Implementation and evaluation of Otsu's thresholding

Project proposal

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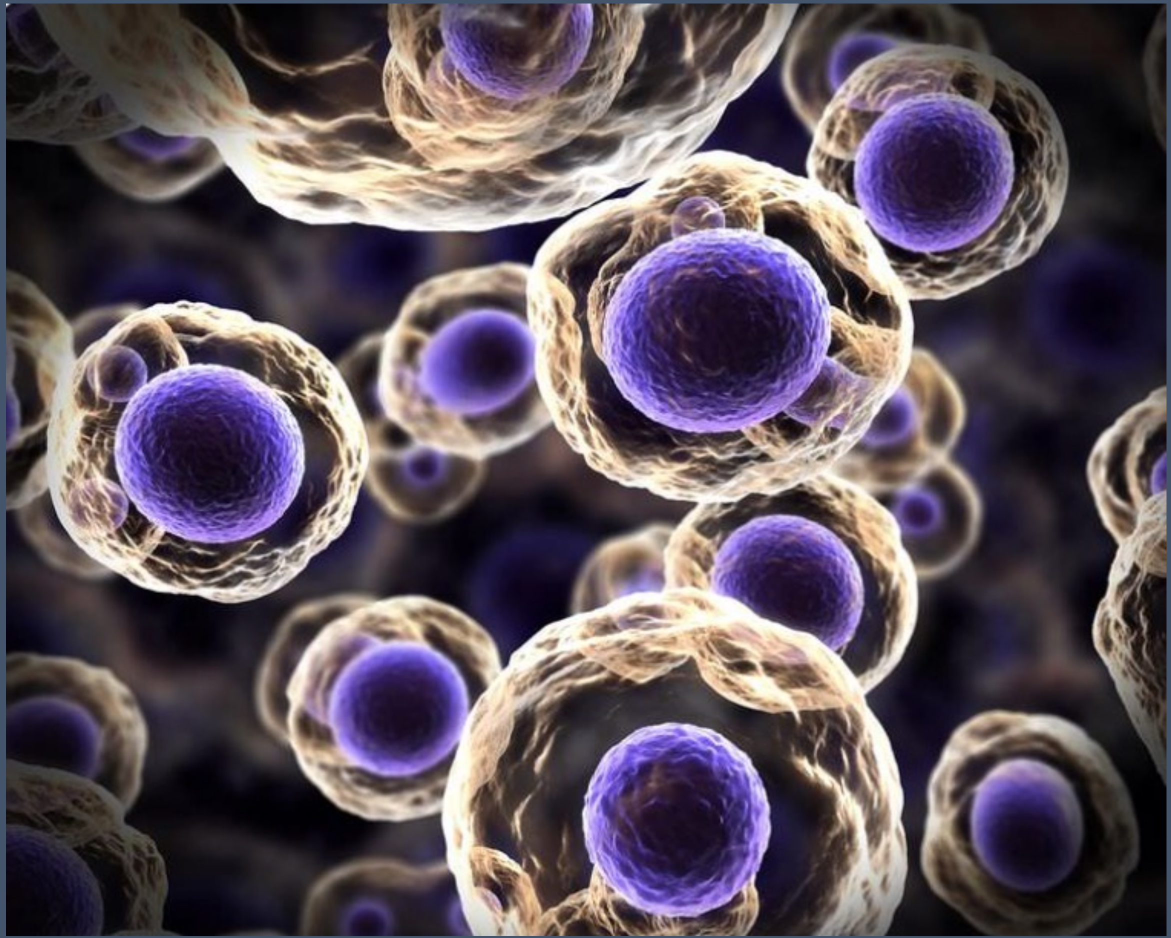
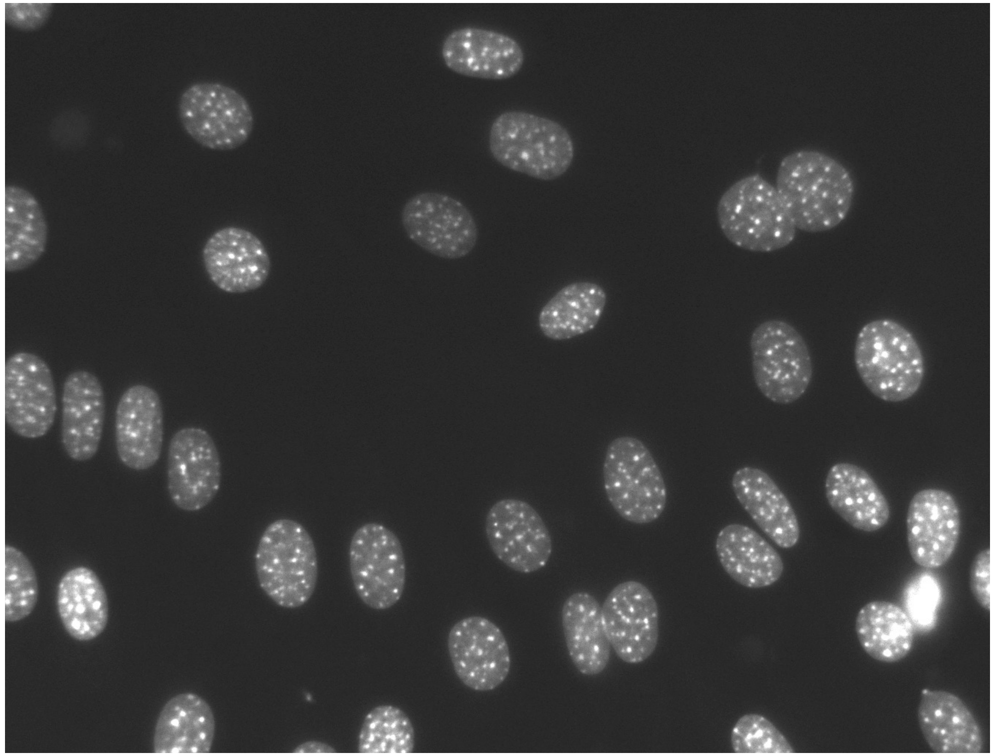
12.05.2021

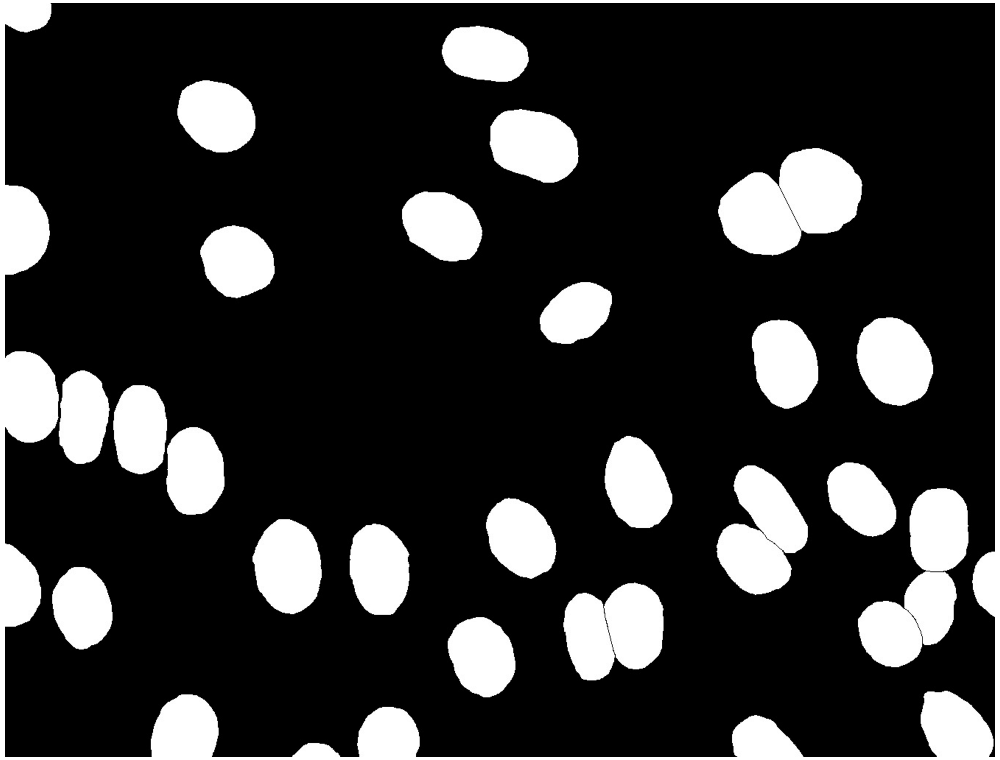


Cell nuclei segmentation

https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_Introductory_Biology_(CK-12)/02%3A_Cell_Biology/2.08%3A_Cell_Nucleus

Image segmentation

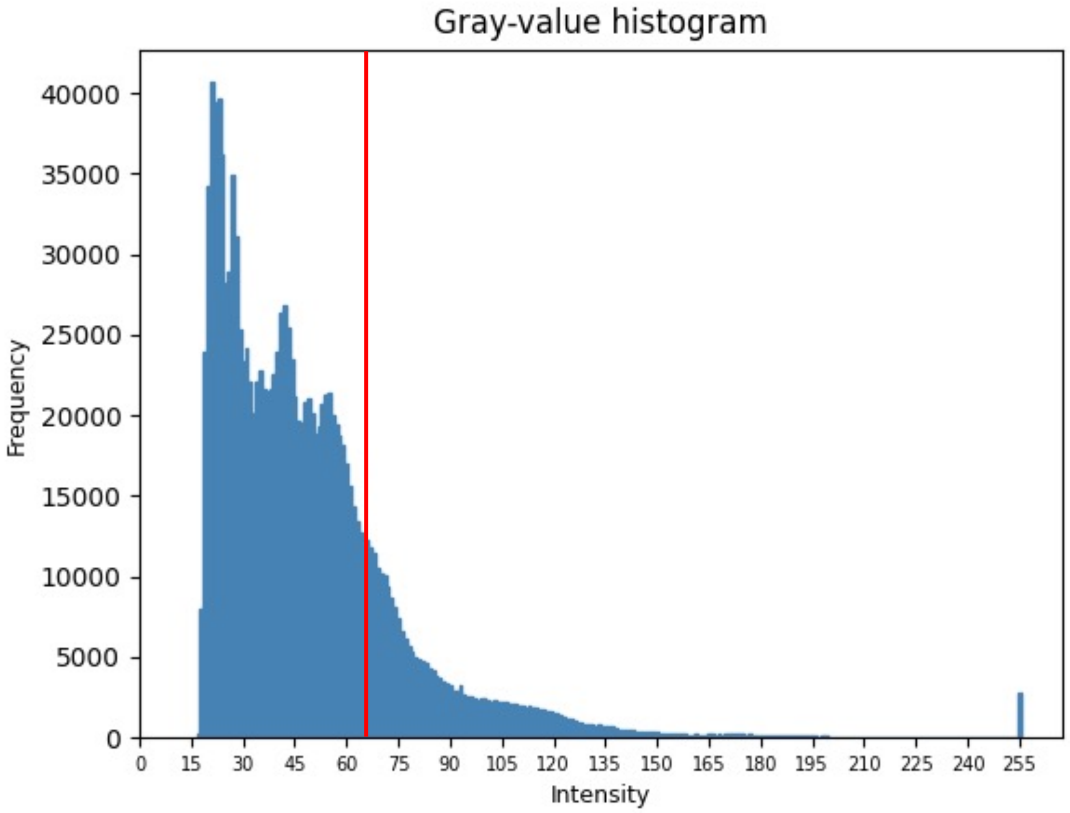




Methods

- Thresholding → Intensity clipping

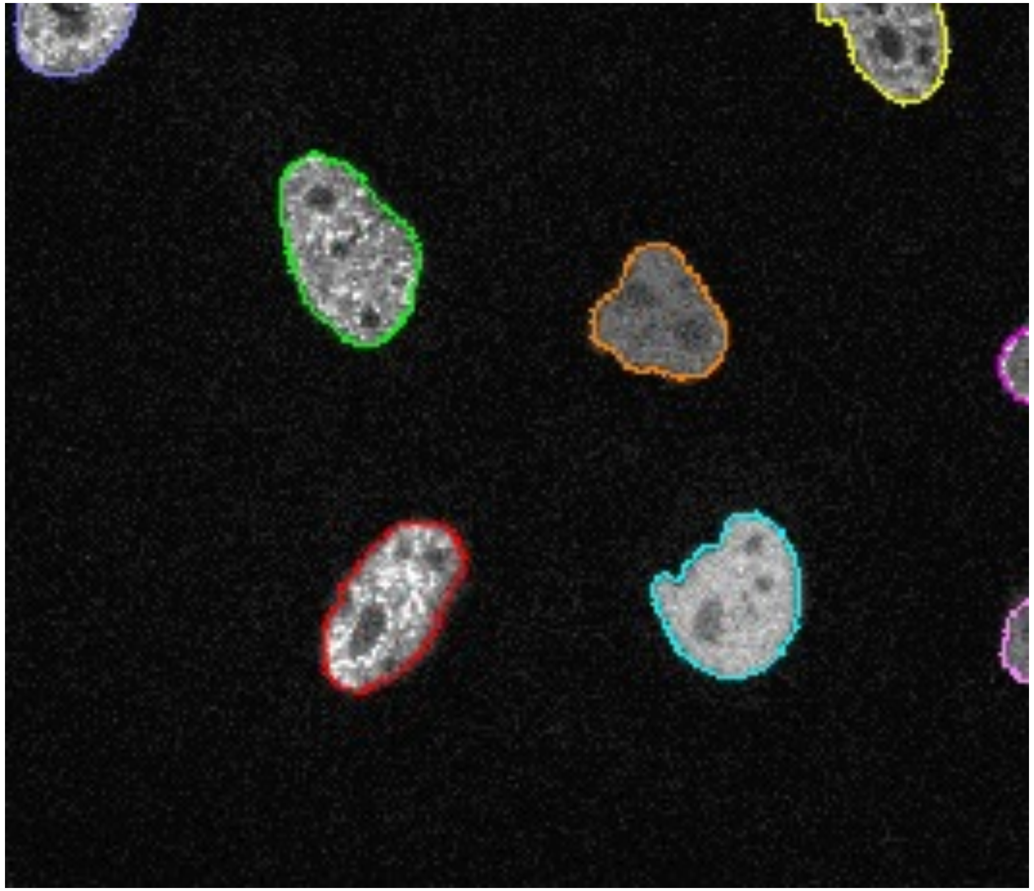
Otsu's thresholding



Applications

- High-throughput cytometry
  - Cell size
  - Cell counting

- Cell tracking



Workflow

01
Input

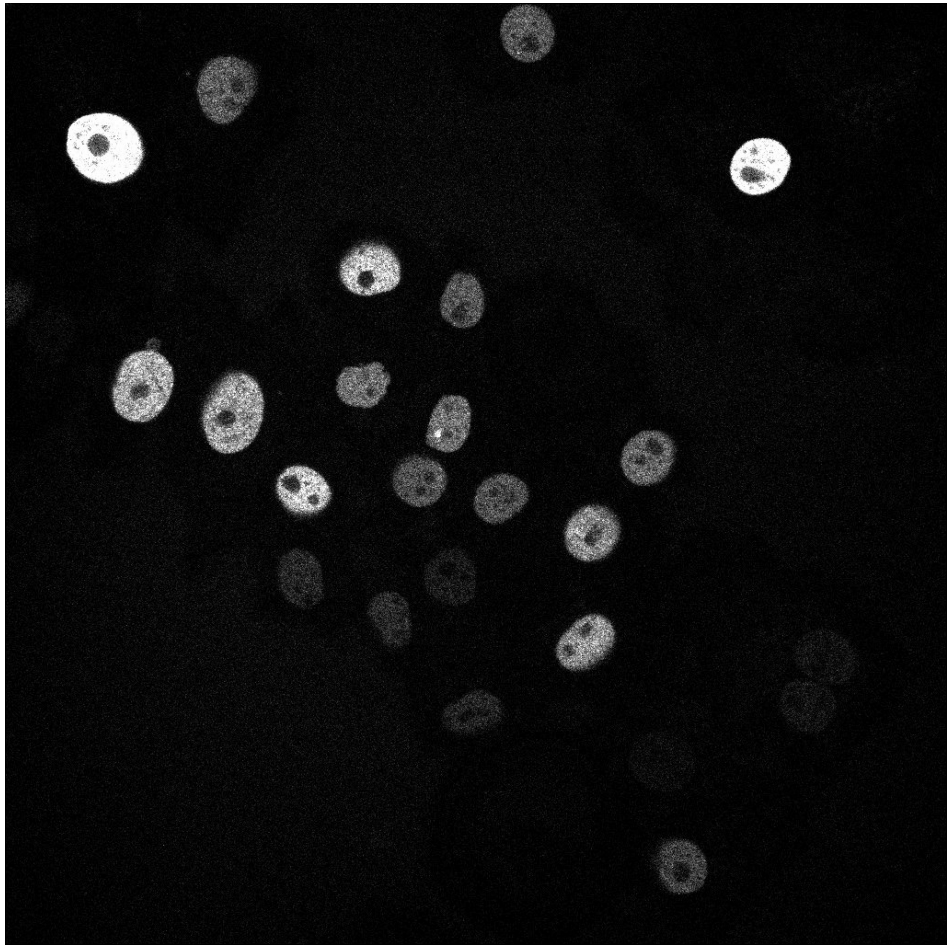
02
Preprocessing

03
Otsu‘s Thresholding

04
Dice Score

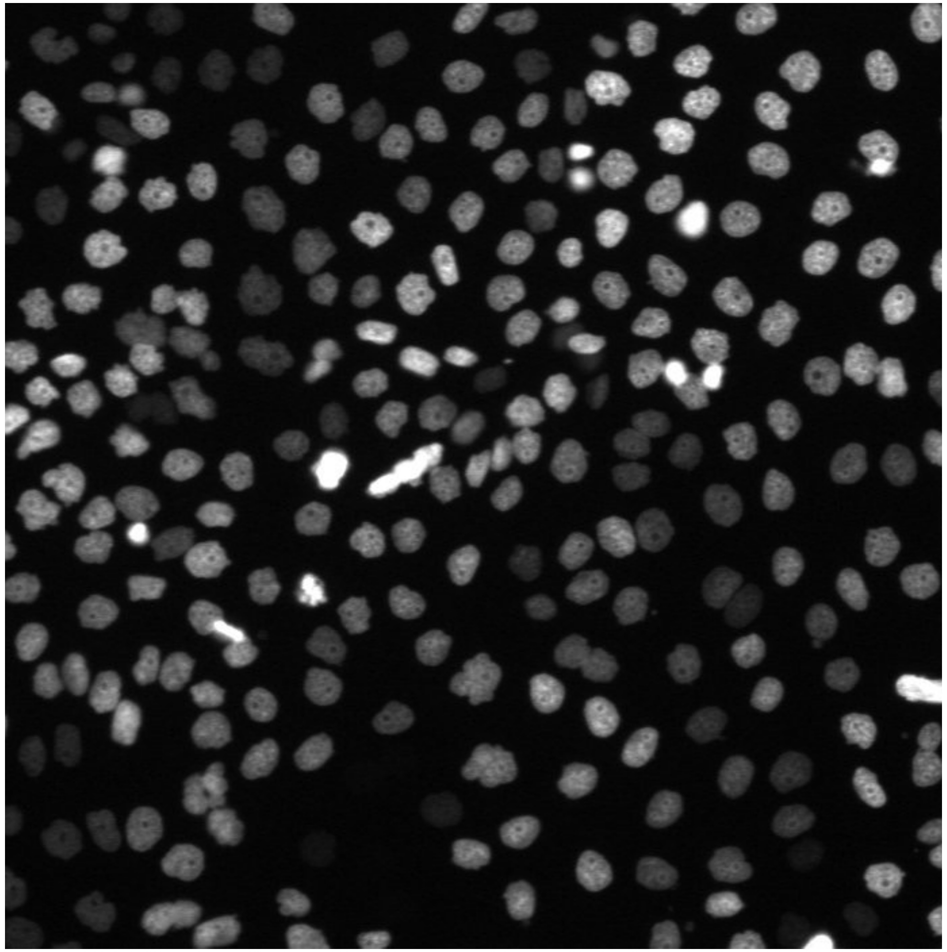
05
Comparison

Input 1: N2DH-GOWT1 cells



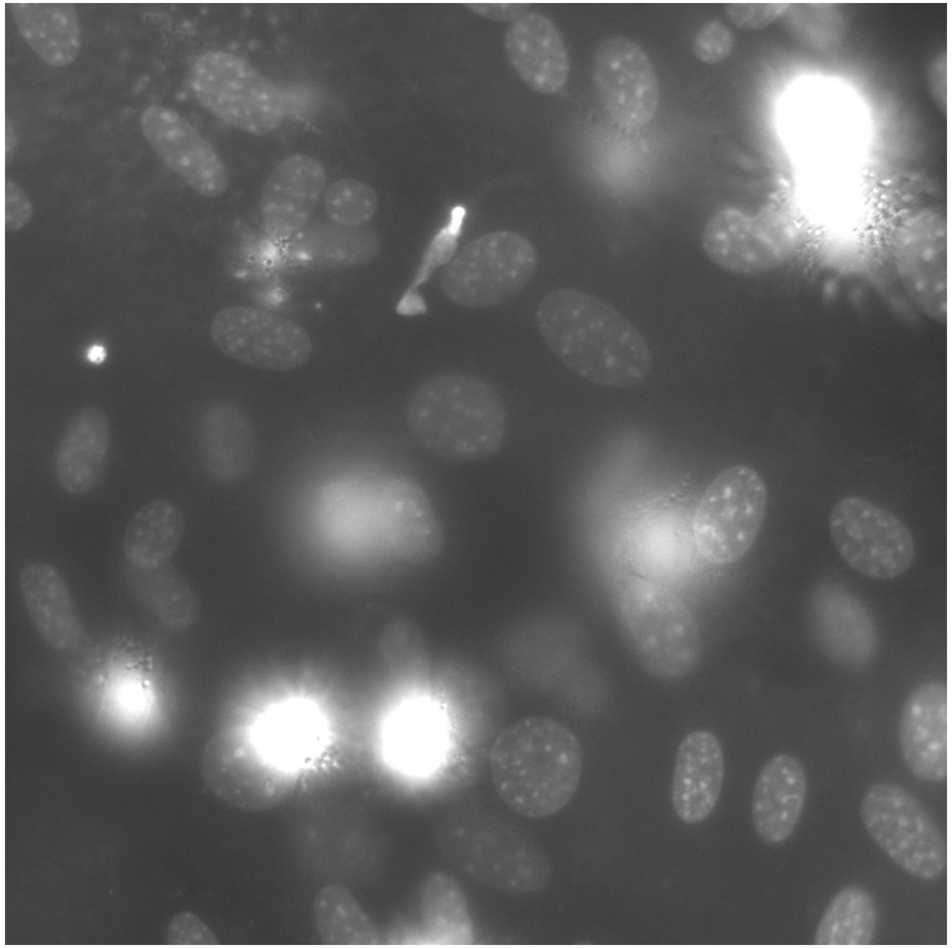
- GFP-Gowt1 mouse embryonic stem cells
- Time-lapse confocal microscopy and GFP-staining
- Investigate genomic integrity of the cells

Input 2: N2DH-HeLa cells



- Human epithelial cells of cervical cancer
- Live imaging of fluorescently labelled chromosomes
- Olympus IX81 microscope
- Phenotypic profiling of the human genome

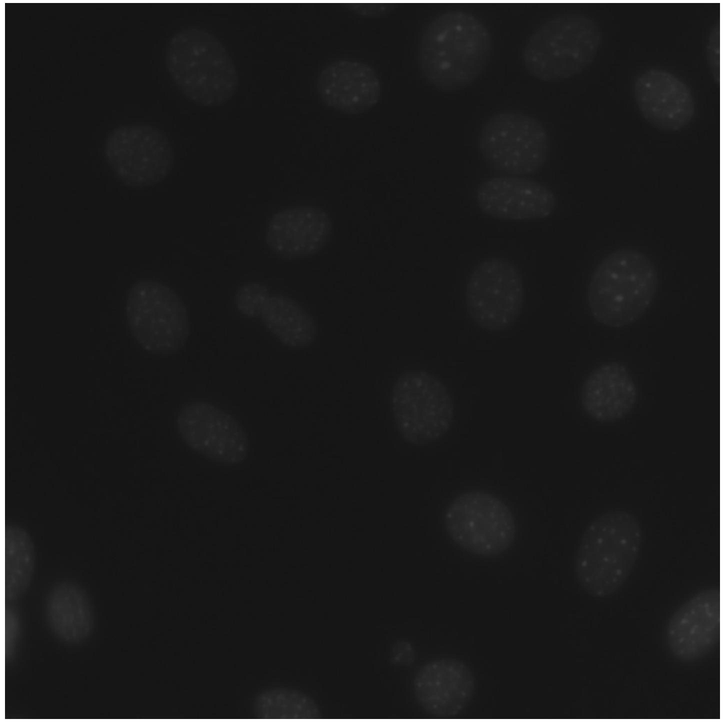
Input 3: NIH3T3 cells



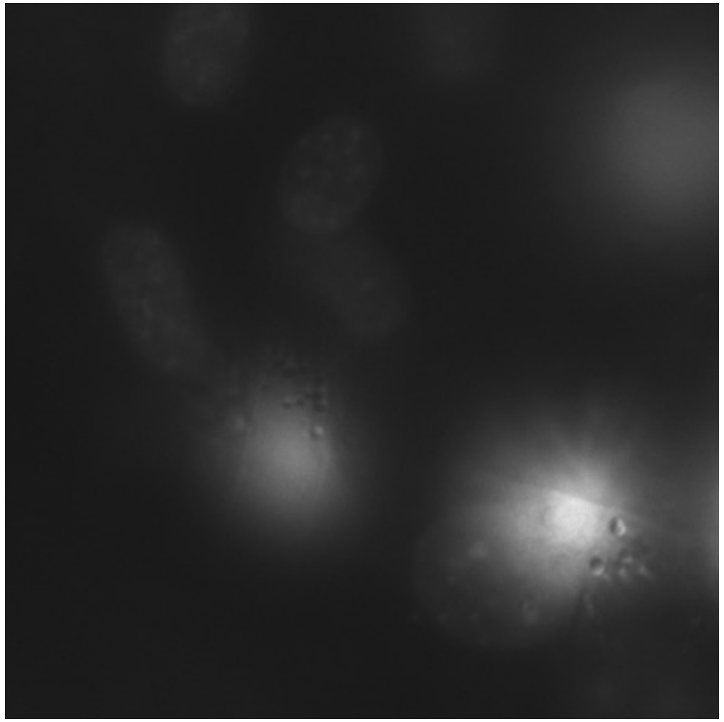
- Several mouse embryonic fibroblast cells
- Fluorescence microscopy images
- Evaluation of image analysis pipelines

Problems

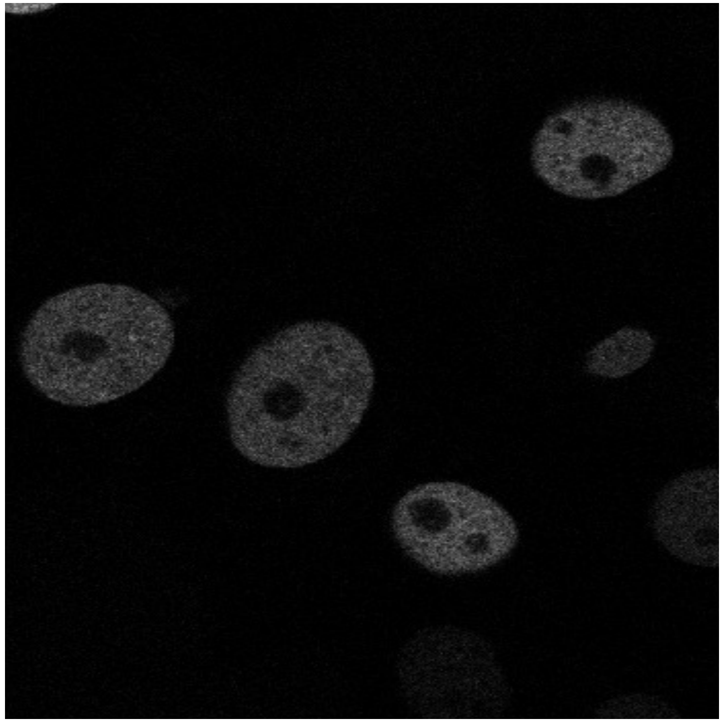
Low contrast



Reflections



Random noise



Preprocessing

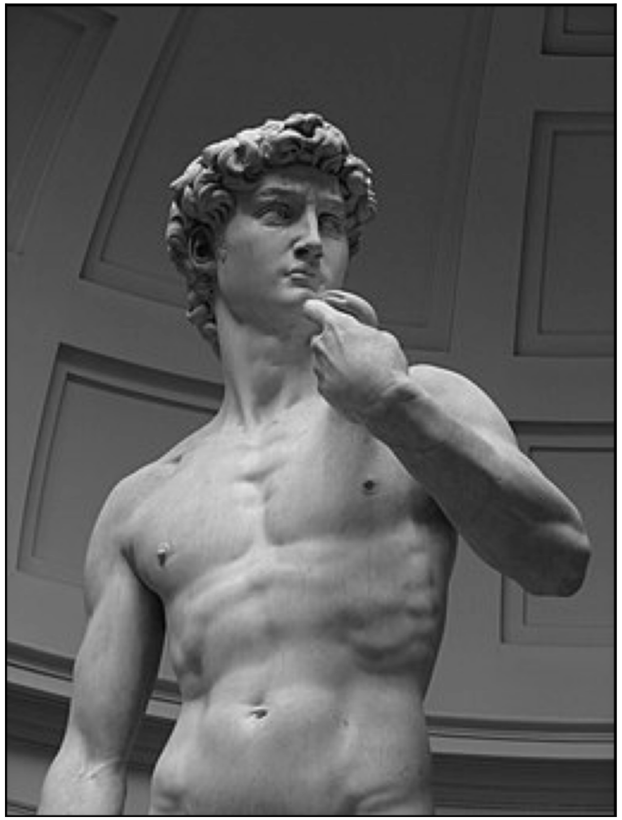
Solutions:

- Random noise

  → **Gaussian filter**

- Reflections

- Low contrast



Original

Gaussian filter ($\sigma = 3$)

Preprocessing

Solutions:

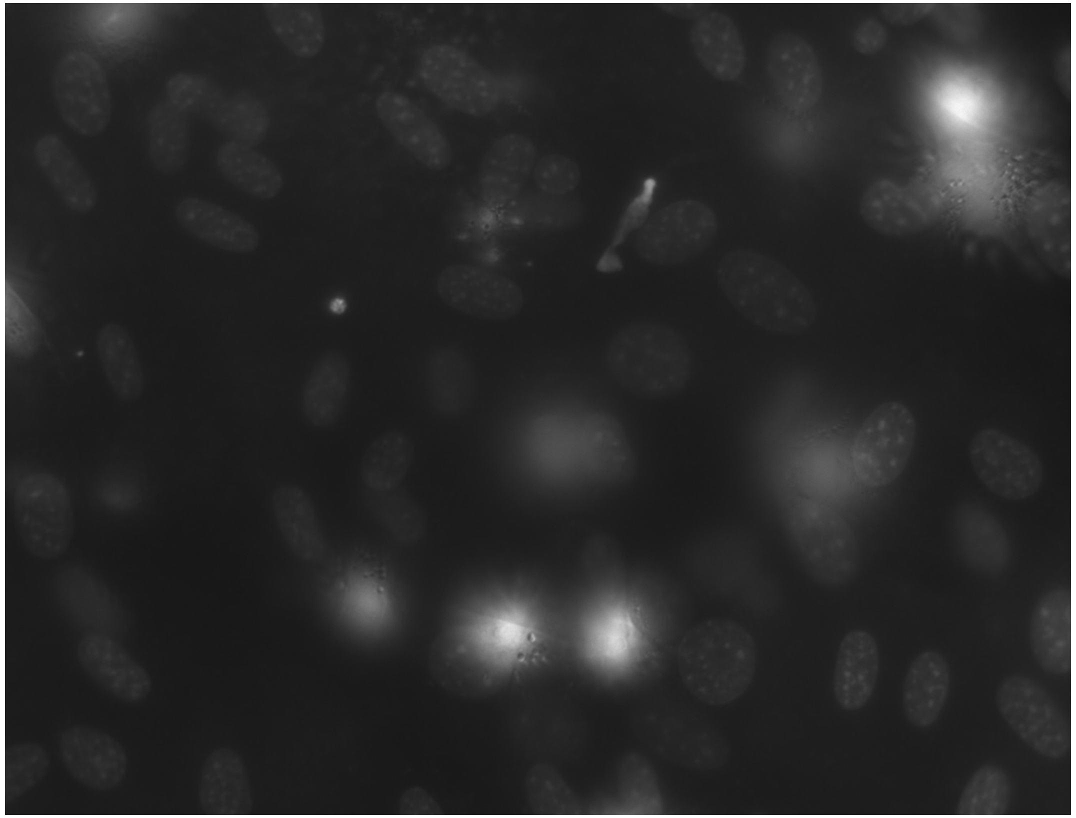
- Random noise

→ **Gauss filter, median filter**

- Reflections

→ **Thresholding**

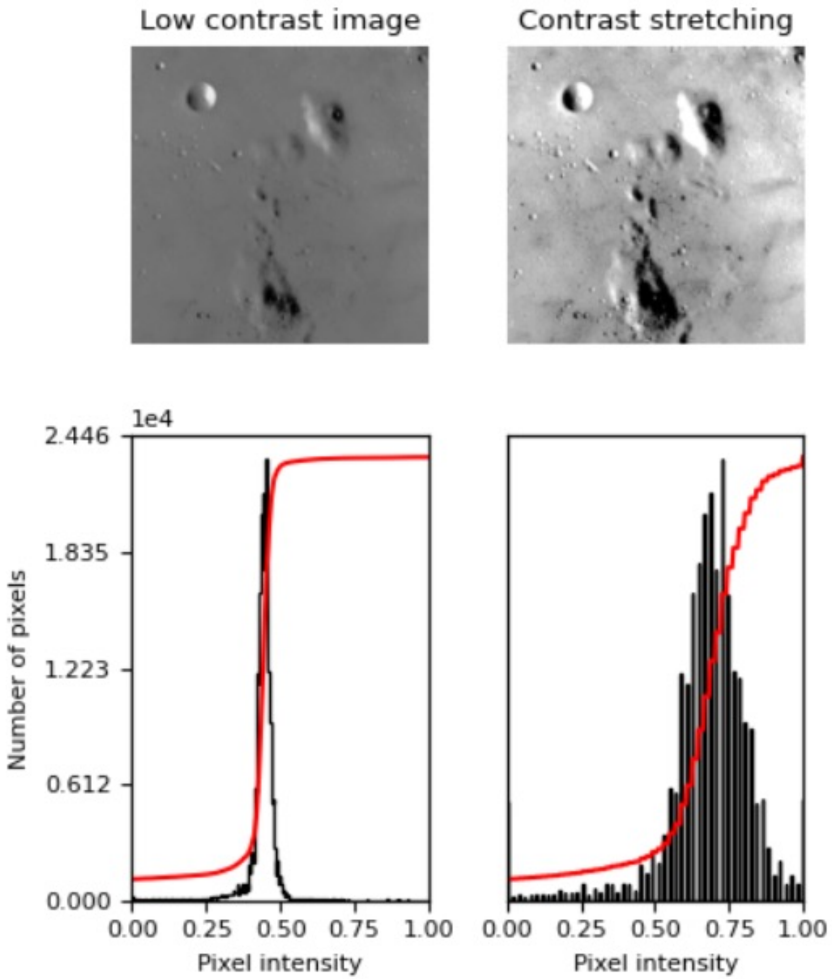
- Low contrast



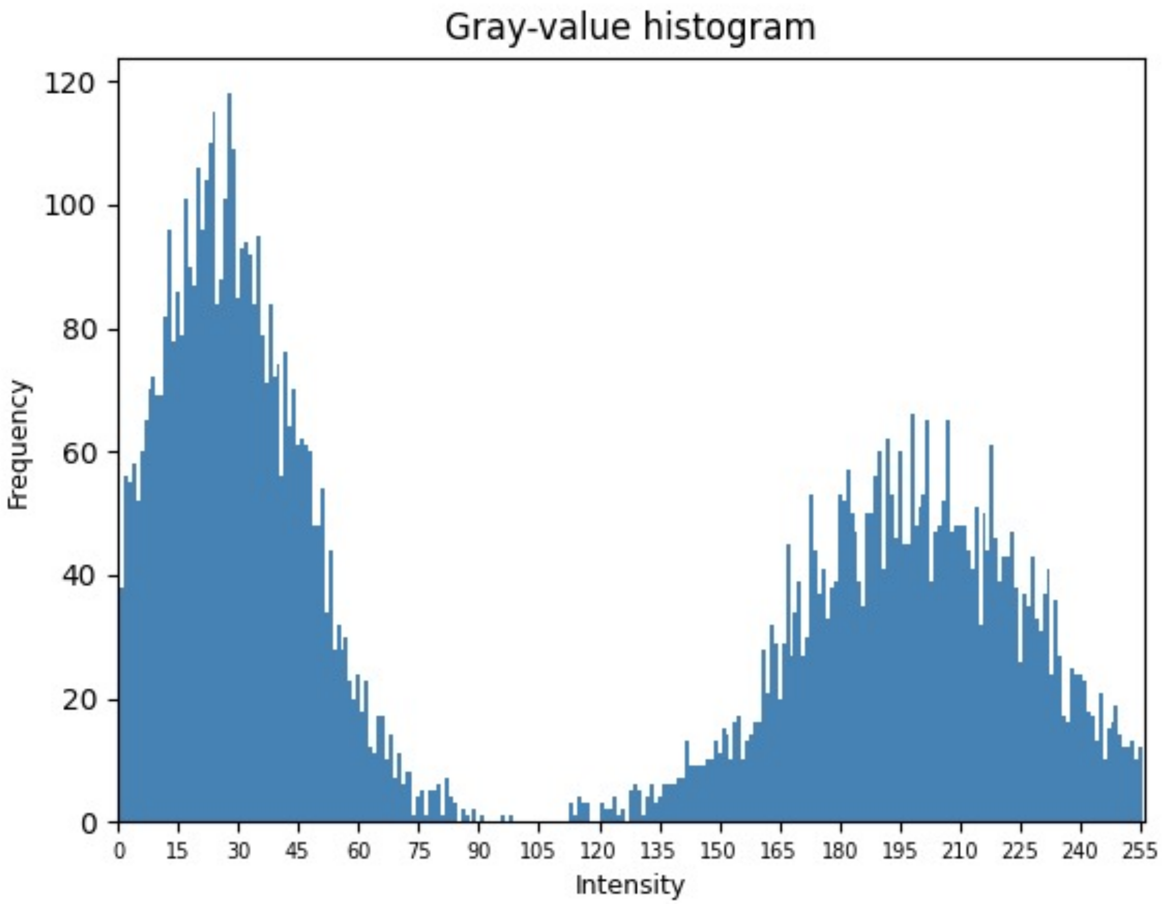
Preprocessing

Solutions:

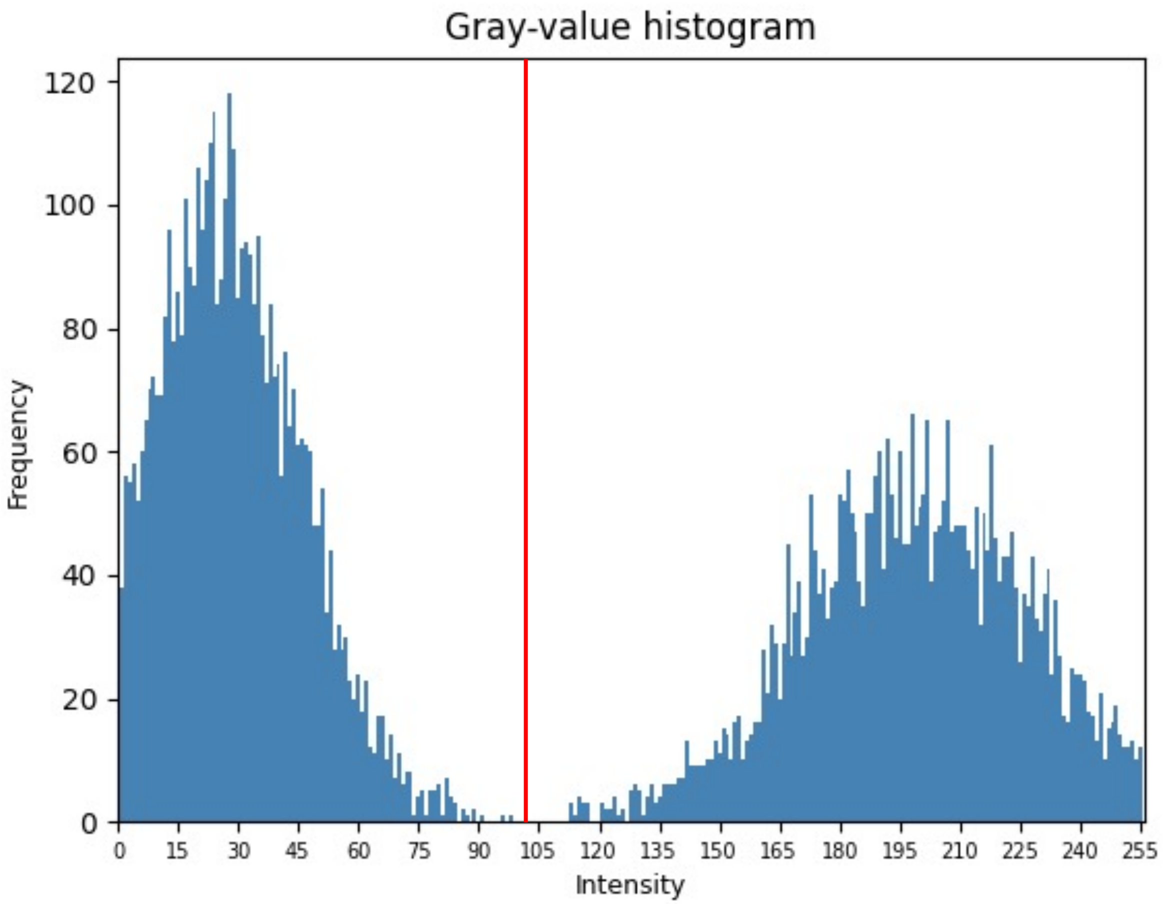
- Random noise
  → **Gauss filter, median filter**
- Reflections
  → **Thresholding**
- Low contrast
  → **Histogram stretching**



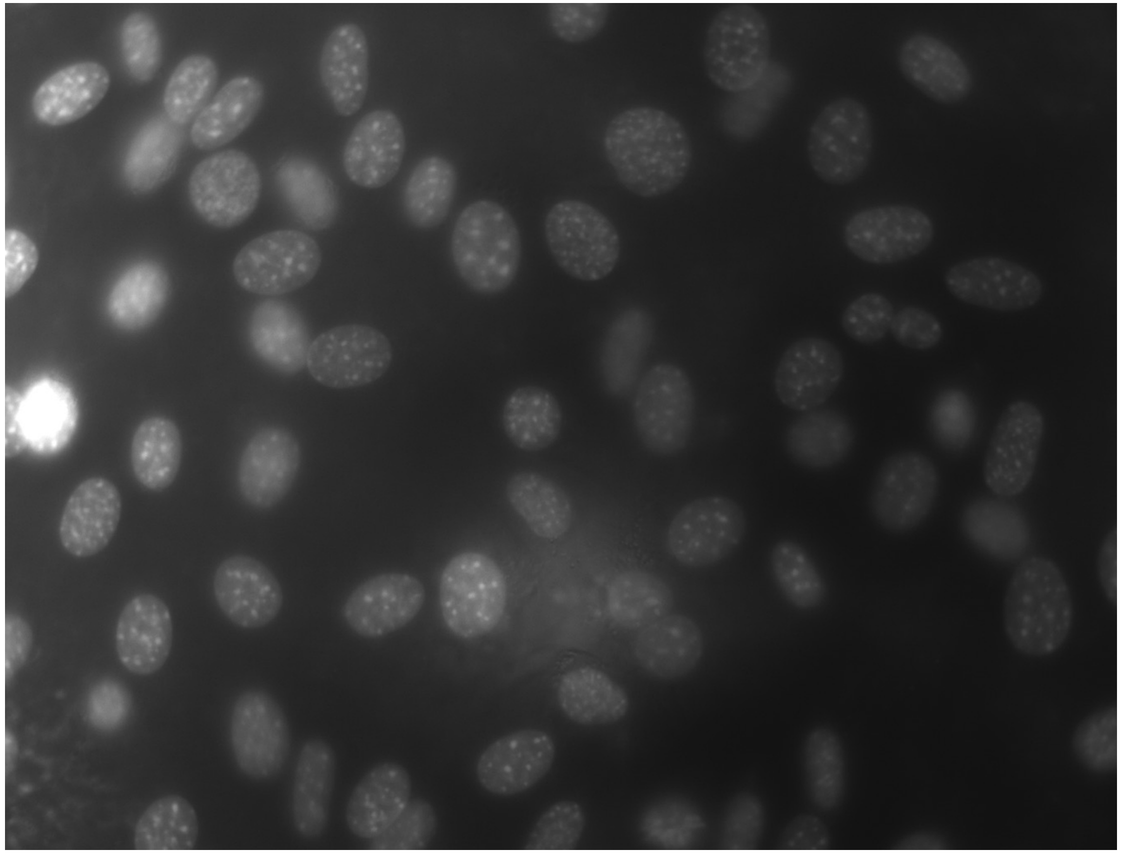
Otsu's Thresholding

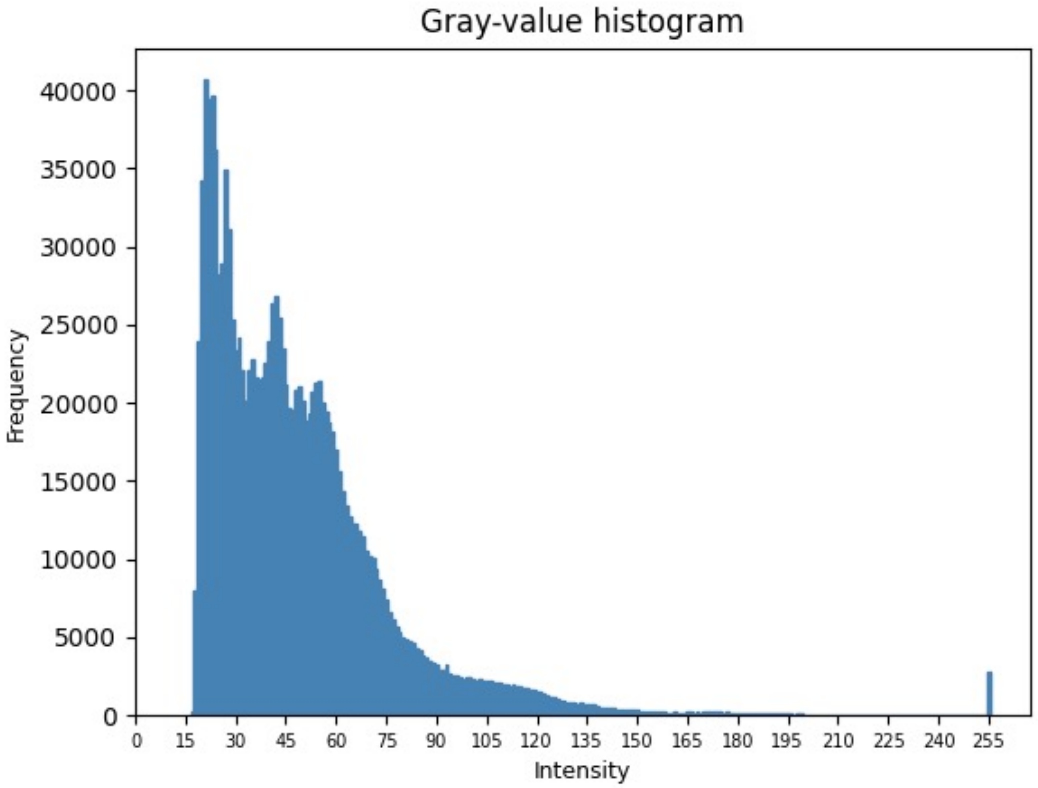


Otsu's Thresholding



Otsu's Thresholding





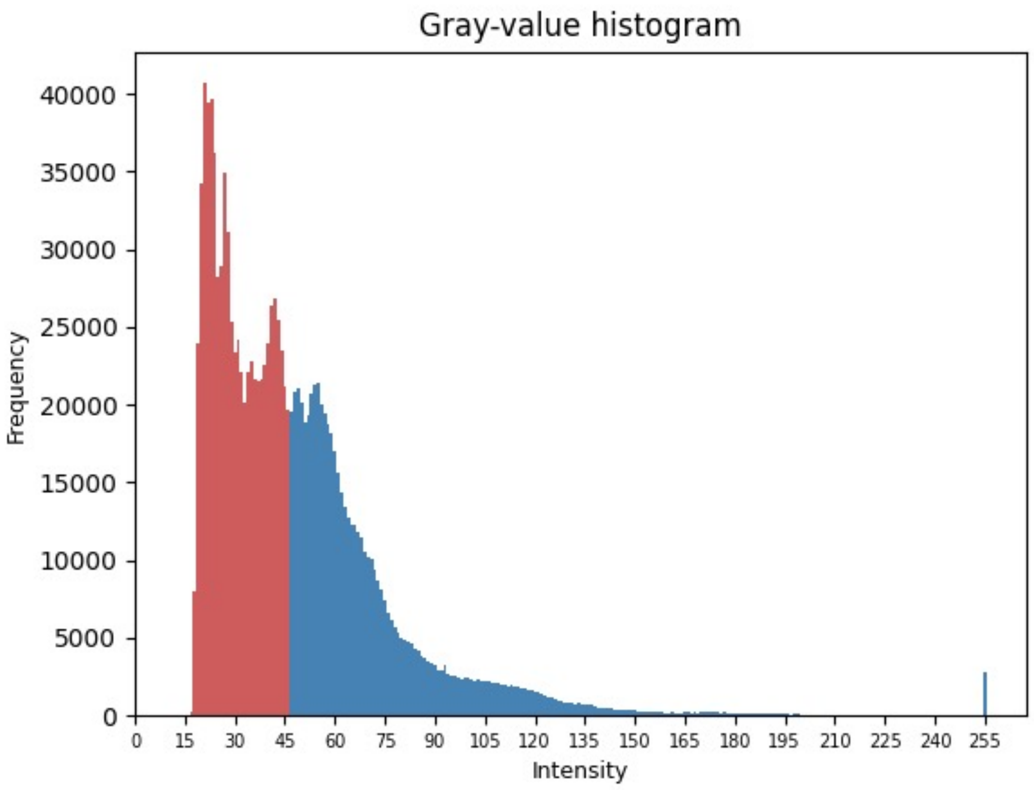
Threshold value k $\in [0,255]$

Otsu's Thresholding

Between-class variance

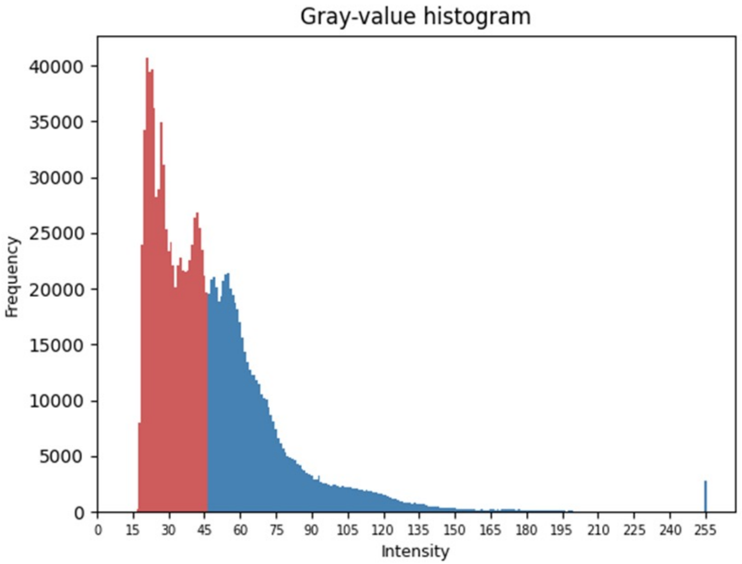
$$\sigma_B = \omega_0 \omega_1 (\mu_1 - \mu_0)^2$$

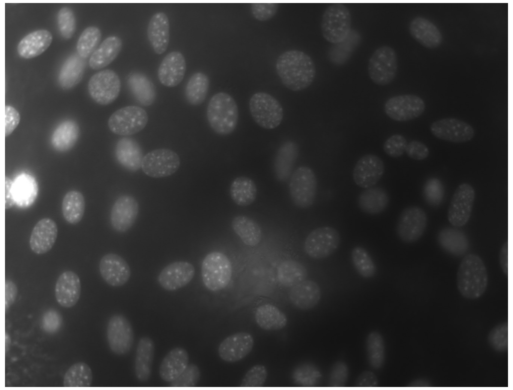
$\omega_{0,1}$ = probability of class occurrence
$\mu_{0,1}$ = mean intensity values



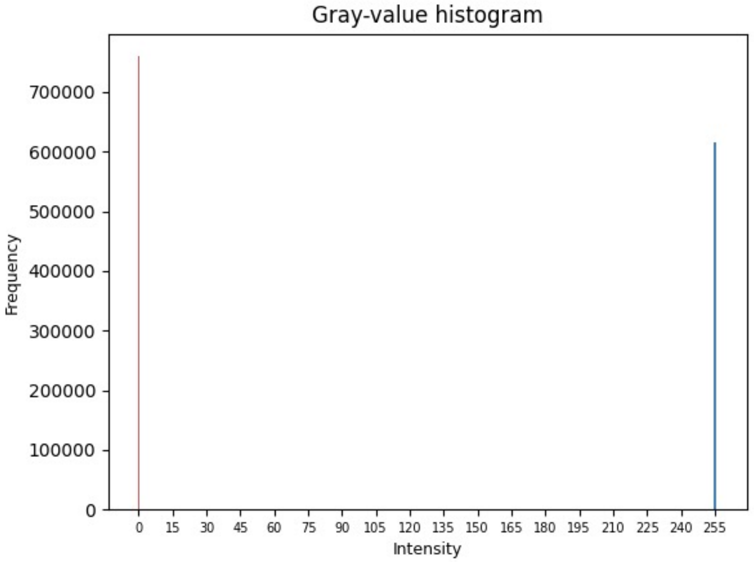
Threshold value k $\in [0,255]$

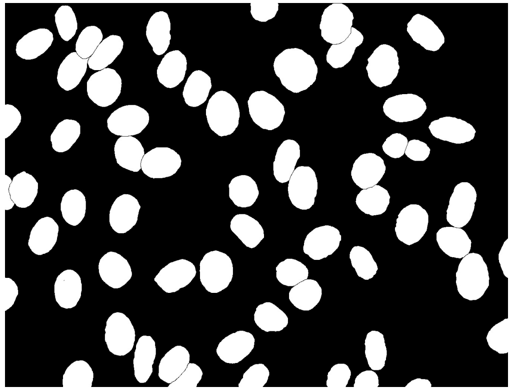
Image clipping



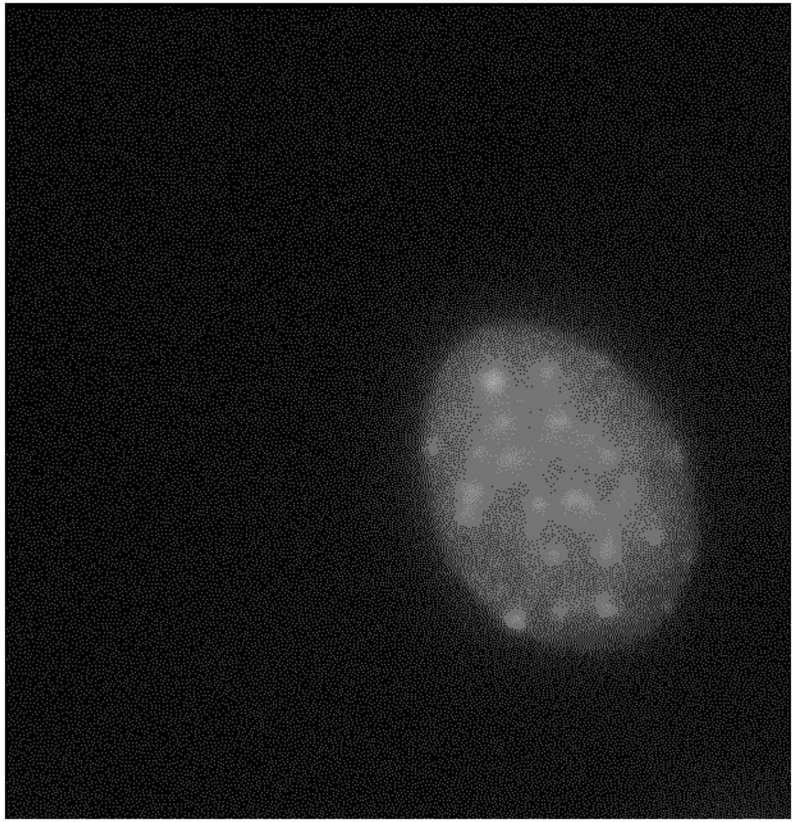


$$g_{clip}(x,y) = \begin{cases} 0 & \text{if } g(x,y) \leq k \\ 255 & \text{if } g(x,y) > k \end{cases}$$

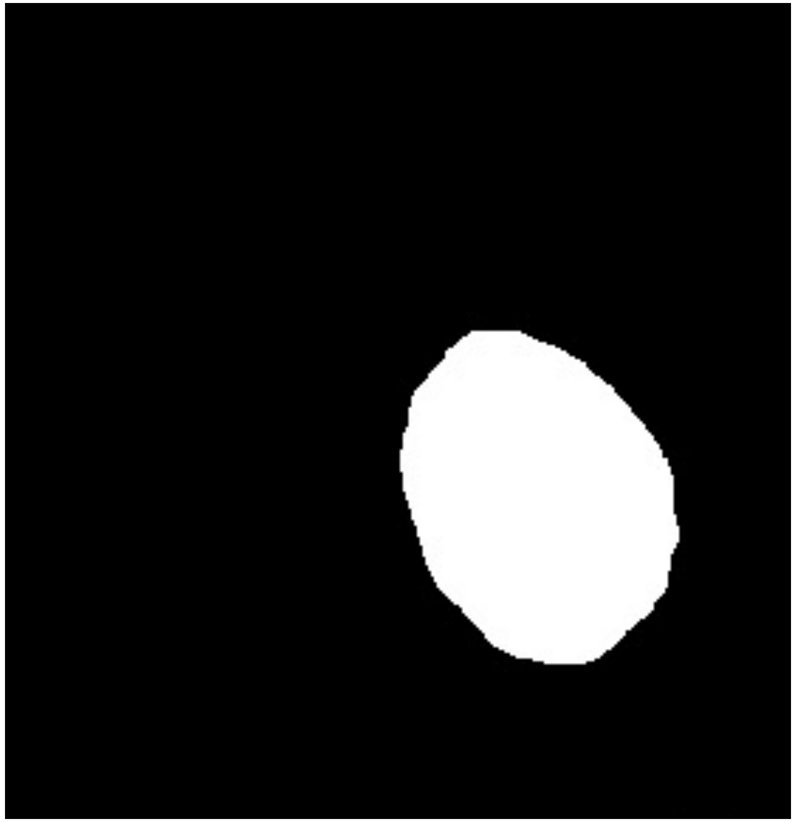




Implementation of the Dice Score



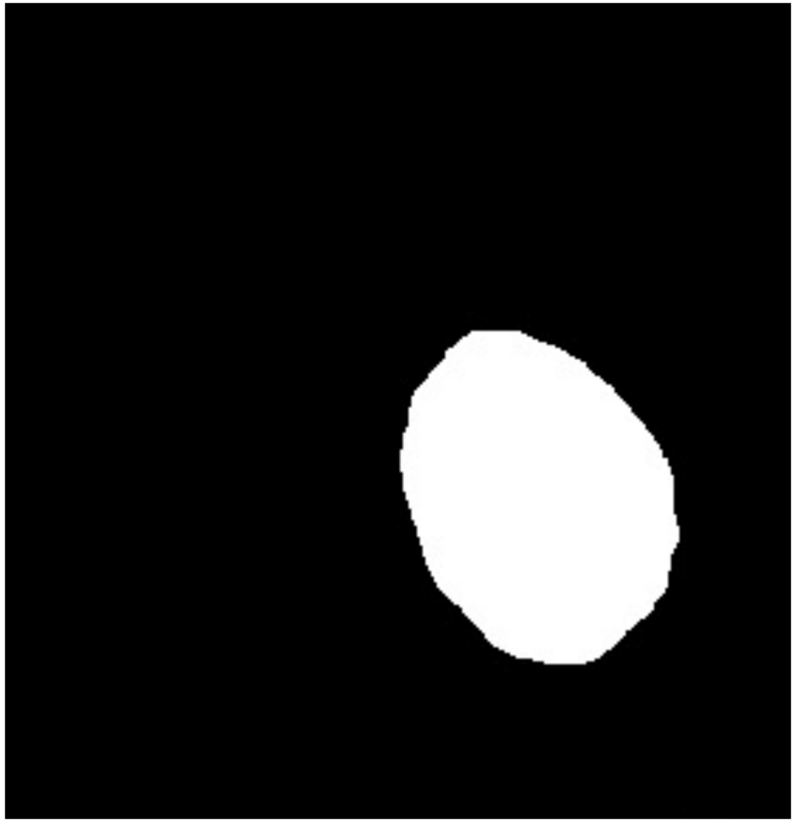
Original image



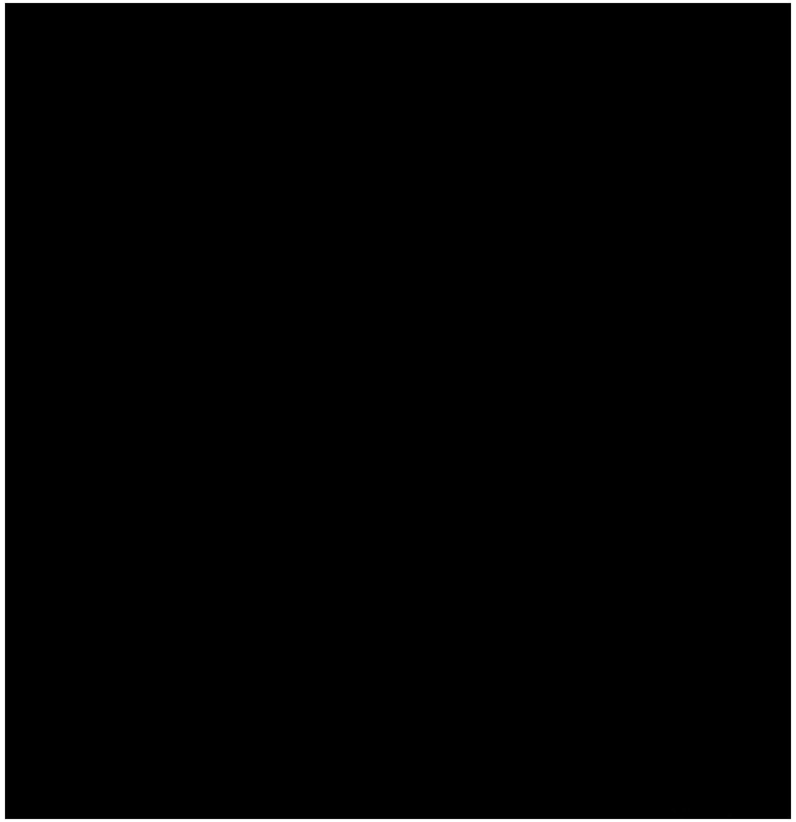
Ground truth

Implementation of the Dice Score

≈80 % accuracy



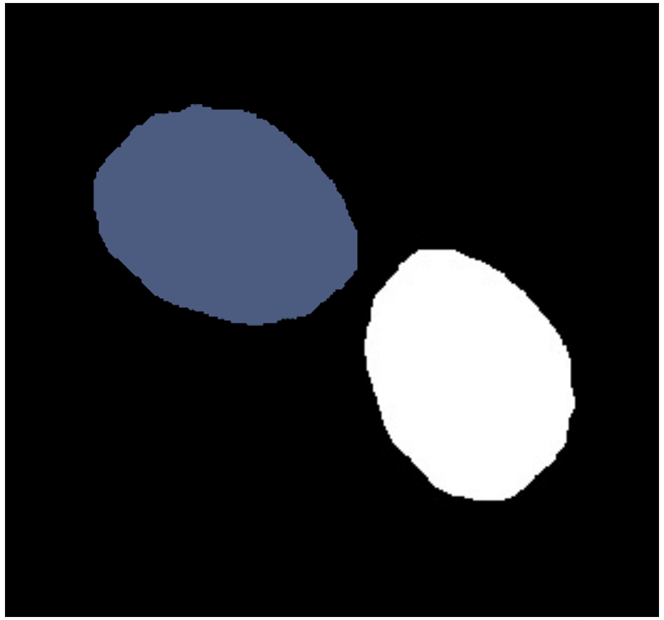
Ground truth



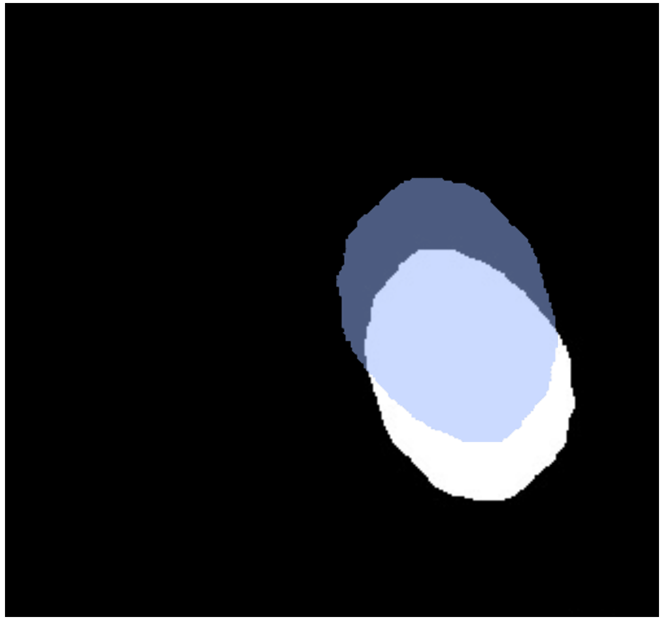
Prediction

Implementation of the Dice Score

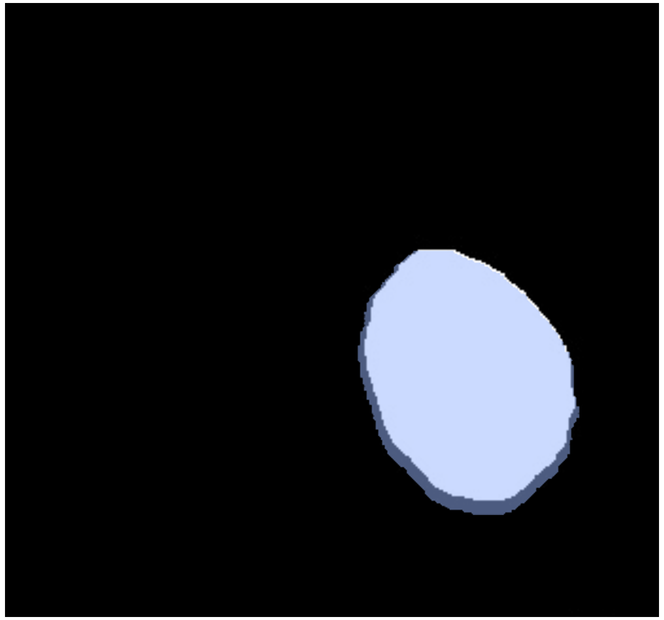
Bad prediction



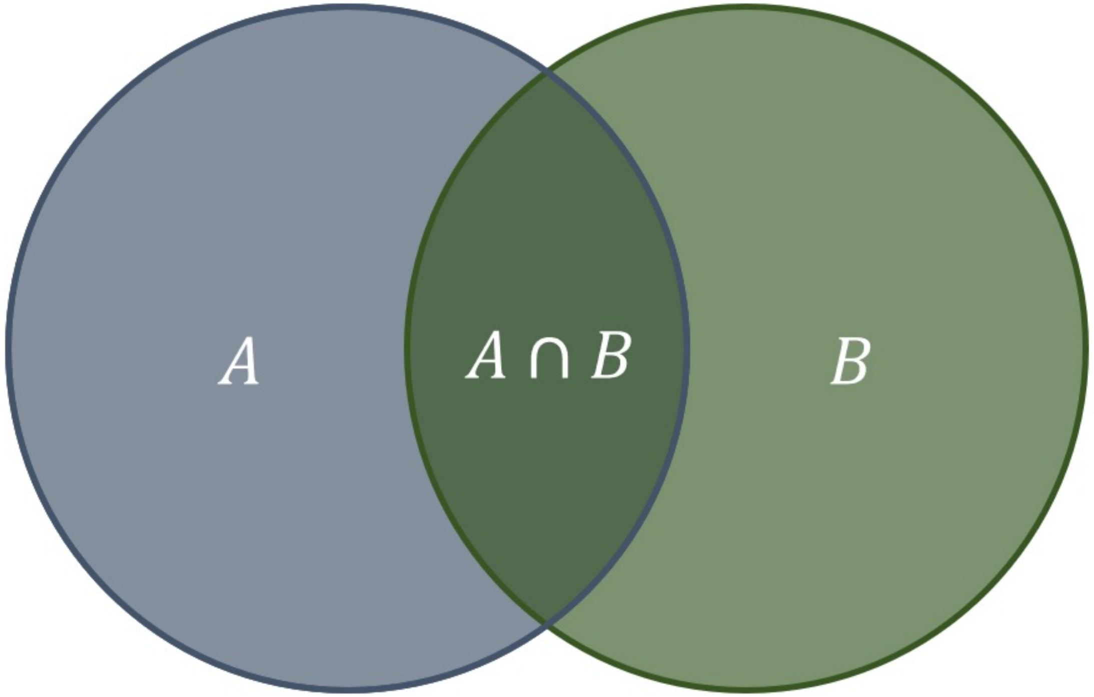
Better prediction



Good prediction



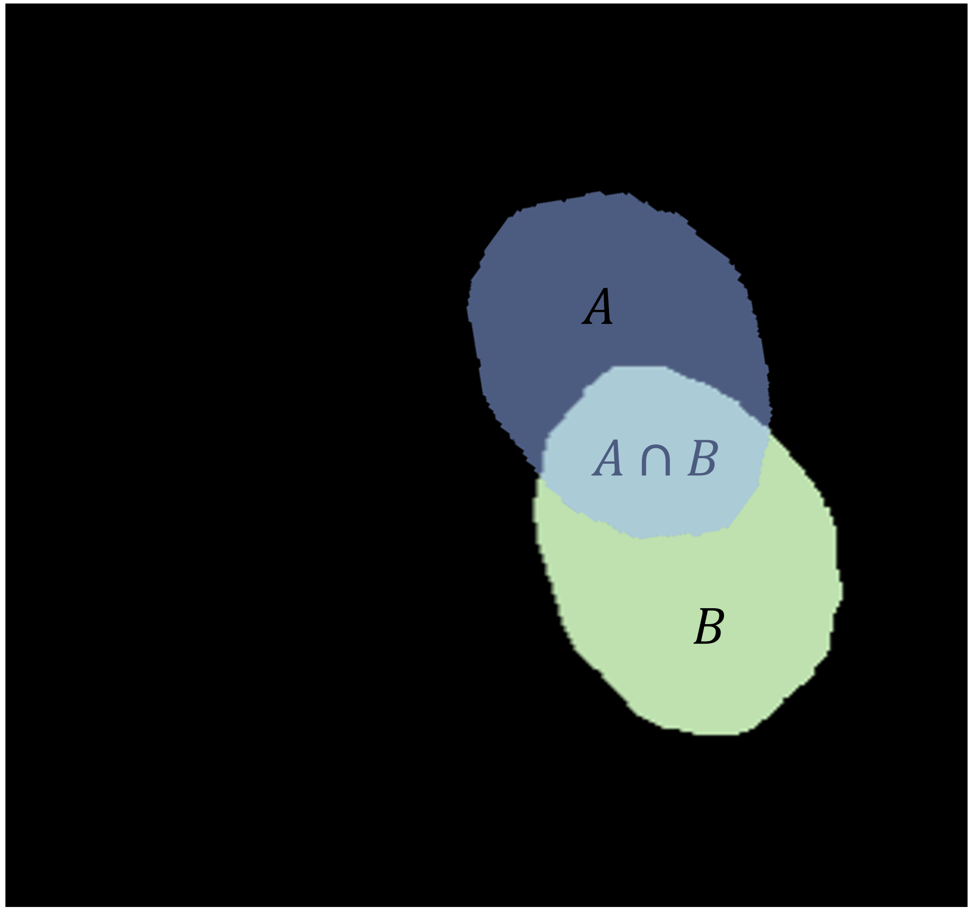
Implementation of the Dice Score



$$DSC = \frac{2 \times |A \cap B|}{|A| + |B|}$$

A: Predicted shape
B: Ground truth

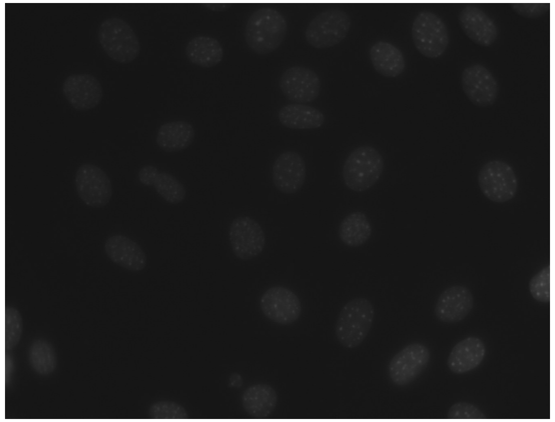
Implementation of the Dice Score



$$DSC = \frac{2 \times |A \cap B|}{|A| + |B|}$$

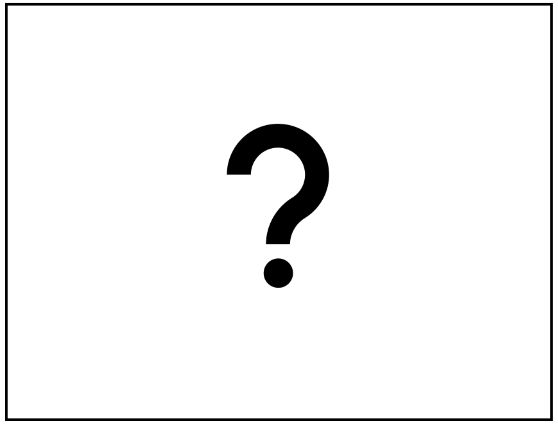
Implementation of the Dice Score

Our goal: compare ground truth images with our results



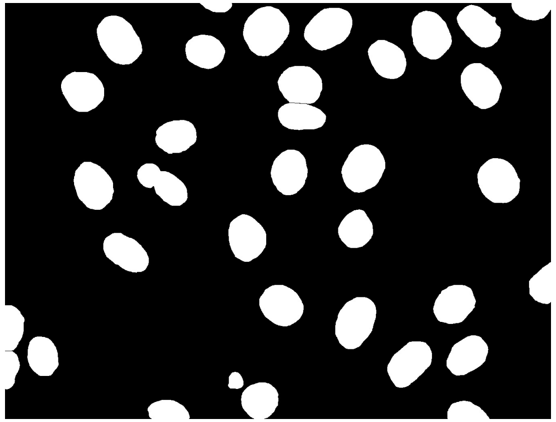
Original image

Otsu’s thresholding



Our result

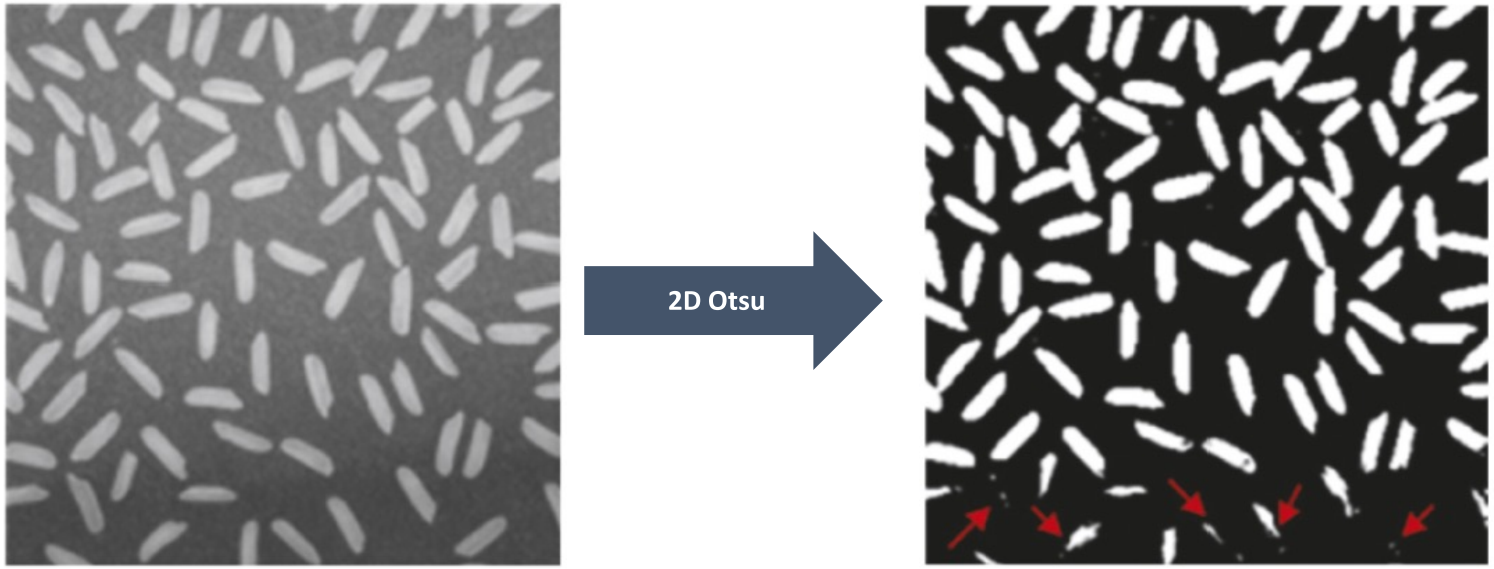
comparison



Ground truth

Further ideas

- 2D Otsu
- Algorithm for counting cells



Timeline

12.05 — Project Proposal
Research Otsu Tresholding and Dice Score
Team

19.05 — Explore Data
Histograms and Data Analysis
Team

26.05 — Code algorithms
Otsu: E.C., H.W.
Dice: L.W., V.S.

02.06 — Pipeline Assembly
And testing of further preprocessing methods
Team

09.06 — Research
Alternative evaluation methods
Code optimization
Team

16.06 — Testing on BBBC pictures
Team
Alternative Evaluation Methods
IoU, Pixel Accuracy: Team
Haussdorf: E.C., H.W.
MSD: L.W., V.S.

23.06 — Compare
With other Teams
Team

30.06 — Report
Jupyter Notebook
Team

07.07 — Complete final presentation
Team

Laura Wächter, Hannah L. Winter, Elizaveta Chernova, Veronika Schuler

Thank you for your attention!

Additional slide – Histogram stretching

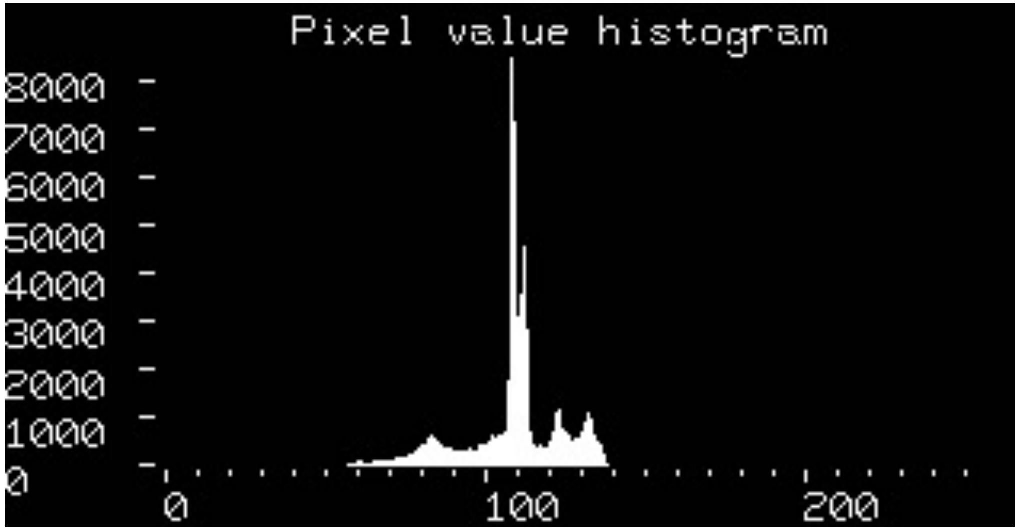
a = 0, b =255

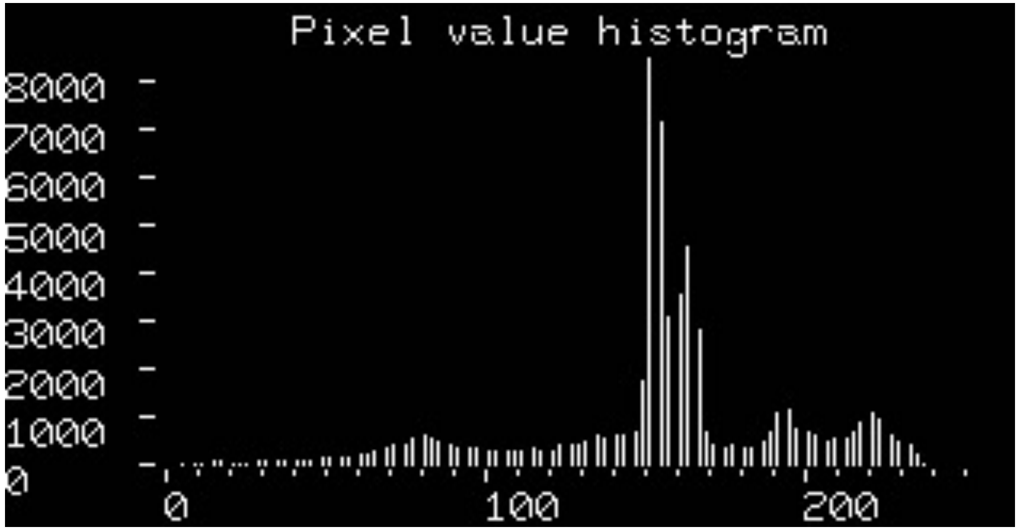
c – lowest pixel intensity in the image

d – highest pixel intensity in the image

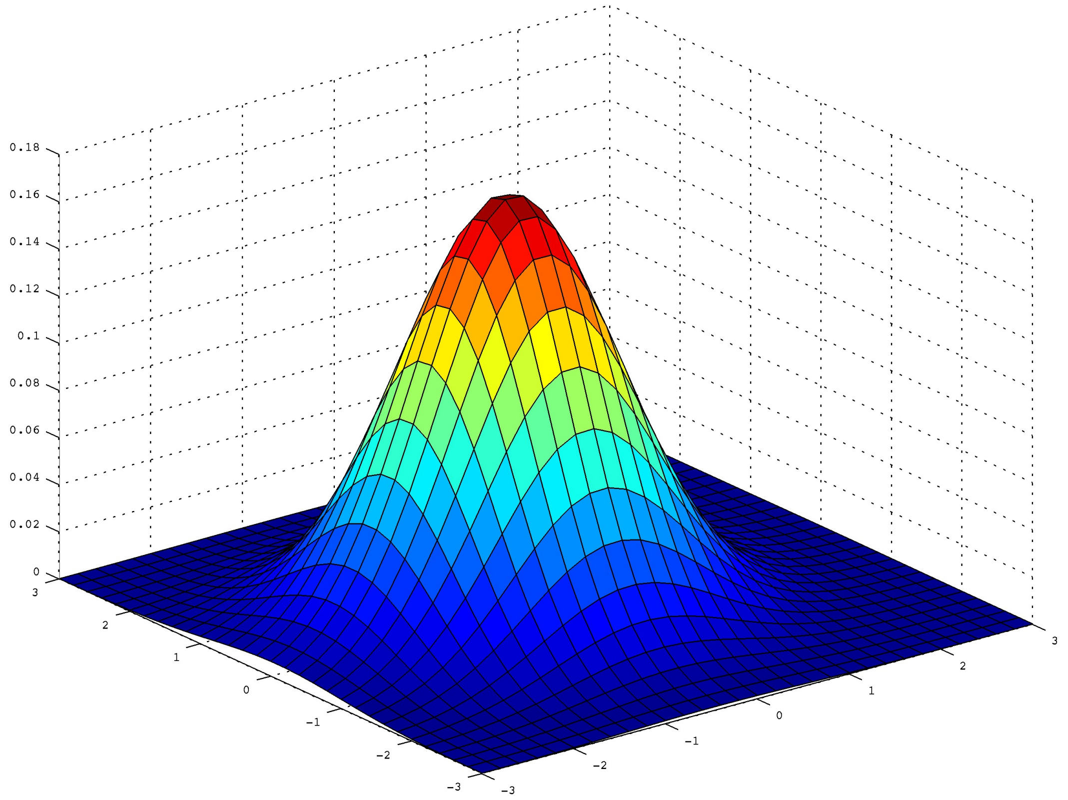
$$P_{out} = (P_{in} - c)\left(\frac{b-a}{d-c}\right) + a$$

Additional slide – Histogram stretching





Additional slide – Gaussian filter



$$G(x,y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}}$$

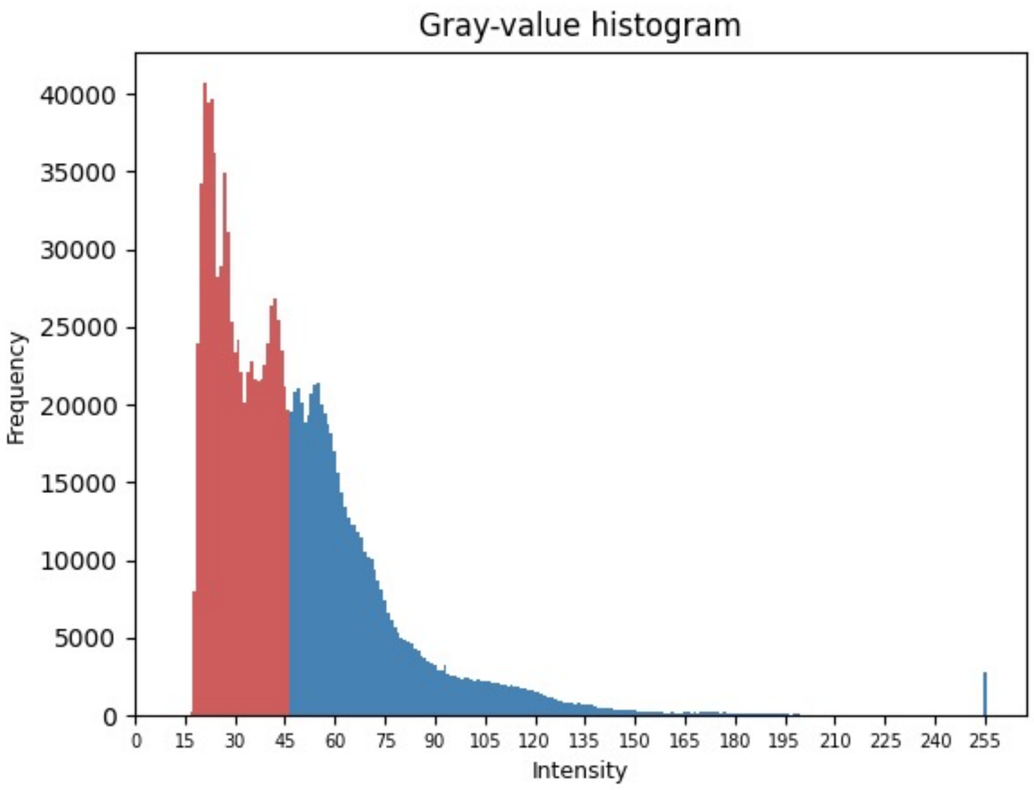
https://stats.stackexchange.com/questions/102632/plot-two-dimensional-gaussian-density-function-in-matlab

Additional slide – criterion measure

Criterion measure

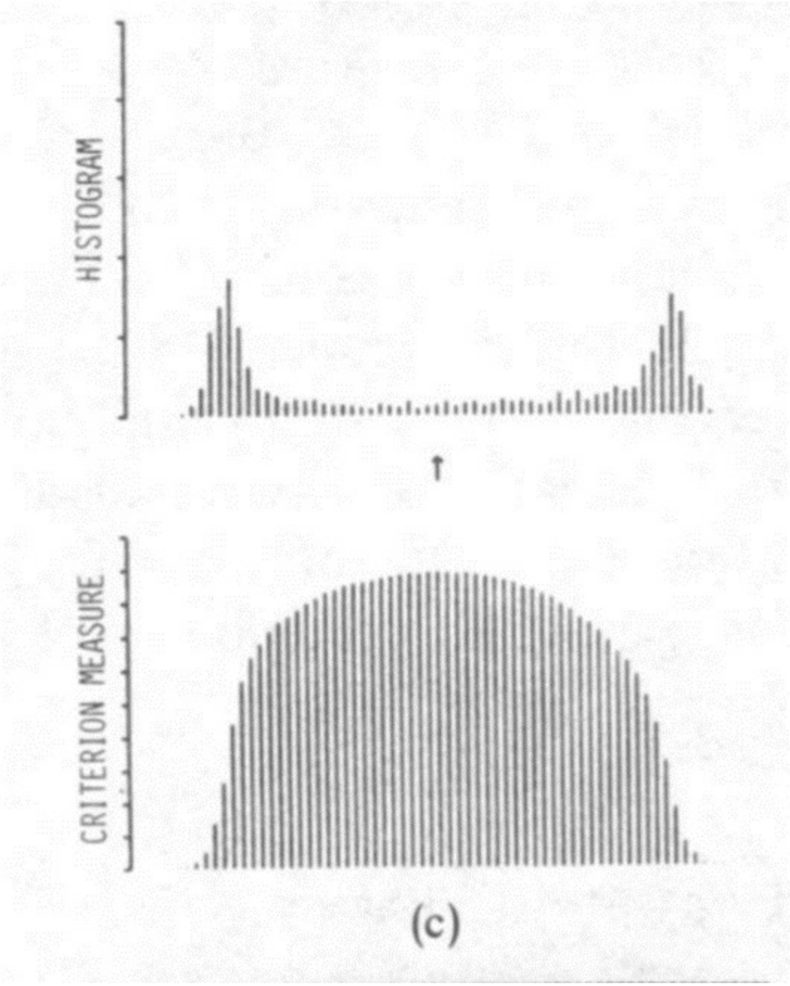
$$\eta(k) = \frac{{\sigma_B}^2(k)}{{\sigma_T}^2}$$

σ_B = between-class variance
σ_T = total variance
$\eta(k) \in [0,1]$



Threshold value $k \in [0,255]$

Additional slide – criterion measure



Otsu, 1979

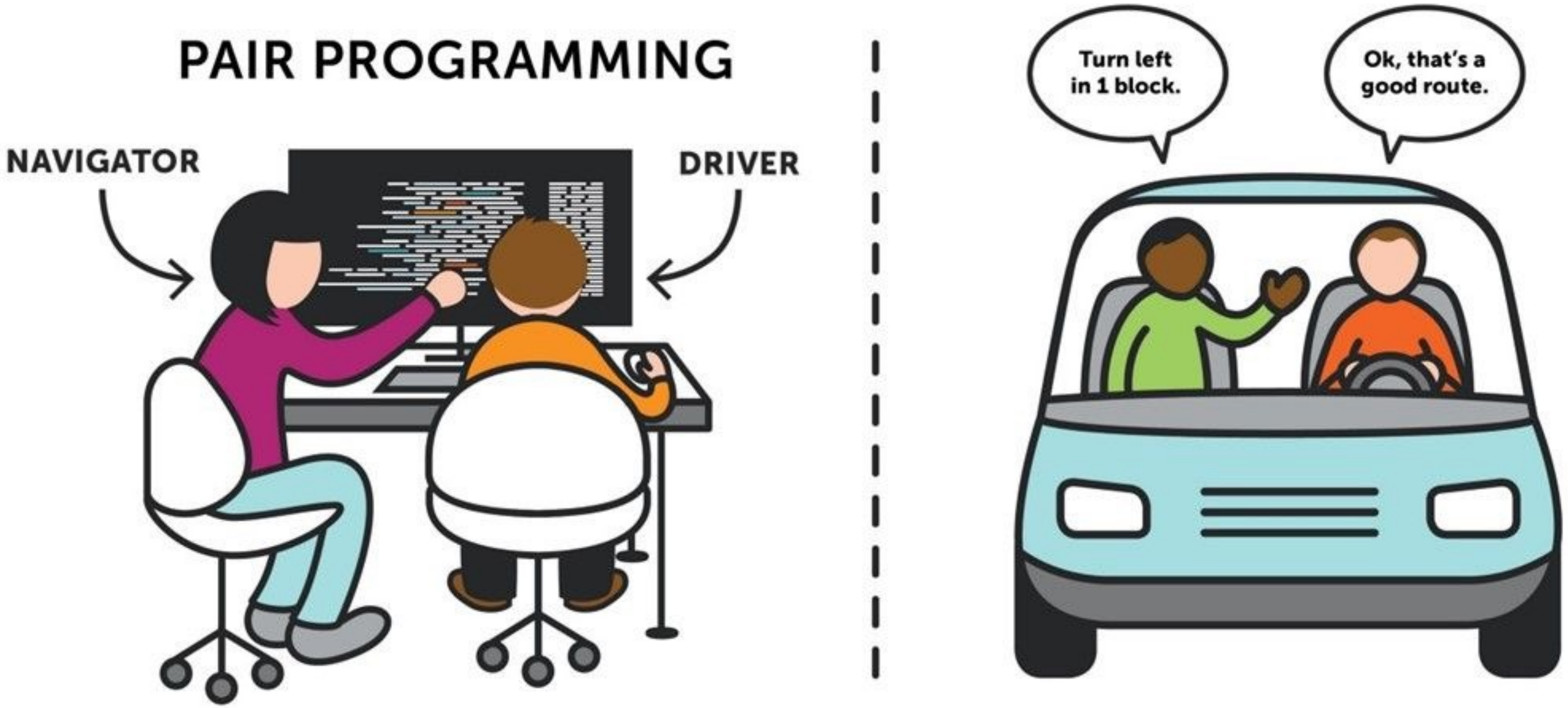
Additional slide – Otsu disadvantages

(a) (b) (c)

(a) (b) (c)

Not suitable for images with high complexity

Additional slide – Team coding



Additional slide – 2D Otsu

Intensity level of pixel is compared with immediate neighborhood pixels

Algorithm:

- For each pixel calculate average gray-level of neighborhood
- Gray level of pixel and average gray levels are divided in L discrete values
- Form pairs: pixel gray level i and neighborhood average j
- There are $L \times L$ possible pairs
- Frequency $f_{i,j}$ of a pair (i, j) divided by the total pixel number N defines probability mass function in a 2D histogram:

$$P_{i,j} = \frac{f_{i,j}}{N} \qquad \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} P_{i,j} = 1$$

Additional slide – IoU

IoU = Intersection-Over-Union

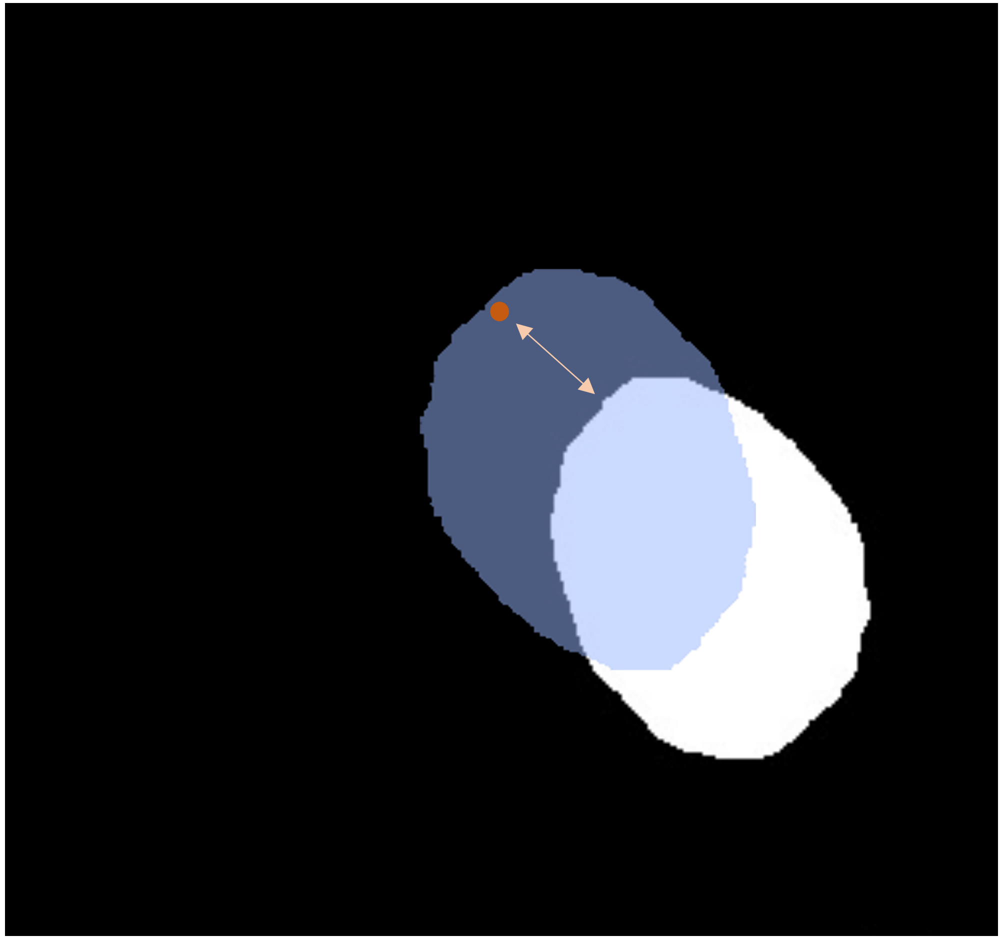
$$\text{IoU} = \frac{\text{Area of Overlap}}{\text{Area of Union}}$$

Additional slide - MSD

MSD = mean surface distance

$$d(p, S') = \min_{p' \in S'} ||p - p'||_2$$

$$\text{MSD} = \frac{1}{n_S + n_{S'}} \left(\sum_{p=1}^{n_S} d(p, S') + \sum_{p'=1}^{n_{S'}} d(p', S) \right)$$



Additional slide – Housdorff

HD=max[d(S,S’),d(S’,S)]