DIGS-BB Dresden International Graduate School for Biomedicine and Bioengineering
"From Nanobiotechnology to Regenerative Medicine"

MPI- CBG IPF
Basics of Image Processing
Image Segmentation

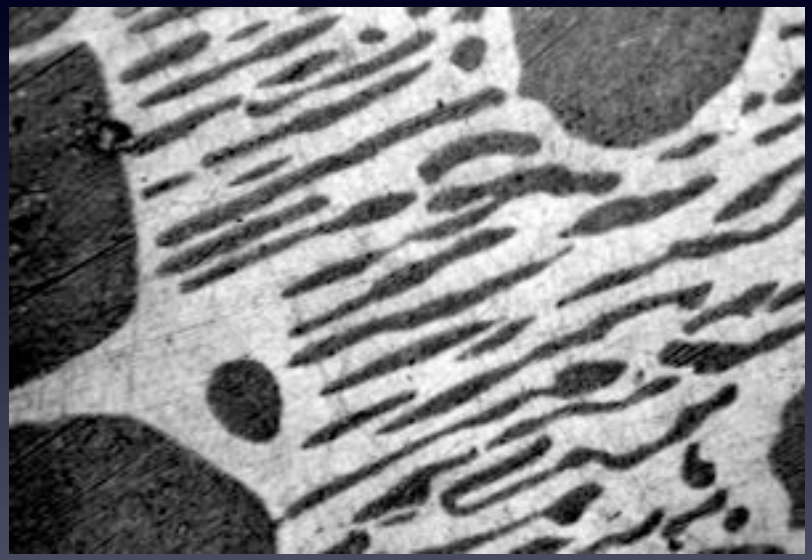
Using ImageJ

Dan White
May 2008

CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

IPF

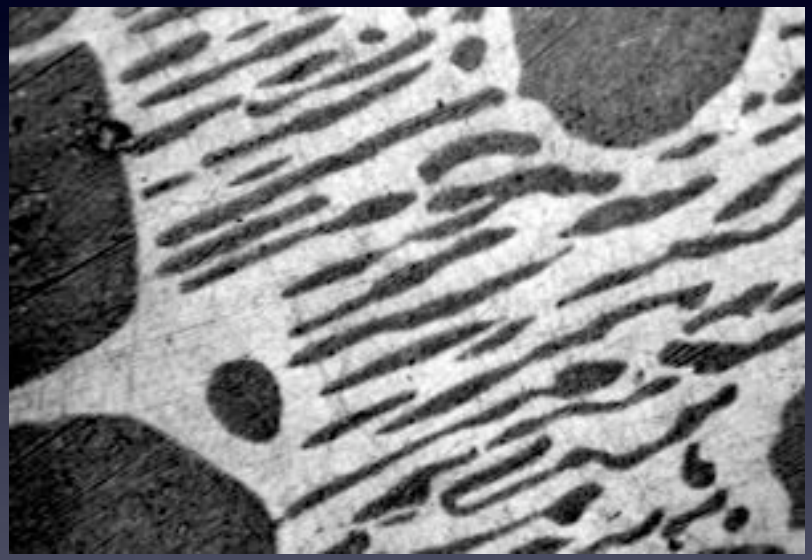
What is "Image Segmentation"?



"Greyscale" image

foreground background

What is "Image Segmentation"?



"Scalar Intensity" image

"Binary" image

What is "Image Segmentation"?

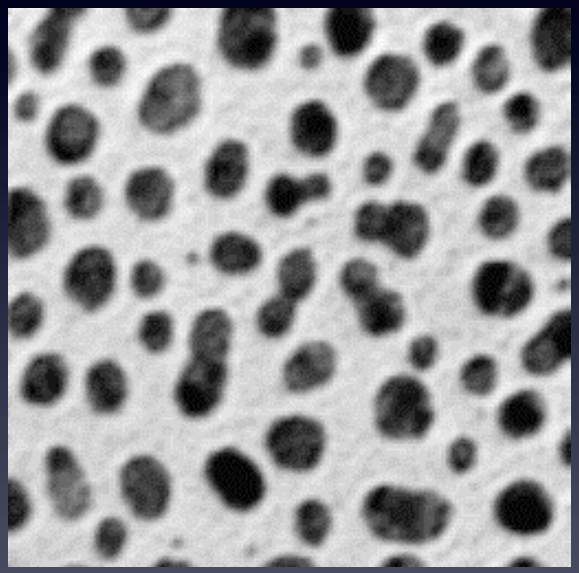
1	65	13	55	2
2	3	34	2	1
4	0	31	1	2
1	33	3	54	3
56	3	2	1	34

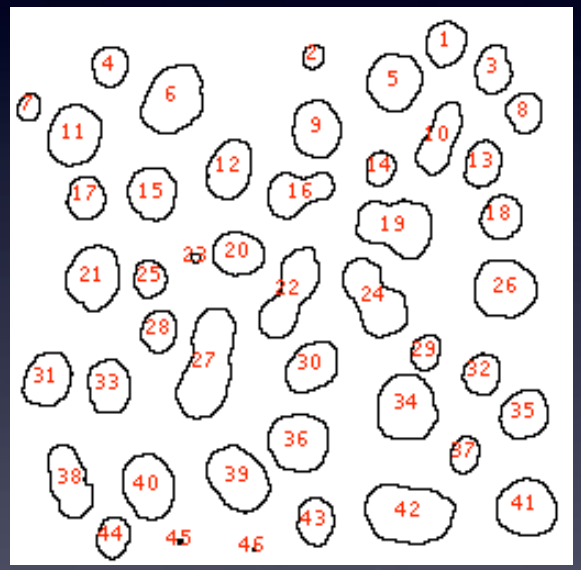
0	1	1	1	0
0	0	1	0	0
0	0	1	0	0
0	1	0	1	0
1	0	0	0	1

"Scalar Intensity" image

"Binary" image

What is "Image Segmentation"?

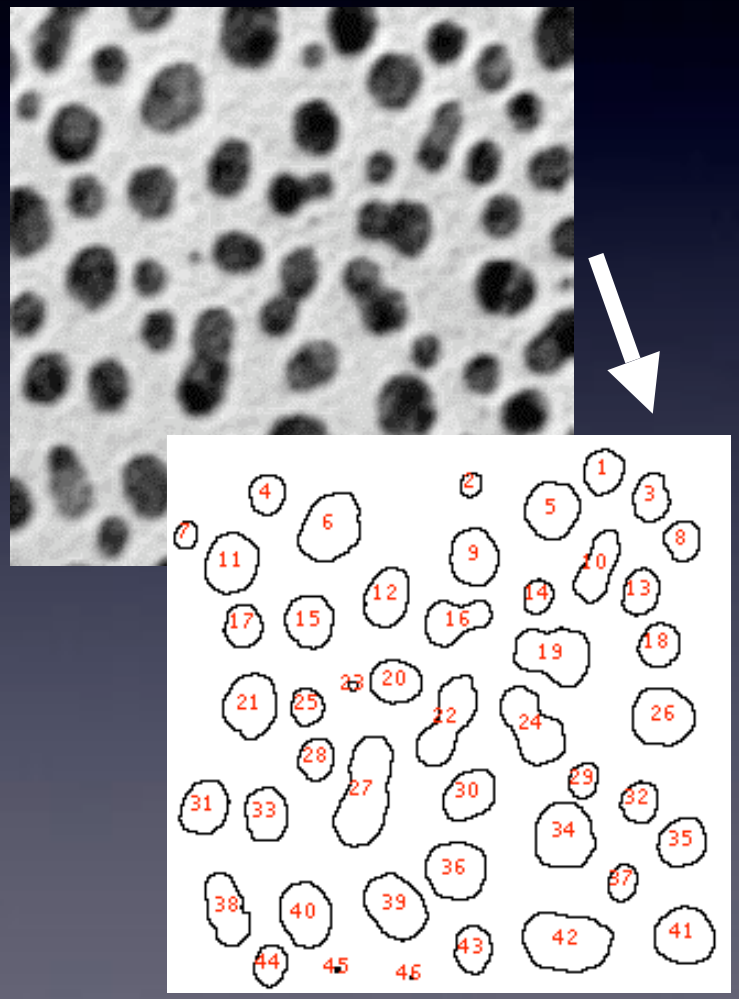




"Scalar Intensity" image

"Labelled Objects"

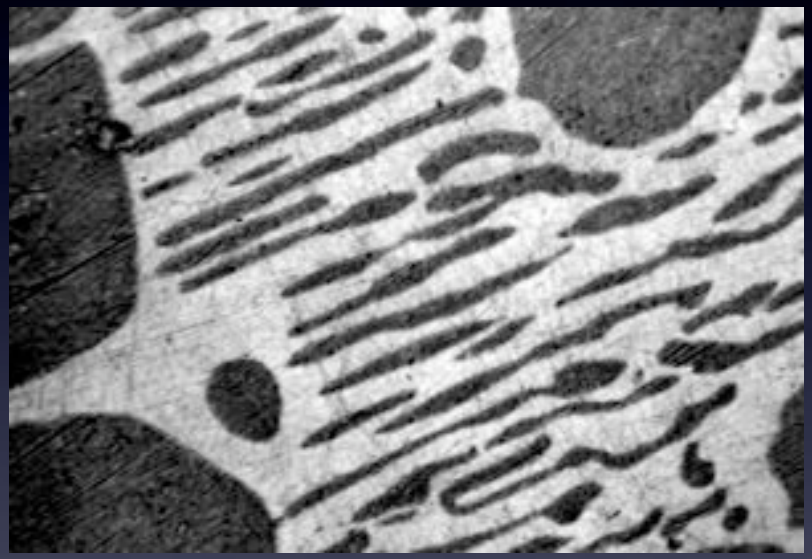
What is "Image Segmentation"?



High Information Content
65536 pixels, 0-255 value

Lower Information Content
But easier to interpret biological meaning:
45 "objects" with properties: size, shape, intensity etc.

“Thresholding” (Intensity Histogram Split)

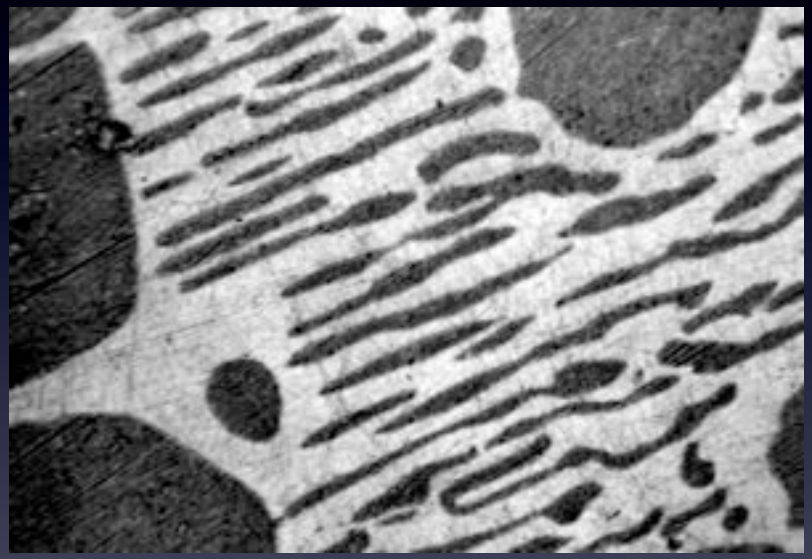


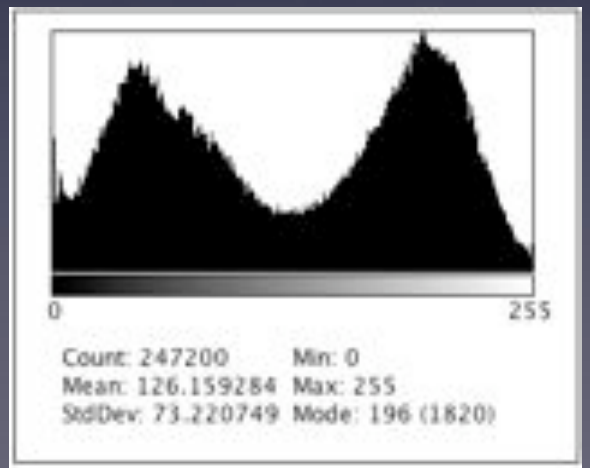
Clear difference between foreground and background?
Image not very noisy?

Choose an intermediate grey value = “threshold”

Determines foreground and background.

“Thresholding” (Intensity Histogram Split)



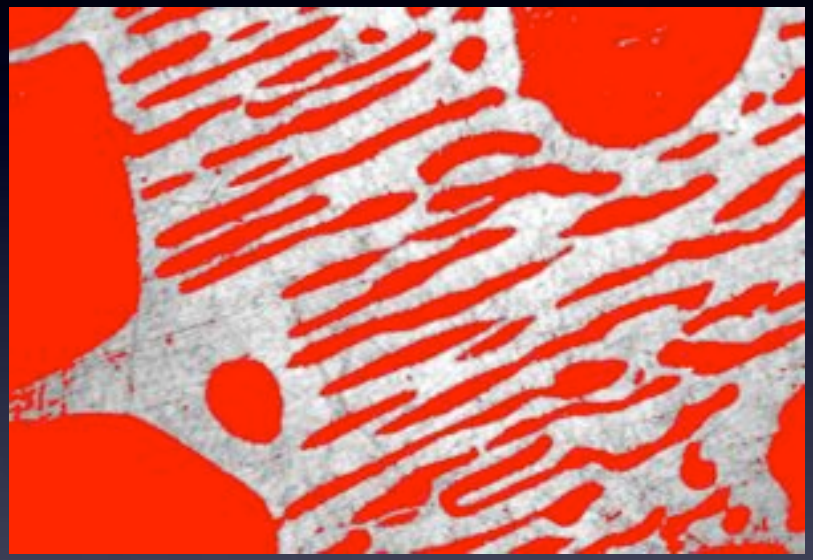


How to choose the grey level for thresholding?

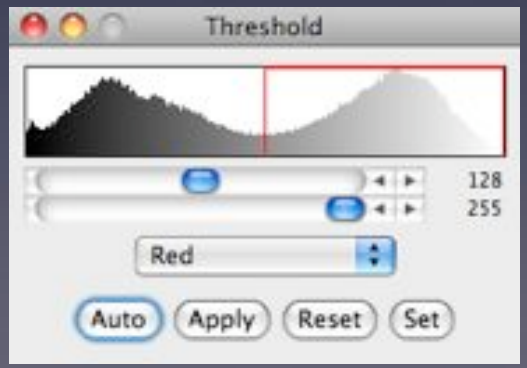
Look at pixel intensity histogram of whole image…

Is there an obvious place?

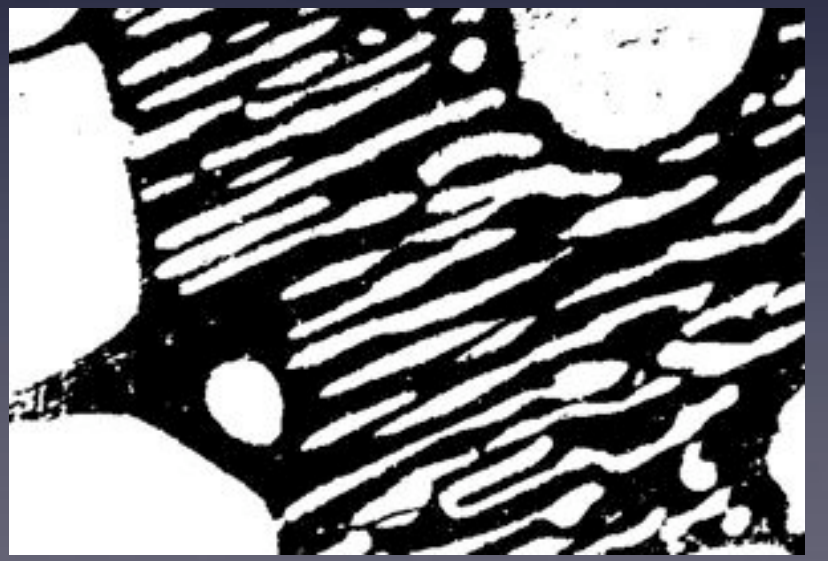
“Thresholding” (Intensity Histogram Split)



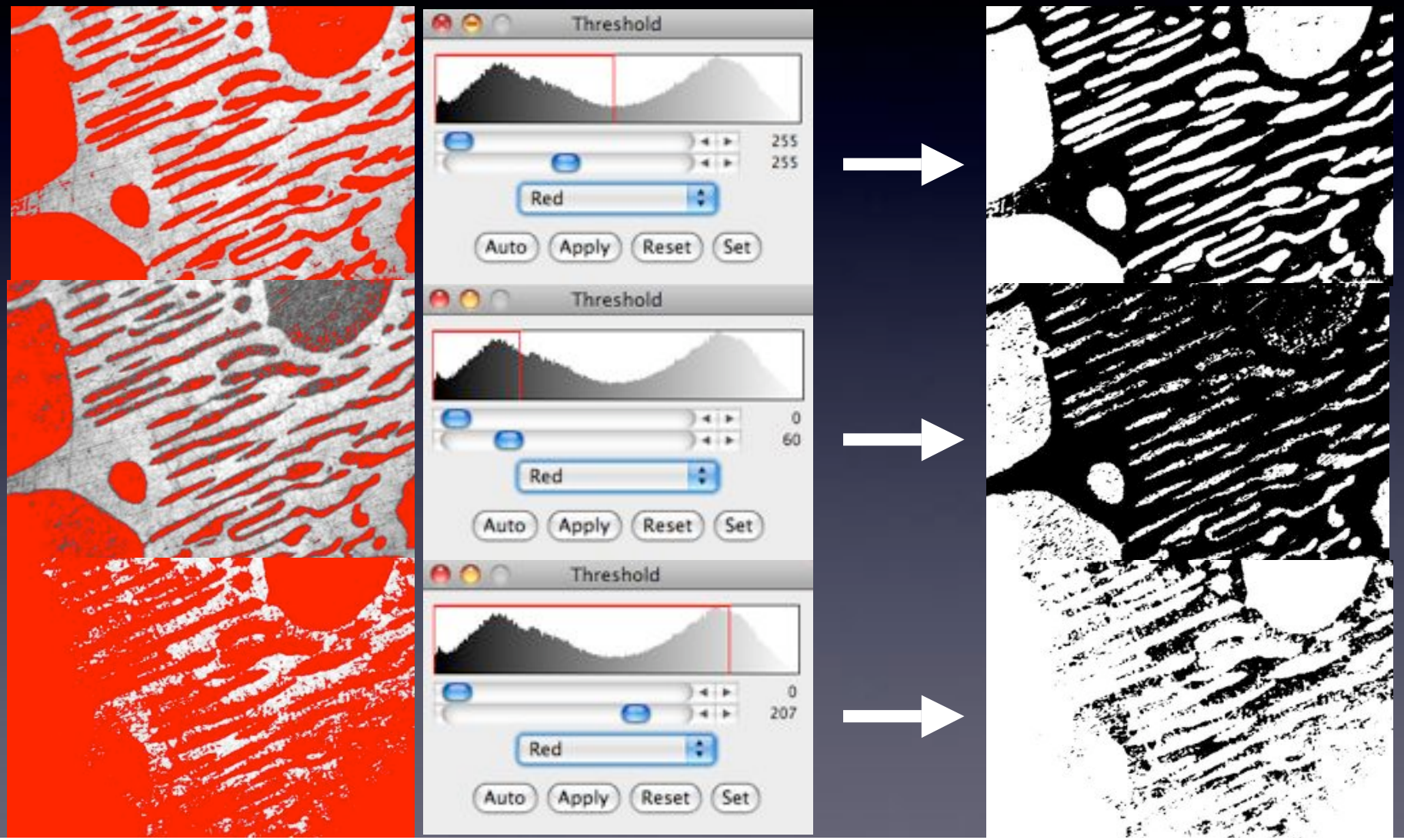
Histogram is bimodal, so put threshold in the trough between the peaks!



Note, in this case:
Foreground = “dim” objects
Background = “bright” objects



“Dumb Global Threshold” (Subjective - User Biased)



Computed Global Threshold
Objective - Reproducible

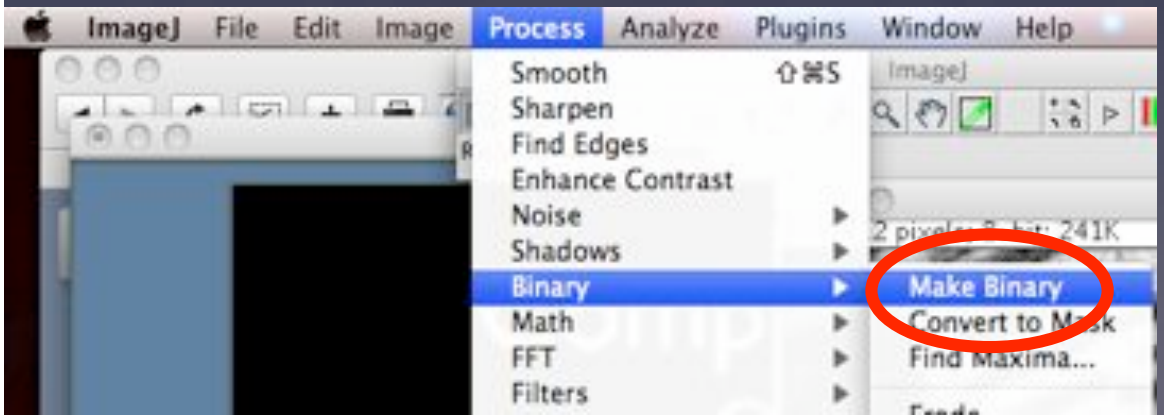
ImageJ - Auto Threshold (and Make Binary):

Initial guess of Threshold, T

Compute mean pixel intensity of background and foreground

Tnew =
1/2 x (mean of foregrnd + mean of bkgrnd)

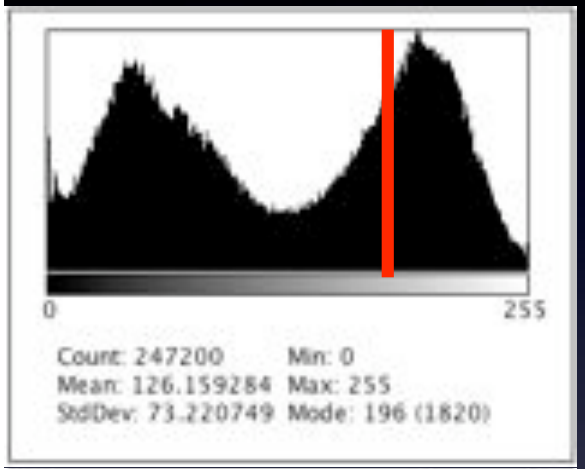
Iterate until Tnew no longer changes.

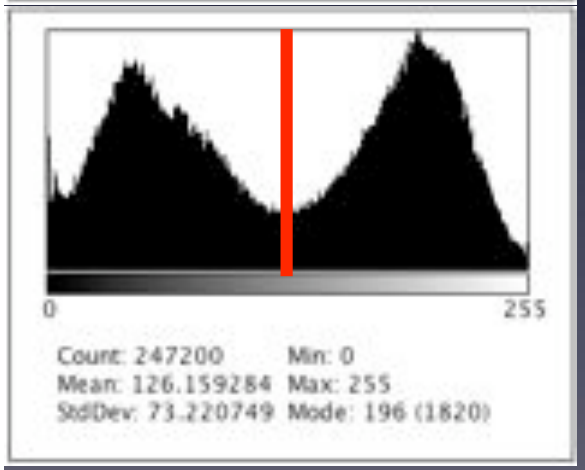


Note:
Manual threshold set?
Make Binary uses
that dumb threshold!

Otsu Threshold

Global - Objective - Reproducible





ImageJ -
Plugins-Segmentation-OtsuThreshold8bit

Another pixel intensity histogram method:

Search for the threshold that minimizes the background and foreground variances, σ^2 (square of S.D.)

Same as maximising variance between background and foreground

http://en.wikipedia.org/wiki/Otsu's_method

Also see “K-means Clustering”, “Maximum Entropy”, “Mixture Modelling” and others.

Segmentation - Practical Exercises.

1) Dumb threshold, example of easy case histogram - bimodal.

Sample data : AuPbSn40.jpg

1) Analyze-Histogram,
2) Image-Adjust-Threshold (set it then Apply),
3) Analyze-Analyze Particles (select options-show outlines)
   try with different thresholds.

Segmentation - Practical Exercises.

2) Computed / Optimised global threshold

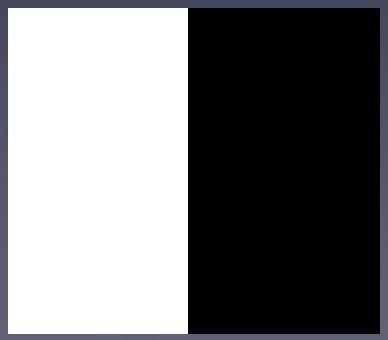
a) Tnew = 1/2 (mean of foreground + mean of background)
Make Binary and Auto threshold use this method:
blobs2.gif
process-binary-make binary, analyse particles

b) Otsu threshold minimise intra class
blobs2.gif
Plugins - segmentation - Otsu Thresholding 8 bit
process-binary-make binary, analyse particles

Edge Detection

What is an “edge” ?

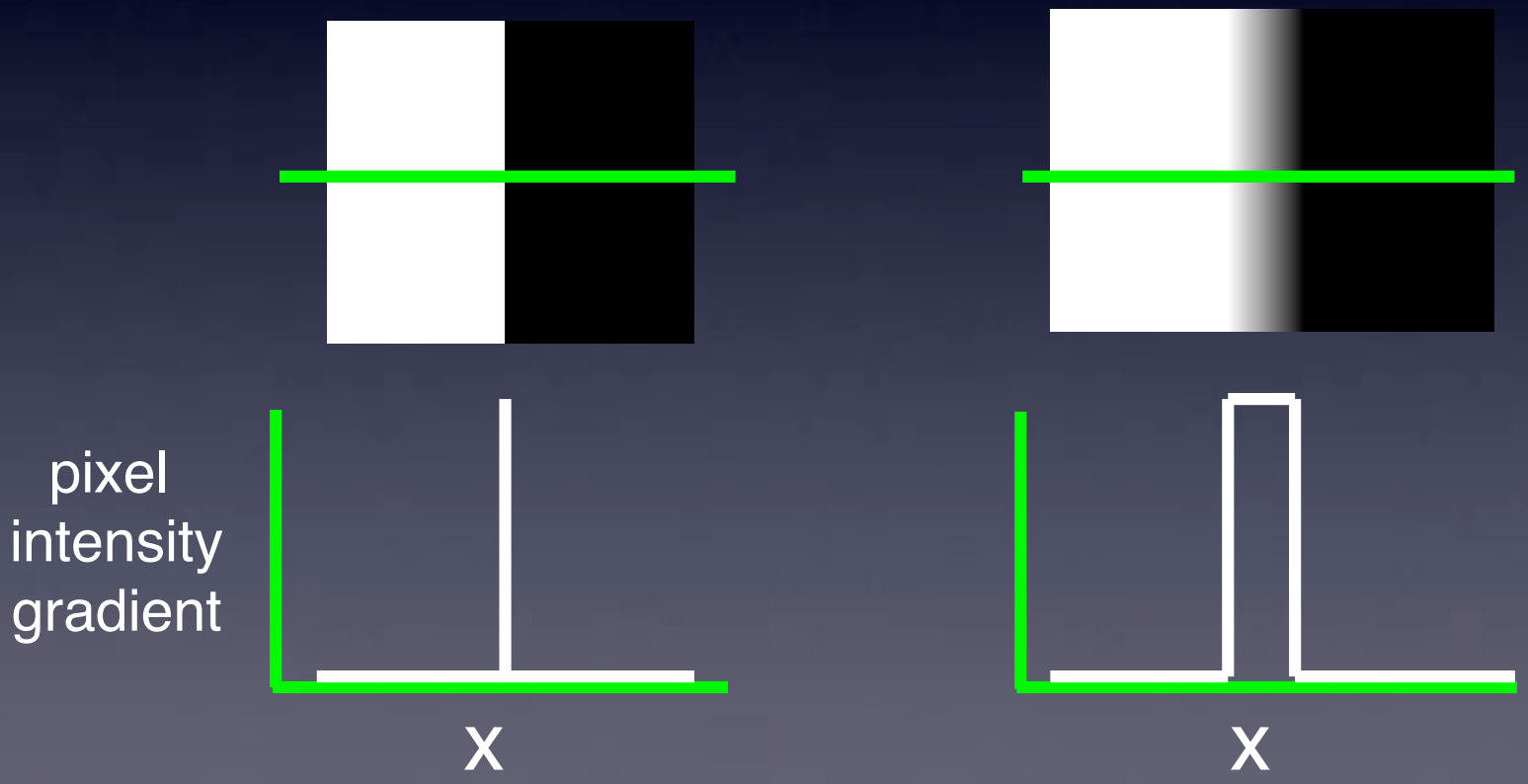
- “Hard Edge” - Adjacent black - white pixels
- “Soft / Fuzzy Edge” - common in images
  - Especially for small diffraction limited objects (vesicles / membranes)
  - Noise makes edges look softer



Edge Detection
”Image Gradient”

What is a “Gradient Image” ?

Rate of change of pixel intensity (1st derivative)



Edge Detection
”Image Gradient”

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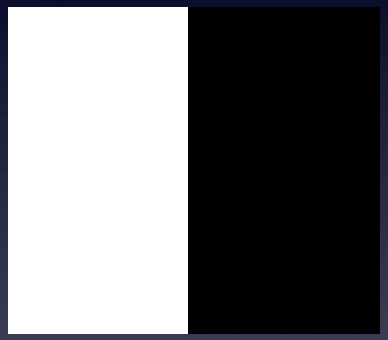
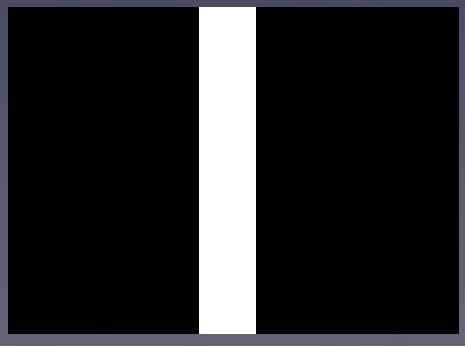


Image

hard edge

soft edge

Gradient Image



”Image Gradient” - How?

Sobel filter - 3x3 convolution filters in x and y

- find edges with x and y components
- compute total gradient magnitude
- approximates 1st derivative of image

-1	0	+1
-2	0	+2
-1	0	+1

+1	+2	+1
0	0	0
-1	-2	-1

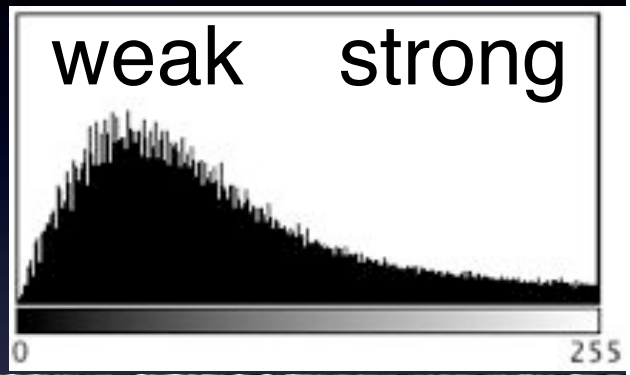
$$|\,G_x\,| \quad + \quad |\,G_y\,| \quad = \quad |\,G\,|$$

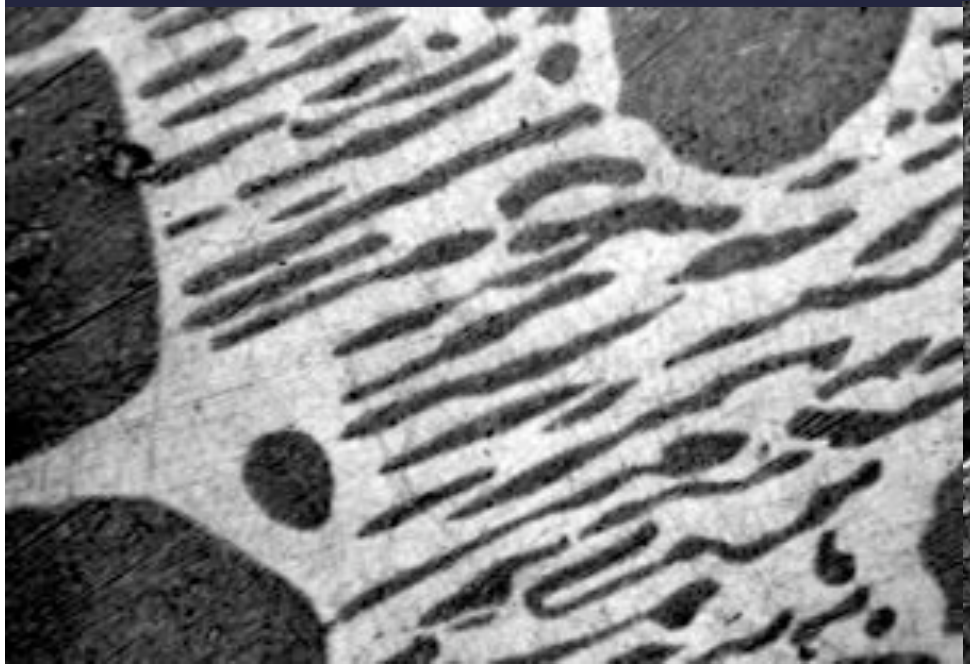
Gradient Image - Real Sample:

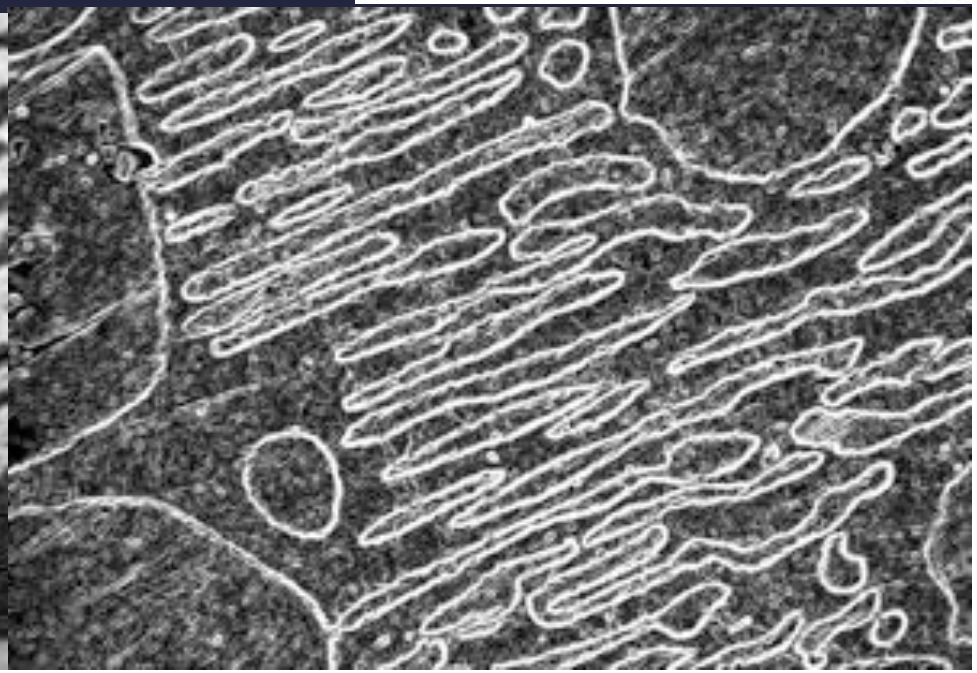
Real / Biological images:

- Sobel filter
- many edges
- many weak edges from noise

gradient image histogram



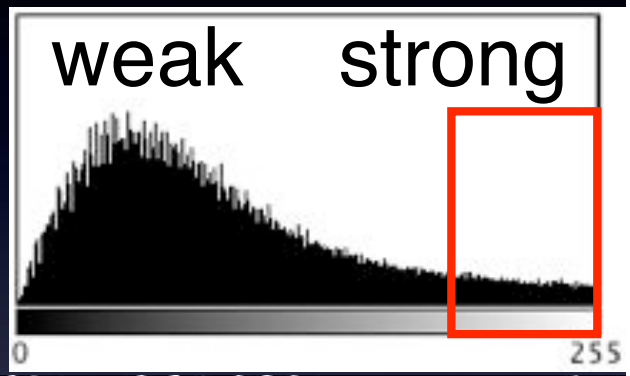


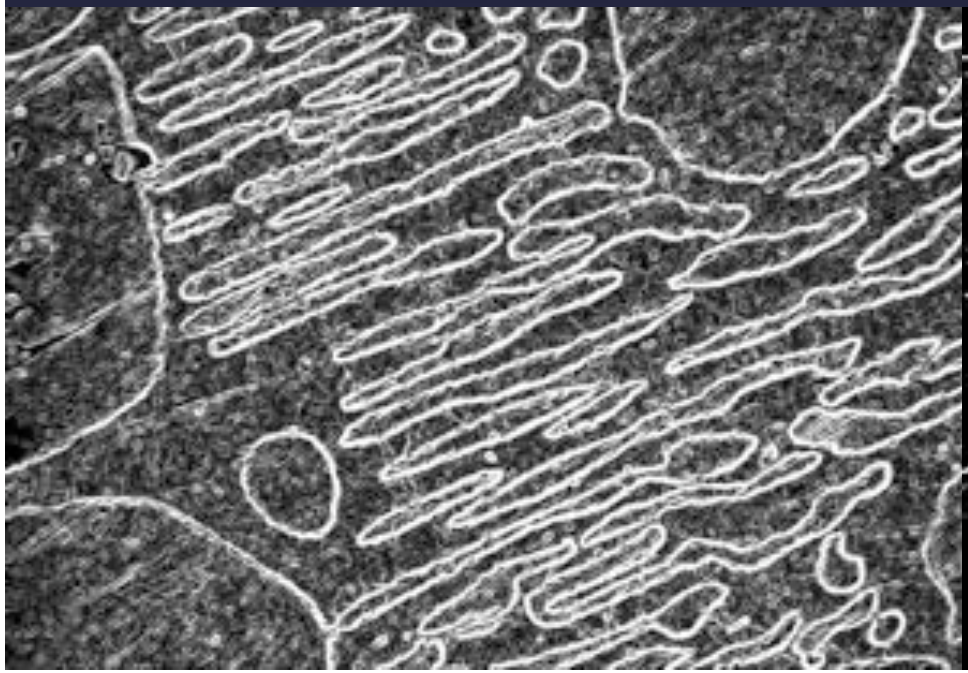


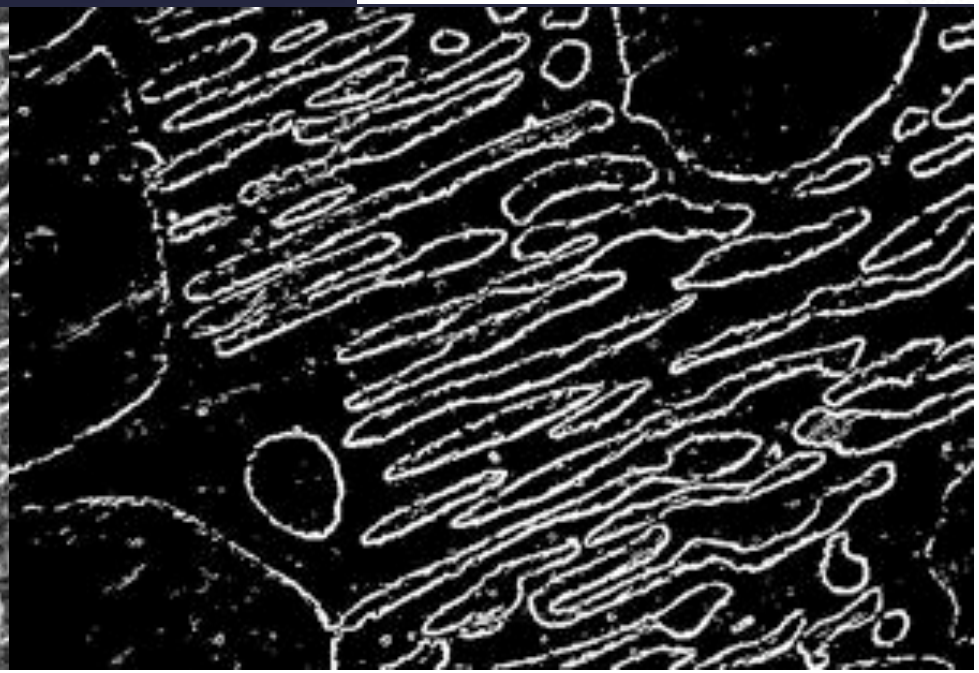
Gradient Image - Strong Edges?

Remove weak edges?

- Threshold the gradient image
- Smoothing filter beforehand

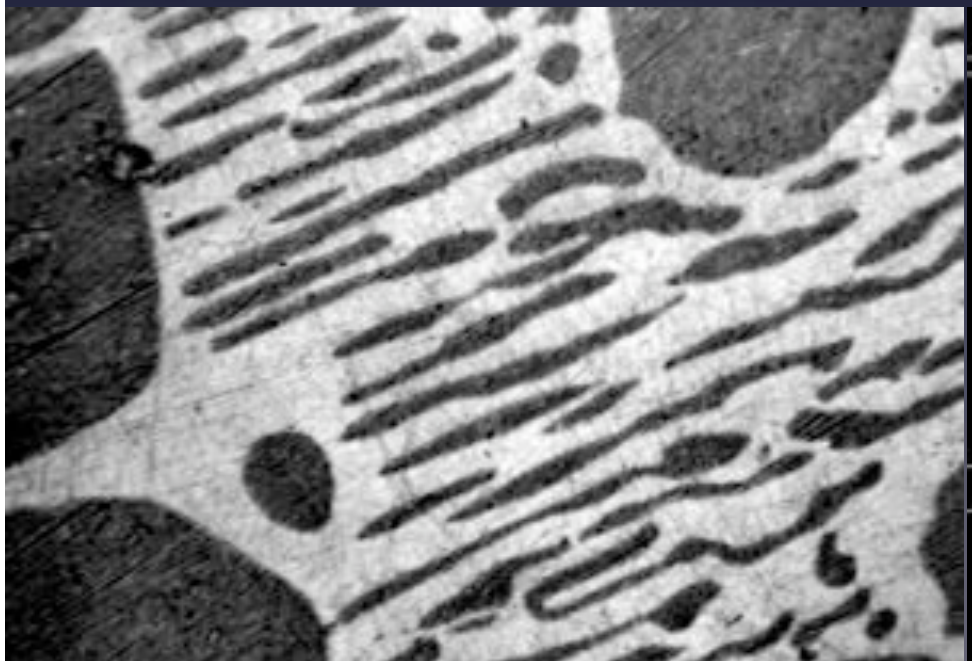


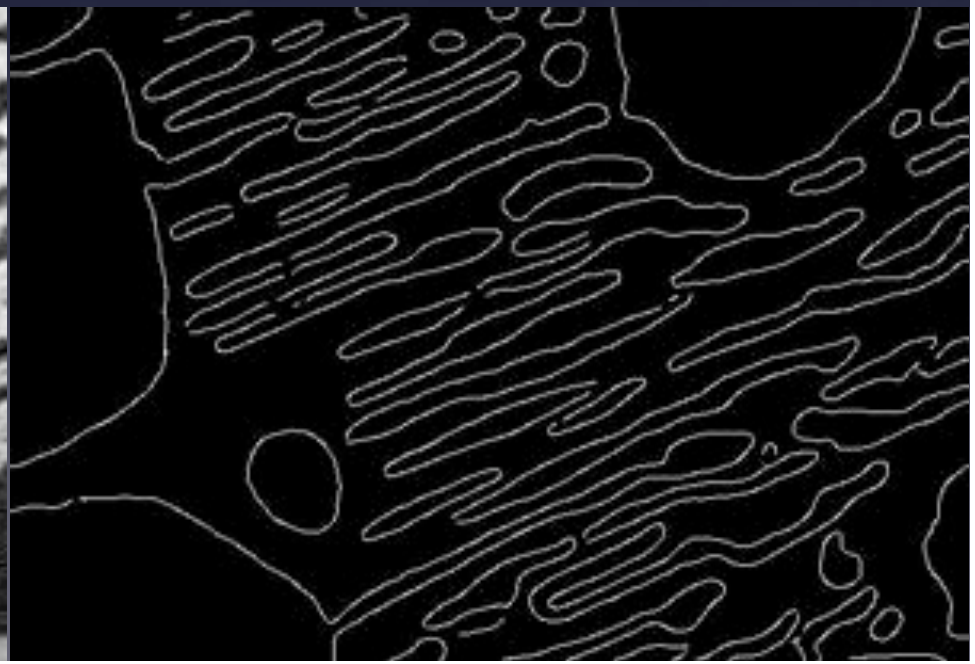




“Canny” Edge Detection

- Remove weak/noisy edges - keep strong
  - Gaussian smooth image + hysteresis threshold gradient image
- Make edges sharp - 1 pixel wide
  - Non maximal suppression of gradient image

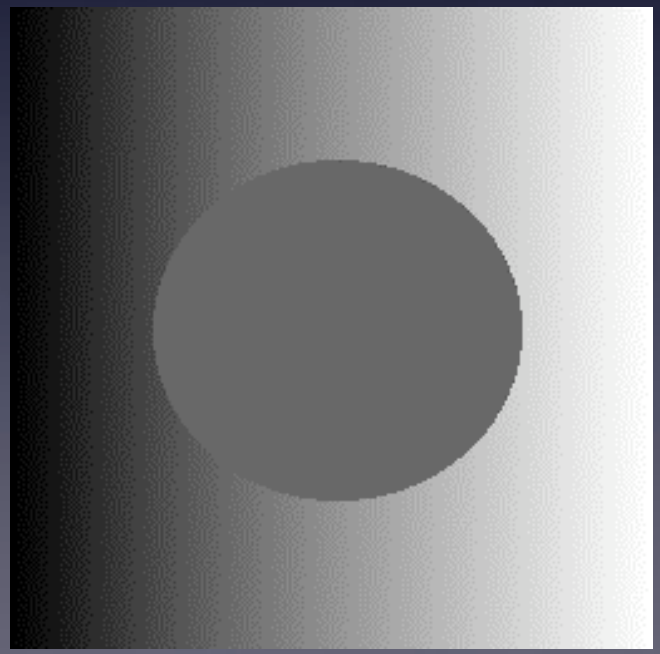


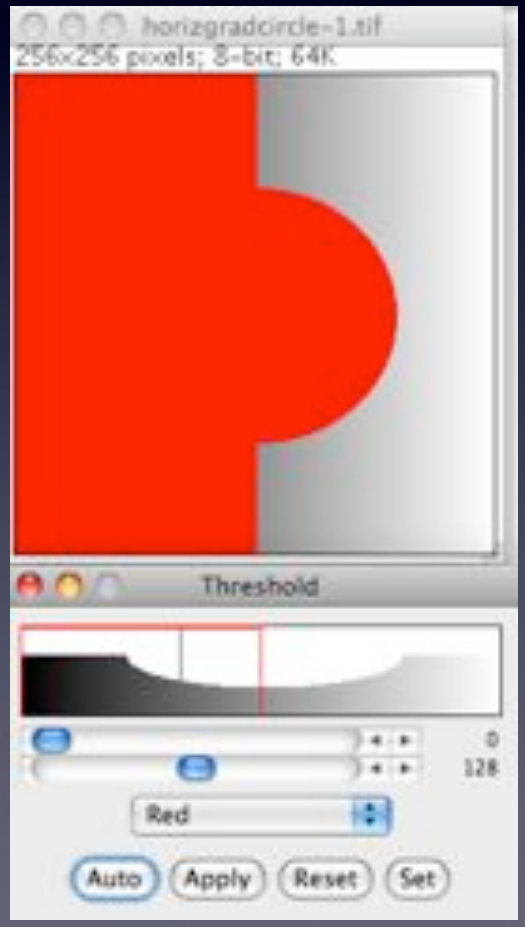


Intelligent Thresholding using Local Adaptive Thresholds

- The problem:
  - Non flat illumination or background
  - Dumb threshold or computed global threshold…

  Doesn't Work!

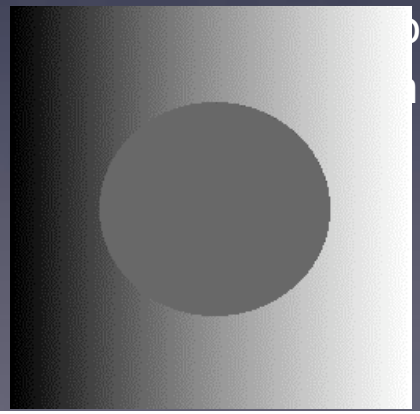




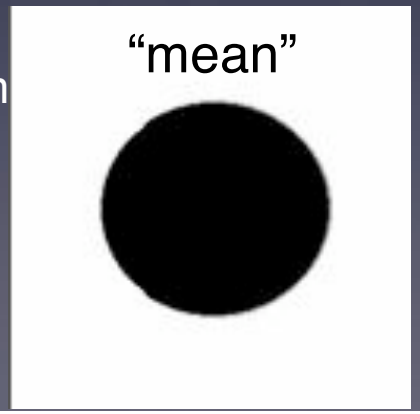
Intelligent Thresholding using Local Adaptive Thresholds

- The Answer : “Adaptive / Dynamic Threshold”
  - Local Thresholding
    - looks at small parts of image
    - determine threshold in that small region
      - http://homepages.inf.ed.ac.uk/rbf/HIPR2/adpthrsh.htm

1. Convolve image with statistical operator: *mean* or *median*.
2. Subtract original from convolved image.

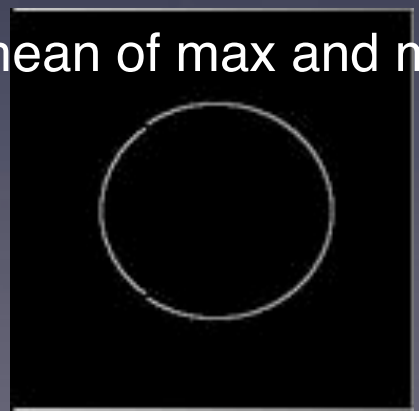


old difference
resholded im



“mean”

“mean of max and min”

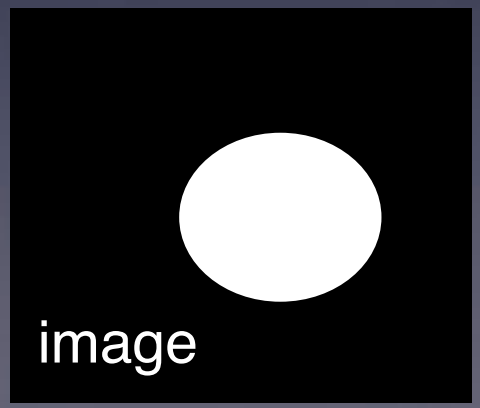


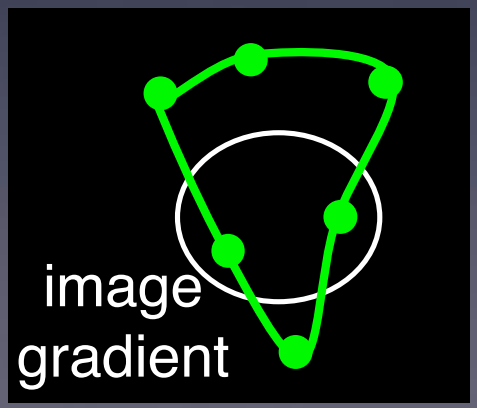
Active Contours - “Snakes”

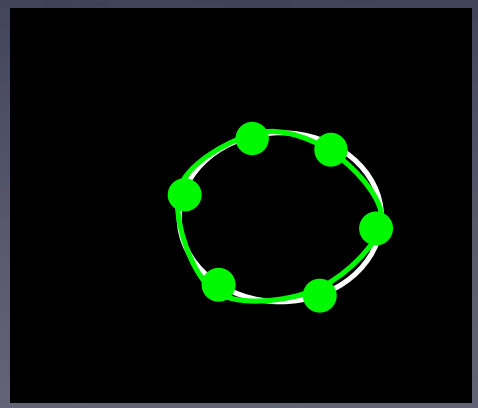
- Physical model for finding edges.
  - Image gradient = “energy landscape”
  - Starting shape = points joined by a closed line
  - Let the points move to lowest energy

Active Contours - “Snakes”

- Physical model for finding edges.
  - Image gradient = “energy landscape”
  - Starting shape = points joined by a closed line
  - Let the points move to lowest energy
    - but add high energy terms for
      - high bending / curvature
      - stretching (distance between points)
        - prevents messy edges and explosion







Segmentation - Practical Exercises.

3) Edge Detection

a) Sobel filter approximates 1st derivative
blobs2.gif
Process - find edges

b) Canny edge detection
AuPbSn40.jpg
Plugins - FeatureJ - FJ edges
smoothing scale 3, lower threshold 10

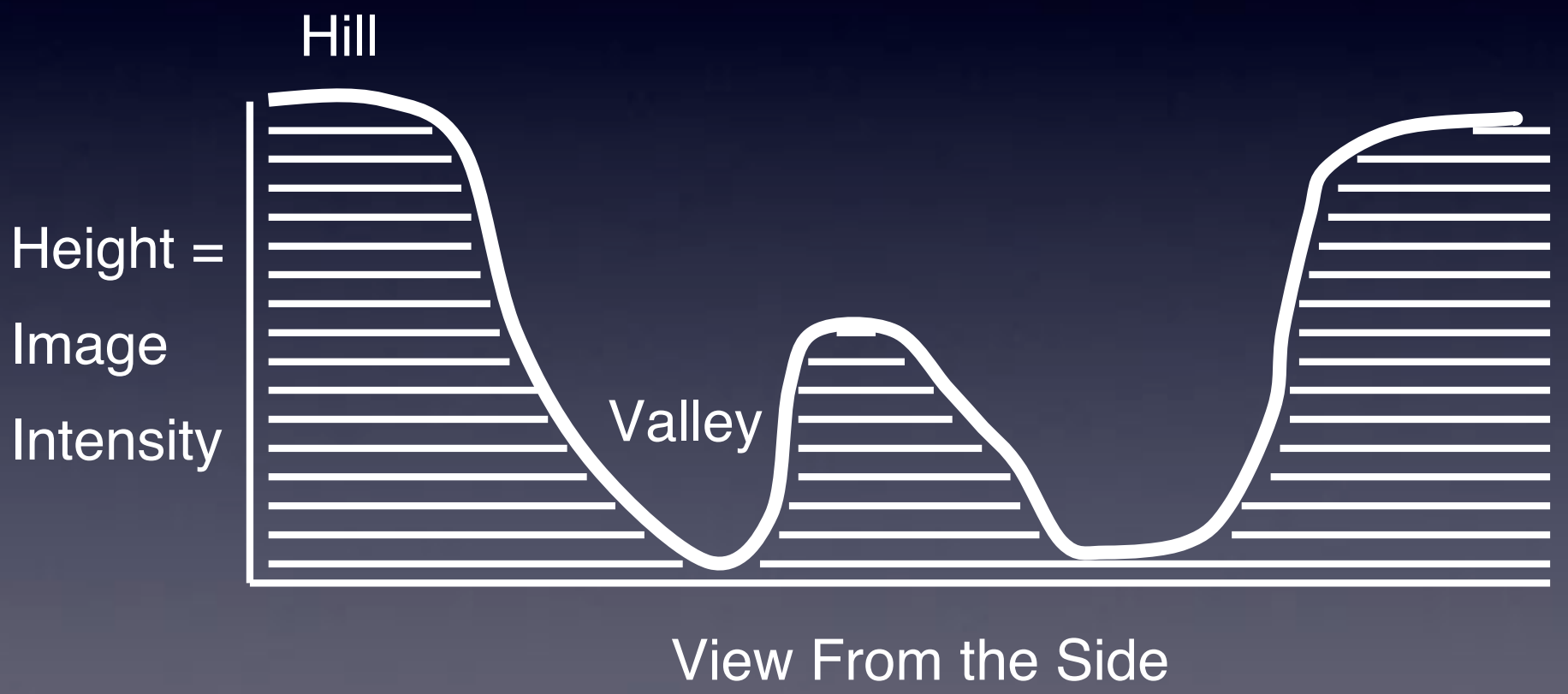
Segmentation - Practical Exercises.

4) Dynamic / Adaptive / Local thresholding

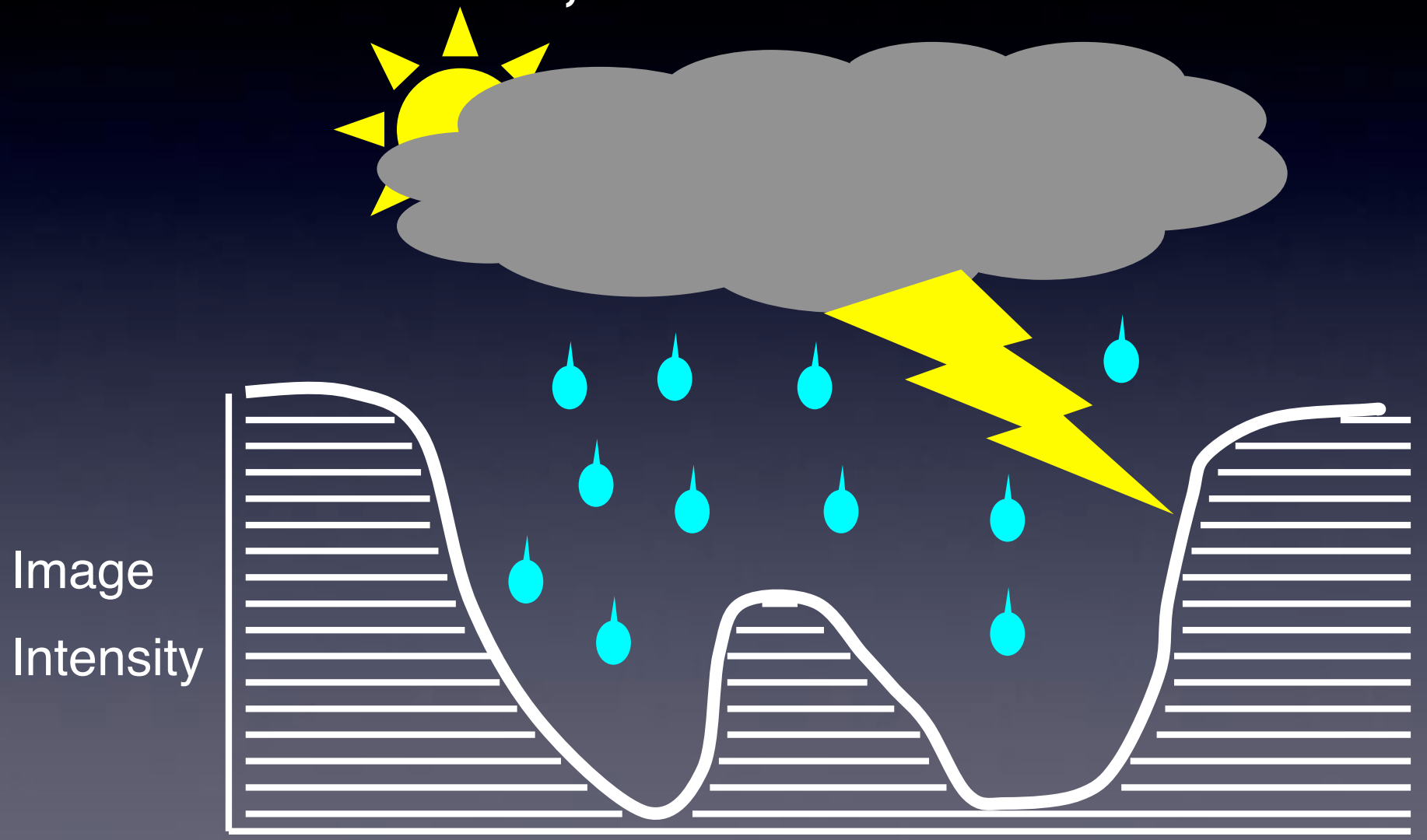
a) dynamic threshold
horizgradcircle.tif
Plugins - dynamic threshold 1b
tick all, mask size 3

b) Active Contours (snakes)
blobs2.gif and blue channel of fluorescent cells
Plugins - ABSnake

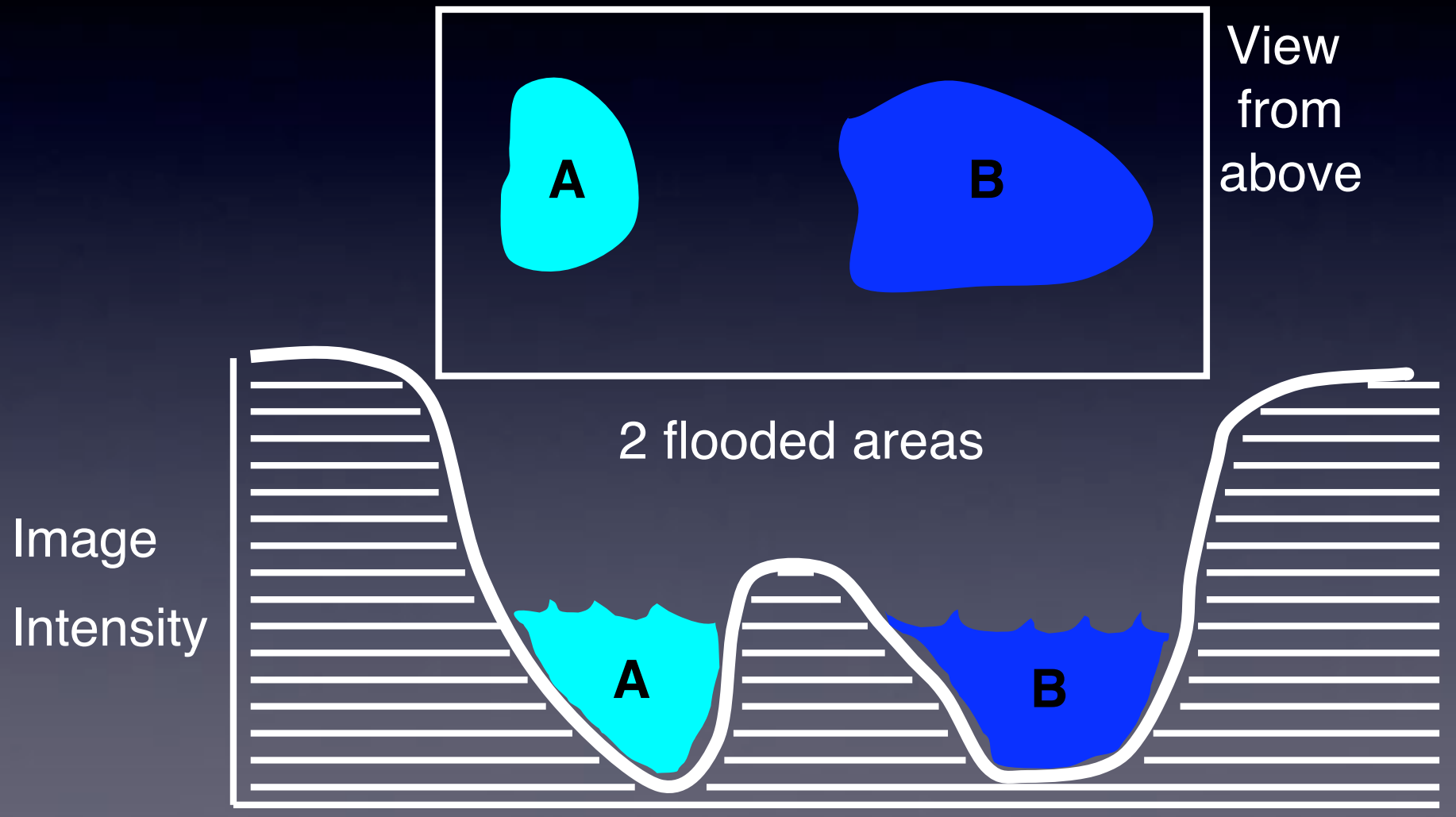
Watershed Algorithm: mountains, lakes and oceans



Watershed Algorithm: mountains, lakes and oceans



Watershed Algorithm: mountains, lakes and oceans

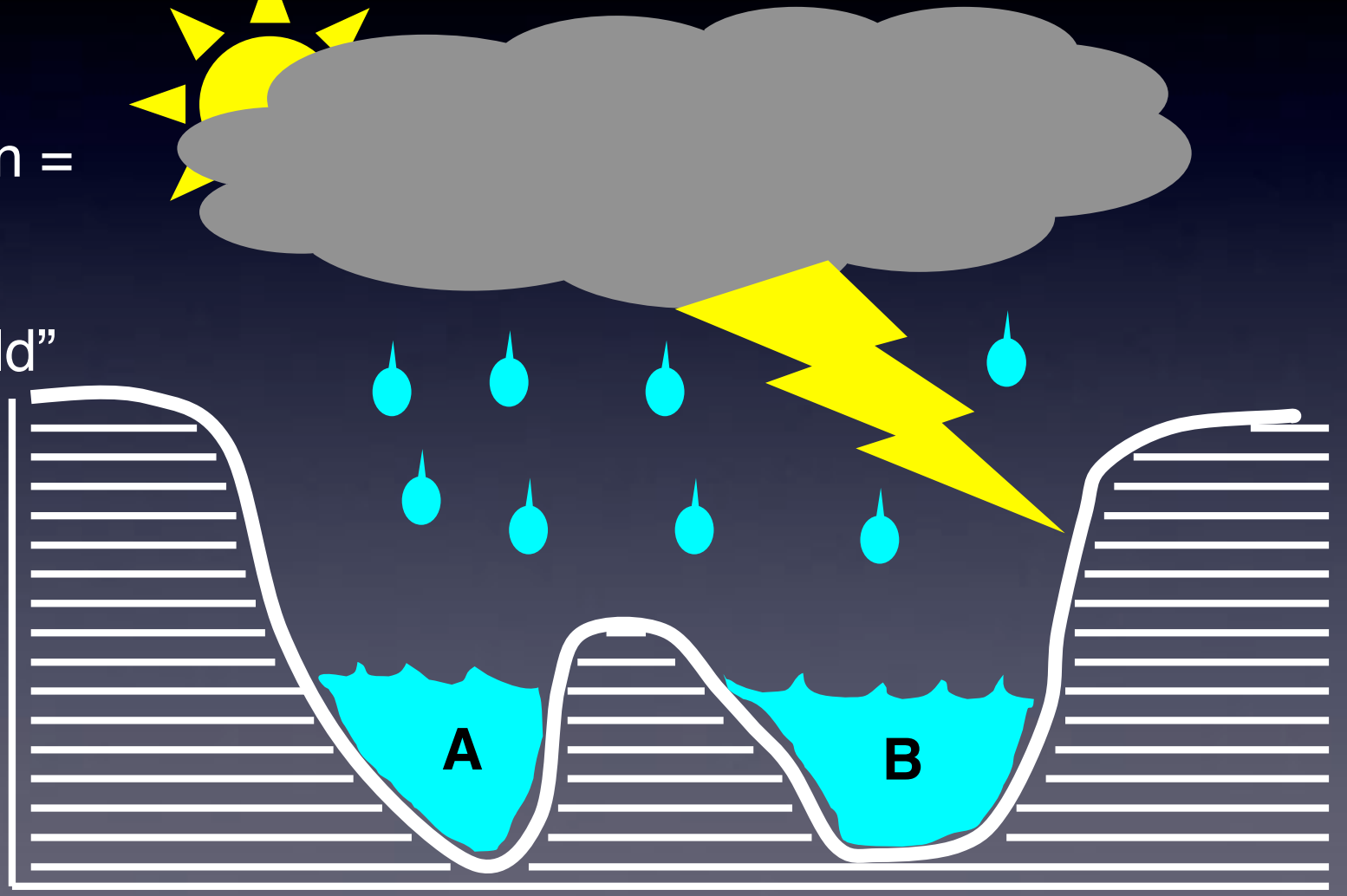


Watershed Algorithm: mountains, lakes and oceans

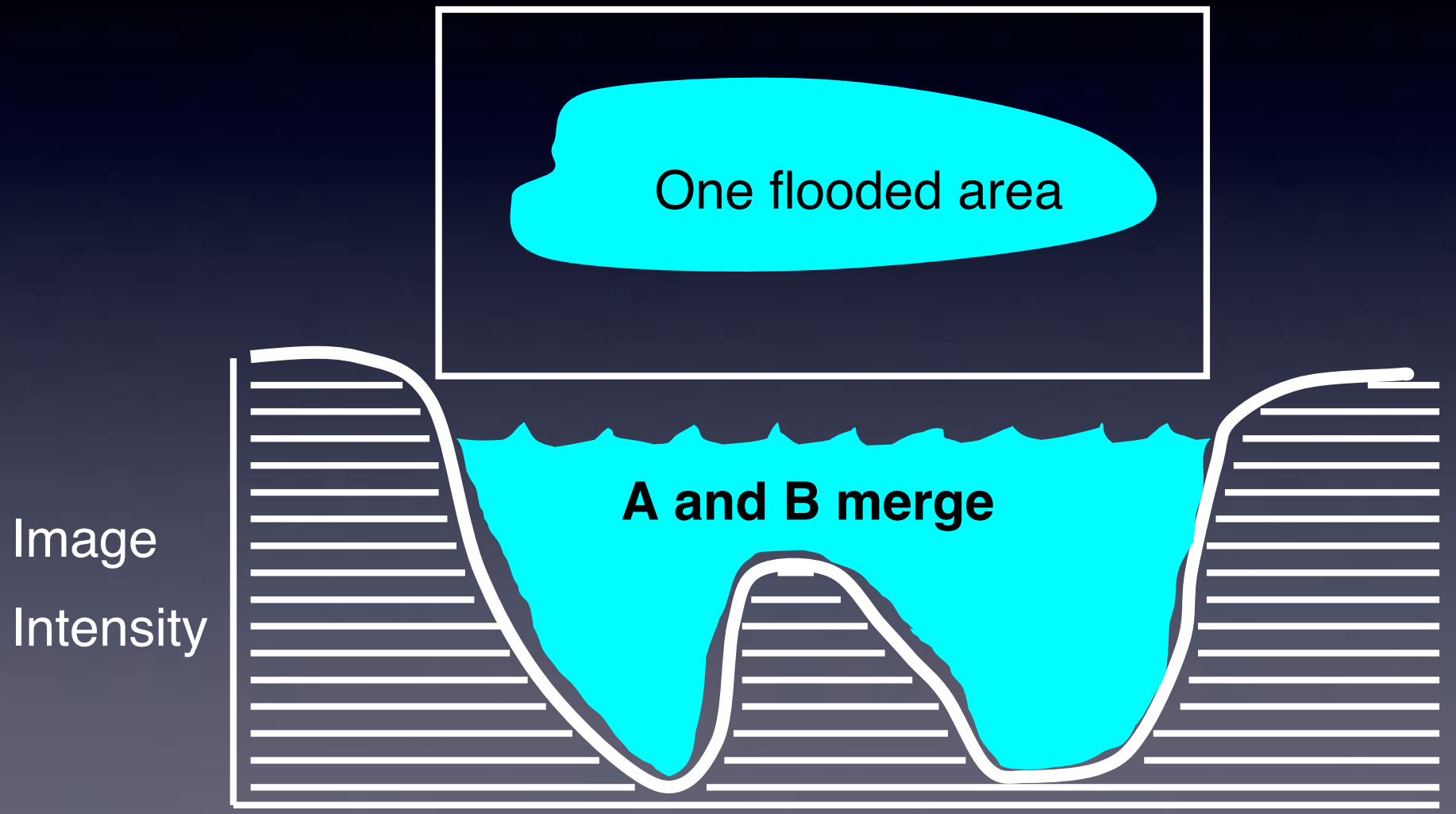
More rain =

increase

“threshold”

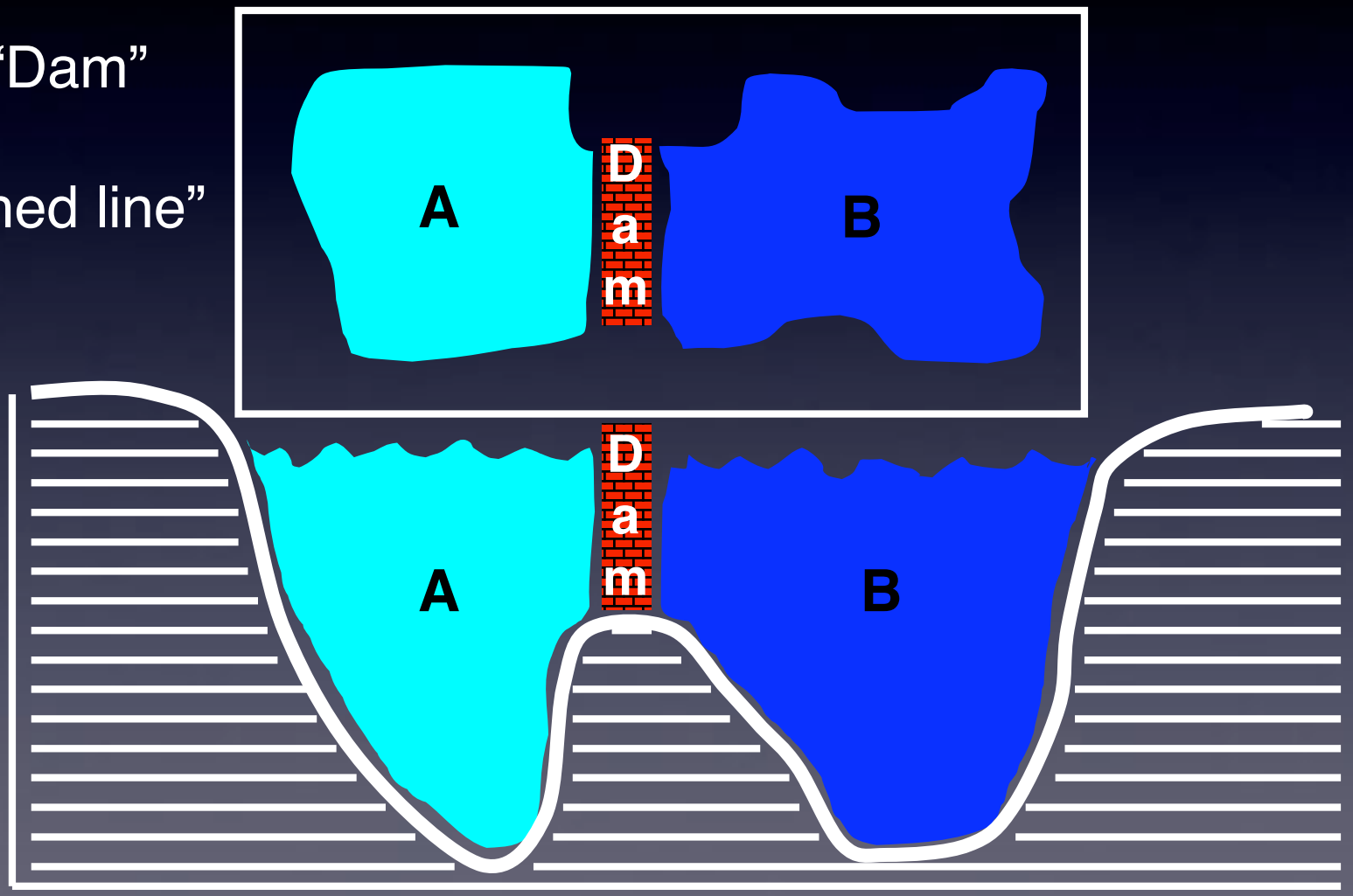


Watershed Algorithm: mountains, lakes and oceans



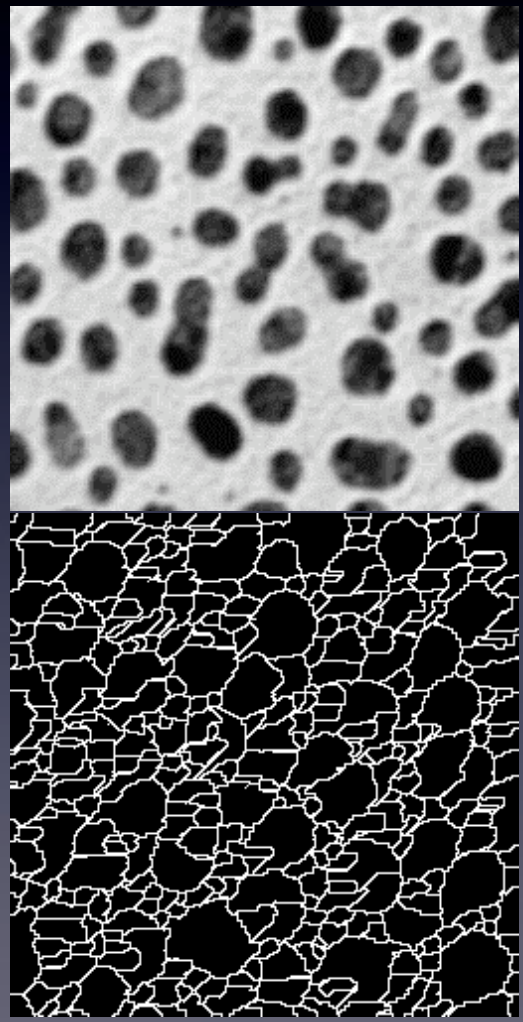
Watershed Algorithm: mountains, lakes and oceans

Make a “Dam” at the “Watershed line”



Watershed on a “biological” image:

- Watershed of blobs2.gif
- Over segmented
  - Why?
  - image is grey scale
  - many ‘low hills”

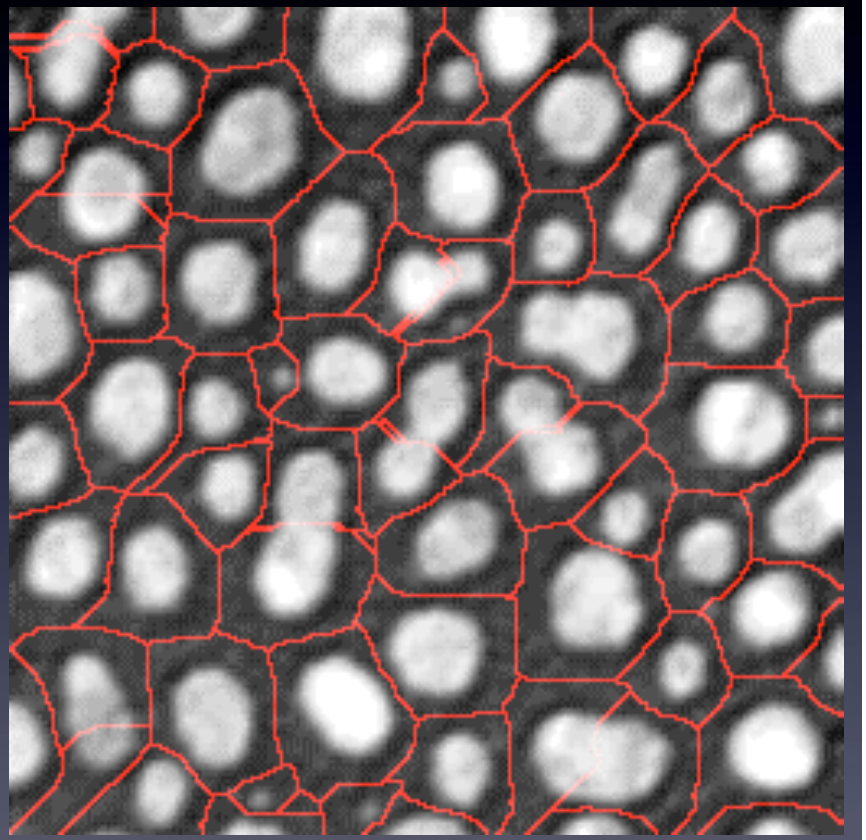


Watershed to find object number

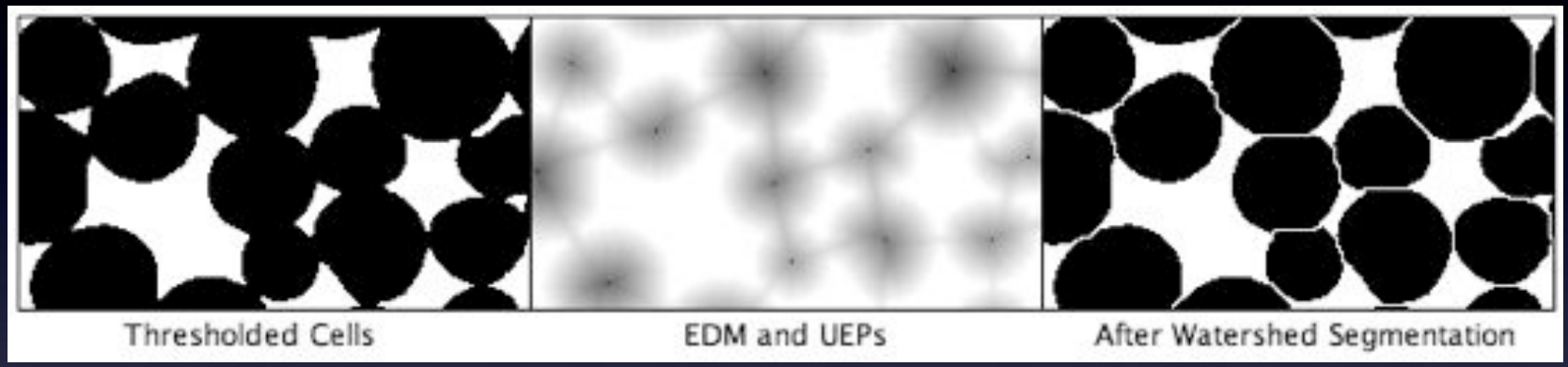
- Blobs2.gif
- Make Binary
- Euclidian Distance Map
- Invert EDM
- Watershed

- Gives number of objects!

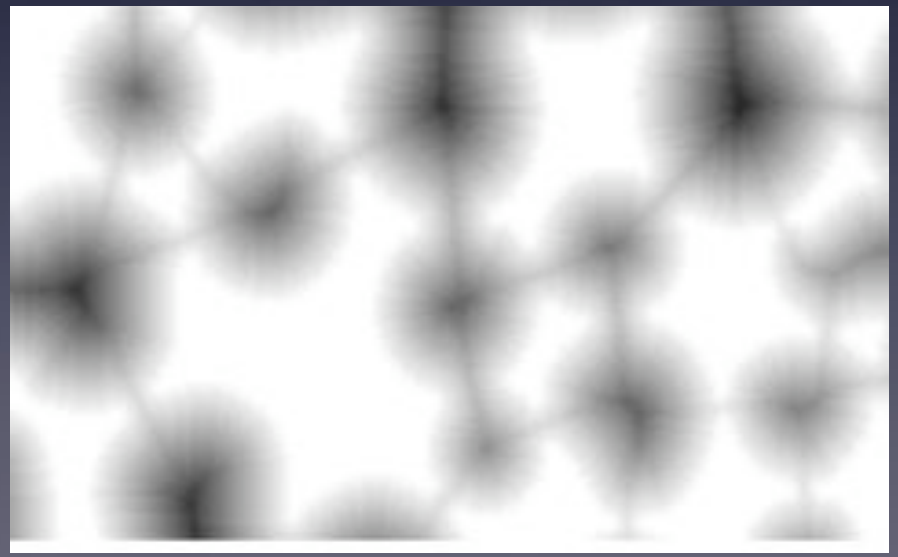
(imagine there were too many to count by hand, eg 2D Gel)



Watershed to separate touching objects



- Euclidian Distance Map
- Ultimate Eroded Points
- Fill with water from UEP
  - until hits edge of object, or dams between objects



Segmentation - Practical Exercises.

5) Watershed

a) Watershed of raw biological image
blobs2.gif
plugins-filters-watershed algorithm
note over segmentation because image is grey scale not binary

b) Manual Binary-EDM-Watershed
blobs2.gif
process-binary-make binary, process-binary-distance map, edit-invert, plugins-filters-watershed algorithm

c) Automatic ImageJ watershed
Whiteoverlaps.tif
(Edit - Invert), Process - binary -watershed, (Edit - Invert), Analyse-Analyse particles (outlines)

Links and Further Reading

- Standard Text Book

  Digital Image Processing 2nd Ed.
  Gonzalez and Woods, Prentice Hall

- Image Processing Facility
  - Intranet - Services and Facilities - Image Processing Facility
  - Wiki - info for beginners - tips - software documentation
  - https://zope.mpi-cbg.de/intranet/services/image-processing-facility

- ImageJ
  - http://rsb.info.nih.gov/ij/
  - MacBioPhotonics plugins collection
    - http://www.macbiophotonics.ca/downloads.htm

- Email: ipf@mpi-cbg.de