Yeast Cells Segmentation & Classification

Student: Nizar Cherkaoui

Advisor: Dr. Chia-Ling Tsai (Computer Science Dept.)

Advisor: Dr. Eric Muller (Biology Dept.)

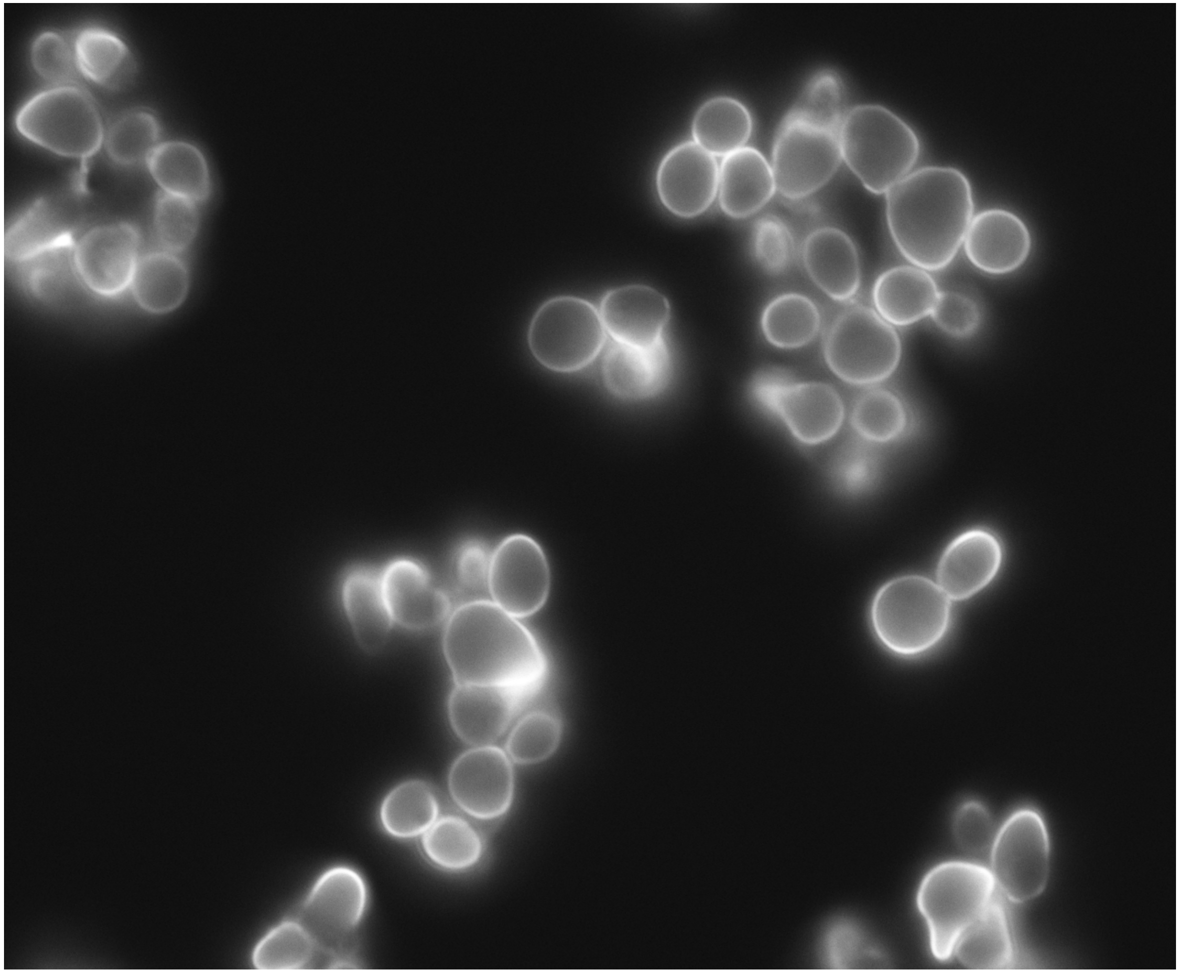
Outline

- Introduction
- Foreground Extraction
- Blob Segmentation and Labeling
- Classification

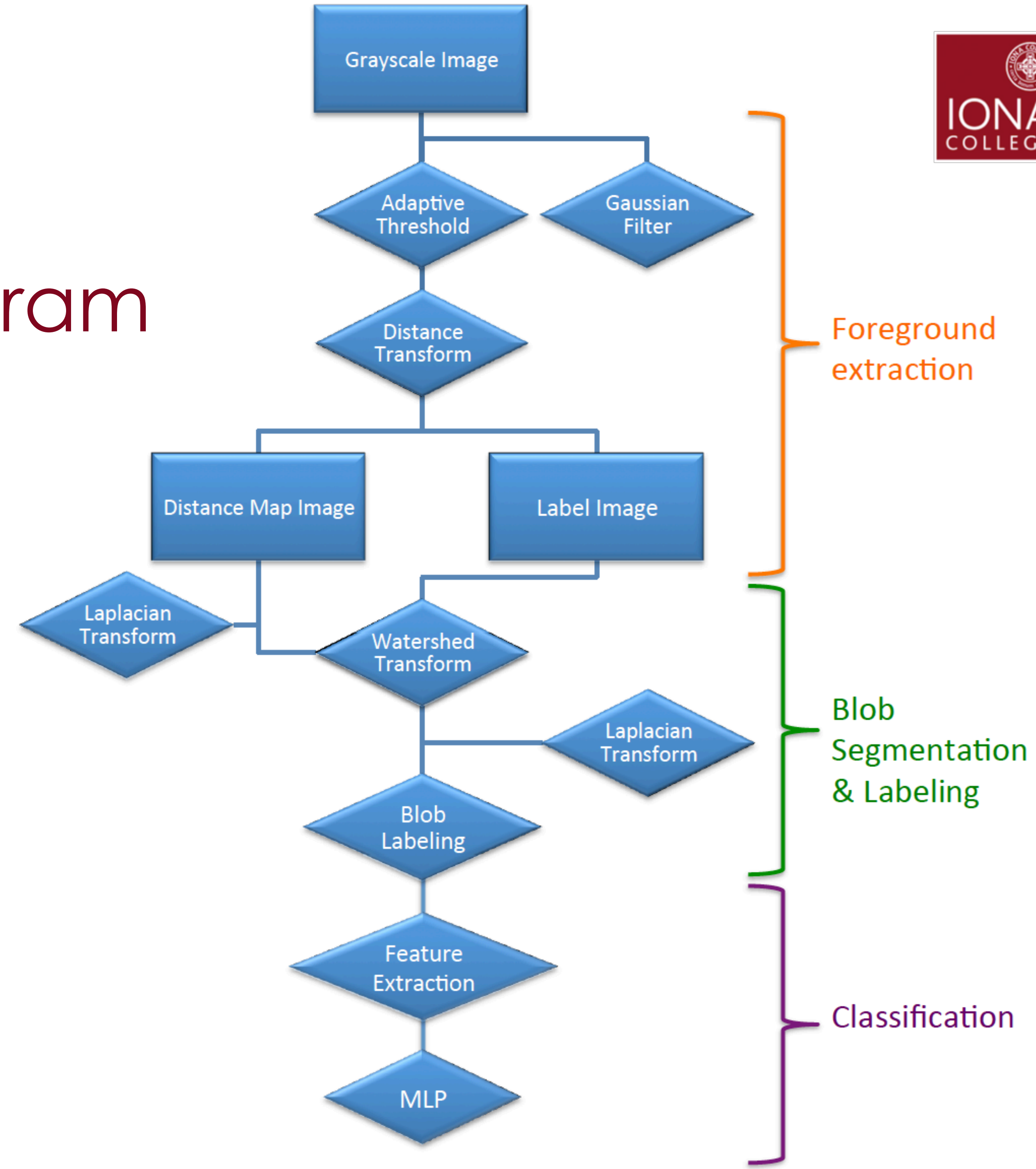
Introduction

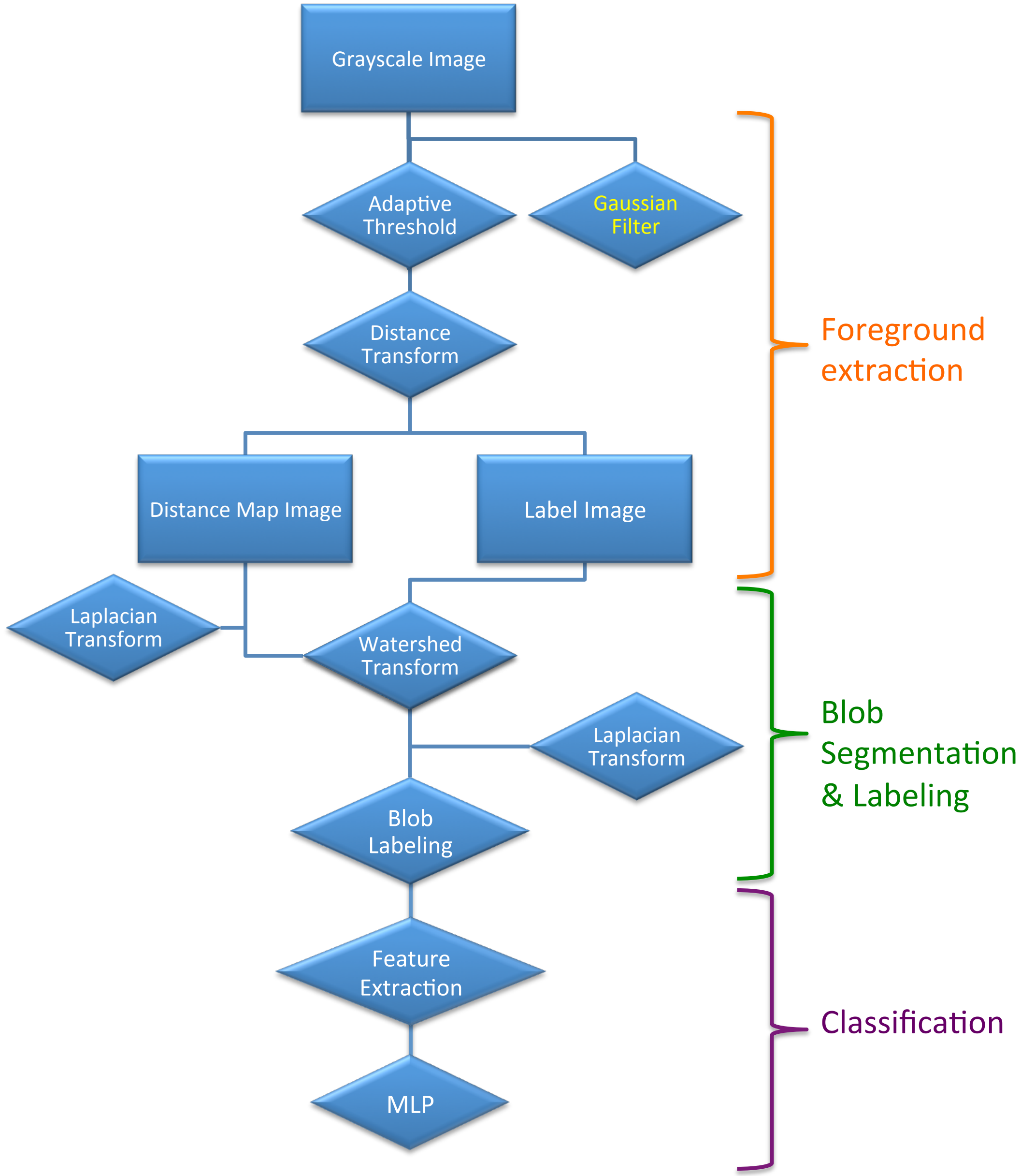
- Image segmentation in Biology has been used in the analysis of microscopy based images.
- The aim is to automatically process huge amount of image samples and produce useful data.
- The challenge lies in:
  - Splitting apart cells that are densely packed and overlap each other.
  - Generate features that can be useful for classification

Introduction



Flow Diagram




Grayscale Image
Adaptive Threshold
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MLP
Foreground extraction
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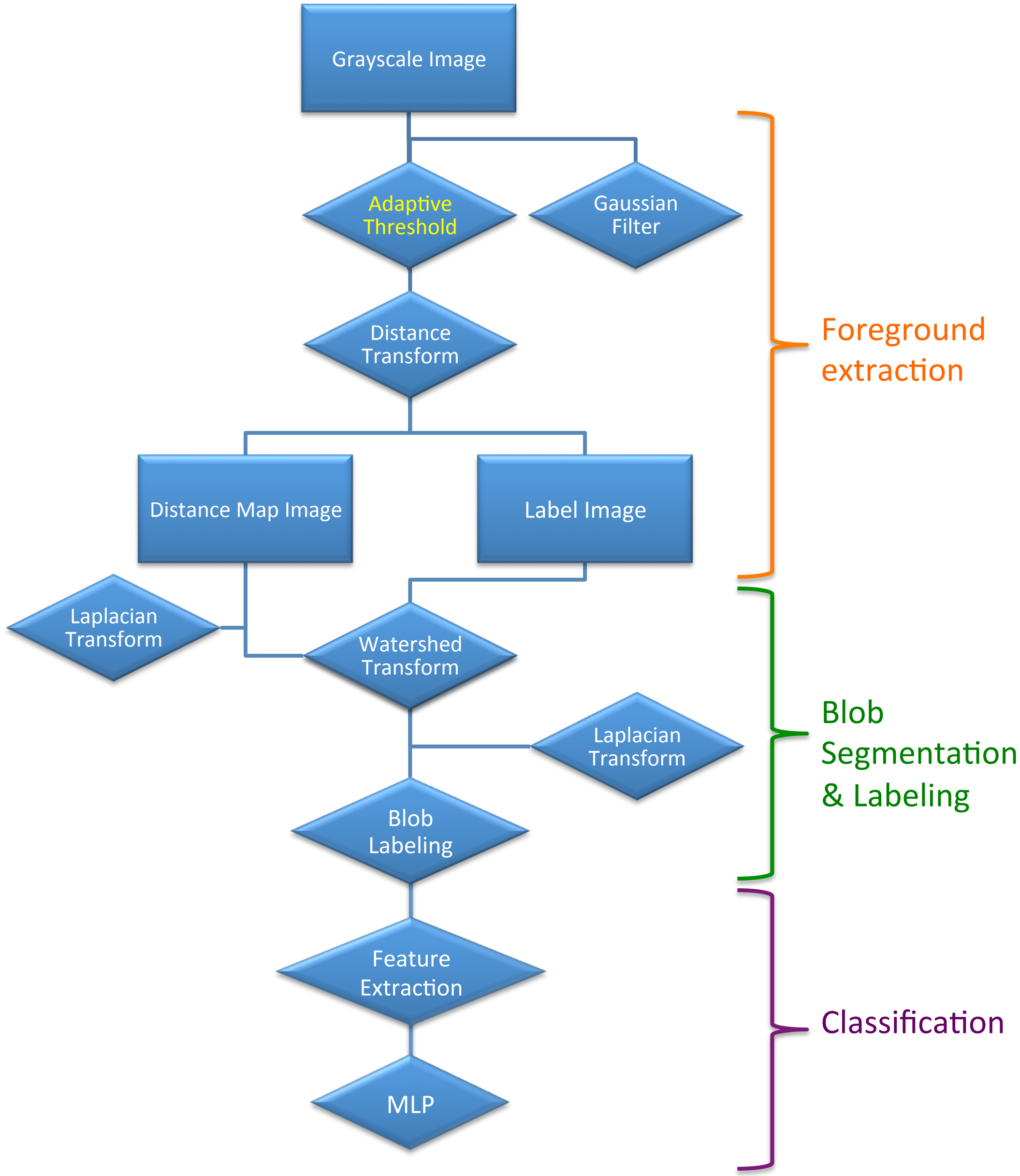
Gaussian Smoothing

- Images have noise (unwanted information)
  - The intensity measured at a pixel is the "true" intensity plus noise.
  - nearby pixels usually have similar "true" intensities.
  - Smoothing the image reduces noise
- To perform smoothing a Gaussian filter is applied to the image.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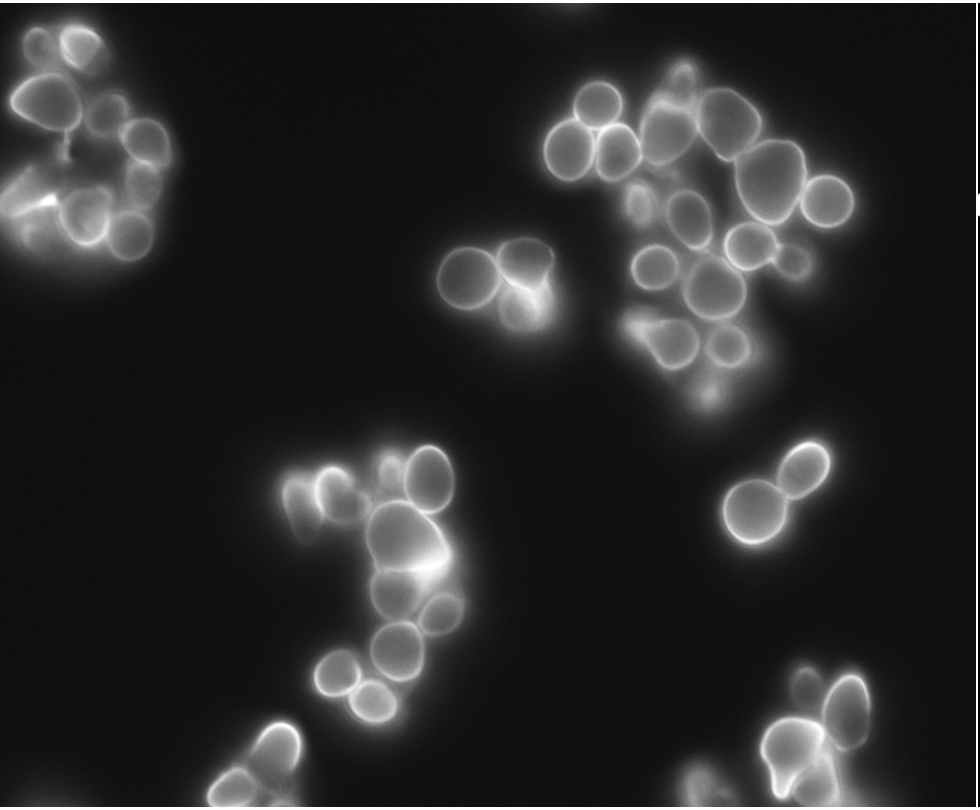
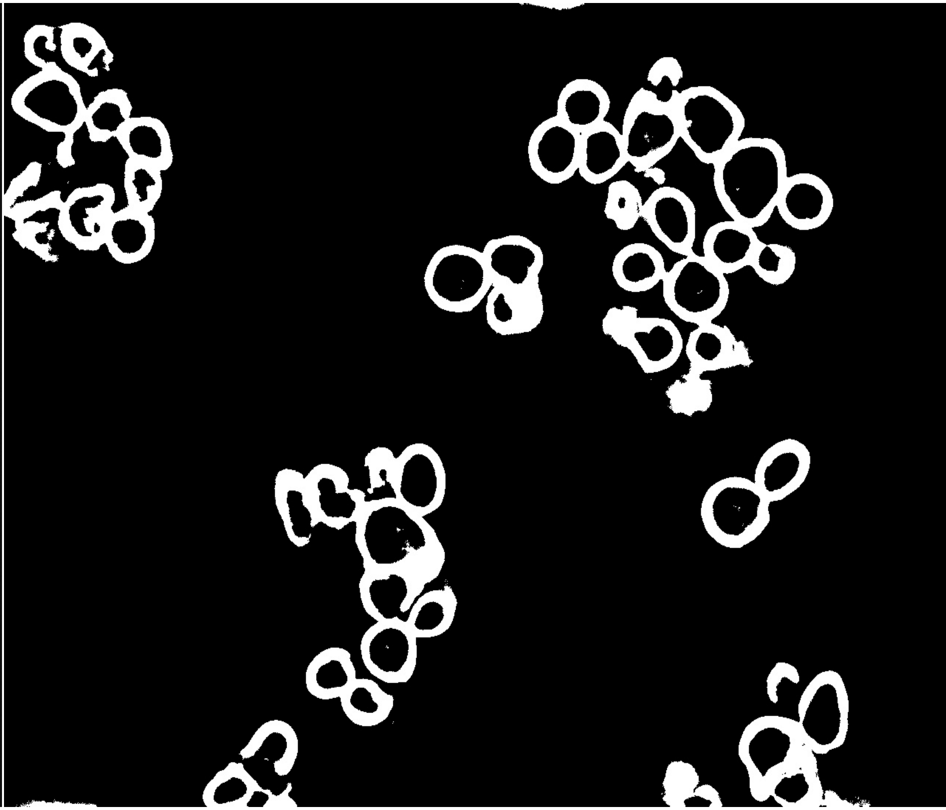
Adaptive Threshold

- To separate foreground objects with background.
- It is used to create a binary image
  - Object pixels are given value of '1'
  - Background pixels are given value of '0'
- Pixels are colored black or white depending on the pixel's label.
- Pixels are processed in a Gaussian window instead of individually.

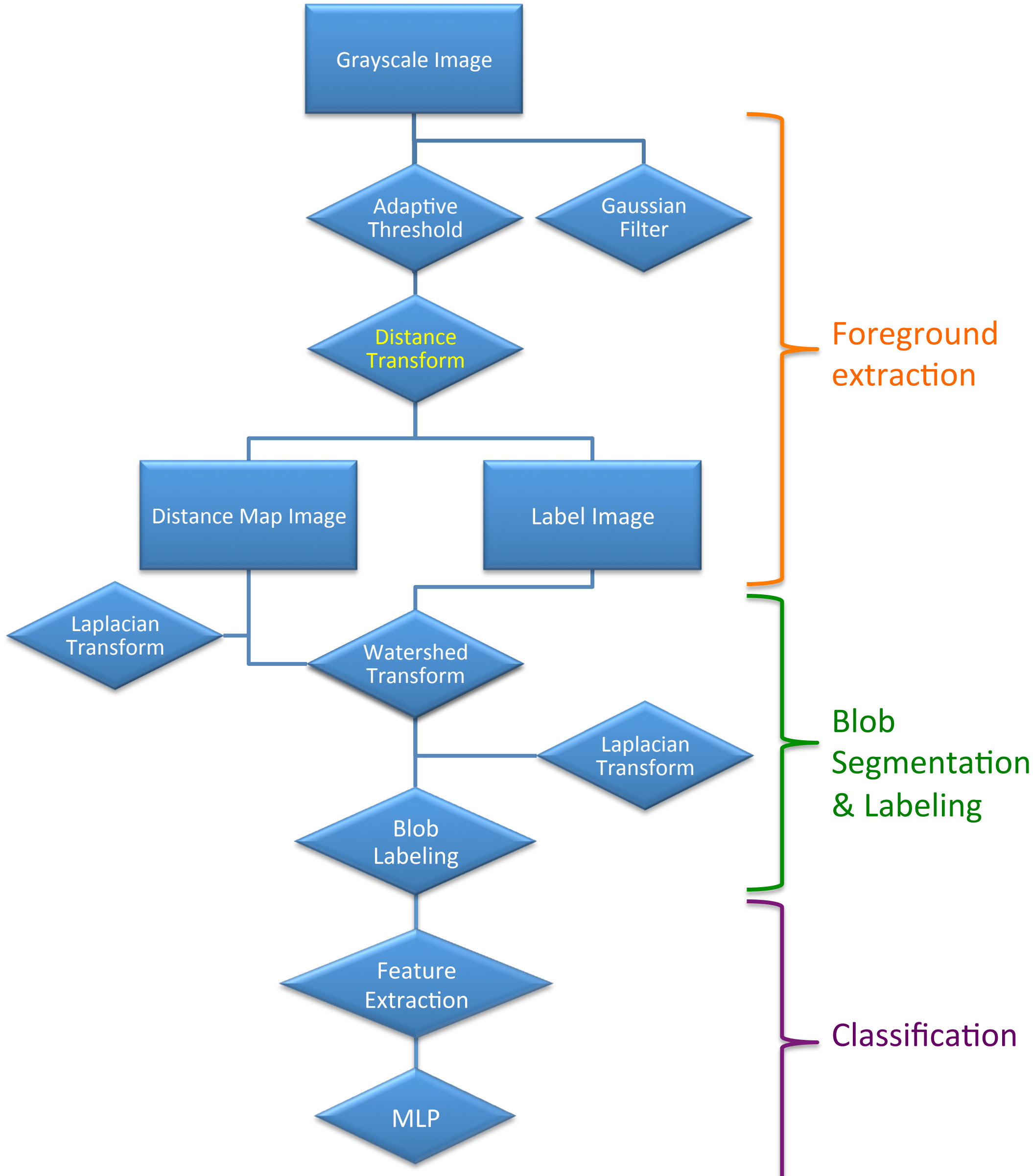
Adaptive Threshold



Original Image



Adaptive Threshold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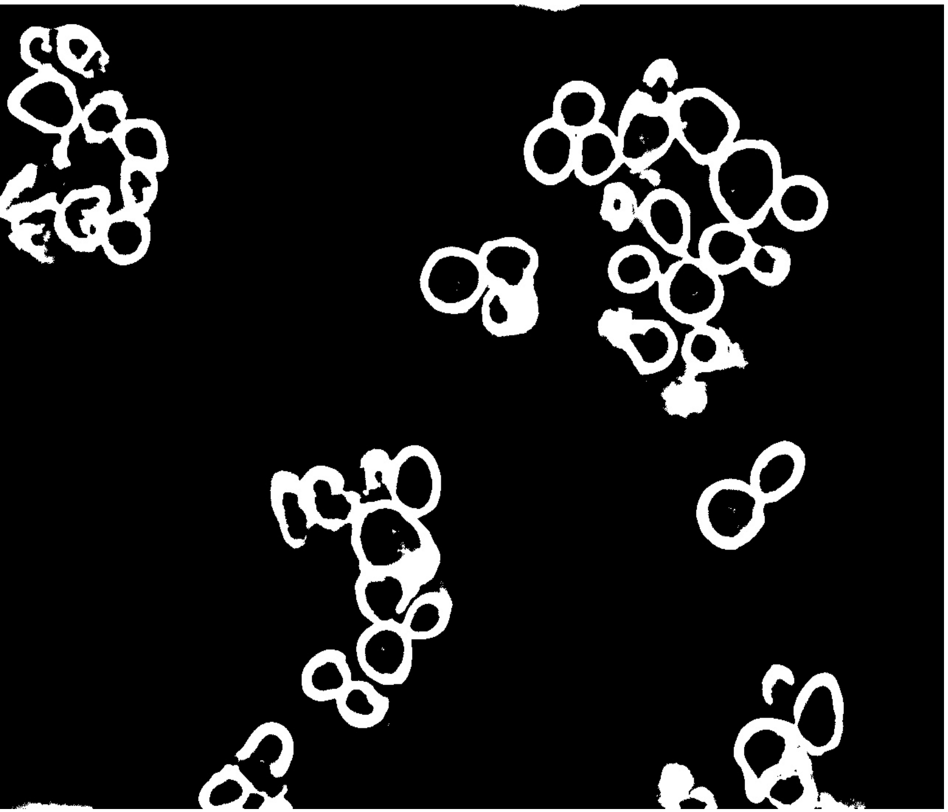
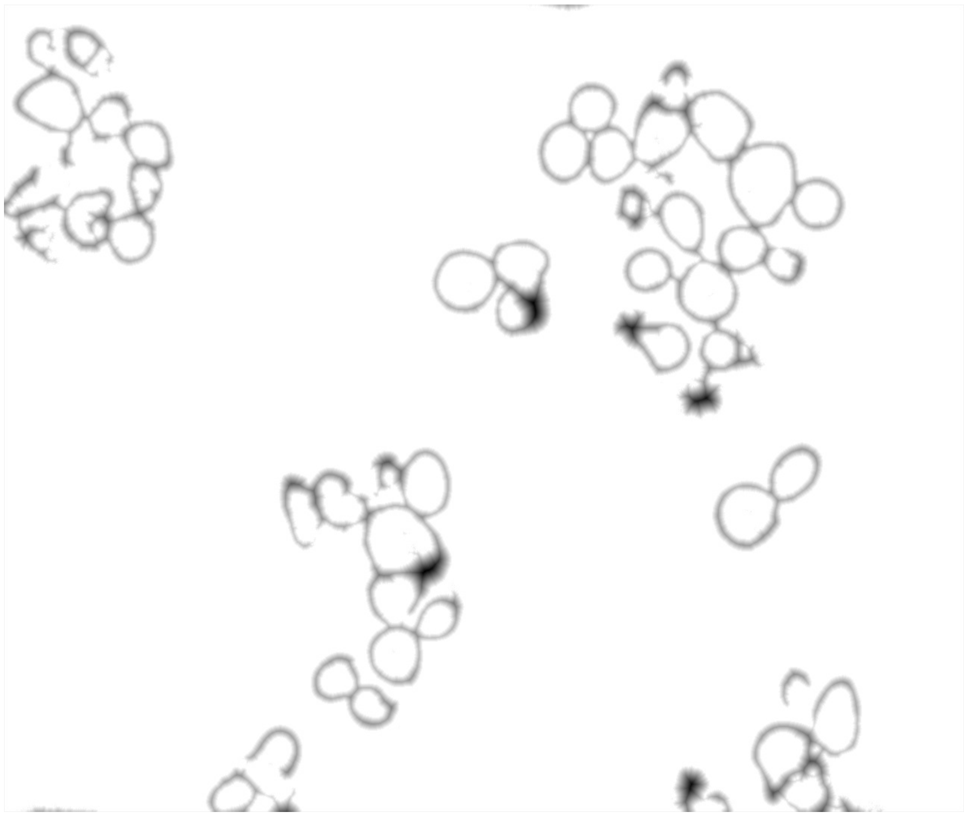
Distance Transform

- The function calculates the approximated distance from every binary image pixel to the nearest background pixel.
  - For background pixels the function sets the zero distance.
  - For foreground pixels the function sets an intensity proportional to the distance calculated.

Distance Transform



Adaptive Threshold

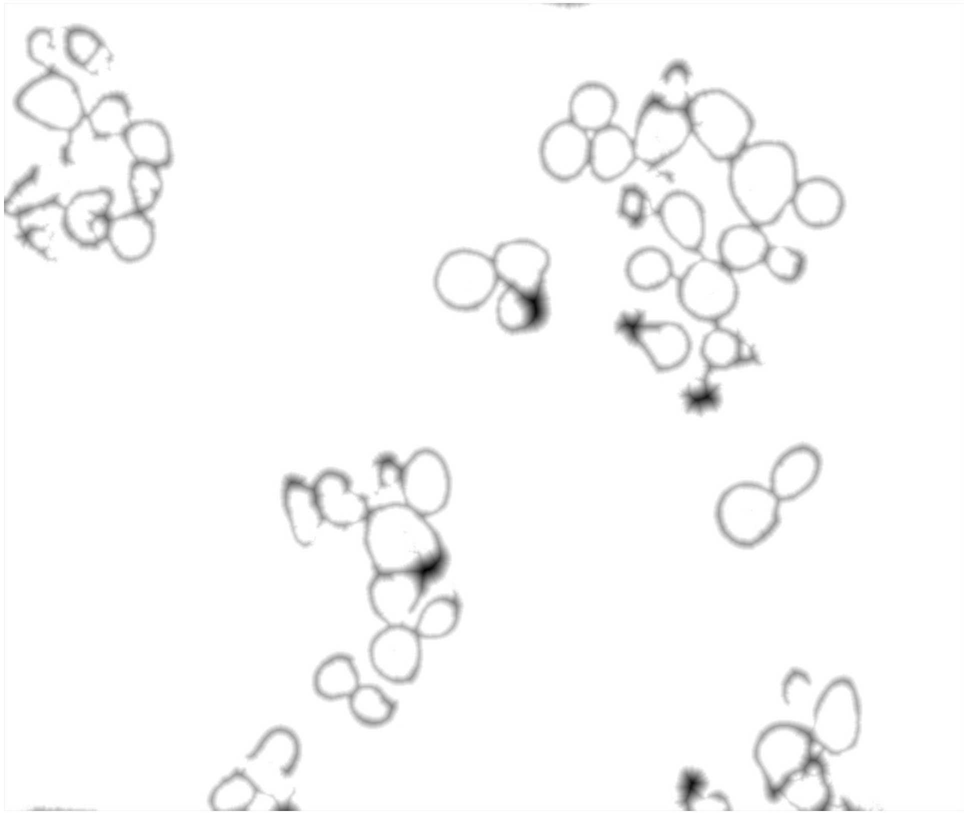


Distance Transform

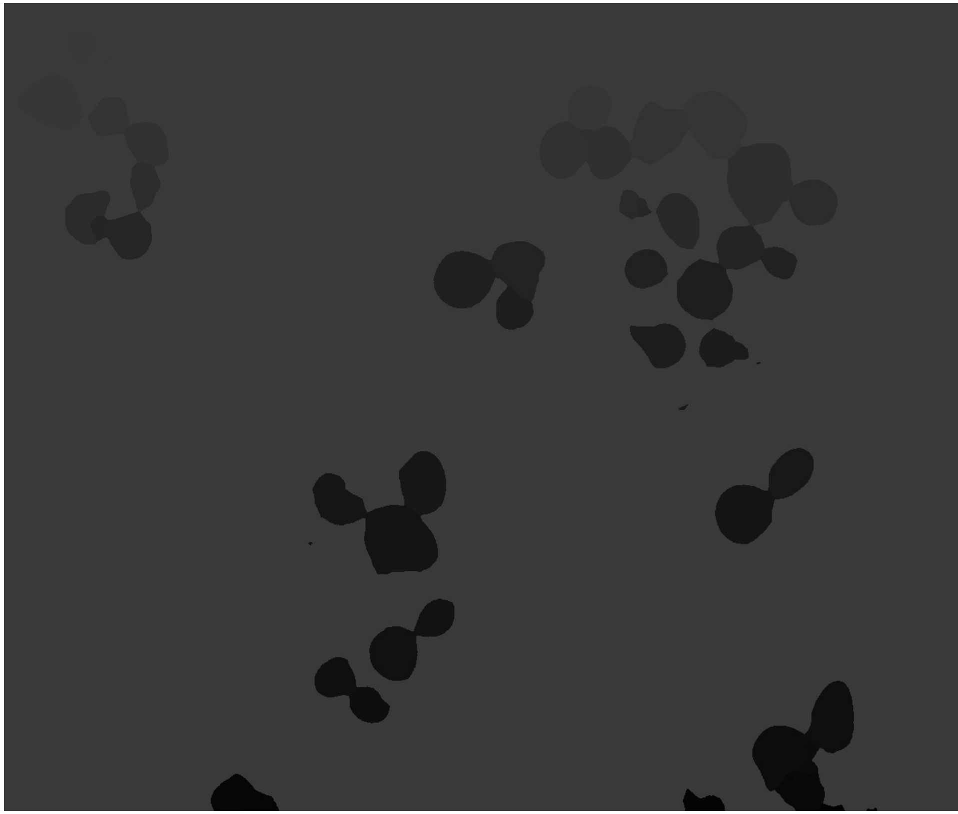
Distance Transform

- The distance Transform also generates an image of Labels.
  - For every foreground object the function finds the nearest connected component consisting of background pixels and uniquely labels it.
  - Using the label image, a gray level image is created containing the labels starting at 1, translated into gray scale intensities.
- The image generated is the result of Foreground and Background Extraction.

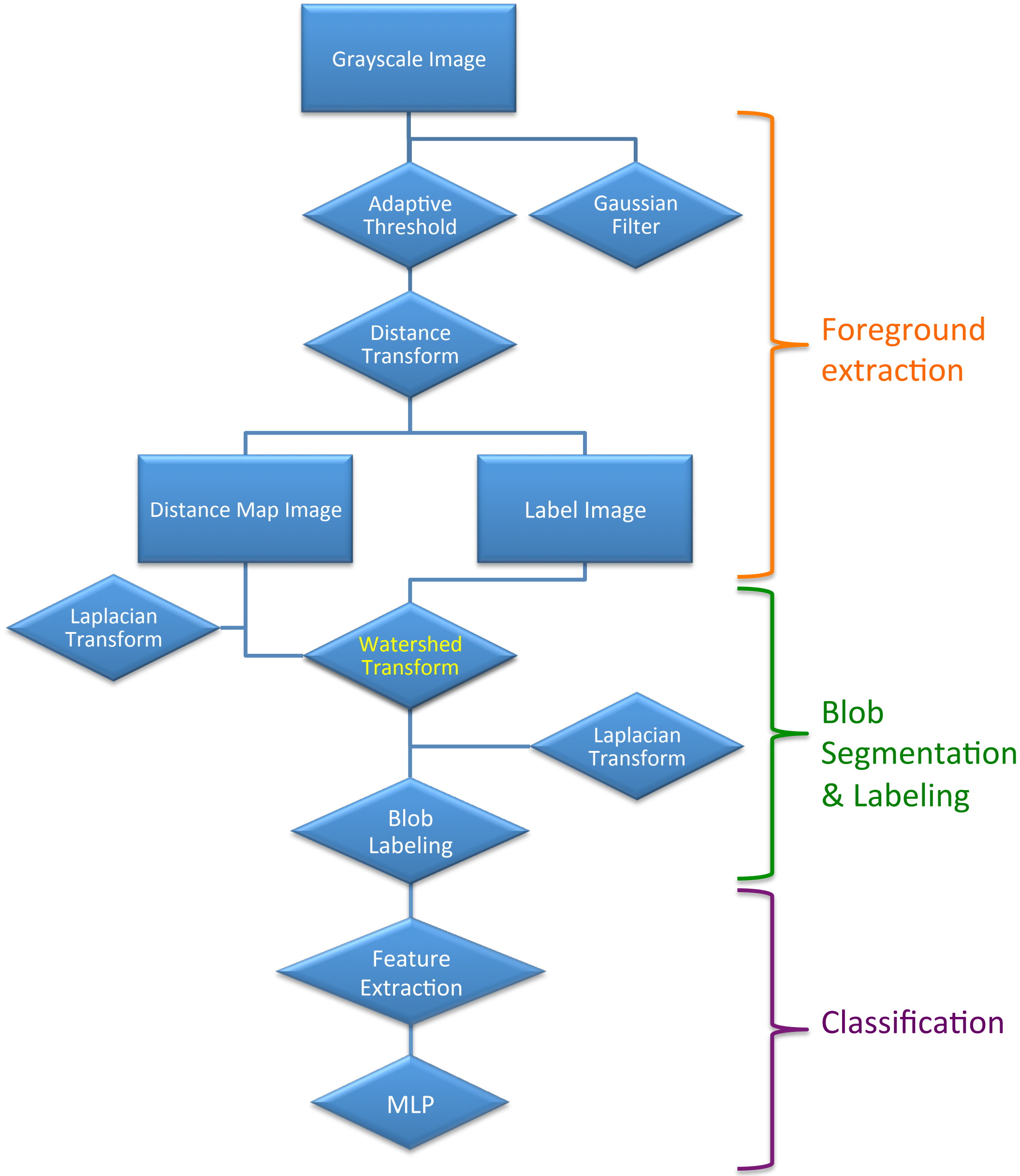
Distance Transform



Distance Transform



Label Image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Marker-based Watershed

- Enhancement of the watershed algorithm
- Treats the input image as a topographic surface where:
  - Dark pixels are high.
  - Light pixels are low.
- Consists of flooding the topographic surface from predefined set of markers.
  - Each foreground object (cell) must have only one set of connected markers.

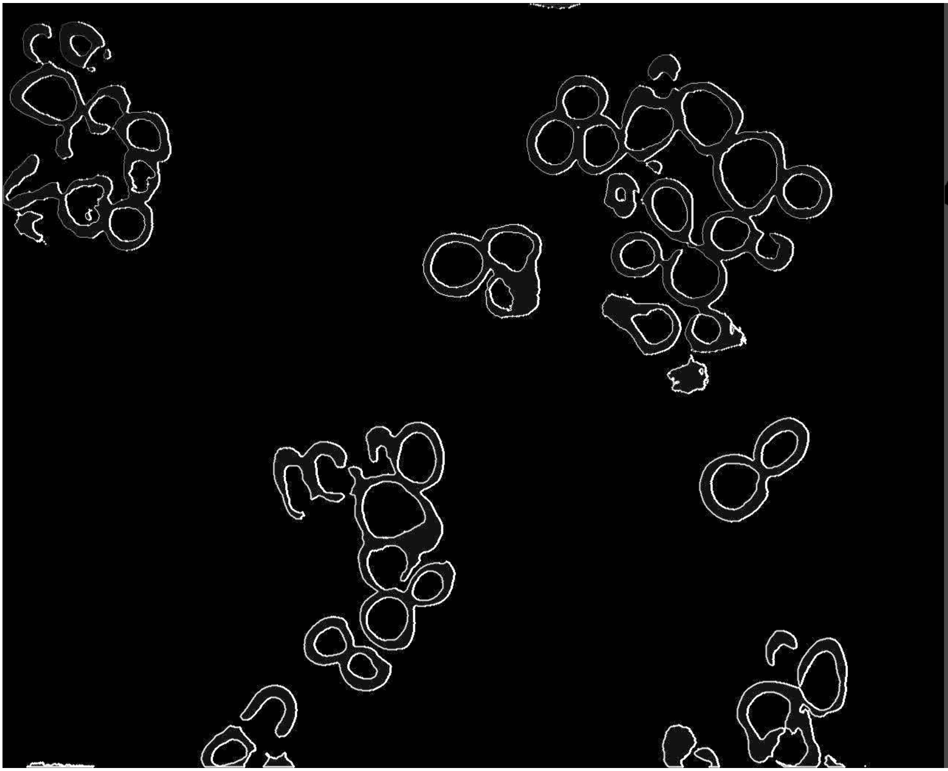
Marker-based Watershed

- To generate the watershed segmentation, pixels that certainly belong to the foreground and pixels that certainly belong to the background have to be identified.
  - The other pixels, that is the ones for which the labeling is unknown, have to be assigned a value 0.
- Foreground and Background pixels were previously labeled from 1 to 255.

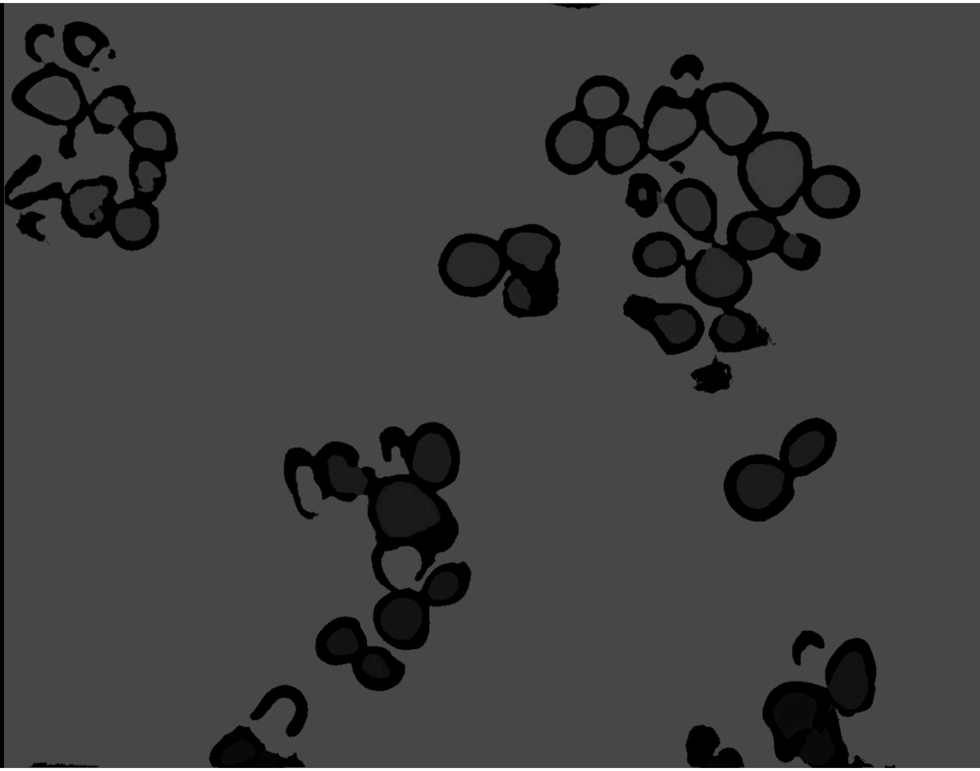
Marker-based Watershed

- In our case, The unknown pixels are those that represent the cell boundaries.
- The idea is to extract the boundaries from the Threshold image and to redraw them on the Foreground and Background labeled image.

Marker-based Watershed

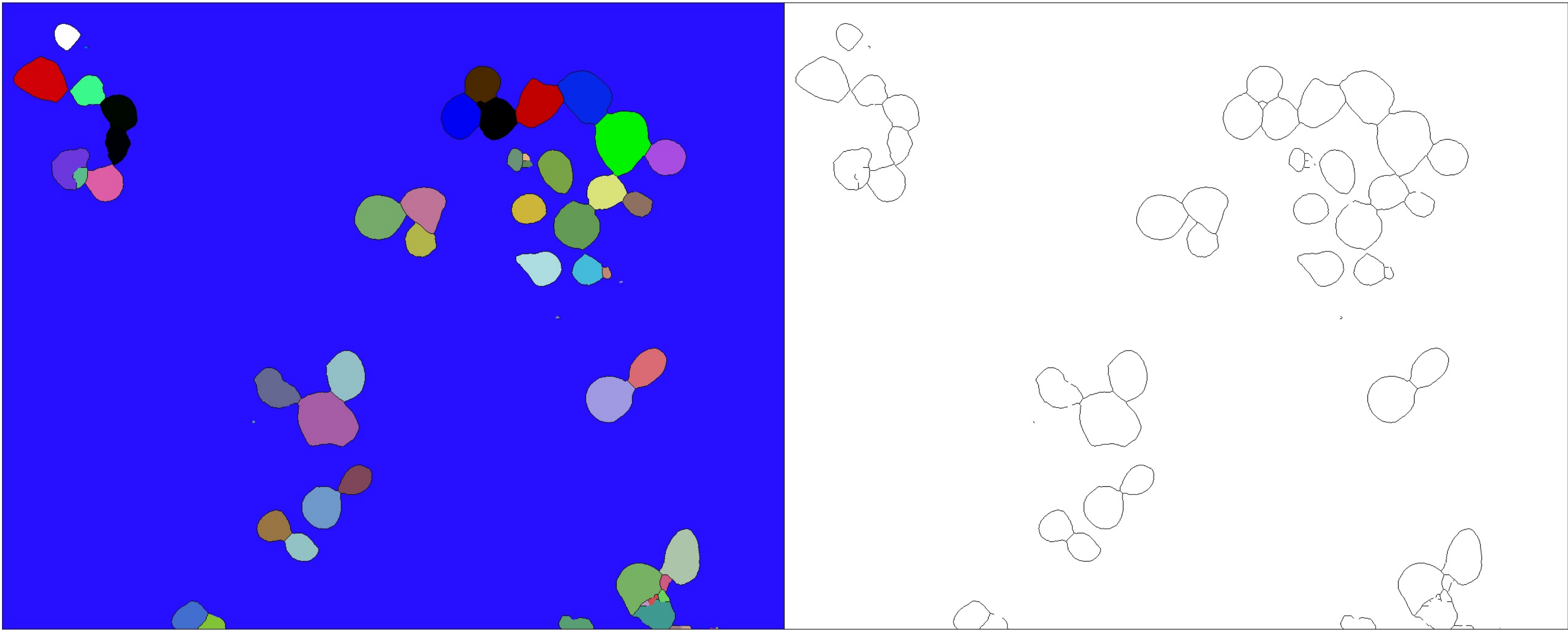


Contour Extraction



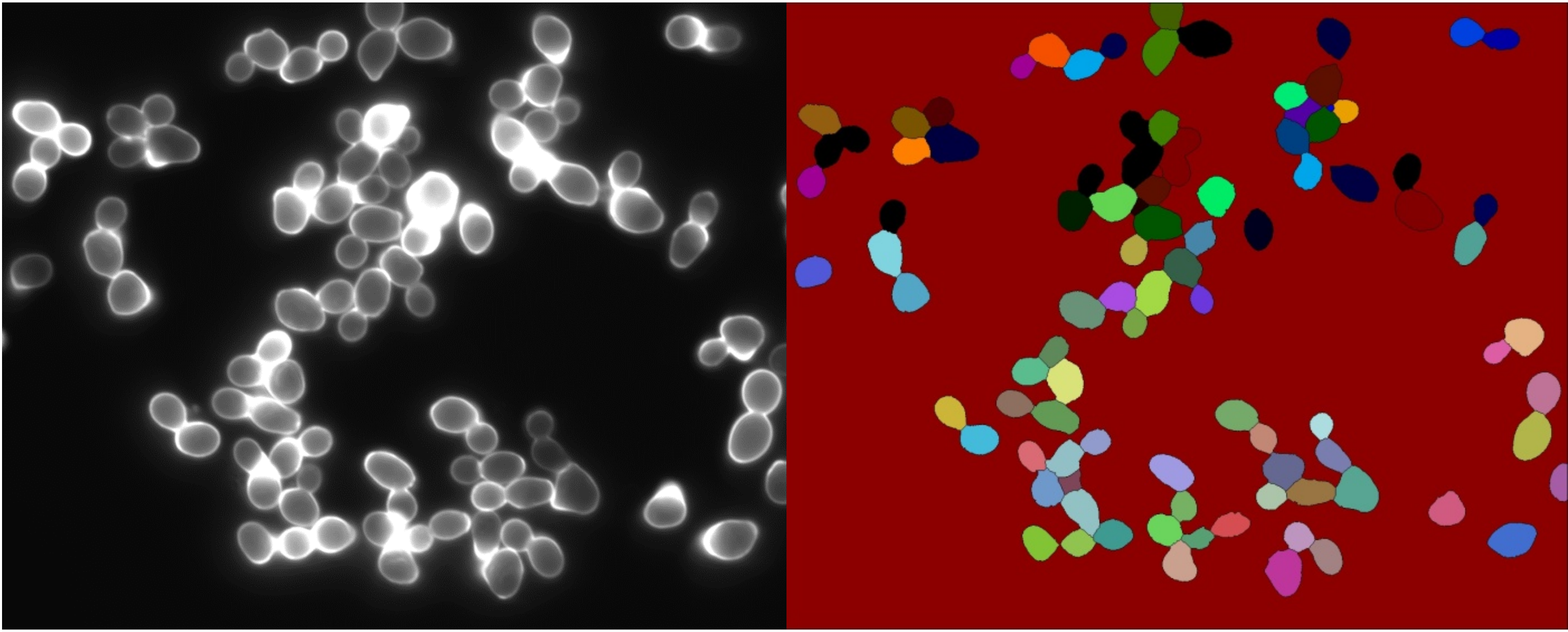
Final Marker Image

Marker-based Watershed

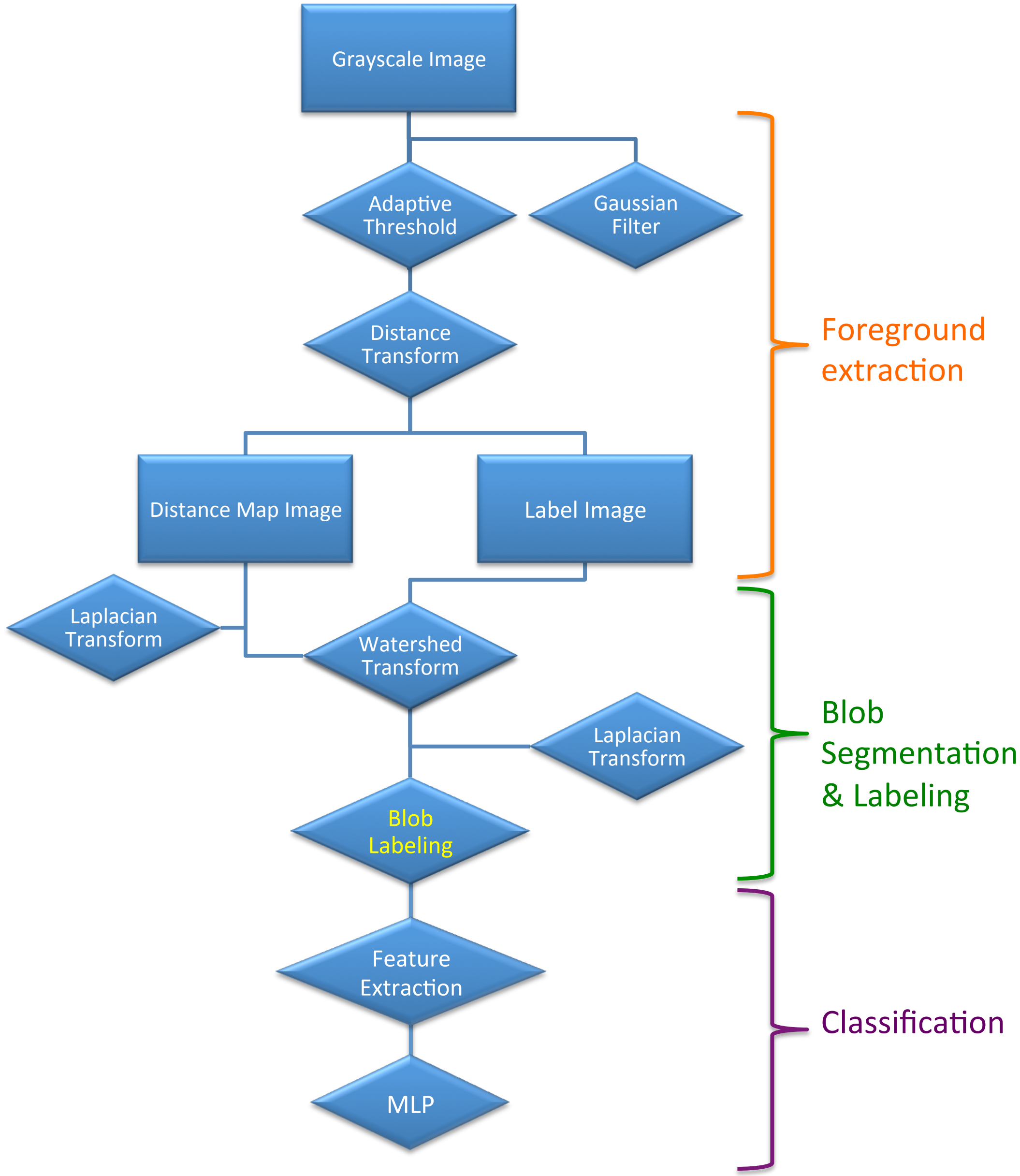


Watershed Segmentation

Marker-based Watershed



Watershed Segmentation



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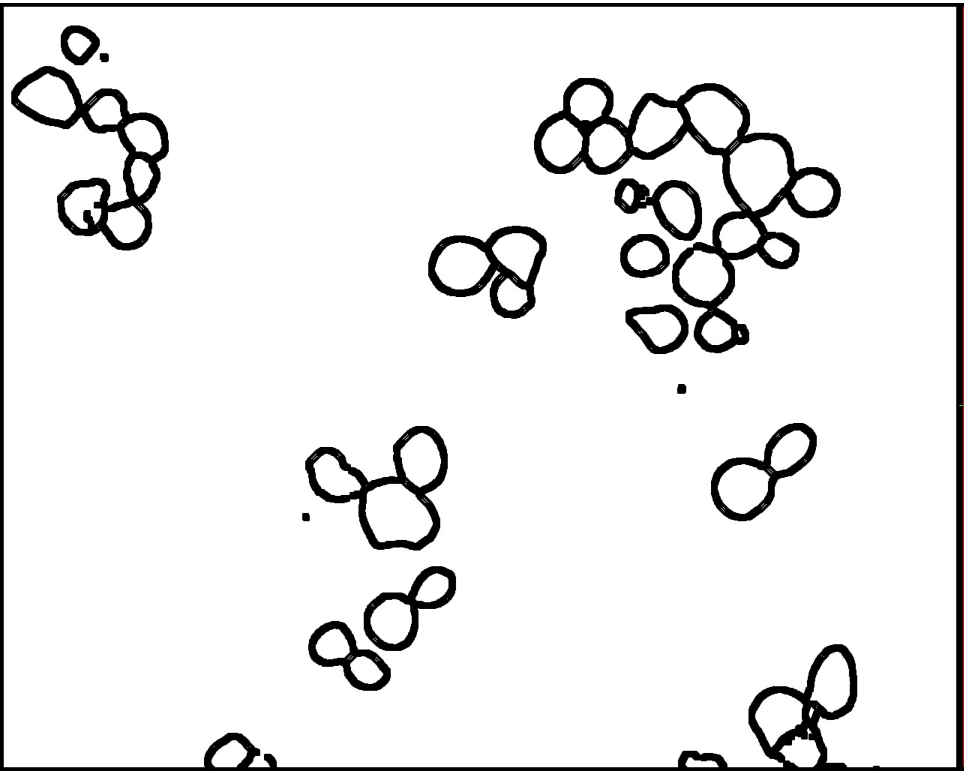
Blob Labeling

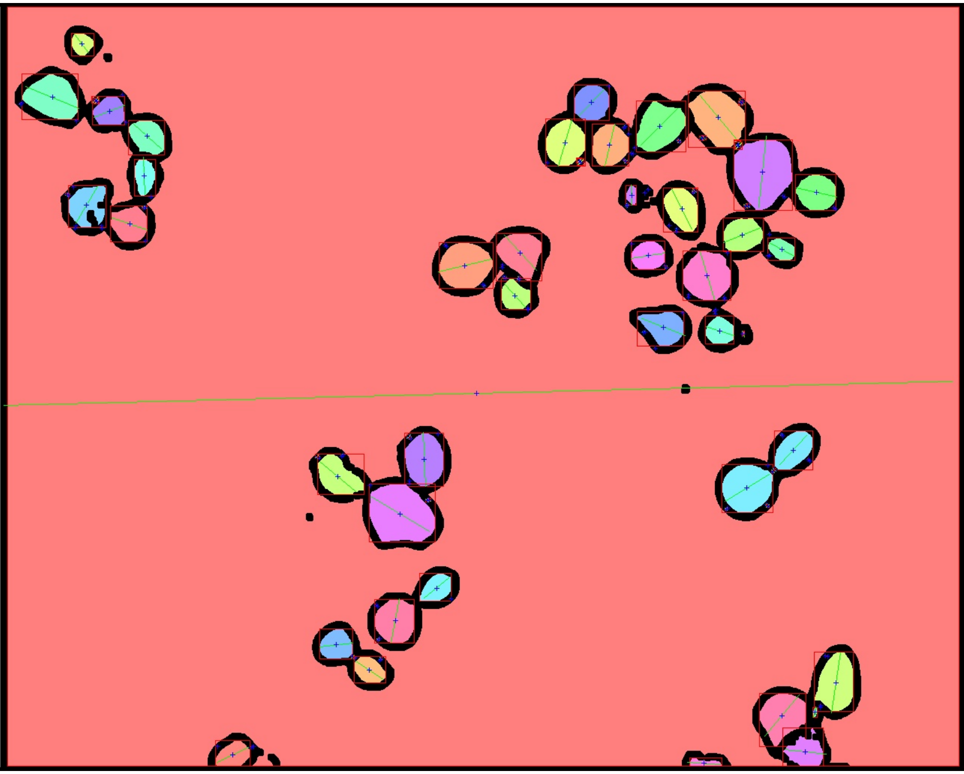
- Region-based component labeling using contour tracing of the segmented blobs.

  - Region-based methods cover more pixels than Edge-based methods.
    - More information available to characterize the blobs.
    - Noisy edges have less negative effects on the feature set.

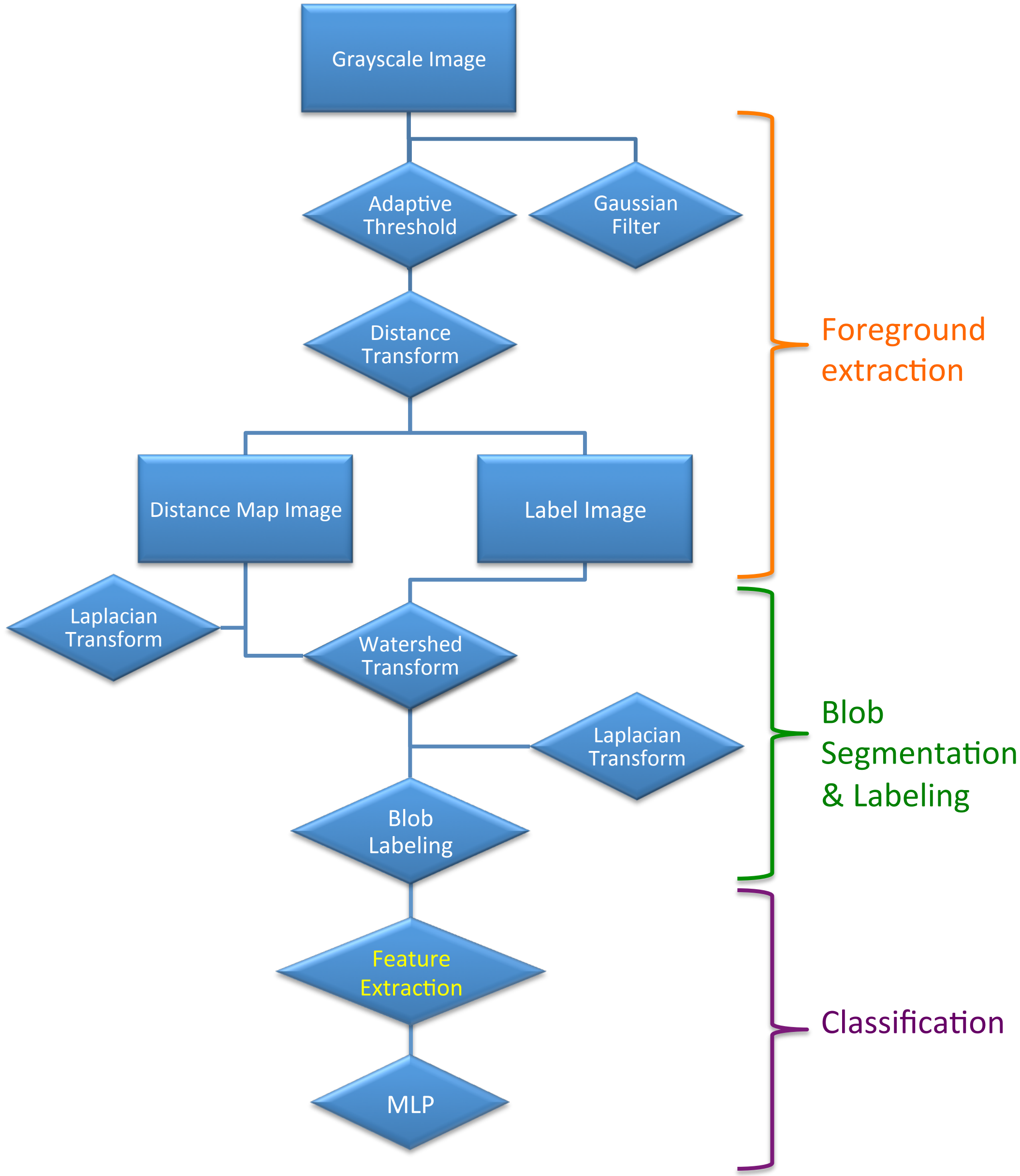
Blob Labeling



Watershed Segmentation



Blob Labeling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Theory of Moments

- Geometrical moment of order $i+j$ for a two-dimensional discrete function $I(x,y)$ is computed by using the formula:

$$M_{ij} = \sum_{x=0}^{M-1}\sum_{y=0}^{N-1} x^i y^j I(x,y)$$

Where M, N are Image Dimensions

- A simple property derived from moments is the area M_{00} .

Theory of Moments

- To be translation invariant the moments are centralized.

$$\mu_{ij} = \sum_{x=0}^{M-1} \sum_{y=0}^{N-1} (x - \overline{x})^i (y - \overline{y})^j I(x, y)$$

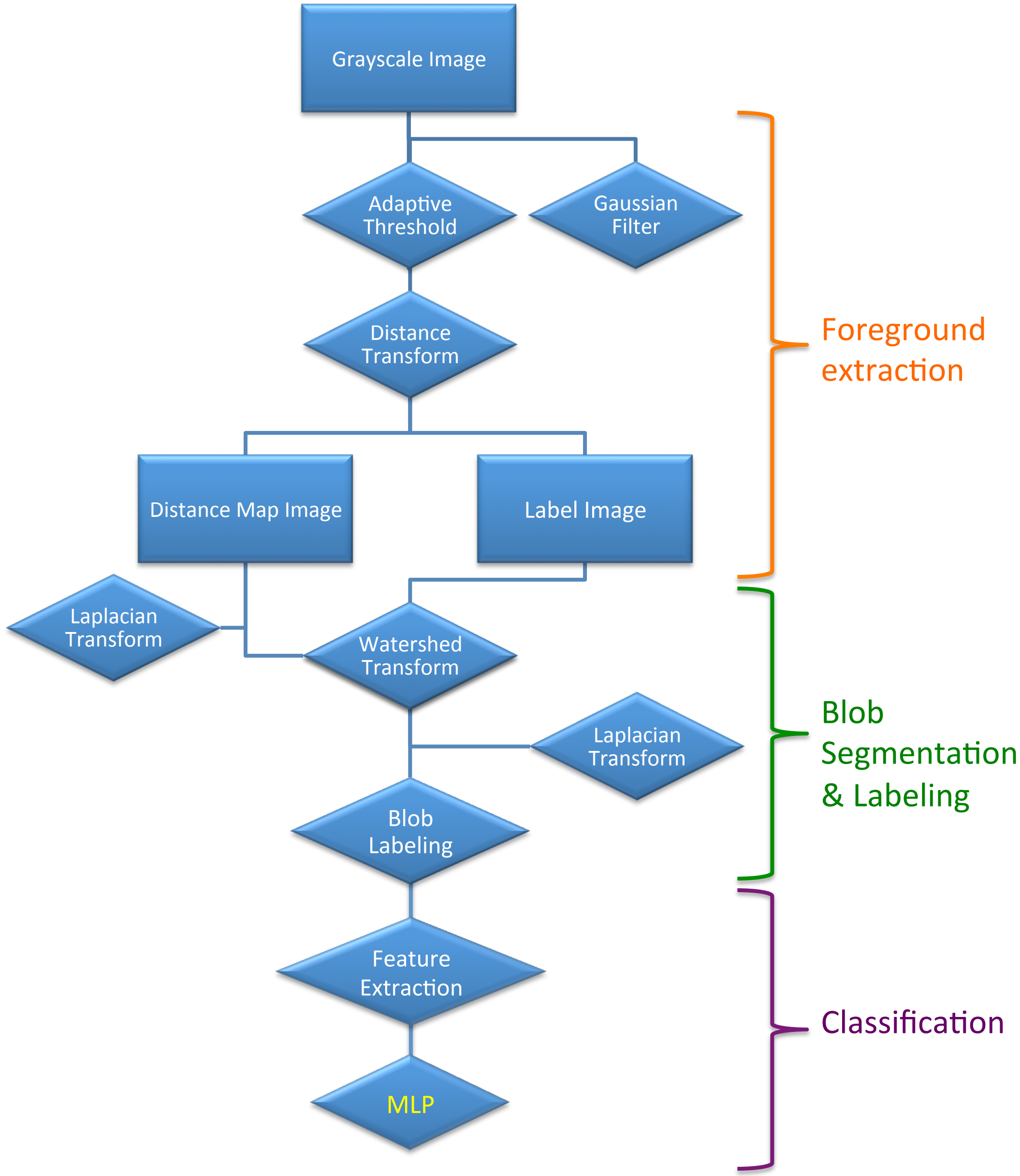
Where $(\overline{x}, \overline{y})$ *is the Blob Center of Mass*

- To be scale invariant the moments are normalized.

$$\eta_{ij} = \frac{\mu_{ij}}{\mu_{00}^{(1+\frac{i+j}{2})}}$$

Theory of Moments

- Using normalized central moments up to the third order, *Hu* introduced eight moments invariant to translation, scale and orientation.
- The eight Hu invariant moments were used as our feature set.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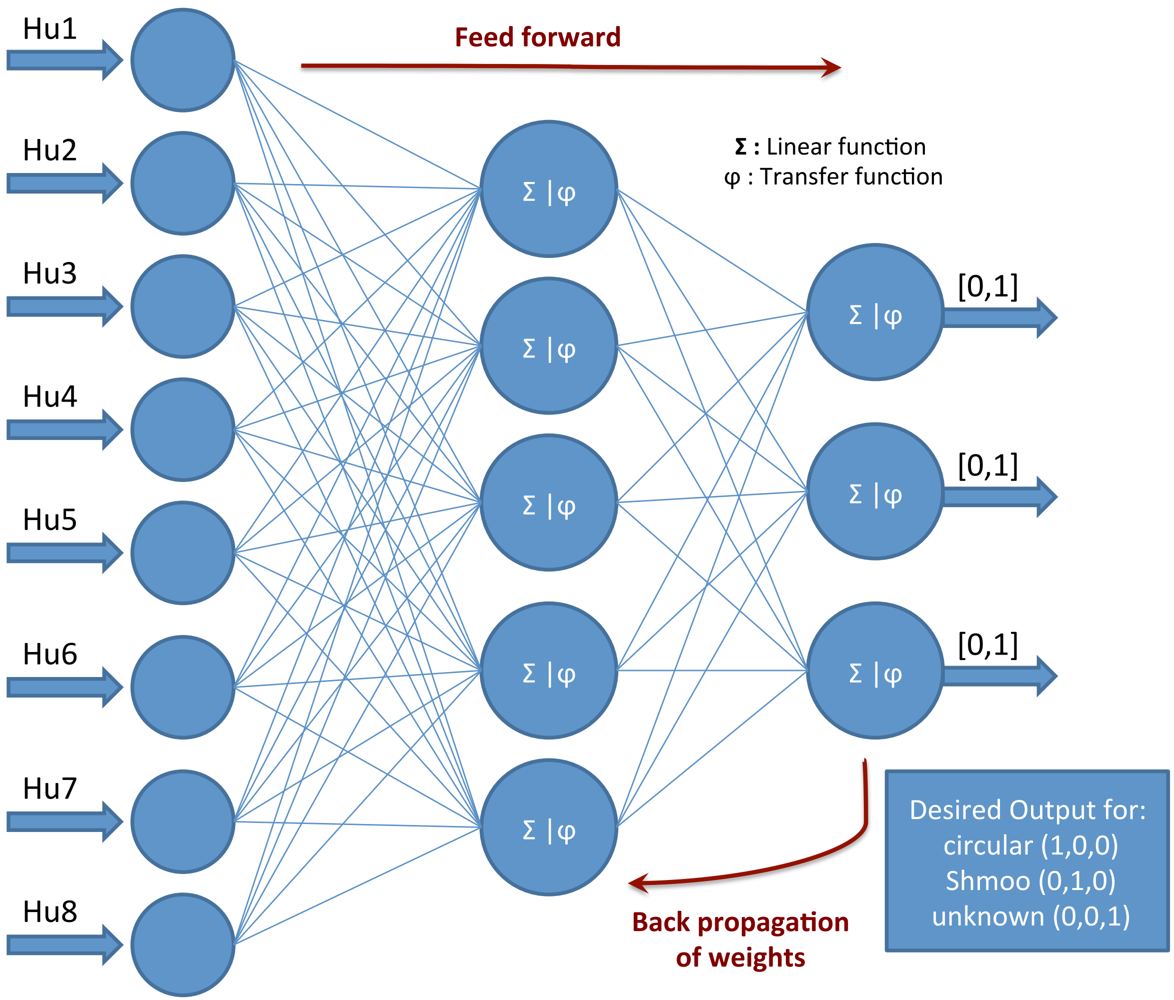
Multi-Layer Perceptron

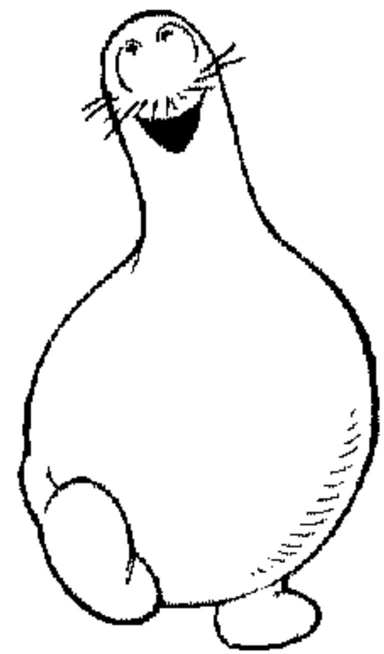
- Artificial Neural Network with interconnected simple computational elements called neurons.
  - Neurons are grouped into layers (input, hidden, output).
  - Useful in mapping an input vector to different classes by optimizing the weights associated with each neuron in the hidden and output layer.
- Optimization of the weights is done using the back propagation learning algorithm.
  - By comparing the actual output with the desired output.

Multi-Layer Perceptron



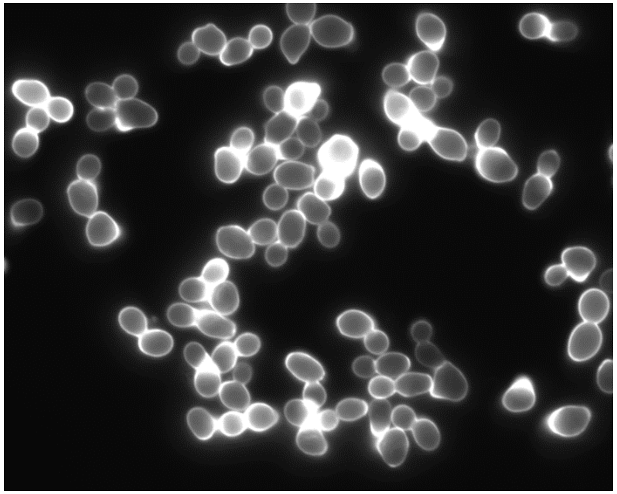
Data Set

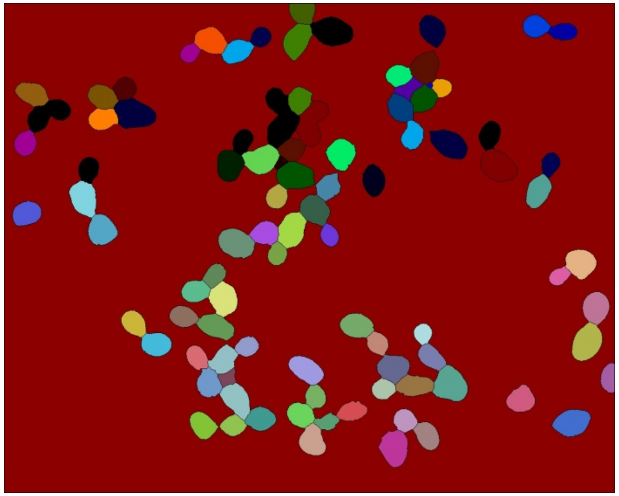
- Three types of cells were extracted from a set of 20 images to test our classifier:
  - Normal (circular): 26 cells.
  - Shmoo : 24 cells.
  - Noisy (unclassified) : 17 cells.
    - Total number : 67 cells.



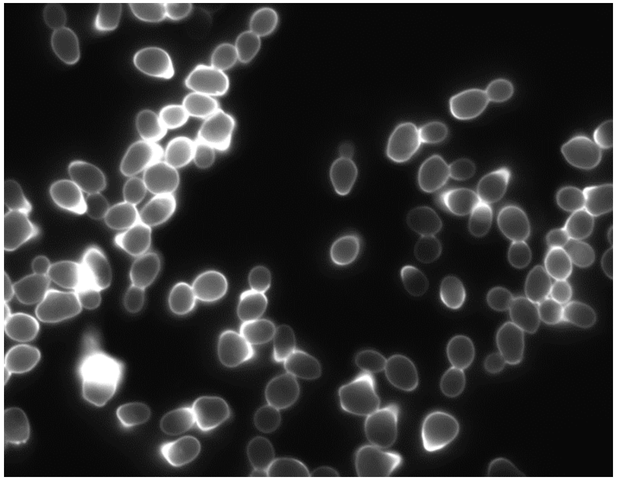
Shmoo Cell

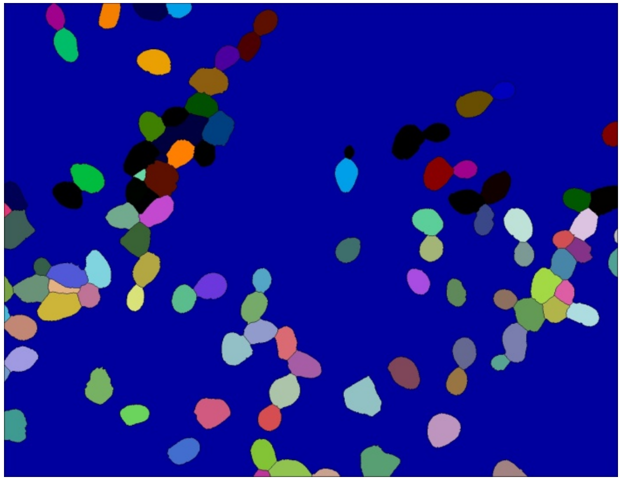
Segmentation Results





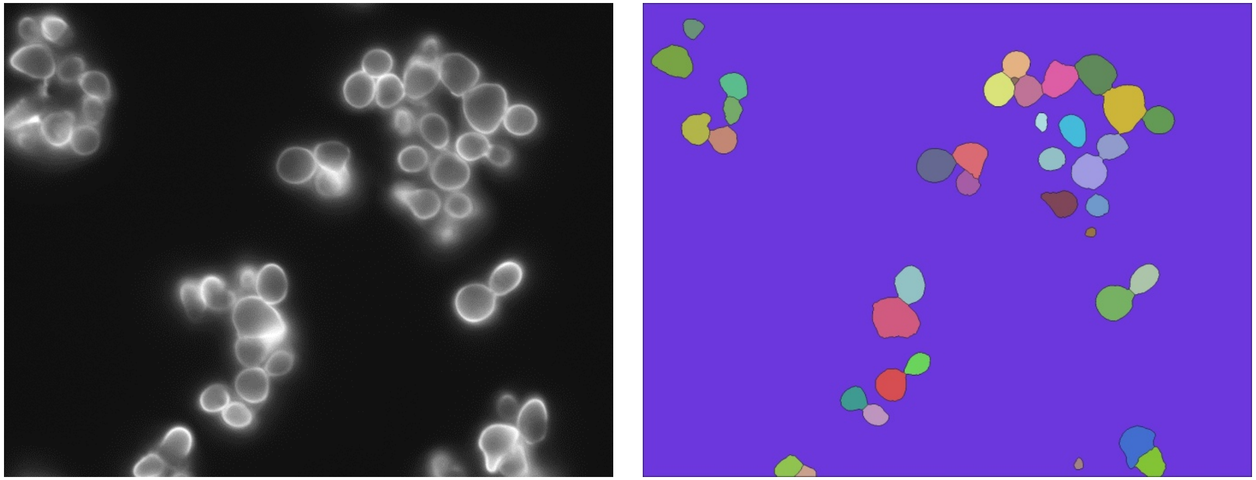
96.77%



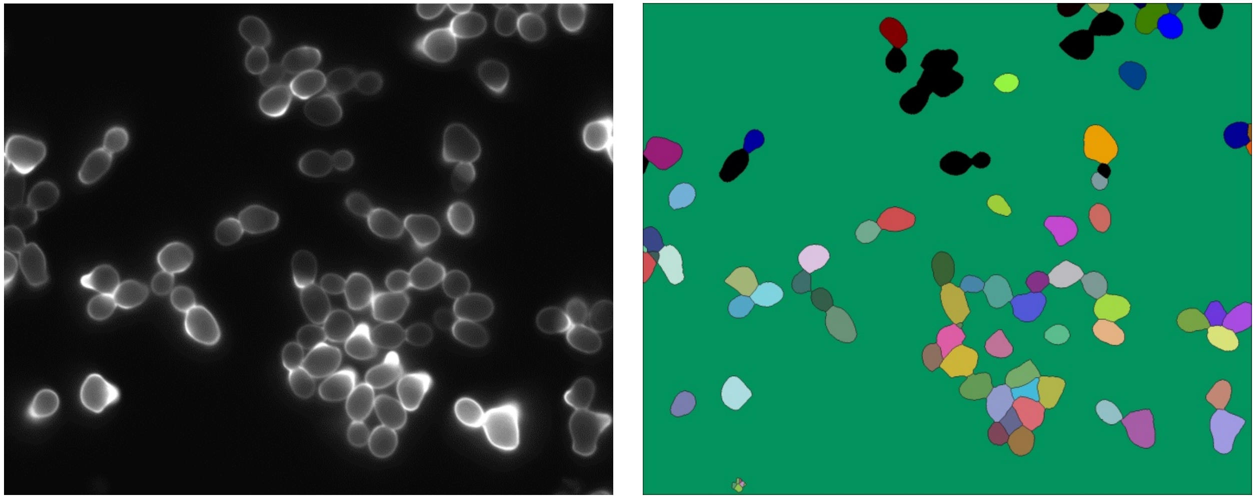


95.01%

Segmentation Results



90.24%

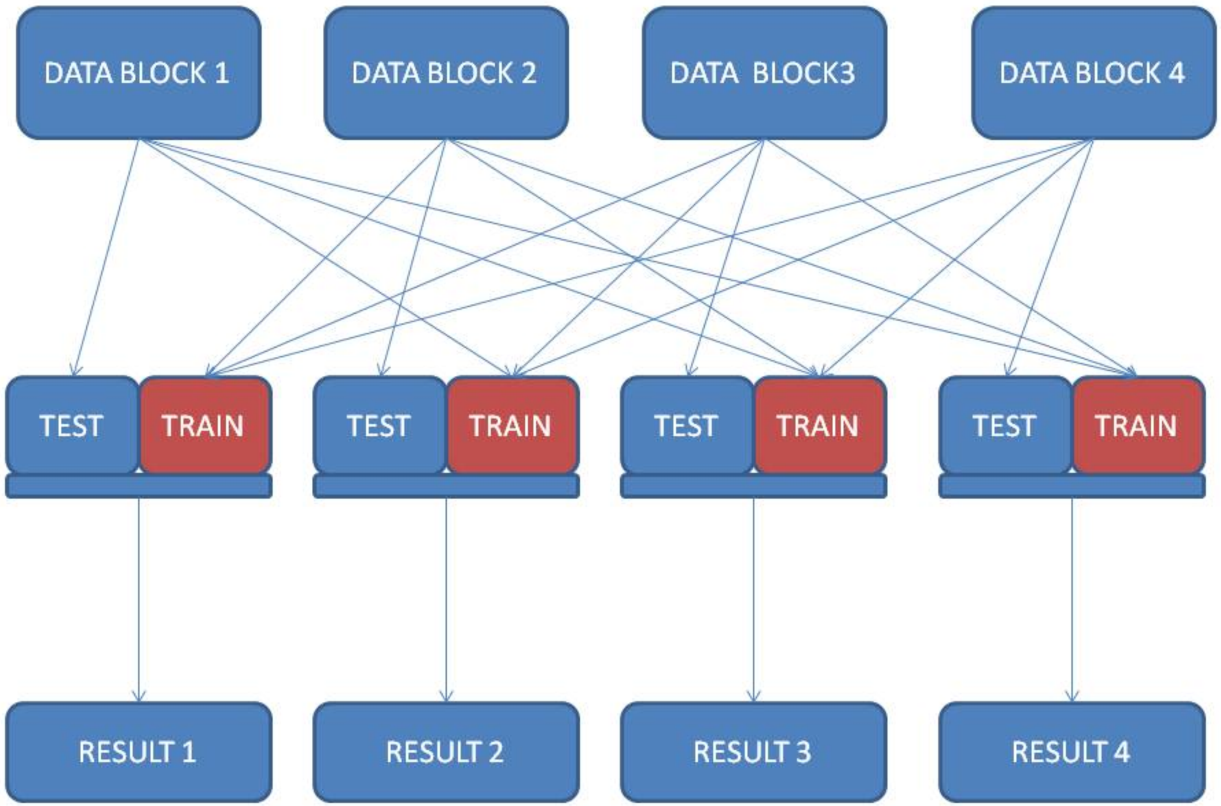


89.21%

Classification Results

- The cells extracted were evenly distributed to four data blocks.
  - Data Block 1 : 6 Normal, 6 Shmoo, 2 Unclassified.
  - Data Block 2 : 6 Normal, 6 Shmoo, 5 Unclassified.
  - Data Block 3 : 7 Normal, 6 Shmoo, 5 Unclassified.
  - Data Block 4 : 7 Normal, 6 Shmoo, 5 Unclassified.

Classification Results



Normal class	100%	100%	100%	86%
Shmoo class	100%	84%	100%	100%

Future Works

- Get more data to further test the method.
- Make the algorithm able to process different kind of images.

Questions?