
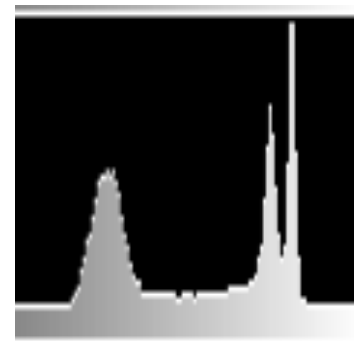
Different textures and brightnesses



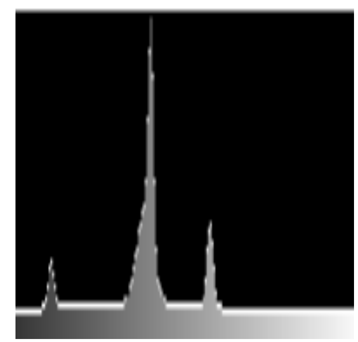
Texture-brightness histogram

Combine



Histogram

Combine



Histogram

1. Segmentation and thresholding

Thresholding

Multiple thresholding criteria

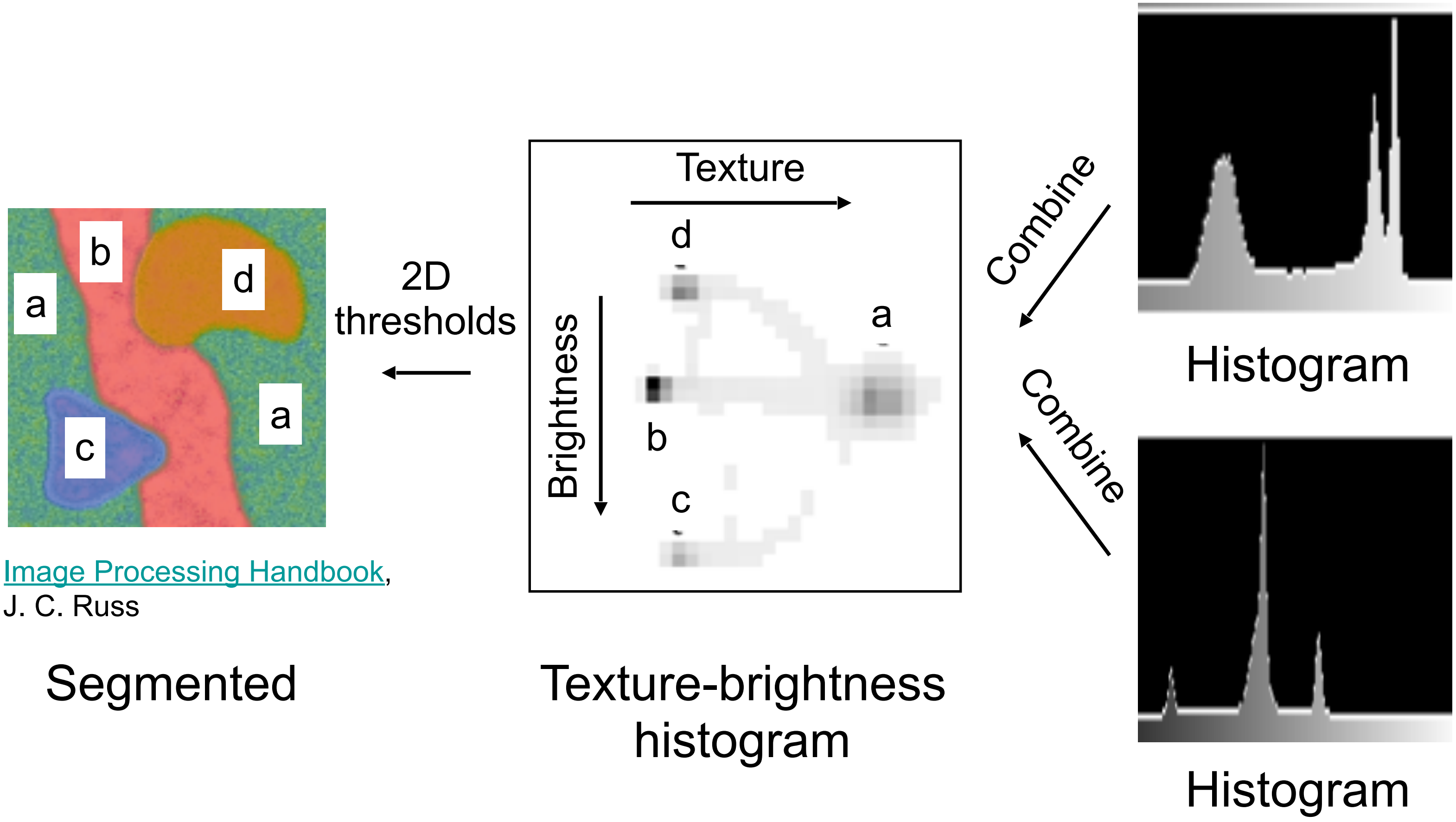


Image Processing Handbook, J. C. Russ

Segmented

Texture-brightness histogram

2. Processing binary images

Masks

Creation

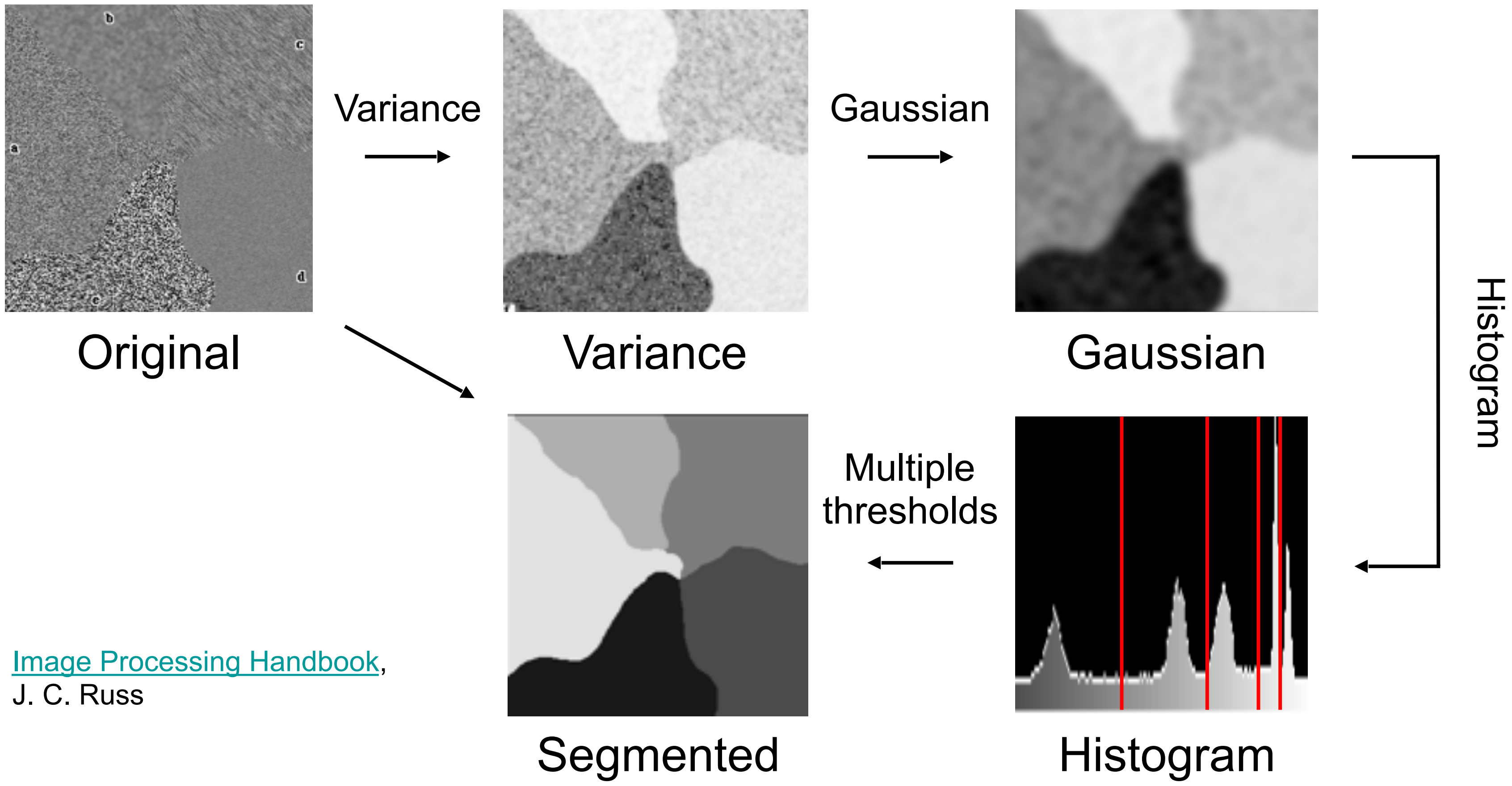
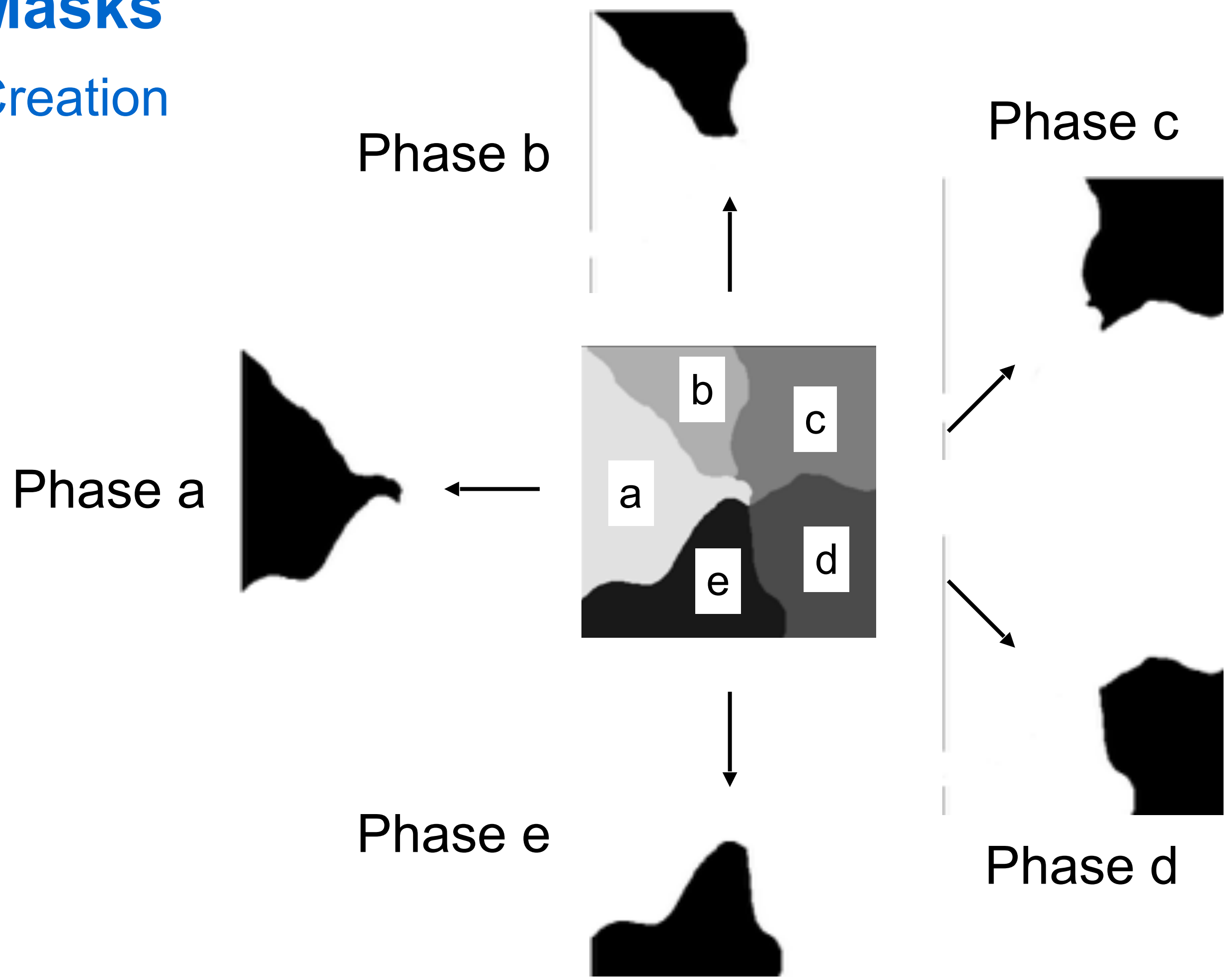


Image Processing Handbook,
J. C. Russ

2. Processing binary images

Masks

Creation



2. Processing binary images

Masks

Application

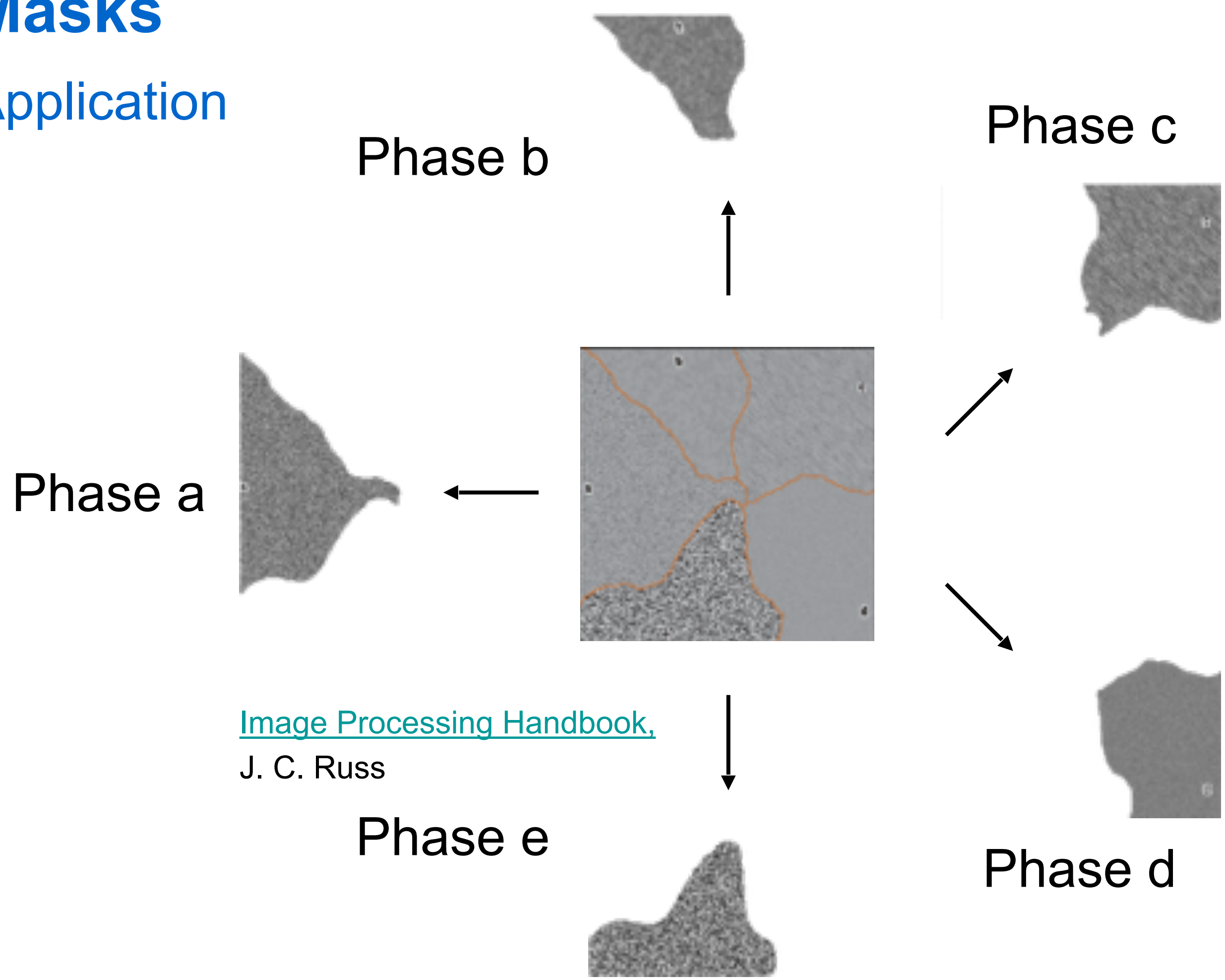


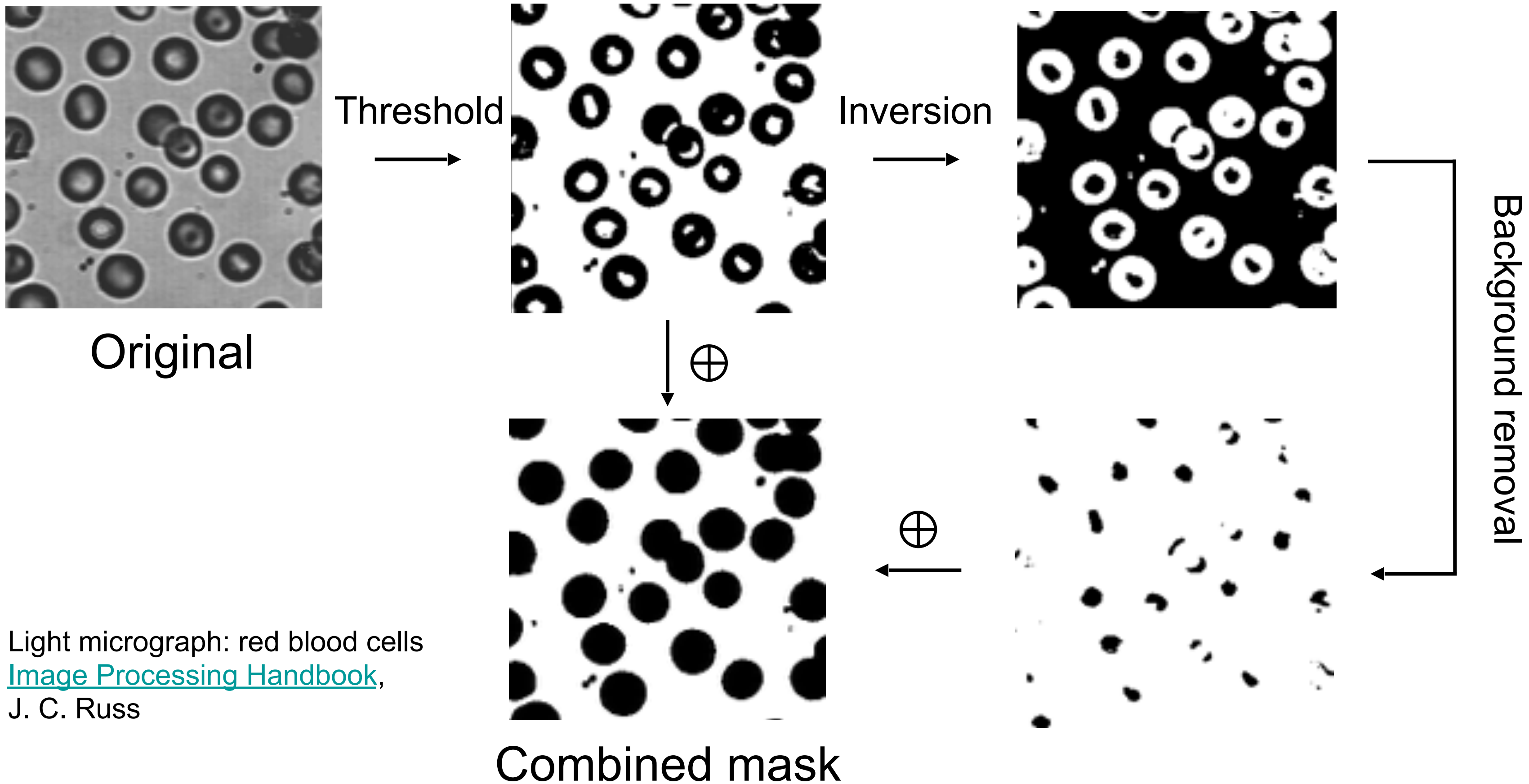
Image Processing Handbook, J. C. Russ

=> Analysis of single regions

2. Processing binary images

Masks

Combining masks

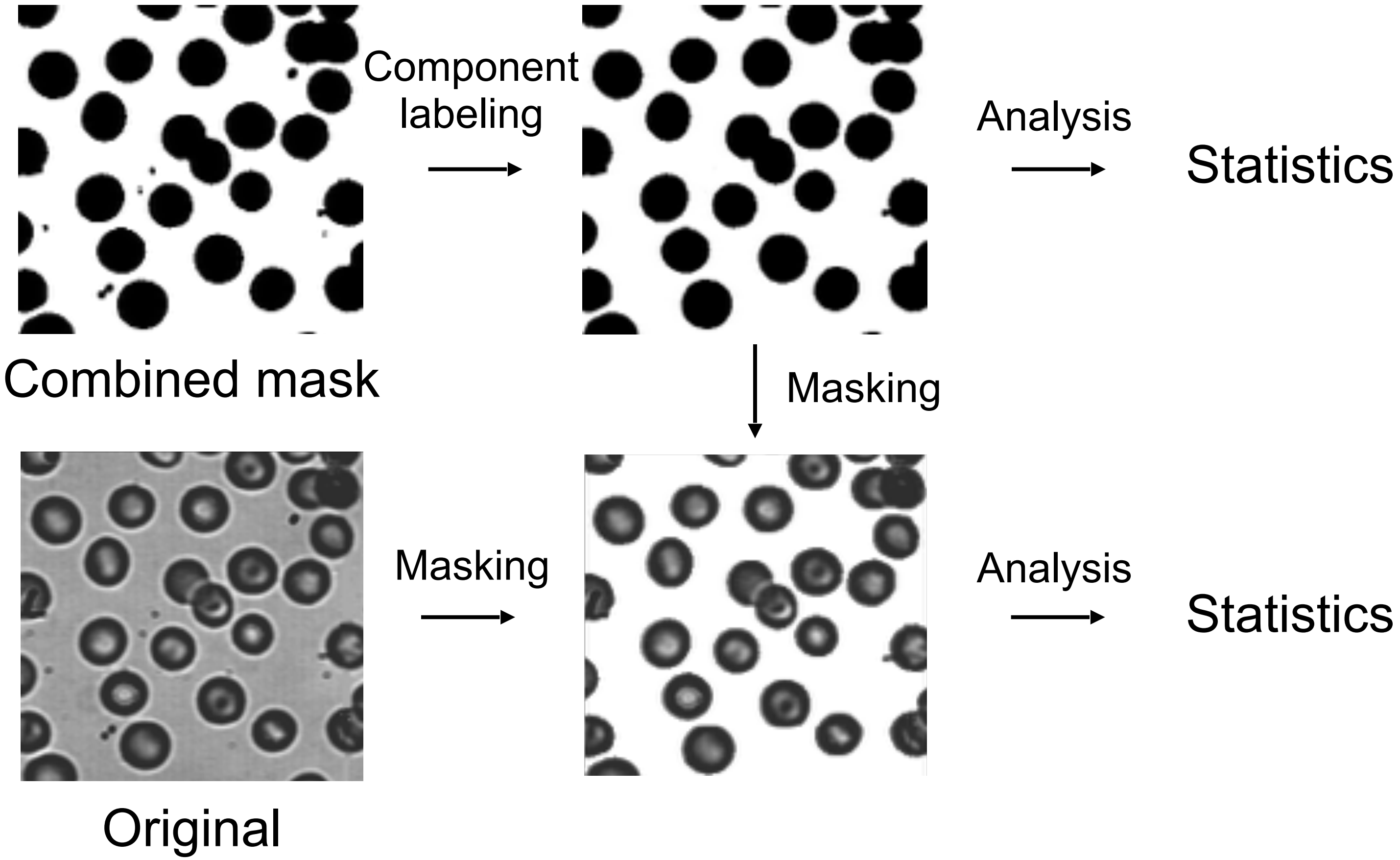


Light micrograph: red blood cells
Image Processing Handbook,
J. C. Russ

2. Processing binary images

Masks

Combining masks



2. Processing binary images

Morphological operations

Foreground and background

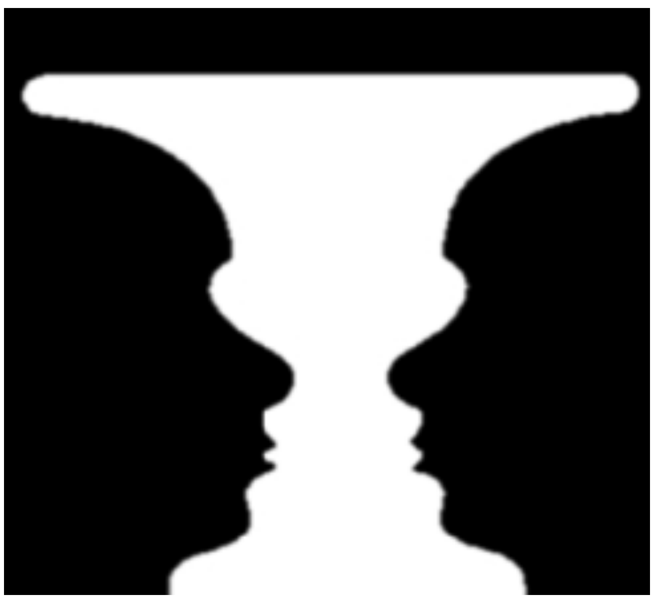


Image Processing Handbook, J. C. Russ

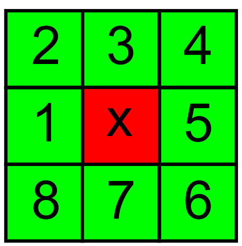
1) Definition of foreground and background is arbitrary

2) Human vision interprets an image in terms of relationships between structures
<=>
For computer-based image analysis systems, classification of foreground-background must be made at the level of individual pixels/voxels

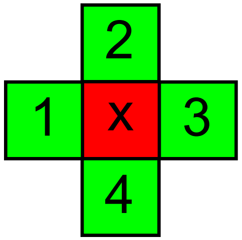
2. Processing binary images

Morphological operations

Neighborhood in 2D



8-adjacent

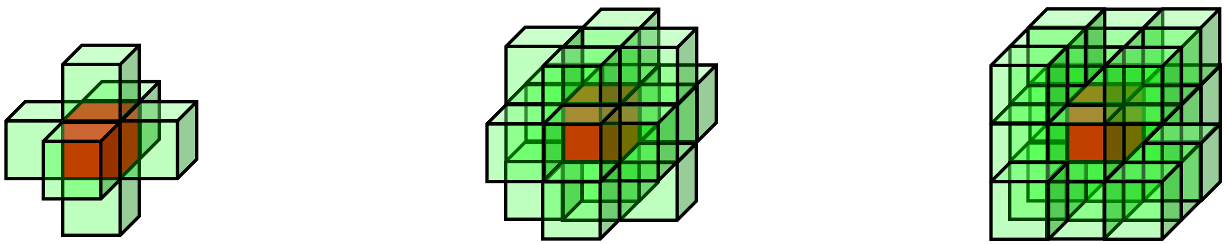


4-adjacent

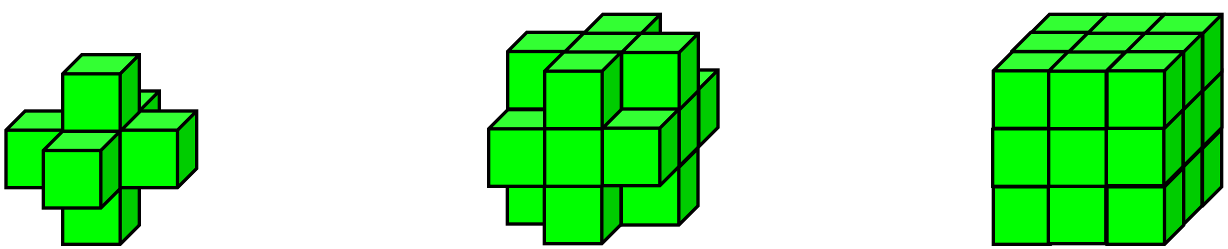
2. Processing binary images

Morphological operations

Neighborhood in 3D



6-adjacent (faces shared) | 18-adjacent (faces or edges shared) | 26-adjacent (faces or edges or corners shared)



Cubic voxels: standard

2. Processing binary images

Morphological operations

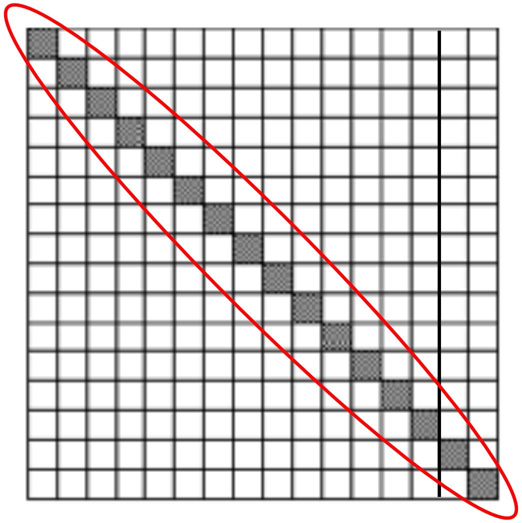
Foreground/background-connectivity dualism

- Foreground: 8-adjacent
- Background: 4-adjacent

=> 1 object ( )
background separated

- Foreground: 4-adjacent
- Background: 8-adjacent

=> 16 objects ( )
background connected



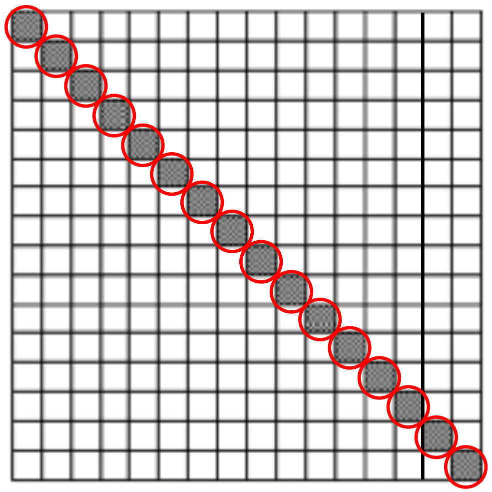


Image Processing Handbook, J. C. Russ

2. Processing binary images

Morphological operations

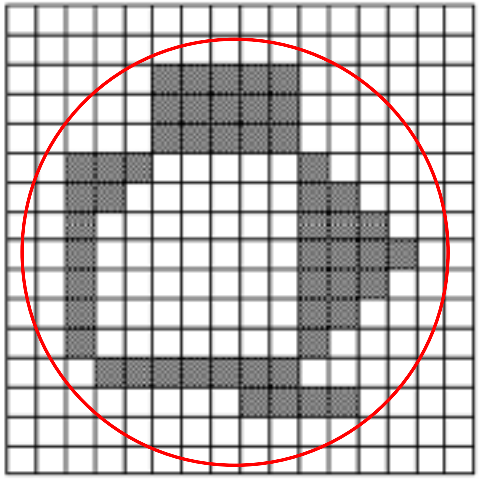
Foreground/background-connectivity dualism

- Foreground: 8-adjacent
- Background: 4-adjacent

=> 1 object ( )
background separated

- Foreground: 4-adjacent
- Background: 8-adjacent

=> 4 objects (,)
background connected



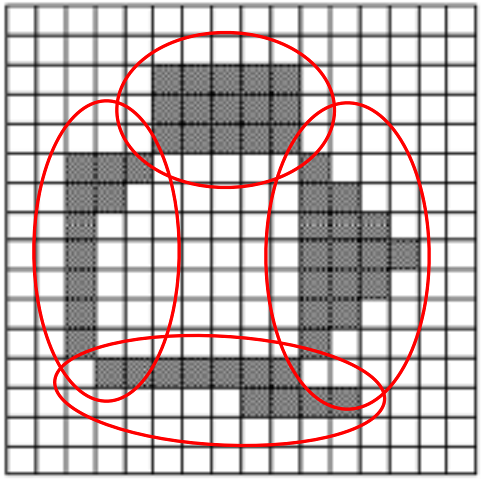
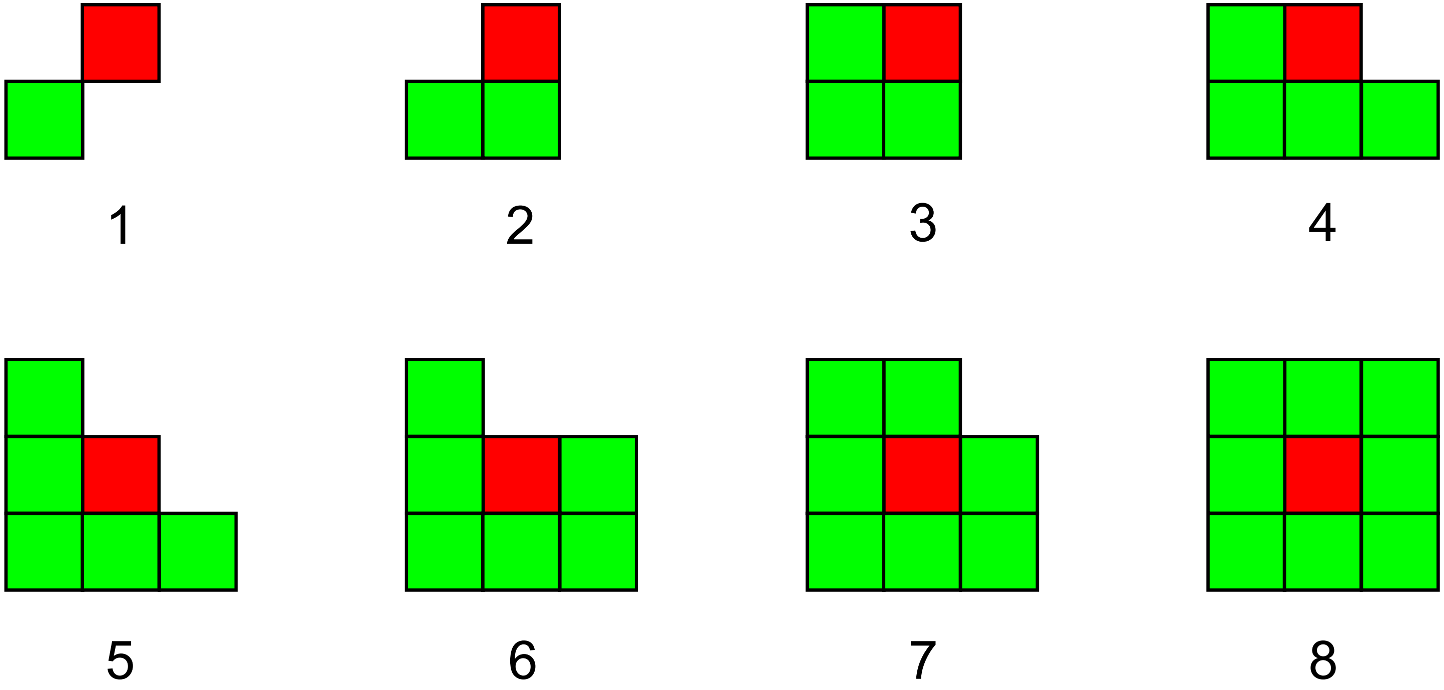


Image Processing Handbook, J. C. Russ

2. Processing binary images

Morphological operations

Neighbor coefficient



2. Processing binary images

Morphological operations

Erosion and dilation / opening and closing

- Erosion:
  Turn pixel off (foreground -> background)
  if touching any background pixel (neighborhood coefficient > 0)
- Dilation:
  Turn pixel on (background -> foreground)
  if touching any foreground pixel (neighborhood coefficient > 0)

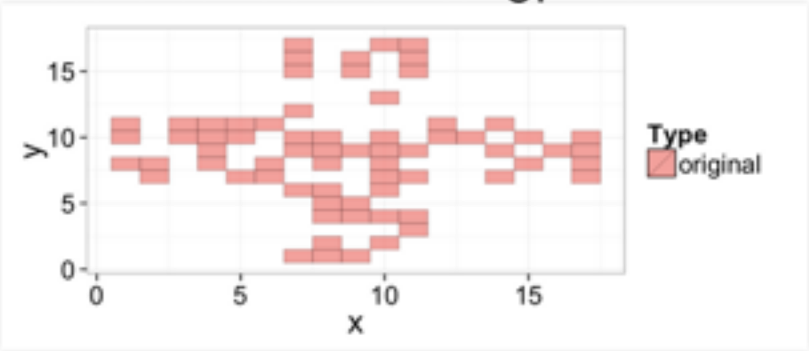
- Opening:
  Erosion followed by dilation
- Closing:
  Dilation followed by erosion

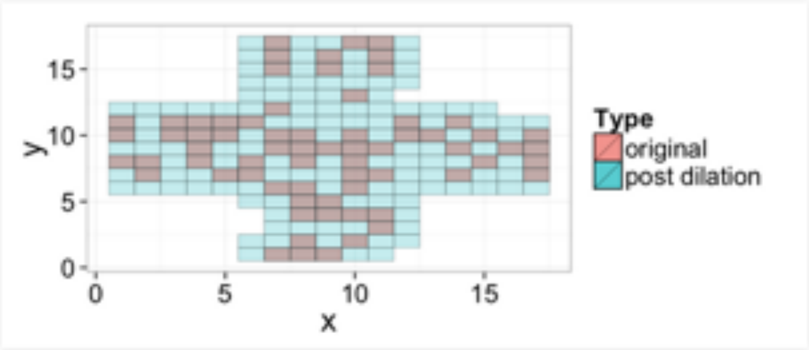
2. Processing binary images

Applied Erosion and Dilation

Erosion

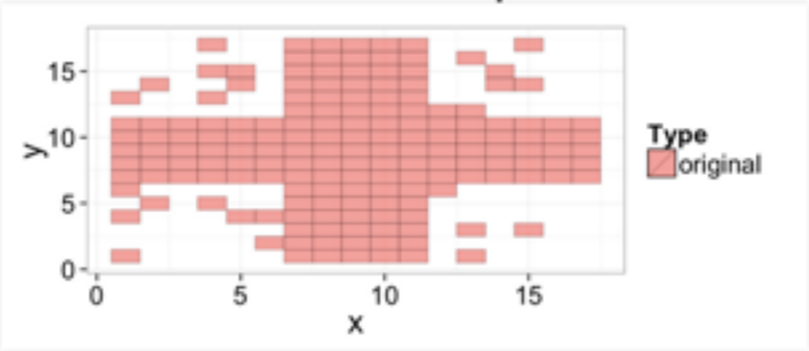
Taking an image of the cross at a too-high threshold, we show how dilation can be used to recover some of the missing pixels

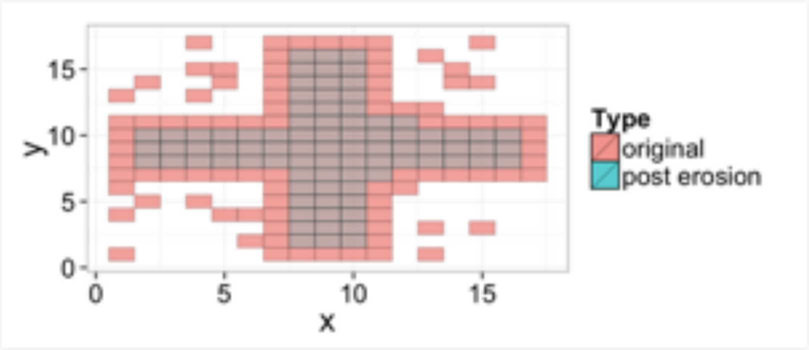




Dilation

Taking an image of the cross at a too-low threshold, we show how erosion can be used to remove some of the extra pixels



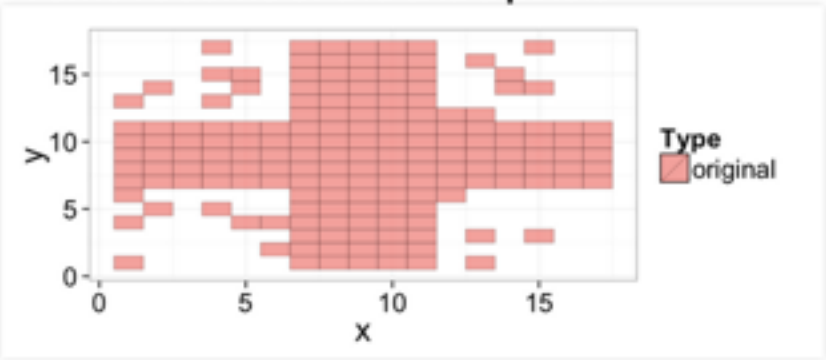


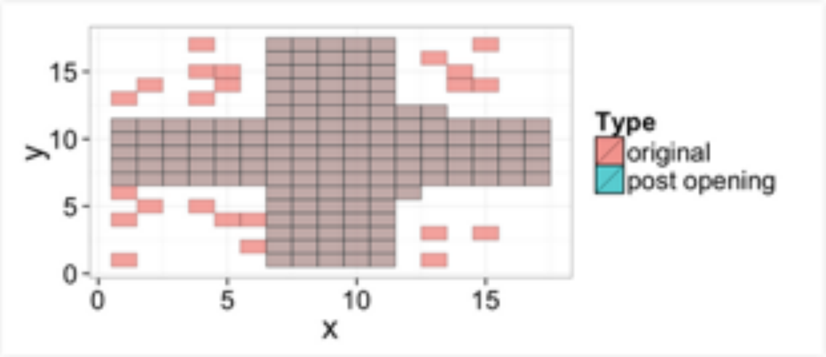
2. Processing binary images

Applied Opening and Closing

Opening

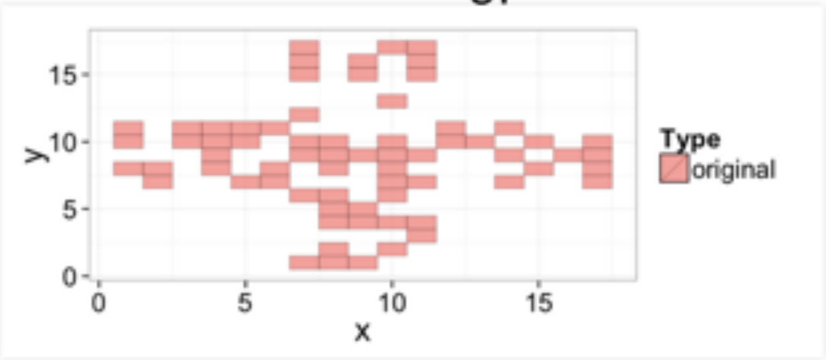
Taking an image of the cross at a too-low threshold, we show how opening can be used to remove some of the extra pixels

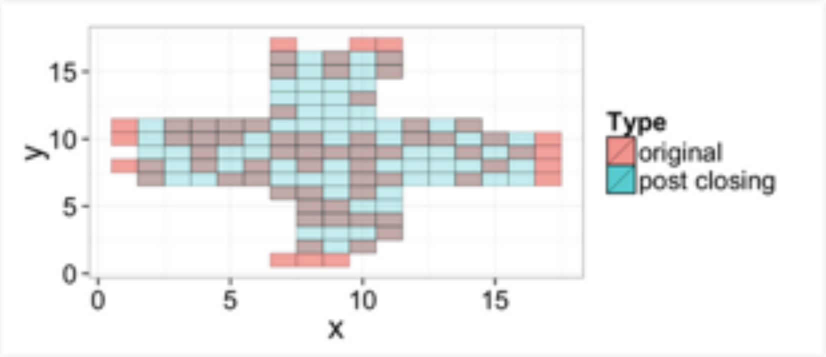




Closing

Taking an image of the cross at a too-high threshold, we show how closing can be used to recover some of the missing pixels





2. Processing binary images

Morphological operations

Erosion and dilation / opening and closing

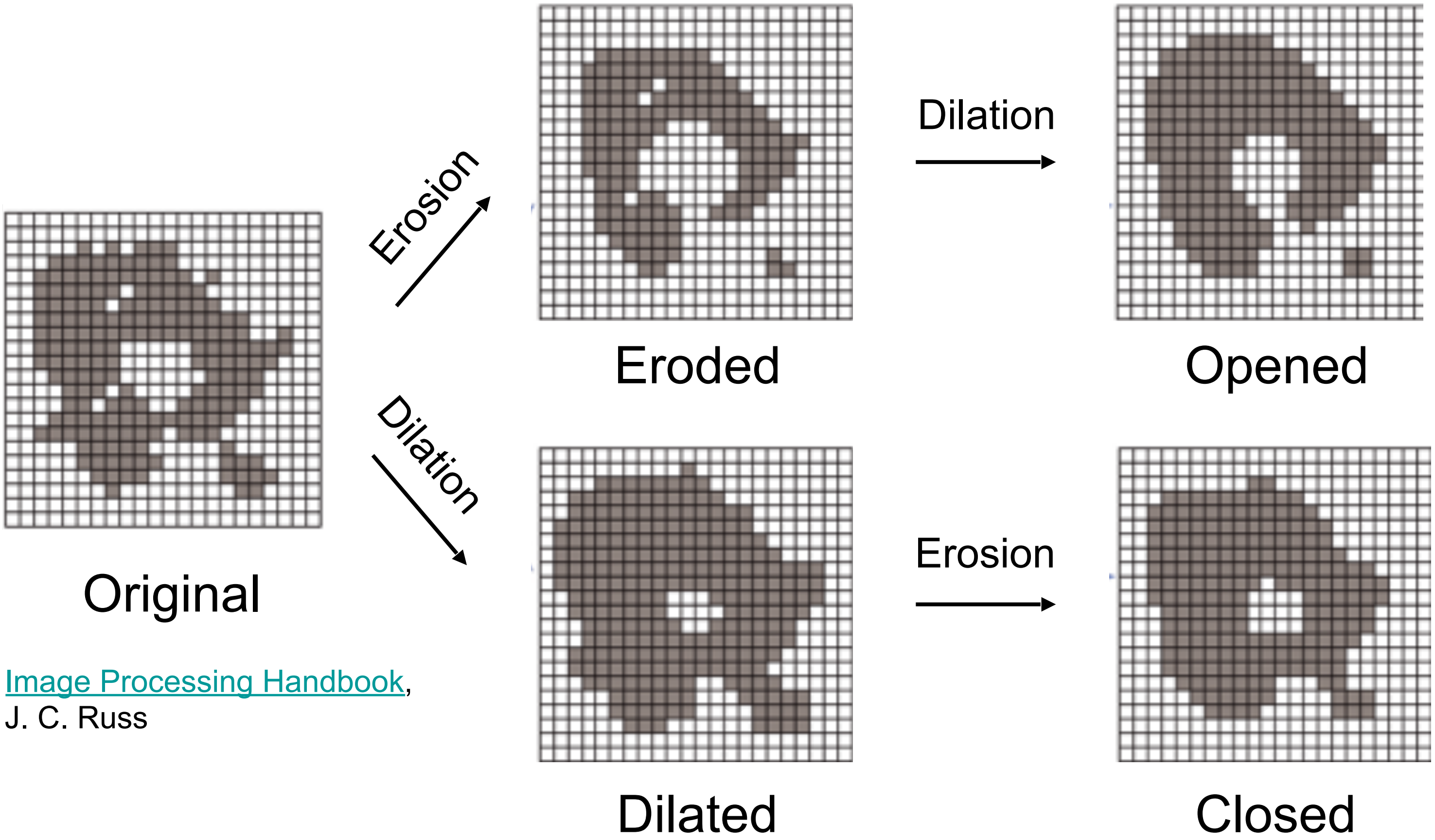
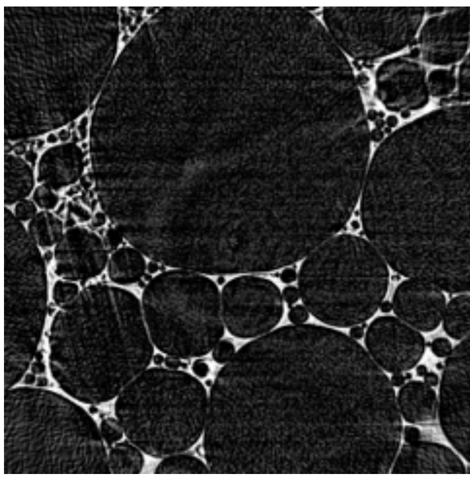


Image Processing Handbook, J. C. Russ

2. Processing binary images

Morphological operations

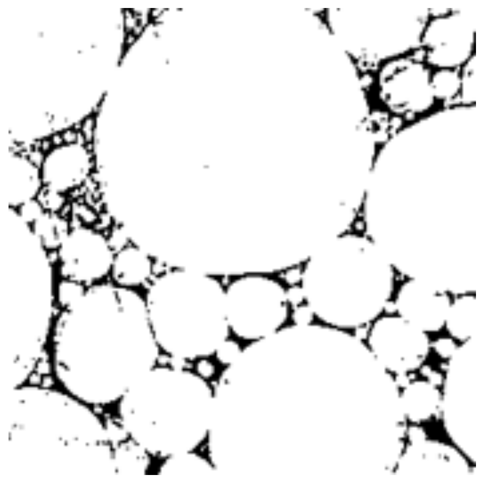
Closing



Original

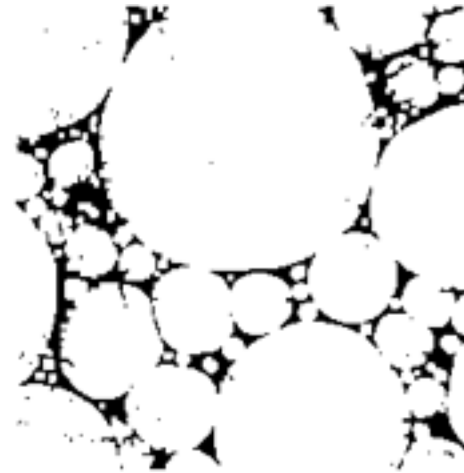
Beer foam, courtesy of R. Mokso

Threshold



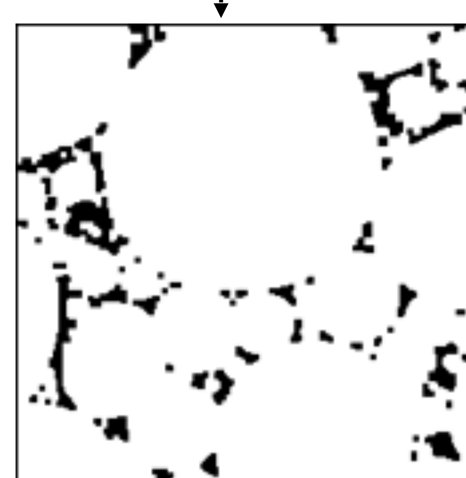
Thresholded

Closing



Closed

Closing

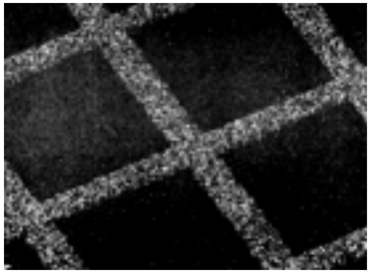


Closed 2x

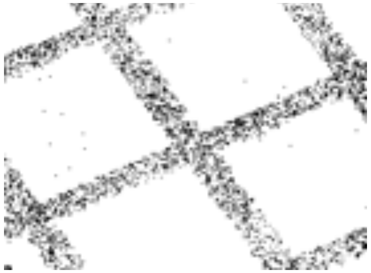
2. Processing binary images

Morphological operations

Masking



Threshold



2 x closing

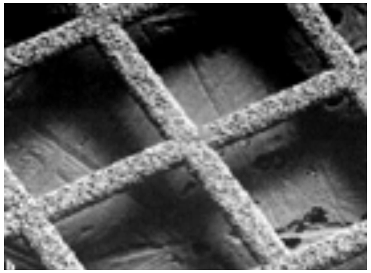
Masking

X-ray dot map:
gold grid above aluminum stub
Image Processing Handbook,
J. C. Russ

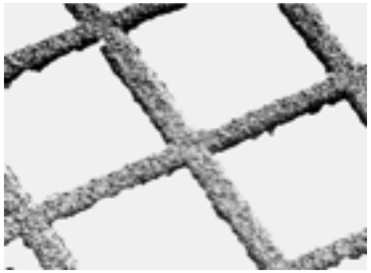
Thresholded

Closed

Originals



Masking



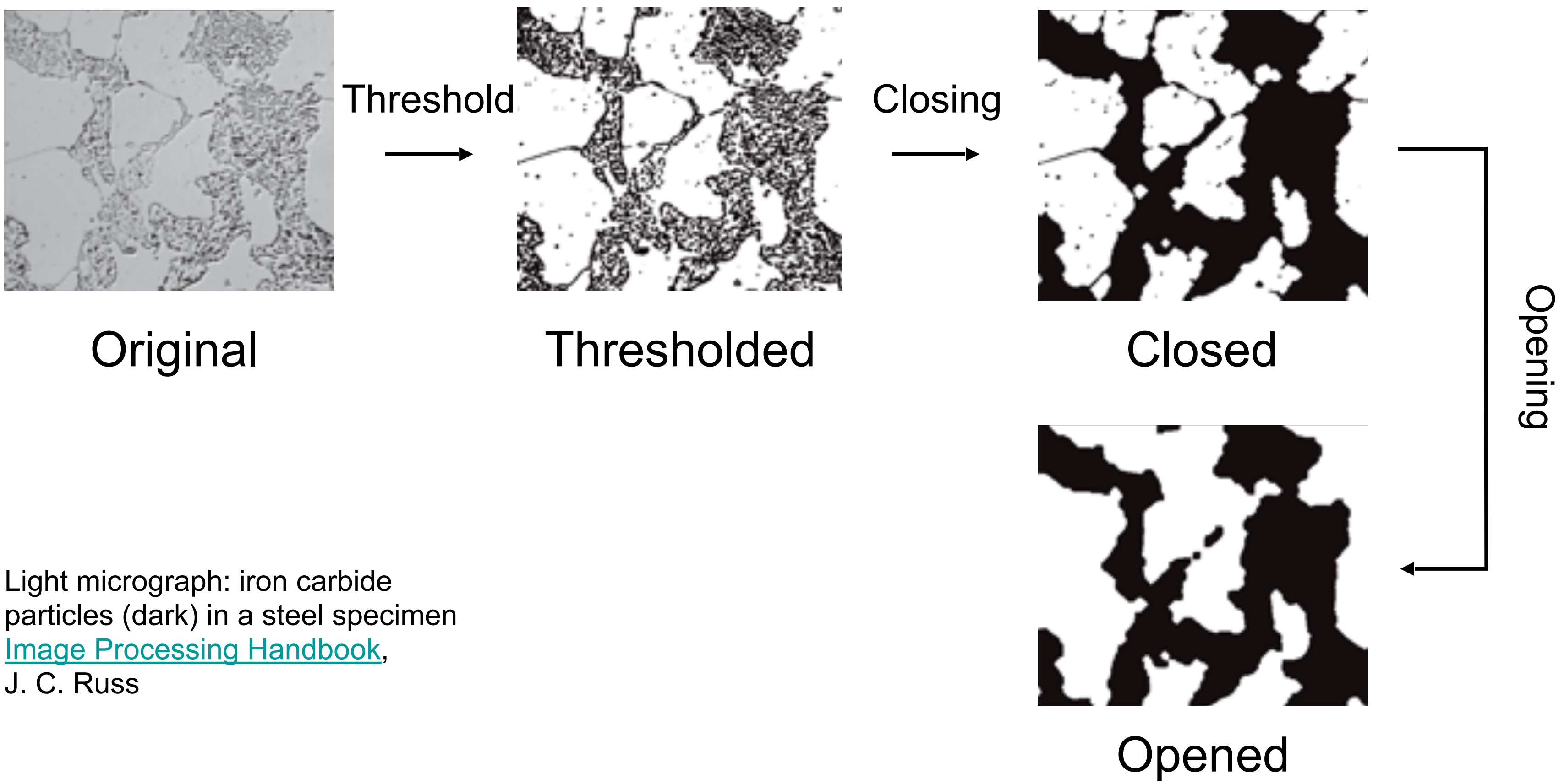
Scanning electron micrograph:
gold grid above aluminum stub
Image Processing Handbook,
J. C. Russ

Masked SEM original

2. Processing binary images

Morphological operations

Segmentation



Light micrograph: iron carbide particles (dark) in a steel specimen
Image Processing Handbook,
J. C. Russ

2. Processing binary images

Morphological operations

Component separation

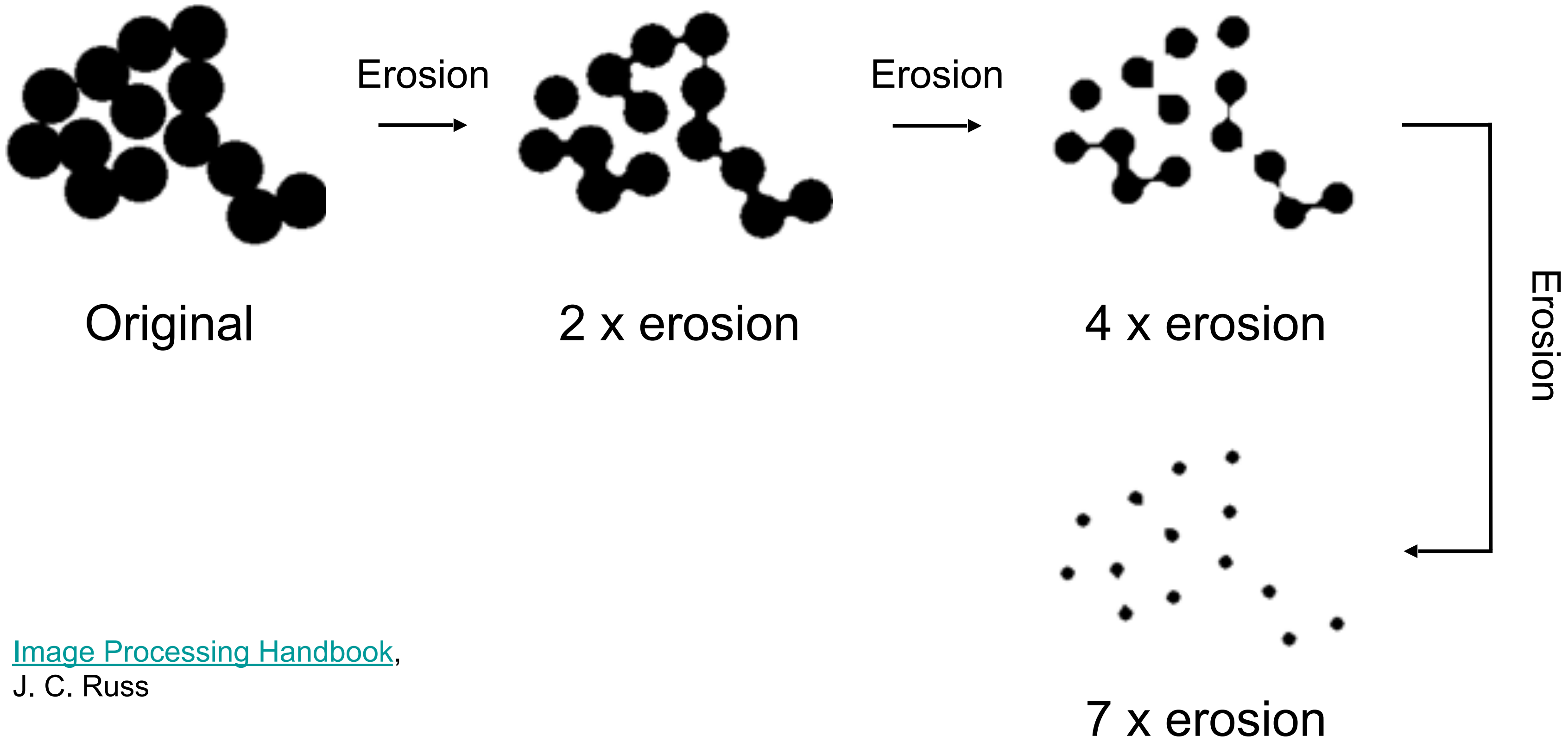


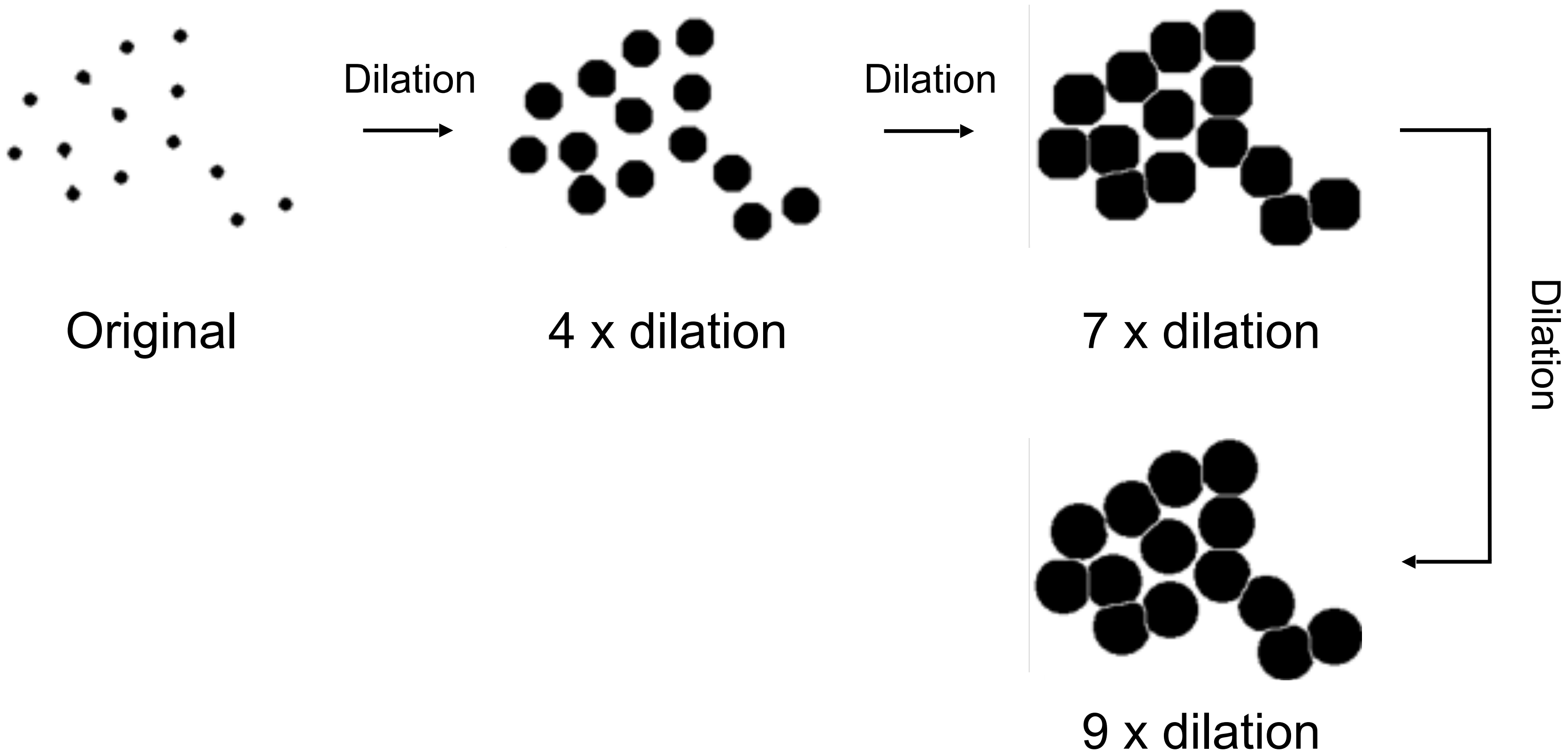
Image Processing Handbook,
J. C. Russ

2. Processing binary images

Morphological operations

Component separation

Additional rules applied here:
- Do not merge distinct structures
- Structures restricted to original size



2. Processing binary images

Morphological operations

Anisotropy

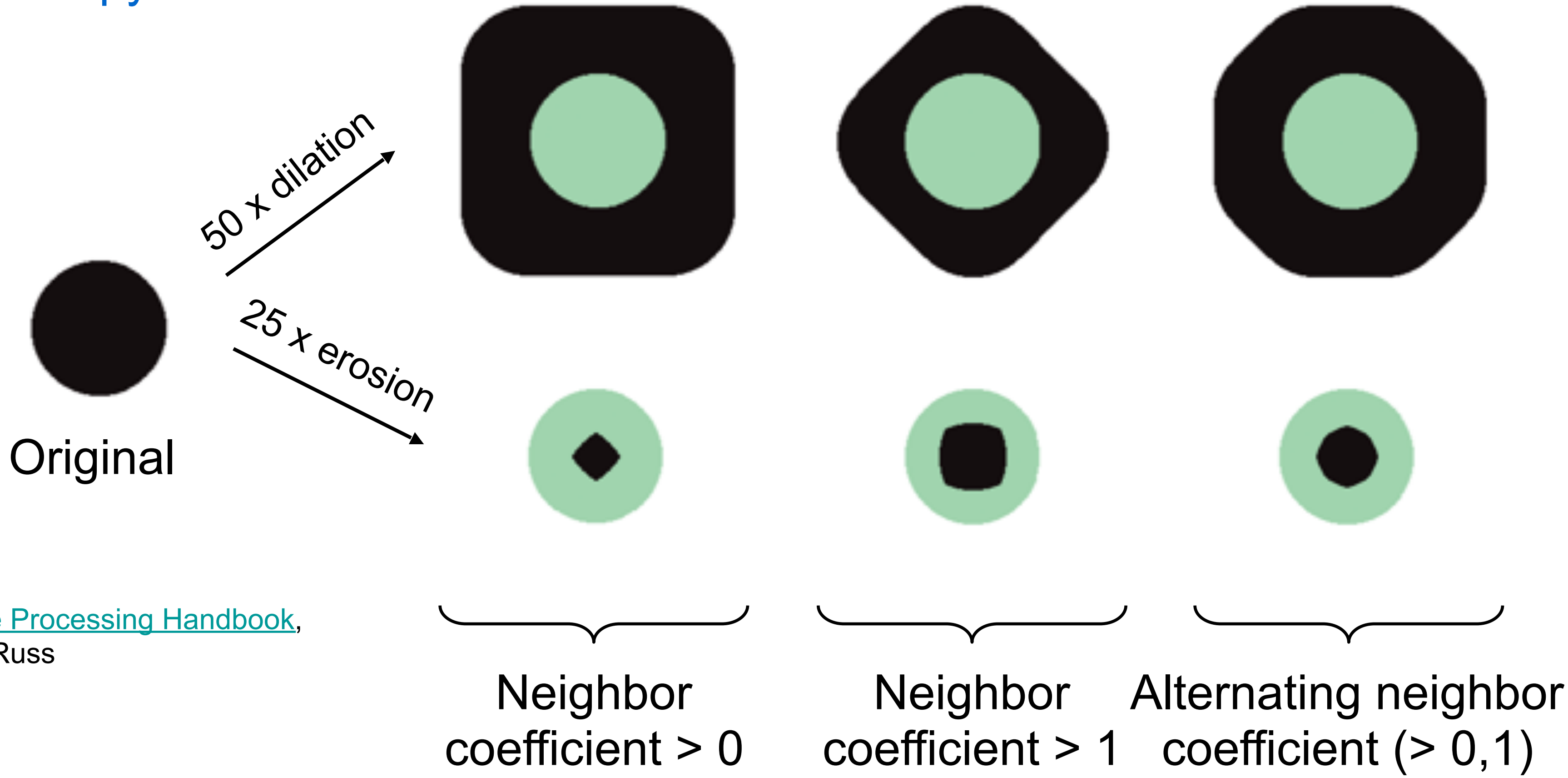


Image Processing Handbook, J. C. Russ

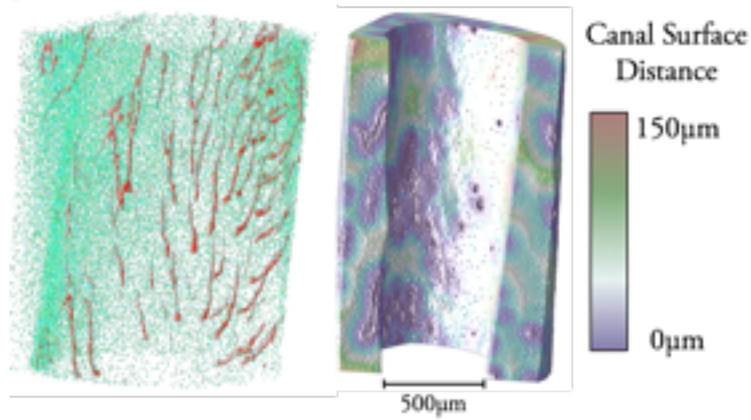
2. Processing binary images

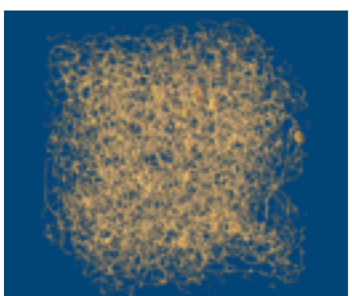
Distance Map / Distance Transforms

Motivation

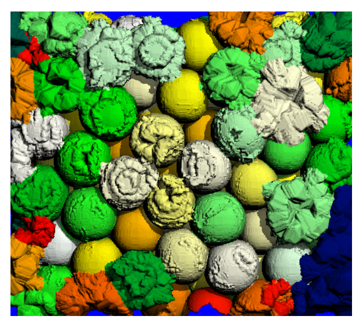
1. Measuring distances between structures
2. Characterizing the thickness of structures
3. Labeling touching structures
4. Identifying skeleton / backbone of structures

Distances

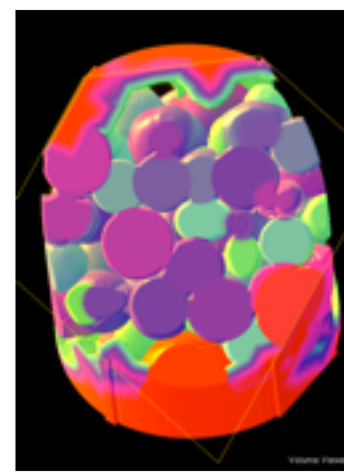




Calculating Skeleton



Labeling Connected Structures



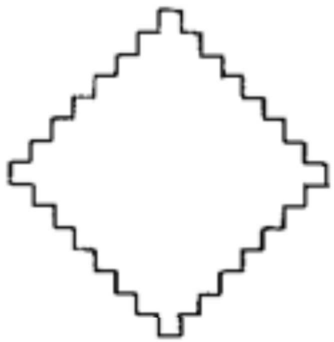
Thickness

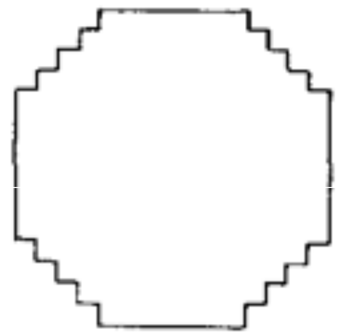
2. Processing binary images

Euclidean distance transformation (EDT)

Distance

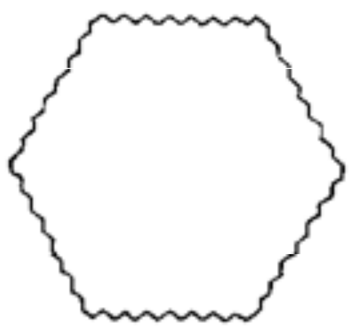
4-neighbor distance

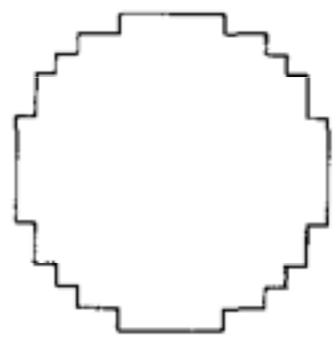




Octagonal distance

Hexagonal distance





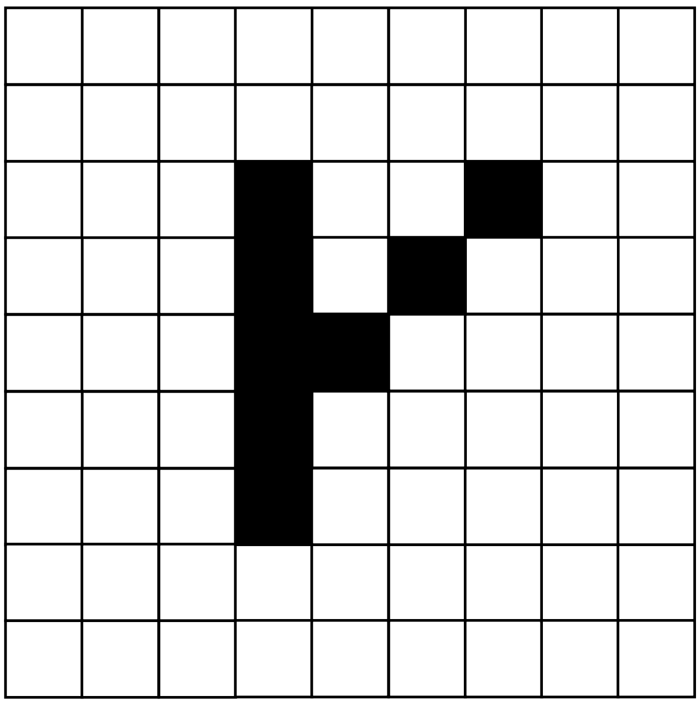
Euclidean distance

G. Borgefors, *Computer Vision, Graphics, and Image Processing* **27**:321-45 (1984)

2. Processing binary images

Euclidean distance transformation (EDT) / map (EDM)

Definition



EDT →

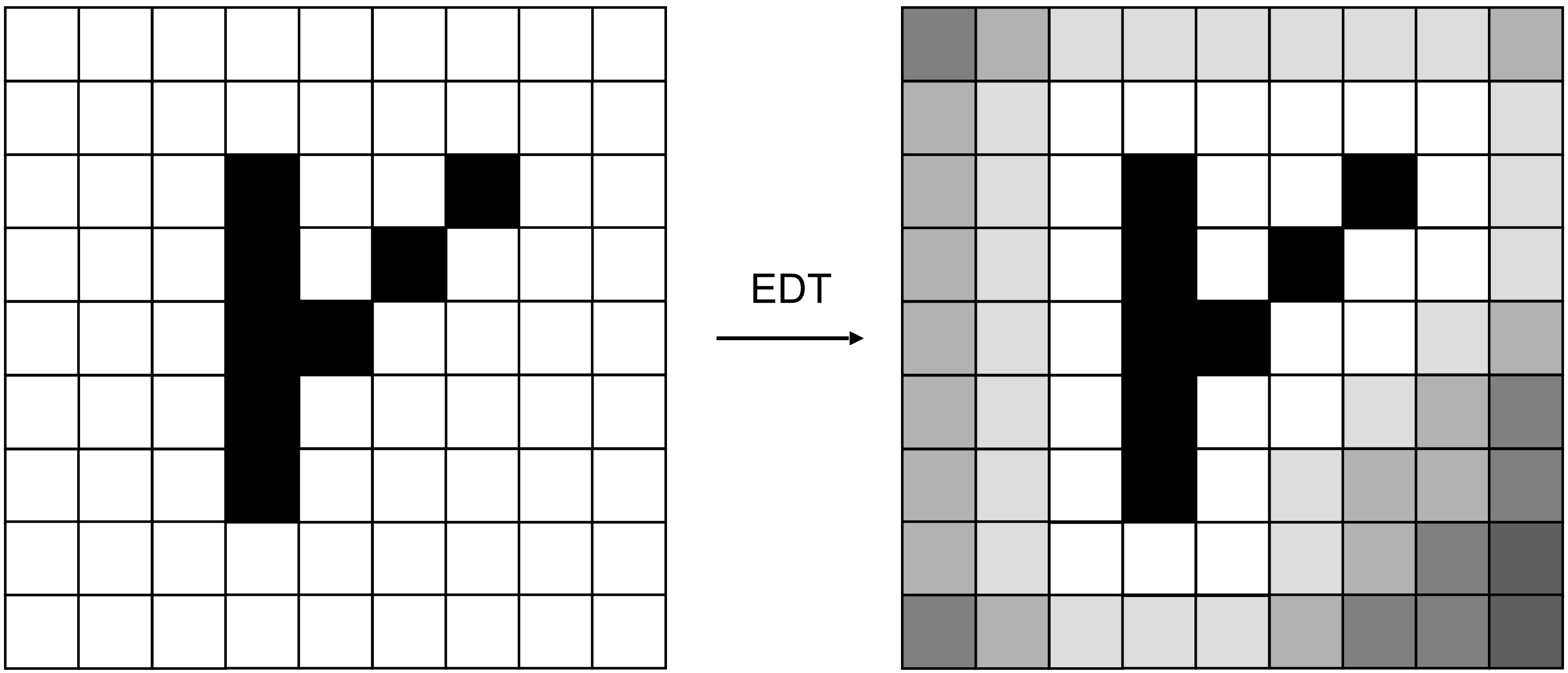
4	3	2	2	2	2	2	2	3
3	2	1	1	1	1	1	1	2
3	2	1		1	1		1	2
3	2	1		1		1	1	2
3	2	1			1	1	2	3
3	2	1		1	1	2	3	4
3	2	1		1	2	3	3	4
3	2	1	1	1	2	3	4	5
4	3	2	2	2	3	4	4	5

Adapted from G. Borgefors, *Computer Vision, Graphics, and Image Processing* **27**:321-45 (1984)

2. Processing binary images

Euclidean distance transformation (EDT) / map (EDM)

Definition



Adapted from G. Borgefors, *Computer Vision, Graphics, and Image Processing* **27**:321-45 (1984)

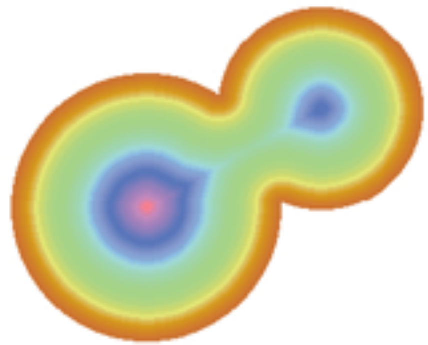
2. Processing binary images

Euclidean distance map (EDM)

Example

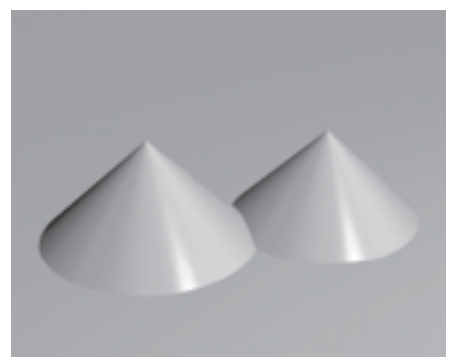
Original

EDT



Euclidean distance map (color-coded)

EDT



Euclidean distance map (topographic map)

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

2. Processing binary images

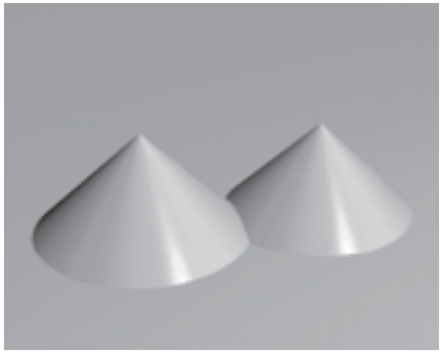
Watershed Transformation

Example

Original

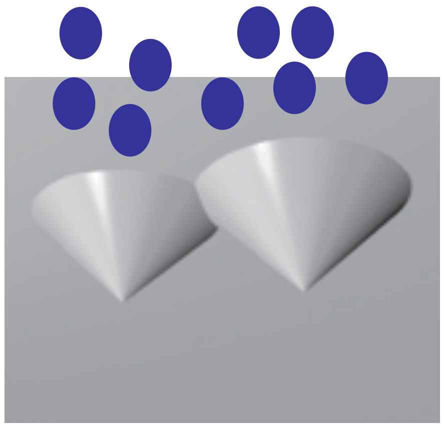
EDT

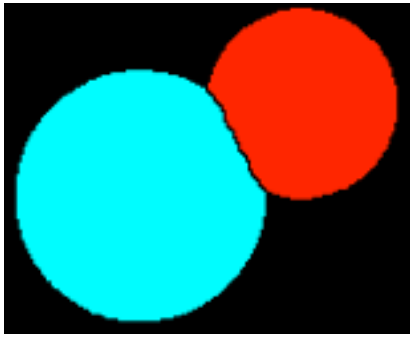
Euclidean
distance map
(topographic map)



Watershed
Transformation

Rain drops -> in which basin
do they collect





2. Processing binary images

Euclidean distance map (EDM)

Erosion and dilation

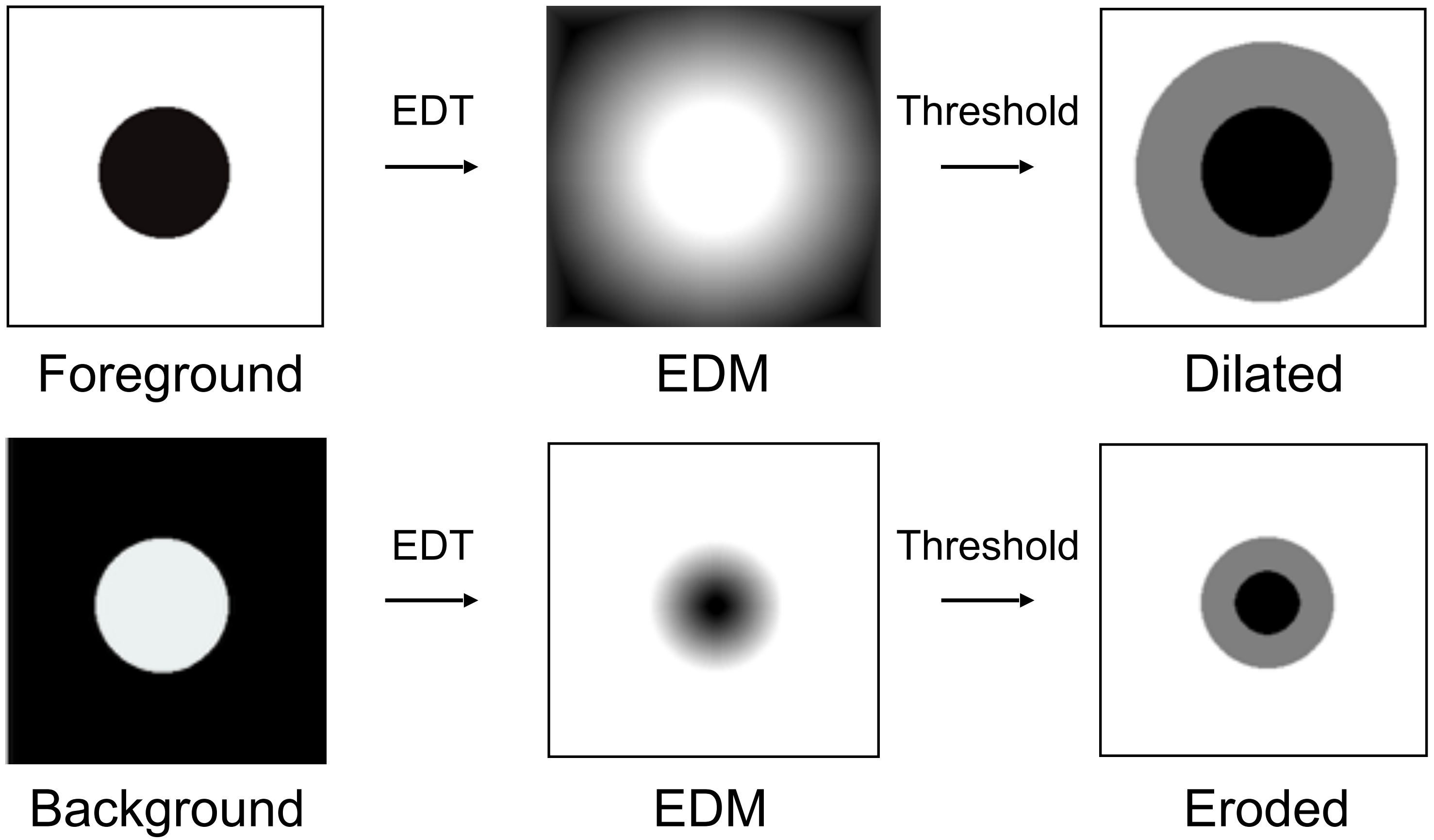


Image Processing Handbook, J. C. Russ

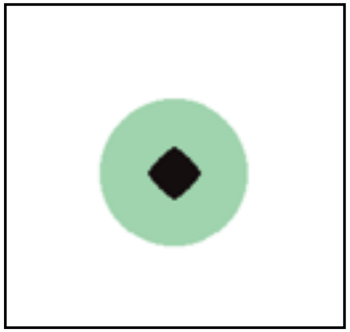
2. Processing binary images

Euclidean distance map (EDM)

Erosion and dilation

Conventional erosion and dilation

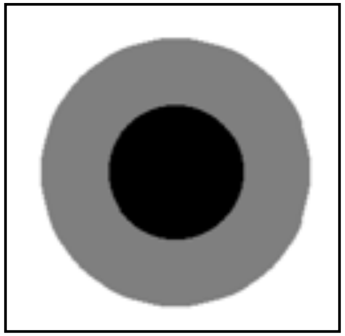
Dilated



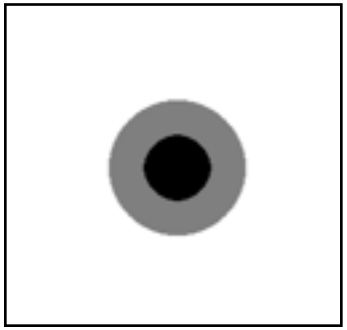
Eroded

=> Erosion and dilation more isotropic when using EDM

Erosion and dilation using EDM



Dilated



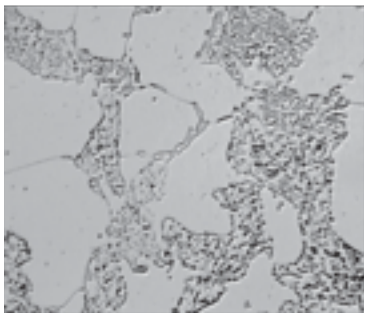
Eroded

Image Processing Handbook, J. C. Russ

2. Processing binary images

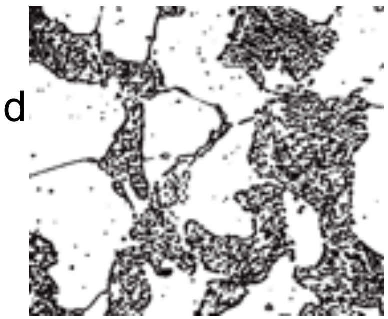
Euclidean distance map (EDM)

Segmentation



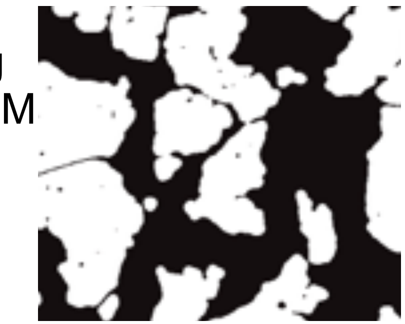
Original

Threshold →



Thresholded

Closing using EDM →



Closed

Opening using EDM

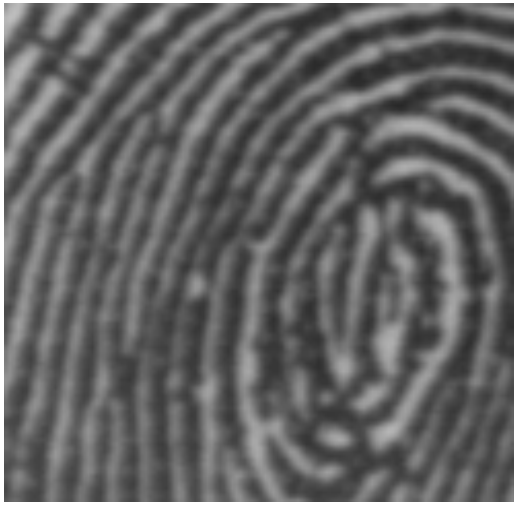
Opened

Light micrograph: iron carbide particles (dark) in a steel specimen
[Image Processing Handbook](),
J. C. Russ

2. Processing binary images

Skeletonization

Examples



Original

Skeleton

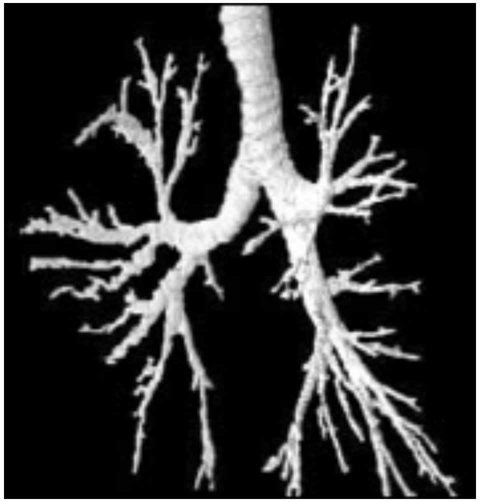
- Reflects topology
- Has a compact form

Light micrograph: fingerprint
Image Processing Handbook, J. C. Russ

2. Processing binary images

Skeletonization

Examples



Original

Skeleton

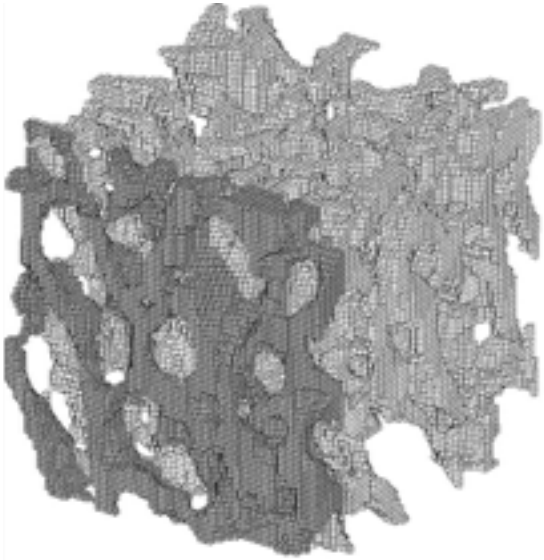
Computed tomography: human bronchus

J. Toriwaki and K. Mori, *Digital and Image Geometry, Advanced Lectures (Lecture Notes in Computer Science)* **2243** (2001)

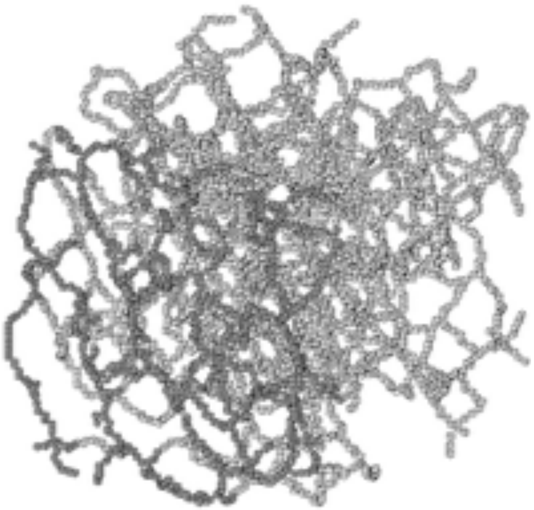
2. Processing binary images

Skeletonization

Examples



Original



Skeleton

Computed tomography: human iliac crest specimen
W. Xie *et al.*, *Pattern recognition* **36**:1529-44 (2003)

2. Processing binary images

Skeletonization

Elements of a skeleton

Original

Skeleton

Image Processing Handbook, J. C. Russ

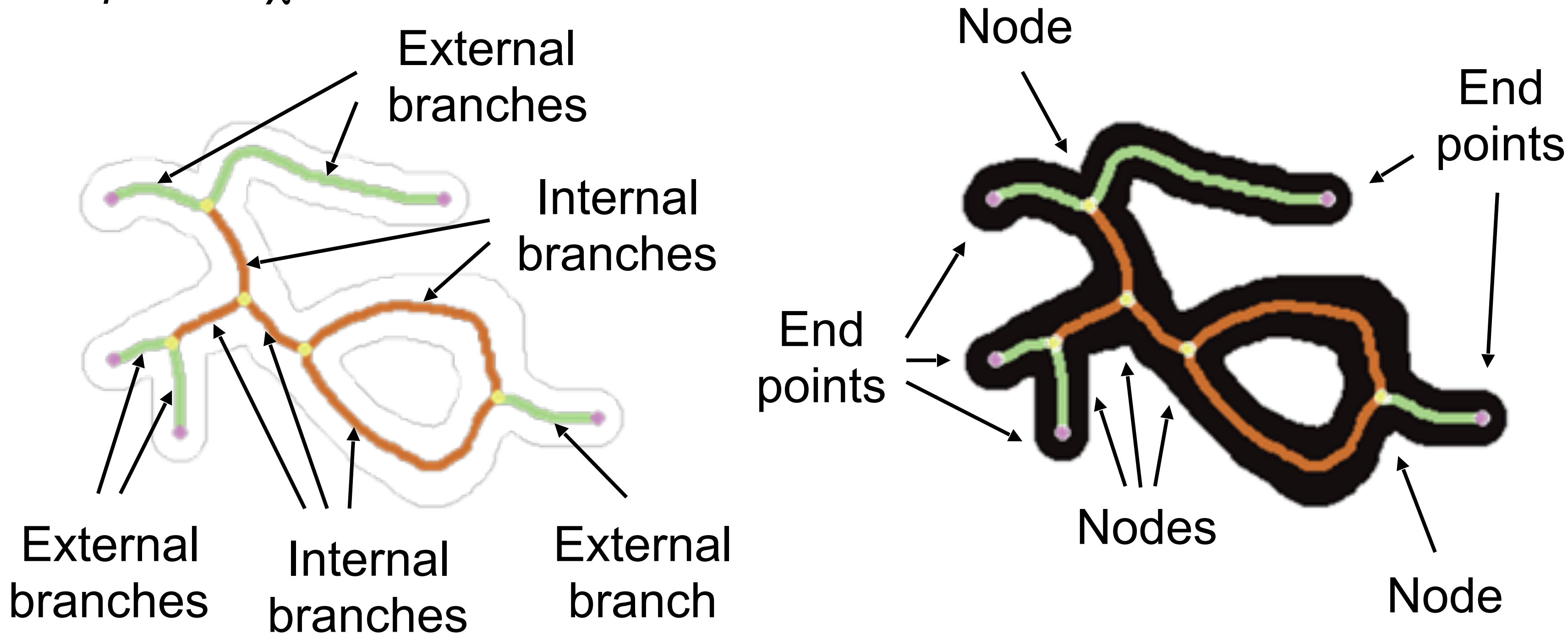
2. Processing binary images

Skeletonization

Euler-Poincaré characteristic

$$\chi = \#Nodes + \#Ends - \#Branches$$

$$\#Loops = 1 - \chi$$



2. Processing binary images

Skeletonization

Requirements for ideal skeleton

- Homotopic (topology preserving): skeleton topologically equivalent to the object (same Euler-Poincaré characteristic)
- Invariant under isometric transformations (distance-preserving transformations): skeleton of transformed object equal to transformed skeleton of original object
- Thin: one-dimensional skeleton (except at nodes)
- Centered: skeleton centered within the object
- Reliable: every boundary point visible from at least one skeleton point
- Robust: insensitive to noise on the boundary of the object
- Smooth
- Others

N. D. Cornea *et al.*, *IEEE Transactions on Visualization and Computer Graphics* **13(3)**:530-48 (2007)

2. Processing binary images

Skeletonization

Thinning classification for discrete data sets

Thinning

Topological thinning
(grassfire propagation)

Distance transform-based thinning
(ridge detection)

N. D. Cornea *et al.*, *IEEE Transactions on Visualization and Computer Graphics* **13(3)**:530-48 (2007)

2. Processing binary images

Skeletonization

Simple points

Human vision interprets an image in terms of relationships between structures
<=>
For computer-based image analysis systems, classification of structures must be made at the level of individual pixels/voxels

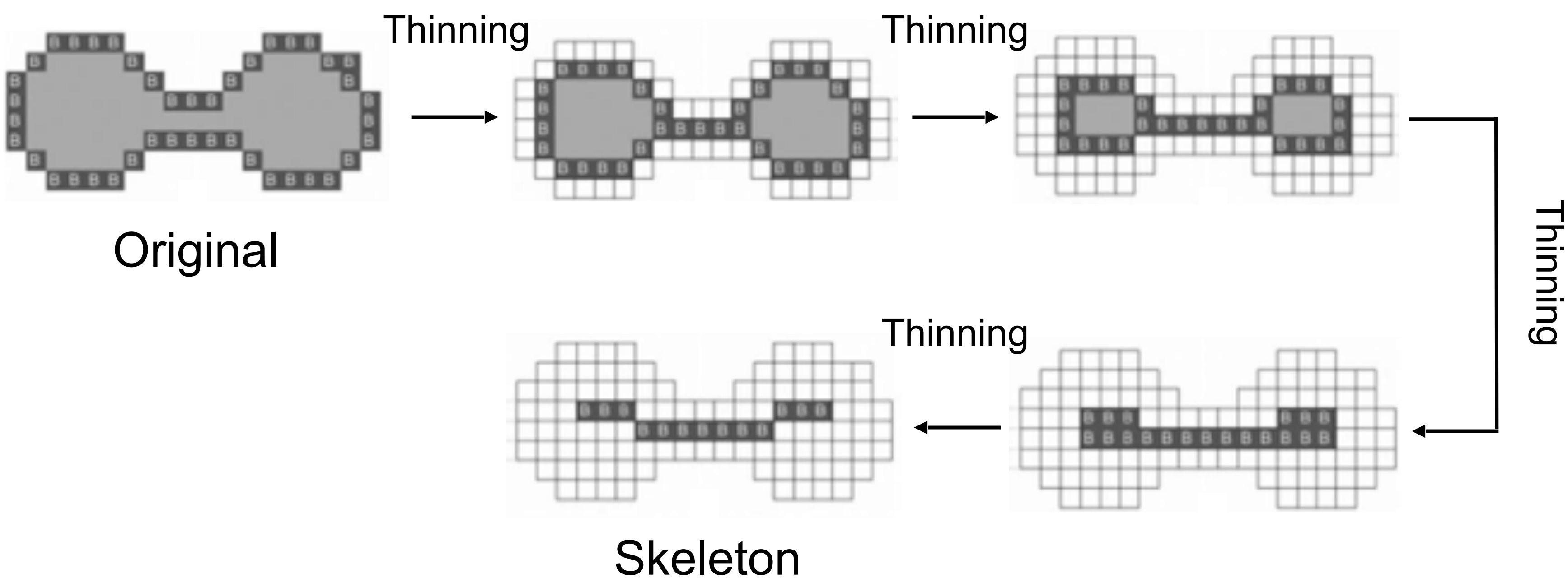
- Thinning algorithms operate in the discrete (pixel/voxel) space and rely on the concept of simple points
- Simple point: object point (pixel/voxel) that can be removed without changing the topology of the object
- Simple points can be locally characterized (-> efficient thinning algorithm)

N. D. Cornea *et al.*, *IEEE Transactions on Visualization and Computer Graphics* **13(3)**:530-48 (2007)

2. Processing binary images

Skeletonization

Topological thinning



=> Erosion with special rule (remove simple boundary points only)

N. D. Cornea *et al.*, *IEEE Transactions on Visualization and Computer Graphics* **13(3)**:530-48 (2007)

2. Processing binary images

Skeletonization

Distance transform-based thinning

Original → Distance map → Skeleton within original

L. J. Latecki *et al.*, *IEEE International Conference on Image Processing* **V**:349-52 (2007)

2. Processing binary images

Skeletonization

Distance transform-based thinning

1) Find ridge points (detection)

↓

2) Pruning

↓

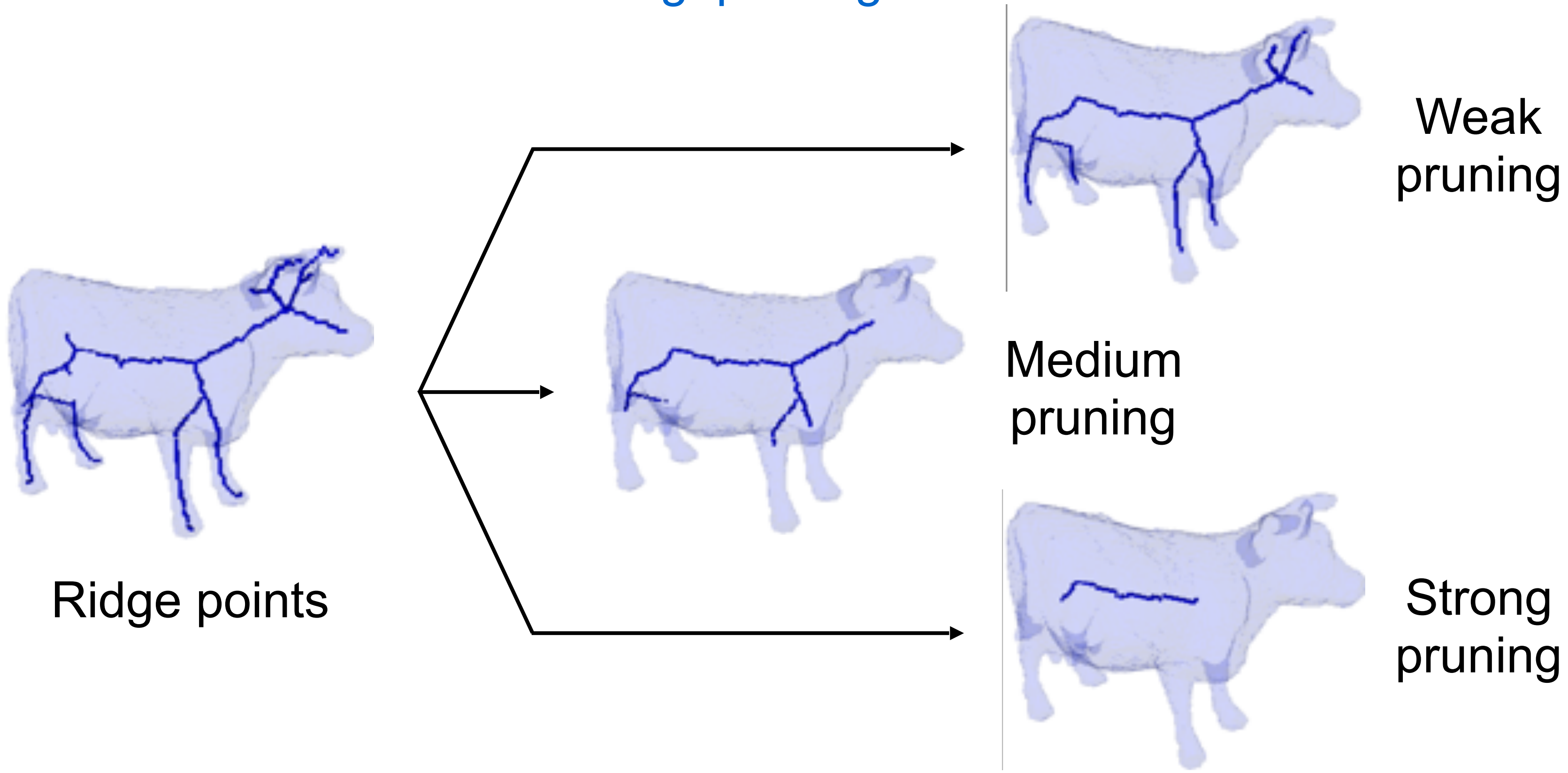
3) Connecting

N. D. Cornea *et al.*, *IEEE Transactions on Visualization and Computer Graphics* **13(3)**:530-48 (2007)

2. Processing binary images

Skeletonization

Distance transform-based thinning: pruning

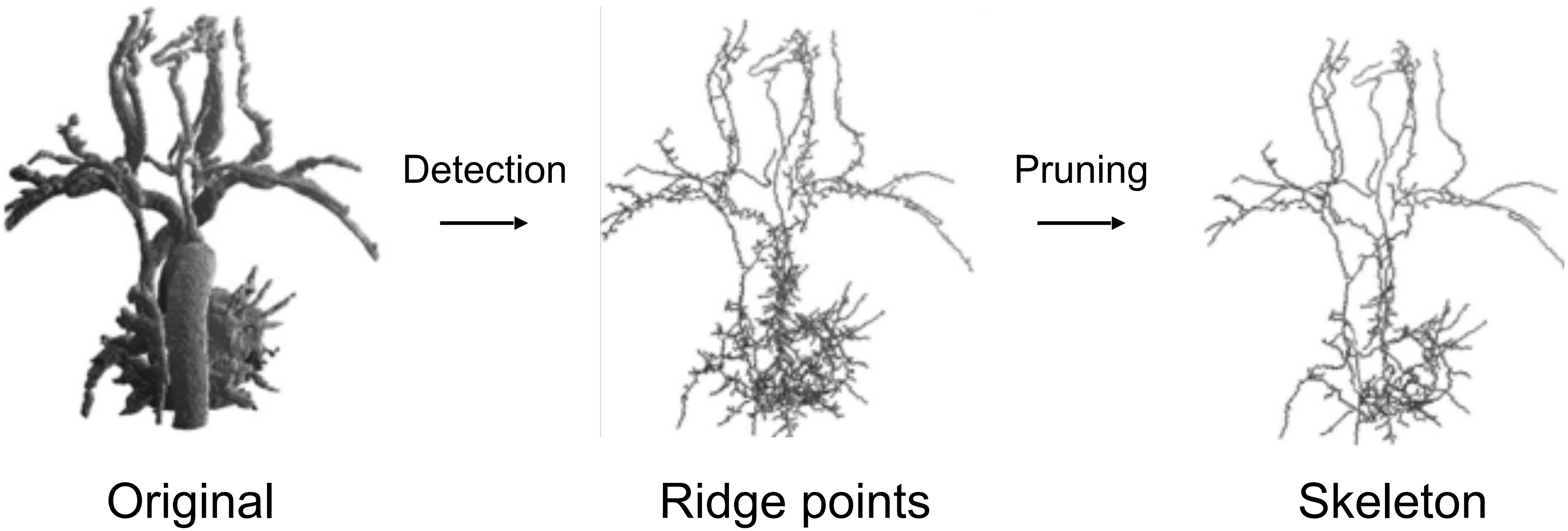


A. Telea *et al.*, *Proceedings of the Symposium on Data Visualisation* **40**:185-94 (2003)

2. Processing binary images

Skeletonization

Distance transform-based thinning: pruning



Magnetic resonance angiography: thoracic vessel system
S. Svensson *et al.*, *Computer Vision and Image Understanding* **90**:242-57 (2003)
I. Nyström *et al.*, *Proceedings of the 11th International Conference on Image Analysis and Processing*: 495-500 (2001)

2. Processing binary images

Skeletonization

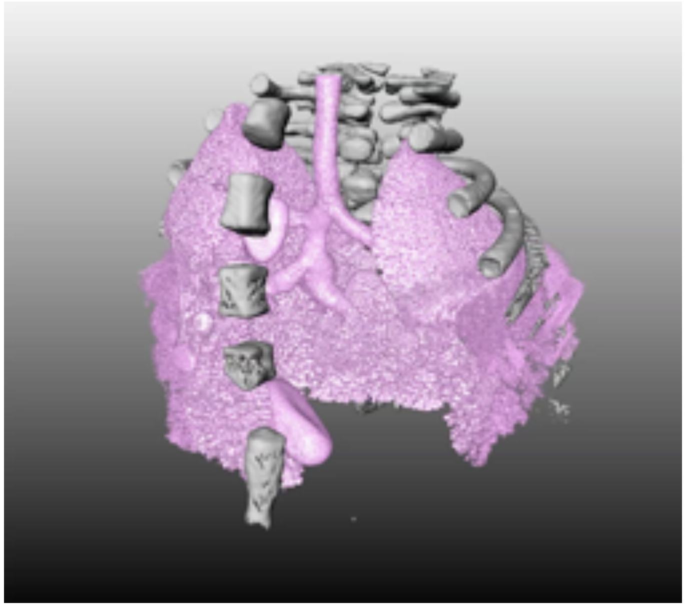
Distance transform-based thinning: connecting

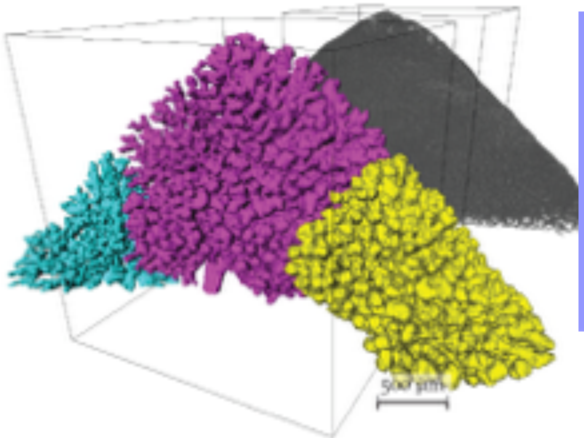
A. Telea *et al.*, *Proceedings of the Symposium on Data Visualization* **40**:185-94 (2003)

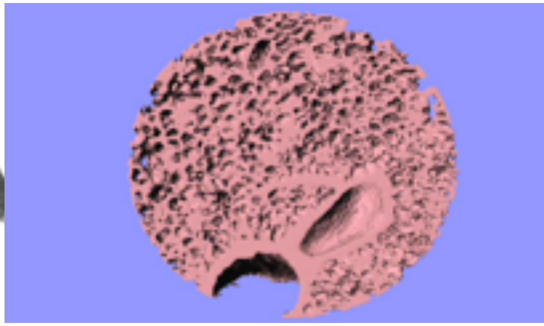
3. Demonstration

Baby Rat Lung Structure

Micron-scale aveolar structure





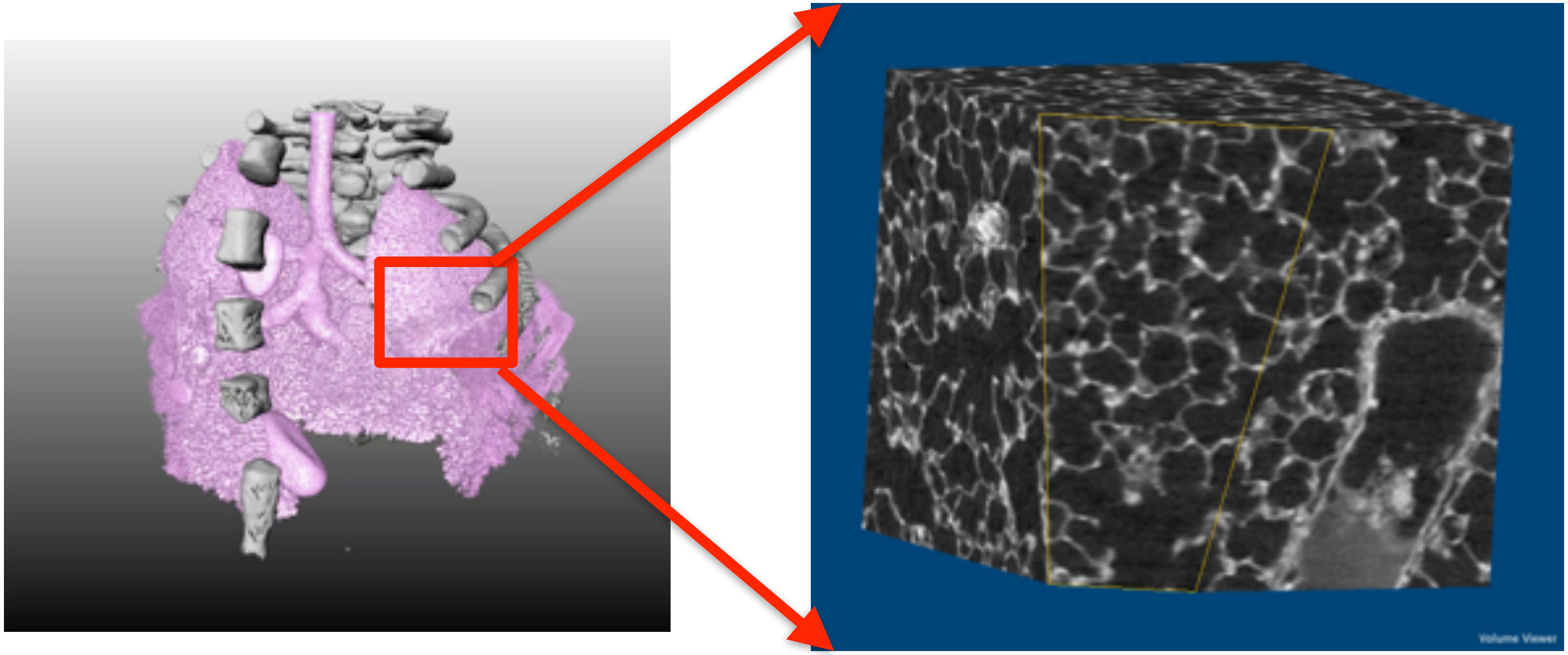


Morphology of lung acini
Haberthür et al., Journal of Synchrotron Radiation, 17(5), 2010
Schittny et al., American Journal of Physiolgy 294 (L246), 2008

3. Demonstration

Baby Rat Lung Structure

Micron-scale aveolar structure

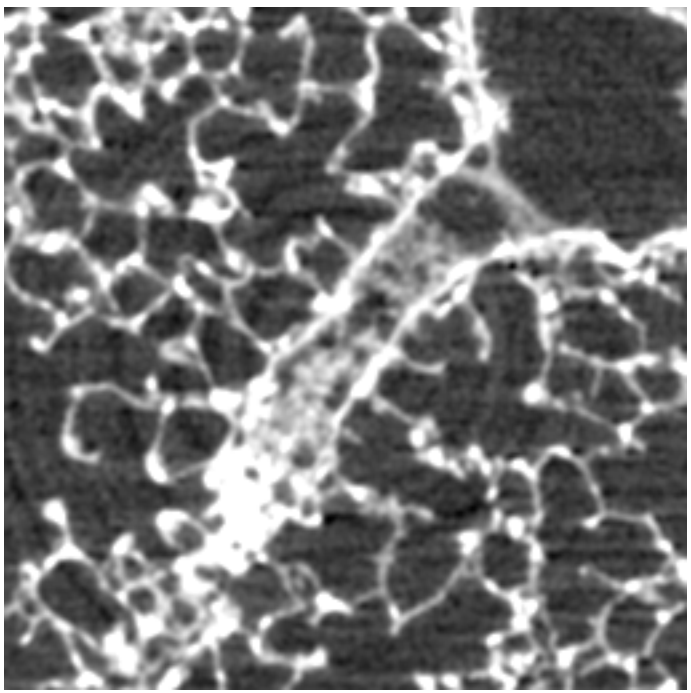


Lung measurement
courtesy of G. Lovric

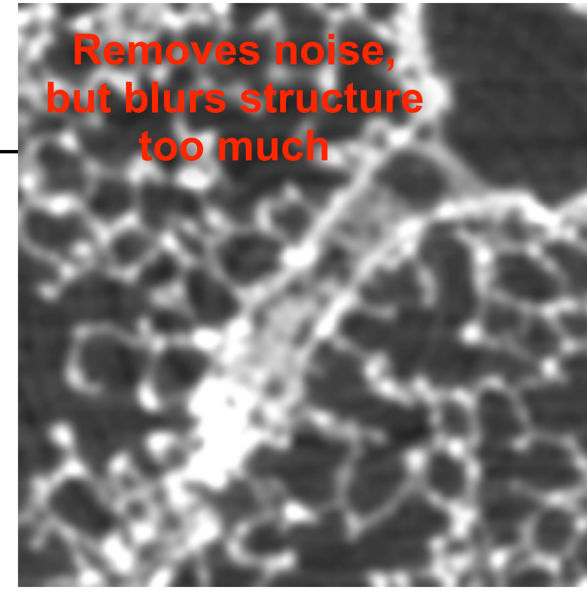
3. Demonstration

Baby Rat Lung Structure

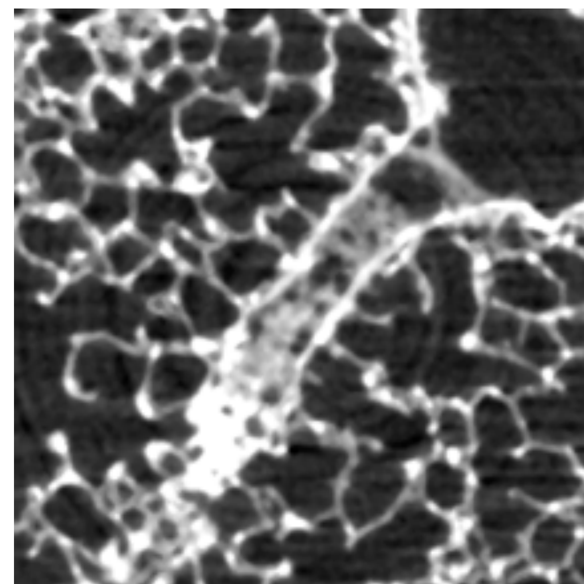
Micron-scale aveolar structure



Gaussian Filtering



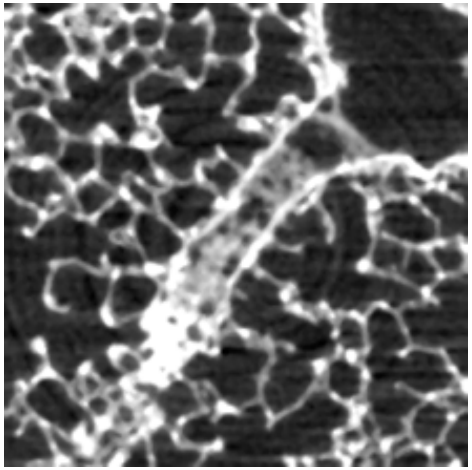
Median Filtering

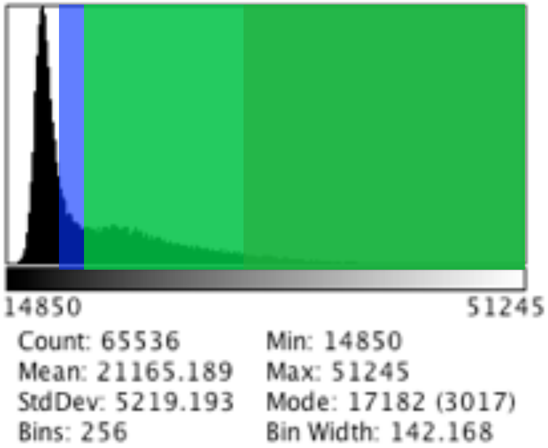


3. Demonstration

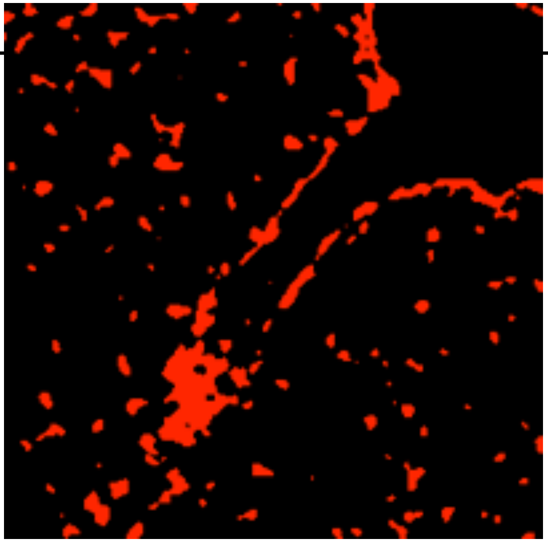
Baby Rat Lung Structure

Micron-scale aveolar structure

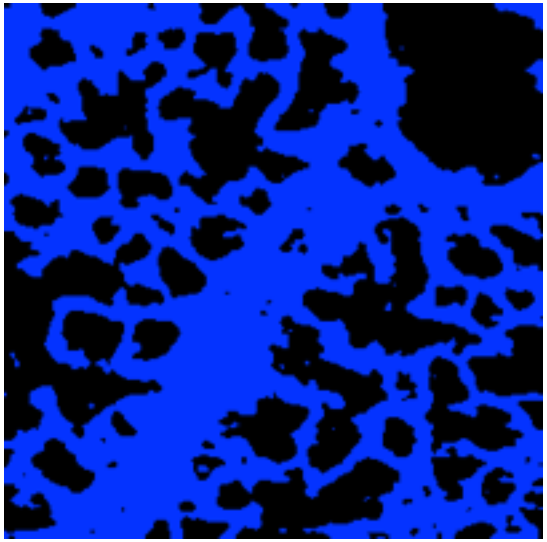




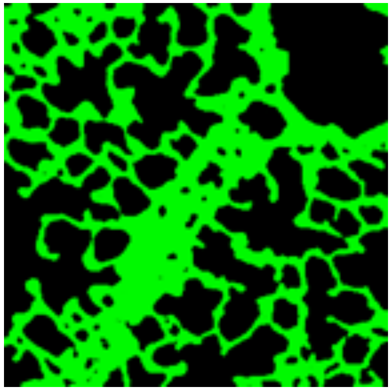
Too strict a threshold



Too lax



Just right

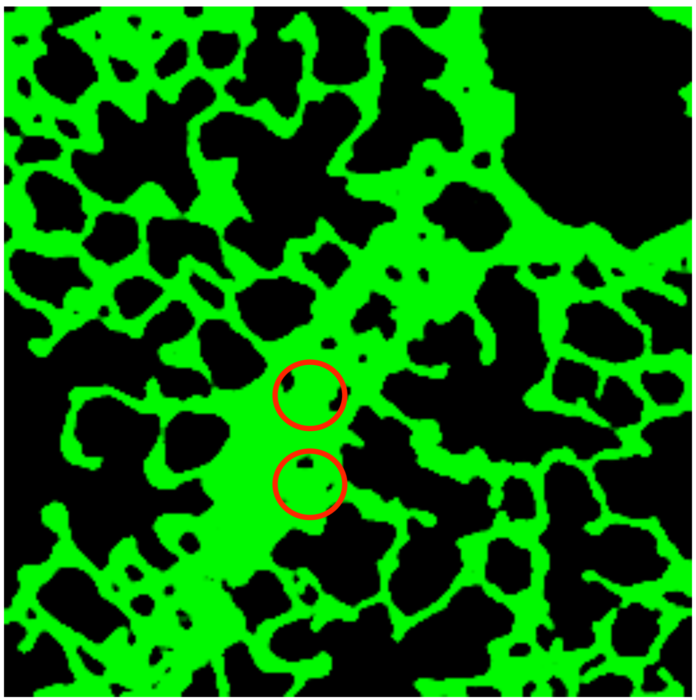


3. Demonstration

Baby Rat Lung Structure

Micron-scale aveolar structure

Some detail is lost!



Remove Small Holes

3x Dilation
3x Erosion
= 3x Close

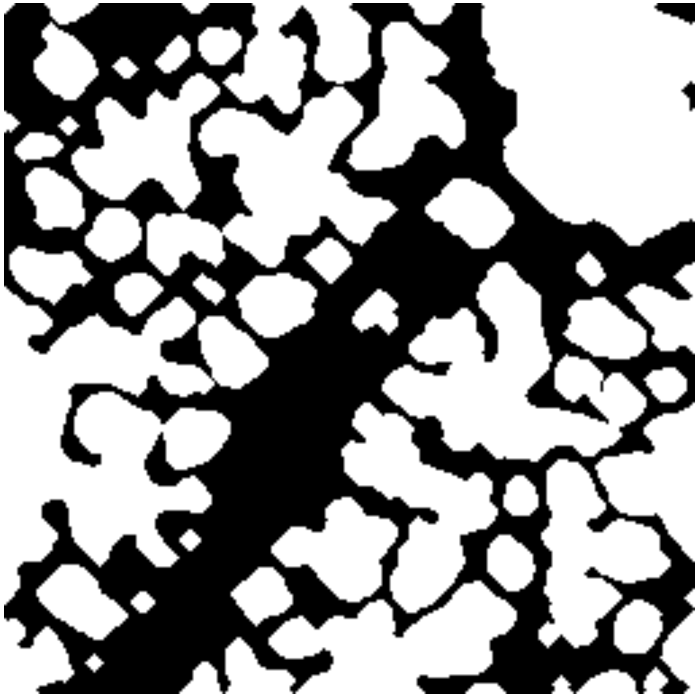
Threshold

Image without small holes

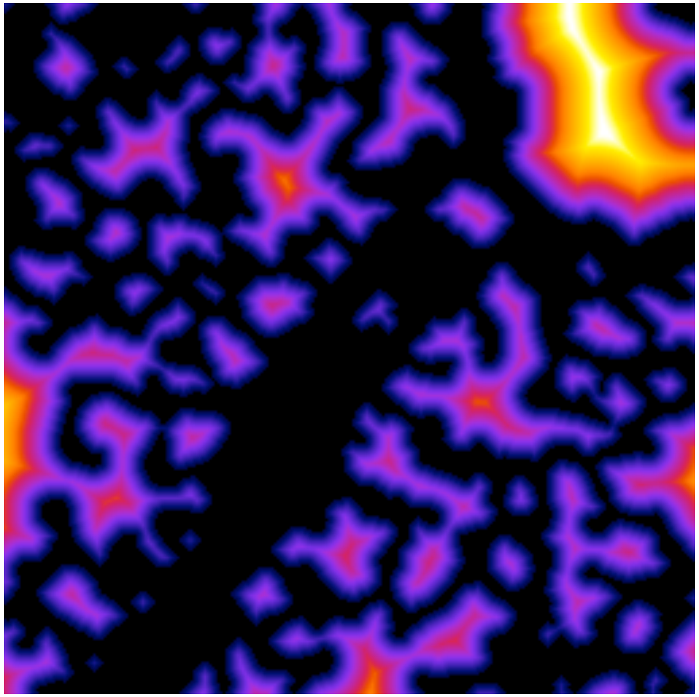
3. Demonstration

Baby Rat Lung Structure

Micron-scale aveolar structure



Euclidean Distance Transform

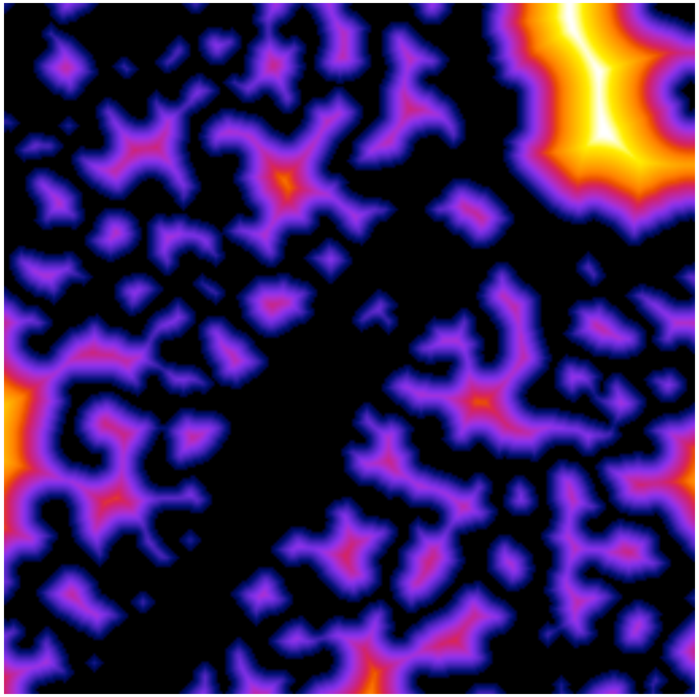


Distance to nearest aveolar structures

3. Demonstration

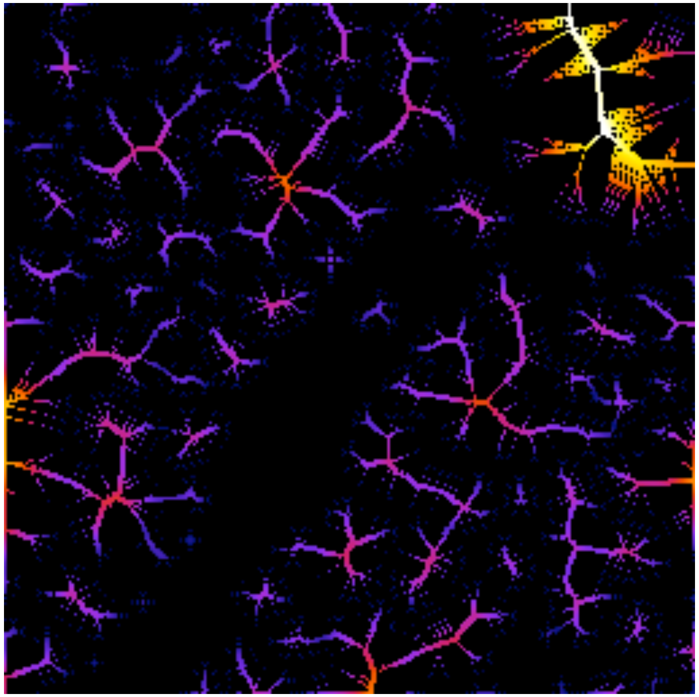
Baby Rat Lung Structure

Micron-scale aveolar structure



Distance Map

Gradient = 0

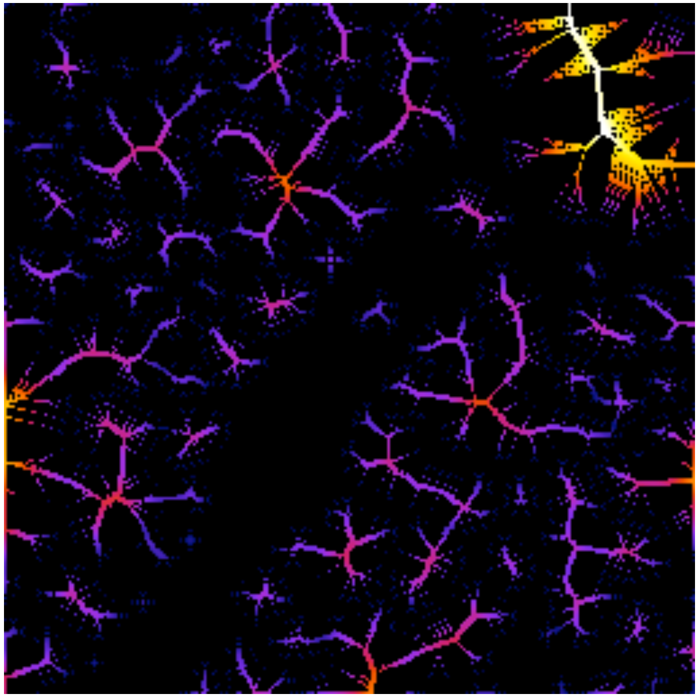


Ridge Map

3. Demonstration

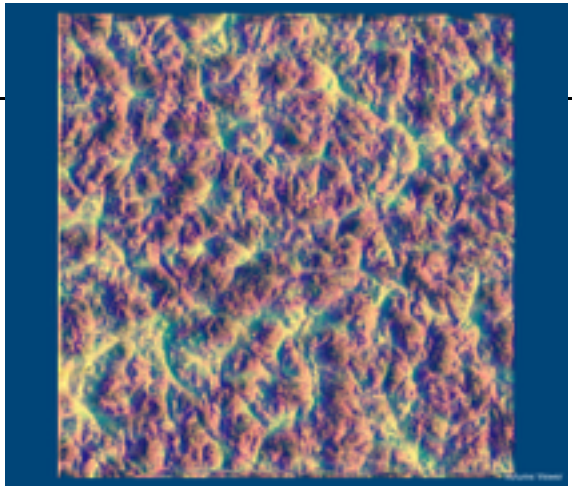
Baby Rat Lung Structure

Micron-scale aveolar structure

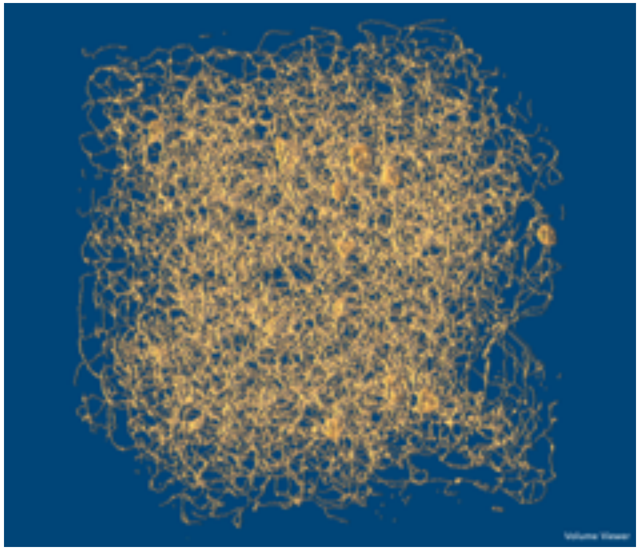


Ridge Map

All structures

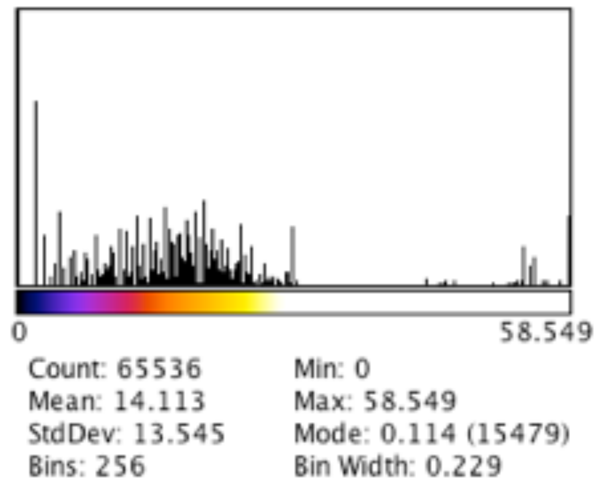


Only structures larger than 5 voxels



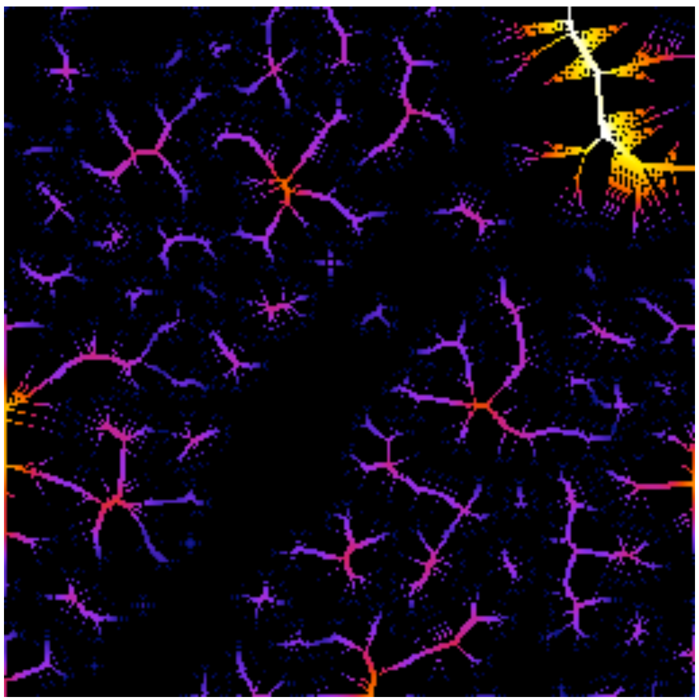
Lung Skeleton / Network

3. Demonstration



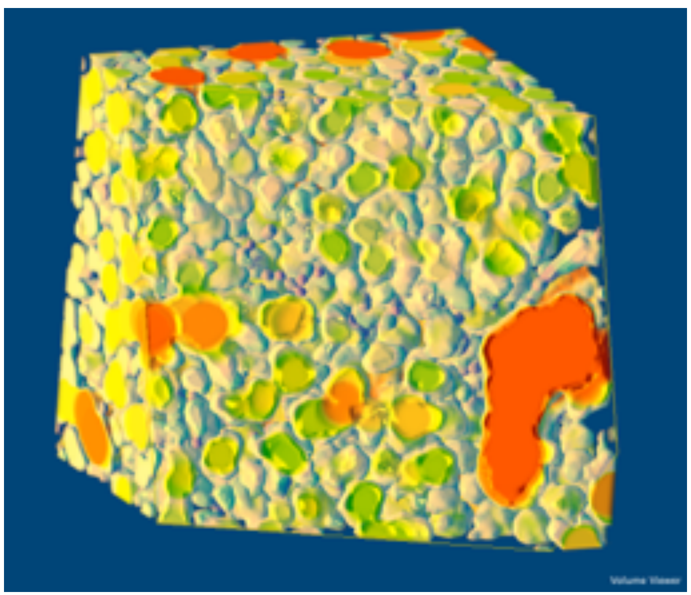
Baby Rat Lung Structure

Micron-scale aveolar structure



Ridge Map

Thickness Filling



Thickness map of alveoli

3. Demonstration

Baby Rat Lung Structure

Reproducibility?

- For this analysis all steps performed with ImageJ / FIJI
- Provides built-in Macro-recording feature
- Acts like a log-book of all the steps done

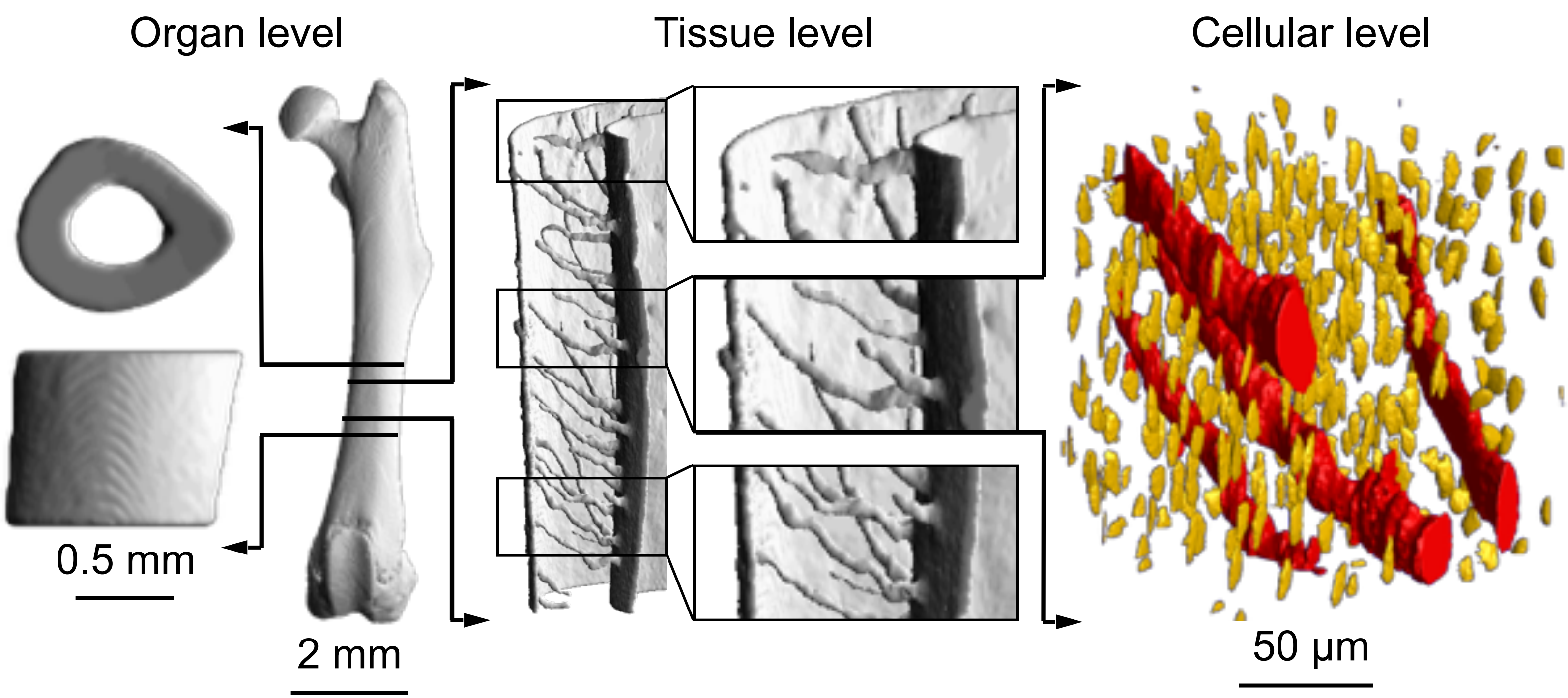
```
run("Median (3D)");
//run("Threshold...");
setAutoThreshold("Default dark");
setAutoThreshold("Default dark");


run("Dilate (3D)", "iso=255");
run("Dilate (3D)", "iso=255");
run("Dilate (3D)", "iso=255");
run("Erode (3D)", "iso=255");
run("Erode (3D)", "iso=255");
run("Erode (3D)", "iso=255");
run("Geometry to Distance Map", "threshold=128 inverse");
run("Distance Map to Distance Ridge");

run("Distance Ridge to Local Thickness");
run("Histogram", "bins=256 use x_min=0 x_max=29.27 y_max=Auto");
```

http://fiji.sc/Fiji

3. Demonstration

Lacuno-canalicular network

Murine bone microstructure

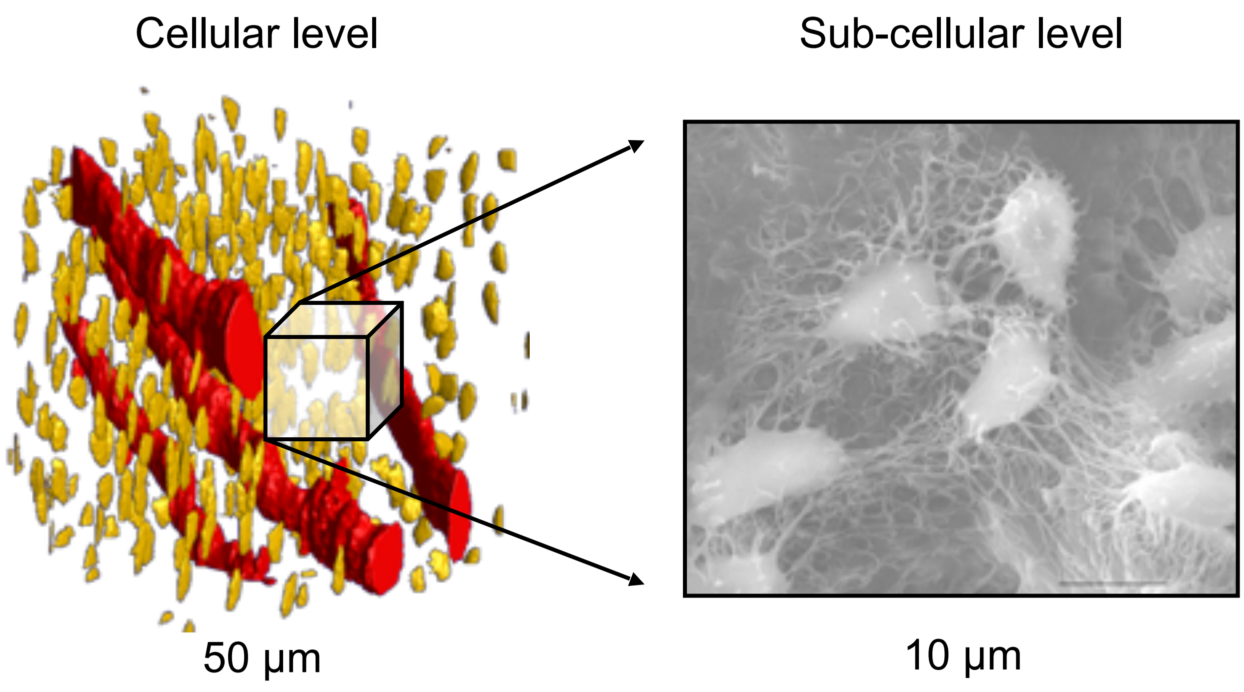


Synchrotron radiation CT: murine cortical bone ultrastructure
P. Schneider *et al.*, *Proceedings of SPIE* **6916**:19-1--19-12 (2008)

3. Demonstration

Lacuno-canalicular network

Osteocytes and canaliculi

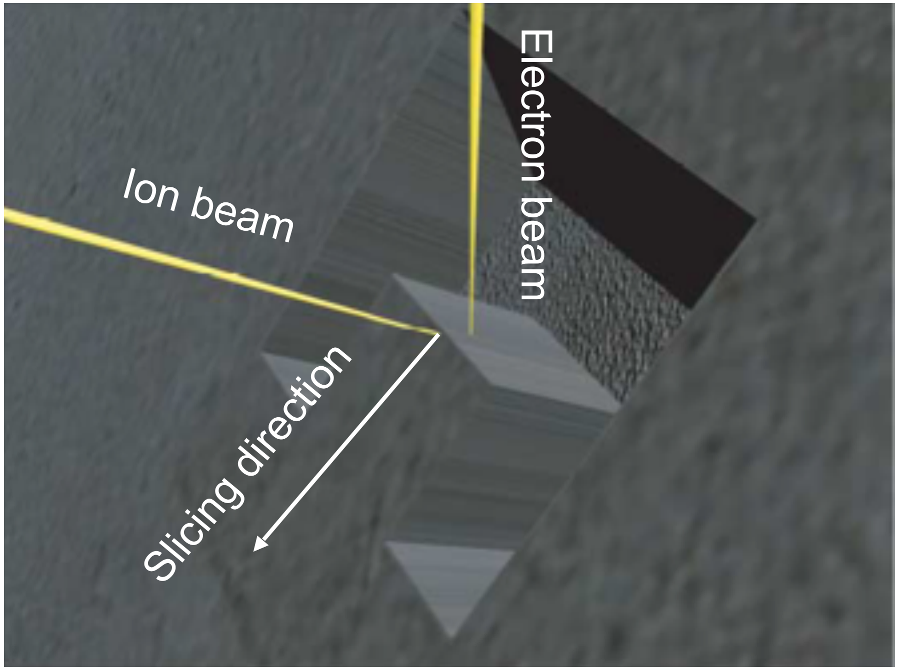


Scanning electron micrograph: murine lacuno-canalicular network
S. C. Cowin, *Journal of Biomechanics* **40**:S105-S109 (2007)

3. Demonstration

Lacuno-canalicular network

Focused ion beam

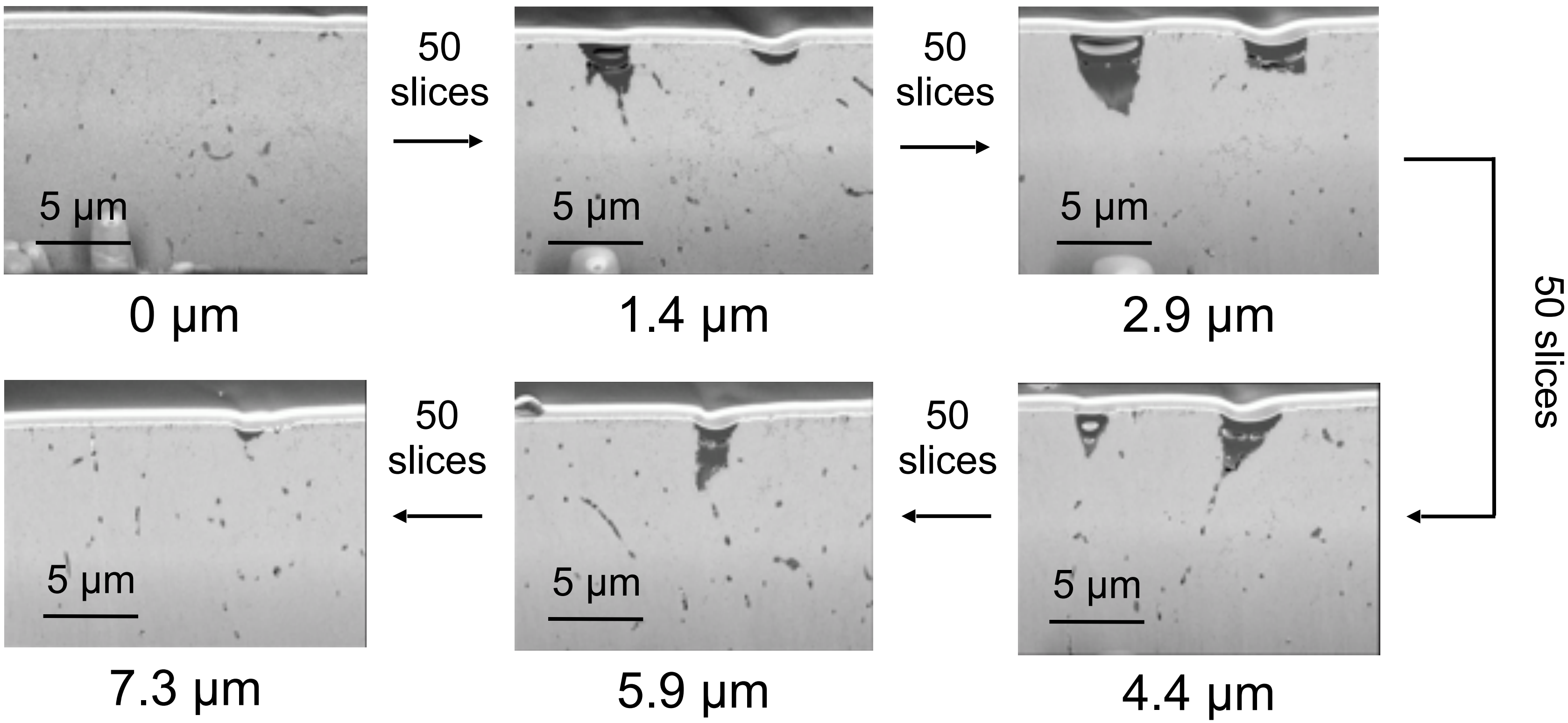


Dual focused ion beam-scanning electron microscopy: serial sectioning and imaging procedure
L. Holzer *et al.*, [*Journal of Microscopy* **216(1)**:84-95 (2004)]

3. Demonstration

Lacuno-canalicular network

Raw data

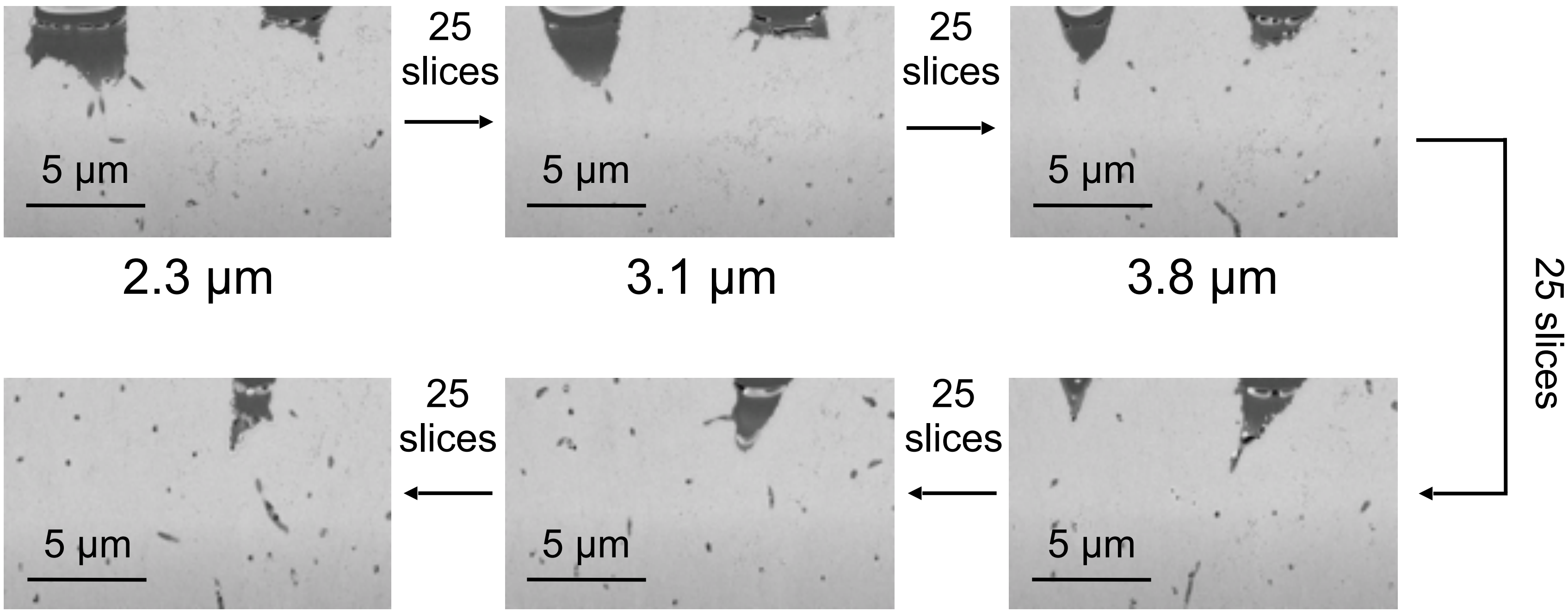


P. Schneider and M. Meier *et al.*, [Bone **49**:304-11 (2011)]

3. Demonstration

Lacuno-canalicular network

Cutting: define volume of interest



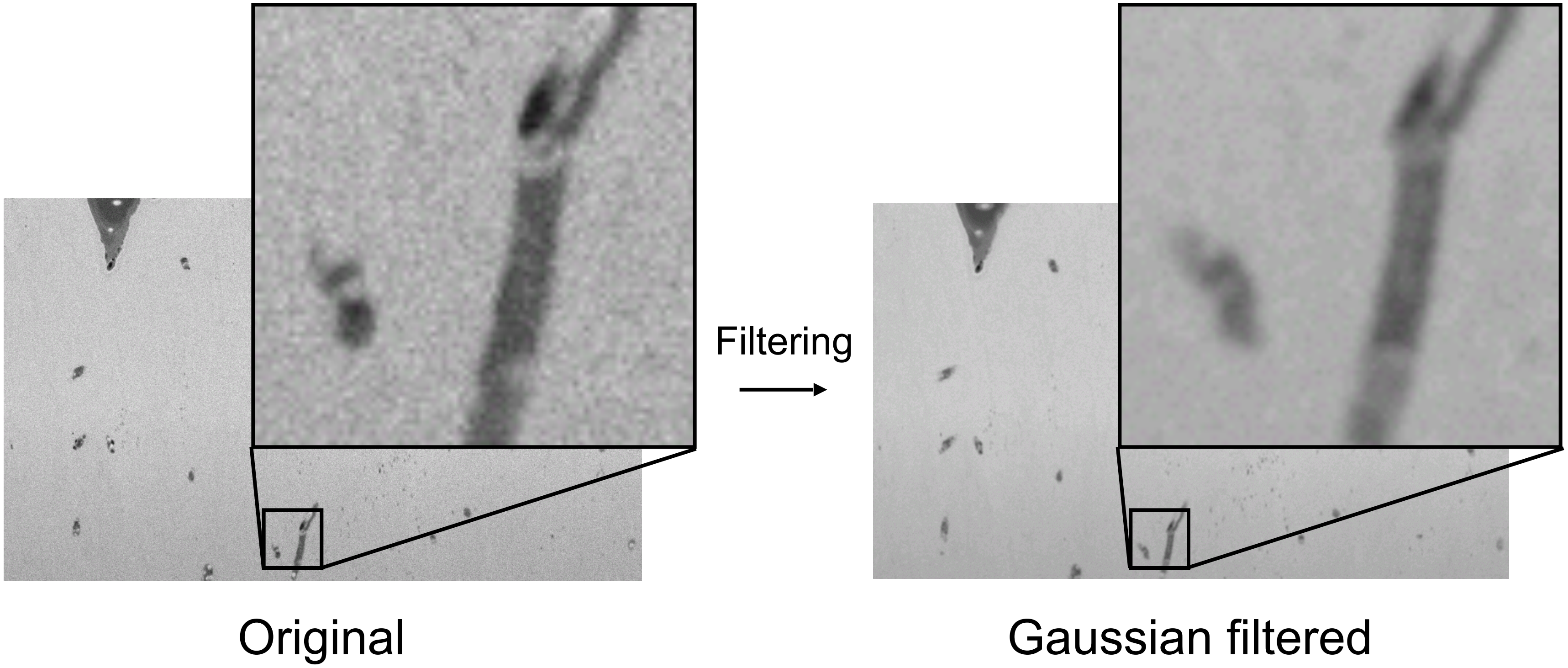
2.3 µm 3.1 µm 3.8 µm

6.0 µm 5.3 µm 4.5 µm

3. Demonstration

Lacuno-canalicular network

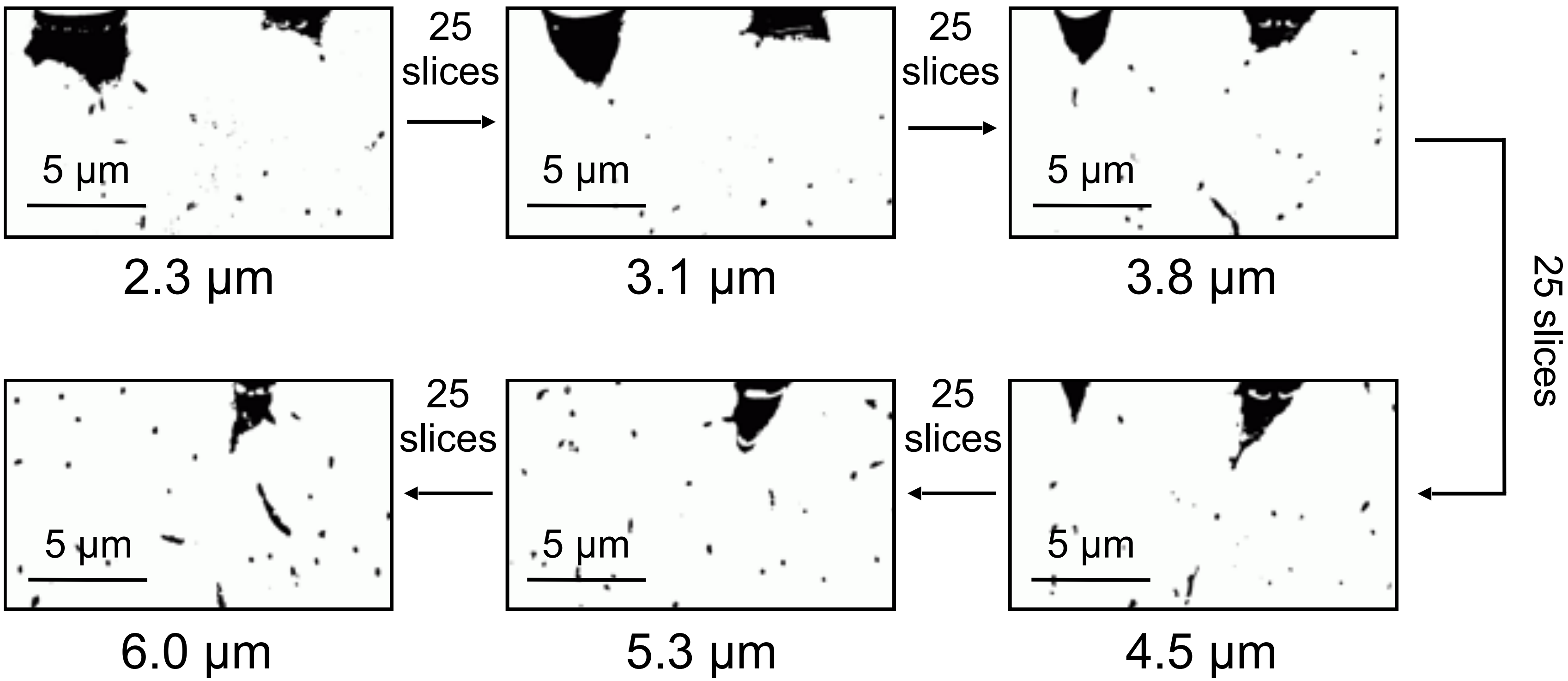
Gaussian filtration: reduce noise



3. Demonstration

Lacuno-canalicular network

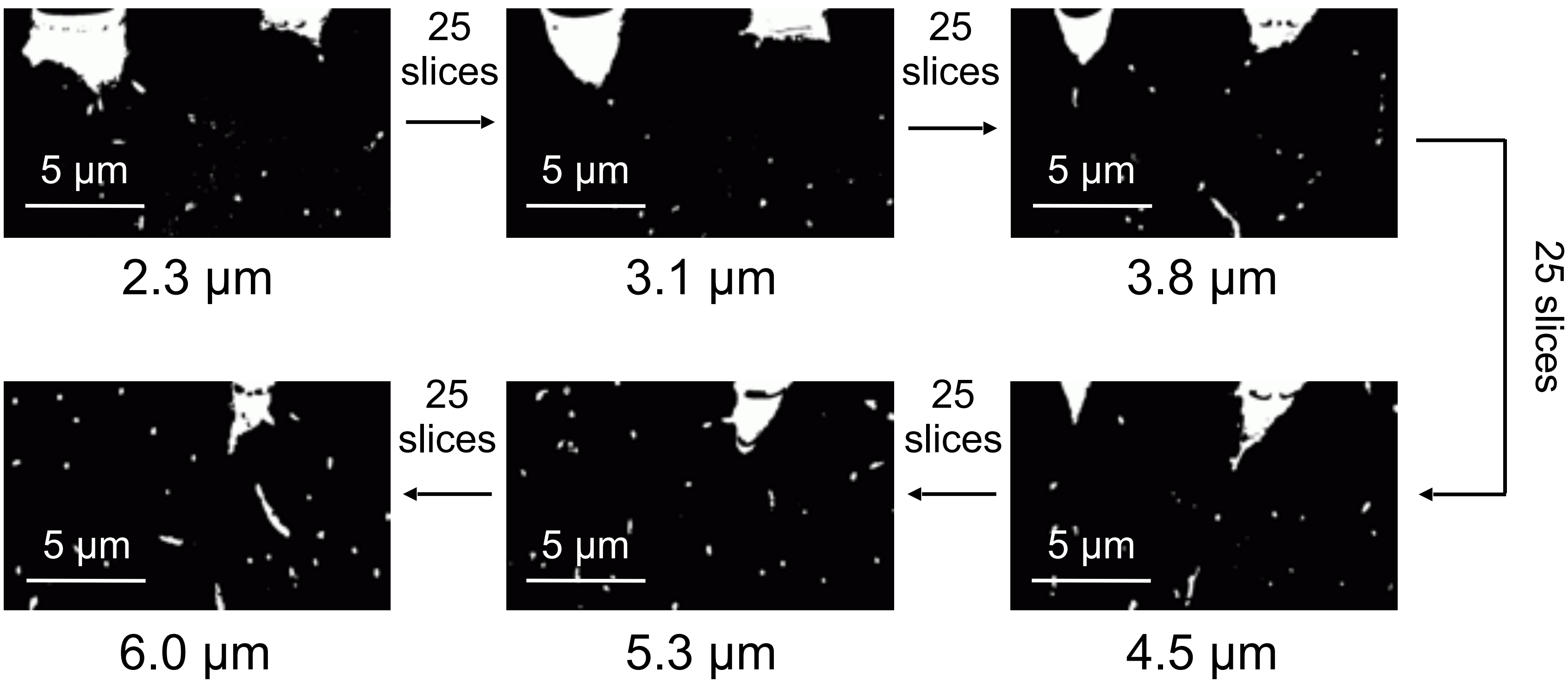
Thresholding: segment data



3. Demonstration

Lacuno-canalicular network

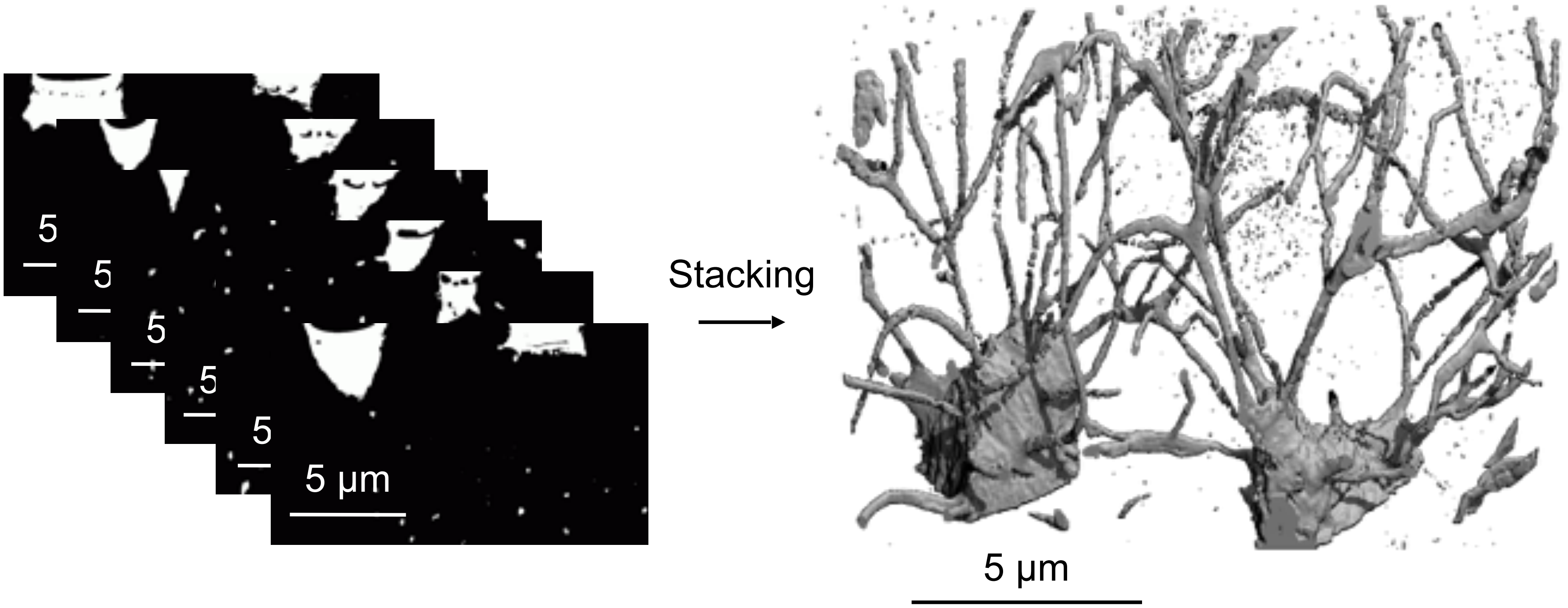
Inverting: access porosity



3. Demonstration

Lacuno-canalicular network

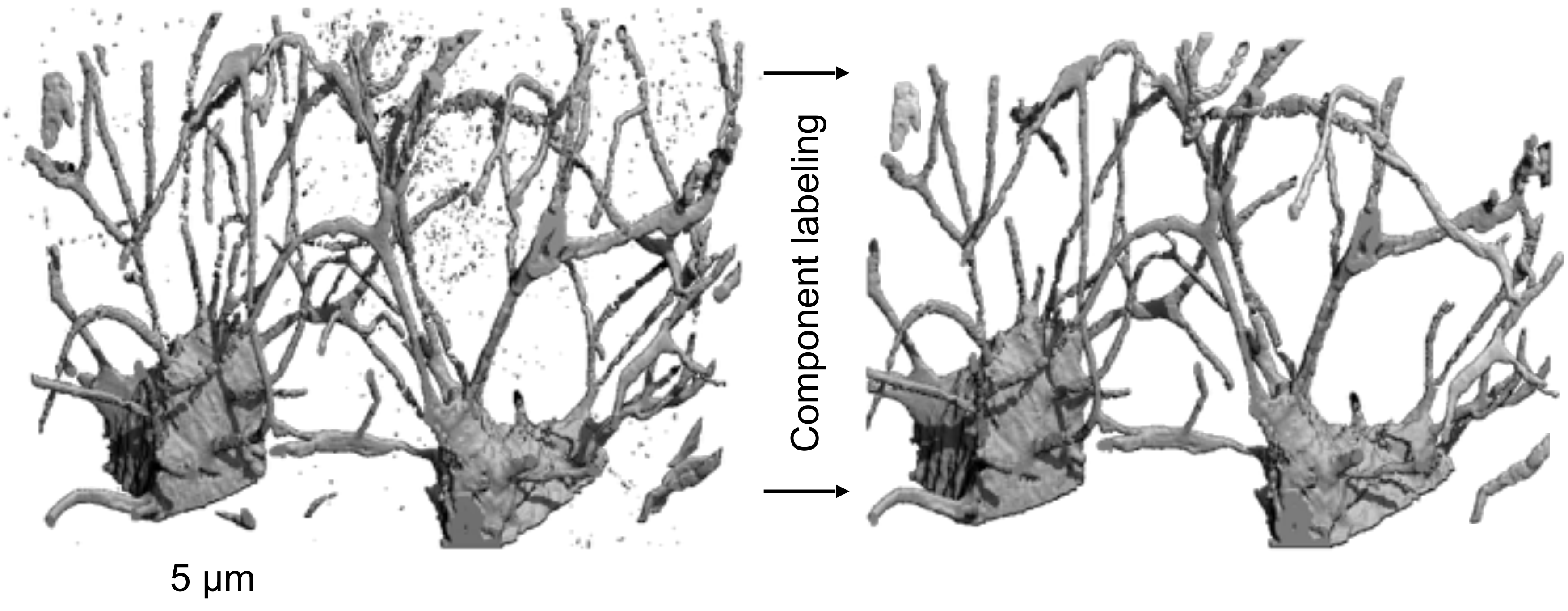
Visualization: 2D -> 3D



3. Demonstration

Lacuno-canalicular network

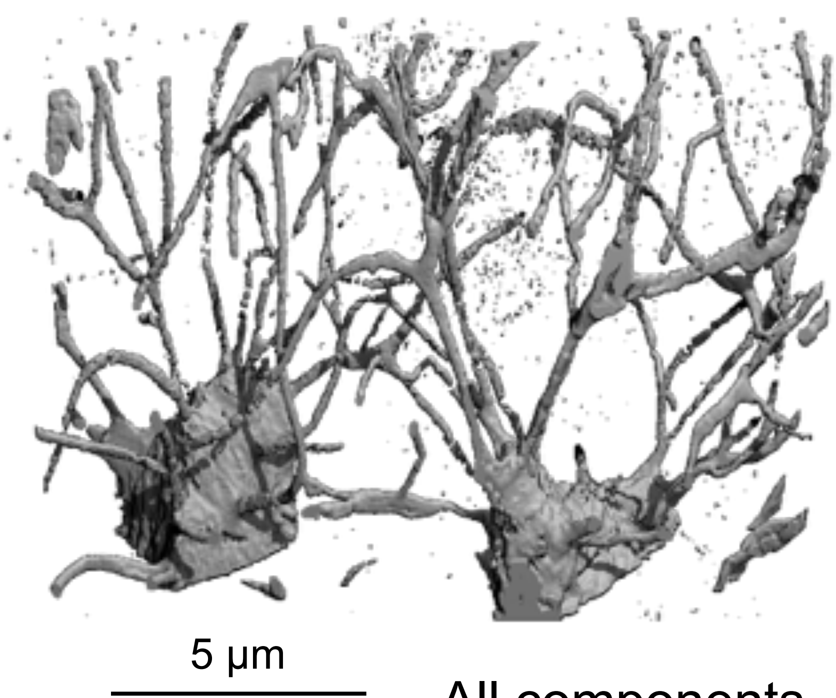
Component labeling: remove noise and small fragments



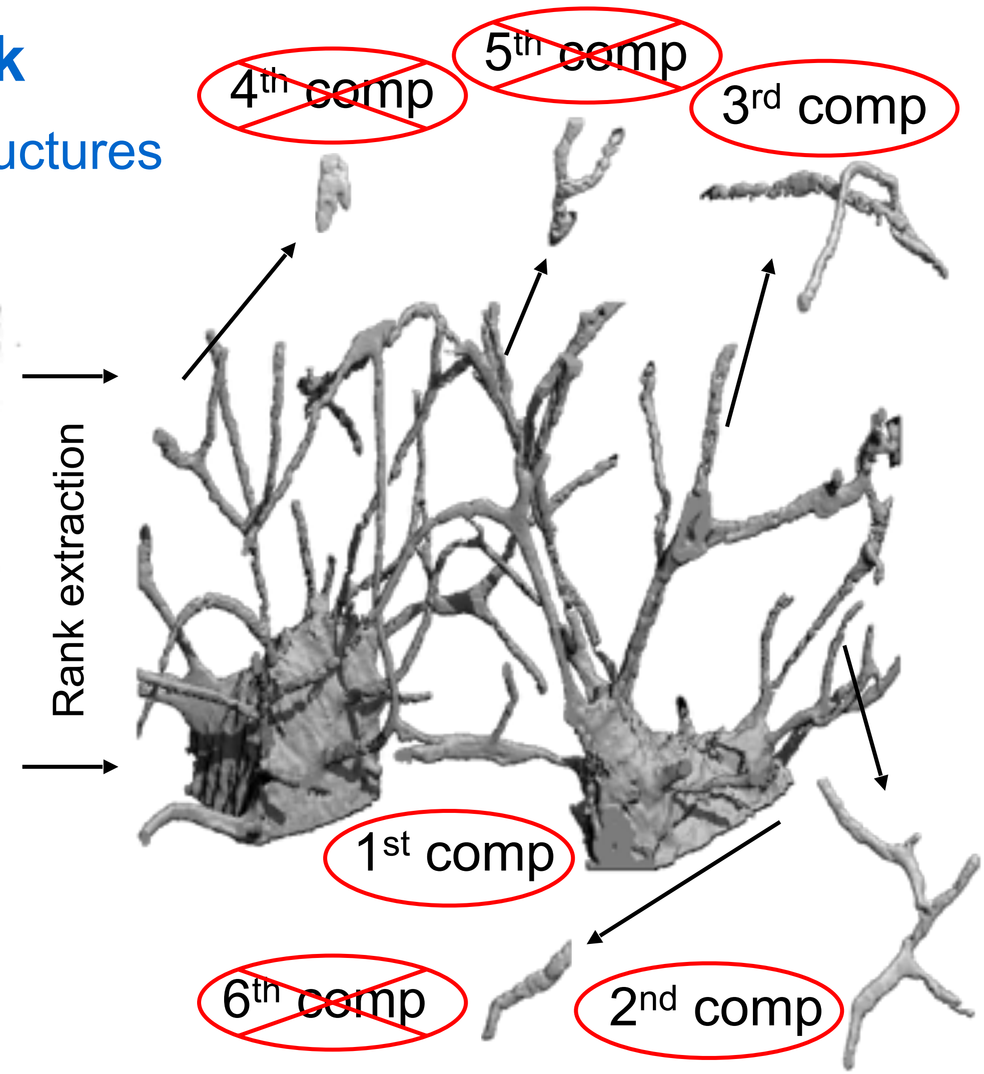
3. Demonstration

Lacuno-canalicular network

Rank extraction: focus on main structures



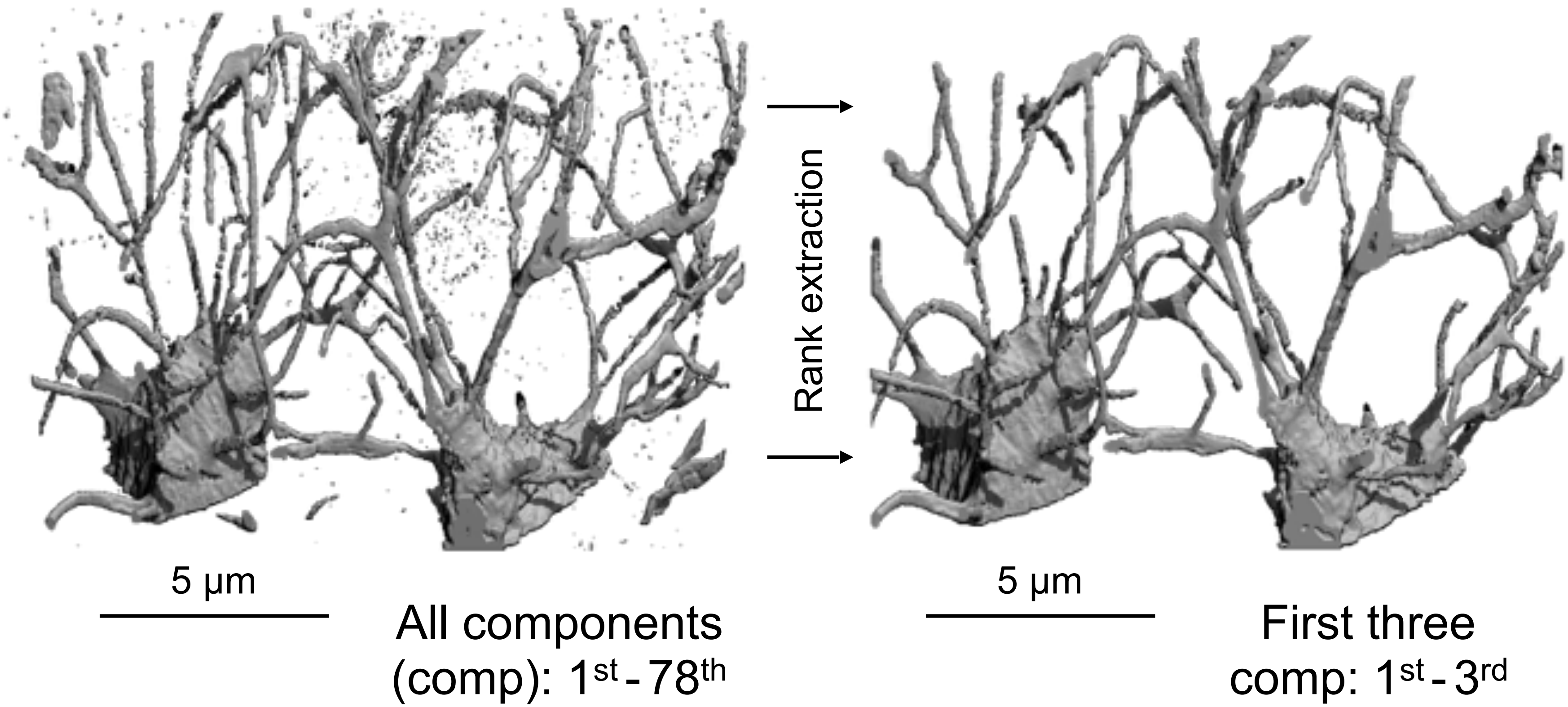
All components (comp): 1st - 78th



3. Demonstration

Lacuno-canalicular network

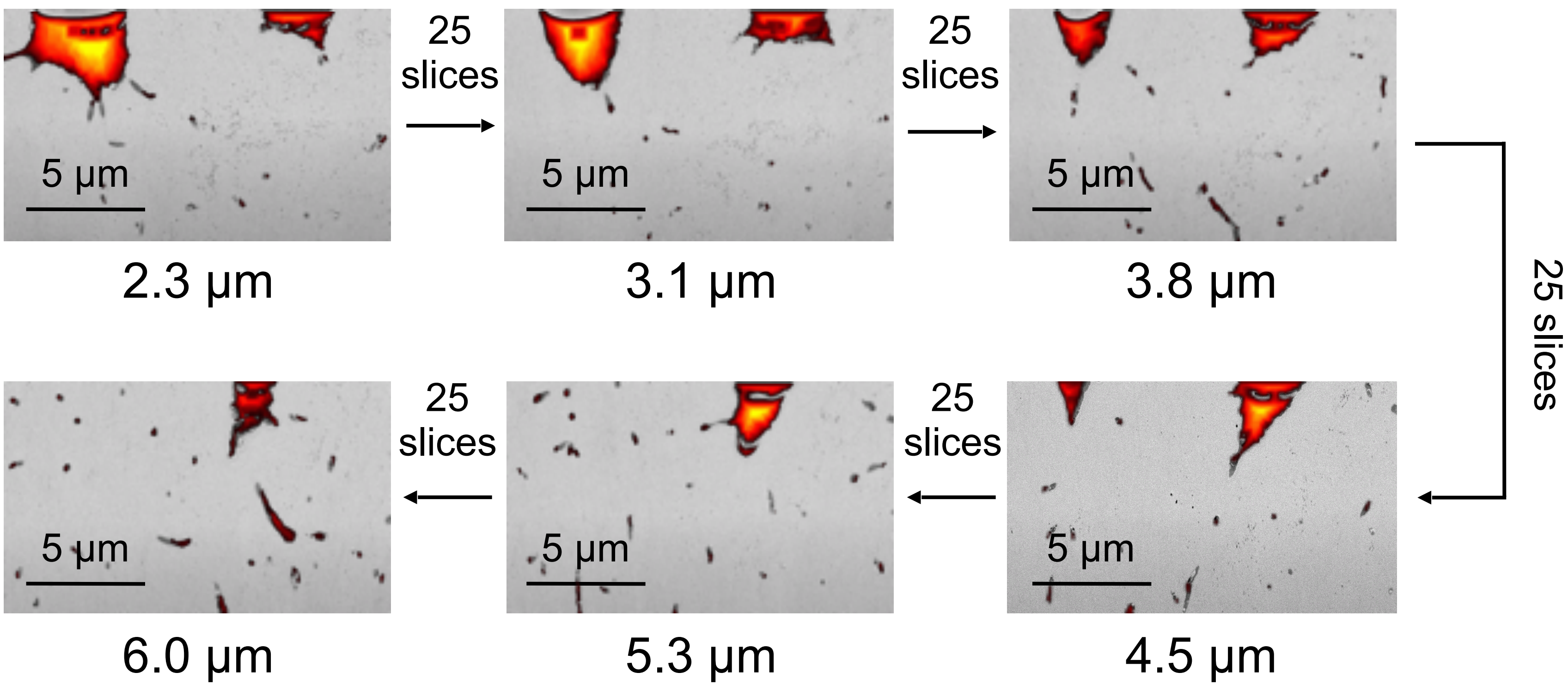
Rank extraction: focus on main structures



3. Demonstration

Lacuno-canalicular network

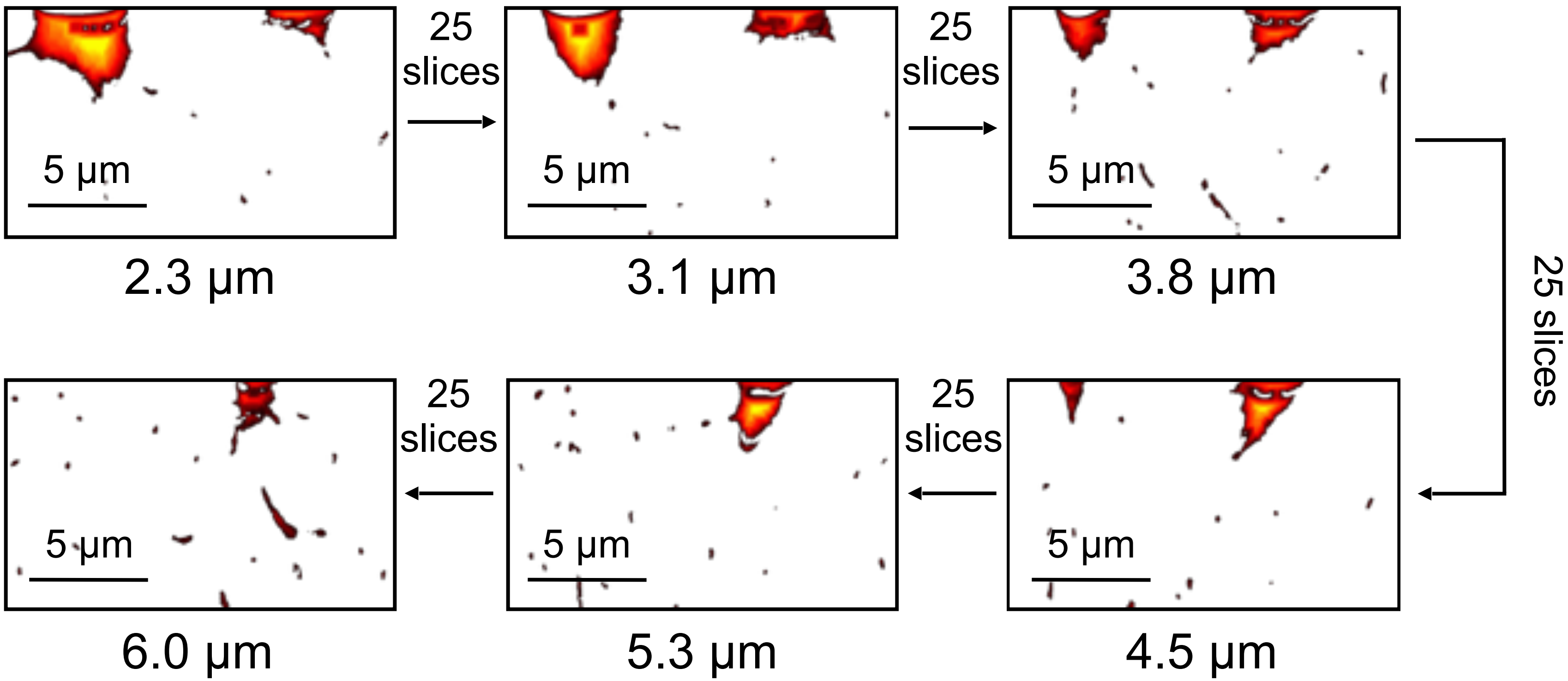
Distance map: extract osteocyte lacunae



3. Demonstration

Lacuno-canalicular network

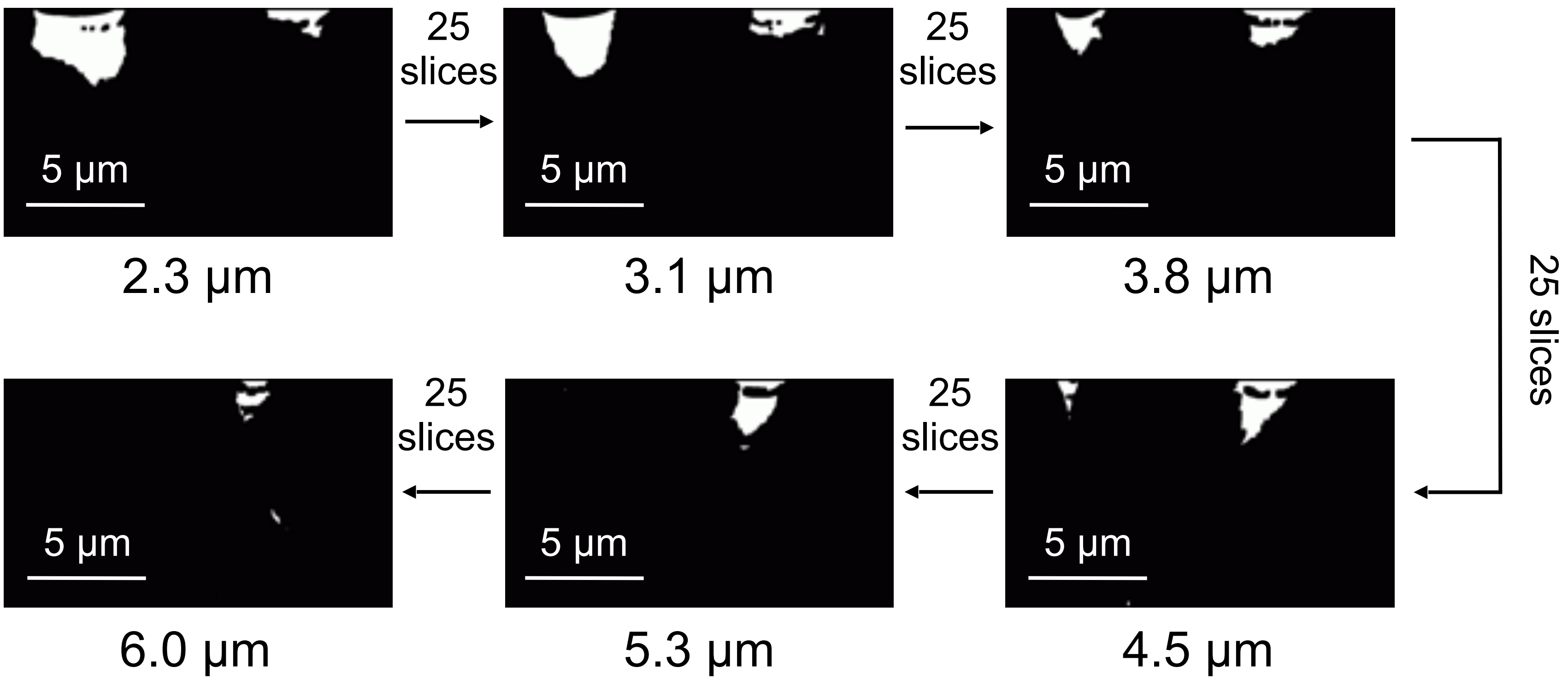
Distance map: extract osteocyte lacunae



3. Demonstration

Lacuno-canalicular network

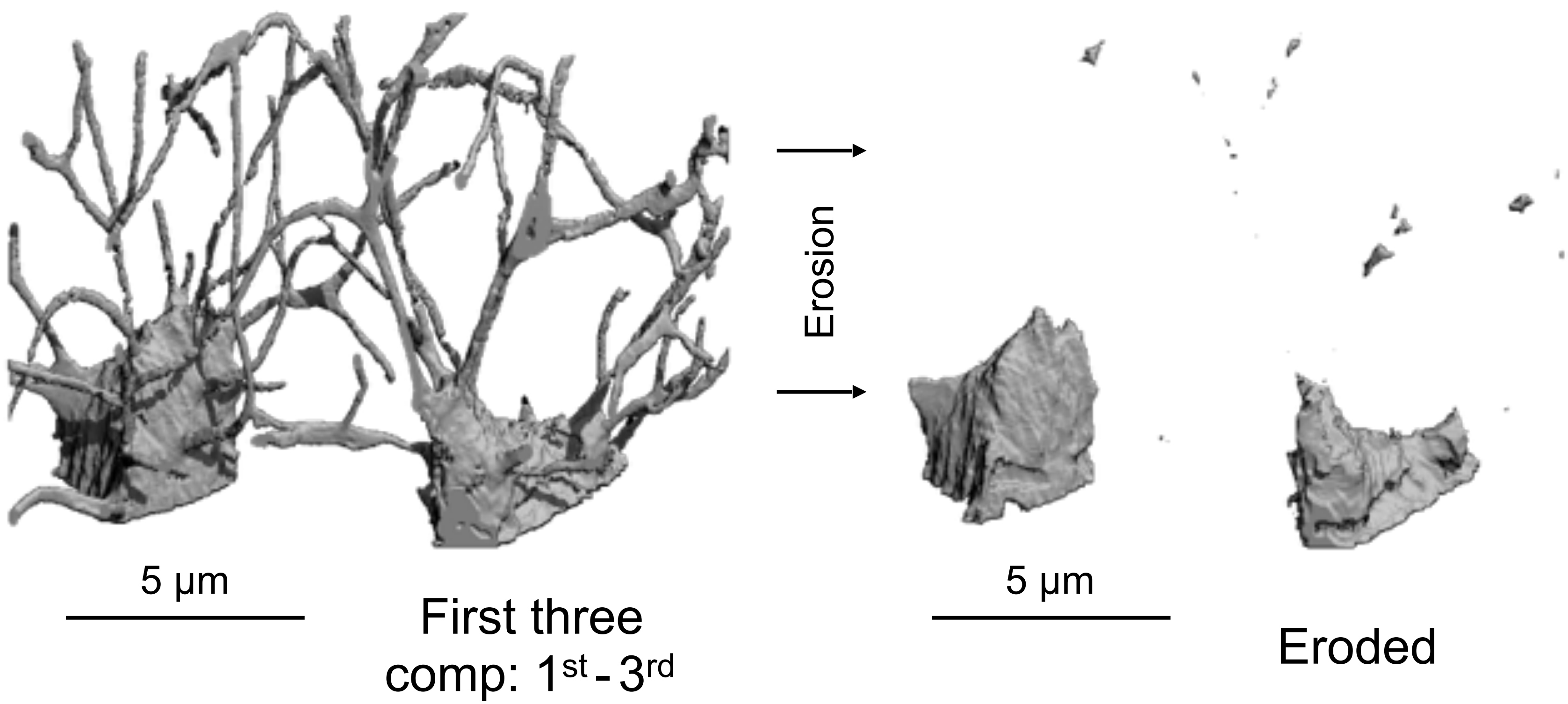
Thresholding distance map: extract osteocyte lacunae



3. Demonstration

Lacuno-canalicular network

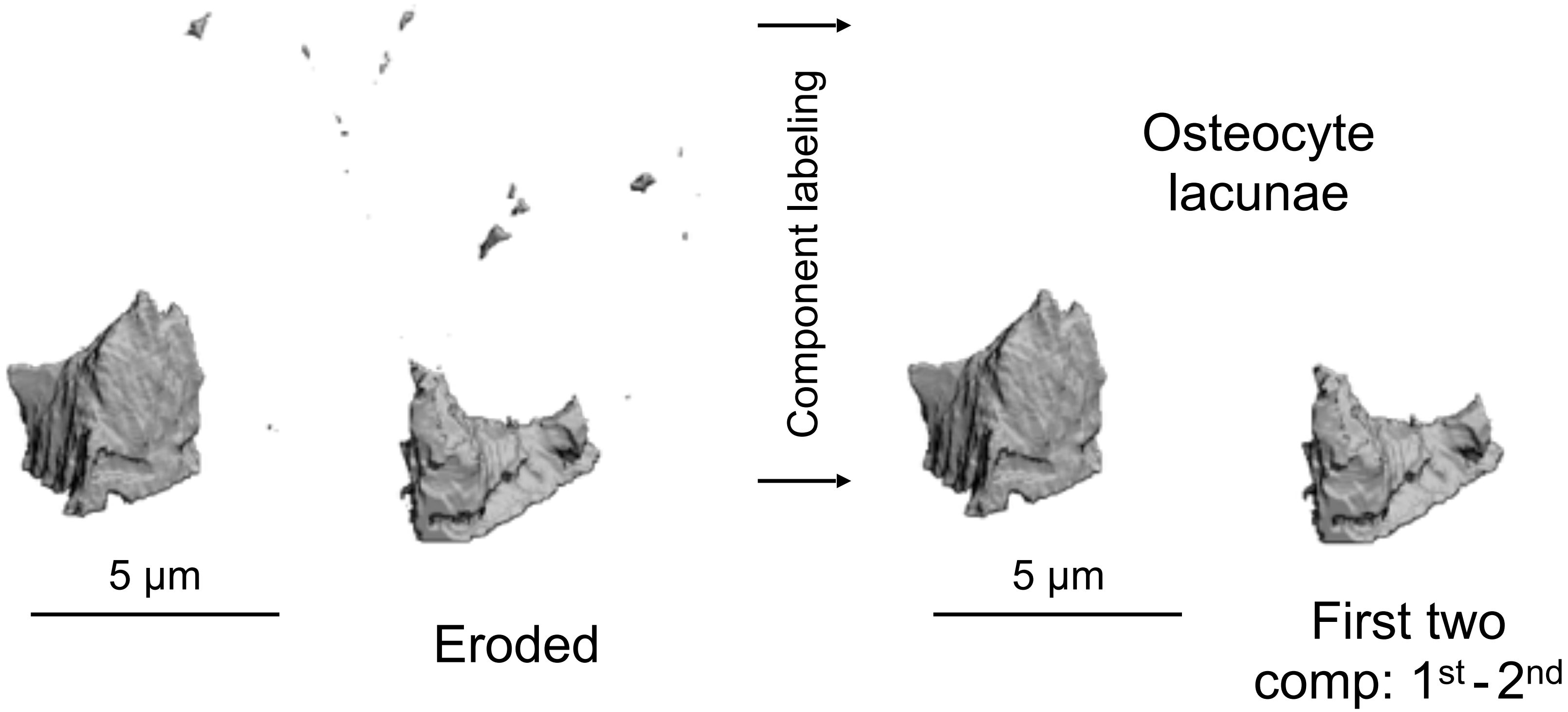
Erosion by thresholding distance map: extract osteocyte lacunae



3. Demonstration

Lacuno-canalicular network

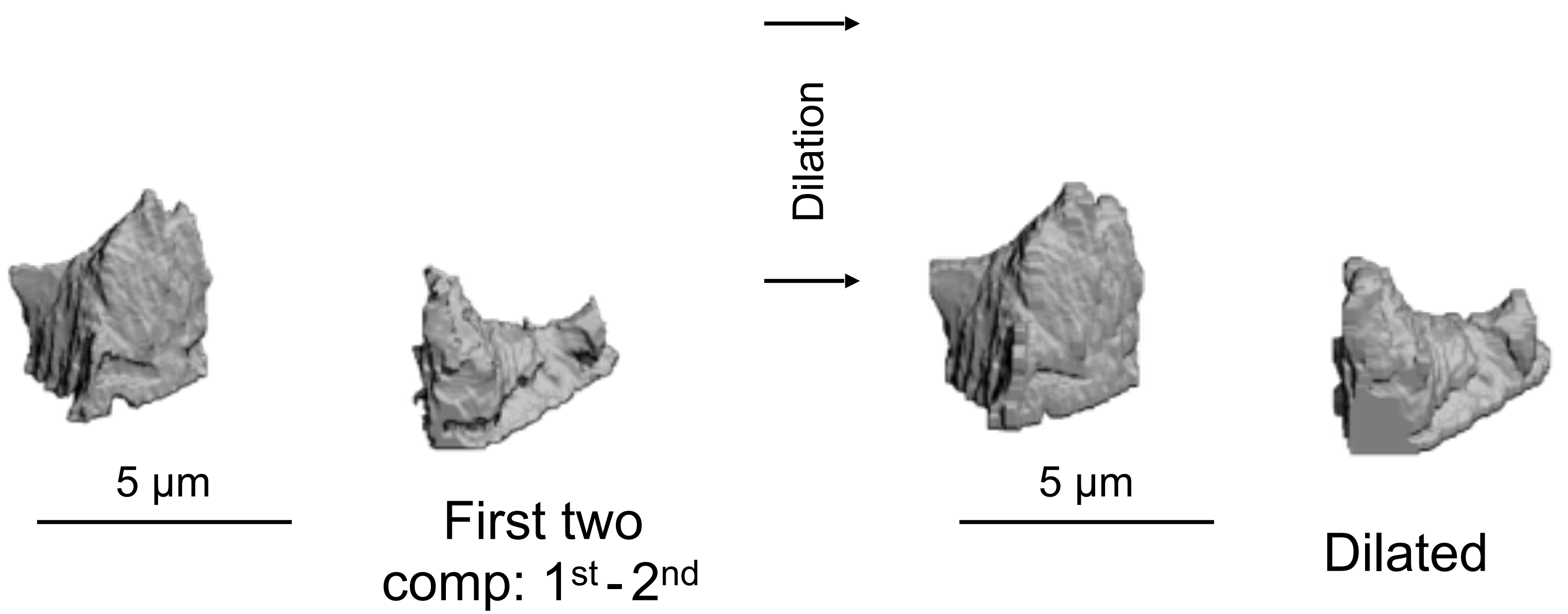
Component labeling: remove remaining canaliculi



3. Demonstration

Lacuno-canalicular network

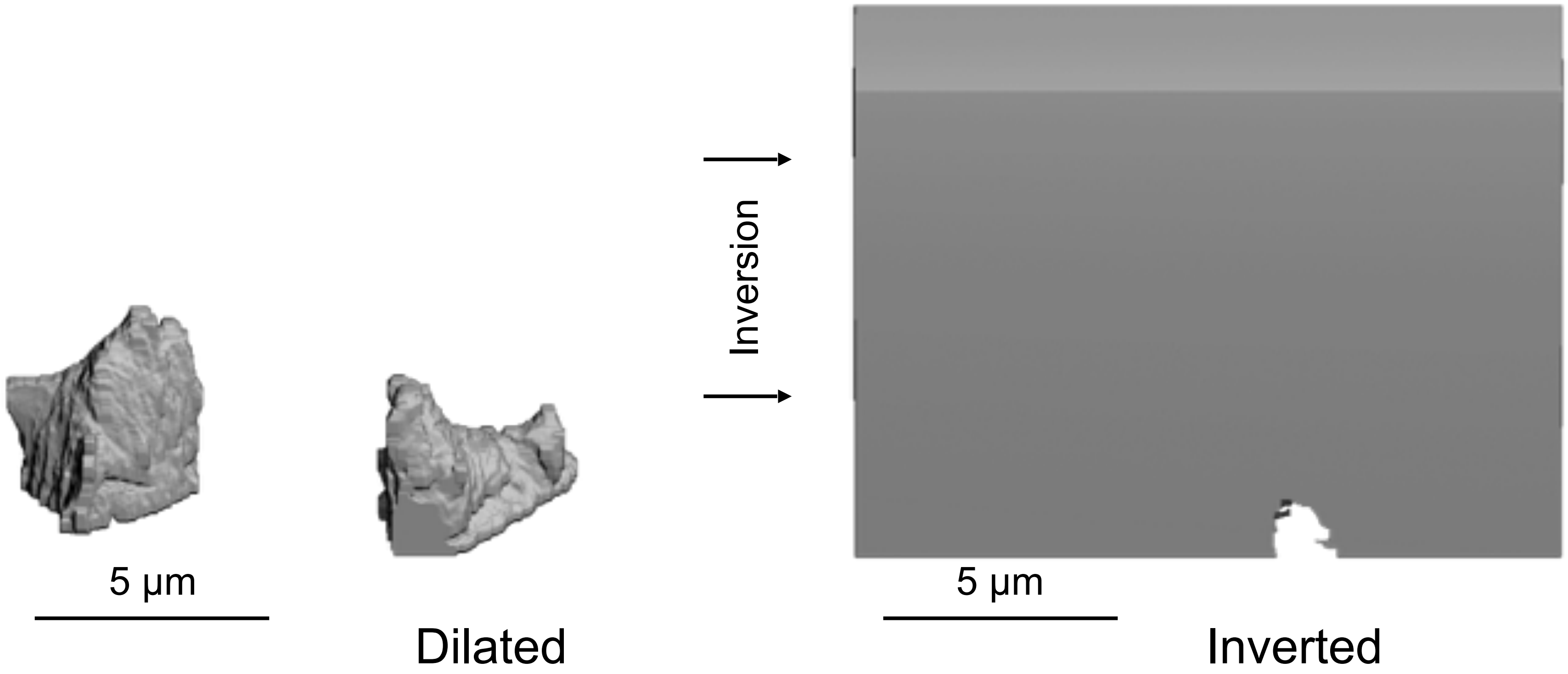
Dilation: recover original extent of osteocyte lacunae



3. Demonstration

Lacuno-canalicular network

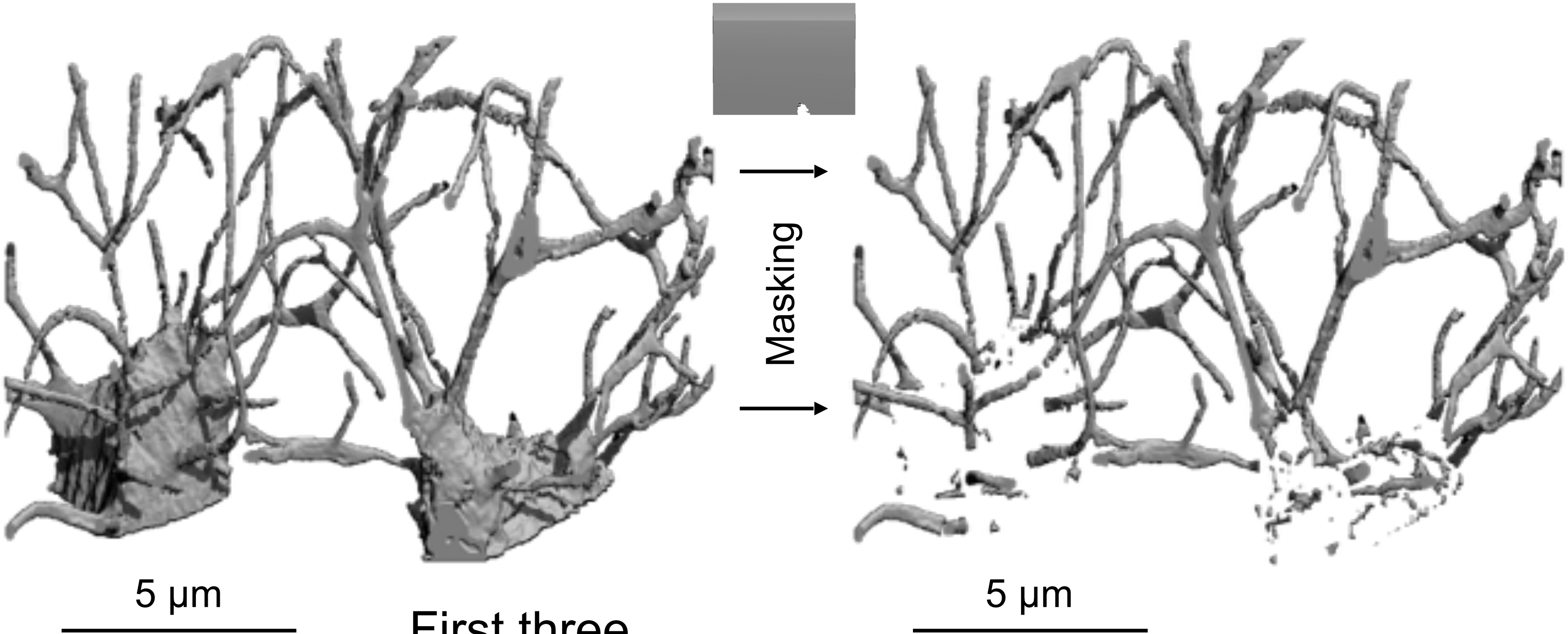
Inversion: create mask to extract canaliculi



3. Demonstration

Lacuno-canalicular network

Masking: extract canaliculi



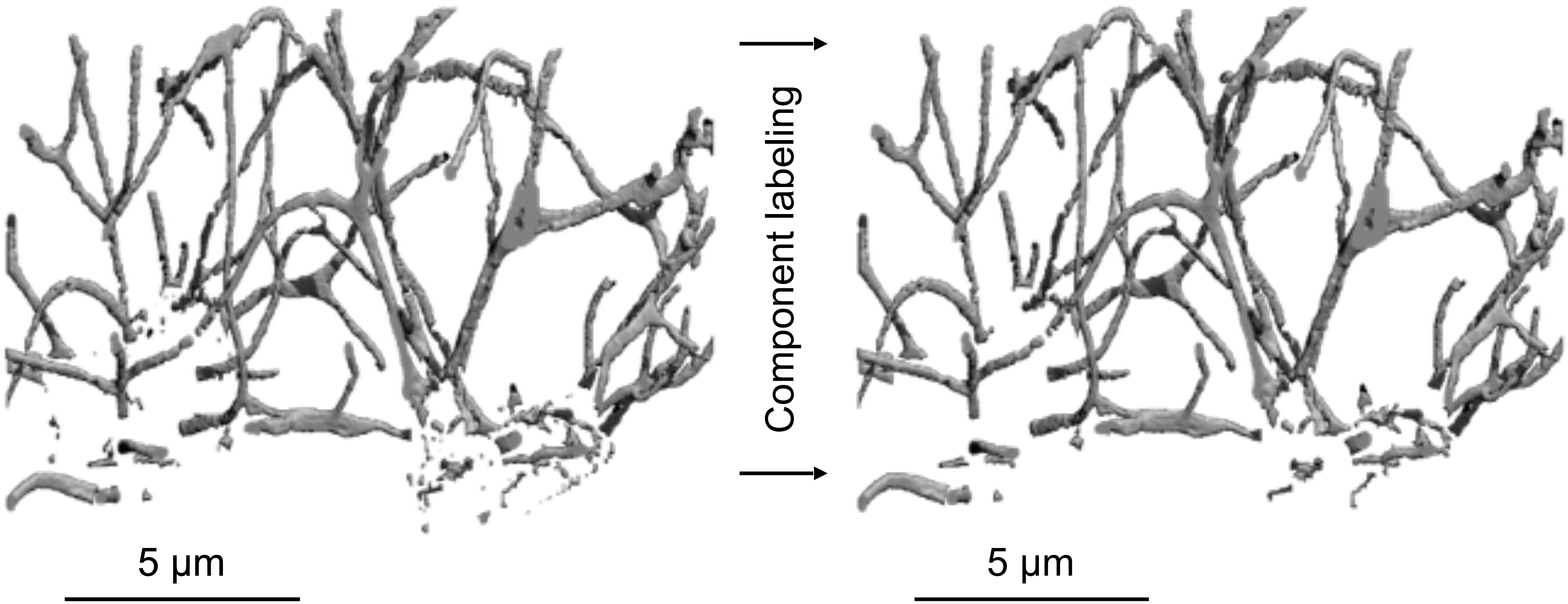
First three comp: 1^{st} - 3^{rd}

Masked canaliculi

3. Demonstration

Lacuno-canalicular network

Component labeling: remove small fragments to extract canaliculi



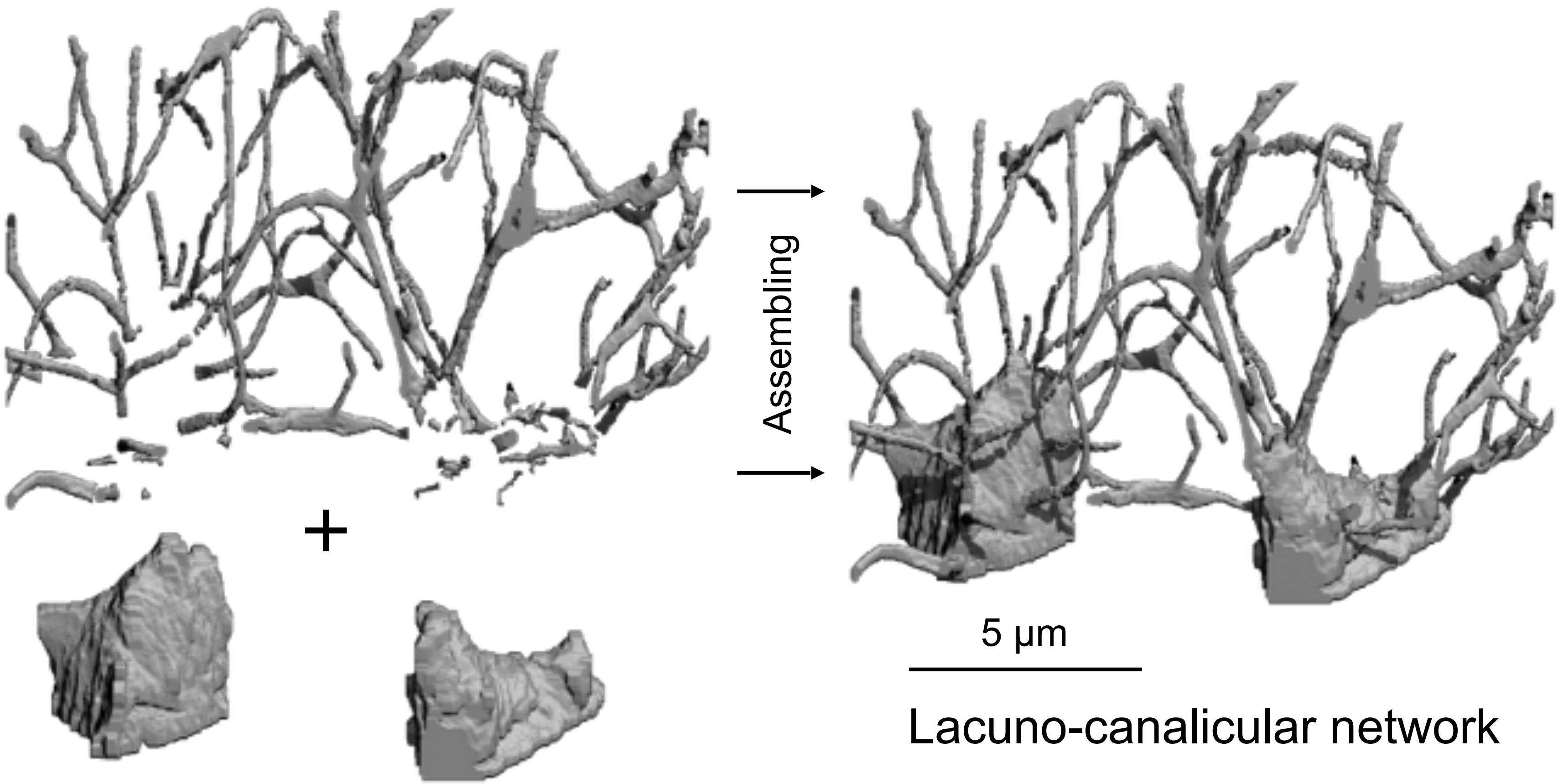
Masked canaliculi

Osteocyte lacunae rests removed

3. Demonstration

Lacuno-canalicular network

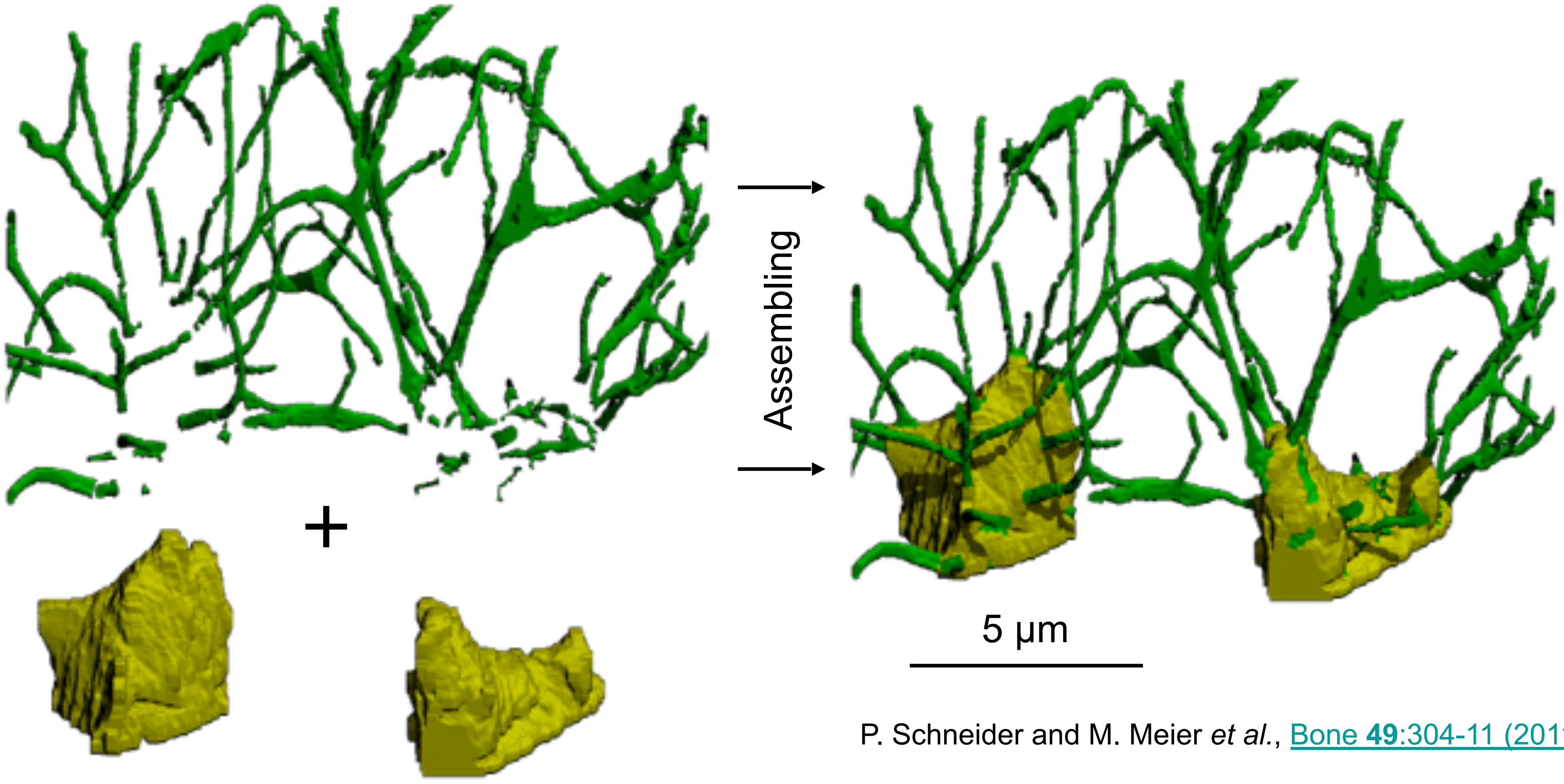
Addition: assemble osteocyte lacunae and canaliculi



3. Demonstration

Lacuno-canalicular network

Visualization: distinguish components optically

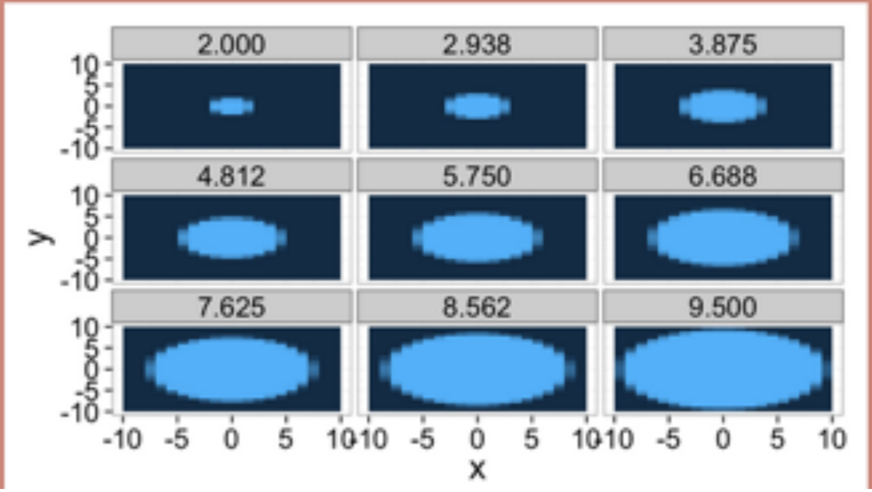


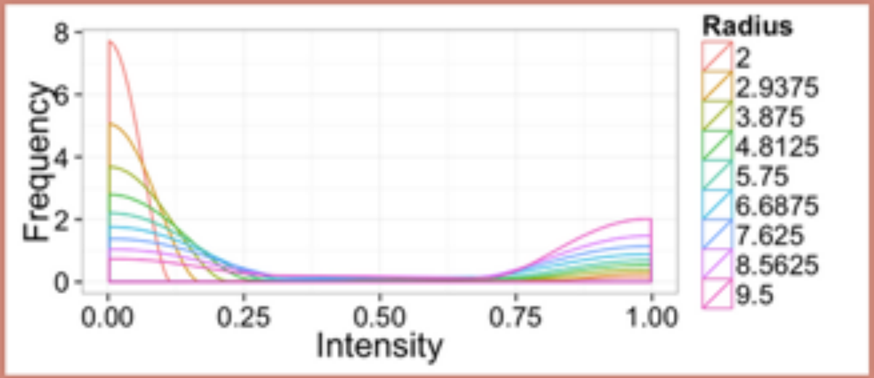
P. Schneider and M. Meier *et al.*, Bone **49**:304-11 (2011)

Pitfalls with Segmentation

Partial Volume Effect

- The partial volume effect is the name for the effect of discretization on the image into pixels or voxels.
- Surfaces are complicated, voxels are simple boxes which make poor representations
- Many voxels are only partially filled, but only the voxels on the surface
- Removing the first layer alleviates issue





- Shown as a function of radius

Radius	Mean Intensity	Sd Intensity
2.000	0.0311	0.1623
2.938	0.0677	0.2370
3.875	0.1177	0.3061
4.812	0.1822	0.3688
5.750	0.2601	0.4196
6.688	0.3511	0.4567
7.625	0.4569	0.4763
8.562	0.5761	0.4690
9.500	0.7073	0.4259