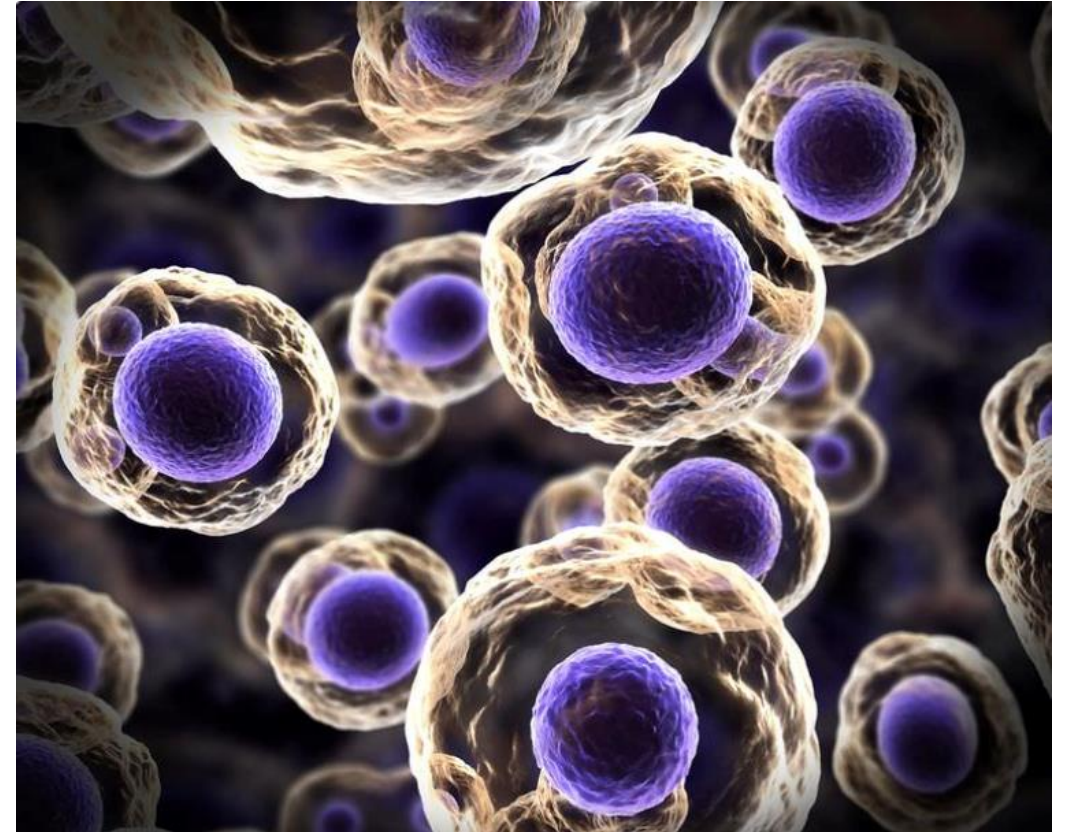# Implementation and evaluation of Otsu's thresholding

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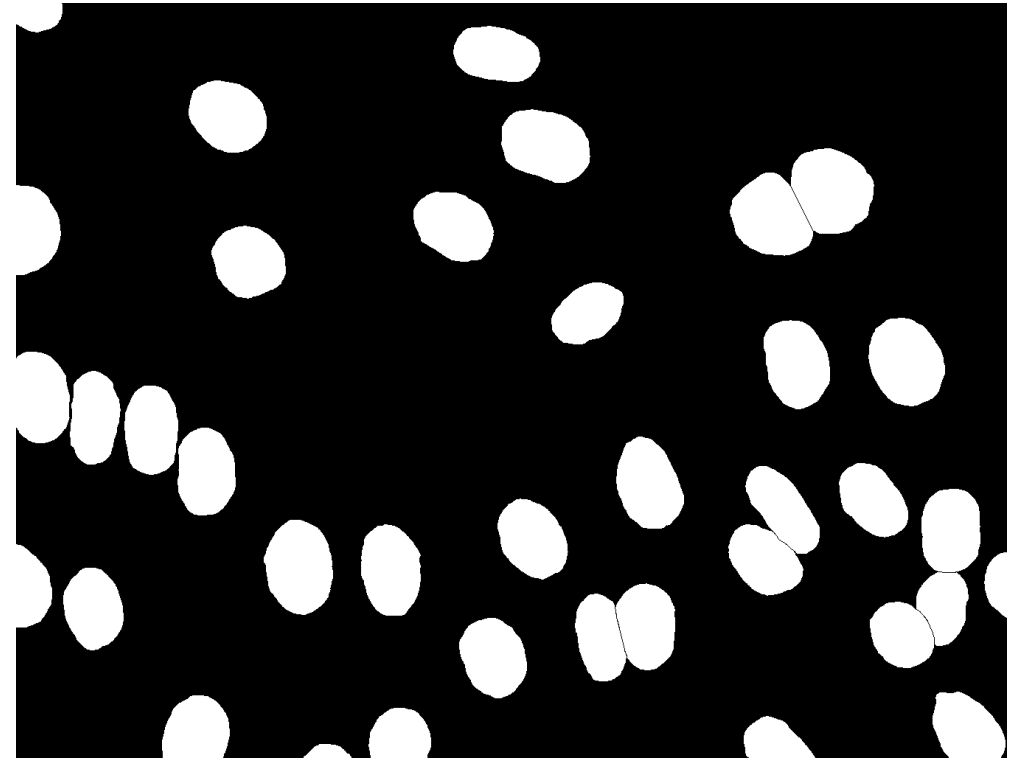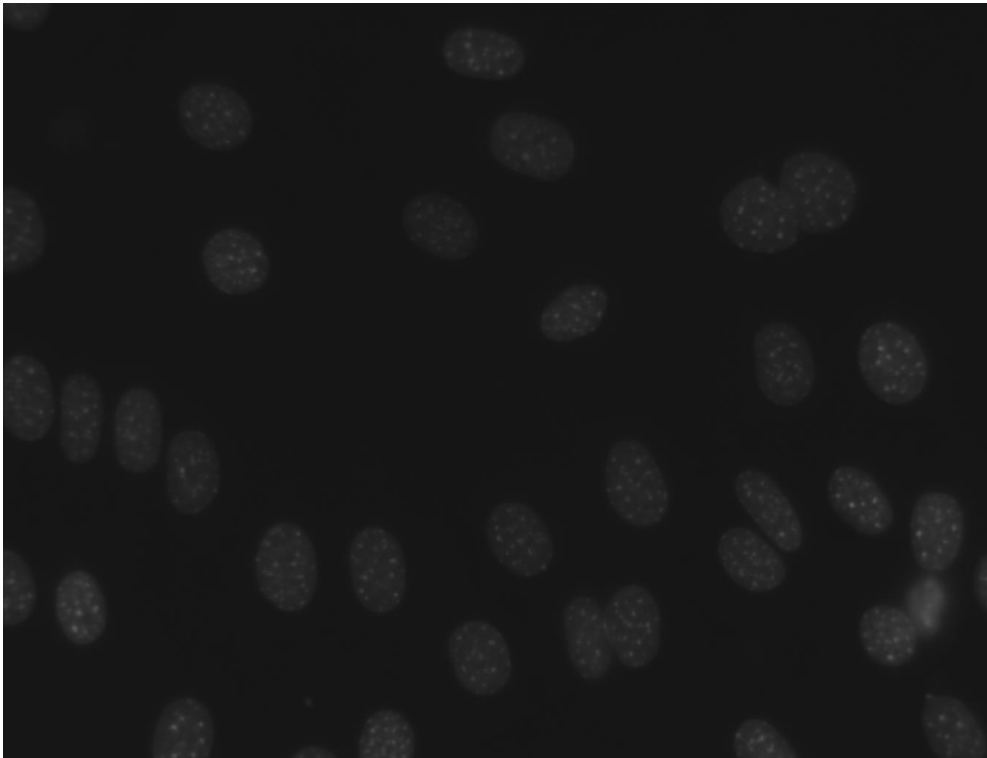
Elizaveta Chernova, Veronika Schuler,
Laura Wächter, Hannah L. Winter

12.05.2021
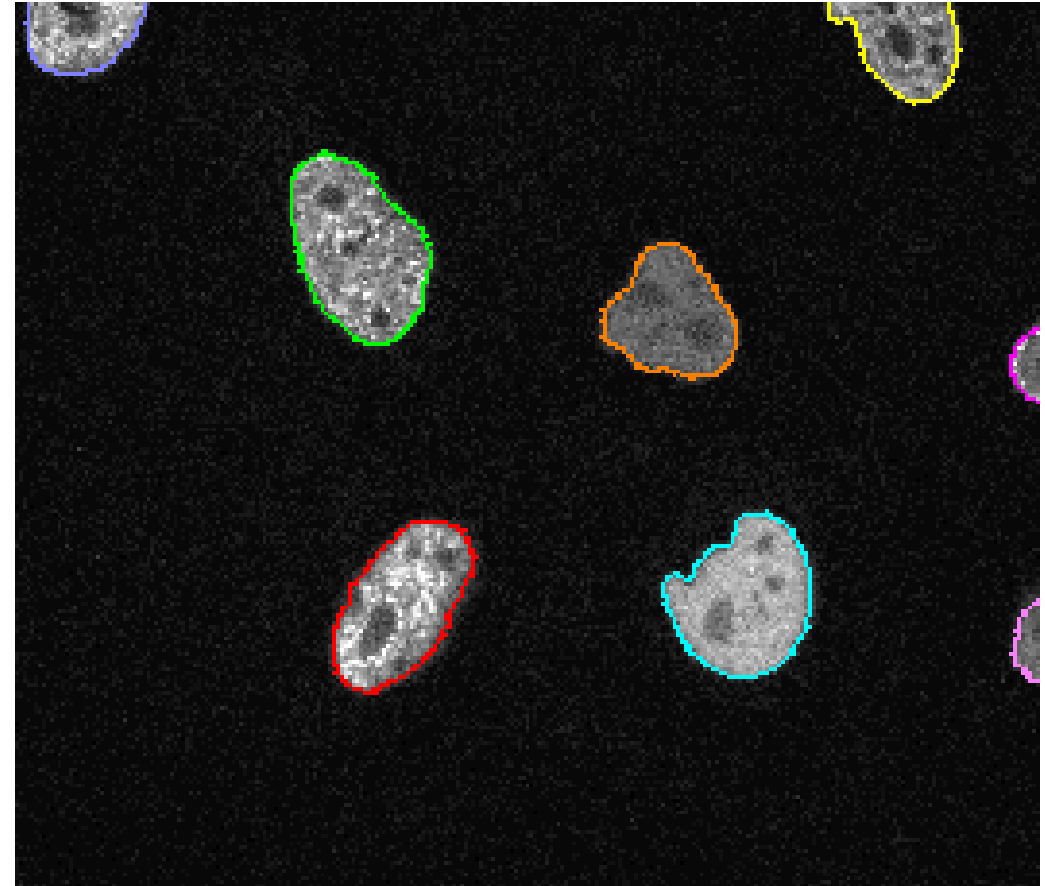


Cell nuclei segmentation
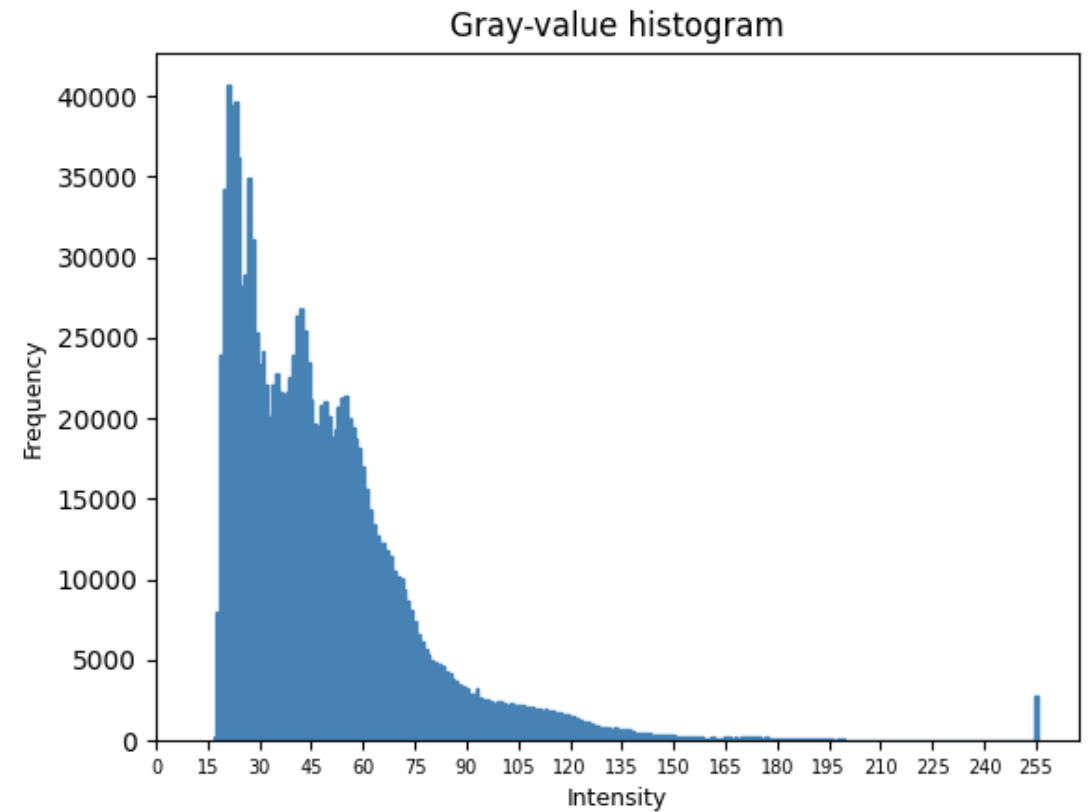
# Image segmentation

# Applications

- High-throughput cytometry
  - Cell size
  - Cell counting
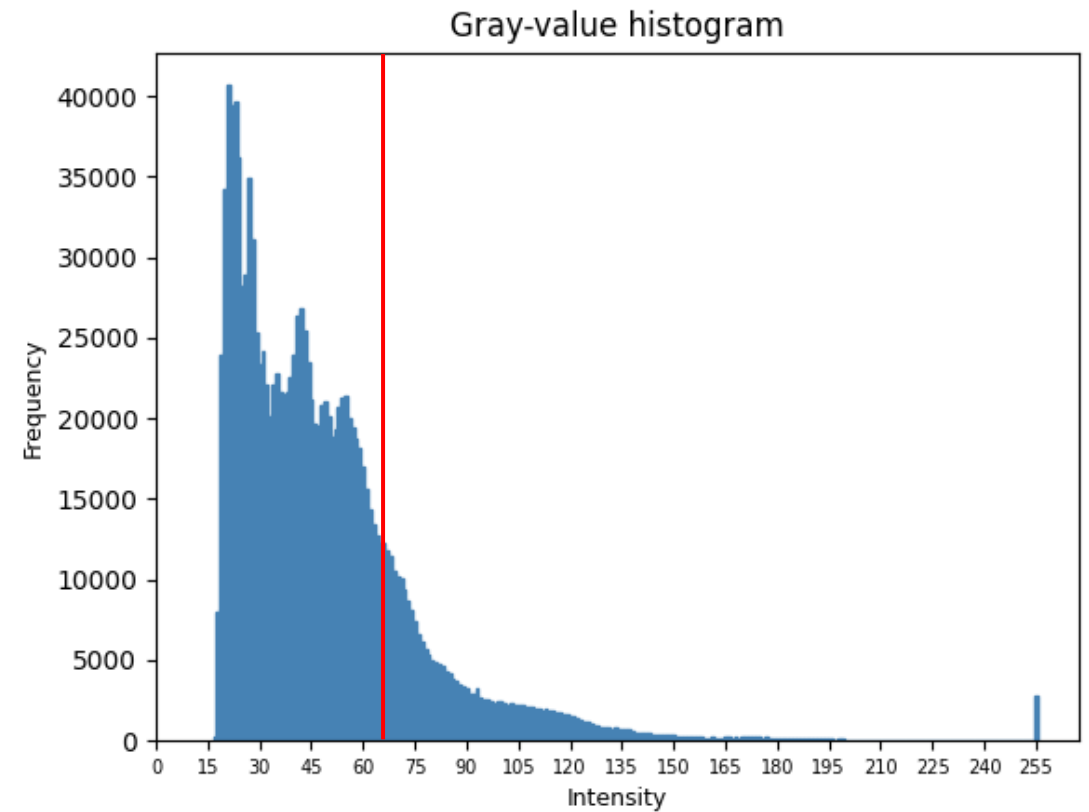  - Cell-cycle determination

- Cell tracking

# Methods

- Thresholding → Intensity clipping

- Region growing
- Machine learning
- etc.



Gray-value histogram

# Methods

- Thresholding ➡ Intensity clipping

**Otsu thresholding**


Gray-value histogram

# Procedure

Input → Pre-processing → Otsu Thresholding → Dice score → Comparison
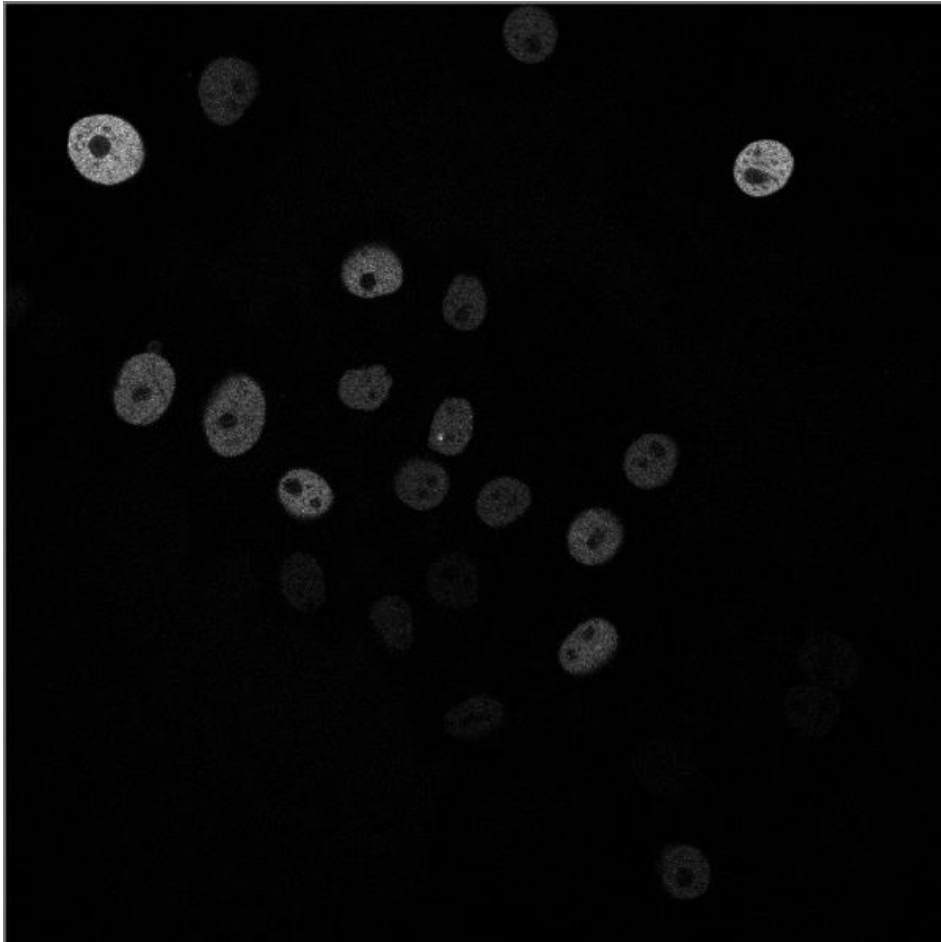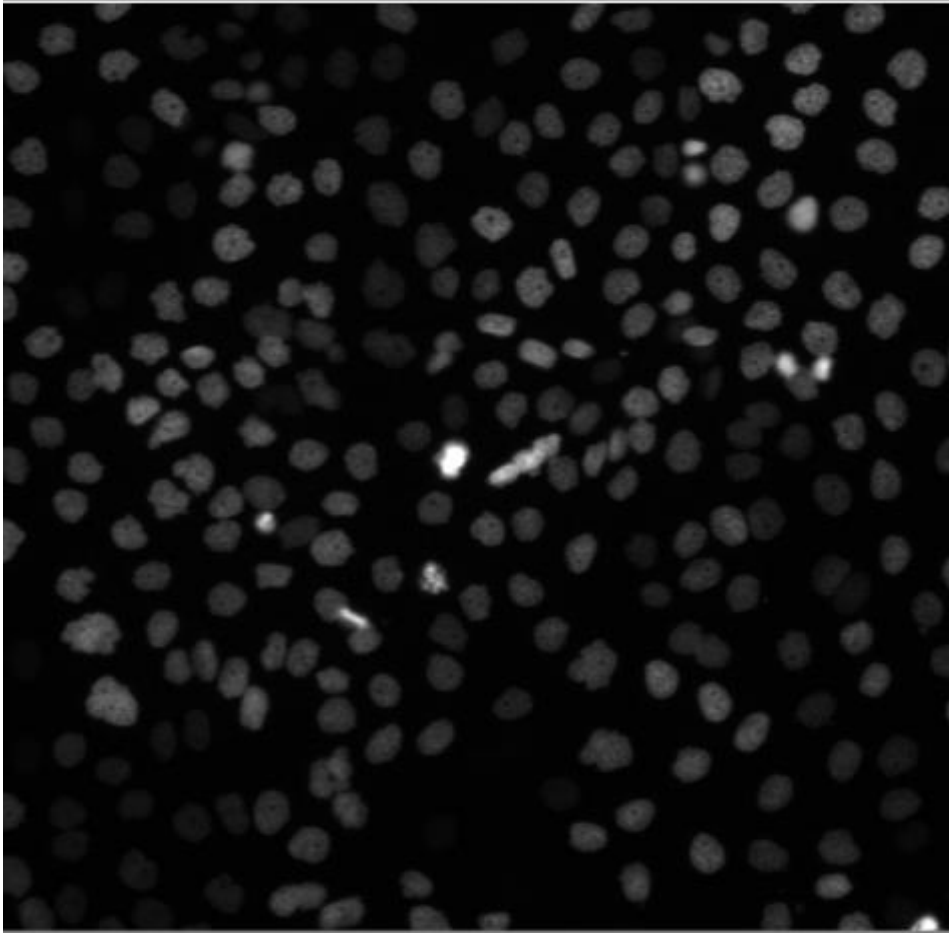
# Input 1: N2DH-GOWT1 cells



- GFP-Gowt1 mouse embryonic stem cells

- Time-laps confocal microscopy and GFP-staining

- Investigate genomic integrity of the cells

- Challenges:
    - Brightness of cells varies
    - Some cells hardly visible

# Input 2: N2DL-HeLa cells



- human epithelial cells of cervical cancer

- live imaging of fluorescently labelled chromosomes

- Phenotypic profiling of the human genome

- Challenges:
  - Some cells hardly can be seen
  - Not easy to distinguish background and cells
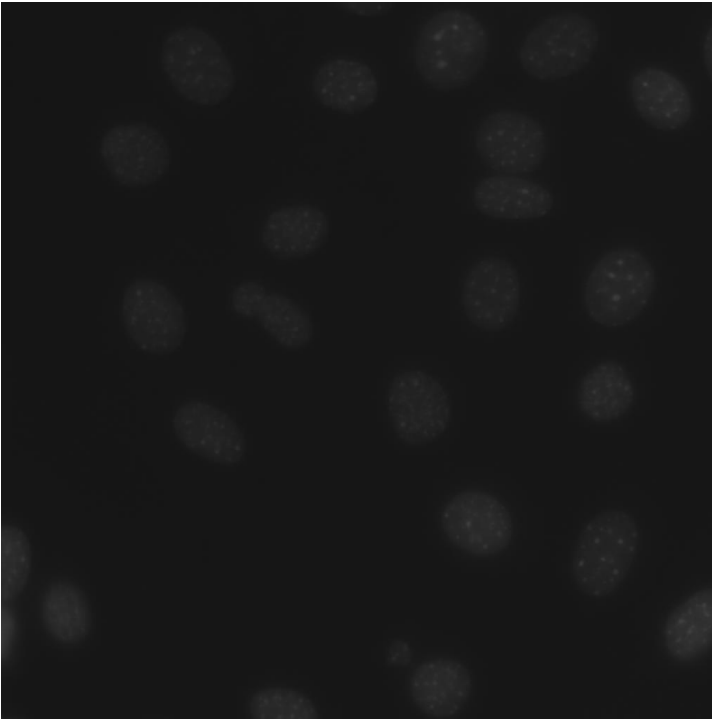  - Not much contrast
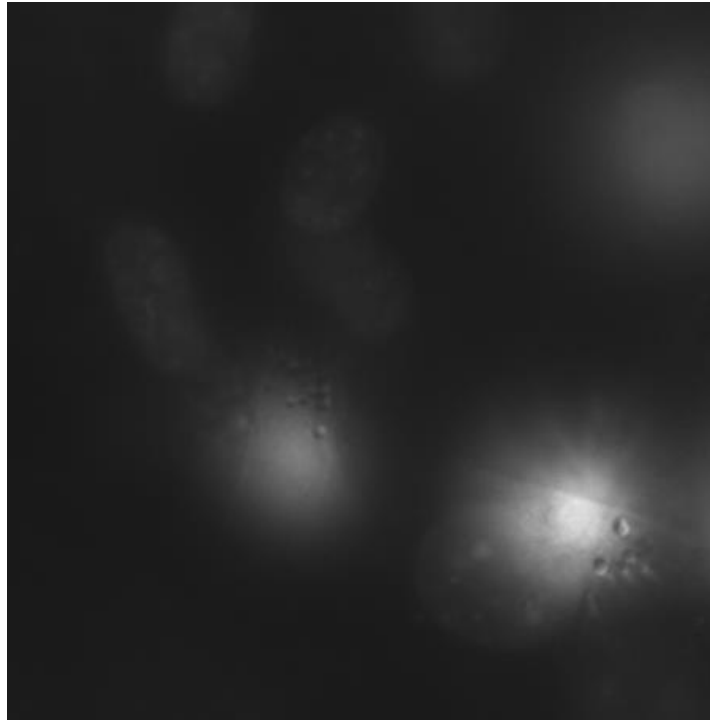
# Input 3: NIH3T3 cells
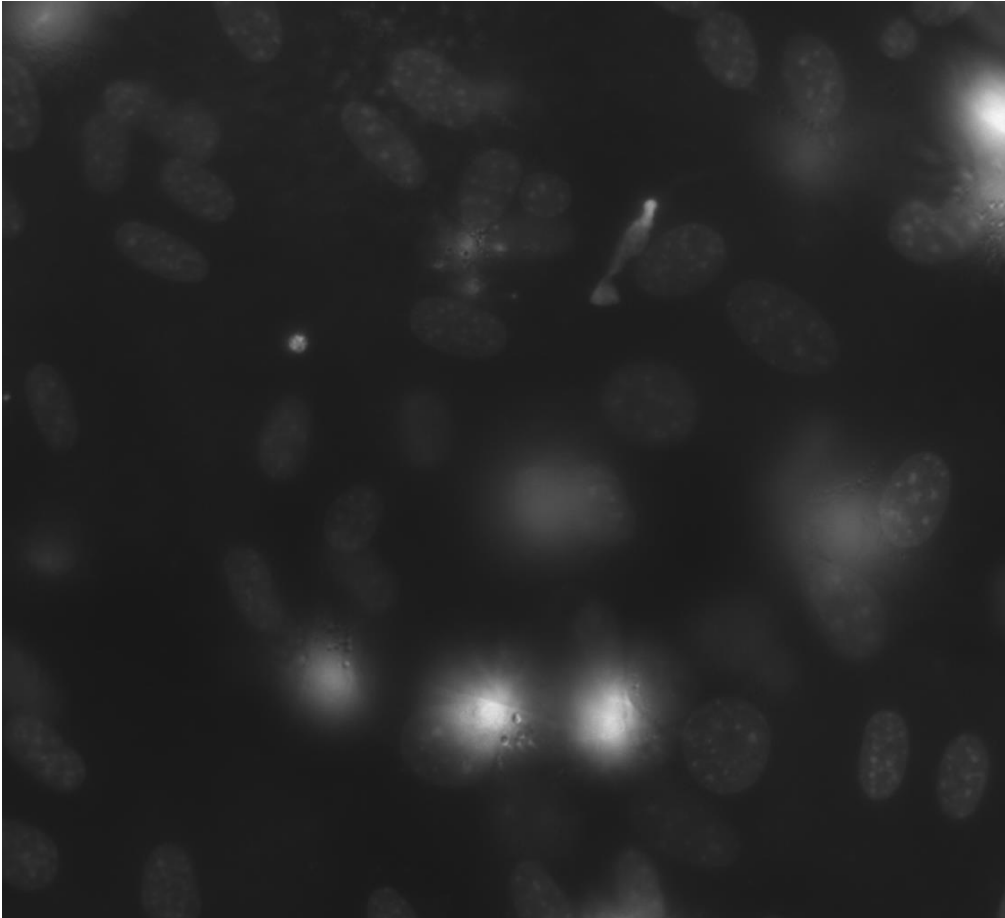


- Several mouse embryonic fibroblast cells

- Fluorescence microscopy images

- Evaluation of image analysis pipelines

- Challenges:
   - Visible debris: light spots
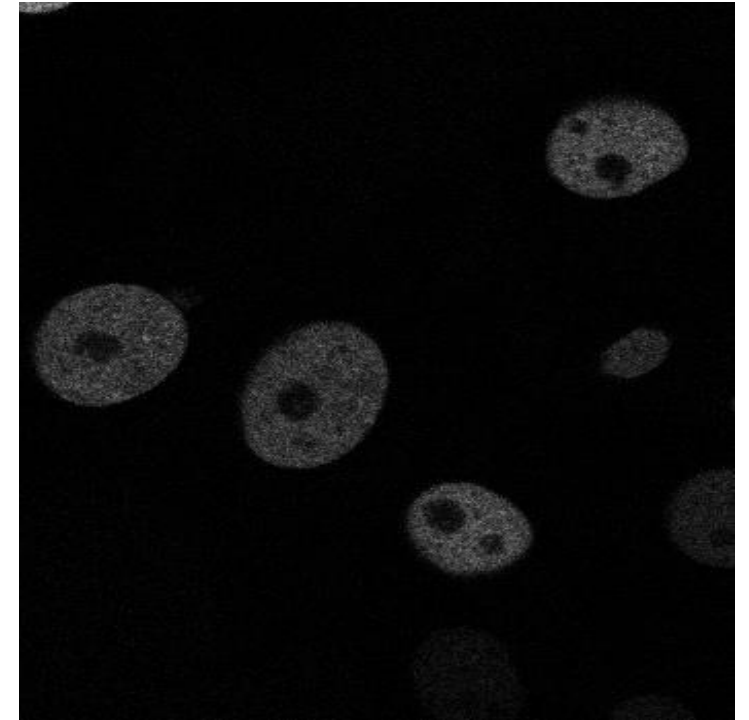   - Nuclei vary in brightness
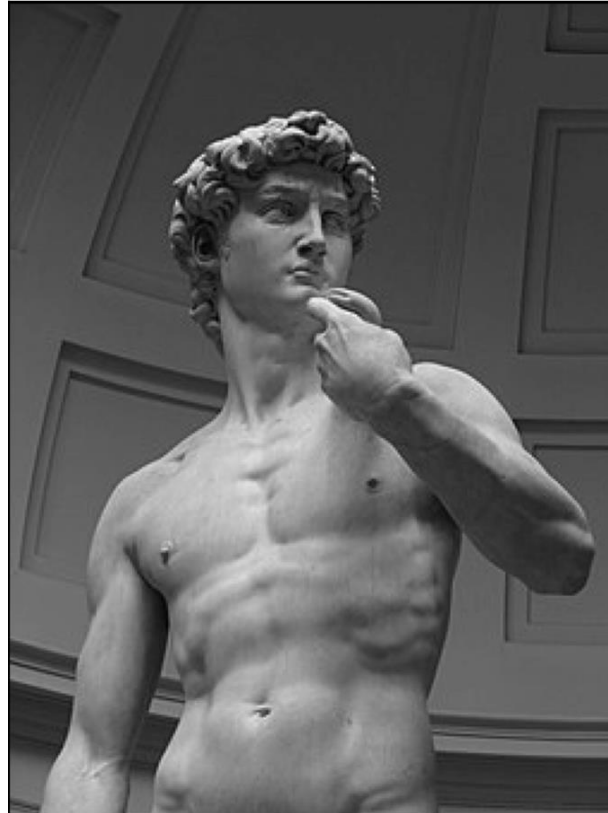
# Problems

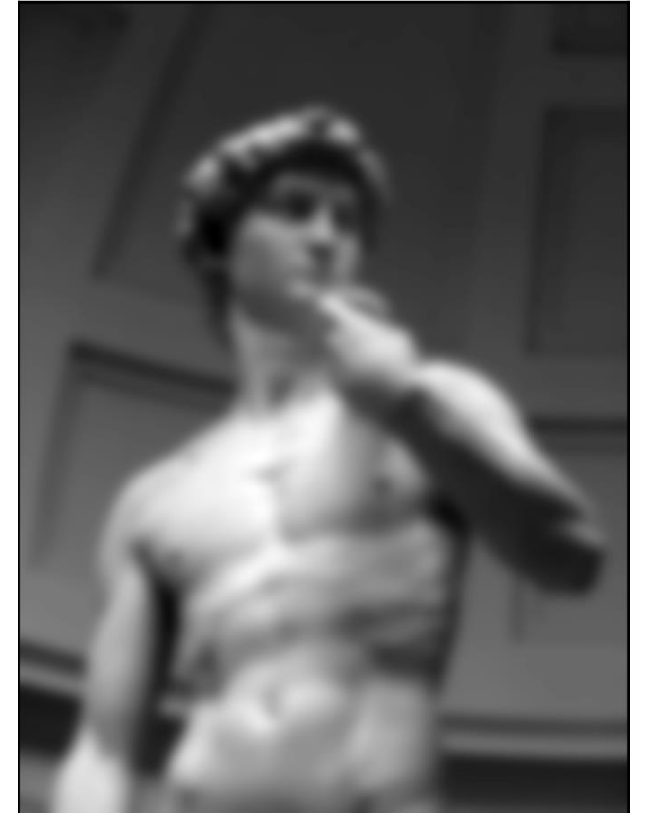Low contrast          Reflections          Random noise

# Preprocessing

Solutions:

- Random noise
  ➡ **Gauss filter, median filter**
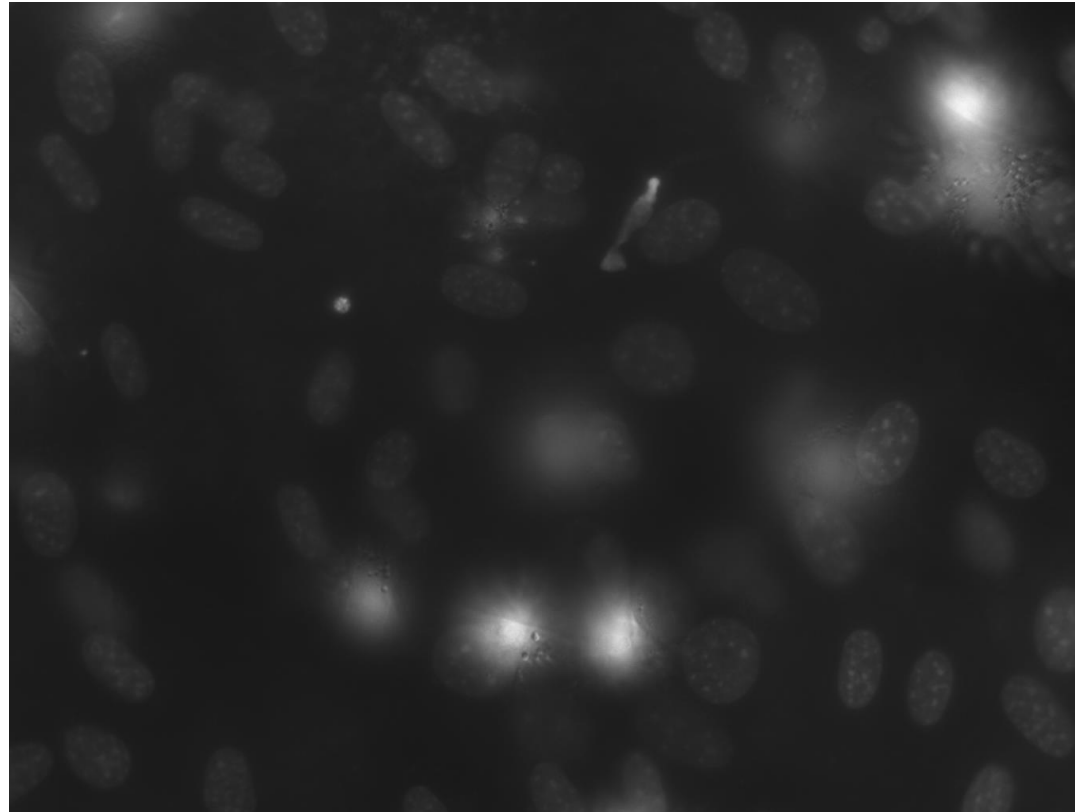
- Reflections

- Low contrast



Original



Gauss filter (σ = 3)
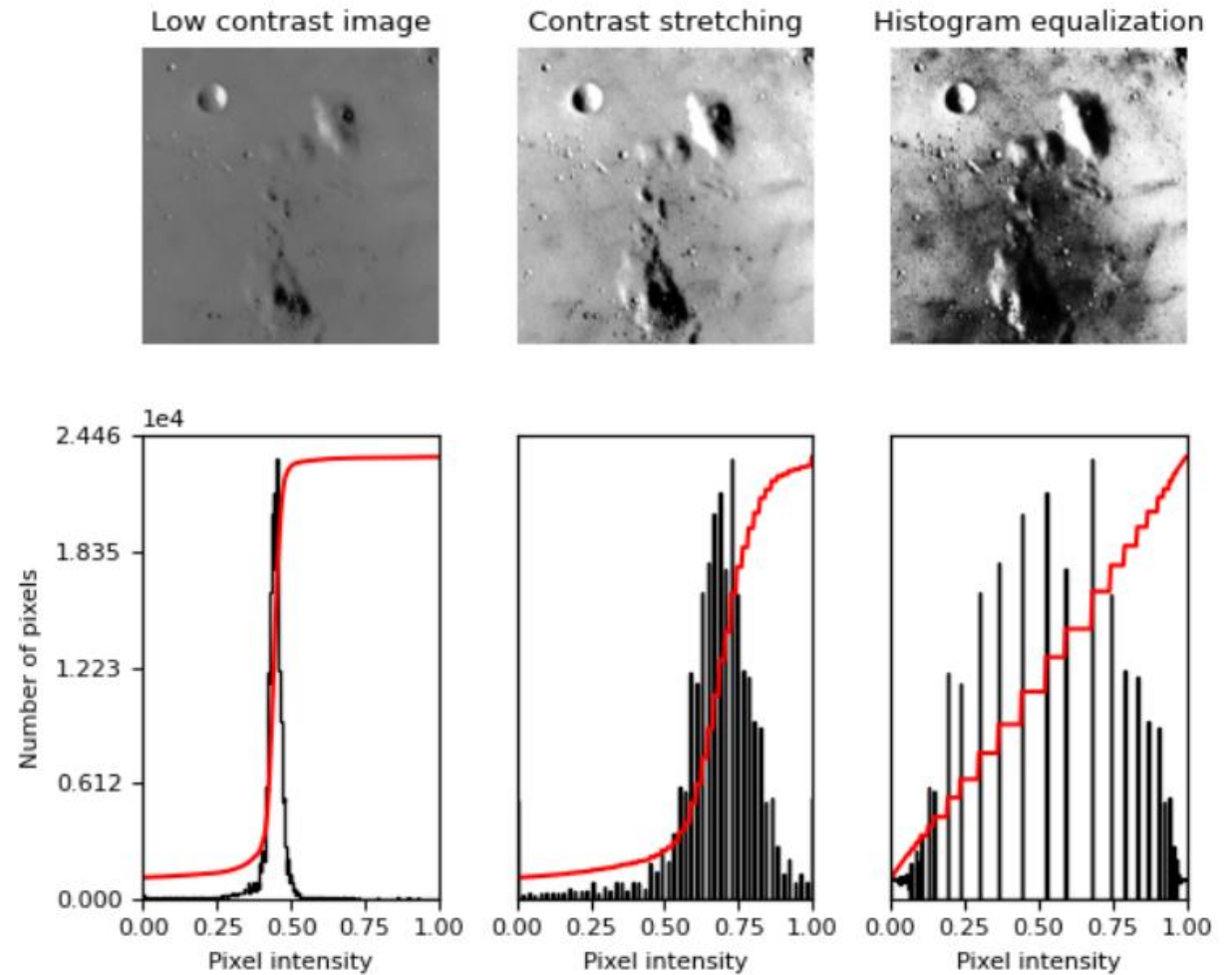
# Preprocessing

Solutions:

- Random noise
  ➡ **Gauss filter, median filter**

- Reflections
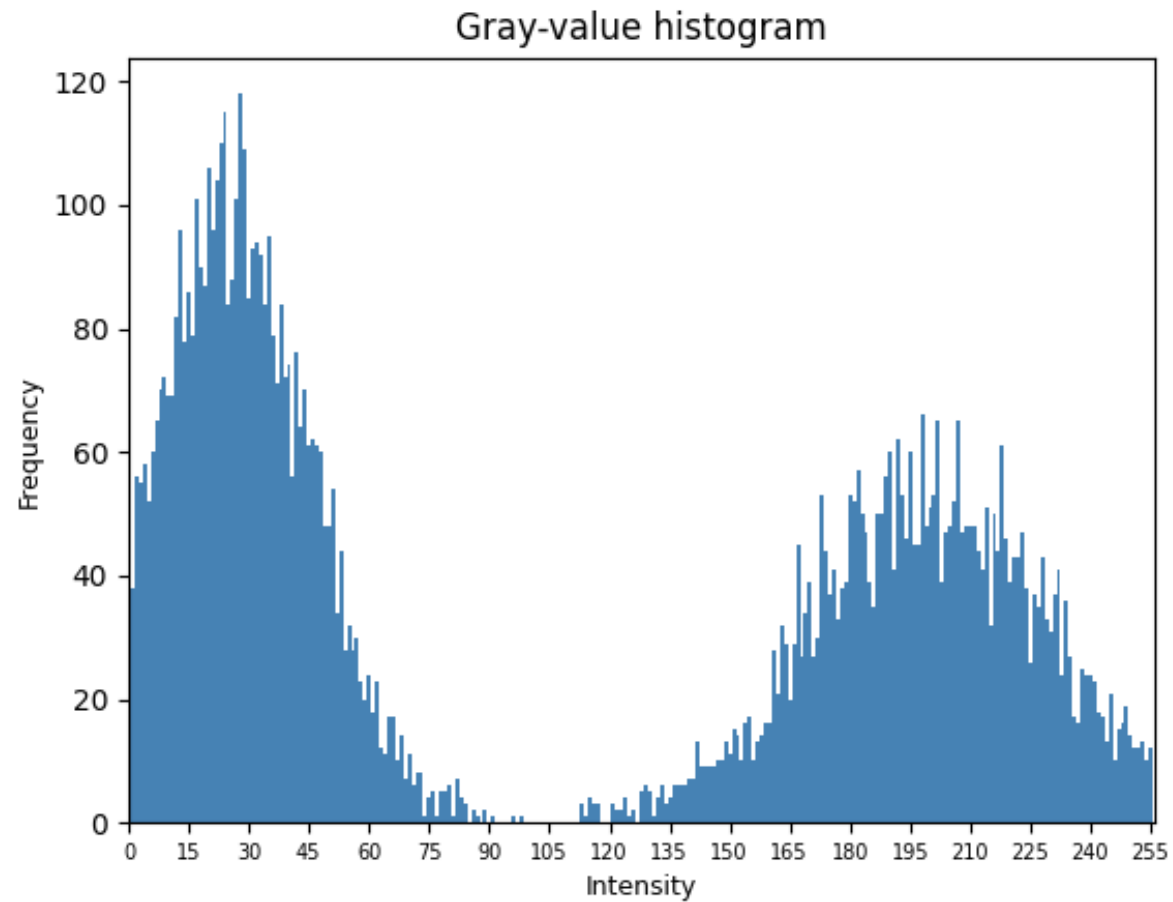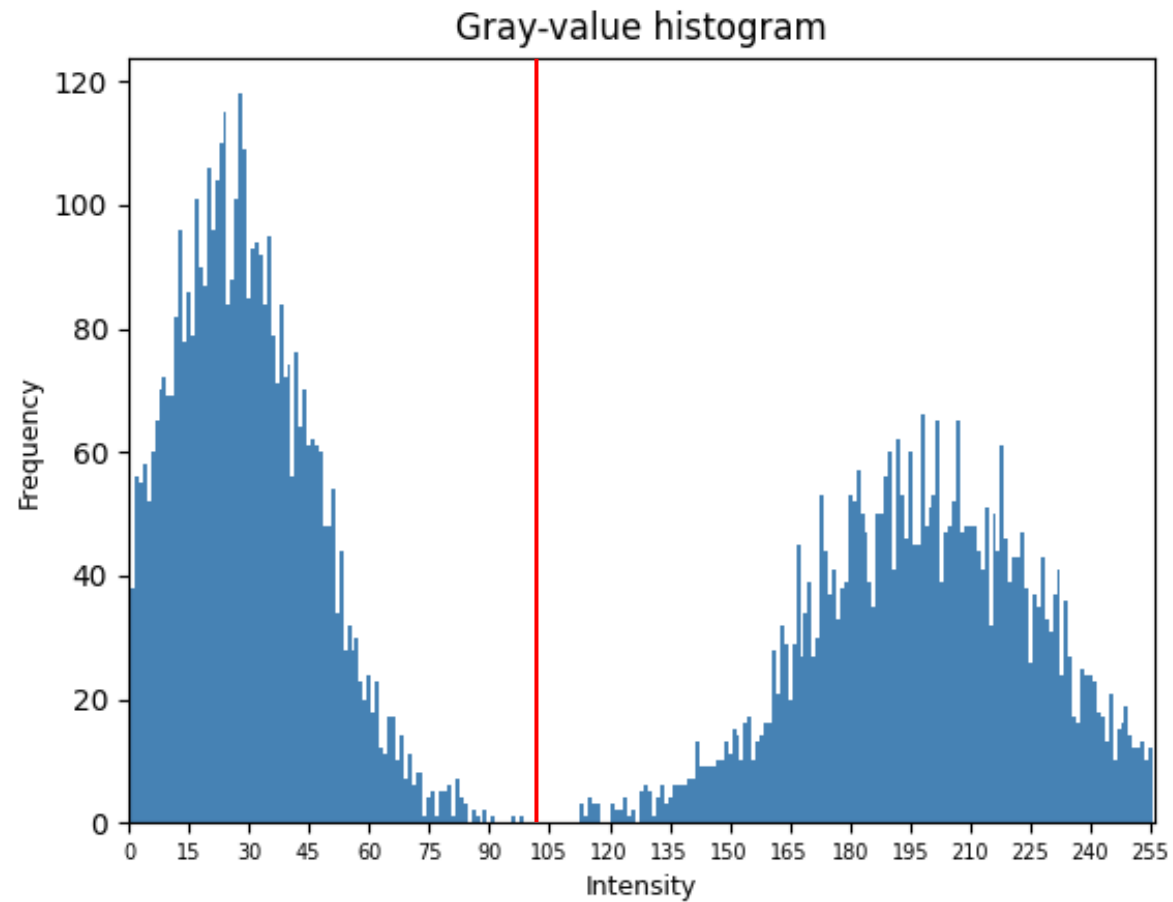  ➡ **Thresholding**

- Low contrast

# Preprocessing

Solutions:

- Random noise
  ➡ **Gauss filter, median filter**

- Reflections
  ➡ **Thresholding**

- Low contrast
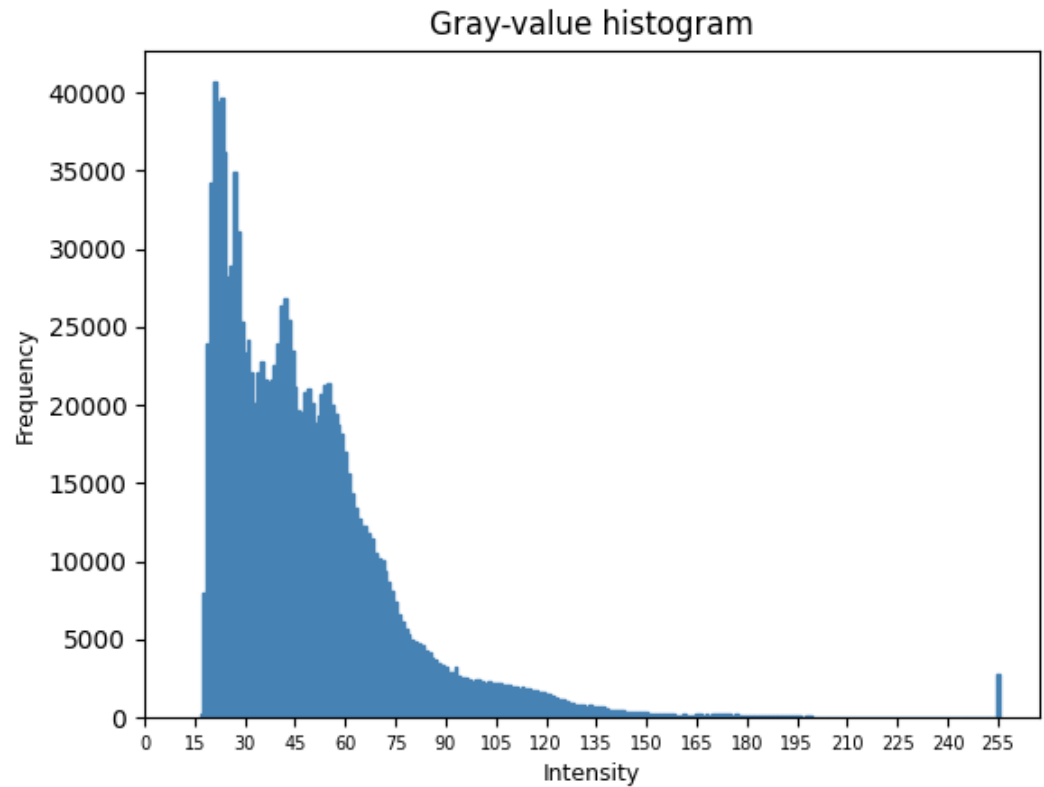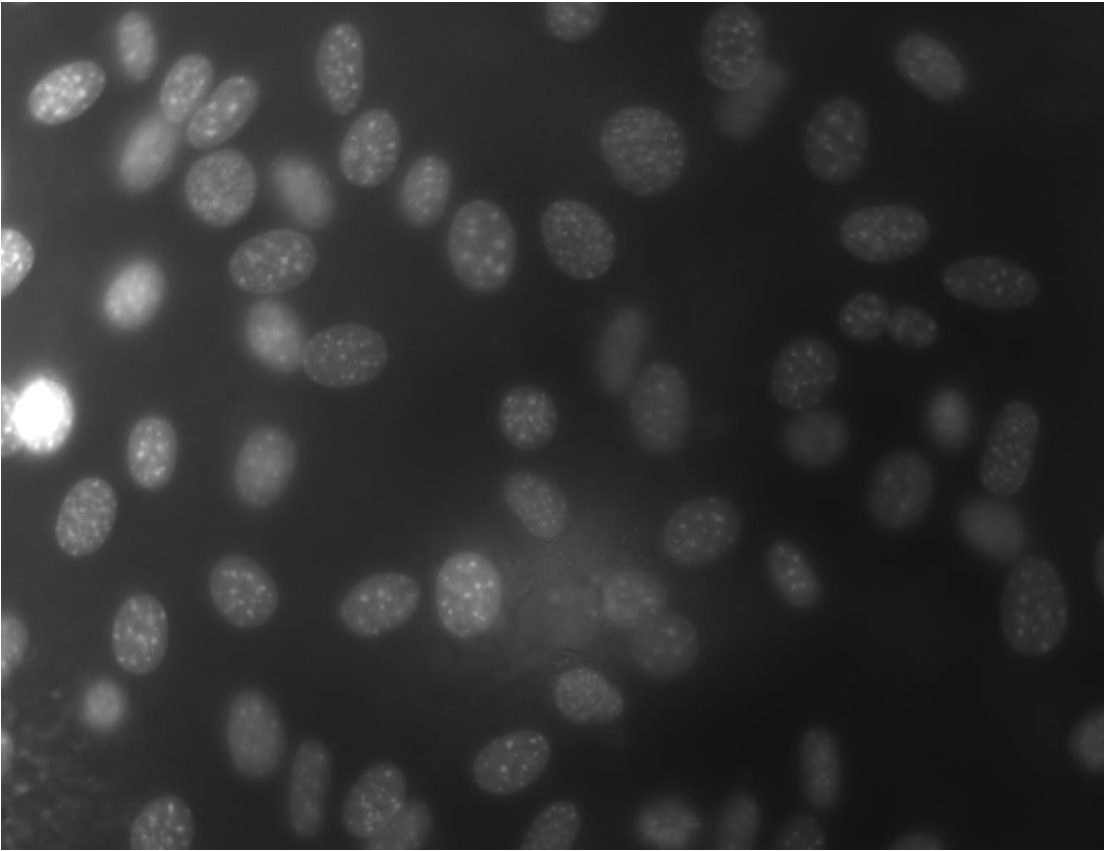  ➡ **Histogram equalization**

# Otsu Thresholding



Gray-value histogram

# Otsu Thresholding



Gray-value histogram

# Otsu Thresholding
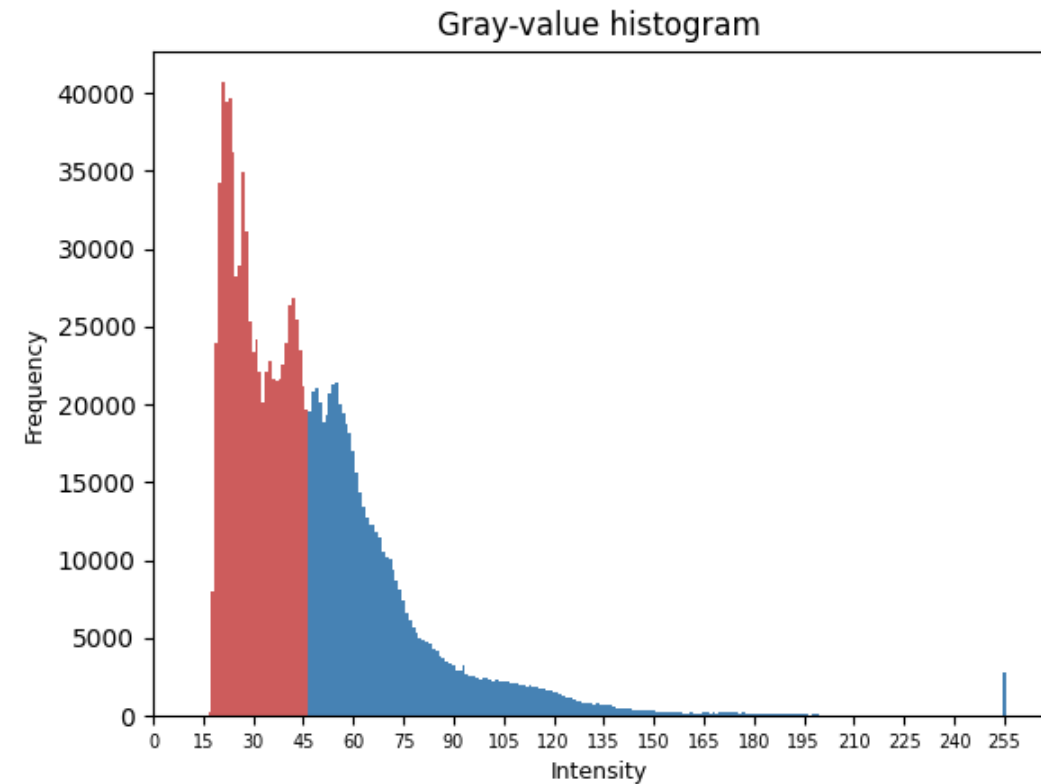




Threshold value k $\in [0,255]$

# Otsu 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
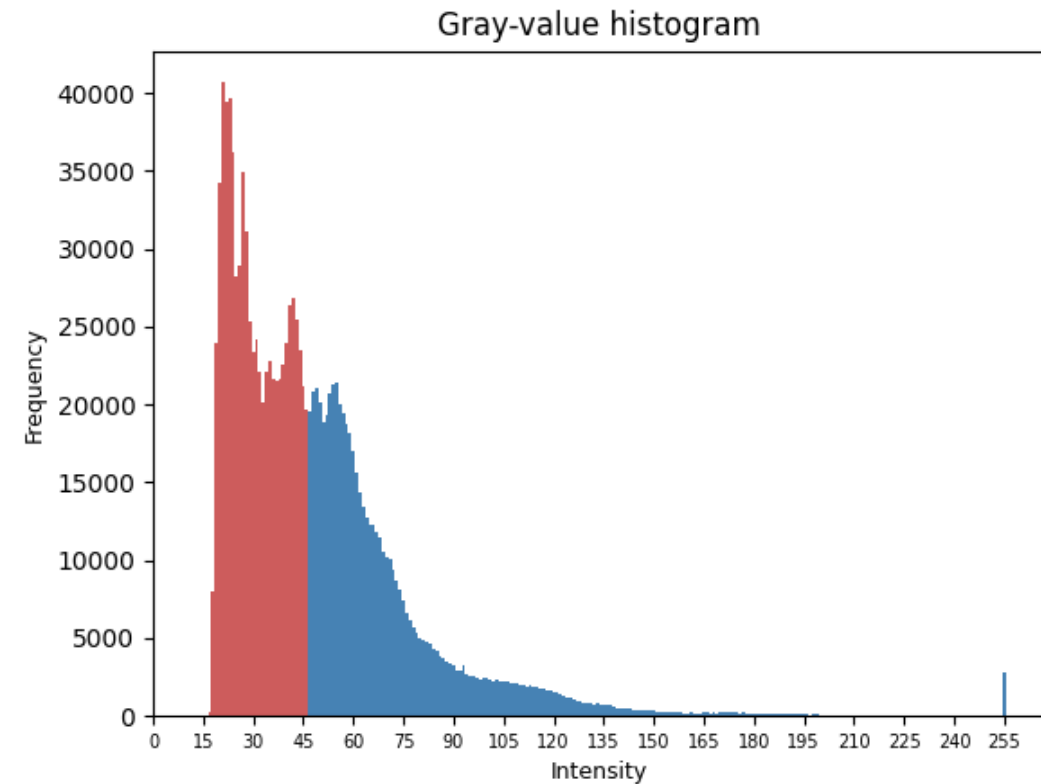


Threshold value k $\in [0,255]$

# Otsu Thresholding

Criterion measure

$$\eta(k) = \frac{\sigma_B{}^2(k)}{\sigma_T{}^2}$$
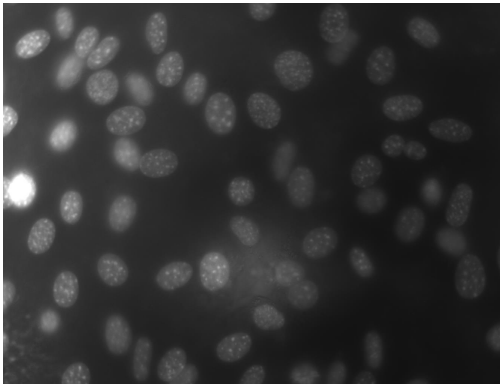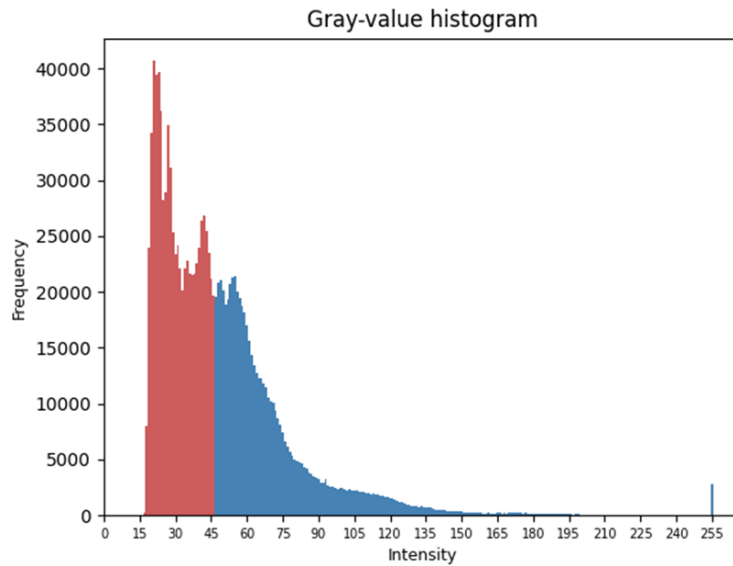
$\sigma_B$ = between-class variance
$\sigma_T$ = total variance
$\eta(k) \in [0,1]$



Gray-value histogram
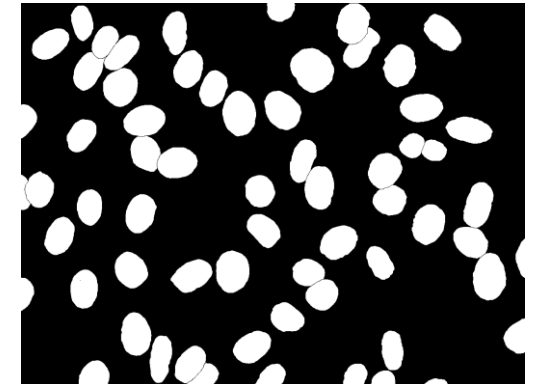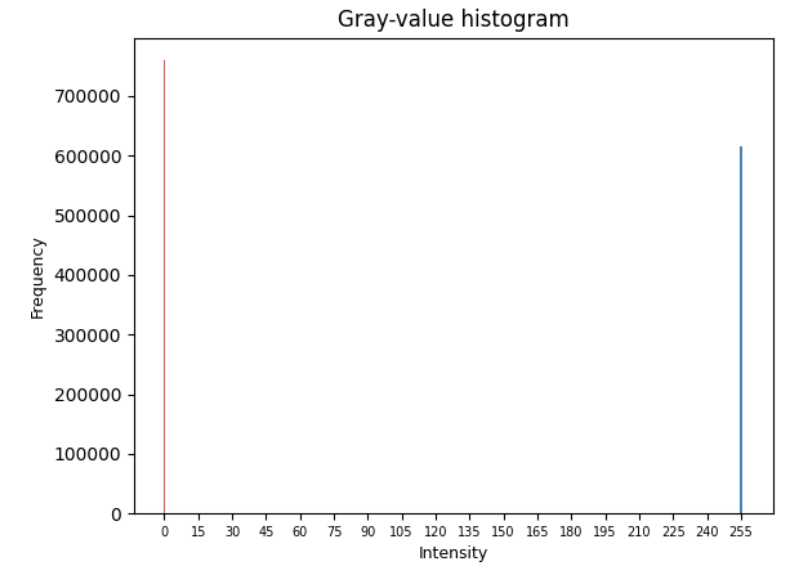
Threshold value k $\in [0,255]$

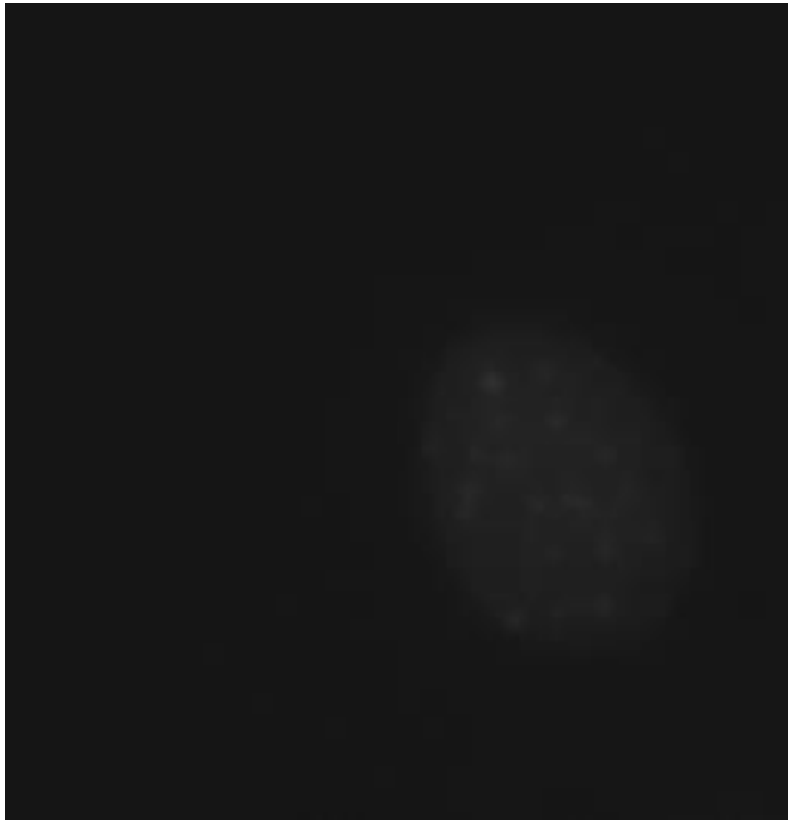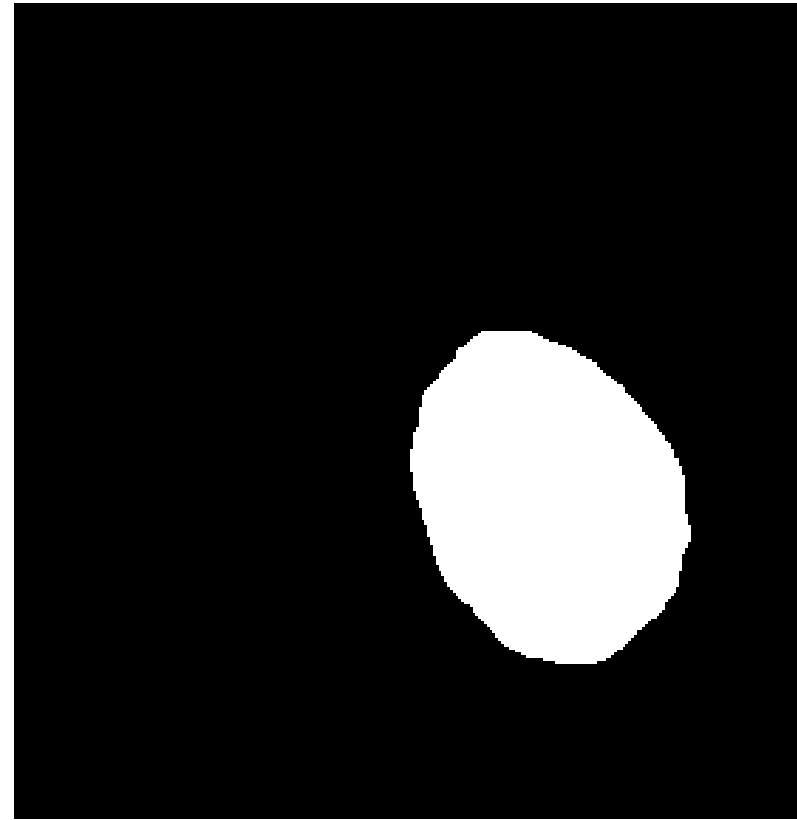# Image Clipping



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

# Implementation of the Dice Score



Original image

Ground truth
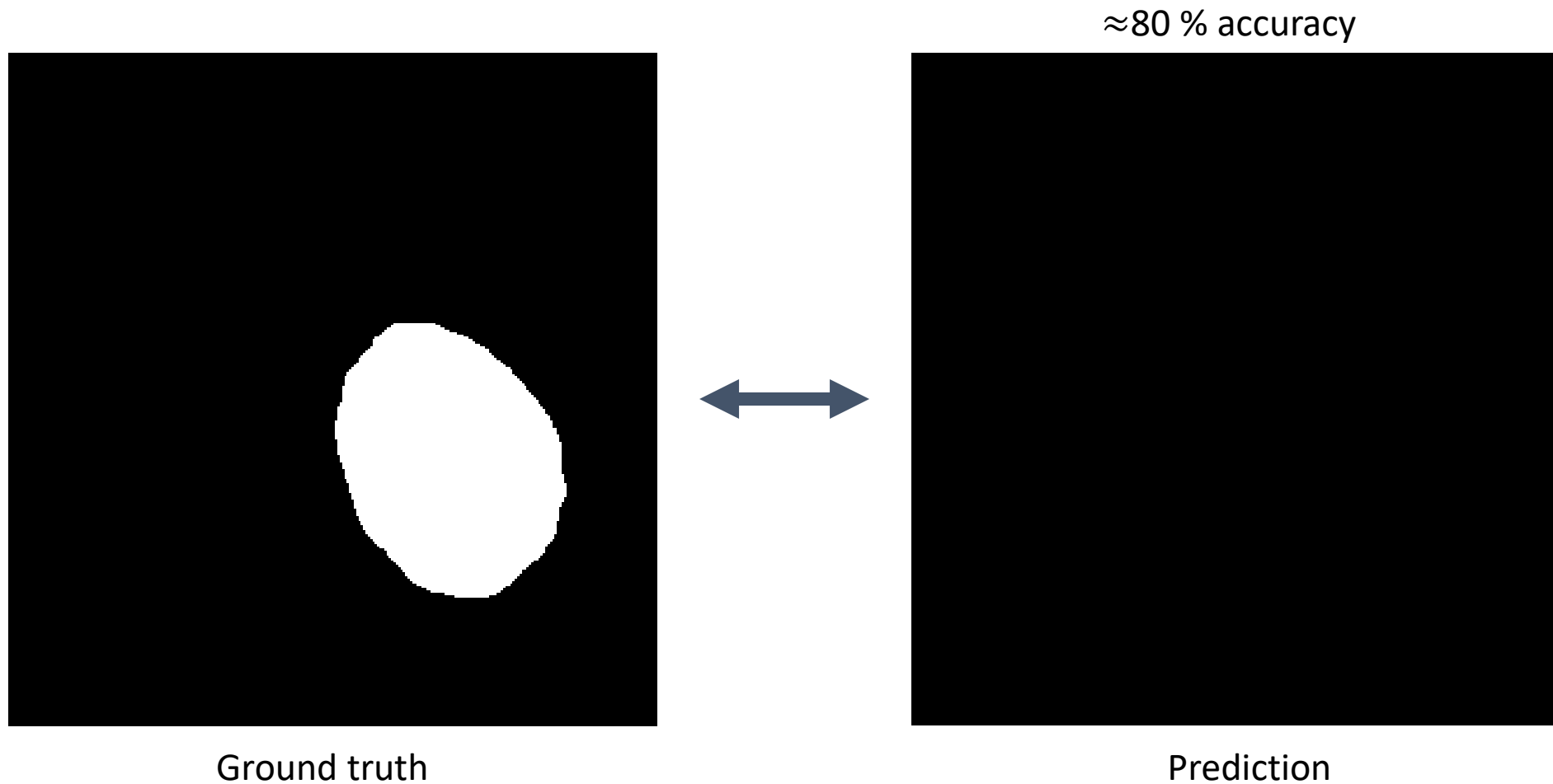
# Implementation of the Dice Score



≈80 % accuracy
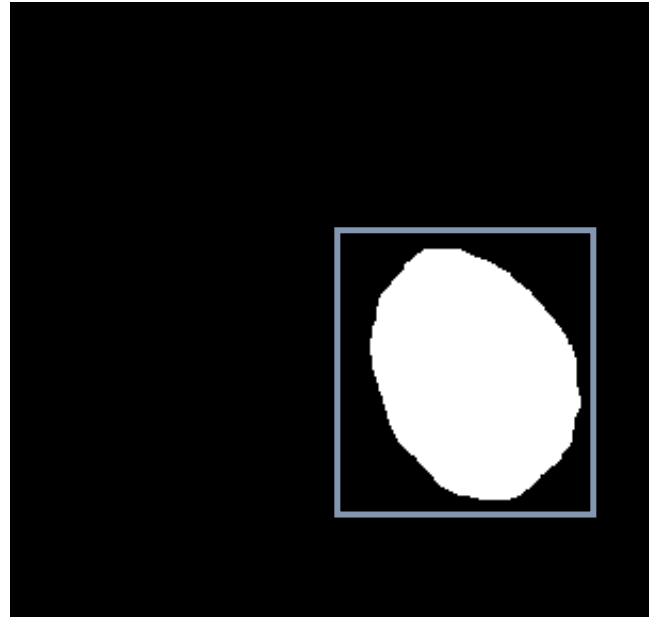
Ground truth

Prediction
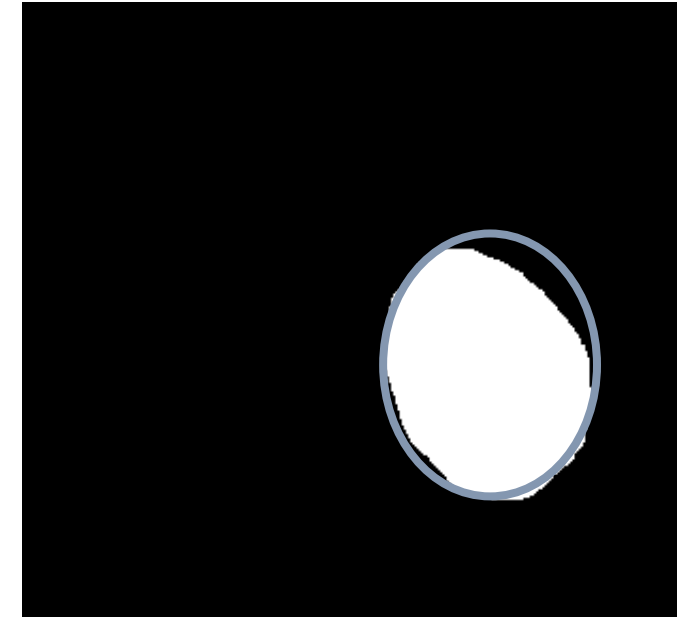
# Implementation of the Dice Score

Bad prediction
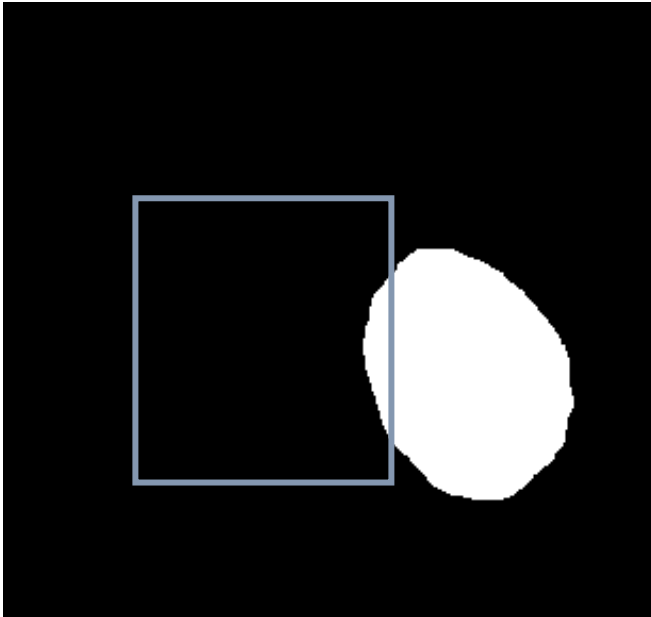
Good prediction

Very good prediction
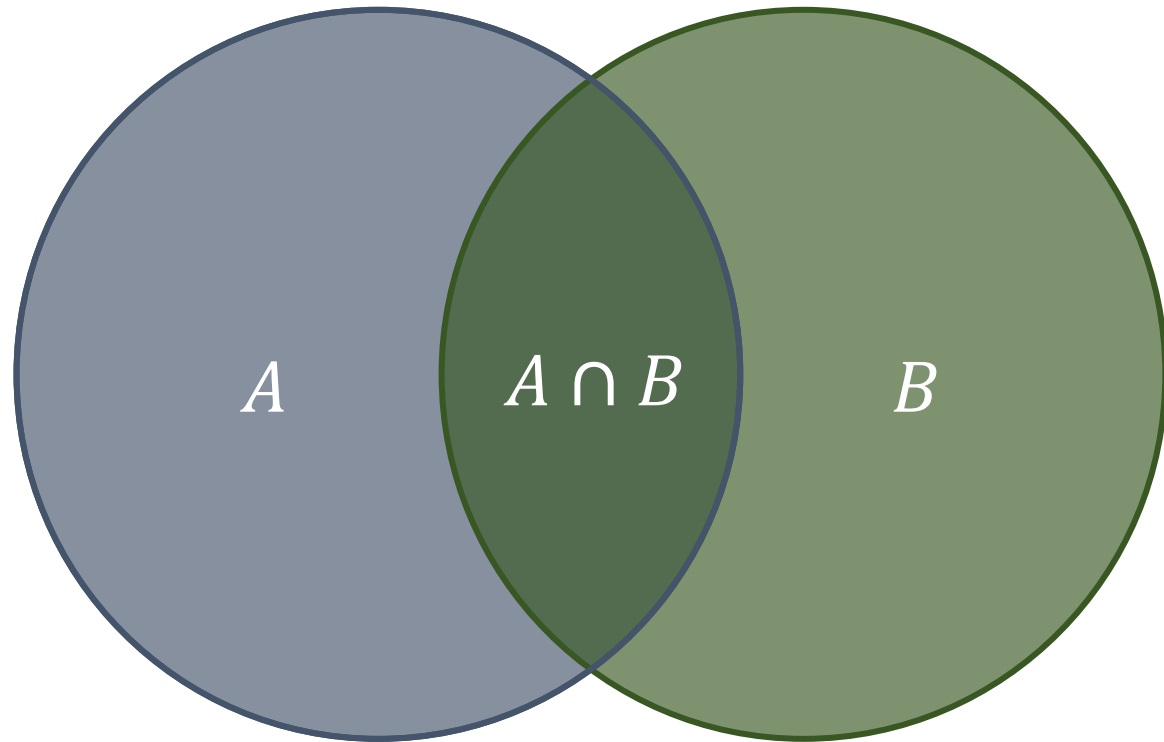
# Implementation of the Dice Score



A: Predicted shape
B: Ground truth

# Implementation of the Dice Score



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

A: Predicted shape
B: Ground truth
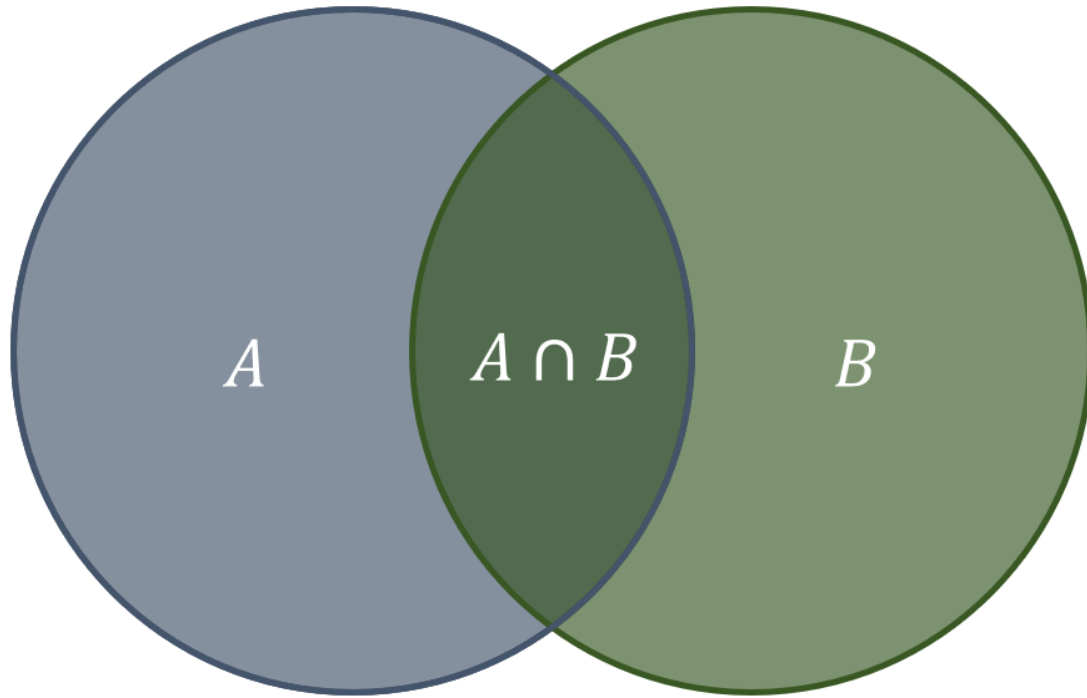
# Implementation of the Dice Score



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

# 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

- Median Otsu

- Algorithm for counting cells

- Algorithm for drawing cell trajectories
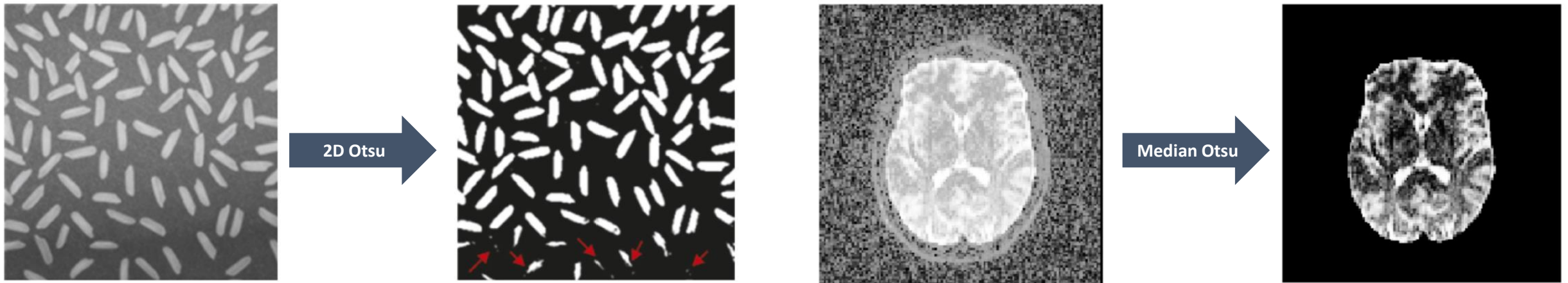
# Timeline

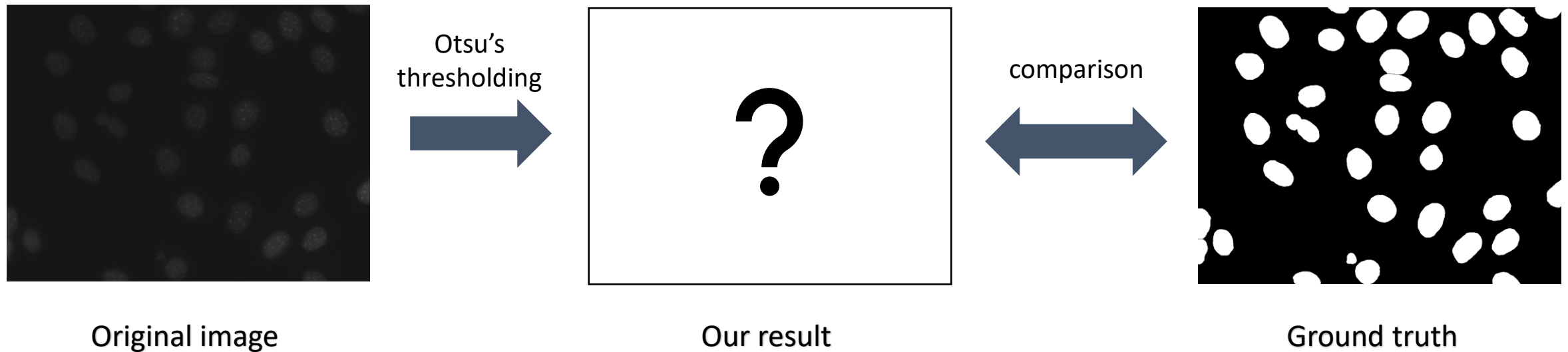| Date | Milestone |
|---|---|
| **Date** | **Milestone** |
| Already done: | Researched Otsu thresholding and Dice scoring; Prepared project presentation |
| 19.05. | Explore data with histograms and similar (All) |
| 26.05. ✳ | Code algorithms for Otsu thresholding (H, E) and Dice scoring (L, V) |
| 02.06. ✳ | Assemble the whole pipeline, test different preprocessing options (All) |
| 09.06. | Research alternative evaluation methods |
| 16.06. ✳ | 1. Test our pipeline on data from the BBBC<br>2. Implement alternative evaluation methods:<br>  • IoU, pixel accuracy (All)<br>  • Hausdorff metric (H, E)<br>  • NSD (L, V) |
| 23.06. | Compare results with group 4.4 and 4.5 |
| 30.06. ✳ | Complete report in Jupyter Notebook (All) |
| 07.07. ✳ | Complete final presentation (All) |

# Thank you for your attention!