

UT Southwestern
Medical Center
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BioHPC

Introduction to ImageJ

[web] portal.biohpc.swmed.edu

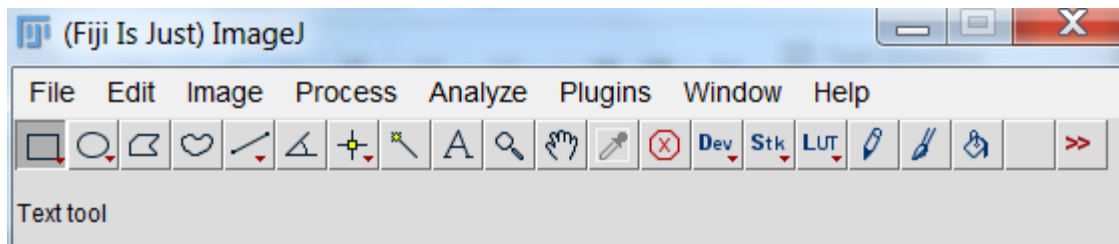
[email] biohpc-help@utsouthwestern.edu

Introduction to ImageJ

ImageJ is a public domain, multi-threaded Java-based image processing program developed at the National Institutes of Health.

ImageJ was designed with an open architecture that provides extensibility via Java **plugins** and recordable **macros**.

Fiji Is Just (the enhanced version of) **ImageJ**.



Introduction to ImageJ

ImageJ can read and process a large variety of image formats (e.g. TIF, DICOM, HDF5), either individually or in a group.

ImageJ can display multiple spatially or temporally related images in a single window. These image sets are called **stacks**:

File->Import->Image Sequence



Accessing ImageJ Software on BioHPC:

1. Use existing installed ImageJ modules and run the following on the command line interface:
 1. `module av ImageJ`
 2. `module load <imagej-version>`
 3. `ImageJ-linux64`
2. Download ImageJ from <https://imagej.net/software/fiji/downloads> (select **Linux 64-bit**)
 1. Store the ImageJ files in your BioHPC storage (`/work`, `/project` or `/home2`)

Running ImageJ on BioHPC:

1. Reserve a webGUI/webGPU session from BioHPC portal:
<https://portal.biohpc.swmed.edu/terminal/webgui/>
2. Submit a SLURM job via sbatch in case you want to run a macro/plugin interactively
 1. Preferably use plugins/scripts rather than macros
 2. In case of macros, use virtual buffers: <https://imagej.net/learn/headless>

Main Components of ImageJ

<https://imagej.nih.gov/ij/docs/menus/>

Selection
tools

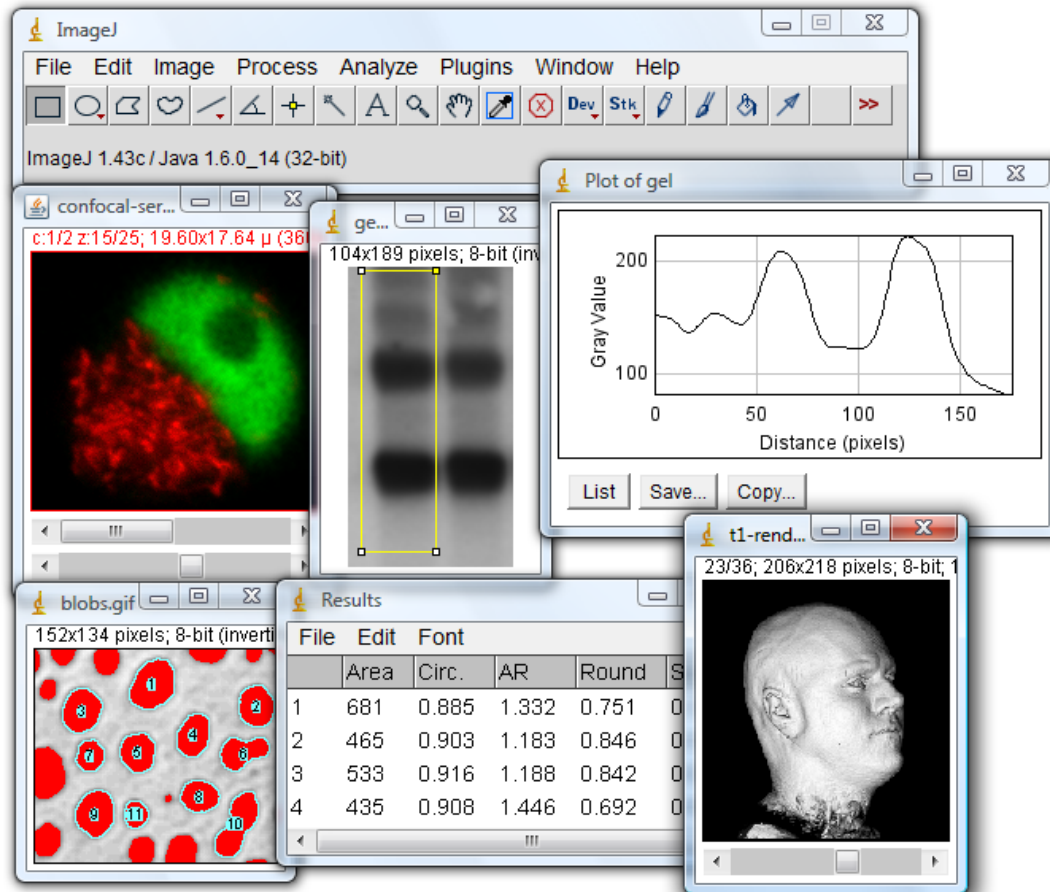
Image
menu

Process
menu

Edit
menu

Analyze
menu

Plugins
menu



Selection Tools and Edit Menu

- Define region of interest (ROI)
- Apply tools to a selection area:
 - Set all the pixels inside ROI to zero (Clear)
 - Set all the pixels outside of ROI to zero (Clear outside)
 - Set all the pixels inside the ROI to one (Fill)
 - Set all the pixels on the ROI boundary to one (Draw)
 - Replace the intensity of the pixels inside of the ROI with the intensity of the pixels outside of ROI (Invert)
 - Create a mask from ROI

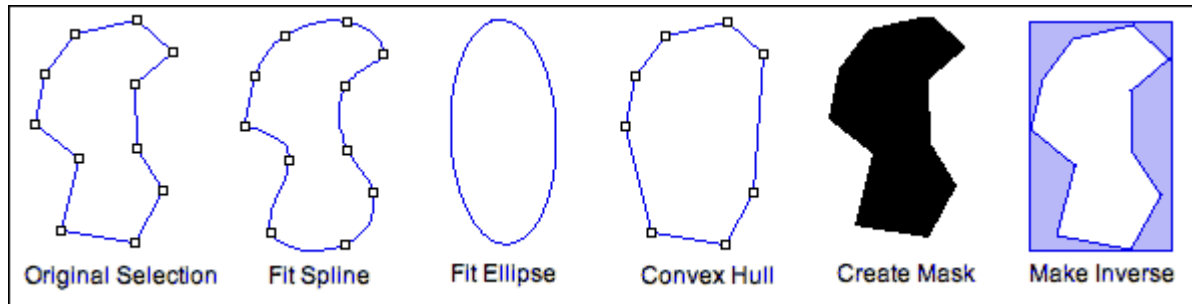
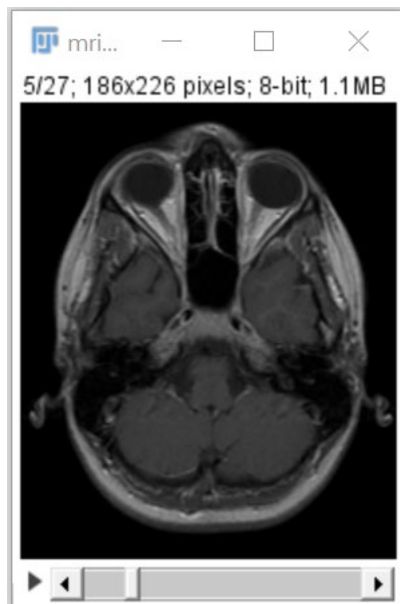


Image Menu

- Determine the type (e.g. 8bit grayscale) of the active image or to convert it to another type
- Adjust brightness/contrast
- Perform different transformations (zooming, scaling, vertical/horizontal flip, rotating)

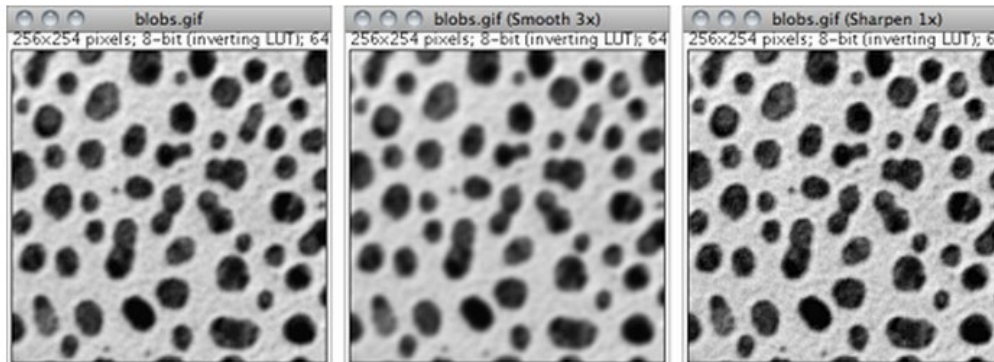
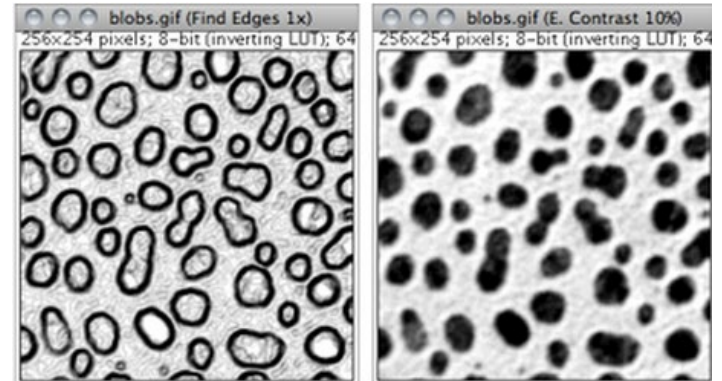


Contrast enhanced



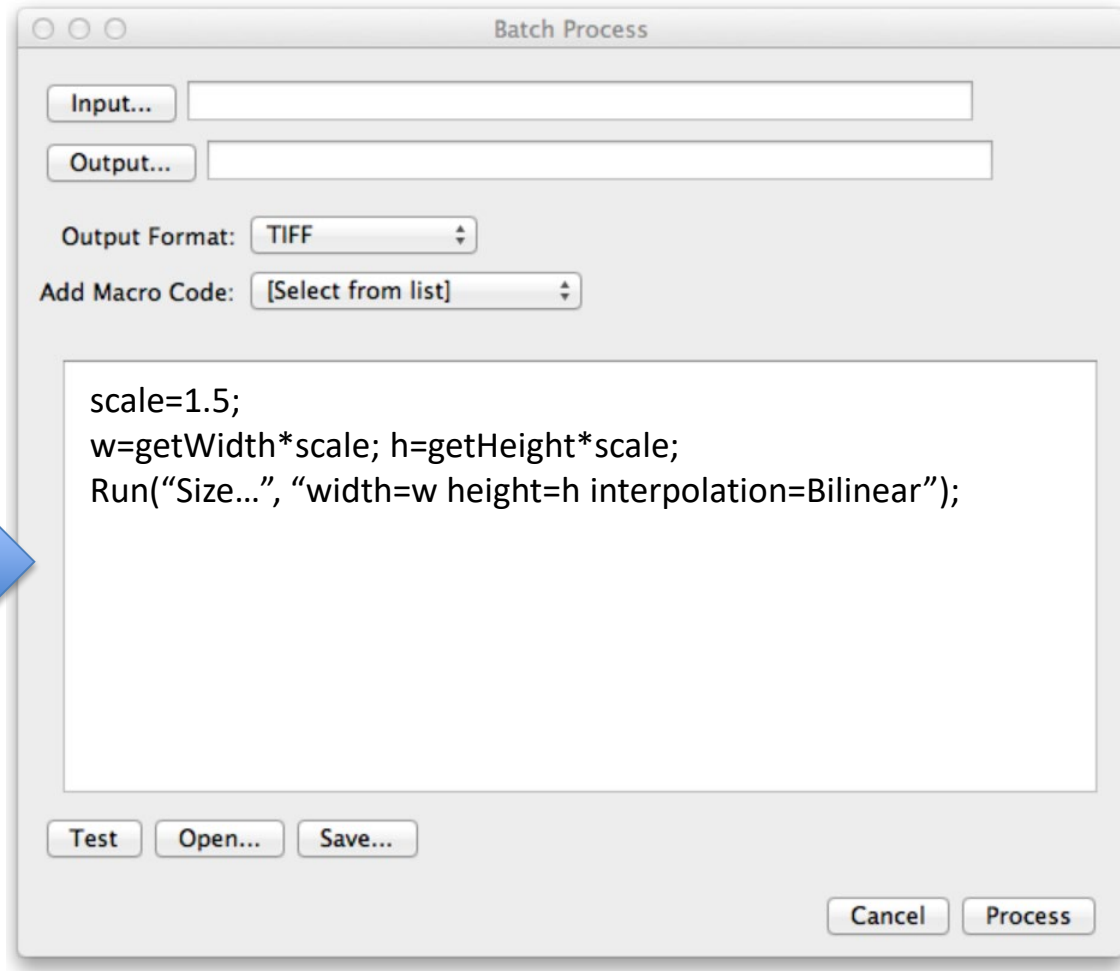
Process Menu

- Smoothing
- Sharpening
- Edge detection
- Apply different types of filter for denoising purpose
- FFT

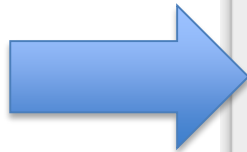


Process Menu

Process -> Batch -> Macro



Write macro code!



Sample ImageJ Macro Code

```
function action(input, output, filename) {
    open(input + filename);
    makeRectangle(10, 10, 300, 180);
    run("Crop");
    saveAs("Jpeg", output + filename);
    close();
}
```

```
input = "/home/fiji/input/";
output = "/home/fiji/images/";
```

```
setBatchMode(true);
```

```
list = getFileList(input);
for (i = 0; i < list.length; i++){
    action(input, output, list[i]);
}
```

```
setBatchMode(false);
```

<https://imagej.nih.gov/ij/macros/examples/>

Process Menu: Morphological filtering

Original

Make Binary ↓

Erode ↓

ImageJ ImageJ ImageJ

Dilate ↓

Open ↓

Close-- ↓

ImageJ ImageJ ImageJ

Outline ↓

SKELETONIZE ↓

ImageJ ImageJ

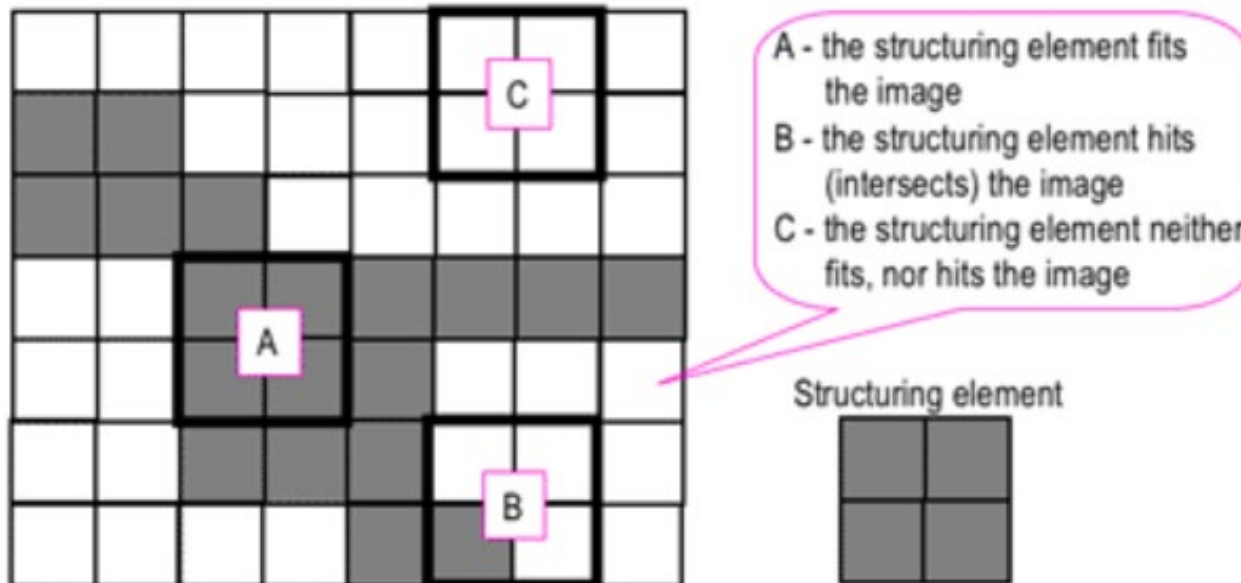
1pixel wide
outline

1pixel wide
skeleton

Morphological operations: Structuring element

The structuring element is a small binary image or matrix such that:

- The matrix dimensions specify the size of the structuring element.
- The pattern of ones and zeros specifies the shape of the structuring element.

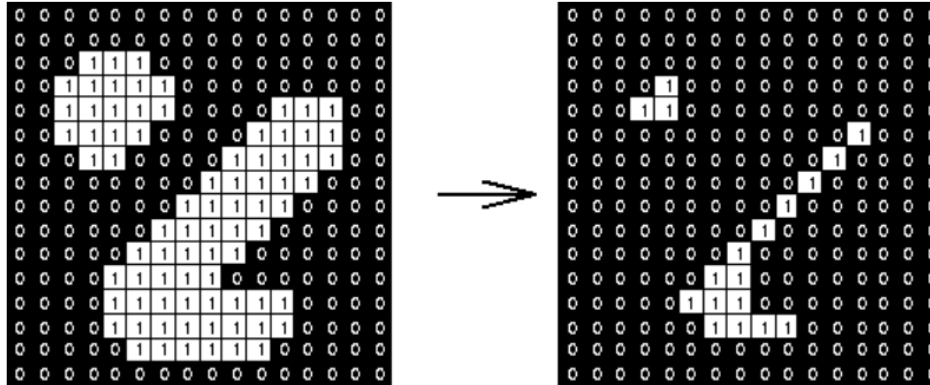


Probing of an image with a structuring element
(white and gray pixels have zero and non-zero values, respectively).

<https://www.cs.auckland.ac.nz/courses/compsci773s1c/lectures/ImageProcessing-html/topic4.htm>

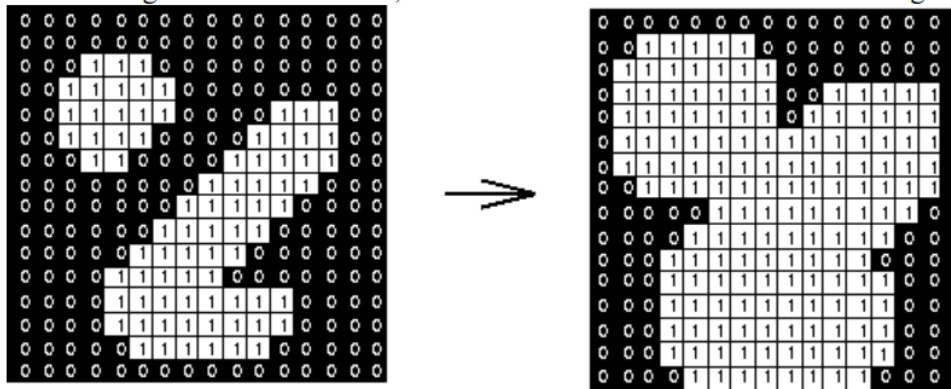
Morphological operations: Dilation and Erosion

Erosion:



Erosion: a 3×3 square structuring element
(www.cs.princeton.edu/~pshilane/class/mosaic/).

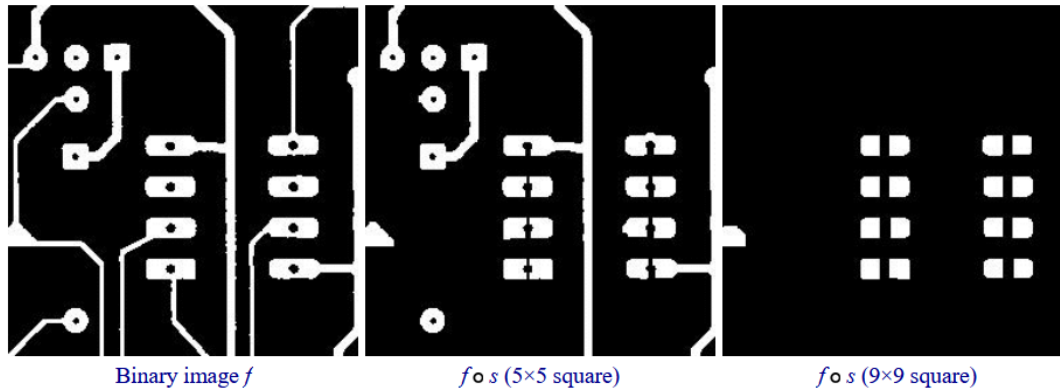
Dilation:



Dilation: a 3×3 square structuring element
(www.cs.princeton.edu/~pshilane/class/mosaic/).

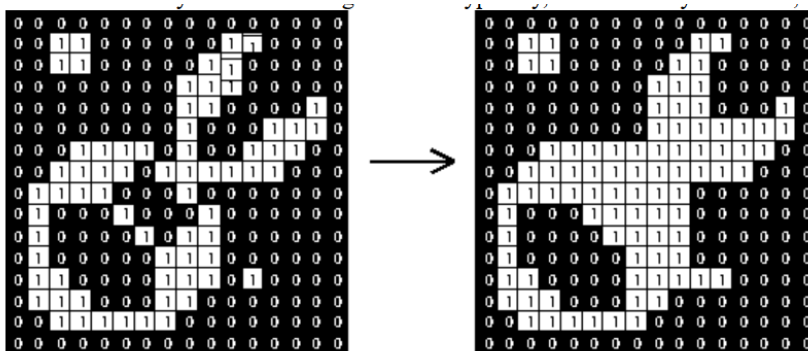
Morphological operations: Open and Close

Opening: erosion followed by a dilation



Results of opening with a square structuring element (www.mmorph.com/html/morph/mmopen.html/).

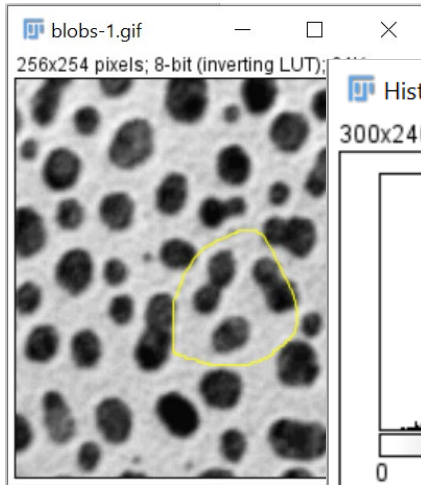
Closing: dilation followed by a erosion



Closing with a 3×3 square structuring element
(www.cs.princeton.edu/~pshilane/class/mosaic/).

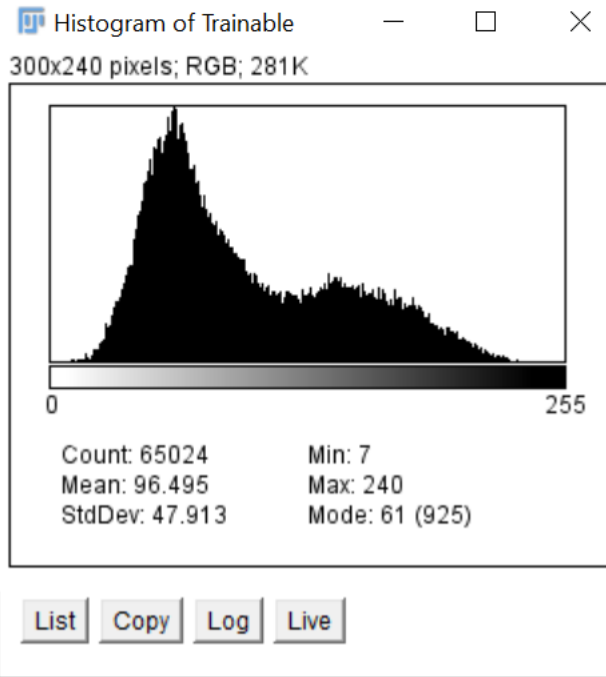
Analyze Menu

Measure

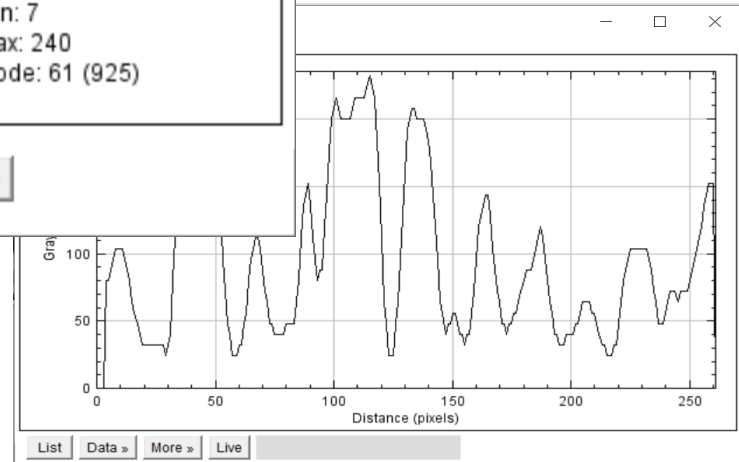
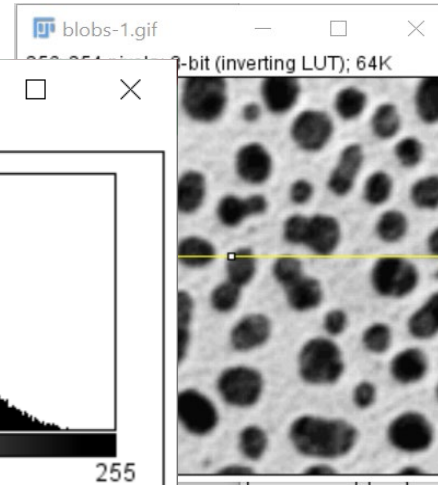


Results

| File | Edit | Font | Results |
|------|------|--------|---------|
| Area | Mean | Min | Max |
| 1 | 5268 | 98.217 | 24 248 |



Plot profile



There are three tiers of plugins:

- Core ImageJ/Fiji plugins, bundled with the base ImageJ/Fiji distribution.
- Plugins installable from an ImageJ update site: **Help ► Update**.
- Additional plugins available from various online sources, which must be manually installed.

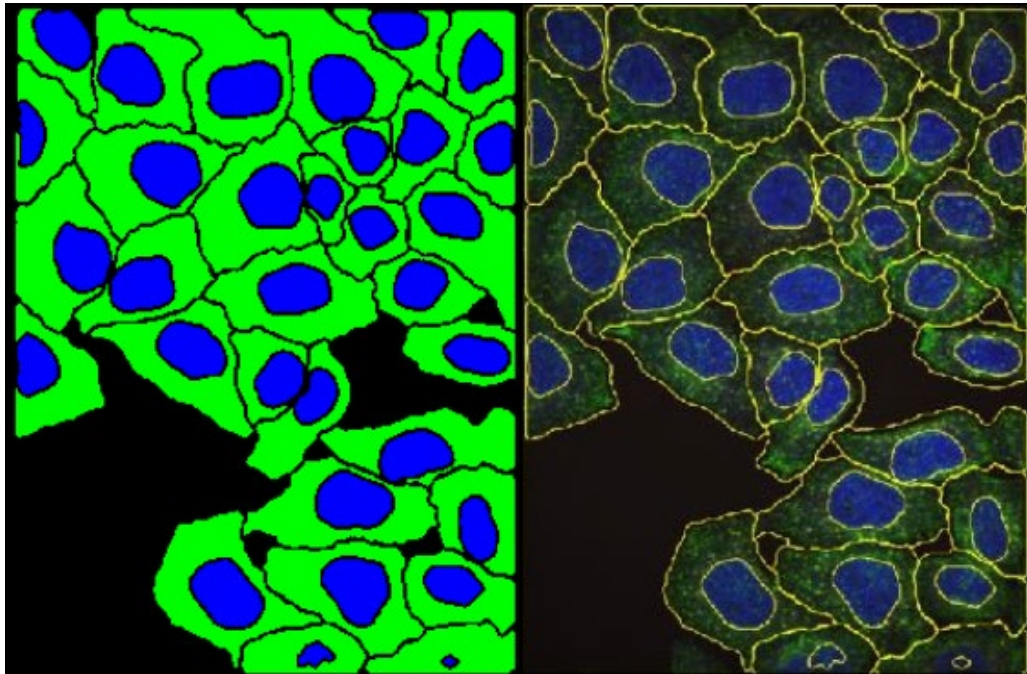
Instruction for manually install plugins:

1. Download the plugin and place it in the plugins folder inside the ImageJ directory
 1. If it is .jar or .class file, restart the ImageJ and it will atomically be listed in the Plugins menu.
 2. If it is a .java file, it first needs to be compiled via a Java compiler and then place it in the plugins folder

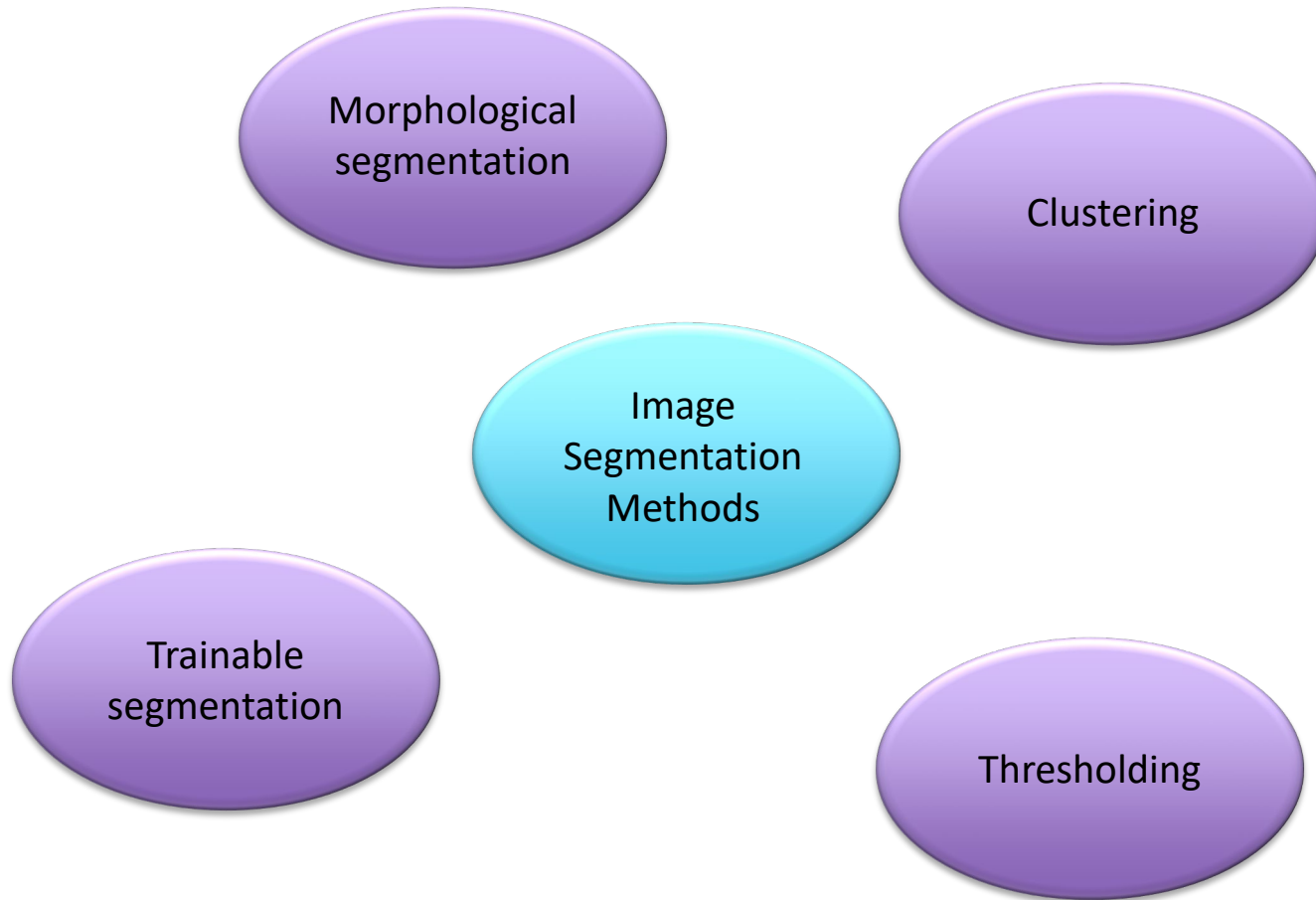
**List of plugins: <https://imagej.net/list-of-extensions>
(BigDataViewer, CLIJ, and many more...)**

Image Segmentation with ImageJ

- Segmentation is a popular image processing technique which is used to locate objects and boundaries.

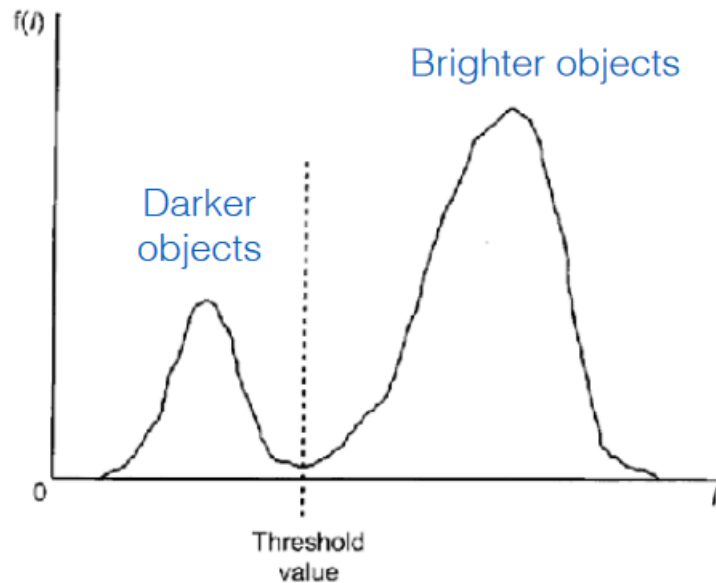


Segmentation Methods



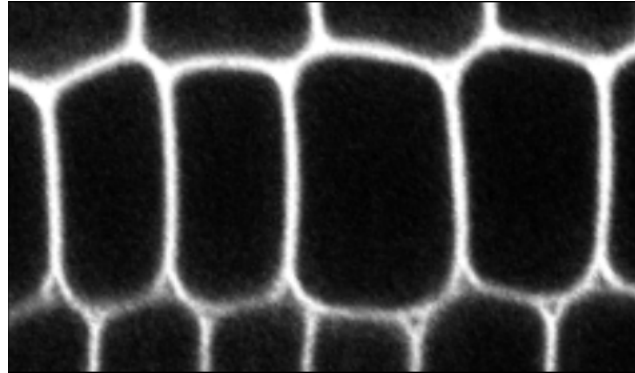
Thresholding

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



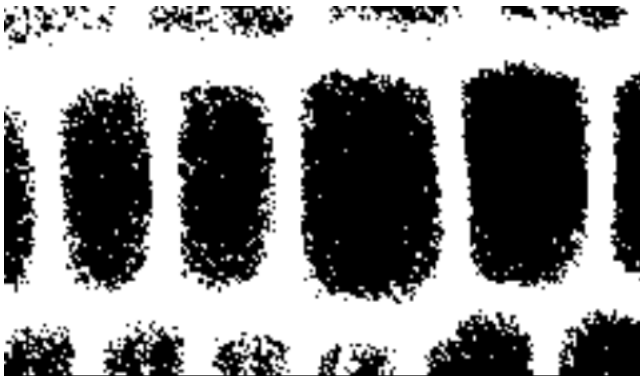
Manual Thresholding

In the ImageJ menu: Image ► Adjust ► Threshold.
Problem: threshold might be too low or too high



Original Image

Threshold too low



Threshold too high



Solution: Plot the histogram and find the best threshold value

Automatic Thresholding

- Manual thresholding is time consuming
- Same threshold over a collection of images? NOT recommended due to fluctuations in intensity across images.
- Solution: optimizing some objective function that can be:
 - Statistical
 - Probabilistic
 - Structural

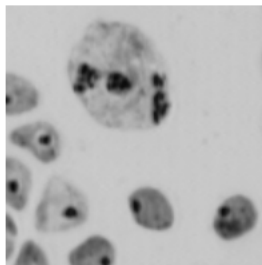
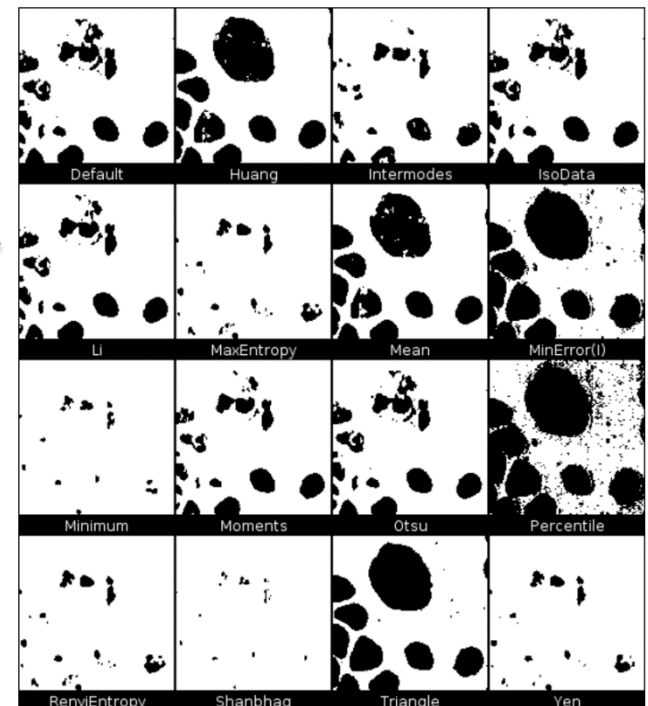


Image ► Adjust ► Auto Threshold

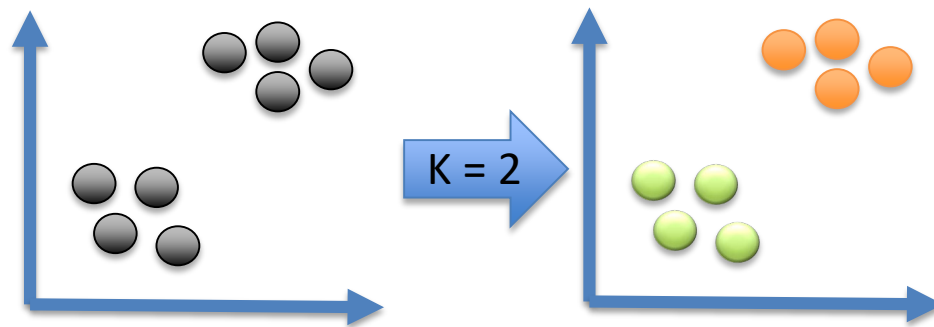


http://imagej.net/Auto_Threshold

Clustering Based Segmentation

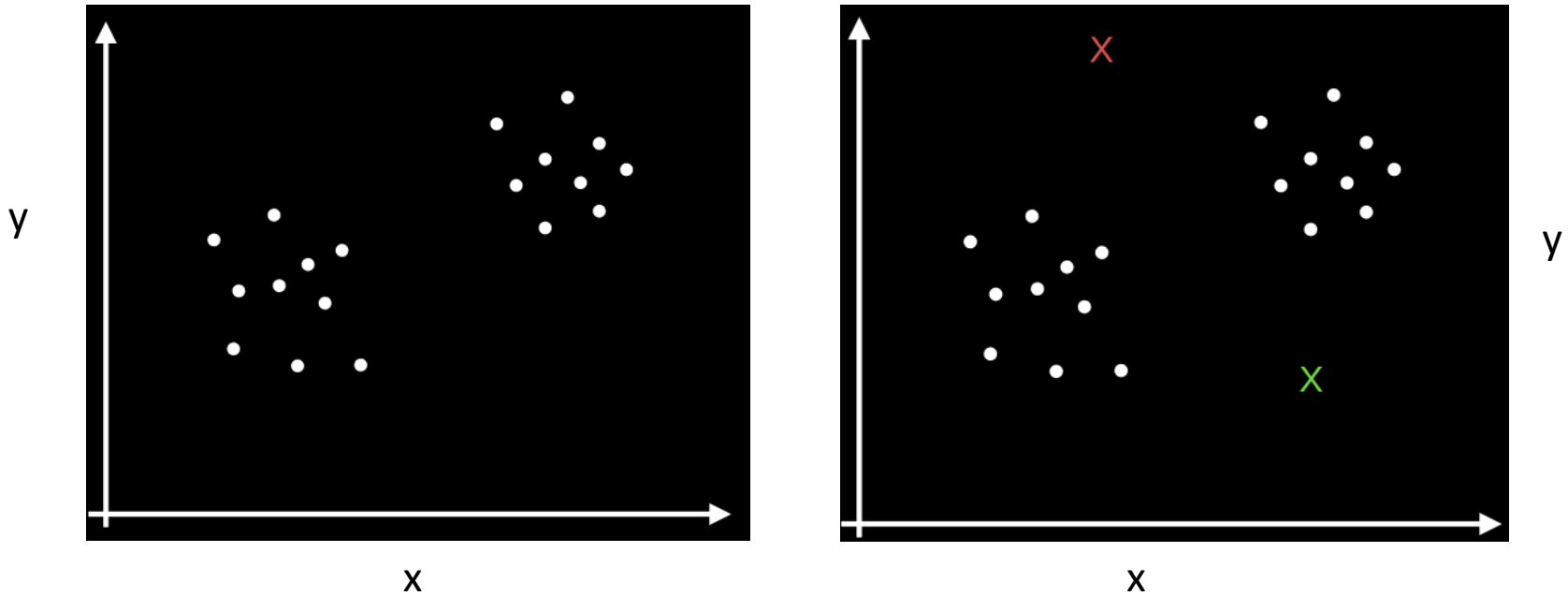
- Organizing data into classes such that:
 - High intra-class similarity
 - Low inter-class similarity
- What is similarity?
 - Intensity
 - Location
 - ...

K-means clustering

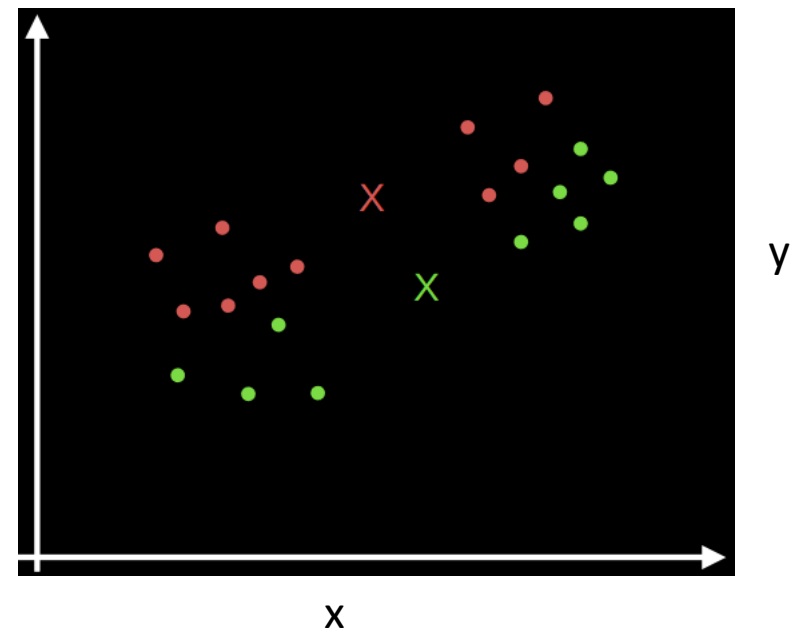
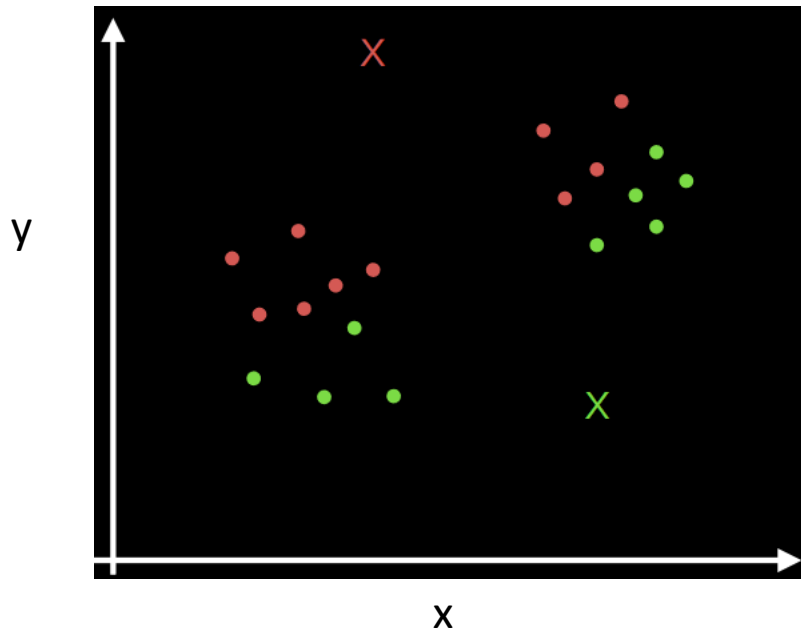


K-means Algorithm Illustration

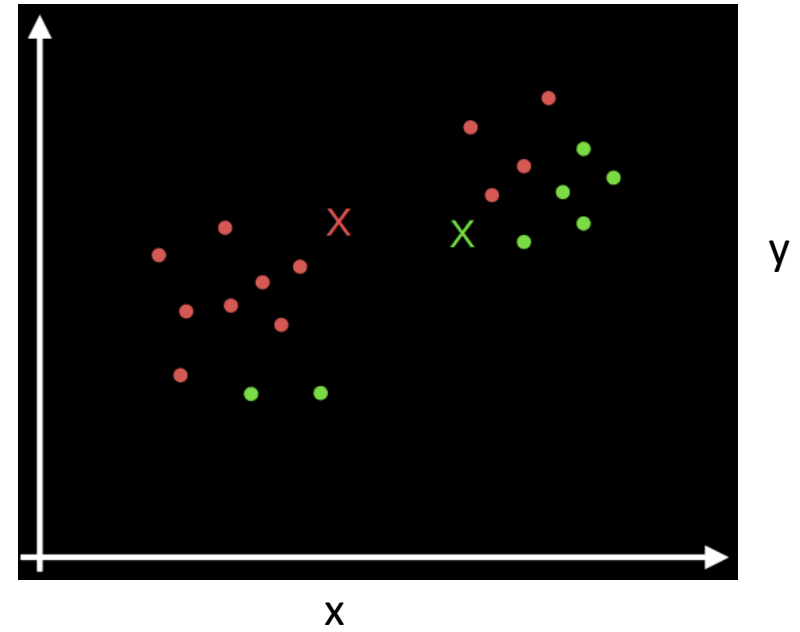
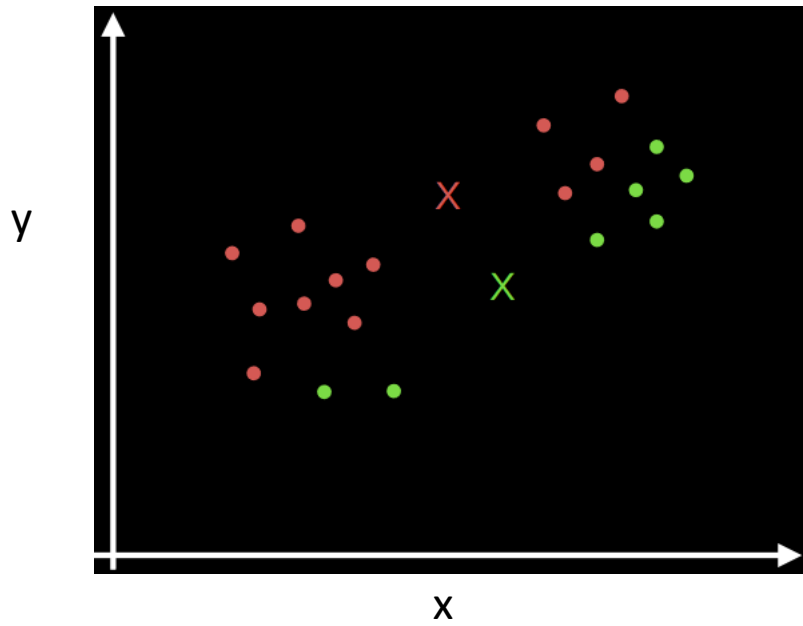
$K = 2$



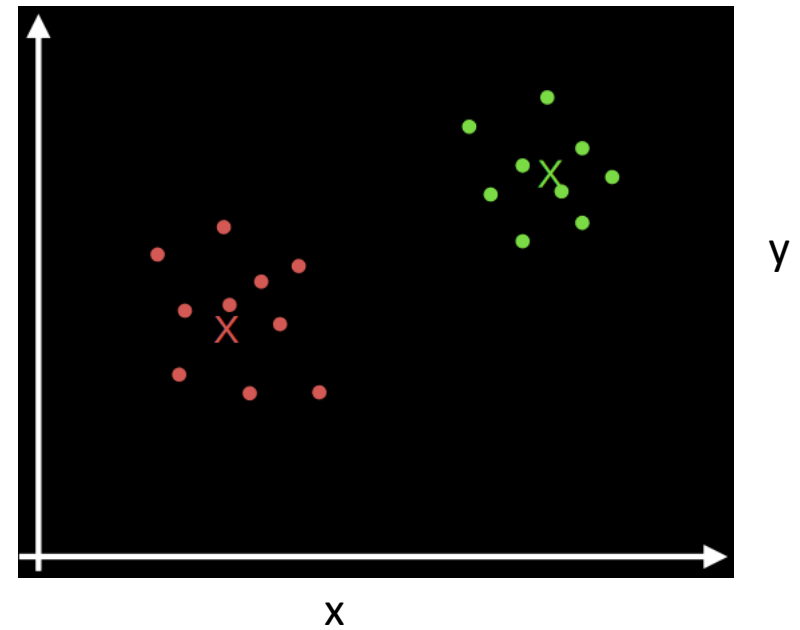
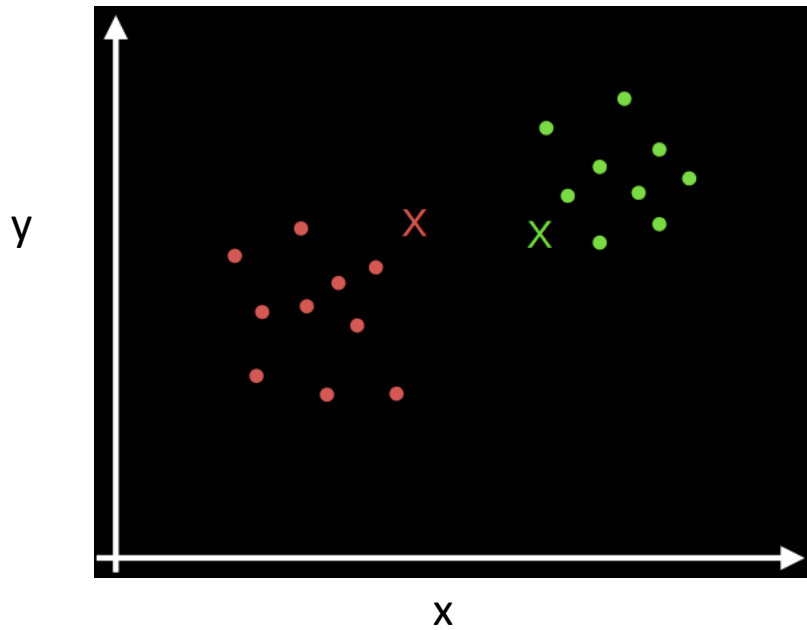
K-means Algorithm Illustration



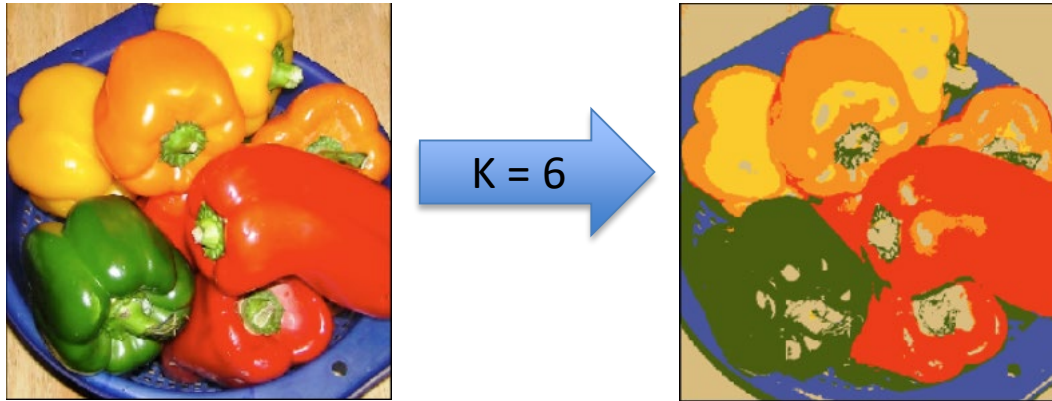
K-means Algorithm Illustration



K-means Algorithm Illustration



Segmentation via K-means in RGB space

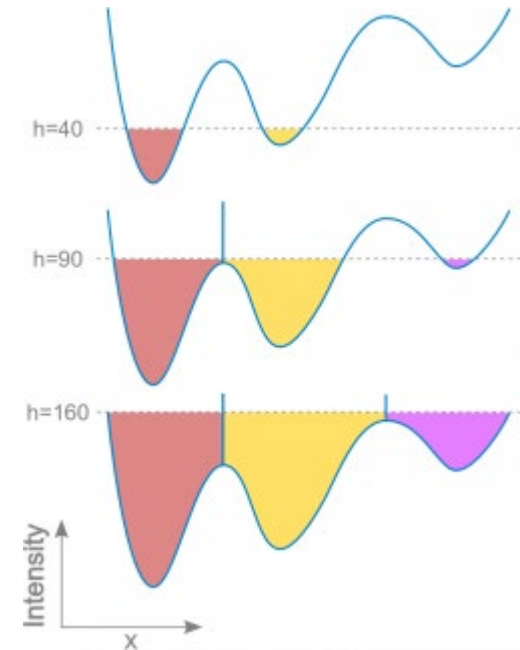


Plugins ► Segmentation ► Color Clustering

Limitations: Heuristic algorithm, there is no guarantee that it will converge to global optimum.

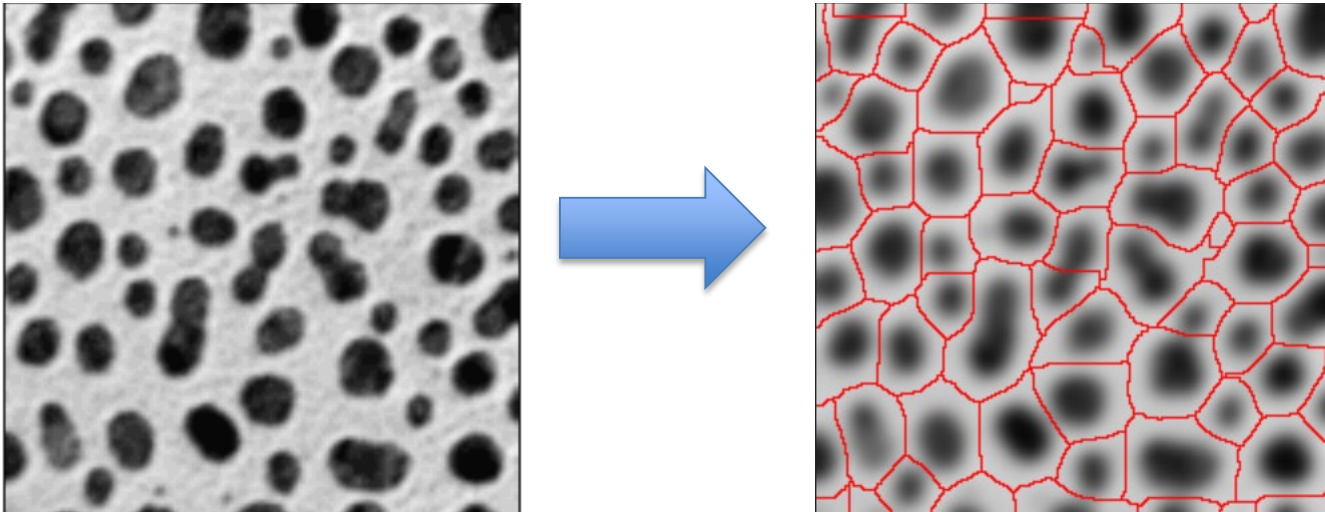
Watershed Segmentation

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



Morphological Segmentation: Watershed

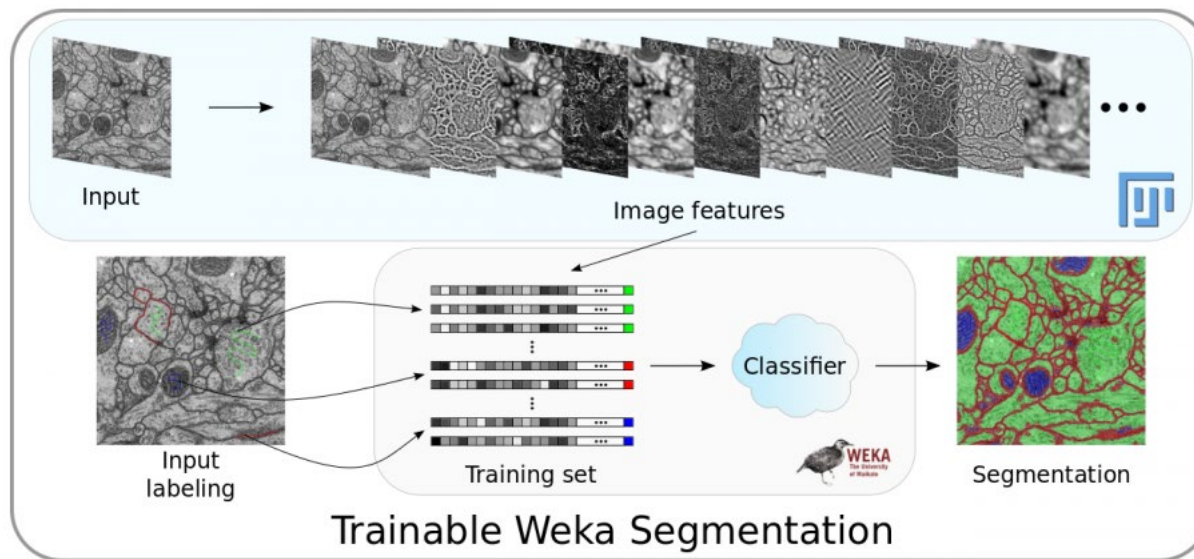
https://imagej.net/Classic_Watershed



Plugins ► MorphoLibJ ► Segmentation ► Morphological Segmentation

Machine Learning Based Segmentation

- Assign labels to objects indicating their class
- Objects represented by a set of measurements or features



Plugins ► Segmentation ► Trainable Weka Segmentation

[http://fiji.sc/Trainable Weka Segmentation](http://fiji.sc/Trainable_Weka_Segmentation)

Thank you!

Please submit your questions about ImageJ to:
Contact: BioHPC-help@UTSouthwestern.edu