MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH
BIMSB THE BERLIN INSTITUTE FOR MEDICAL SYSTEMS BIOLOGY

Biological Image Reconstruction and Analysis
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Introduction to Image Segmentation using ImageJ/Fiji

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DIPC Donostia International Physics Center

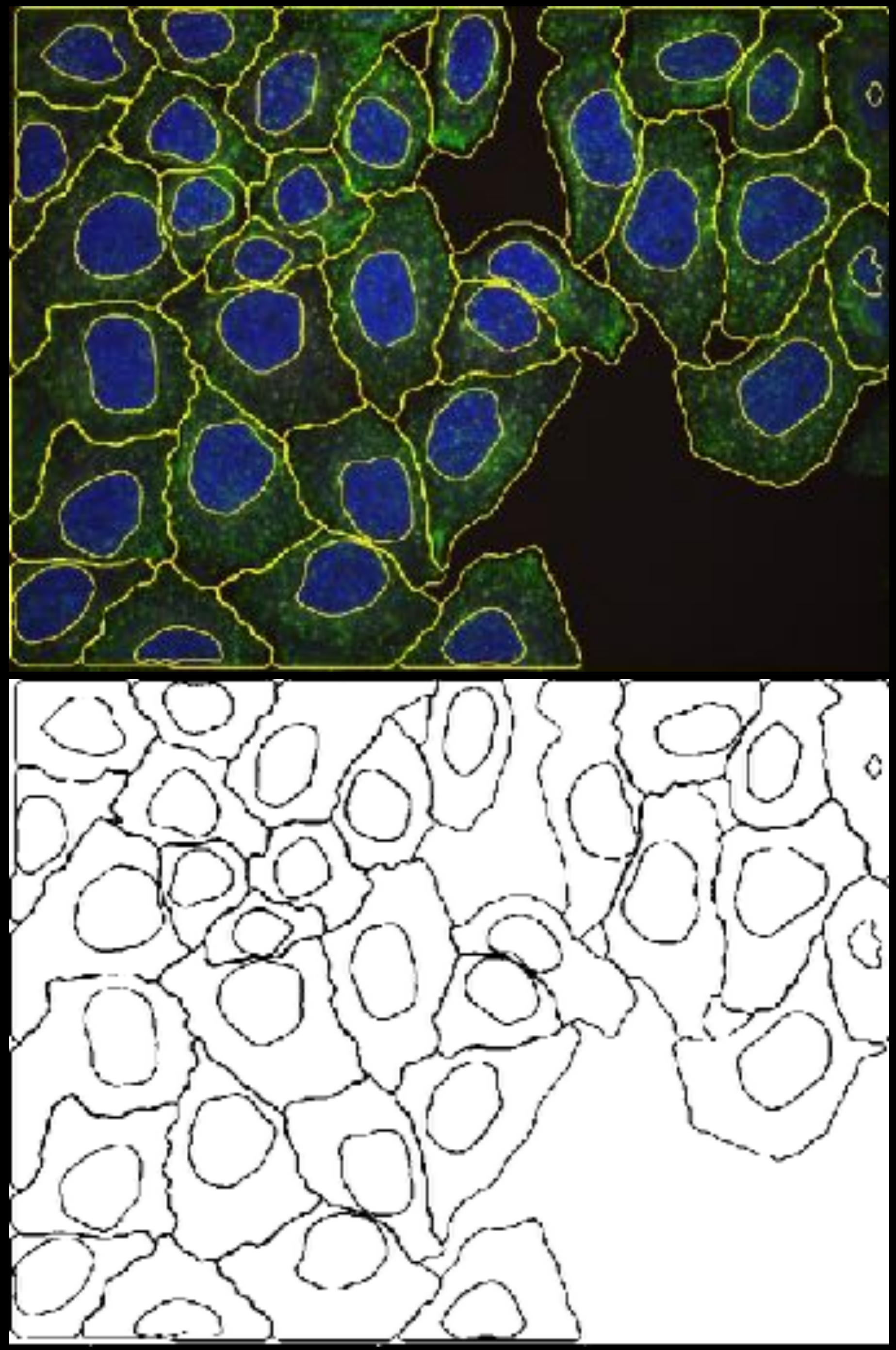
Universidad del País Vasco Euskal Herriko Unibertsitatea

Outline

- Introduction to the problem.
- Solutions:
    - Thresholding.
    - Region growing.
    - Clustering.
    - Morphological segmentation.
    - Trainable segmentation.
- Hands-on tutorial.

Image Segmentation

- “Process of partitioning a digital image into multiple segments”.
- Typically used to locate objects and boundaries.



http://imagej.net/Segmentation

Image Segmentation

- "Process of partitioning a digital image into multiple segments".
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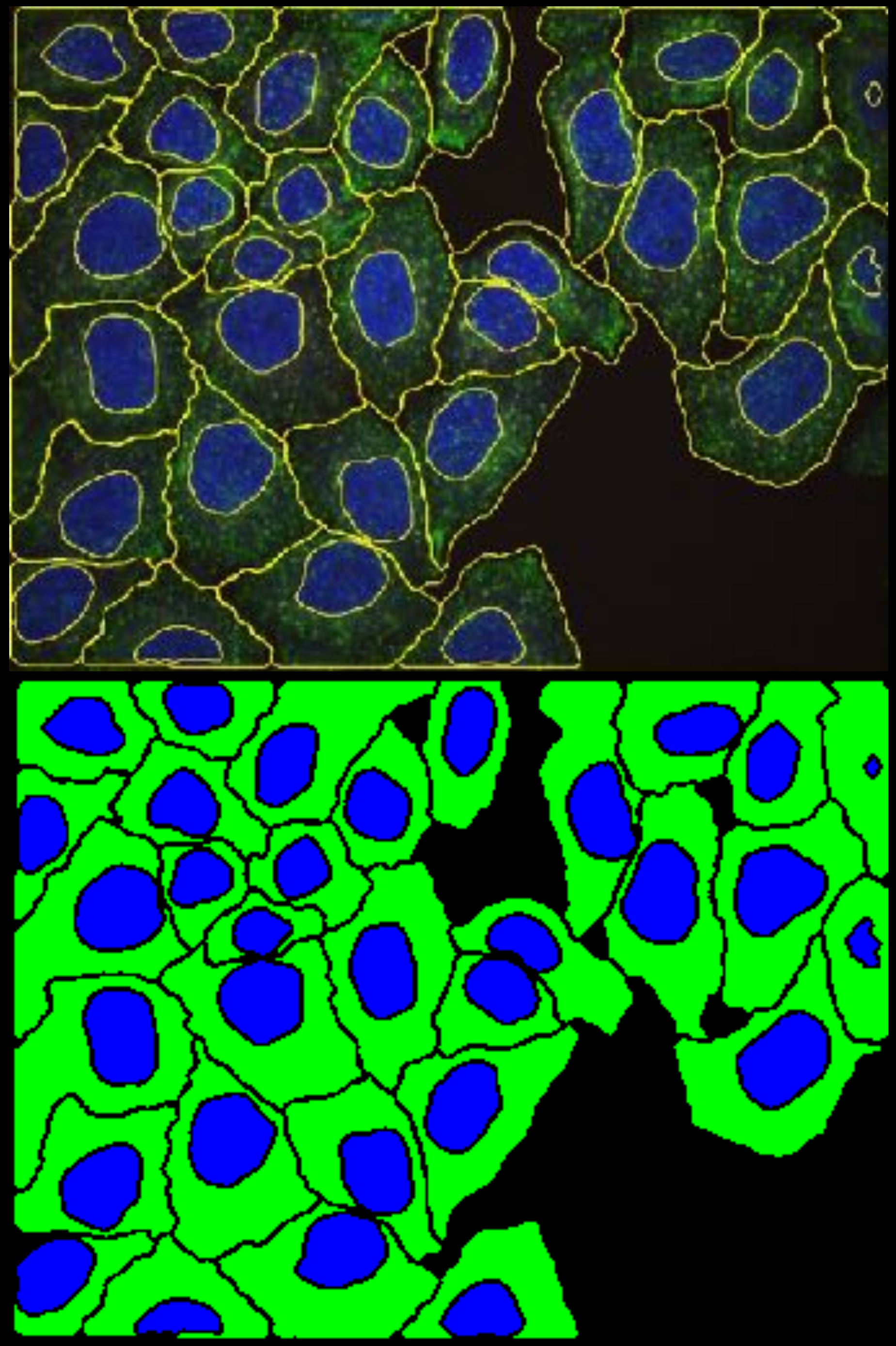
- More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics.



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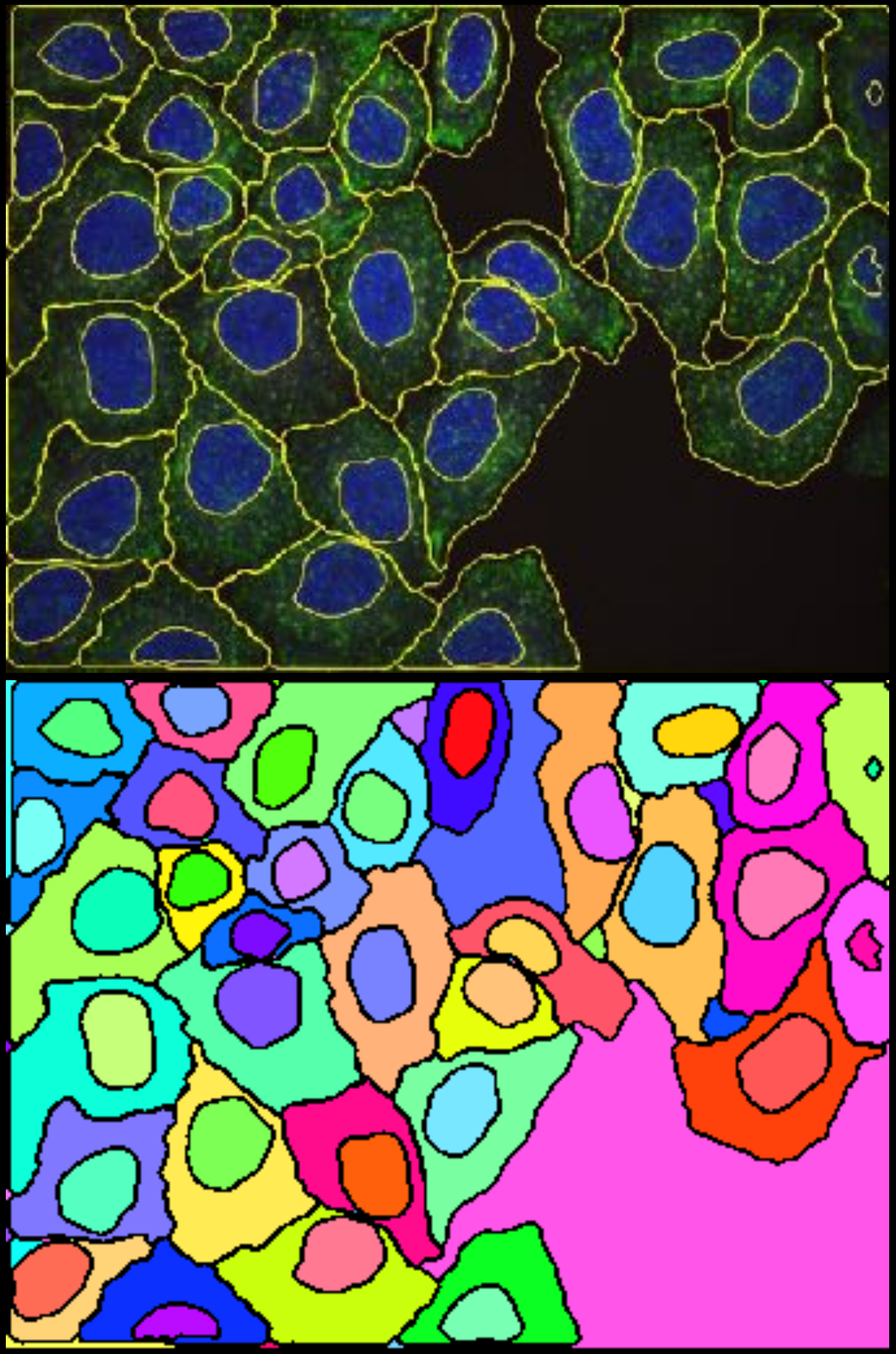

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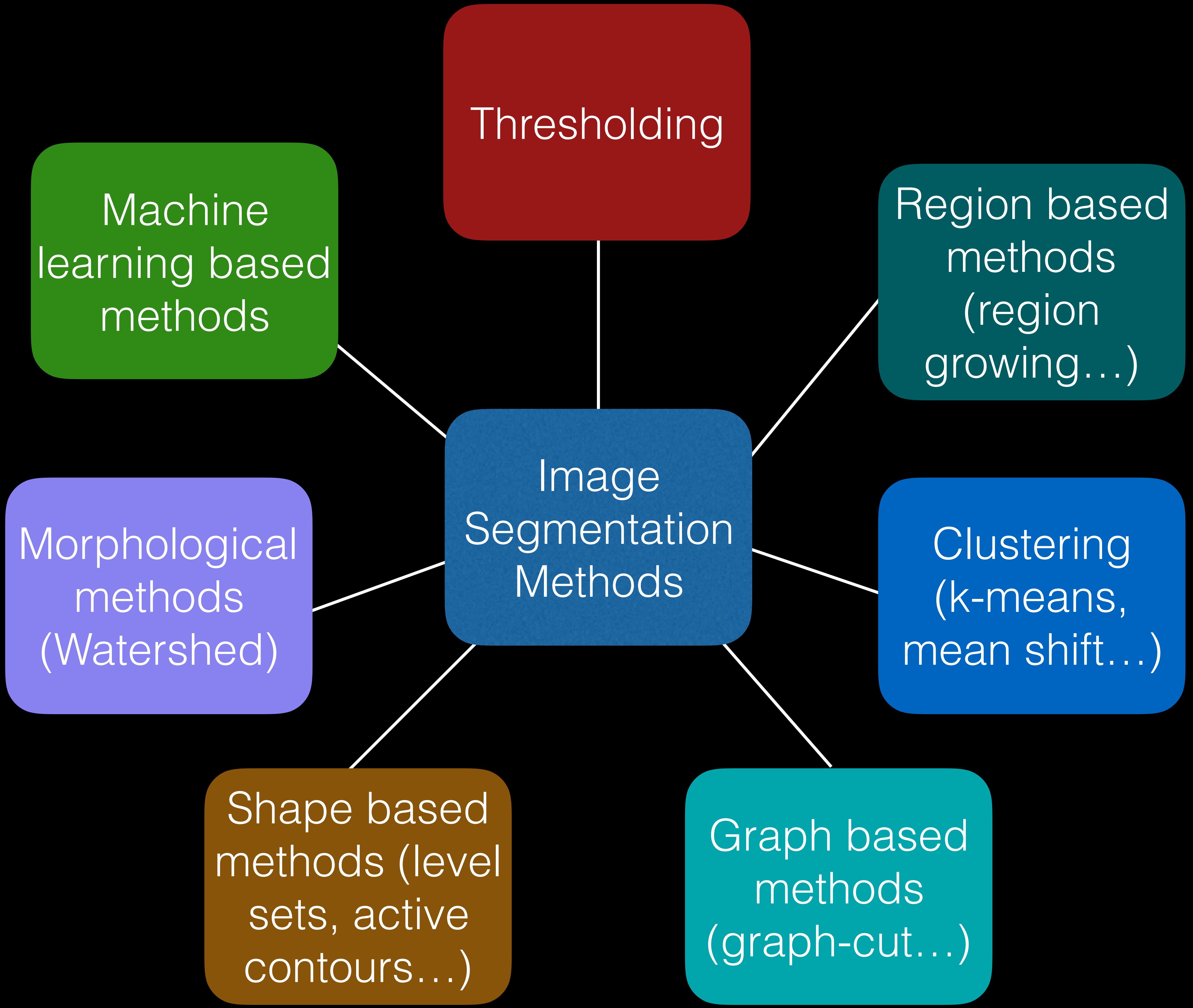
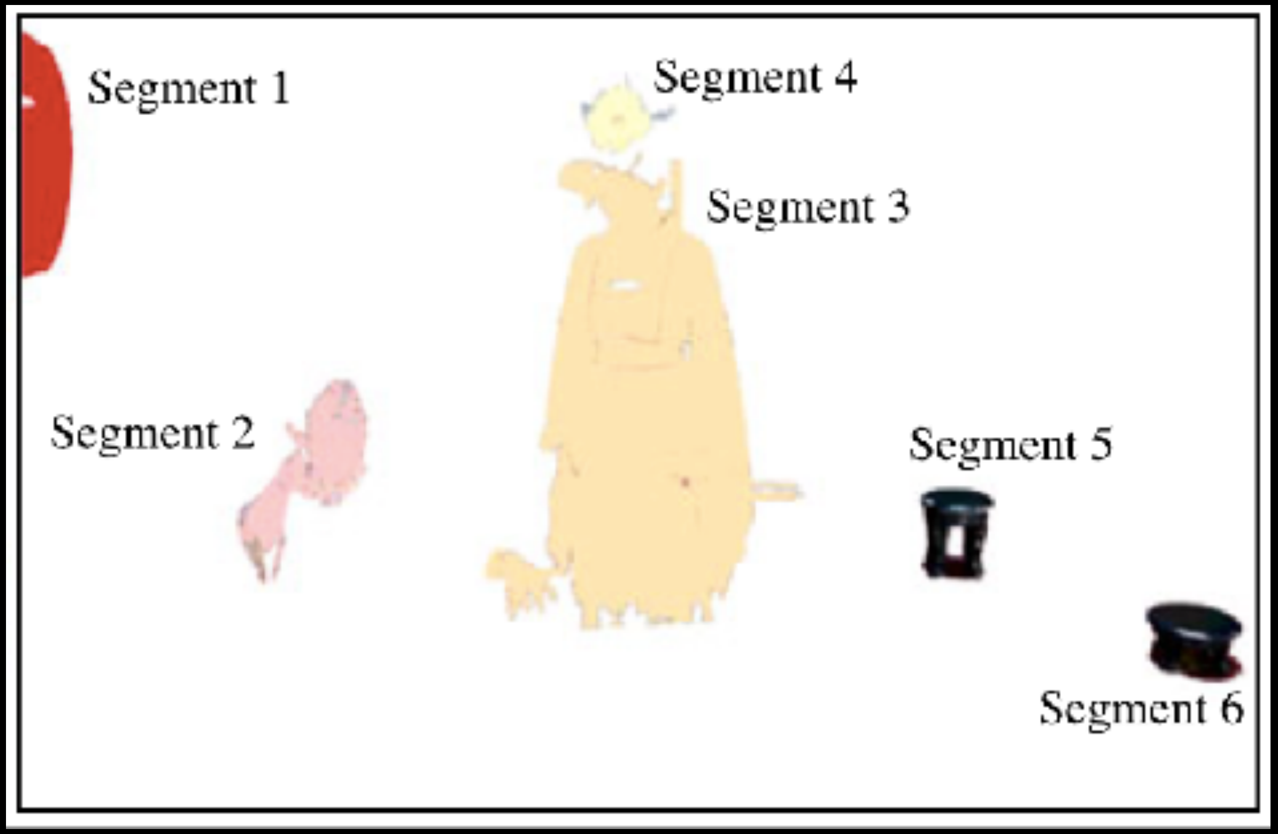


http://imagej.net/Category:Segmentation

Basics of Image Segmentation

- Definition: Image segmentation partitions an image into regions called segments.



- Image segmentation creates segments of connected pixels by analyzing some similarity criteria:
    - *intensity, color, texture, histogram, features…*

Image binarization

- Image binarization applies often just one global threshold p for mapping a scalar image I into a binary image

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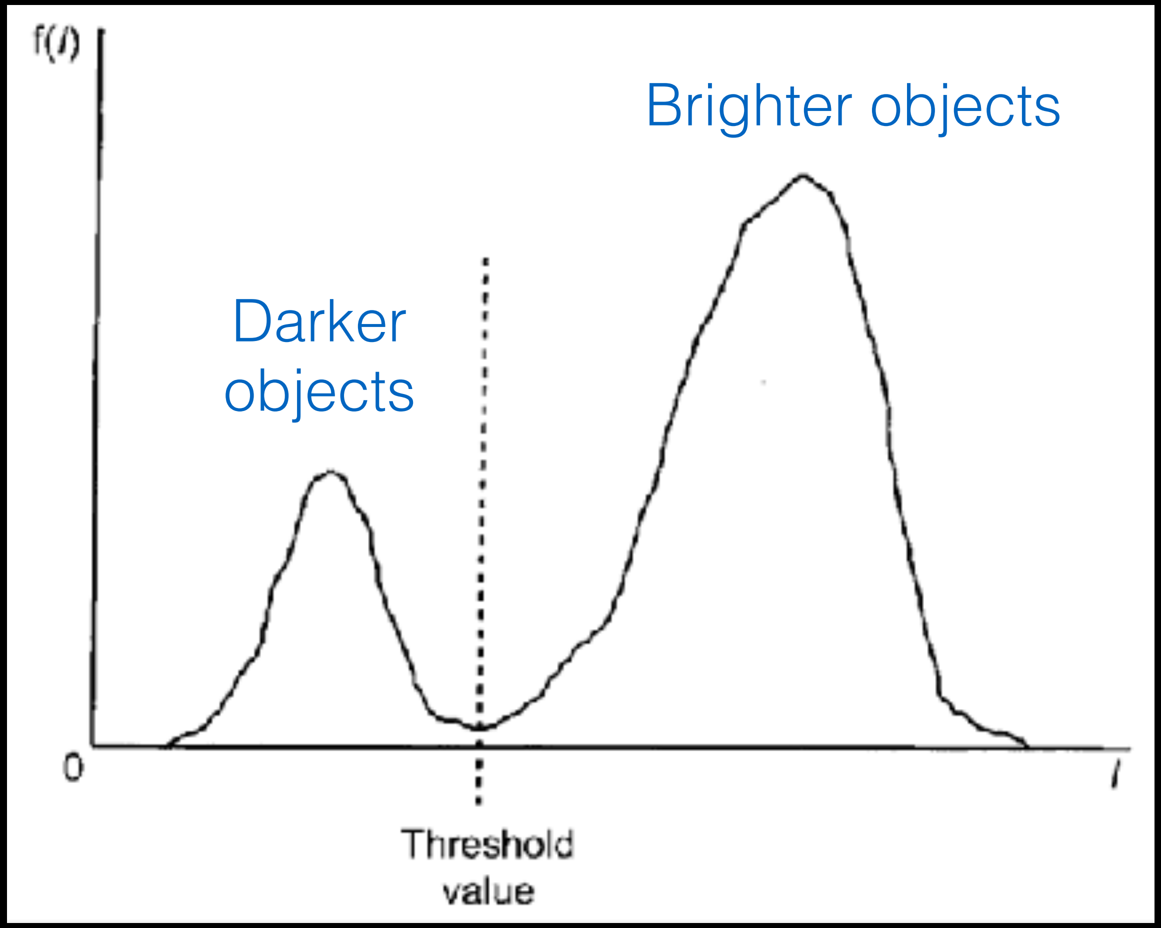
$$O(i,j) = \begin{cases} 0 & if \quad I(i,j) \leq p, \\ 255 & if \quad I(i,j) > p \end{cases}$$

- The global threshold can be identified by an optimization strategy aiming at creating “large” connected regions and at reducing the number of small-sized regions, called *artifacts*.

Image binarization

- Thresholding: Most frequently employed method for determining threshold is based on histogram analysis of intensity levels.



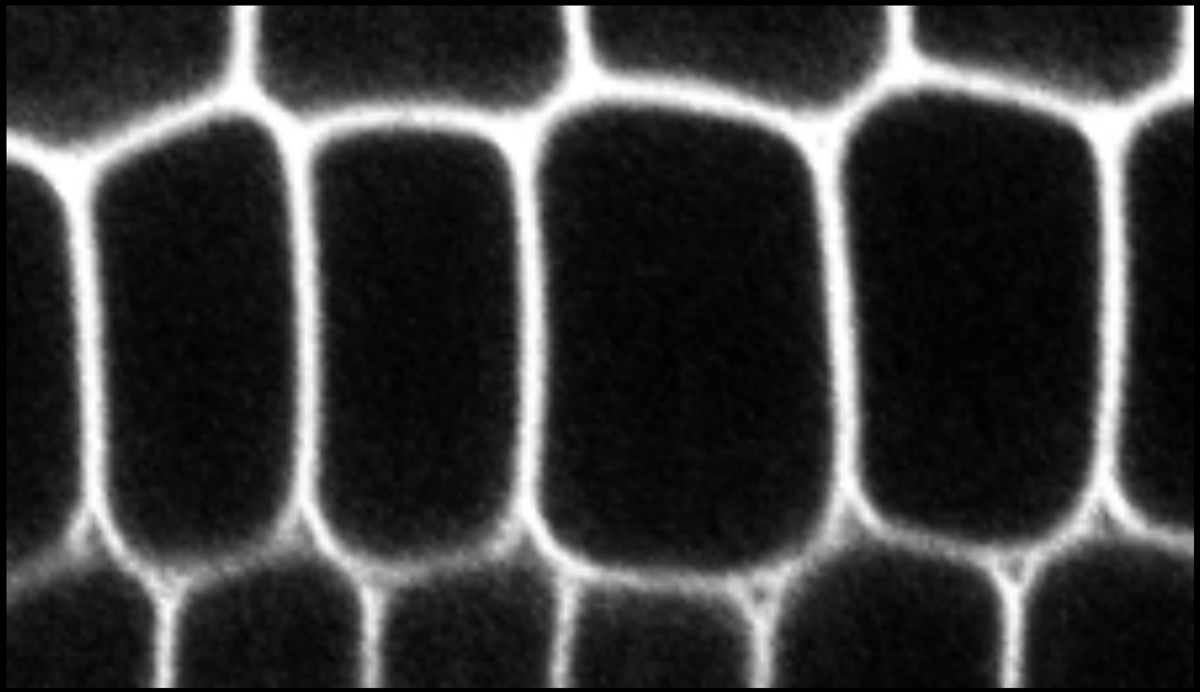
Peak on the left of the histogram corresponds to dark objects

Peak on the right of the histogram corresponds to brighter objects

DIFFICULTIES:

1. The valley may be so broad that it is difficult to locate a significant minimum
2. Number of minima due to type of details in the image
3. Noise
4. No visible valley
5. Histogram may be multi-modal

Manual thresholding example



original image

thresholded image

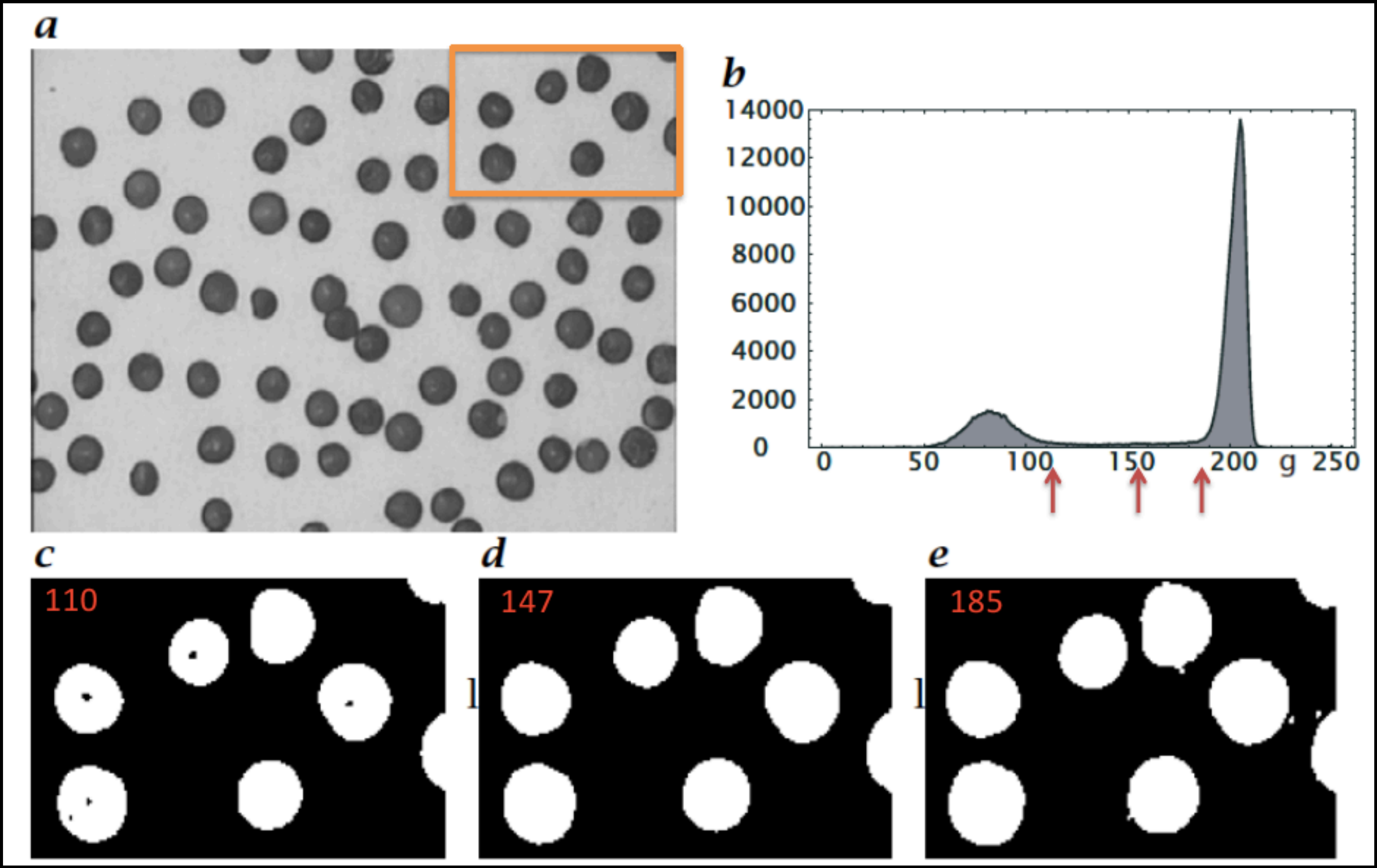
In the ImageJ menu: *Image* ▶ *Adjust* ▶ *Threshold...*

Too low or too high?

threshold too low

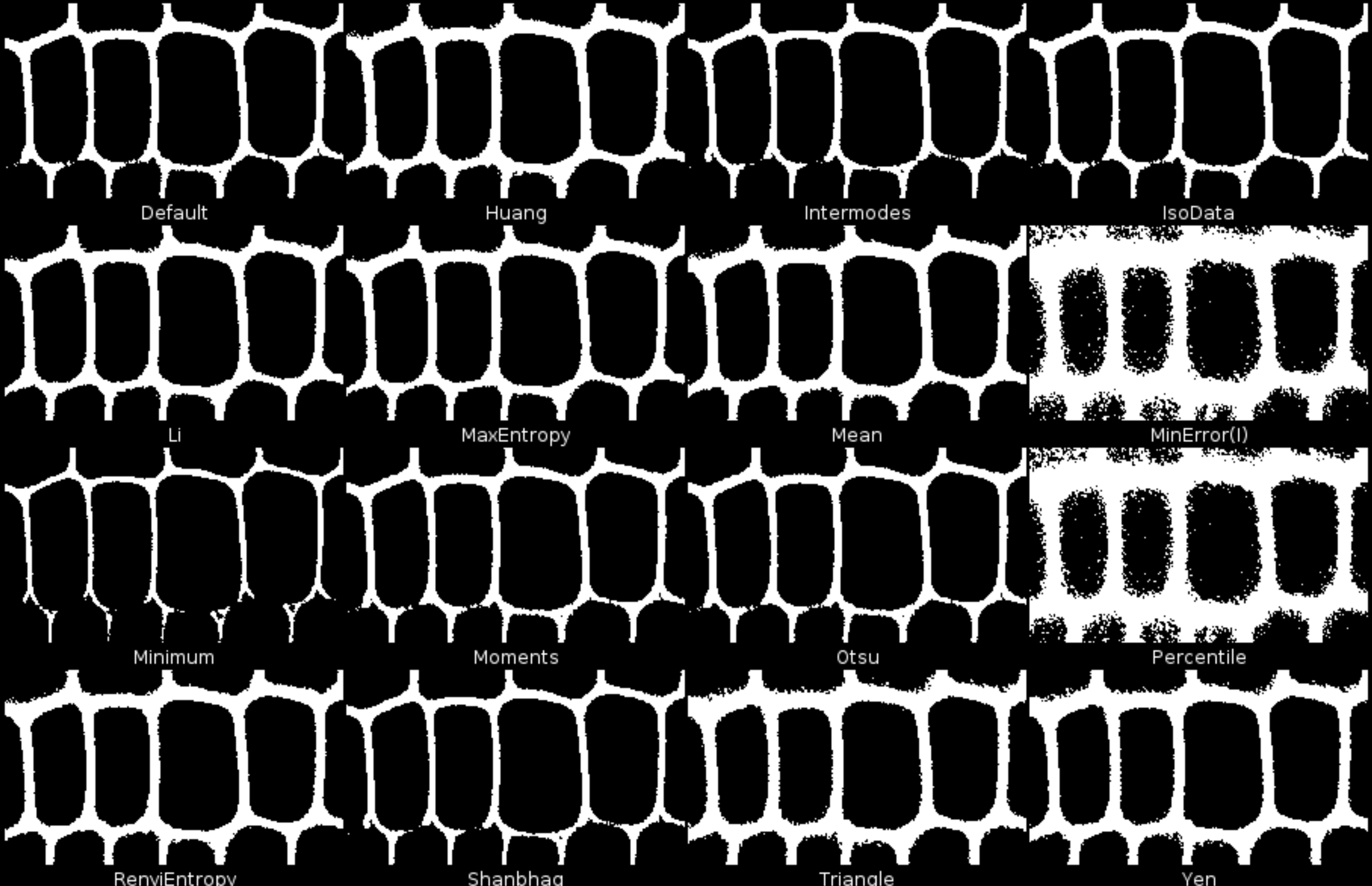
threshold too high

How to choose?



Automatic selection of threshold

- Same threshold over a collection of images? NOT recommended due to fluctuations in intensity across images.
- Automatic threshold methods:
  - optimizing some objective criterion that can be:
    - statistical (e.g., maximization of inter-class variance, entropy…),
    - probabilistic (e.g., minimization of pixel classification error…),
    - structural (e.g., circularity of detected objects…).
- How to choose in Fiji? Try them ALL!

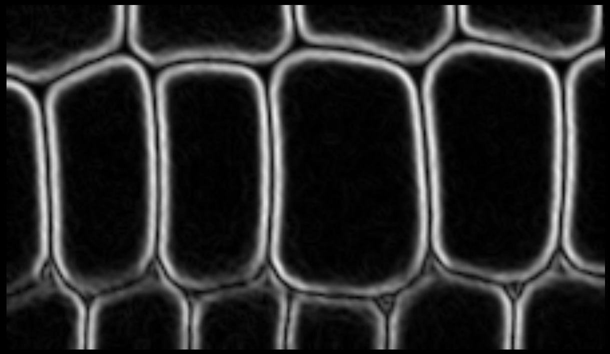


http://imagej.net/Auto_Threshold

Edge detection

- Objects without homogeneous intensities.
- Contours easily identifiable by their contrast difference with the background.
- Task:
  - Segment the image by finding relevant edges.
- Simple Way:
  - Smooth the image (Gaussian blur, median…).
  - Calculate gradient (Sobel operator).
  - Post-process to create continuous borders and close objects (Canny).

Canny edge detector



Gradient magnitude

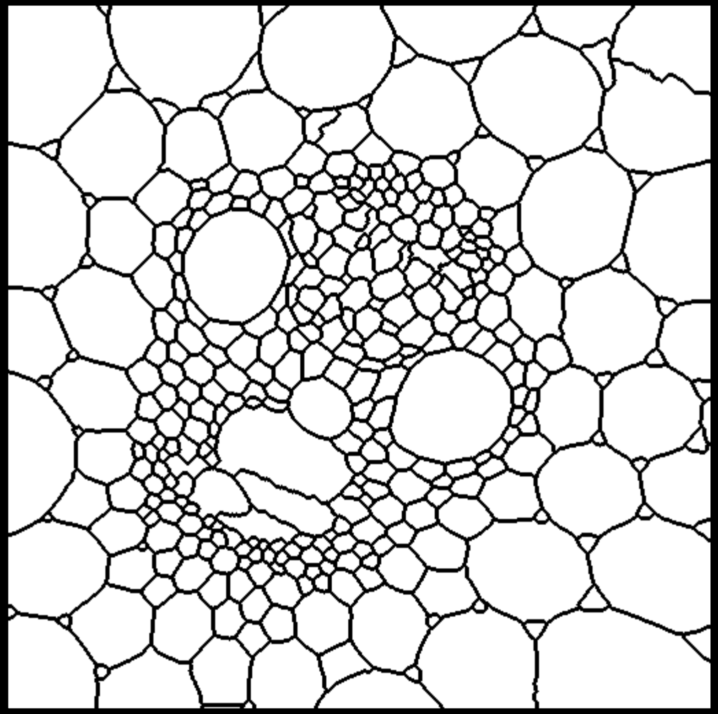
Non-max suppression

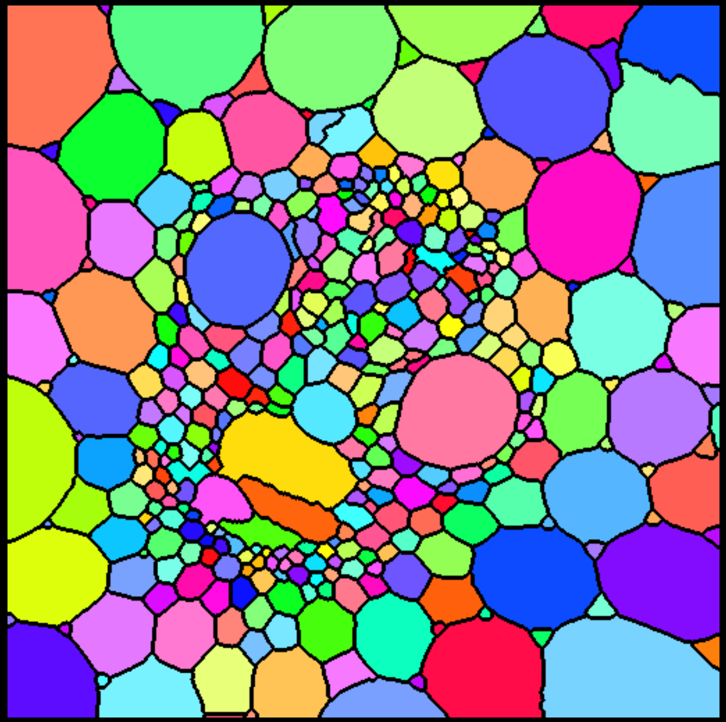
Hysteresis thresholding

http://imagej.net/plugins/canny/index.html

Connected component labeling

- Transform a binary image into a label image
- Label ⇔ particle ID
- Several algorithms:
    - Raster scan + labels merge
    - Flood-fill
        - Breadth-first
        - Depth first
        - Line based
- Need to specify connectivity (2D: 4-8, 3D: 6-26)



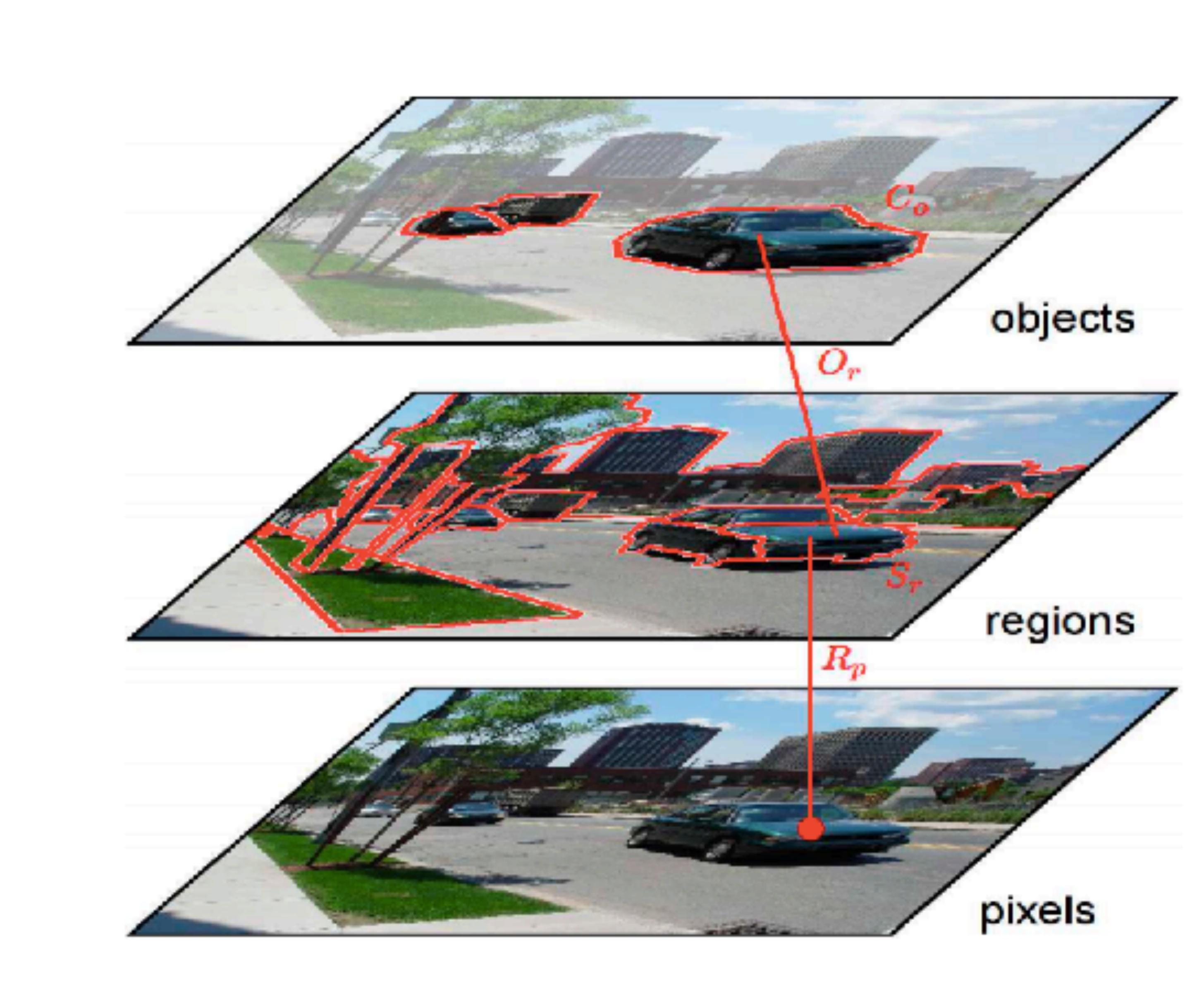


http://imagej.net/MorphoLibJ#Connected_component_labeling

Region based segmentation

Region based segmentation basics

- Region:
  - A group of connected pixels with similar properties
  - Closed boundaries
  - Computation of regions is based on similarity
- Regions may correspond to objects in a scene or parts of objects
- Spatial proximity + similarity



Region growing

- For segment generation in grey-level or color images, we may start at one seed pixel $(x,y,I(x,y))$ and add recursively adjacent pixels that satisfy a “similarity criterion” with pixels contained in the so-far grown region around the seed pixel.

- Defining similarity criteria alone is not an effective basis for segmentation.

- It is necessary to consider the adjacency spatial relationship between pixels.

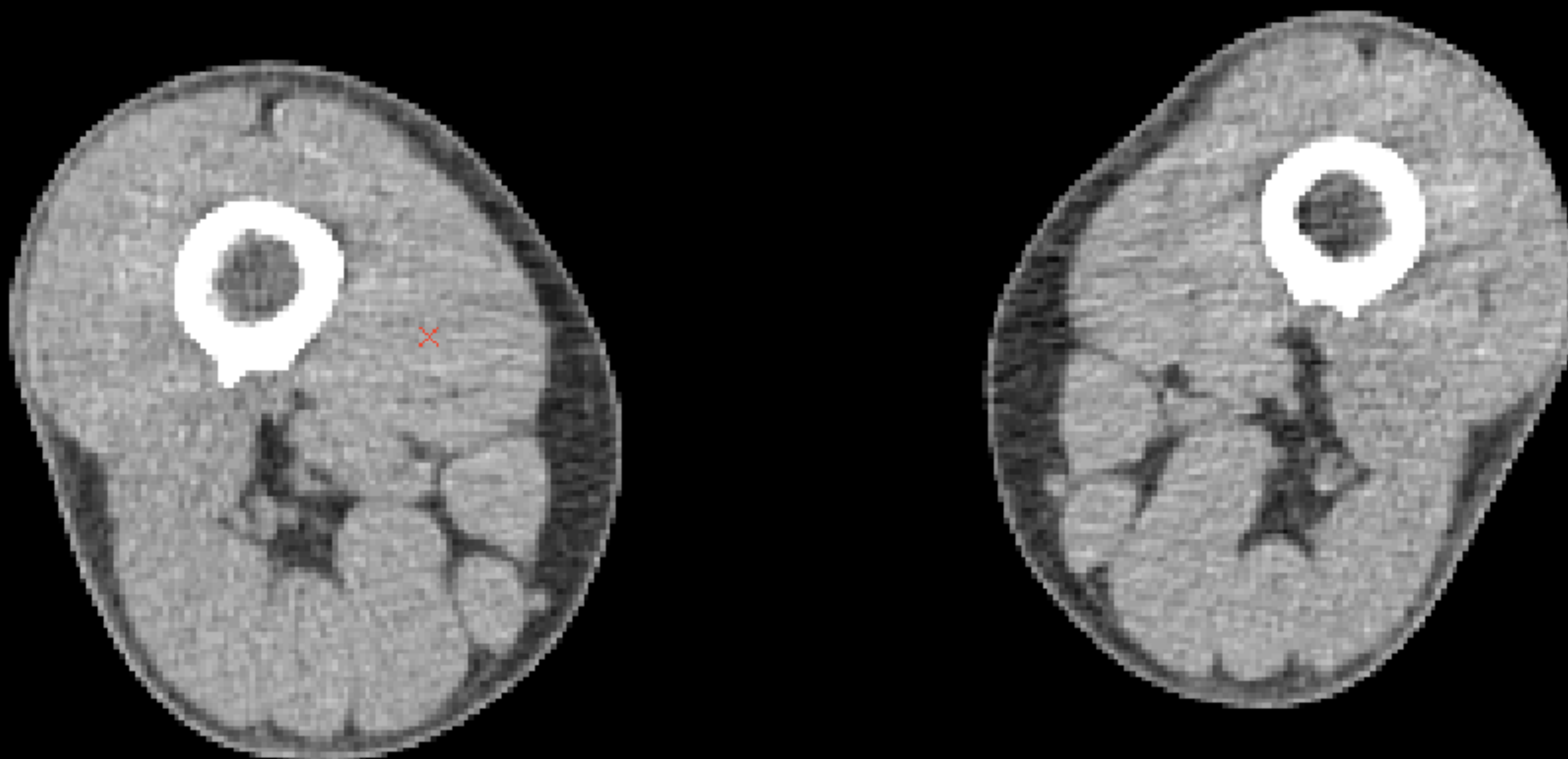
Region growing algorithm

1. The absolute intensity difference between candidate pixel and the seed pixel must lie within a specified range.

2. The absolute intensity difference between a candidate pixel and the running average intensity of the growing region must lie within a specified range.

3. The difference between the standard deviation in intensity over a specified local neighborhood of the candidate pixel and that over a local neighborhood of the candidate pixel must (or must not) exceed a certain threshold.

Ex: Muscle/Bone Segmentation in CT Scans



Threshold

Ex: Muscle/Bone Segmentation in CT Scans

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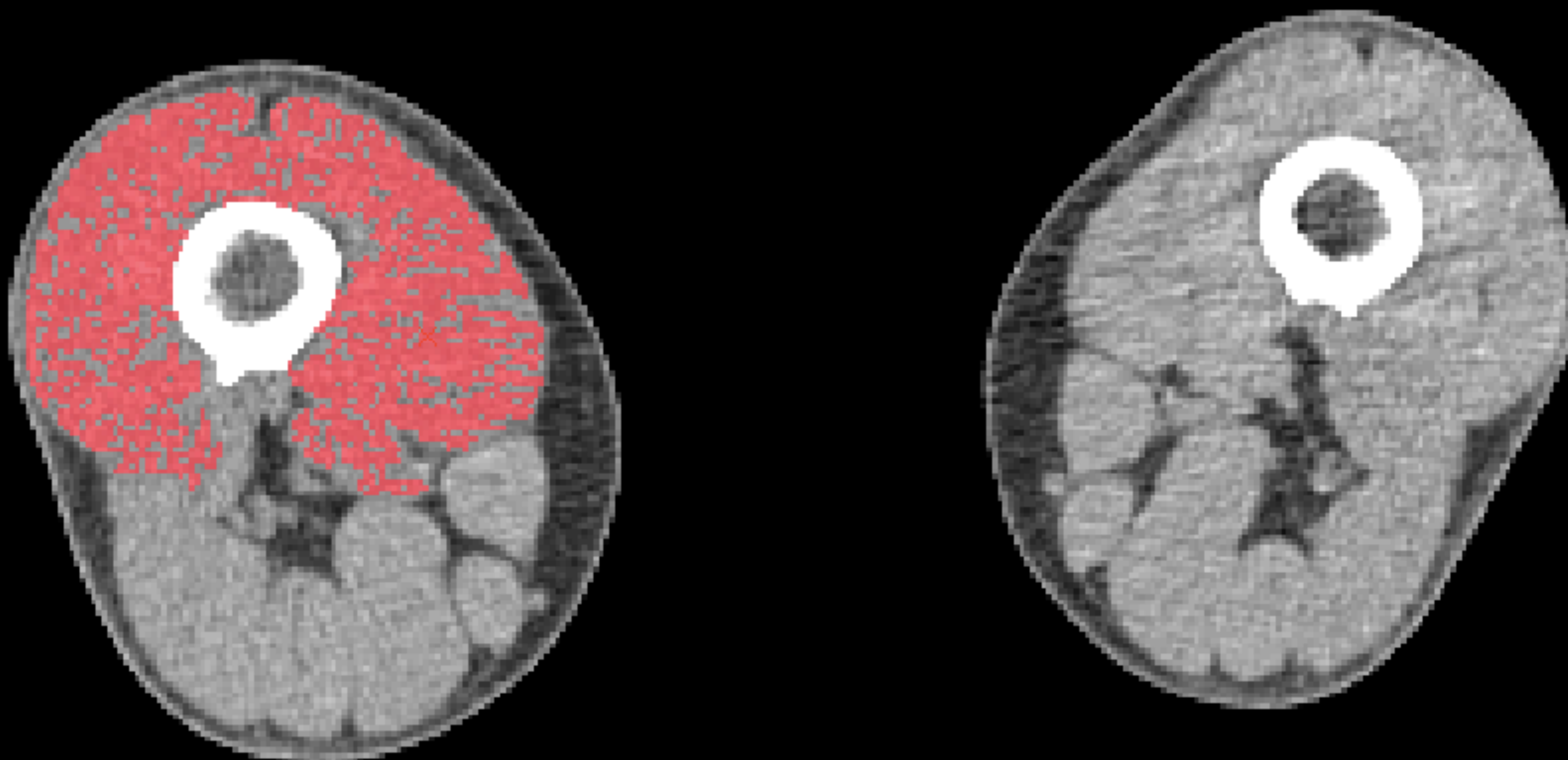

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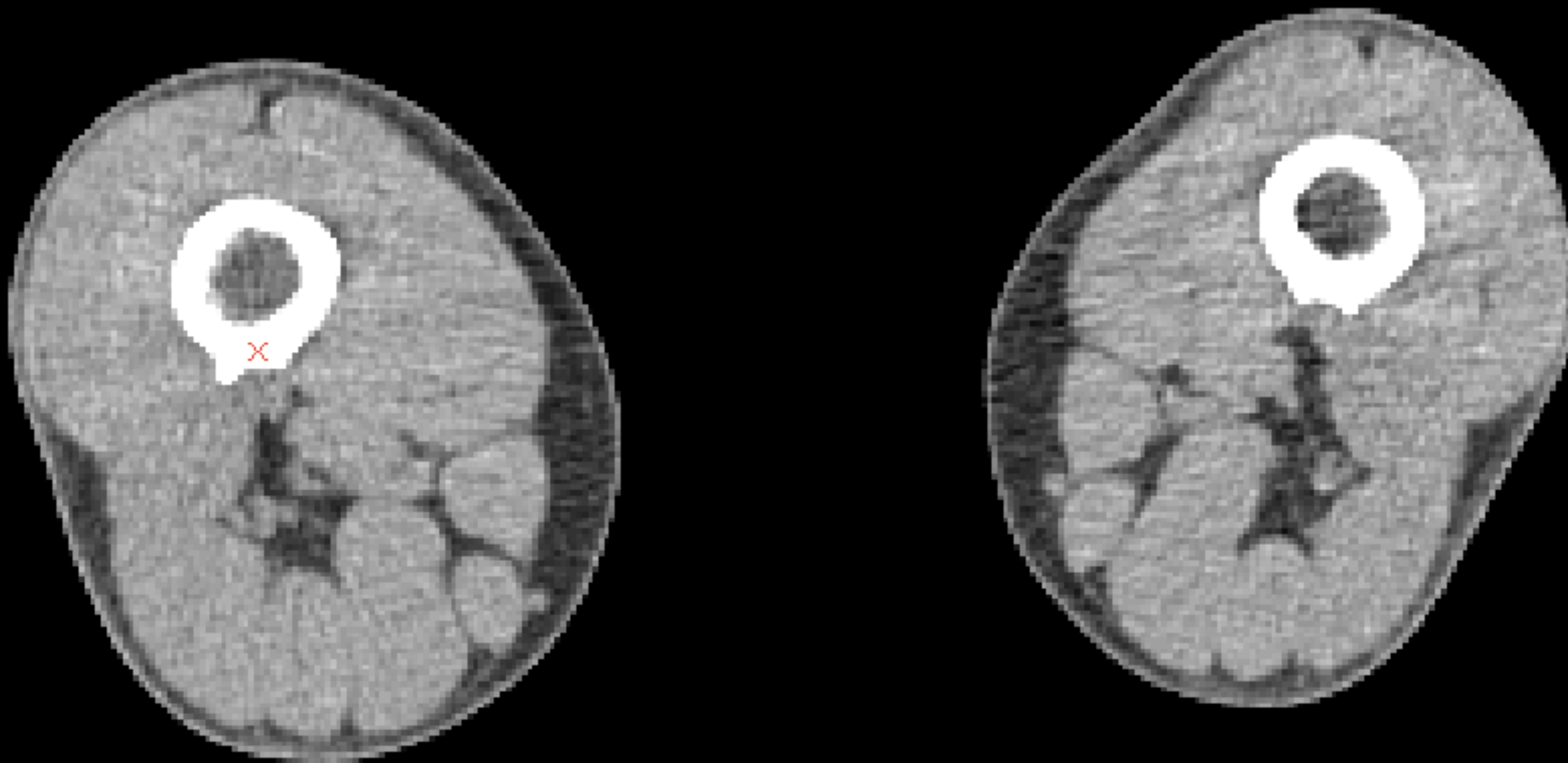

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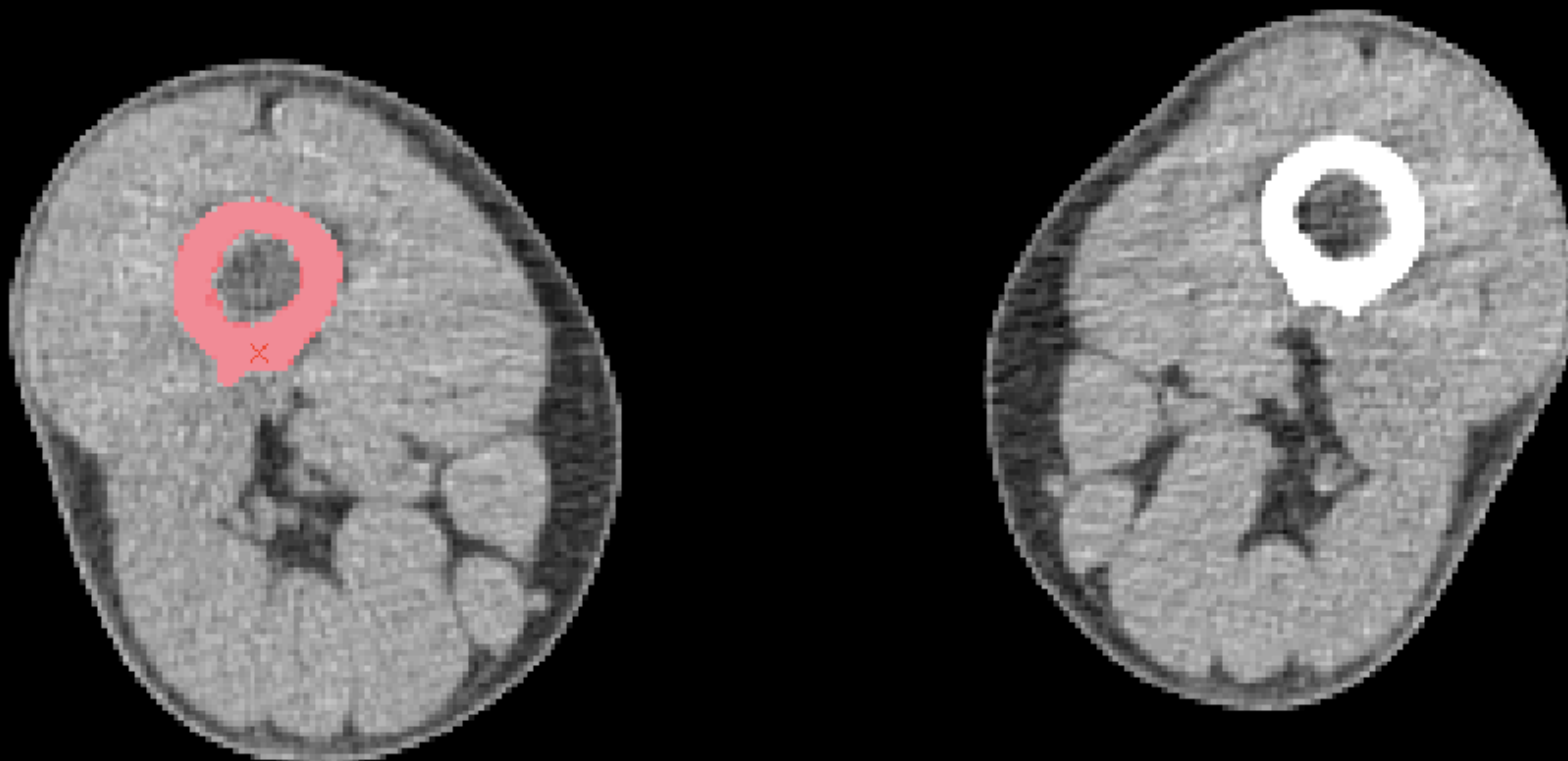
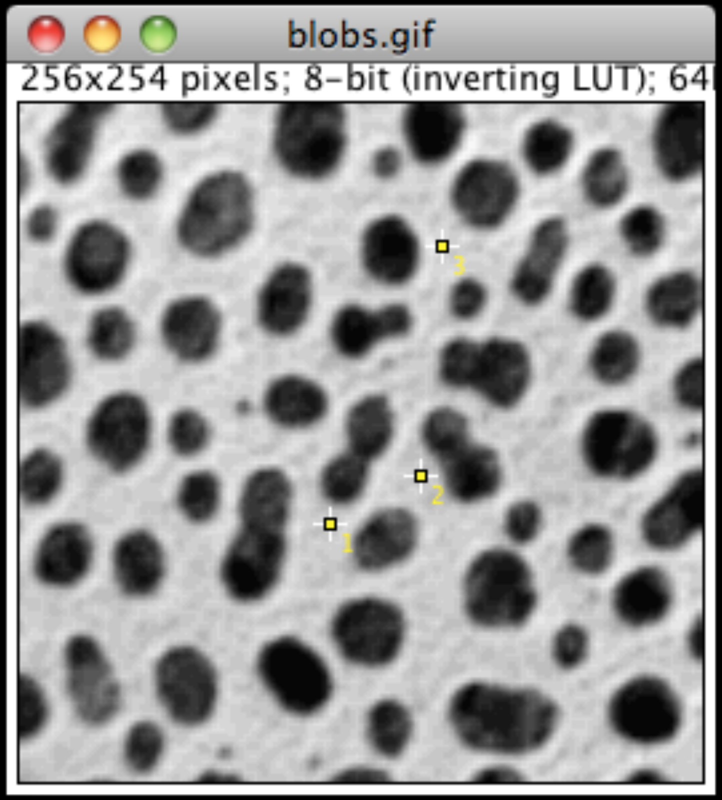


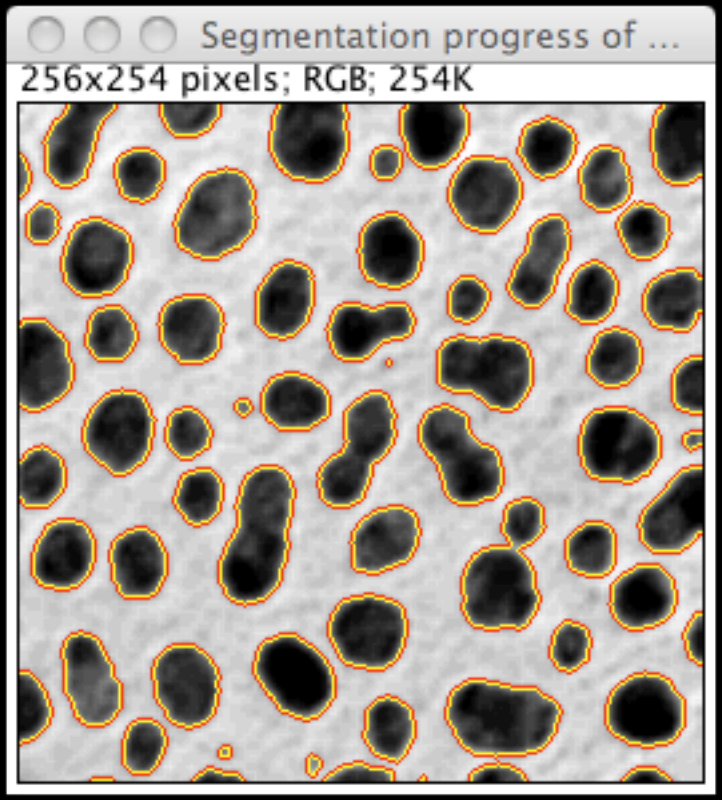
Threshold

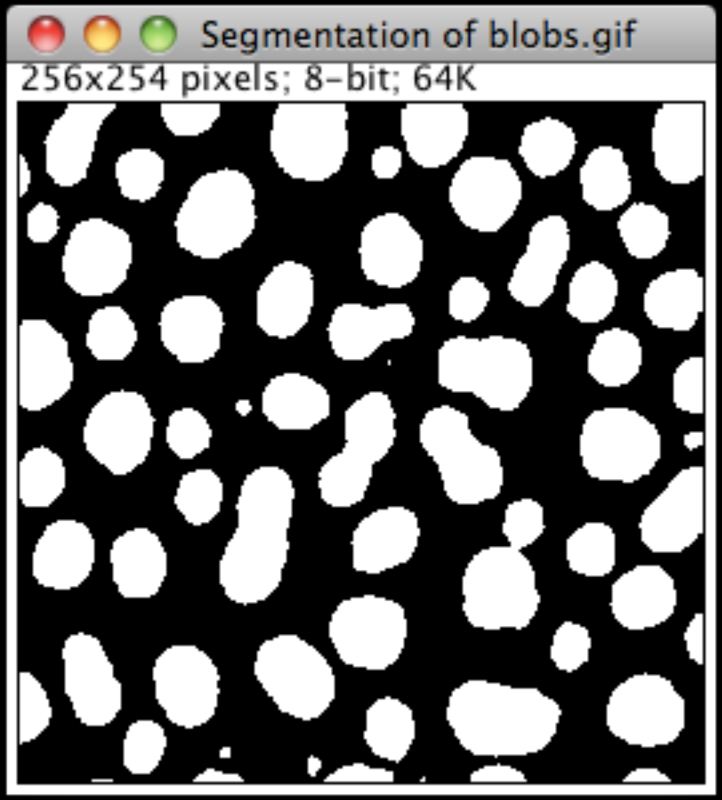
Pros and cons of region growing

- Advantages：
  - Can correctly separate regions that have the same properties we define.
  - Can provide the original images which have clear edges with good segmentation results.
  - Simple. We only need a small number of seed points.
  - We can choose the multiple criteria at the same time.
- Disadvantages：
  - Computationally expensive.
  - It is a local method with no global view of the problem.
  - Sensitive to noise.

Level Sets



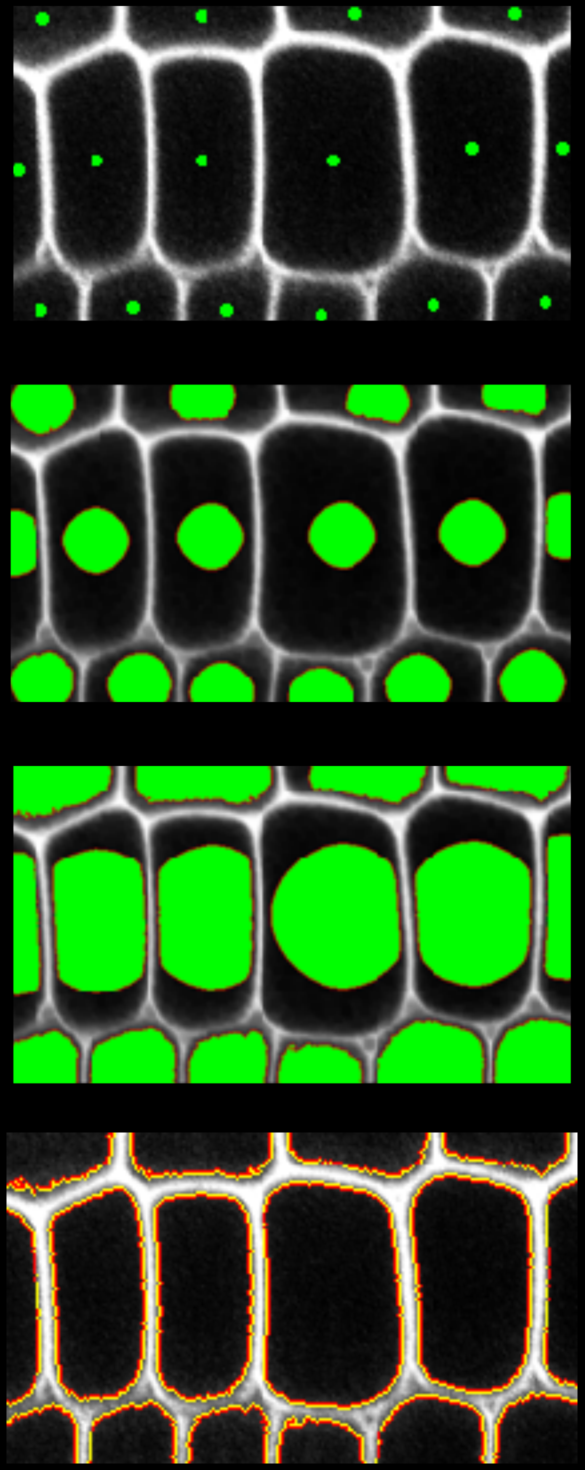




- Based on partial differential equations (PDE).
- Progressive evaluation of the differences among neighboring pixels to find object boundaries.
- Converge at the boundary of the object where the differences are the highest.
- Two methods: Fast Marching and Active Contours.

Fast marching and Active Contours

- Fast marching:
    - Similar to standard flood fill but more sensitive in the boundary detection.
    - Sensitive to leaking.
- Active contours:
    - Slower.
    - Prevents leaking.

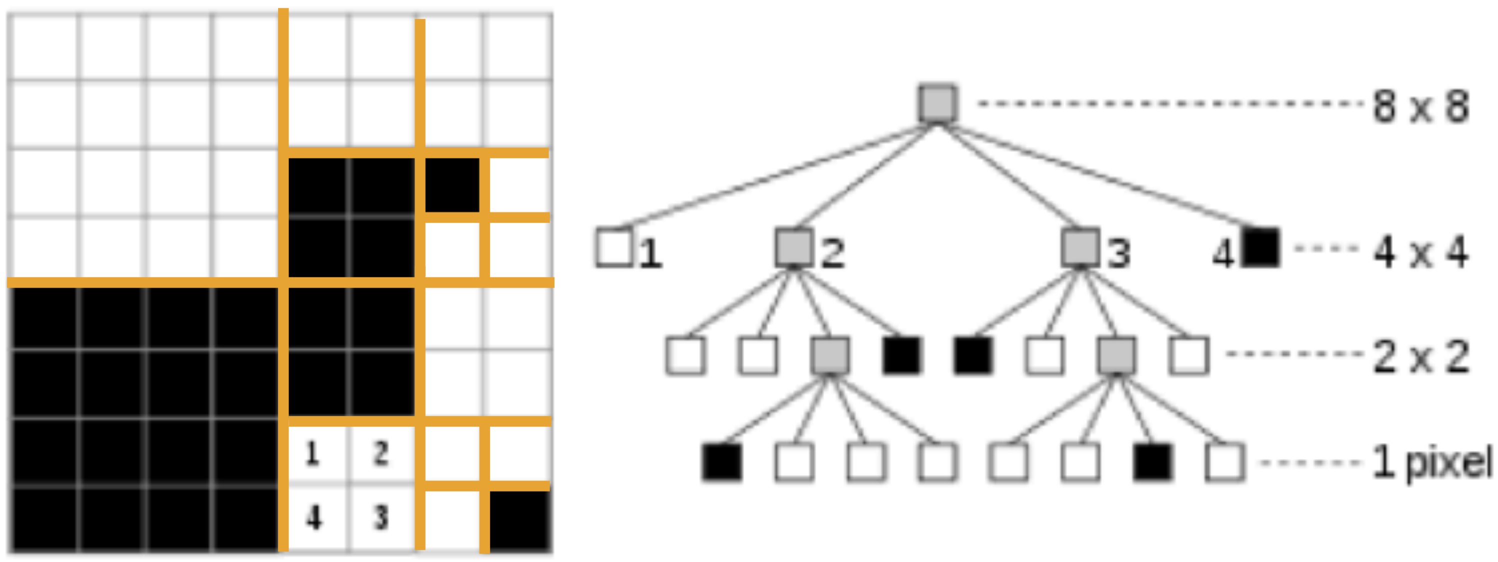


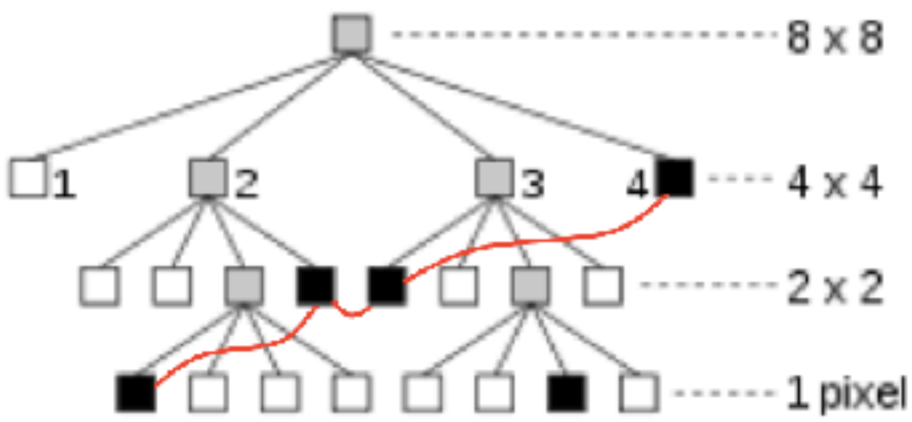
http://imagej.net/Level_Sets

Region splitting and merging Segmentation

- Region splitting:
  - Unlike region growing, which starts from a set of seed points, region splitting starts with the whole image as a single region and subdivides it into subsidiary regions recursively while a condition of homogeneity is not satisfied.
- Region merging:
  - Region merging is the opposite of splitting, and works as a way of avoiding over-segmentation.
  - Start with small regions (2x2 or 4x4 regions) and merge the regions that have similar characteristics (such as gray level, variance).

Region splitting Segmentation





RAG with adjacency relations (in red) for big black region.

- Quadtree for splitting (topdown) procedure
- RAG: region adjacency graph

Split and Merge Region algorithm

1. Start with the entire image as a single region.

2. Pick a region *R*. If it is inhomogeneous ($P(R)$ is false), then split the region into four subregions.

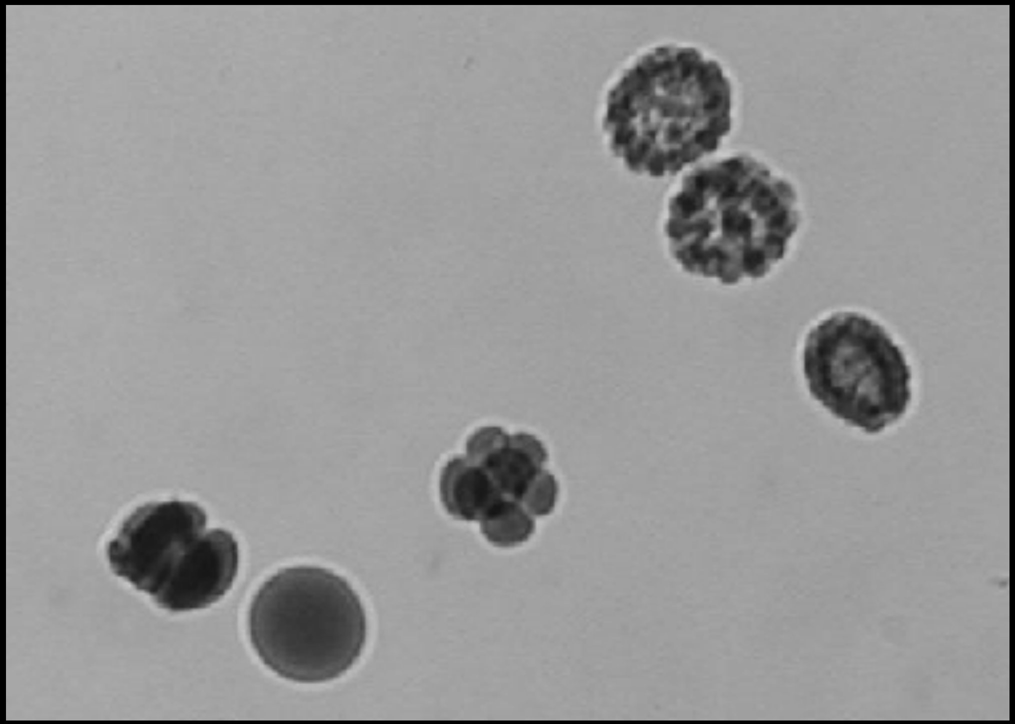
3. Consider any two or more adjacent regions, R_1, R_2,..., R_n, in the image. If they are homogeneous $P(R_1 \cup R_2 \cup \ldots \cup R_n)$ is true), merge the *n* regions into a single region.

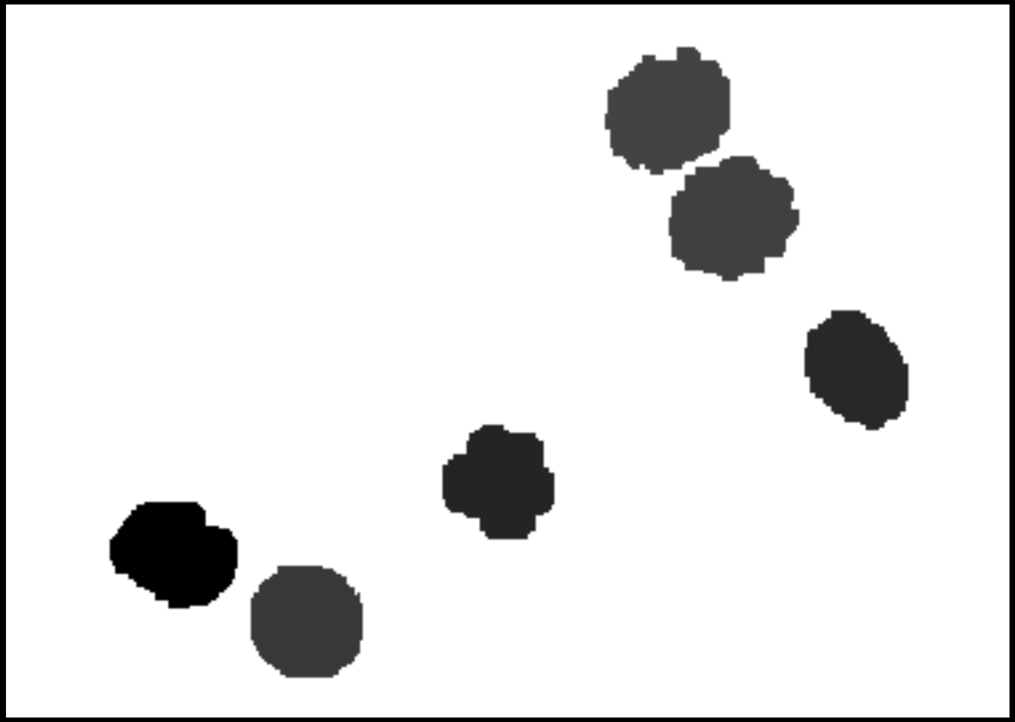
4. Repeat steps 2-3 until no further splits or merges take place.

Statistical Region Merging plugin

- Fast and robust algorithm to segment an image into regions of similar intensity or color.
- Start with one region per pixel.
- Then apply statistical test on neighboring regions to check if mean intensities are similar enough to be merged.
- Only 1 parameter: Q (estimated number of regions).

http://imagej.net/Statistical_Region_Merging





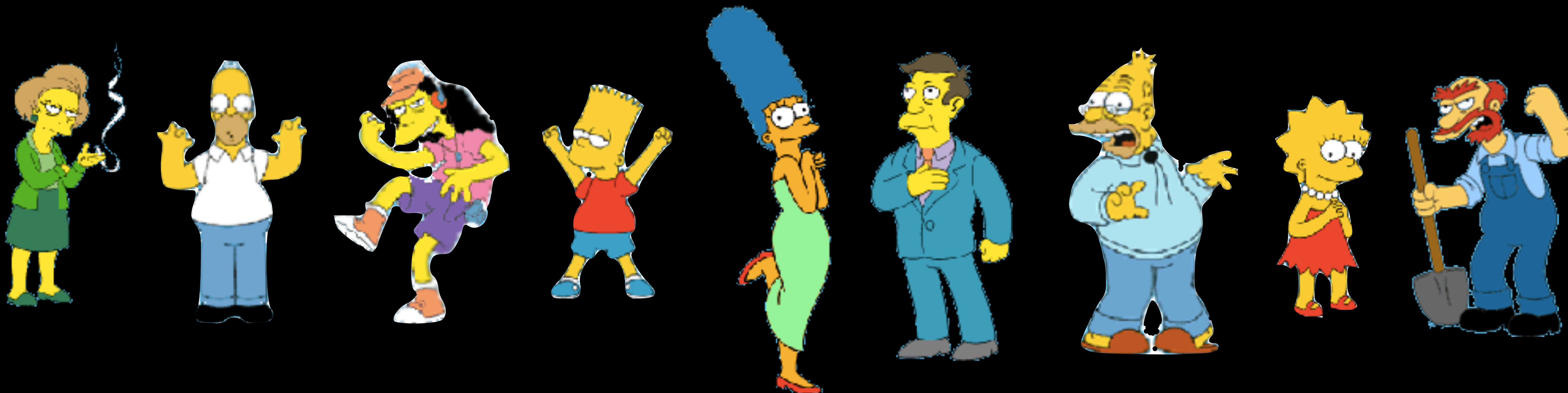
SRM (Q=2)

Clustering based segmentation methods

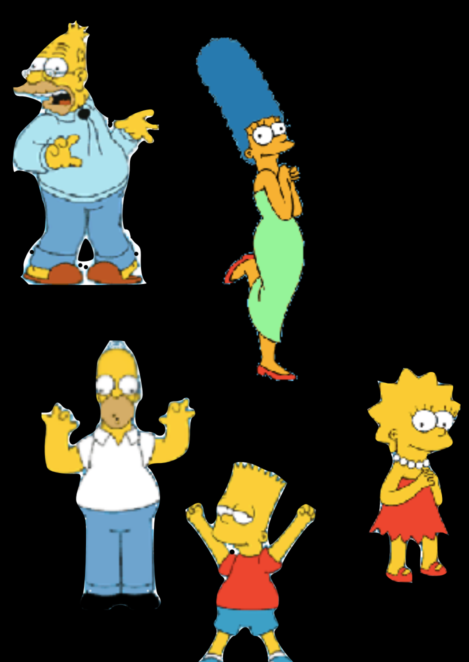
What is Clustering?

- Organizing data into classes such that:
  - High intra-class similarity
  - Low inter-class similarity
- Finding the class labels and the number of classes directly from the data (as opposed to classification tasks)

What is natural grouping?



Clustering is subjective!



Simpson family

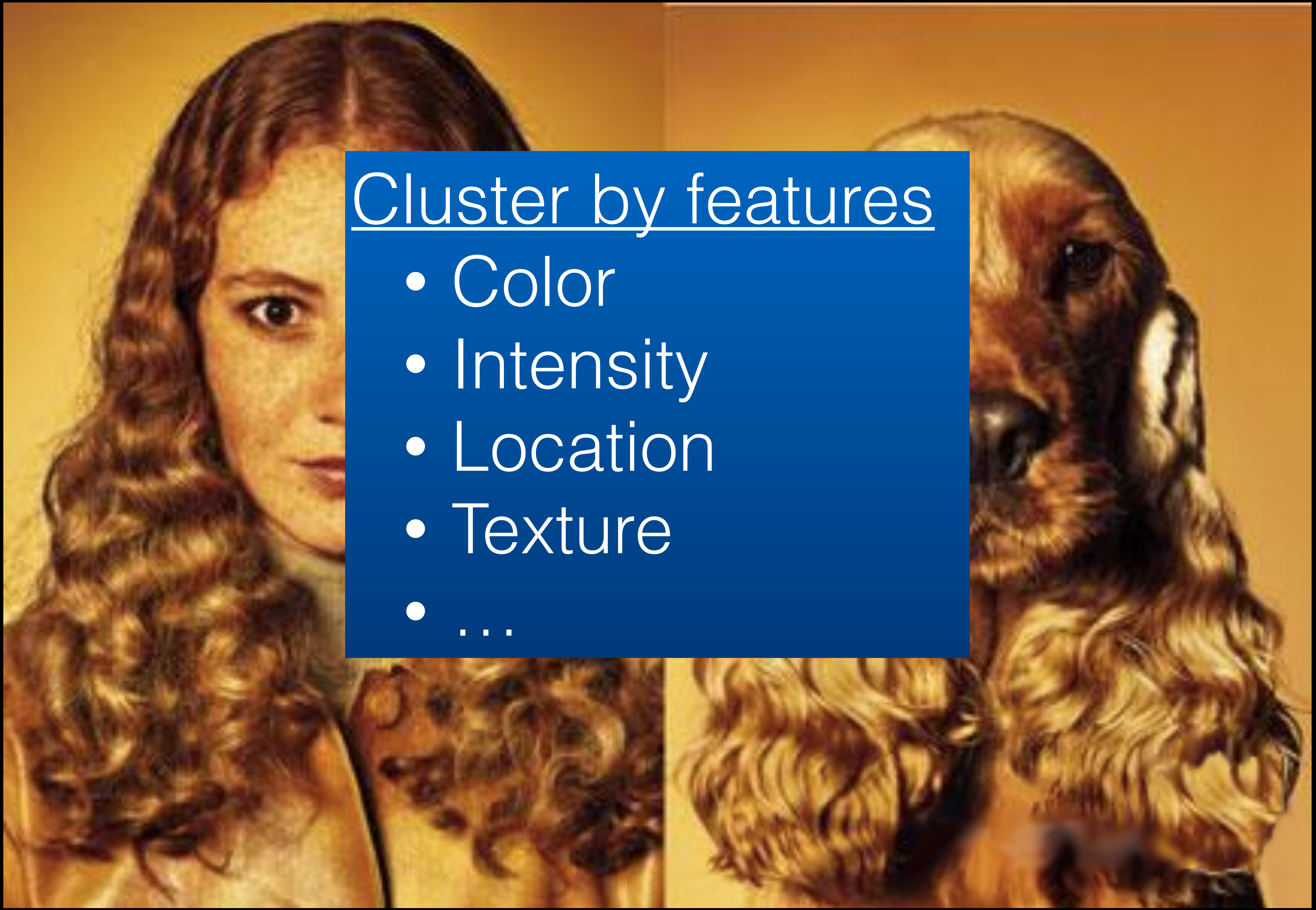
School Employees

Females

Males

What is similarity?

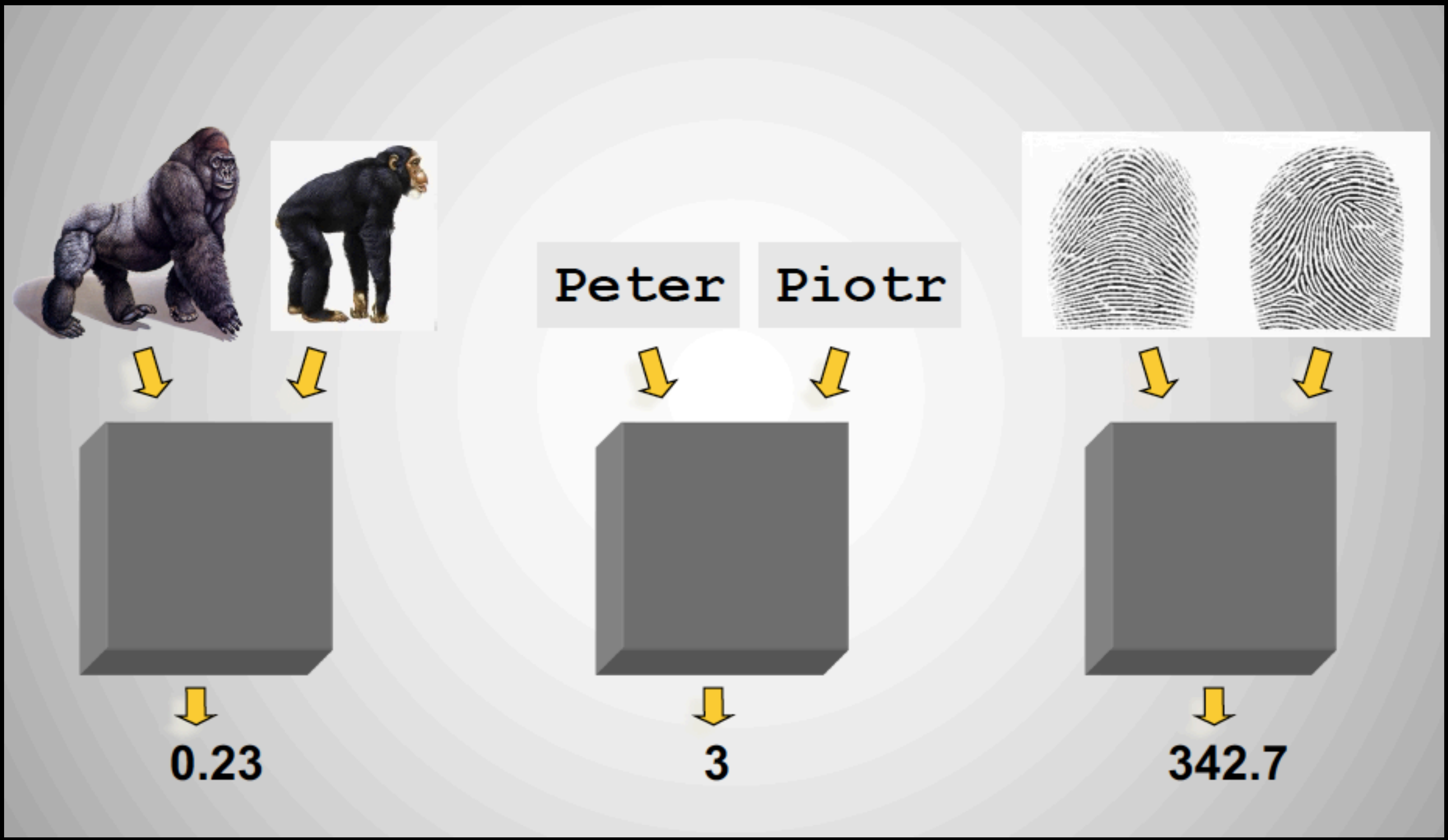
What is similarity?



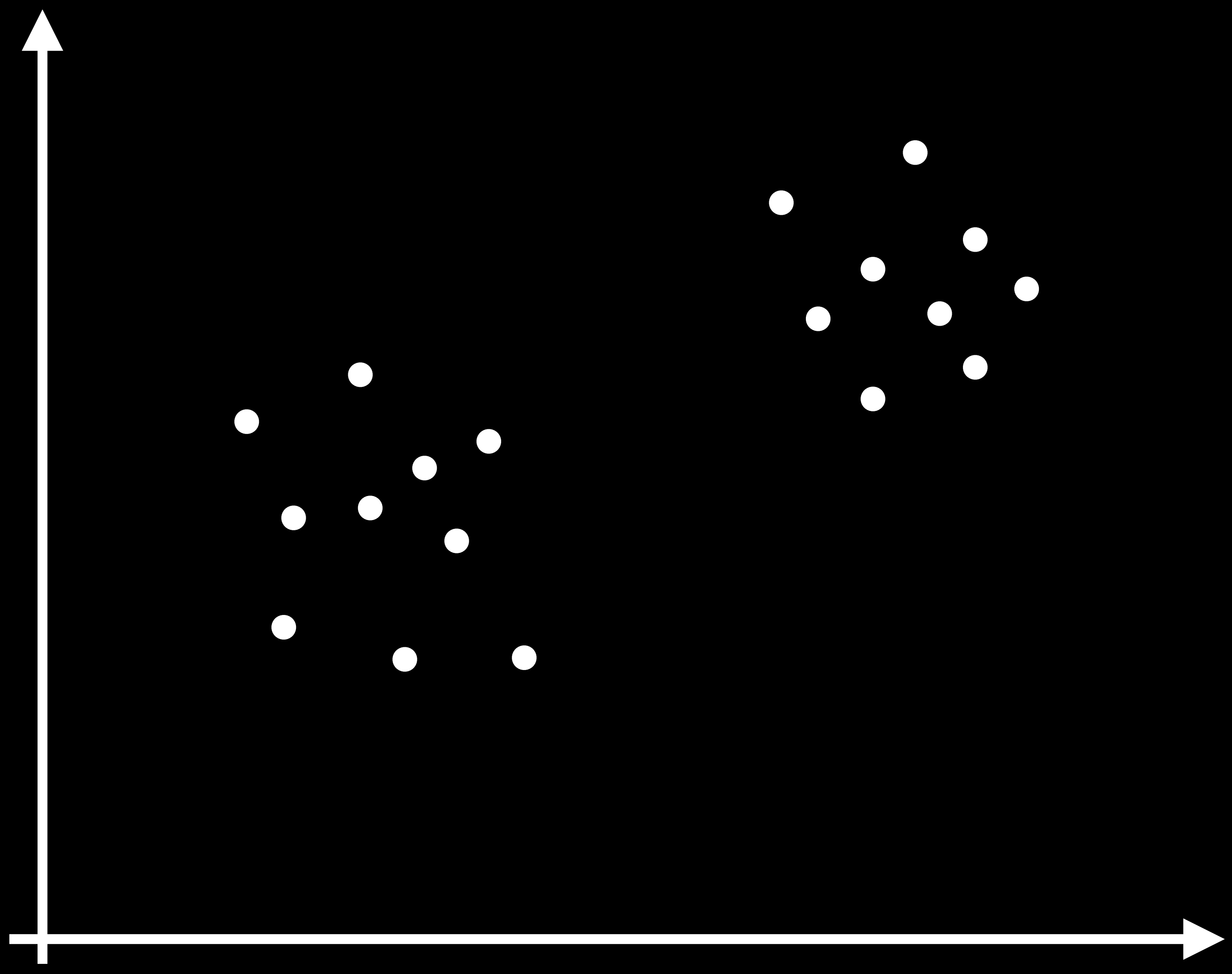
Cluster by features

- Color
- Intensity
- Location
- Texture
- …

Distance metrics



K-means clustering



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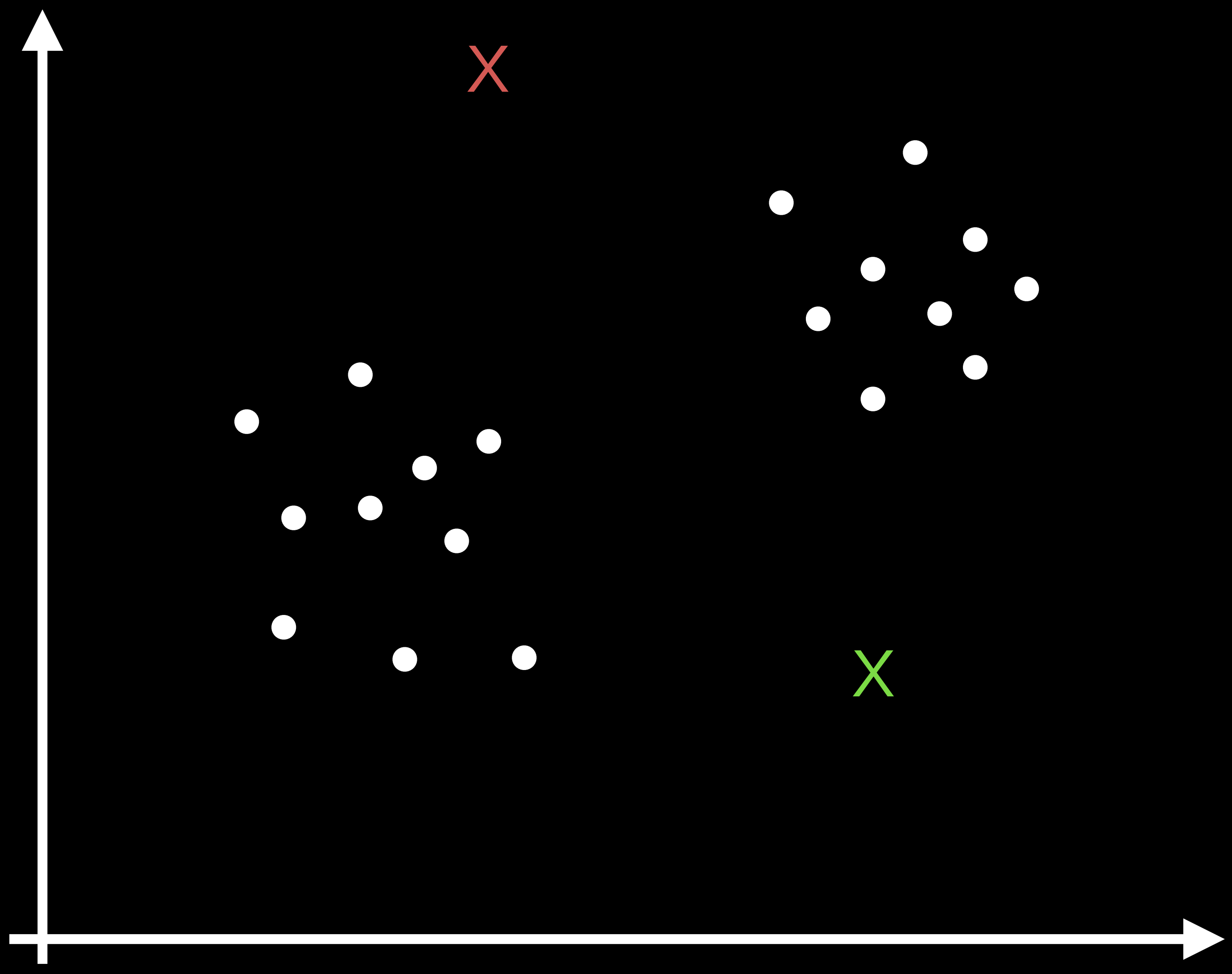

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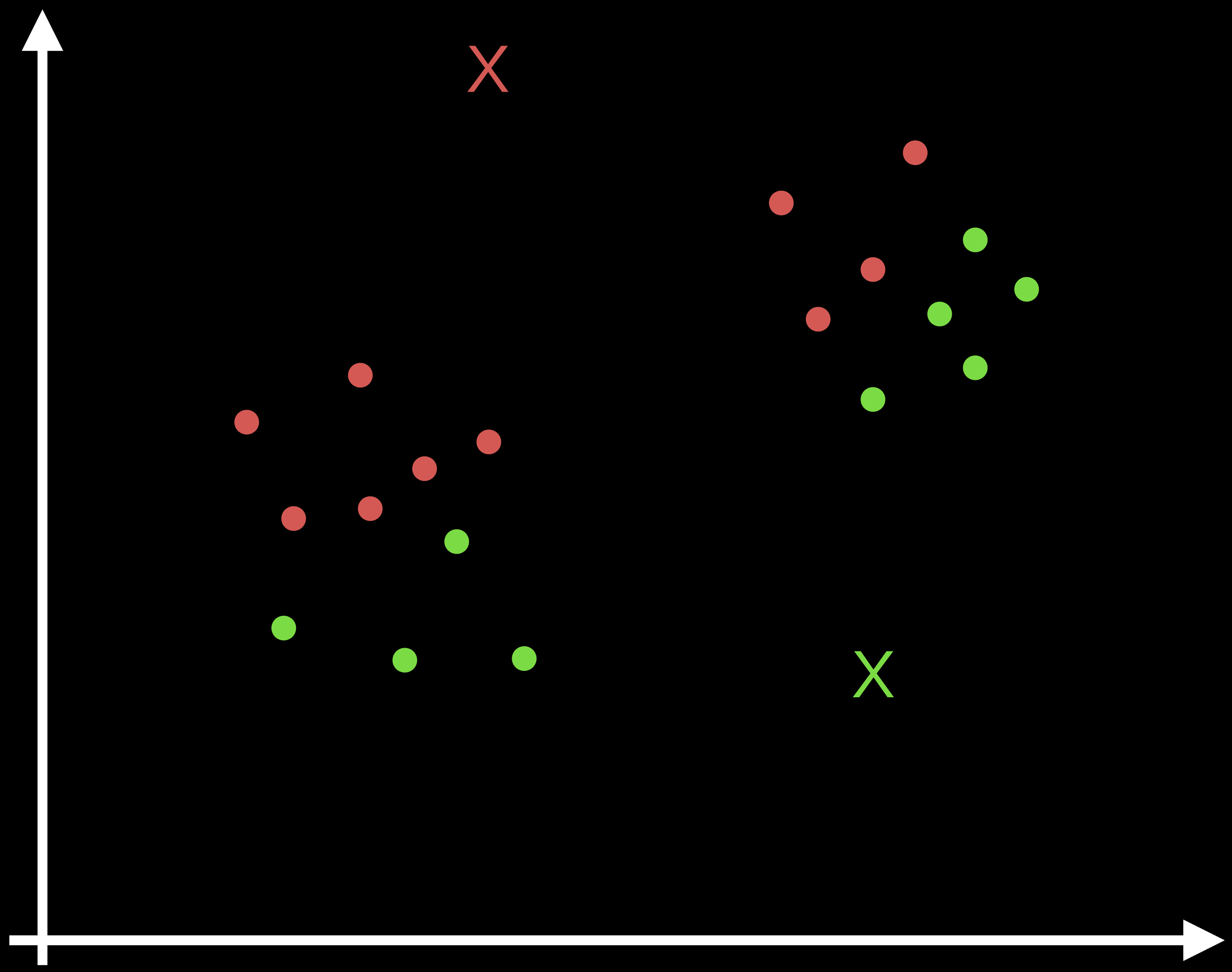

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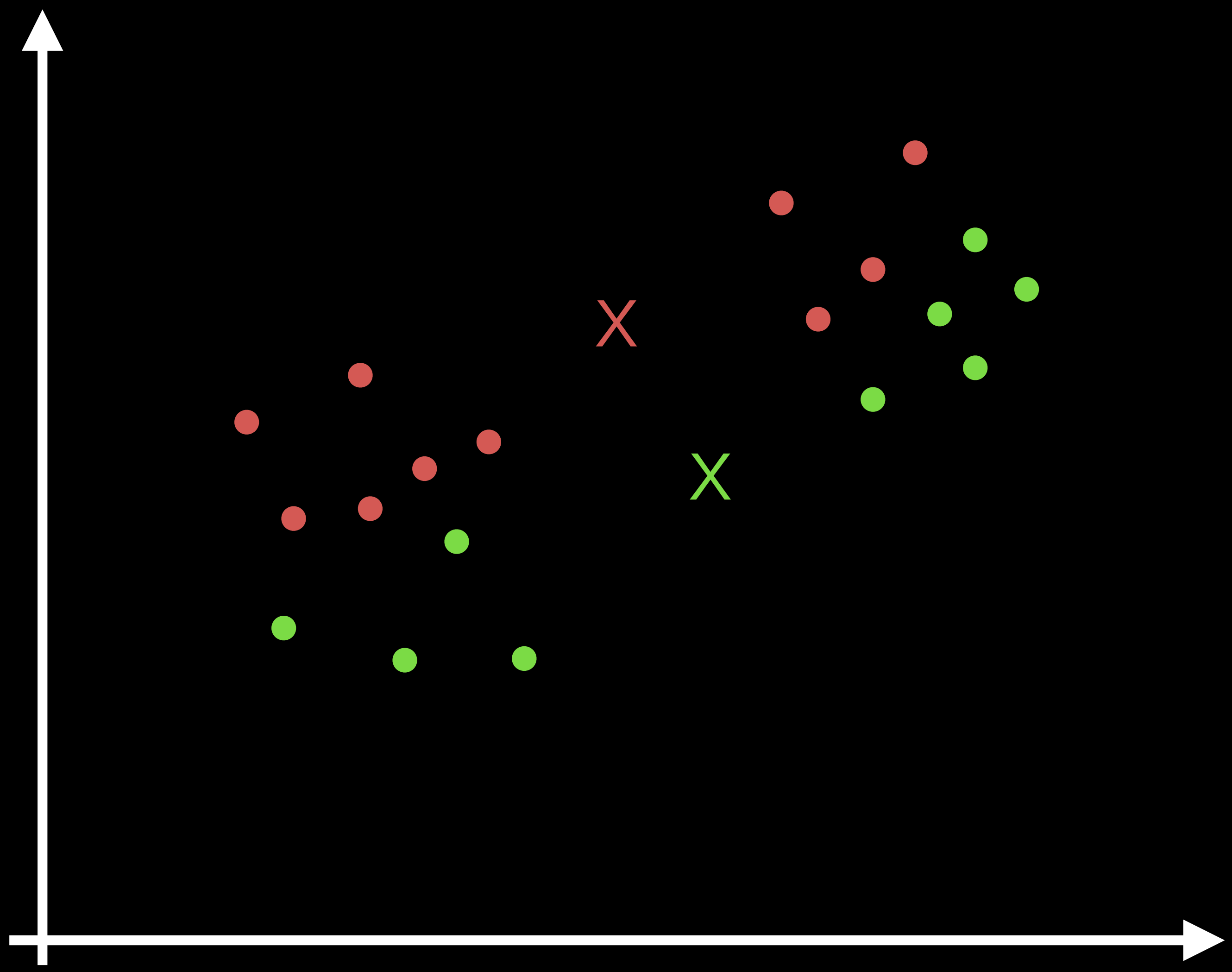

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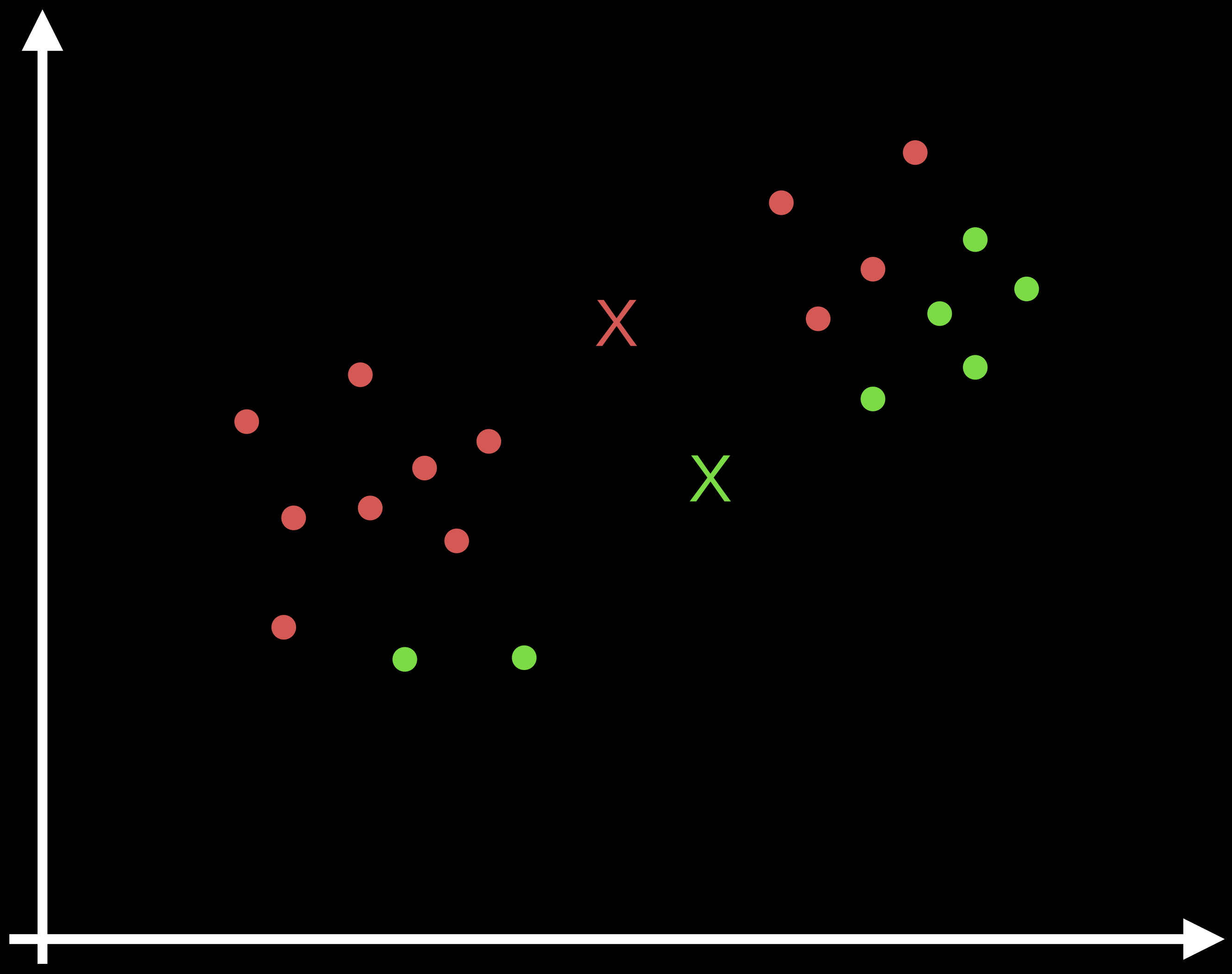

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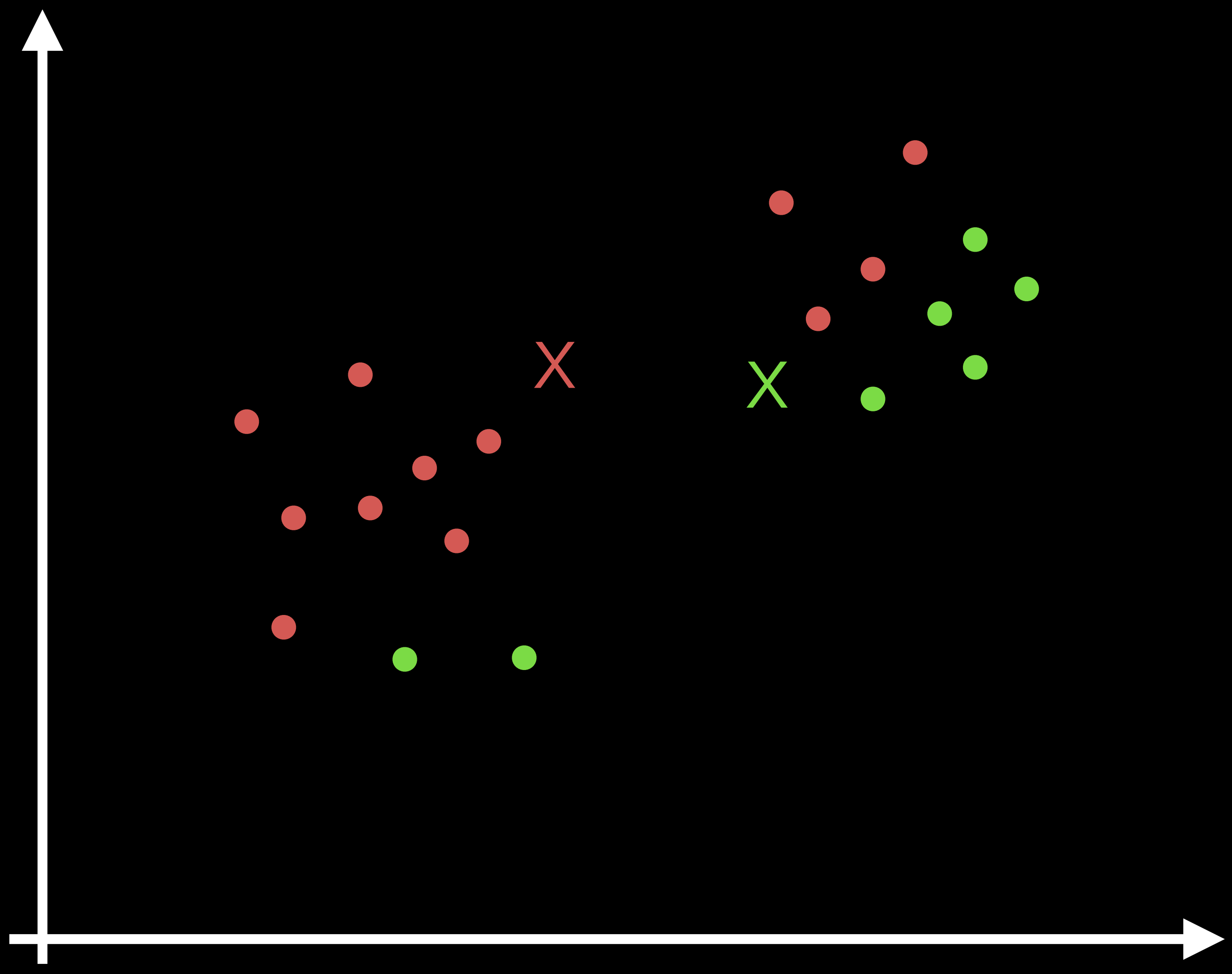

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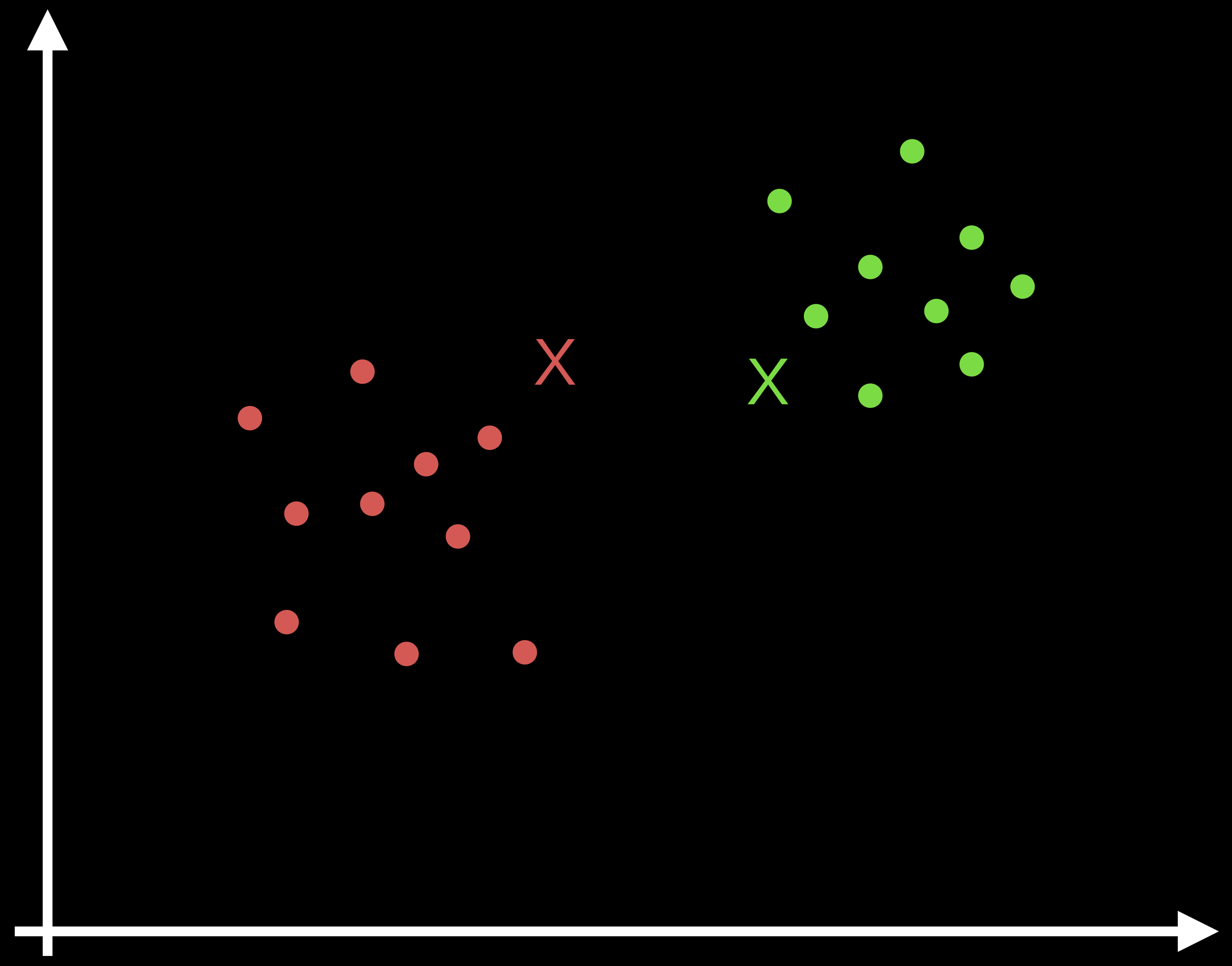

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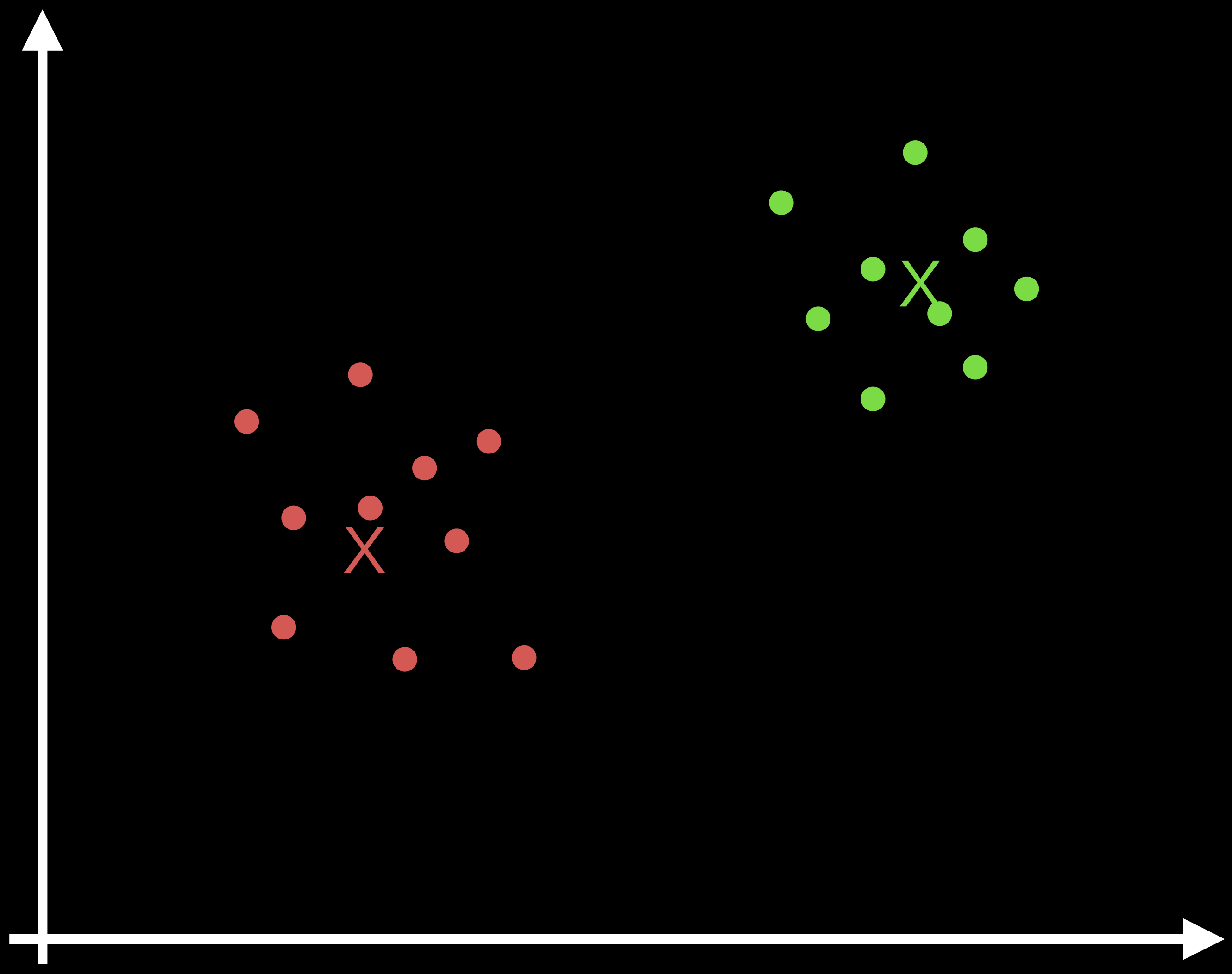
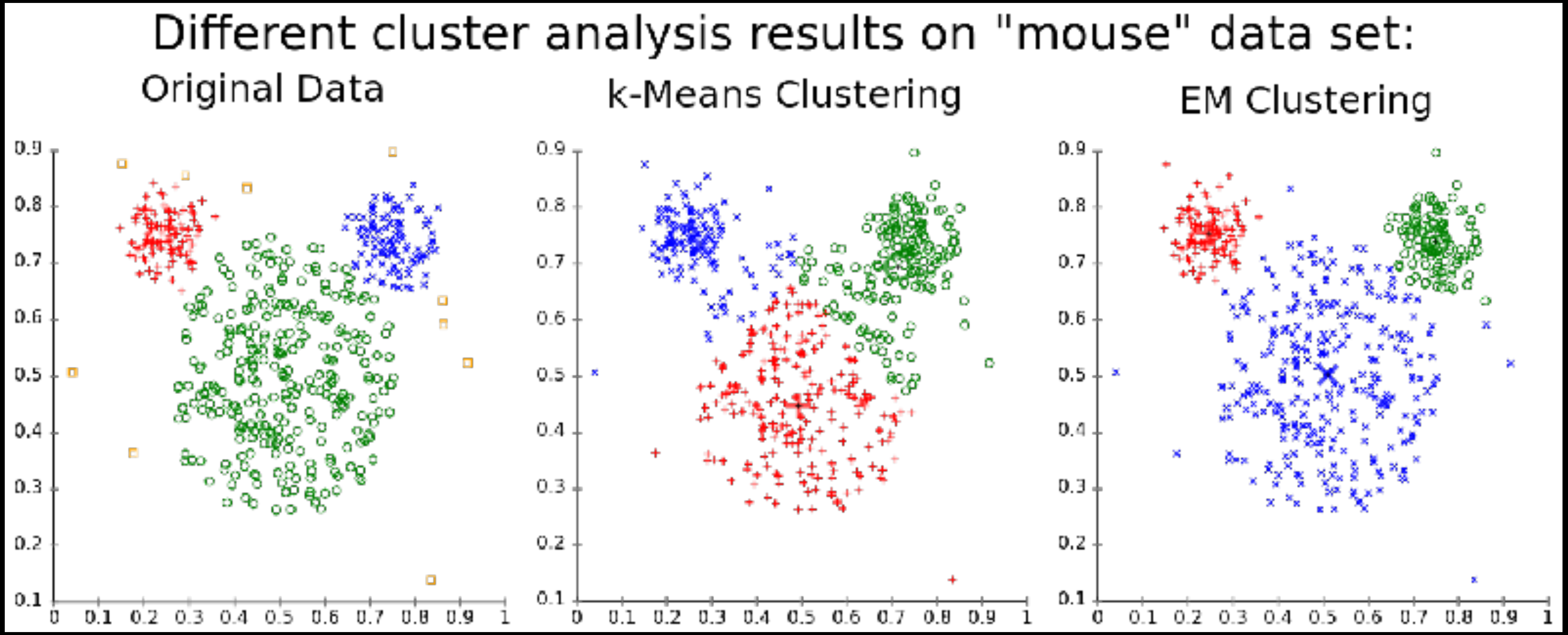


K-means clustering limitations

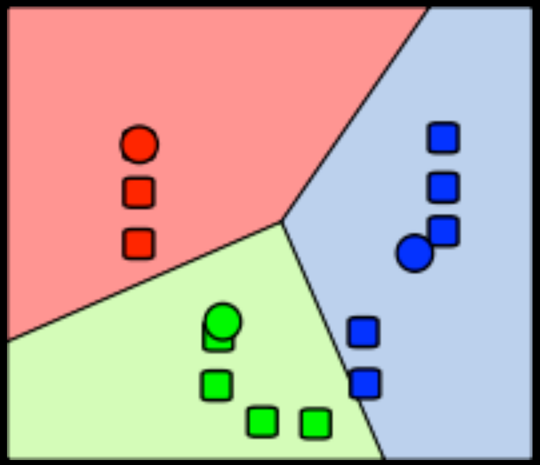
- Heuristic algorithm, there is no guarantee that it will converge to global optimum.
- The result may depend on the initial clusters.
- Based on spherical clusters that are separable in a way so that the mean value converges towards the cluster center.
- The clusters are expected to be of similar size.



Segmentation via K-means in RGB space

Use K-means algorithm in RGB space to assign pixels to K clusters.

K=6



R

G

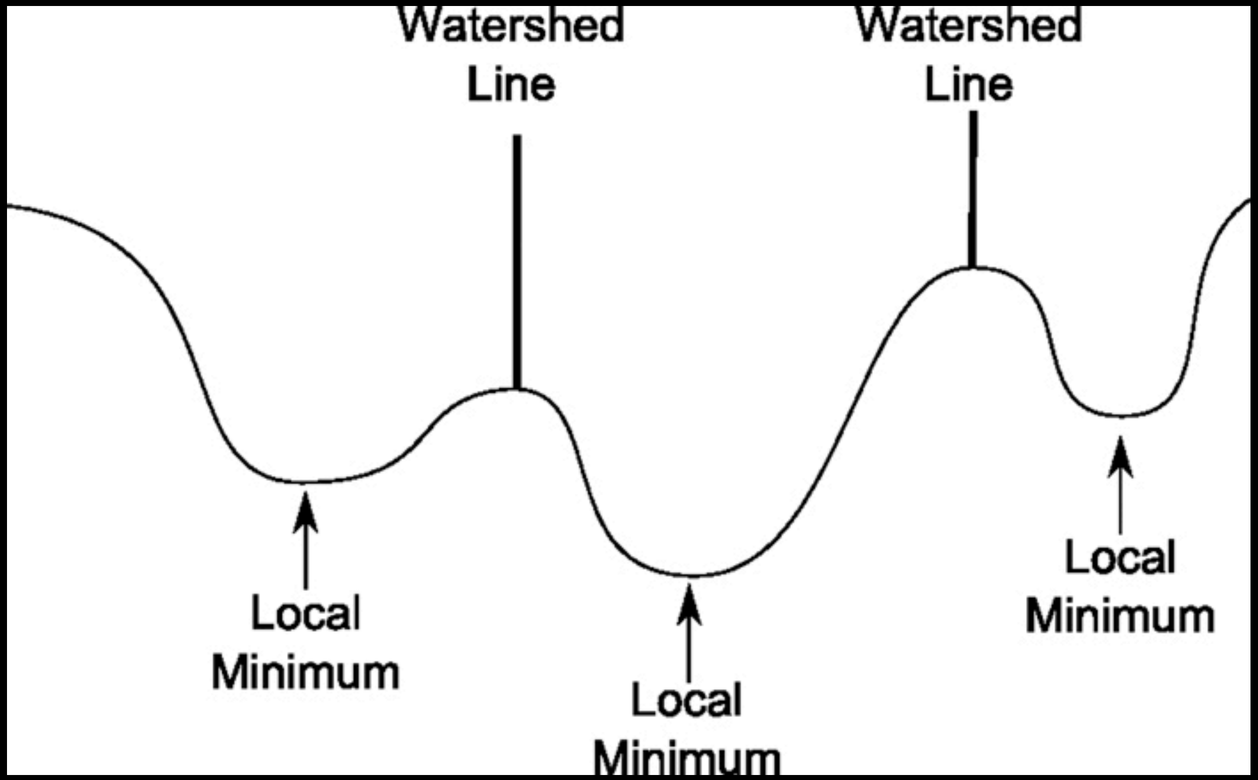
B

http://imagej.net/Trainable_Weka_Segmentation

Morphological segmentation methods

Watershed Segmentation

- Use a topographic analogy
- Principle:
  - Consider grey levels as altitudes
  - Identify local minima
  - Flood basins starting from minima
  - Separate the basins by a “dam” → the watershed



http://imagej.net/MorphoLibJ

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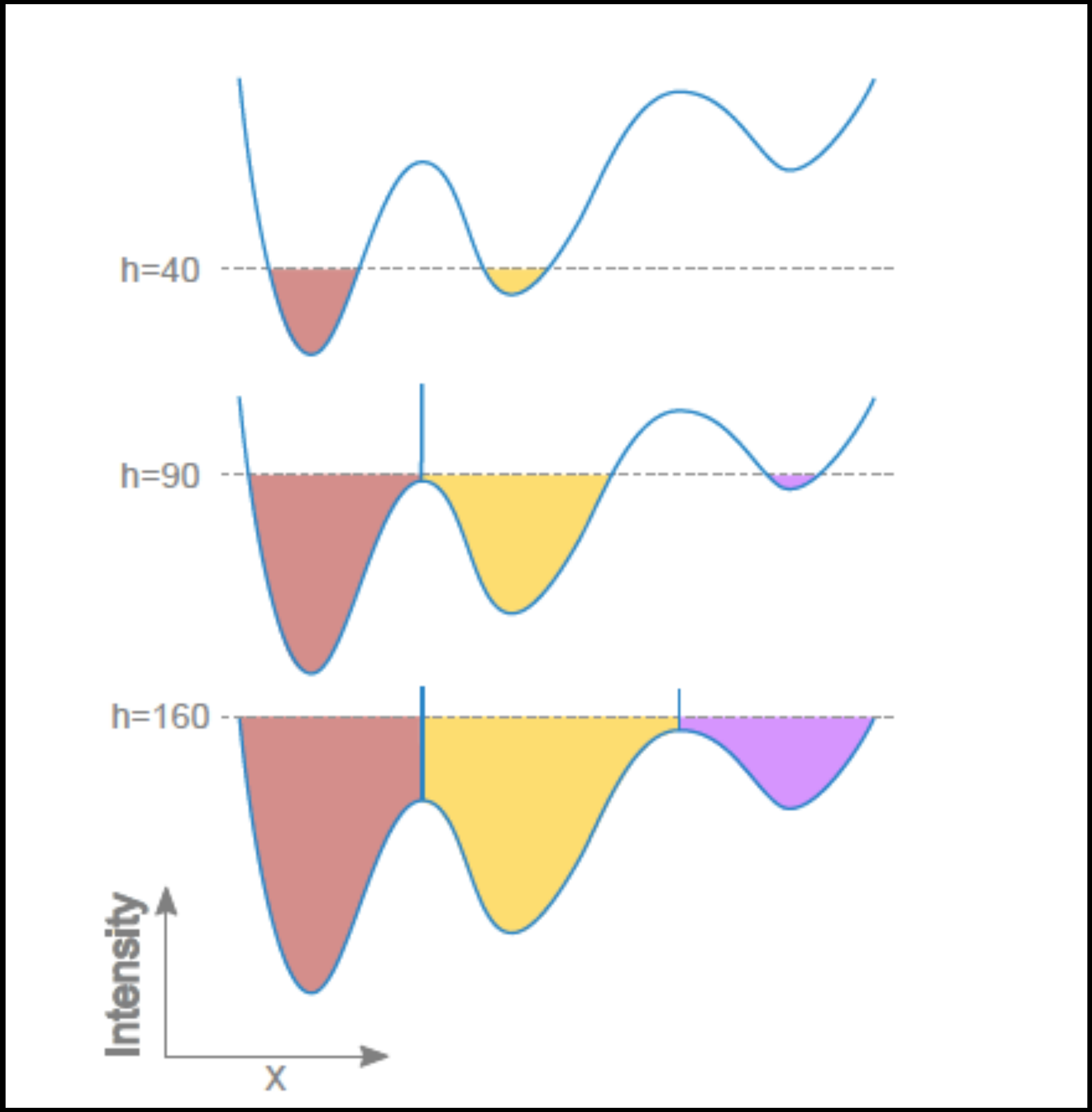

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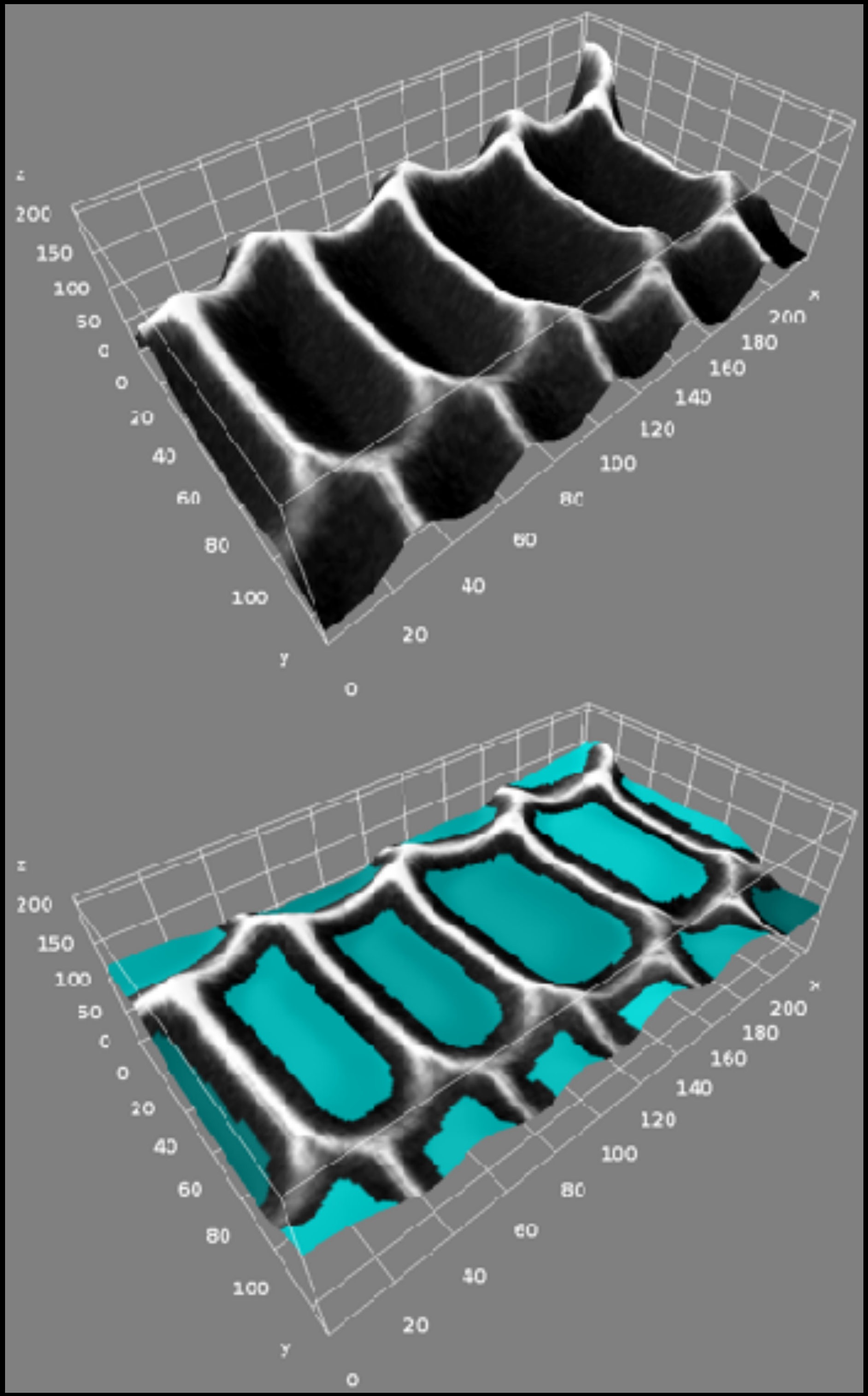
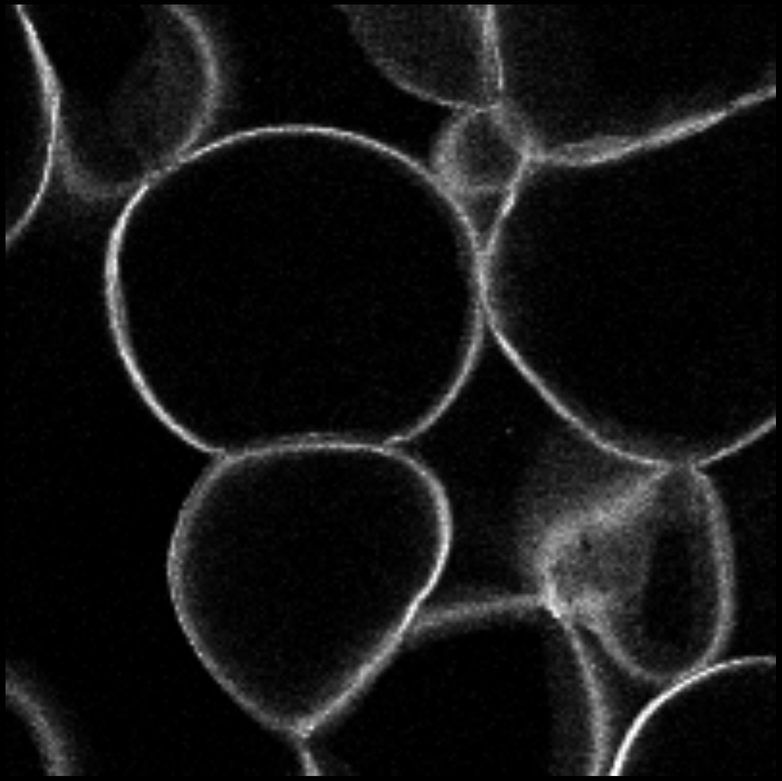


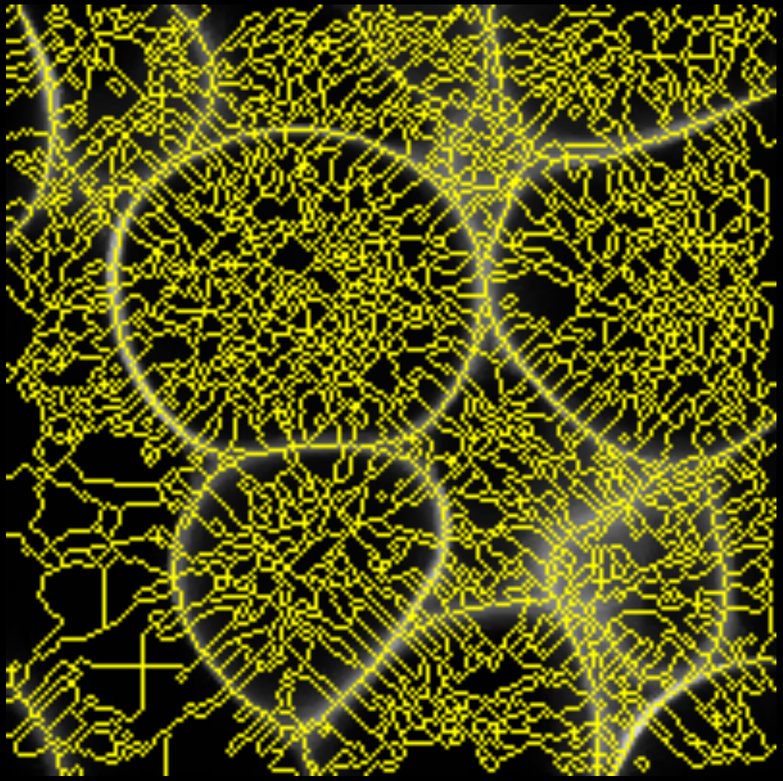
http://imagej.net/MorphoLibJ

Watershed limitations

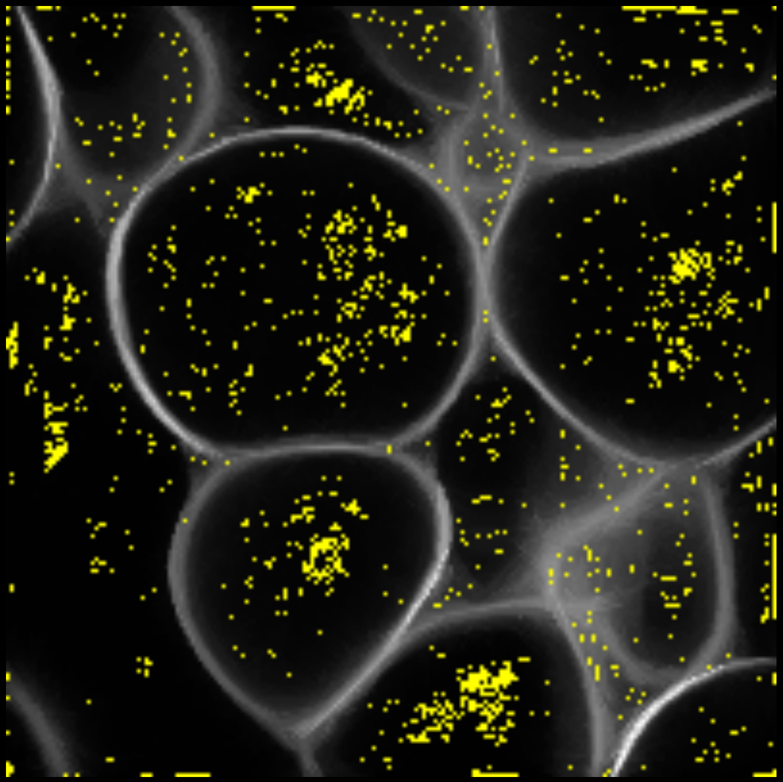
- Over-segmentation (too many regions)
  - due to the presence of many local minima



original image



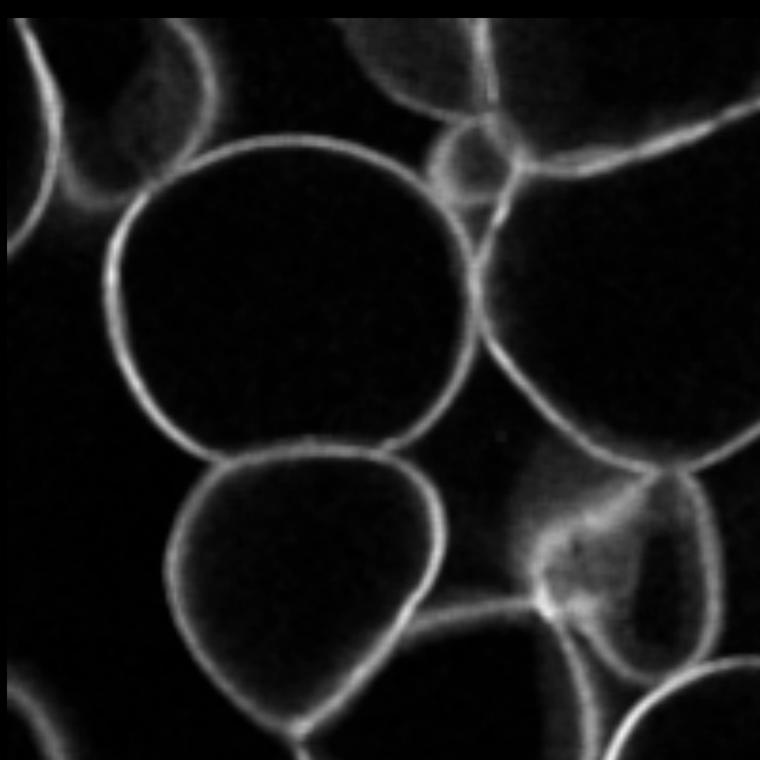
watershed segmentation

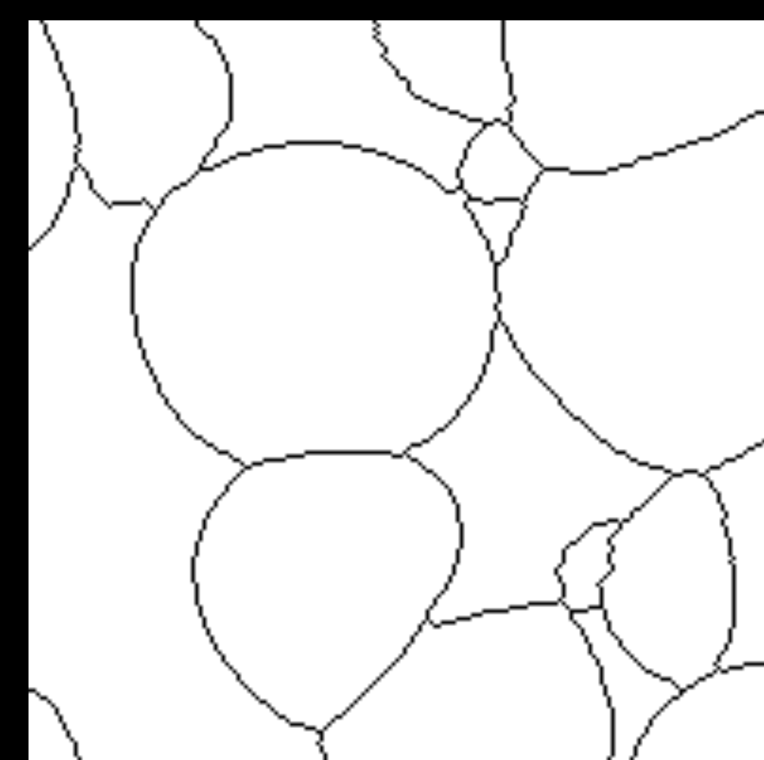


local minima

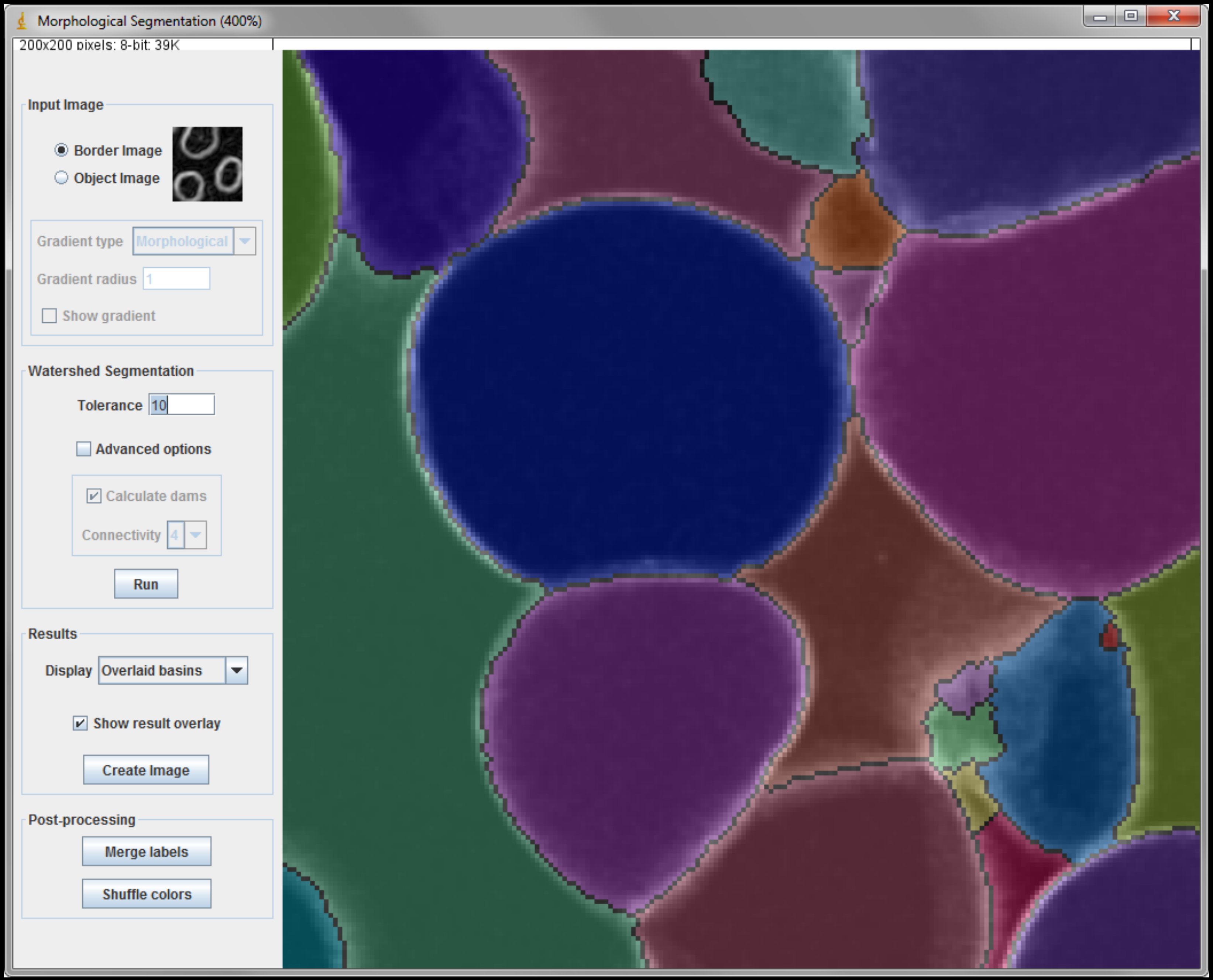
Solutions to over-segmentation

- Idea: remove unwanted minima
  - Filtering of input image (Gaussian, median...)
    - Automatically detect minima
    - Use extended minima





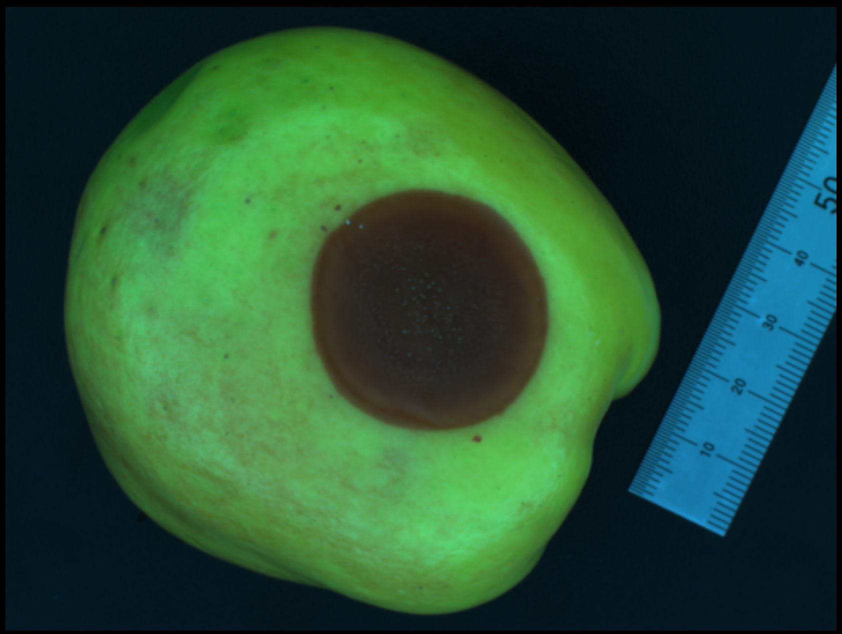
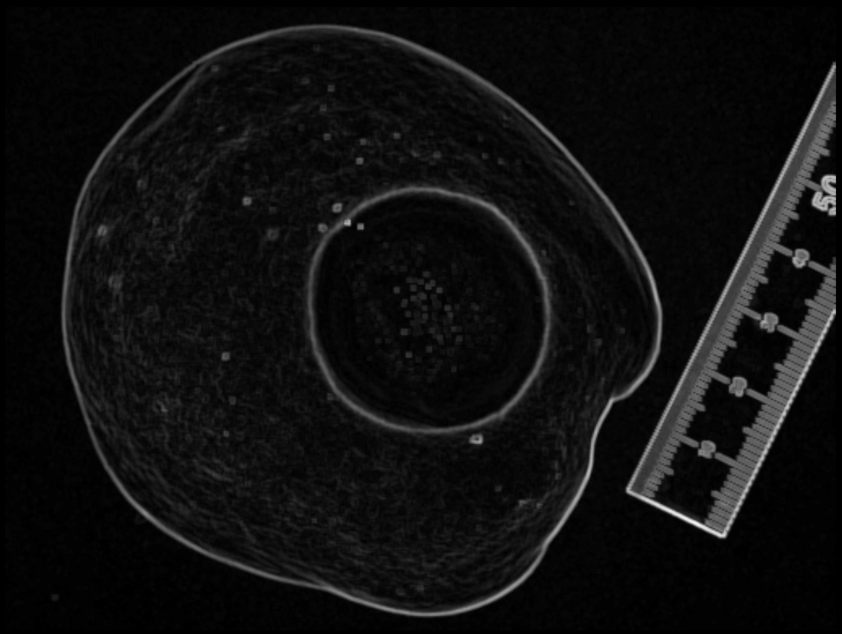
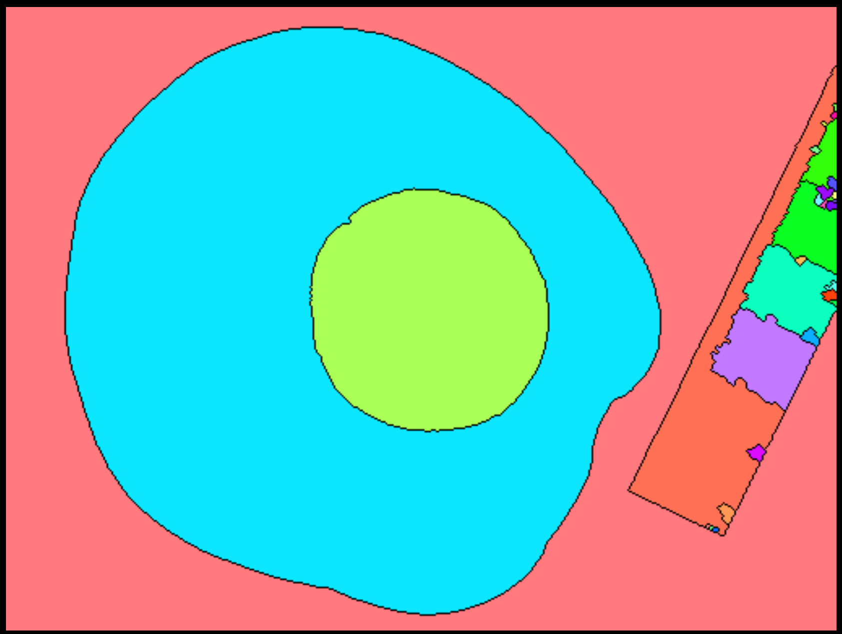
Implemented with GUI



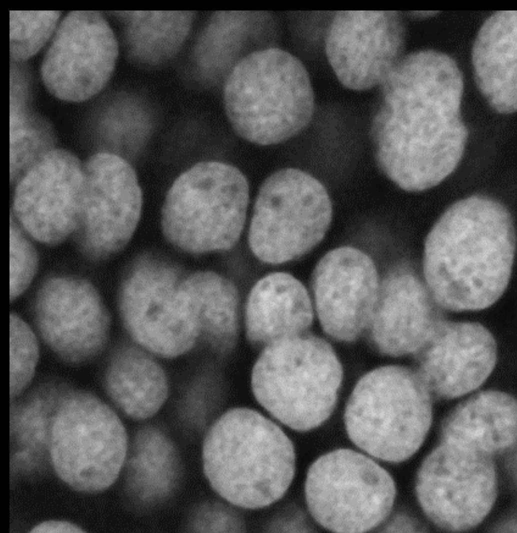
Plugins ▶ MorphoLibJ ▶ Segmentation ▶ Morphological Segmentation

More on watershed: segmentation of contrasted object

- Idea: apply watershed on gradient of image
- Gradient can be of any type (linear, morphological)...

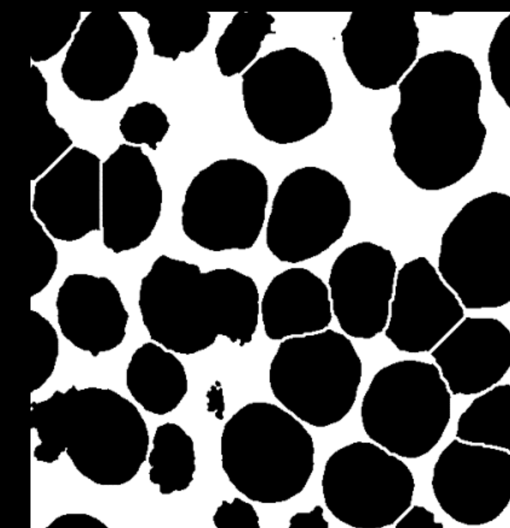




More on watershed: separation of binary particles

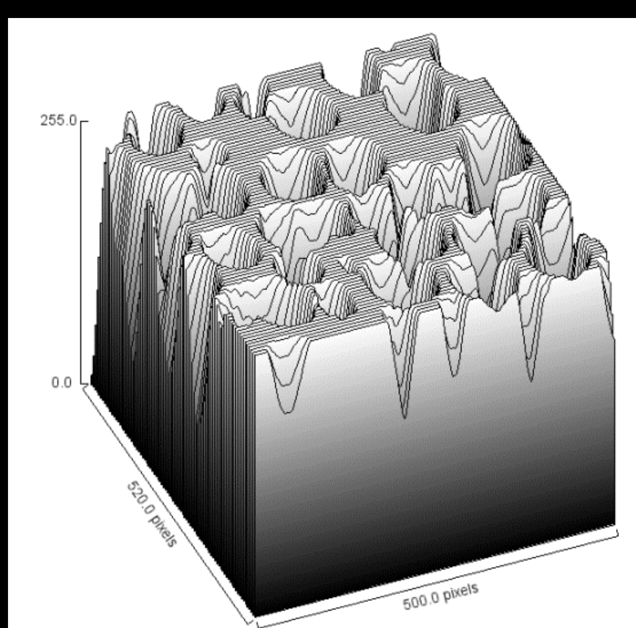


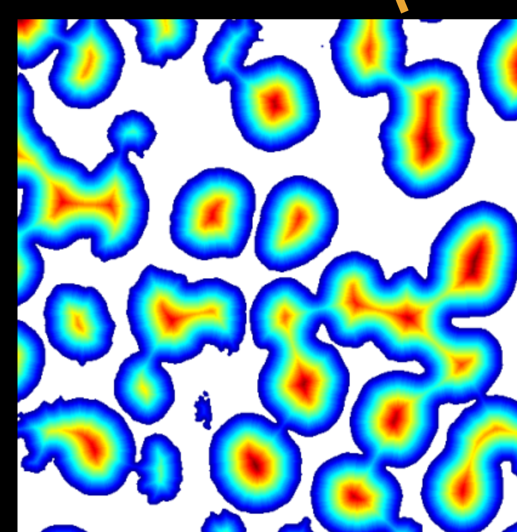
input image

touching nuclei

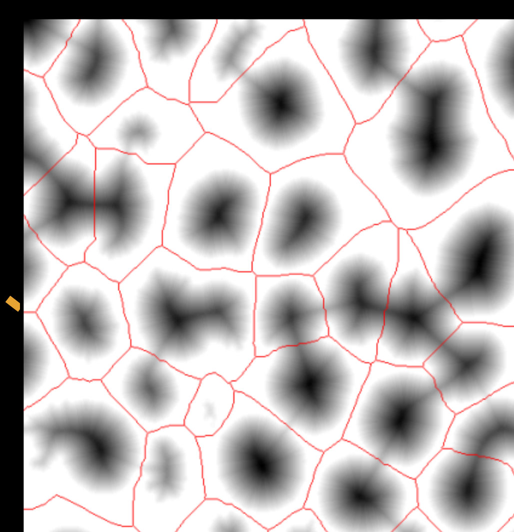


separated nuclei

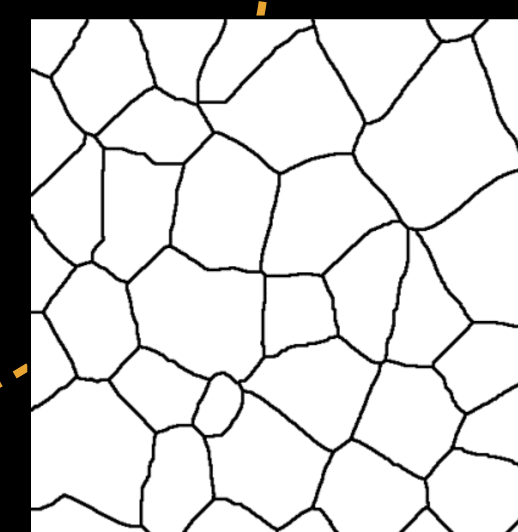




distance map



WS on inverse of distance map



watershed lines

http://imagej.net/Distance_Transform_Watershed

Machine learning based segmentation

Machine learning

- Subfield of computer science that "gives computers the ability to learn without being explicitly programmed" (Arthur Samuel, 1959).

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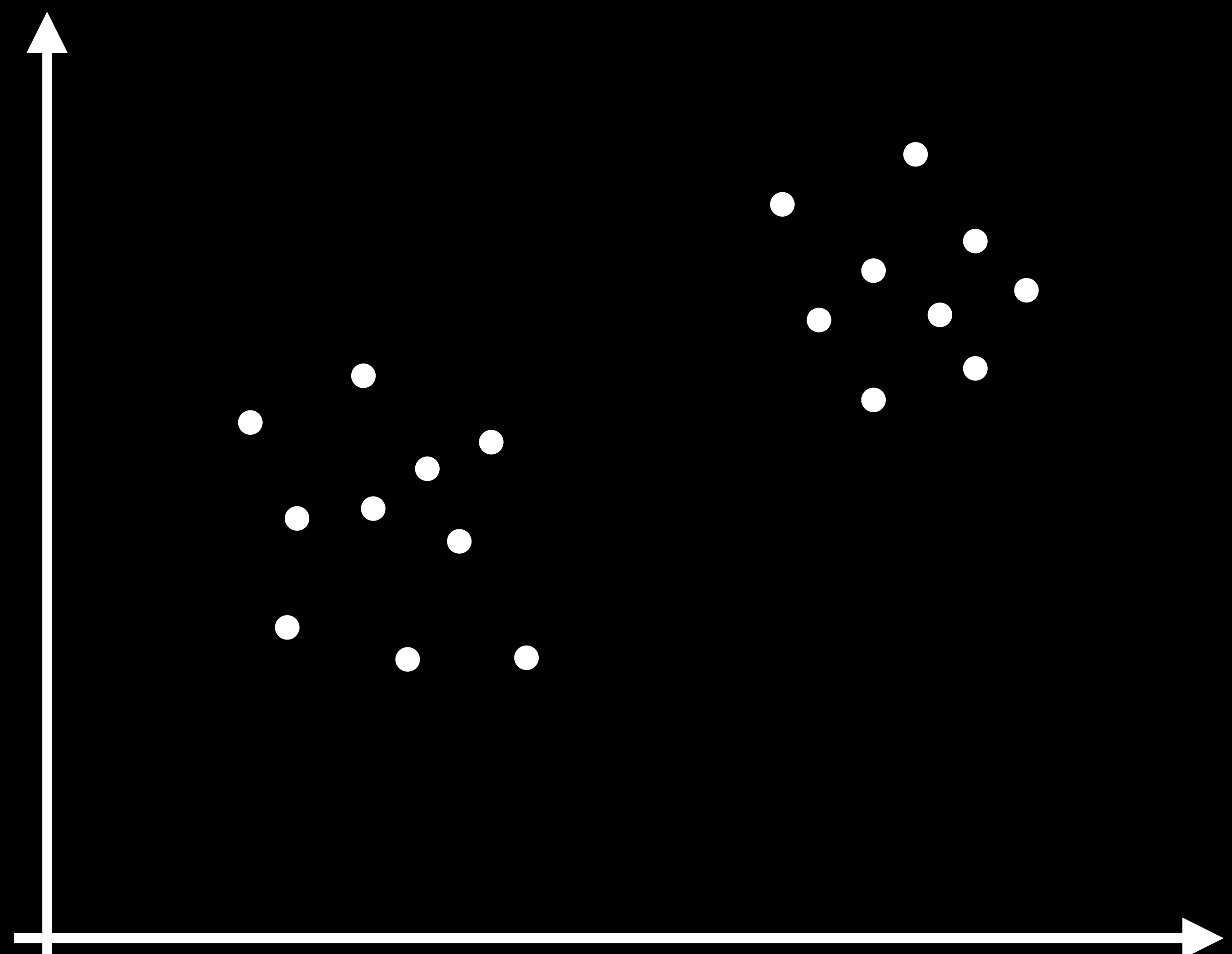
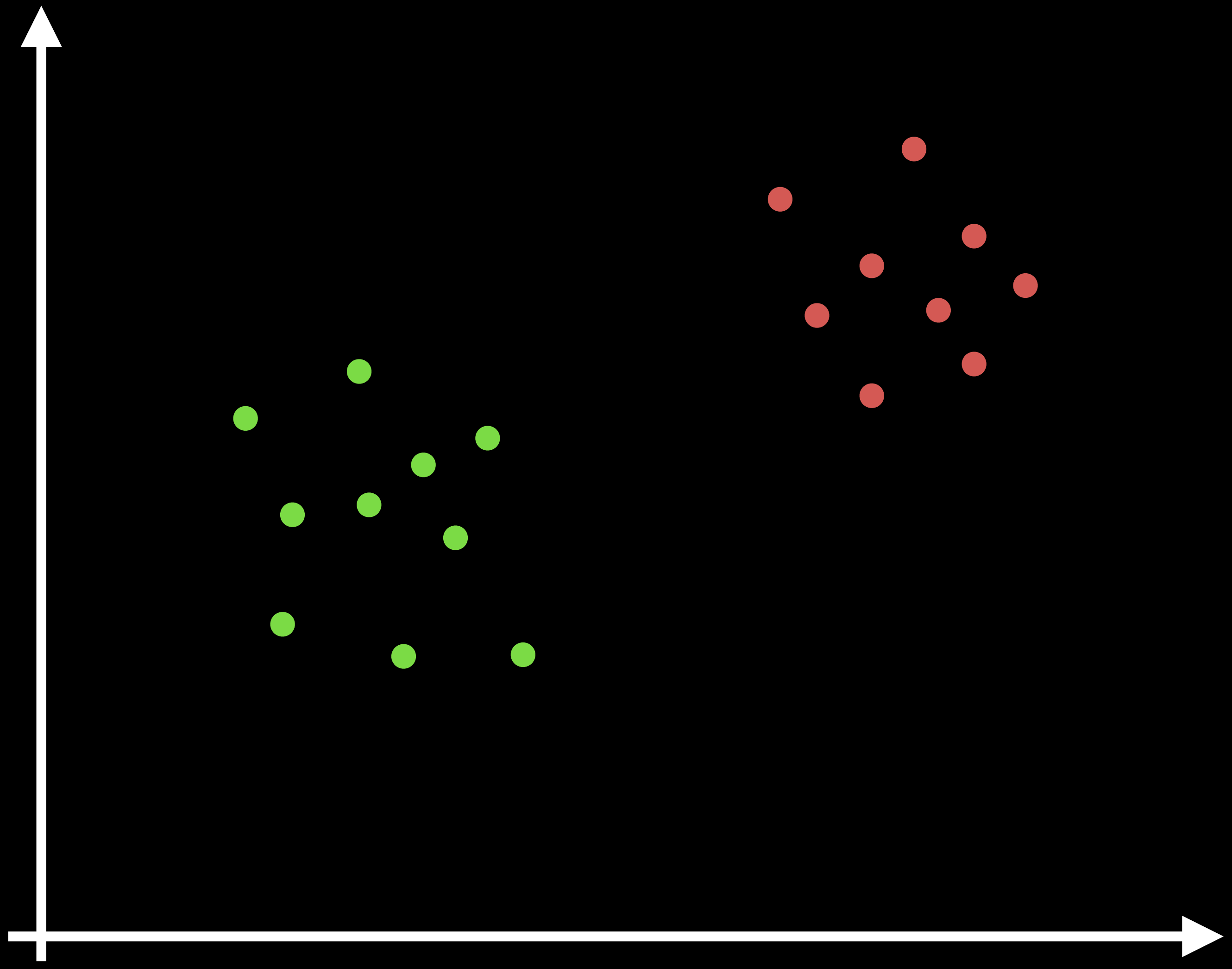


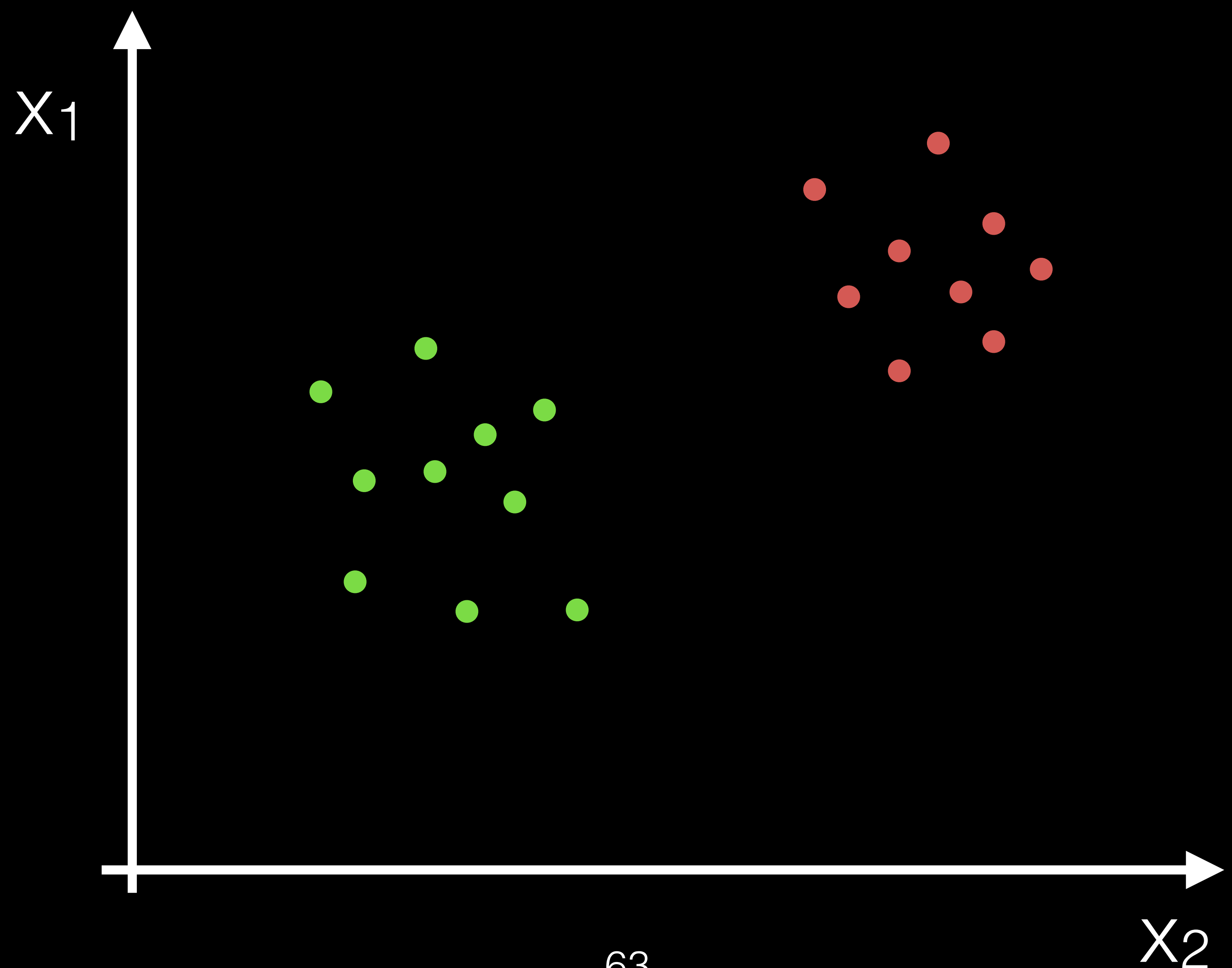
Machine learning

- Subfield of computer science that "gives computers the ability to learn without being explicitly programmed" (Arthur Samuel, 1959).
- Assign labels to objects indicating their class.

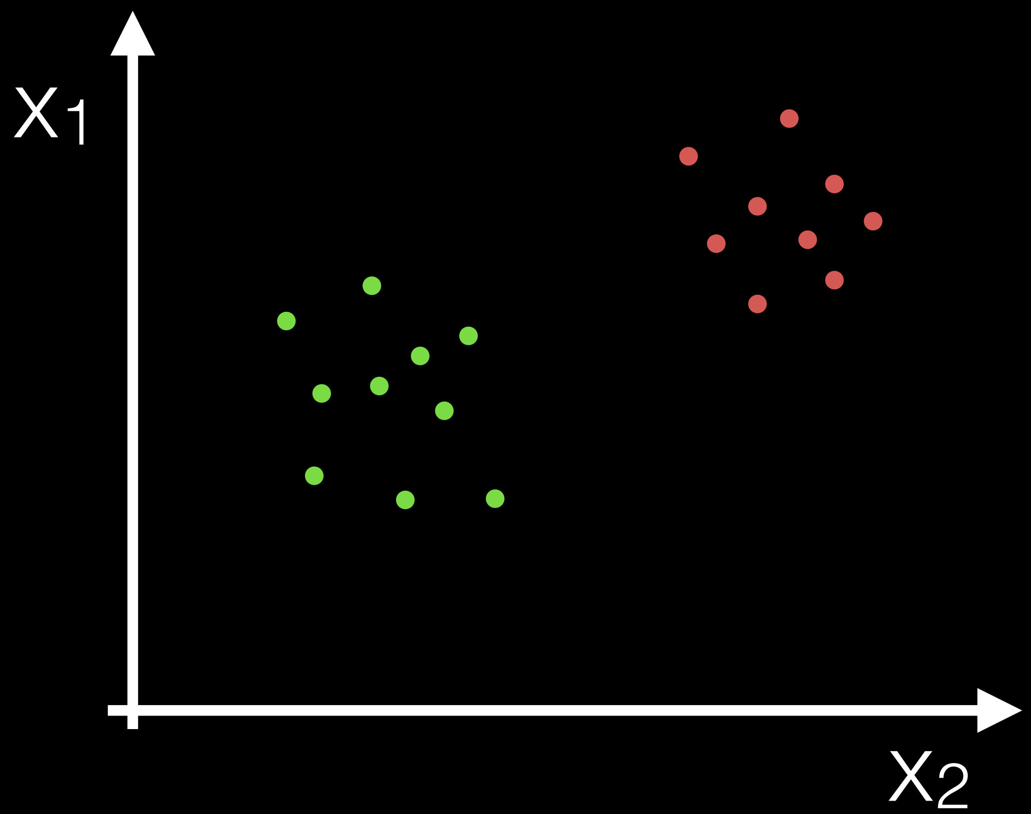


Machine learning

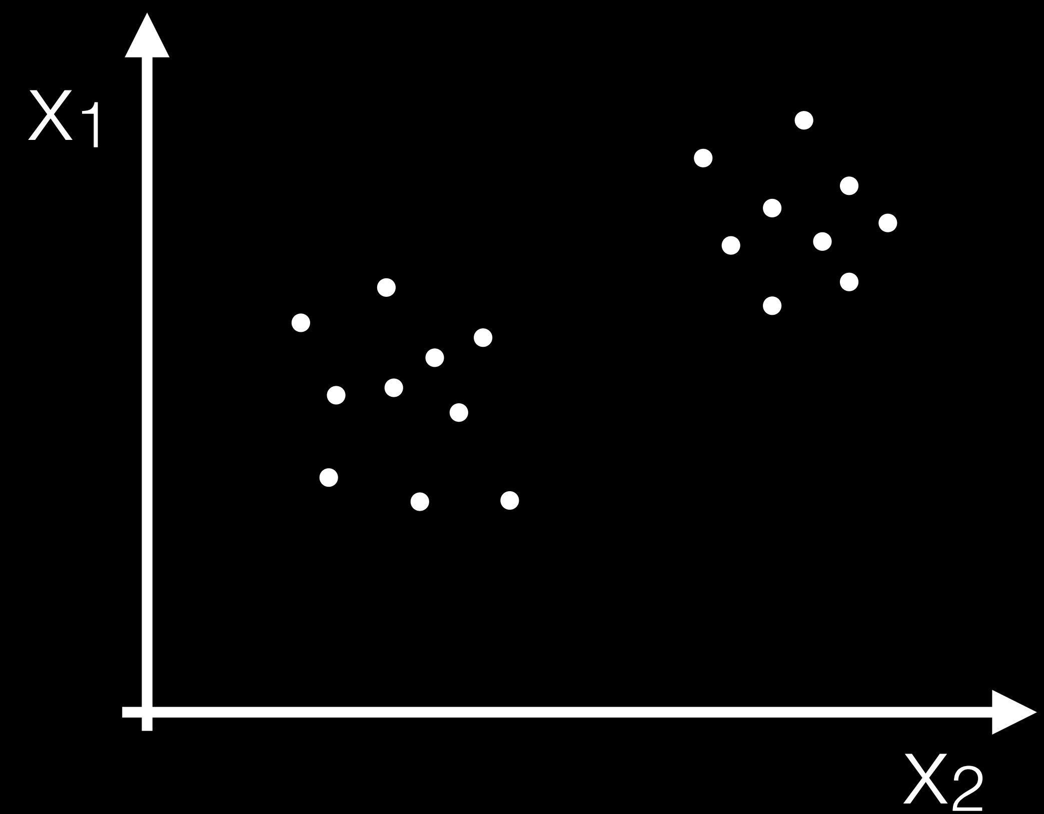
- Subfield of computer science that "gives computers the ability to learn without being explicitly programmed" (Arthur Samuel, 1959).
- Assign labels to objects indicating their class.
- Objects represented by a set of measurements or features.



Supervised vs unsupervised learning

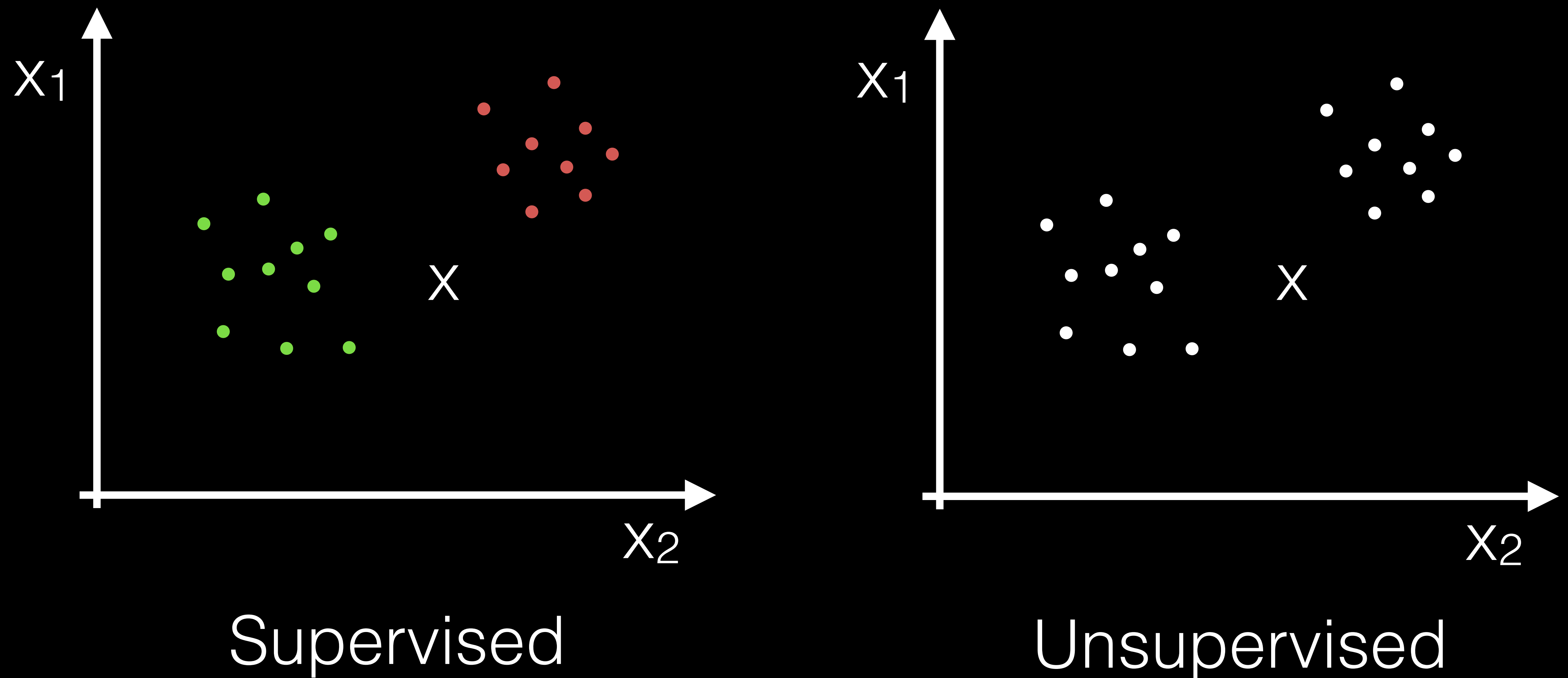


Supervised



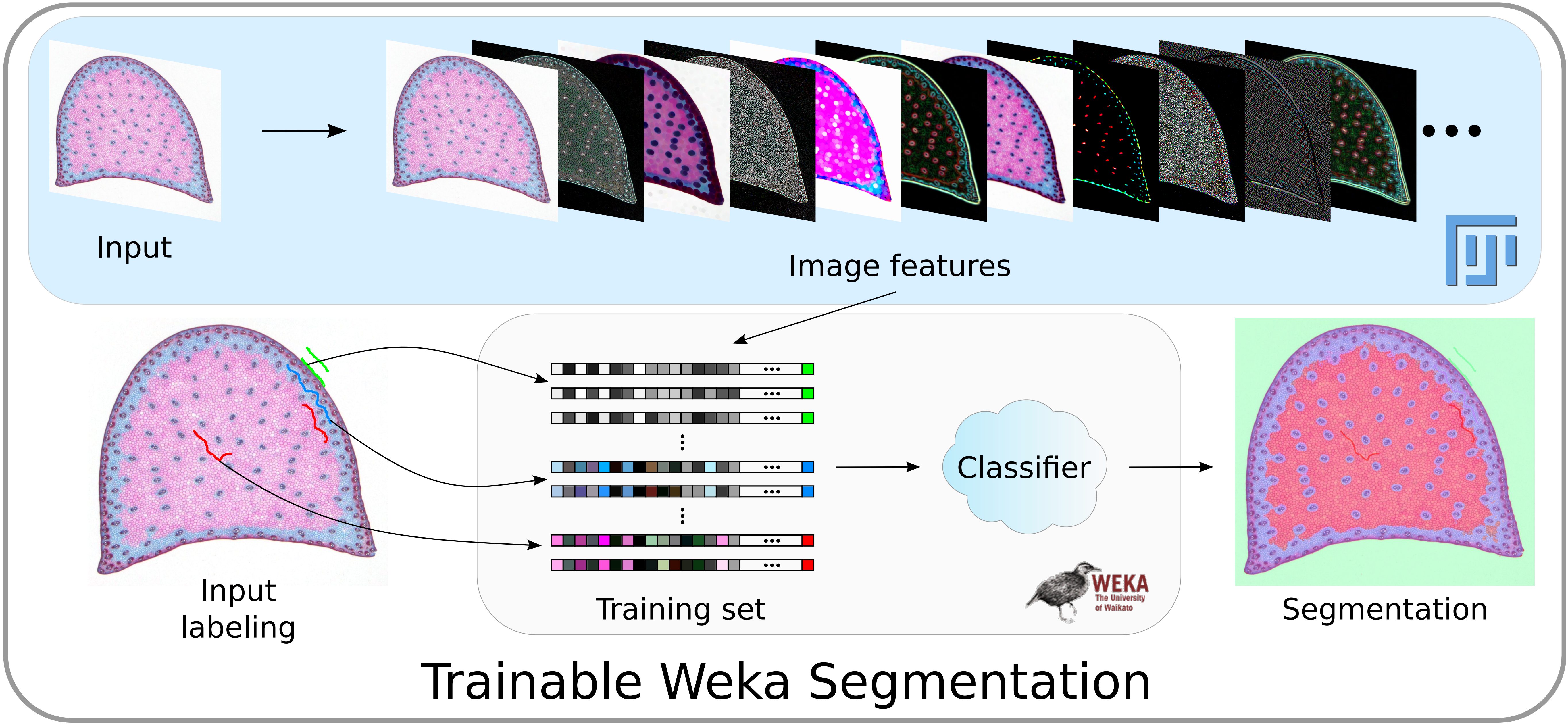
Unsupervised

Supervised vs unsupervised learning



- To which class belongs each new point *x*?

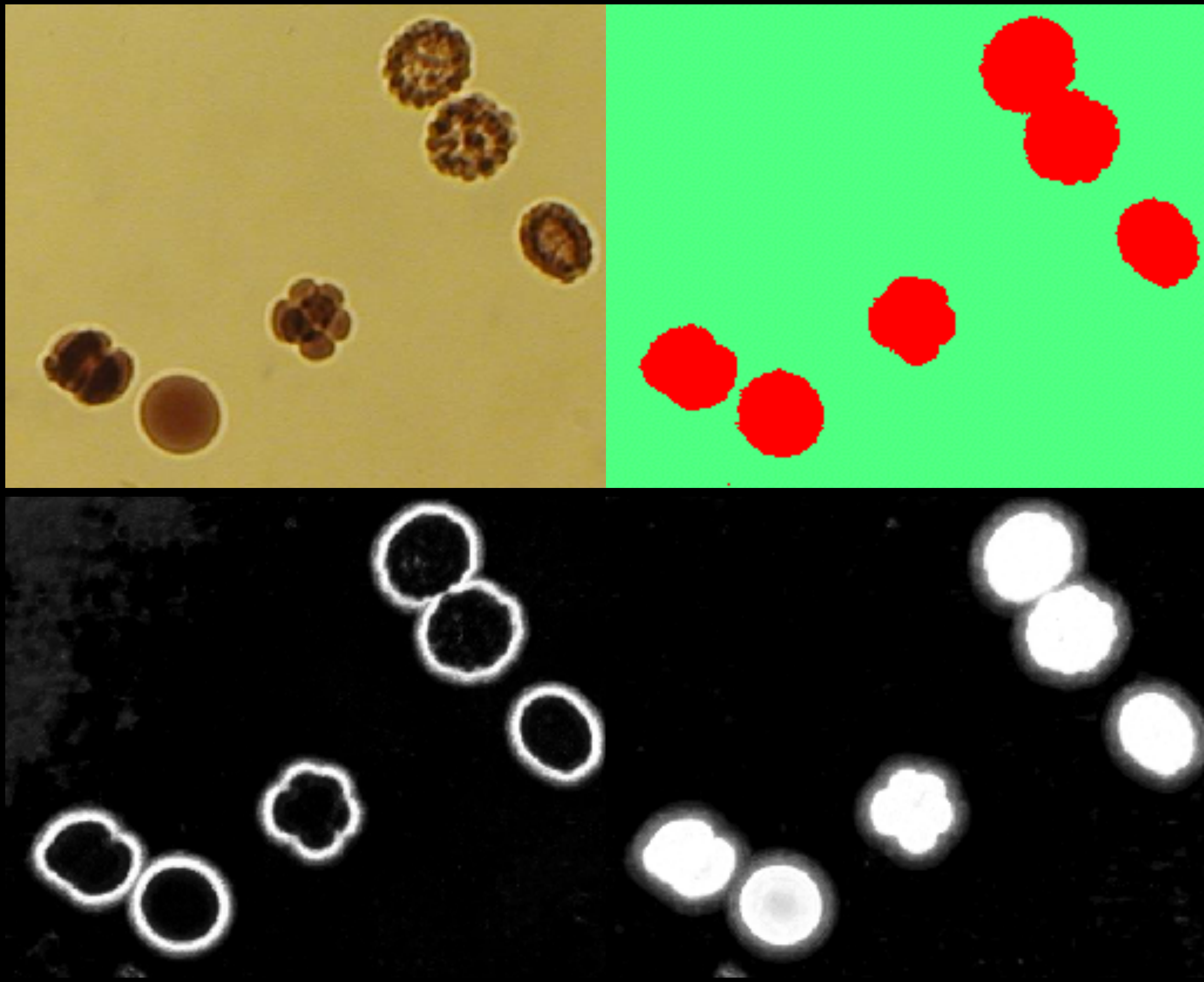
Pixel classification



http://fiji.sc/Trainable_Weka_Segmentation

Pixel classifier versatility

- As a pixel classifier, the Trainable Weka Segmentation presents a wide range of applications:
  - boundary detection,
  - semantic segmentation,
  - object detection,
  - object localization…



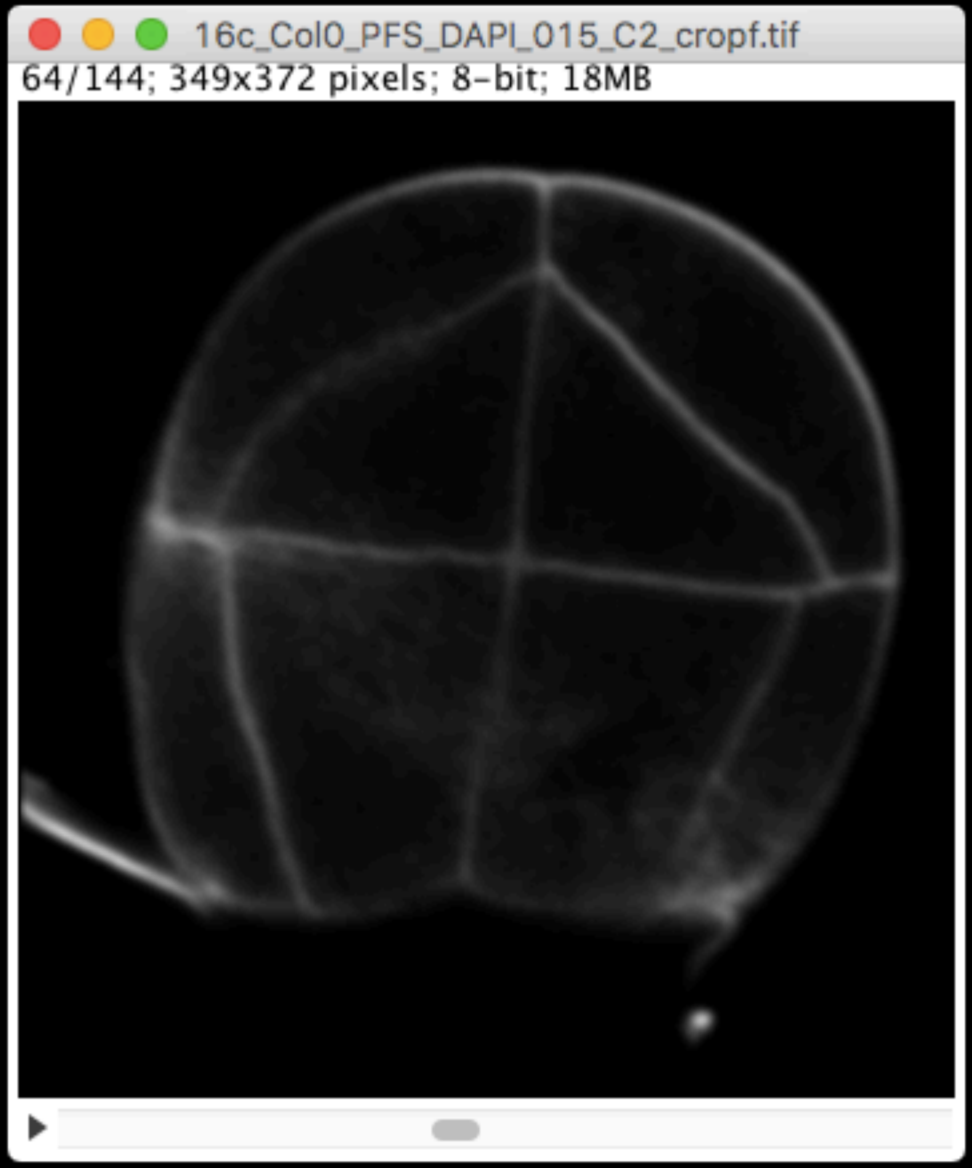
Take home messages

- There is no universal segmentation method.
- It is recommended to try different approaches to determine which one works best for our specific image data.
- Advices:
  - Keep image acquisition conditions fixed.
  - Normalize intensities to reuse successful methods.

Hands-on Tutorial

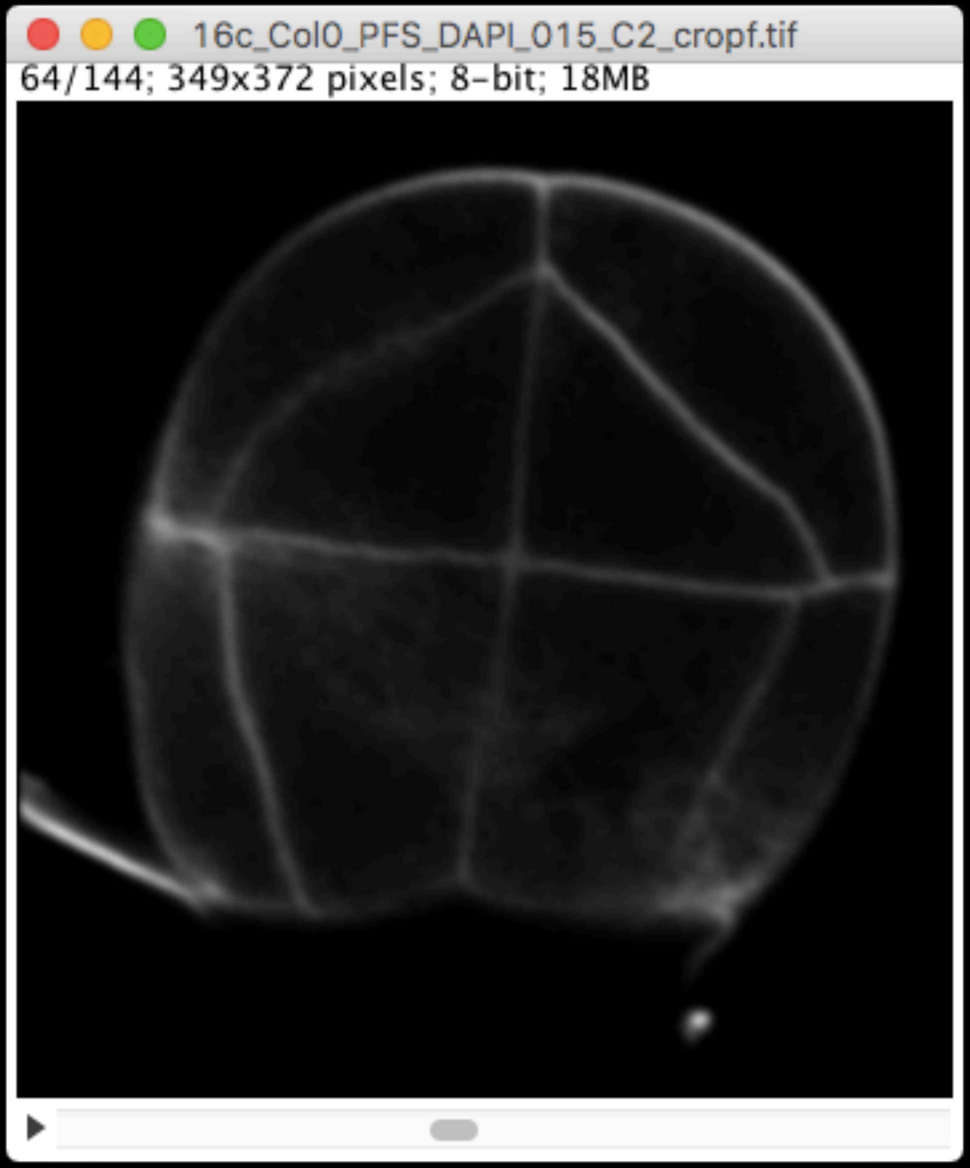
Arabidopsis embryo (LM)

- Open arabidopsis-embryo.zip
- Set correct calibration (1,1,3).



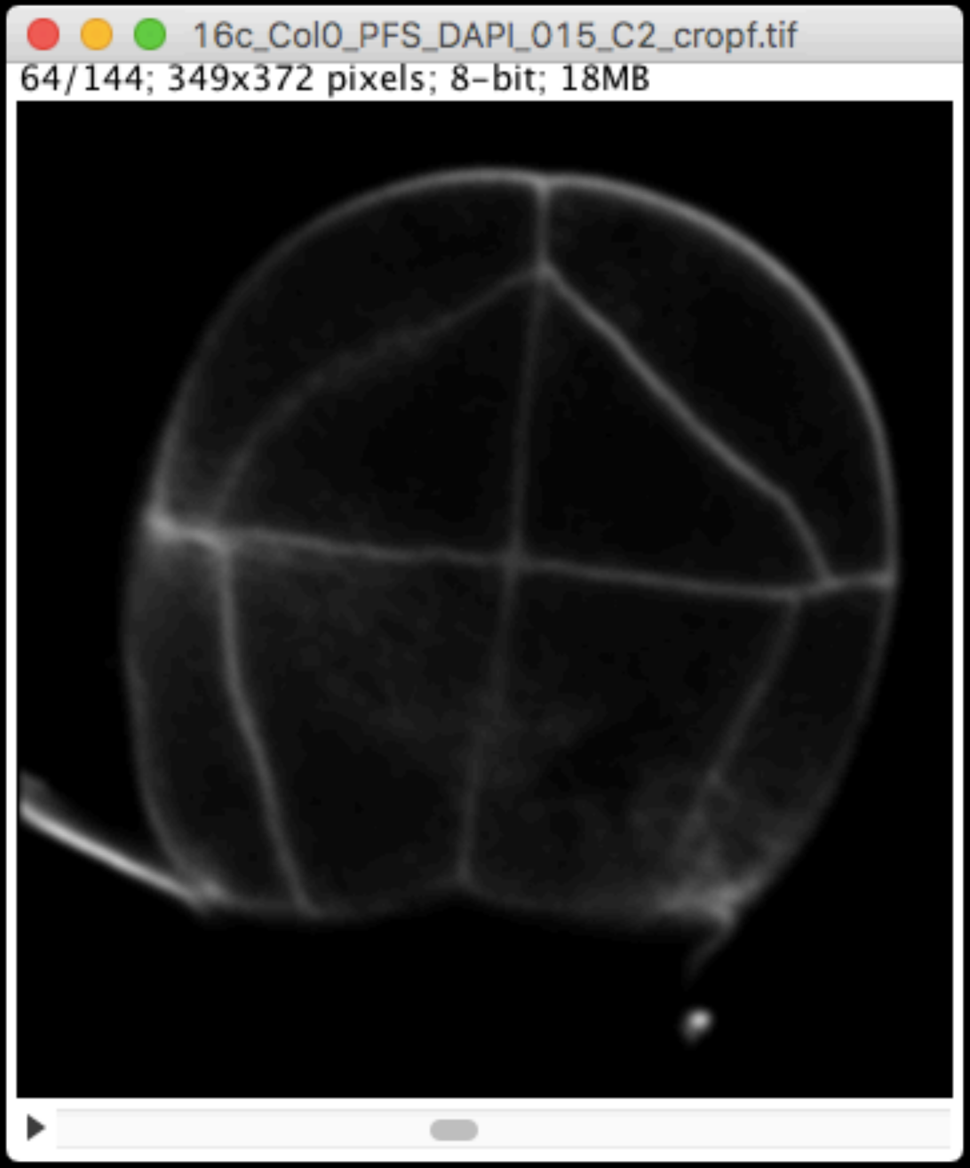
Arabidopsis embryo (LM)

- Open arabidopsis-embryo.zip
- Set correct calibration (1,1,3).
- Try segmenting by manual thresholding (Image > Adjust > Threshold…)



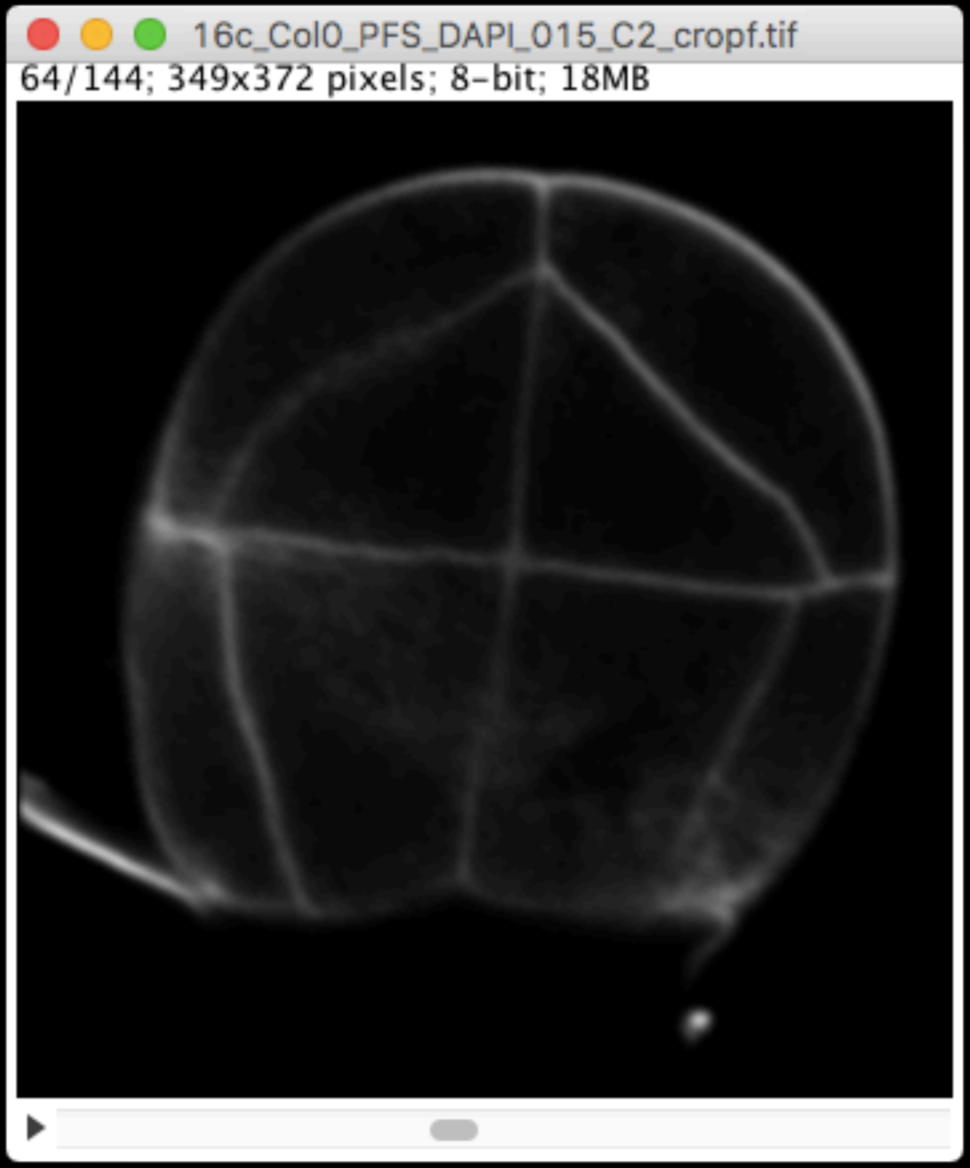
Arabidopsis embryo (LM)

- Open arabidopsis-embryo.zip
- Set correct calibration (1,1,3).
- Try segmenting by manual thresholding (Image > Adjust > Threshold…)
- Try automatic threshold on a slice (Image > Adjust > Auto Threshold)



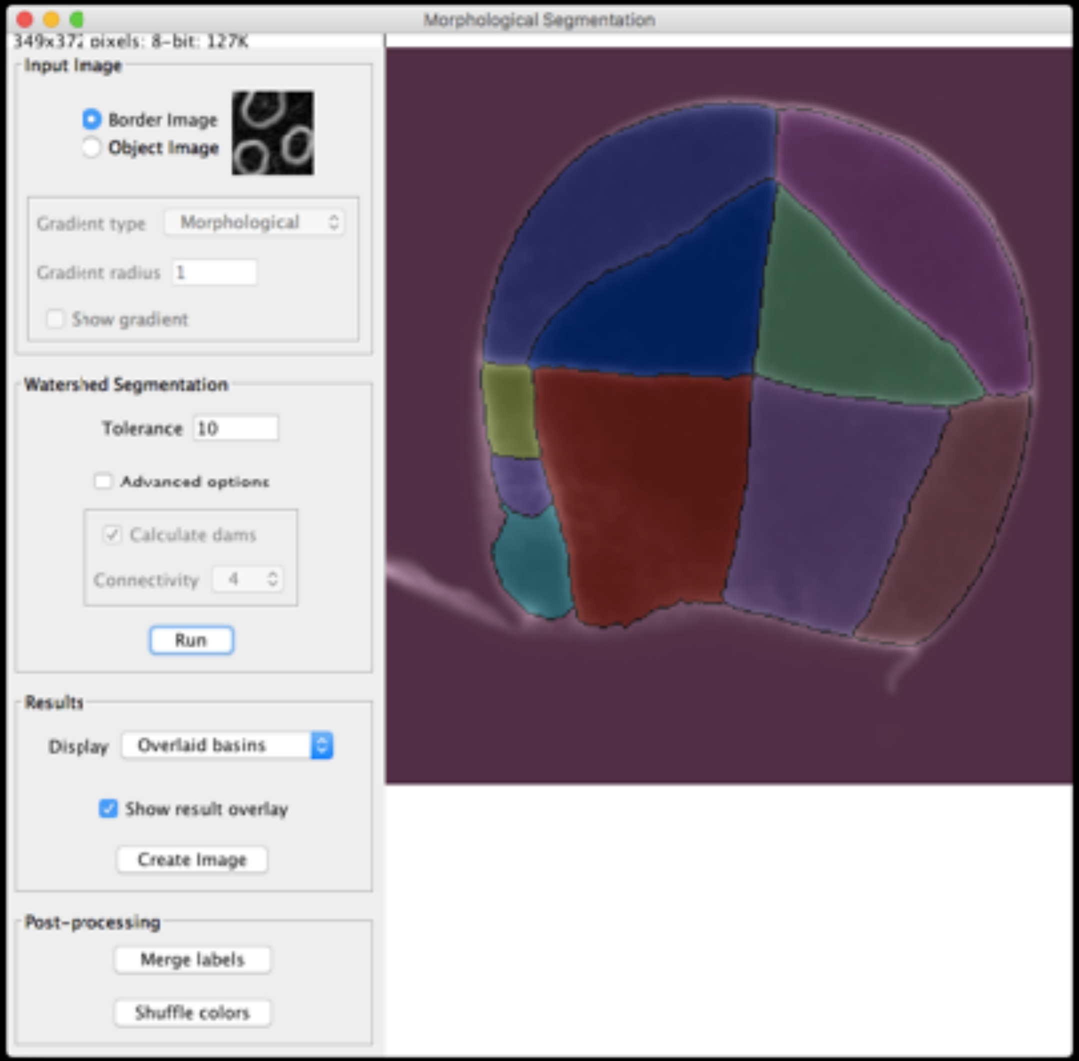
Arabidopsis embryo (LM)

- Try automatic threshold on stack (Image > Adjust > Auto Threshold)



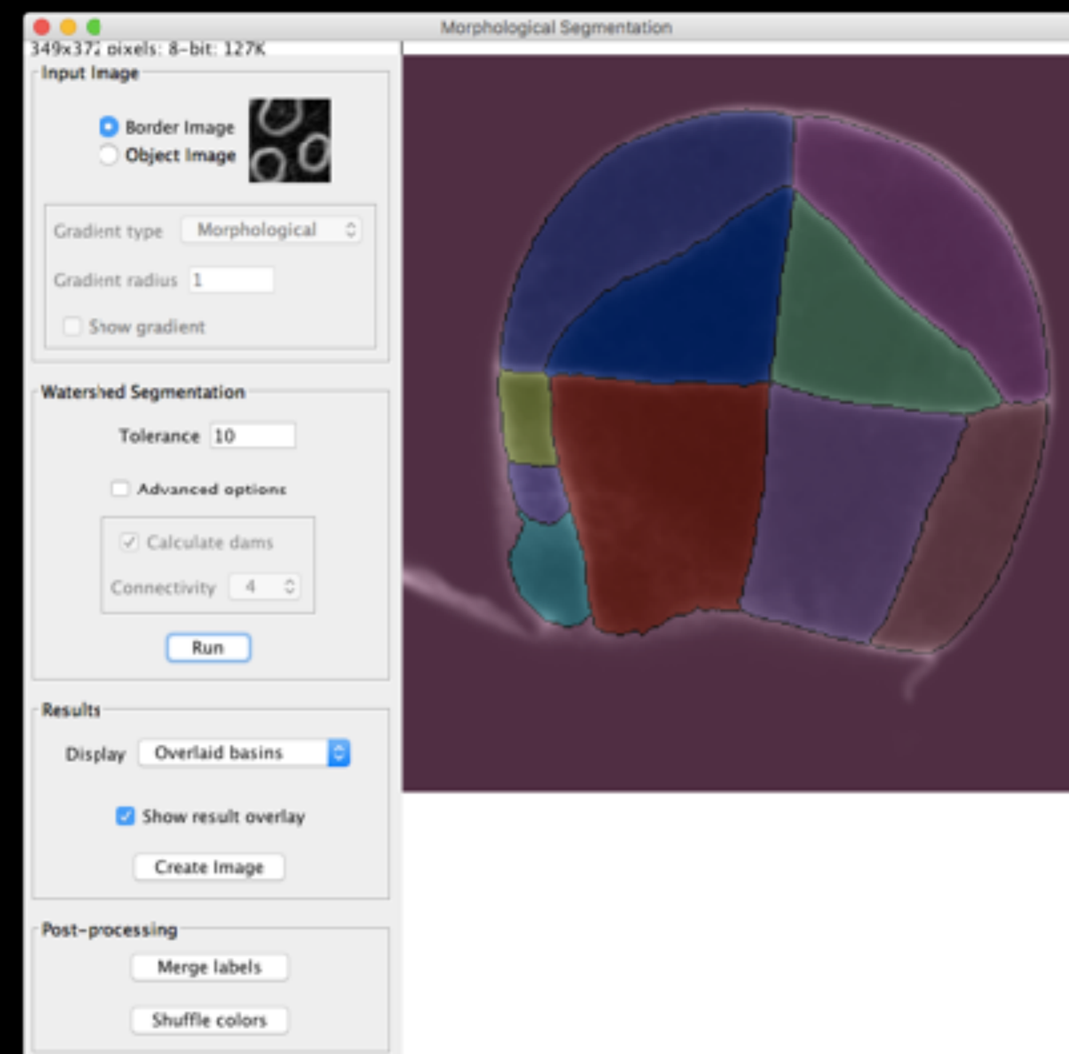
Arabidopsis embryo (LM)

- Try automatic threshold on stack (Image > Adjust > Auto Threshold)
- Try Morphological Segmentation (Plugins > MorphoLibJ > Segmentation) on slice (2D)



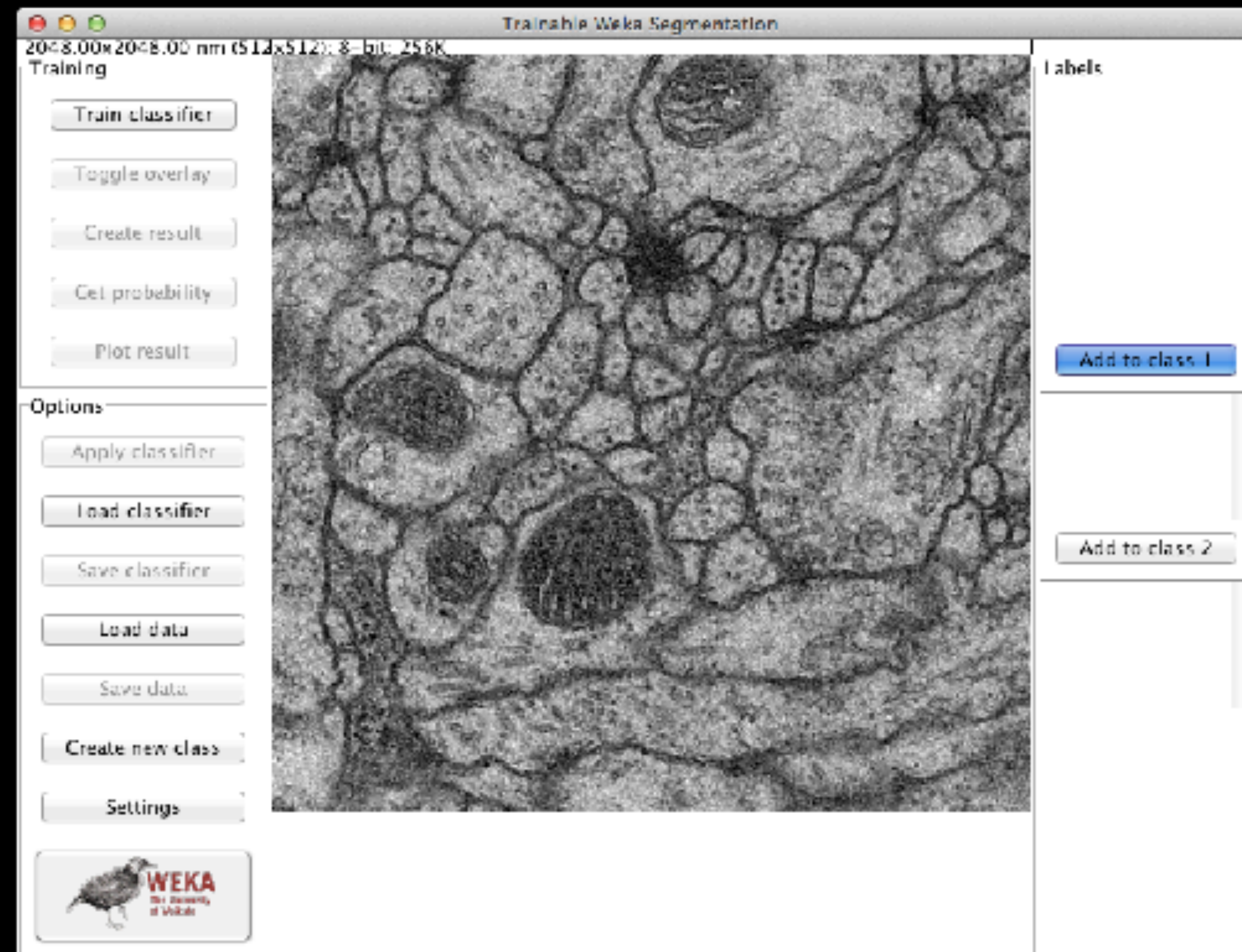
Arabidopsis embryo (LM)

- Try automatic threshold on stack (Image > Adjust > Auto Threshold)
- Try Morphological Segmentation (Plugins > MorphoLibJ > Segmentation) on slice (2D)
- Try Morphological Segmentation (Plugins > MorphoLibJ > Segmentation) on stack (3D)



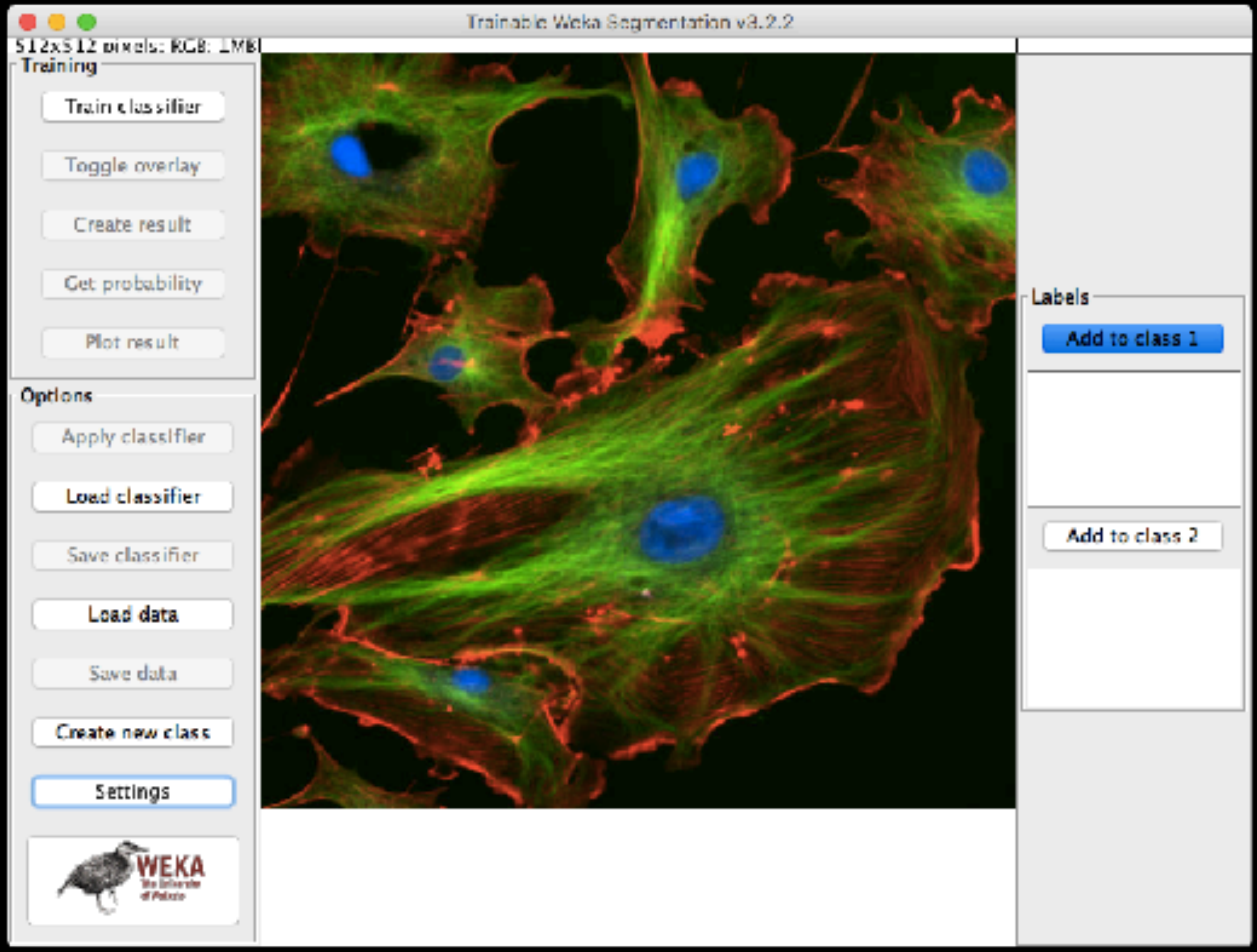
Drosophila embryo (TEM)

- Open test-volume-1.tif
- Call Trainable Weka Segmentation (under Plugins > Segmentation)
- Trace
- Train
- Get results and probabilities
- Apply to test-volume-2.tif
- Balance classes
- …



Unsupervised learning example

- Open sample "Fluorescent Cells".
- Convert to RGB and remove text.
- Call TWS.
- Use ClassificationViaClustering with
  - SimpleKMeans (K=4)
  - EM



Slides credits and references

- Dr. Ulas Bagci, UCF, CAP5415-Computer Vision.
- Dr. David Legland, “Mathematical Morphology for plant sciences”, Microscopie Fonctionnelle en Biologie (2016).
- Ignacio Arganda-Carreras & Philippe Andrey, “Designing Image Analysis Pipelines in Light Microscopy: A Rational Approach”, Light Microscopy: Methods and Protocols (2017).
- ImageJ/Fiji wiki site.