Median filter

Gaussian filter

Histogram stretching

3 datasets

Preprocessing

Postprocessing

Global Otsu thresholding

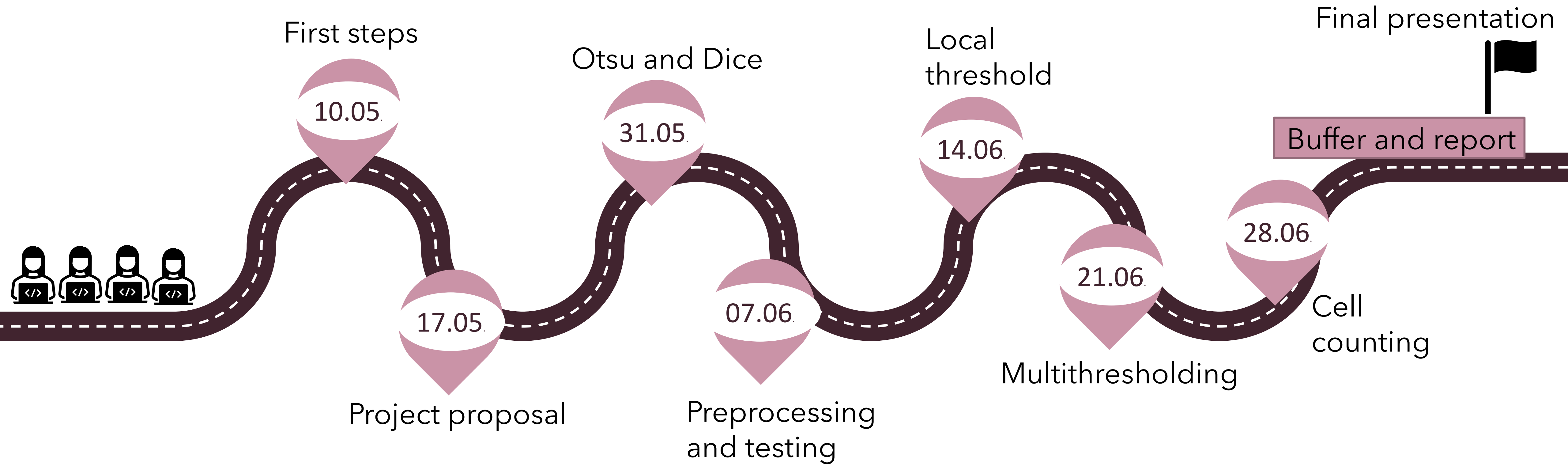
Two-level Otsu thresholding

Local adaptive Otsu thresholding average

Two-level Otsu thresholding clip

Optimal segmented image

Timeline recap



Cell Nuclei Segmentation

IMPLEMENTATION AND EVALUATION OF OTSU THRESHOLDING

Final presentation

Topic 01: Biomedical Image Analysis

Supervisor: PD Dr. Karl Rohr, Christian Ritter, Tutor: Marie Becker
Group 01.04: Marie-Claire Indilewitsch, Helen Jade, Maribel Schneider, Ieva Sorokina-Ozola

Date: 20.07.2022

Overview

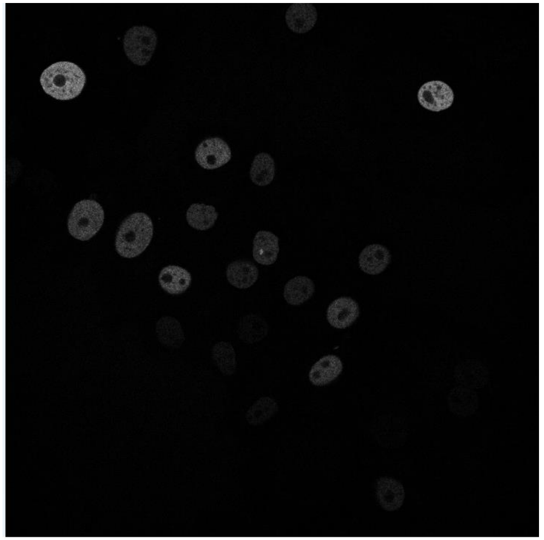
Recap datasets difficulties

Results and Discussion

Challenges

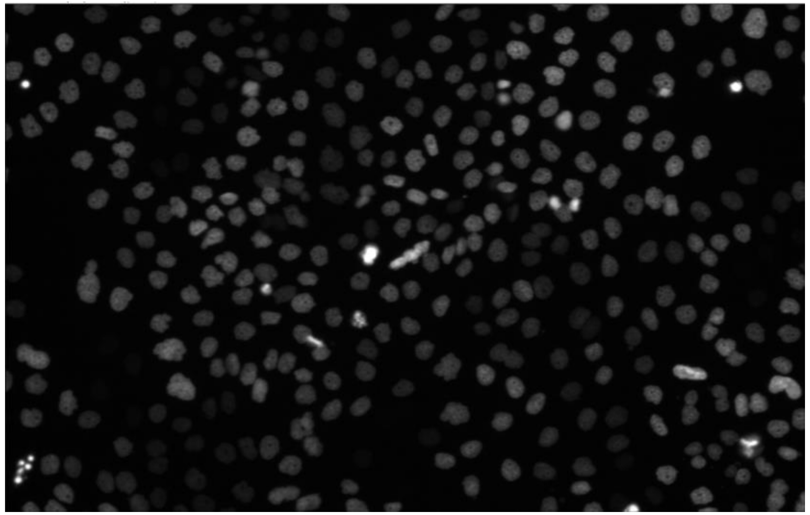
Summary- the perfect recipe

N2DH-GOWT1
(first dataset)



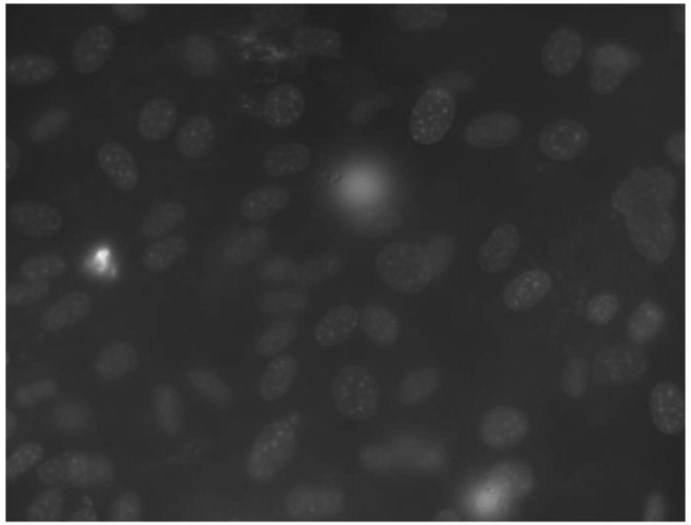
- Noise
- Low contrast
- Holes

N2DL-HeLa
(second dataset)



- Varying brightness of cell nuclei
- Low contrast

NIH3T3
(third dataset)



- Non-uniform illumination
- Reflections

N: nuclear 2D: two-dimensional H: high resolution L: low resolution

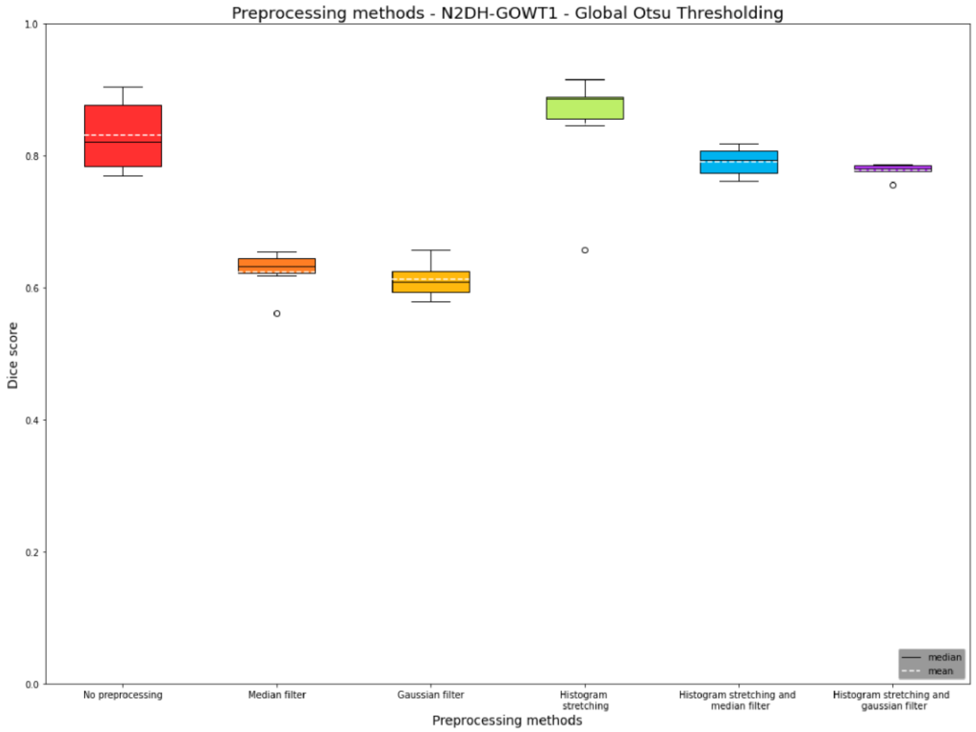
Expectations

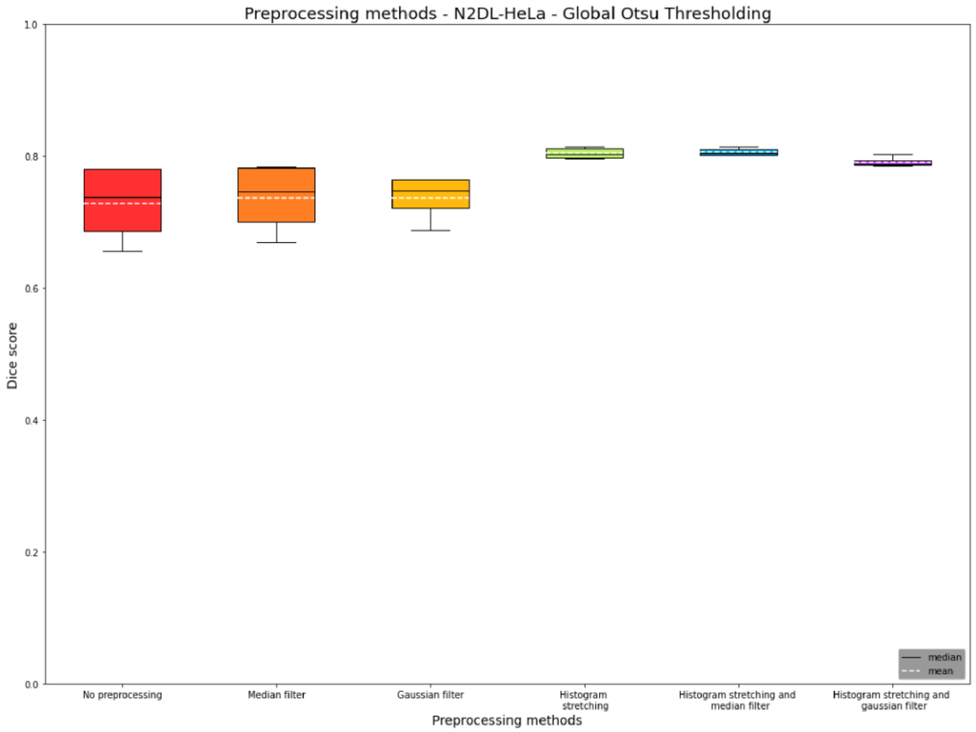
	Challenge	Preprocessing method	Otsu thresholding variation
N2DH-GOWT1	Noise Low contrast Holes	Filters Histogram stretching and combinations +postprocessing	Global Otsu Two-level Otsu
N2DL-HeLa	Varying brigtness of cell nuclei Low contrast	Filters Histogram stretching and combinations	Global Otsu Two-level Otsu Local adaptive thresholding
NIH3T3	Non-uniform illumination Reflection	Filters Histogram stretching and combinations	Global Otsu Two-level Otsu Local adaptive thresholding

→ *Does the combination between preprocessing method and Otsu thresholding variation solve/improve the challenge?*

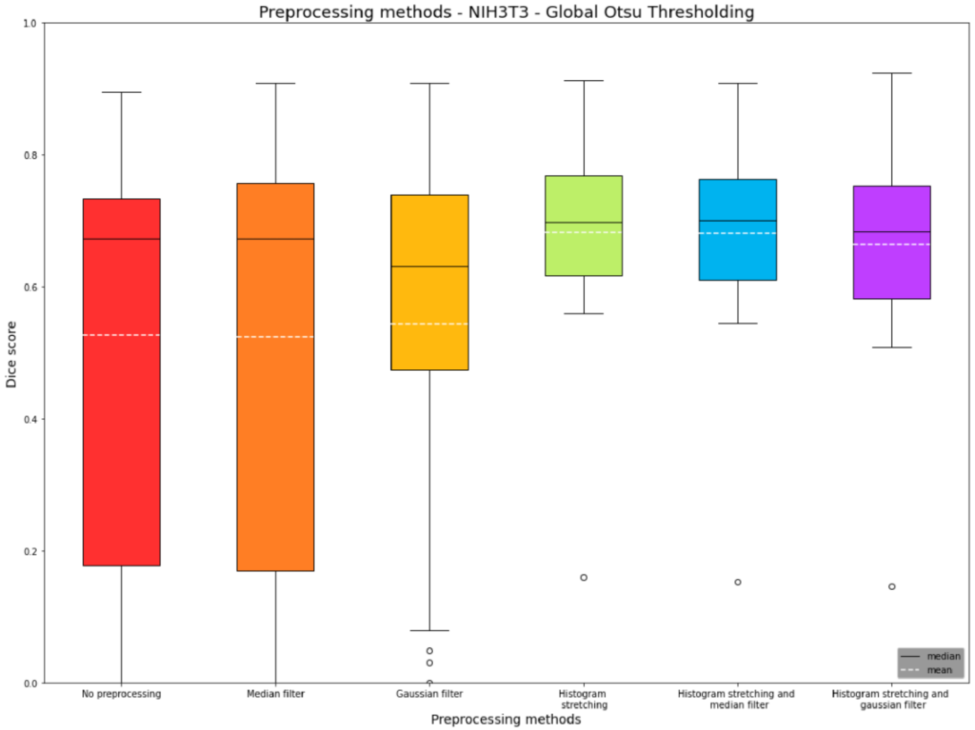
Global Otsu thresholding

Preprocessing methods N2DH-GOWT1 and N2DL-HeLa

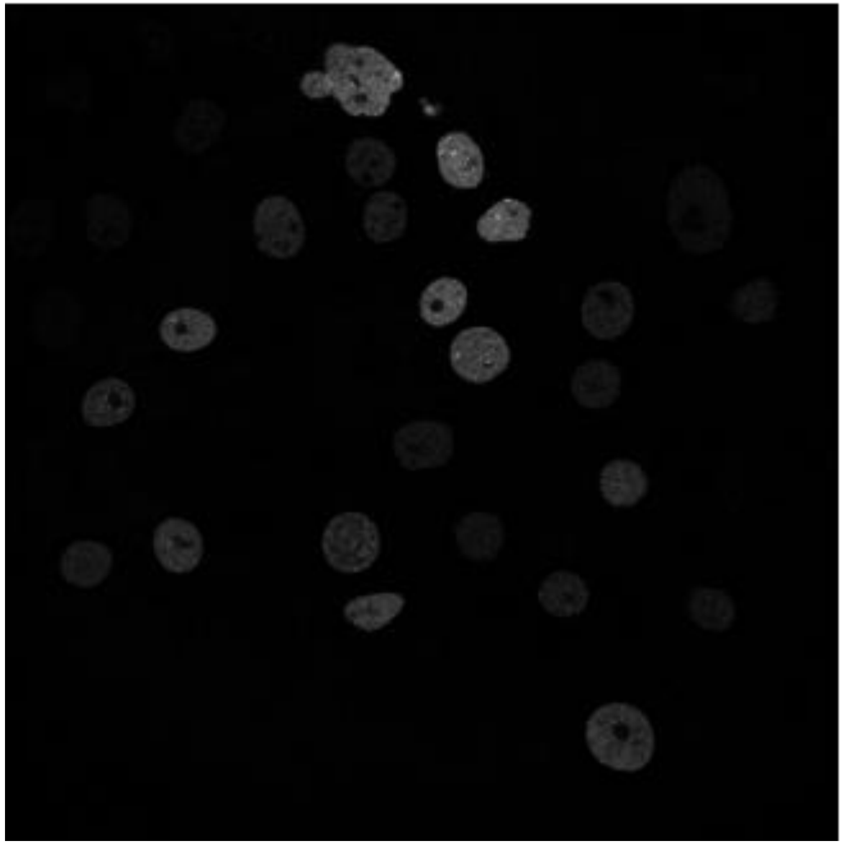


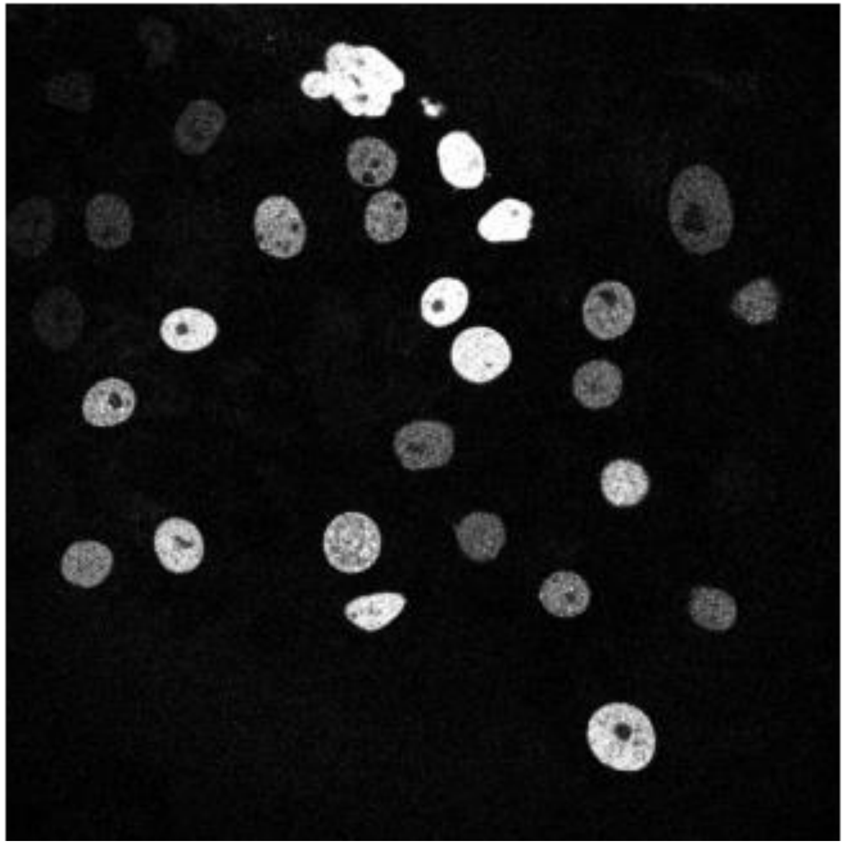


Preprocessing methods NIH3T3

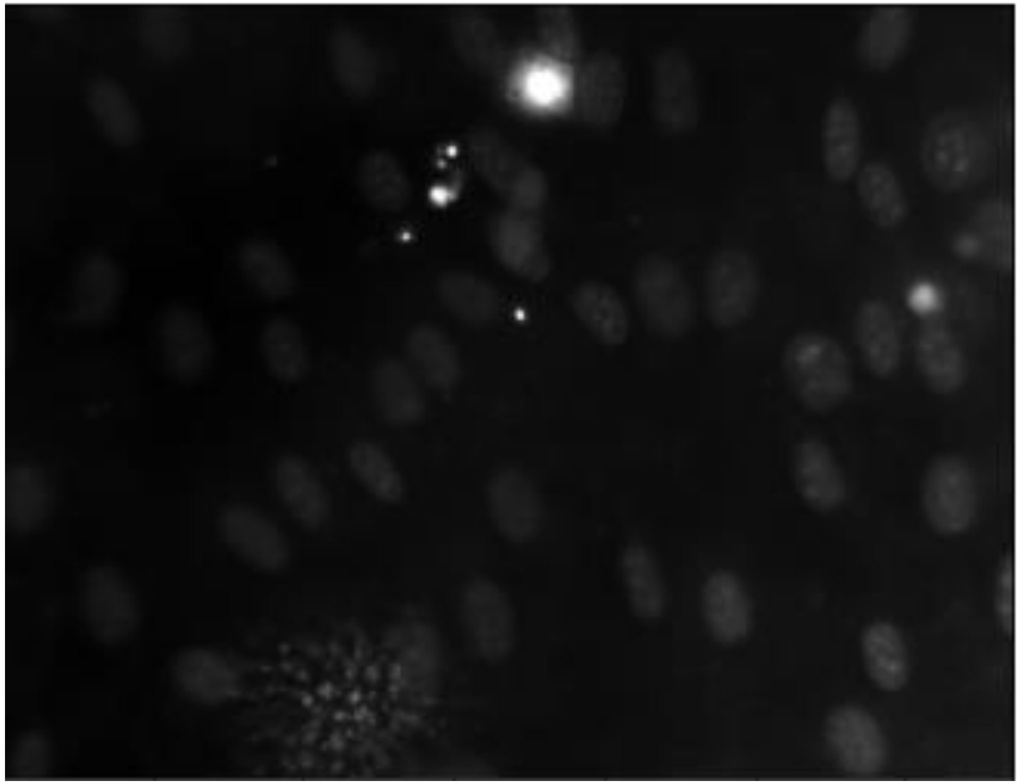


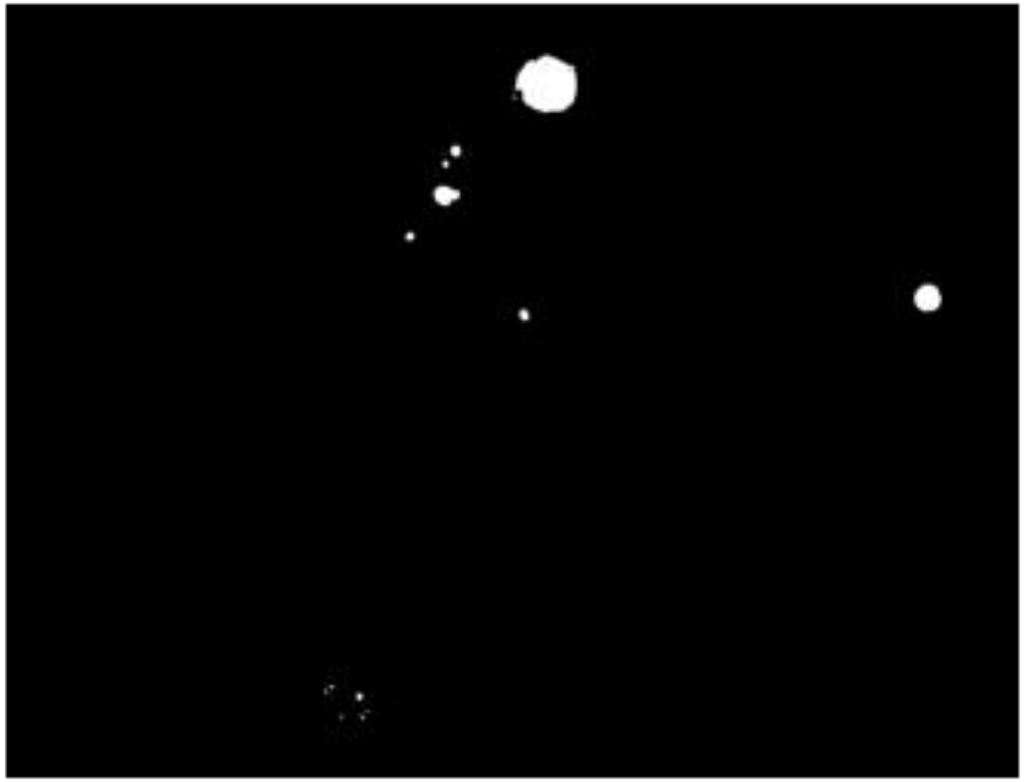
N2DH-GOWT1: Effects of histogram stretching





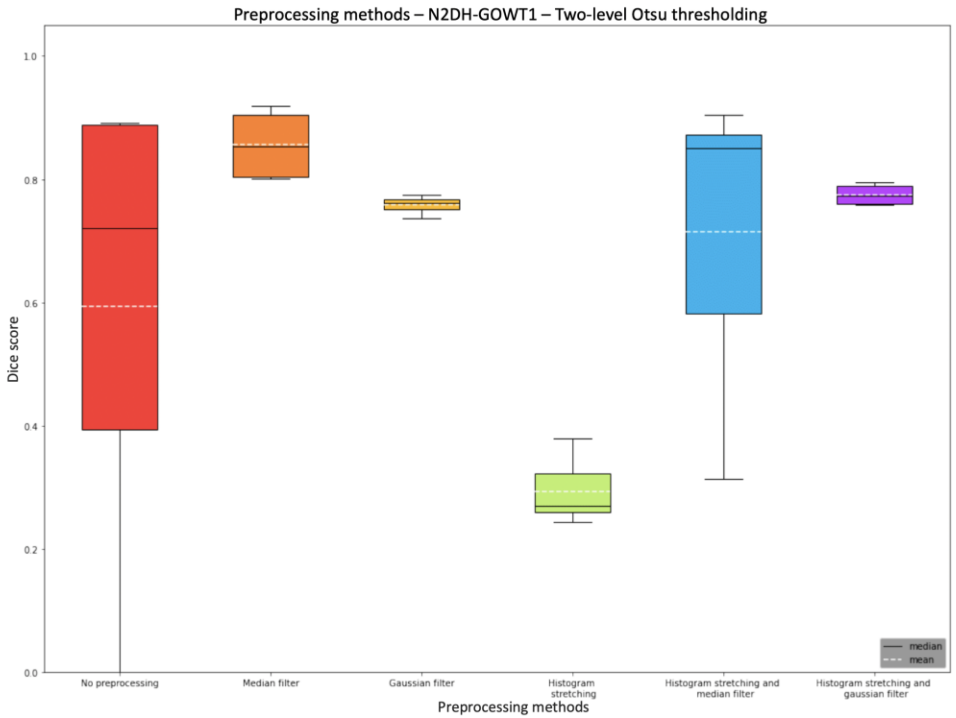
NIH3T3: Reflections

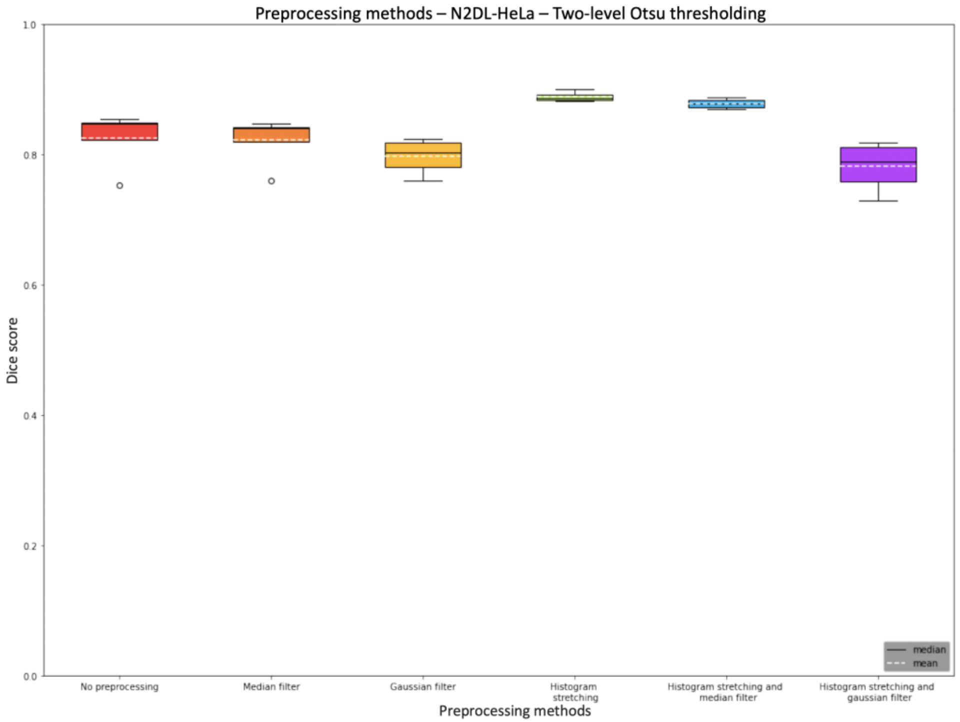




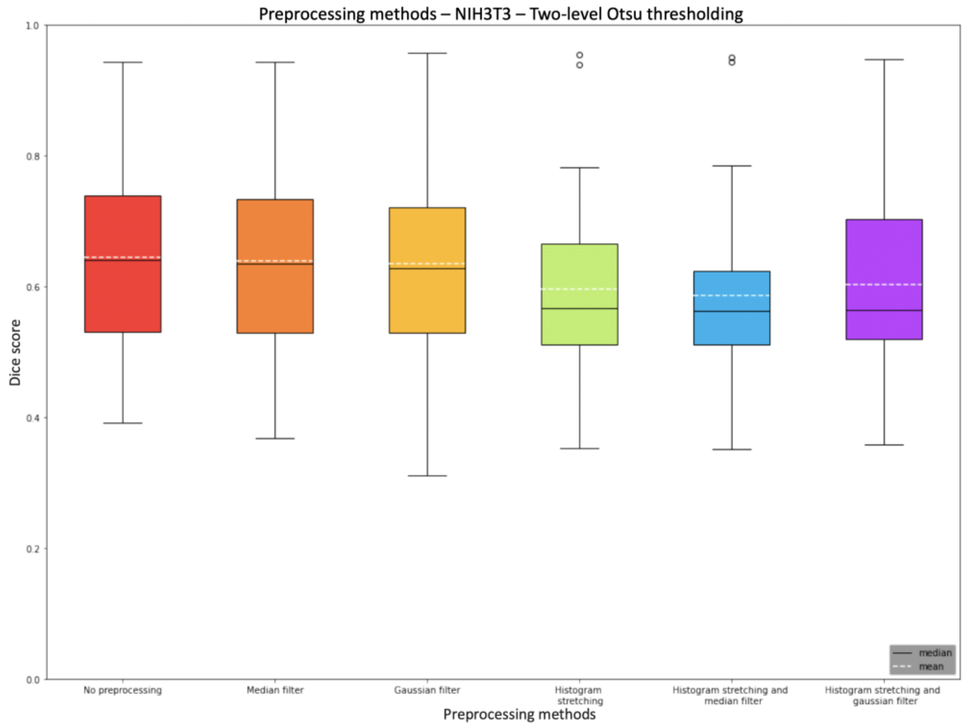
Two-level Otsu thresholding

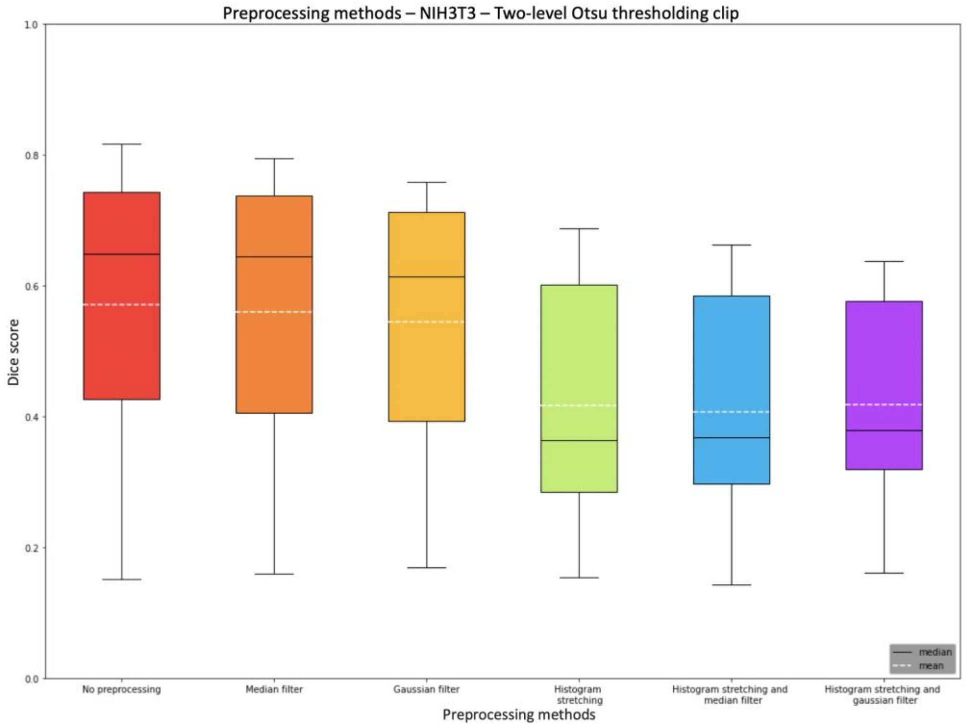
Preprocessing methods N2DH-GOWT1 and N2DL-HeLa



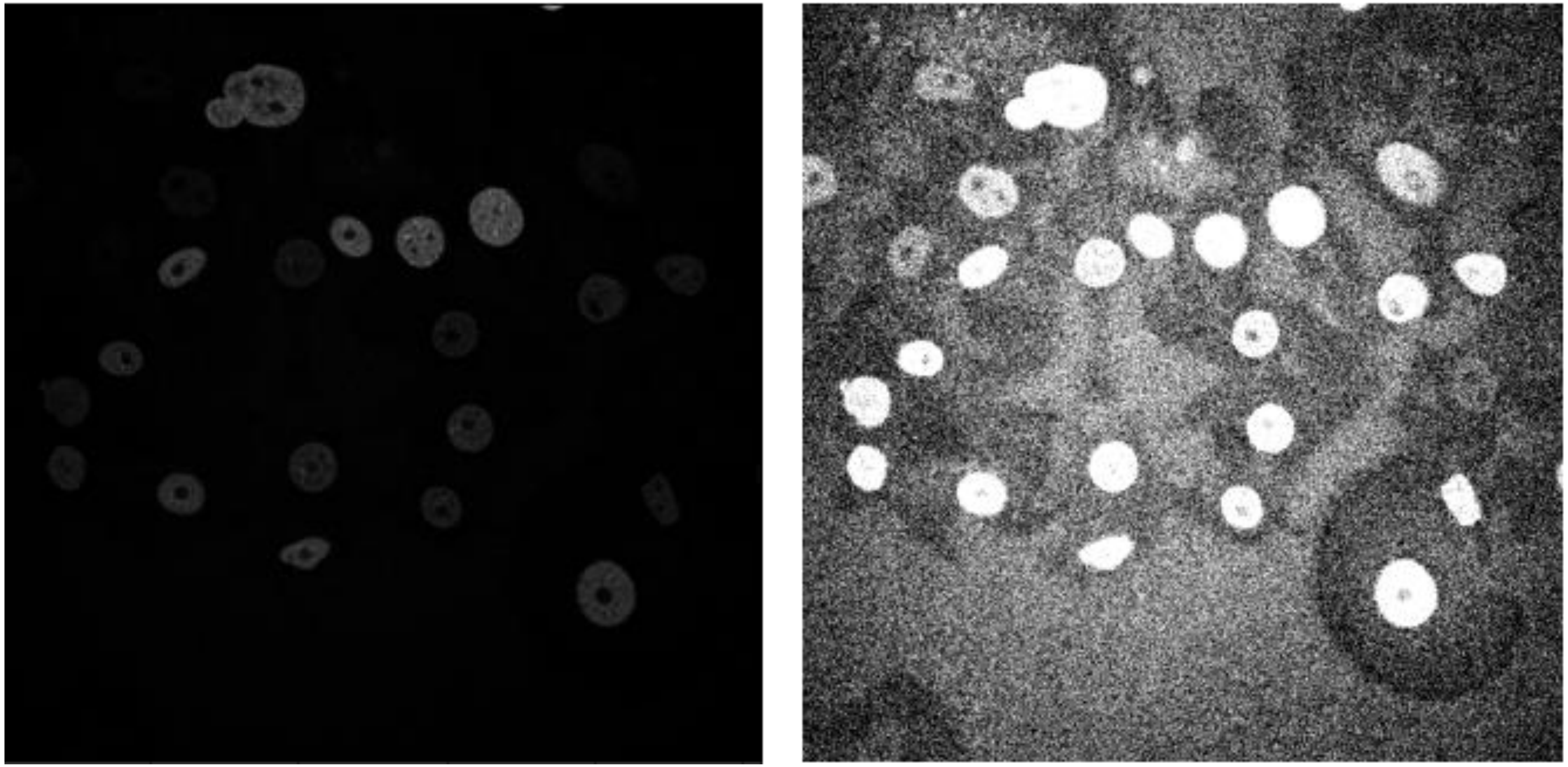


Preprocessing methods NIH3T3 two-level Otsu and clip

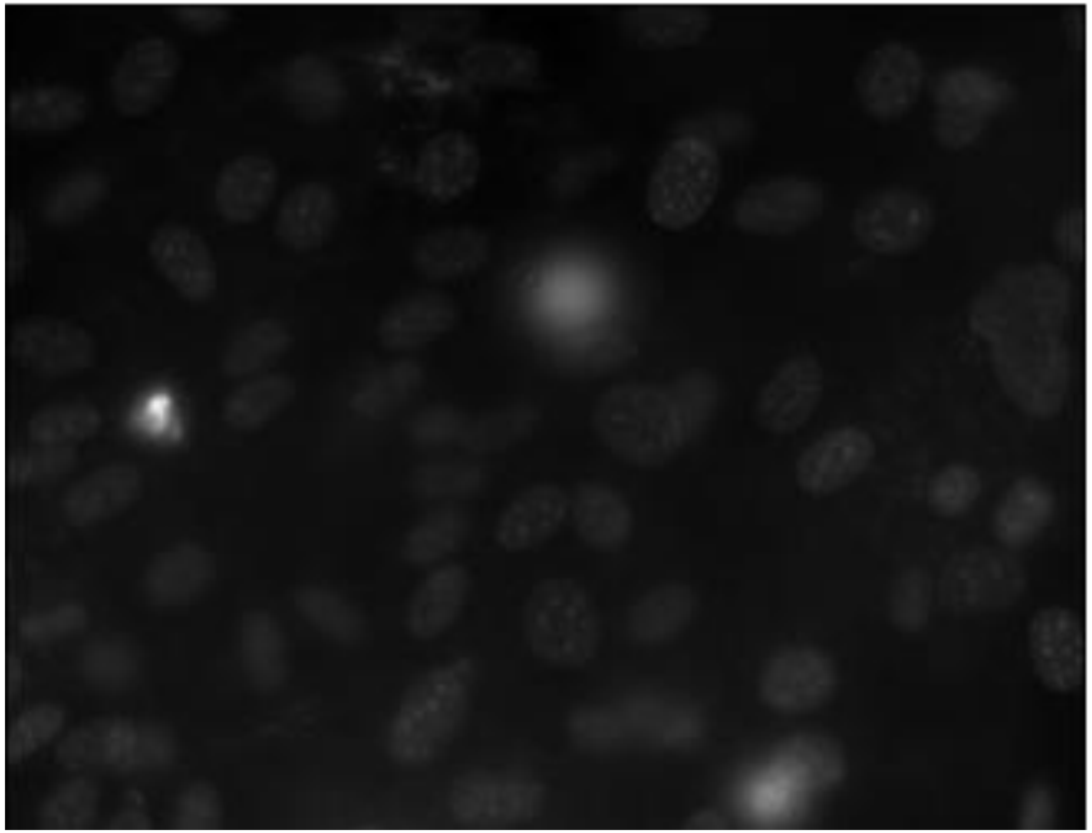


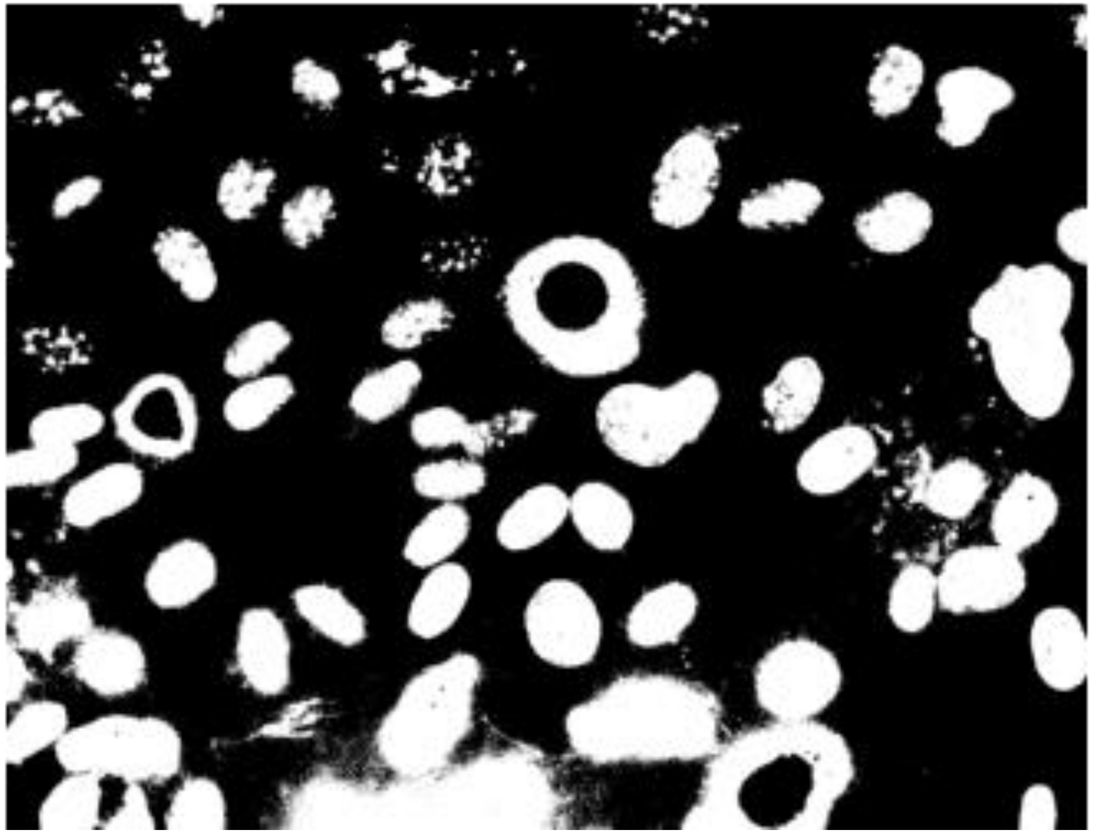


N2DH-GOWT1: Effects of histogram stretching



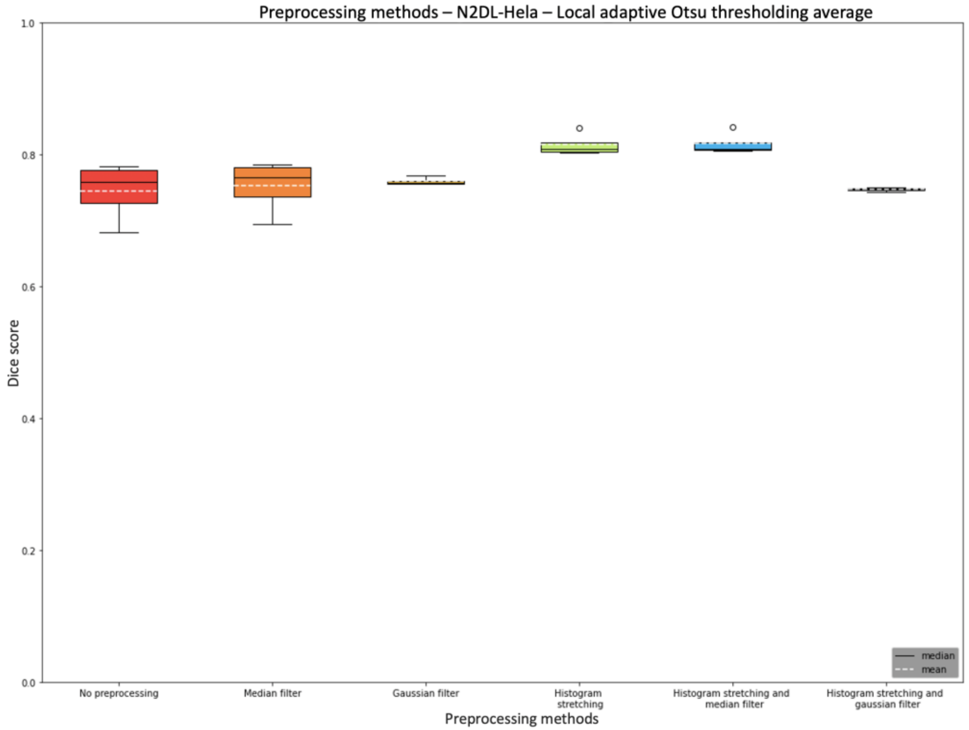
NIH3T3: Two-level Otsu thresholding clip

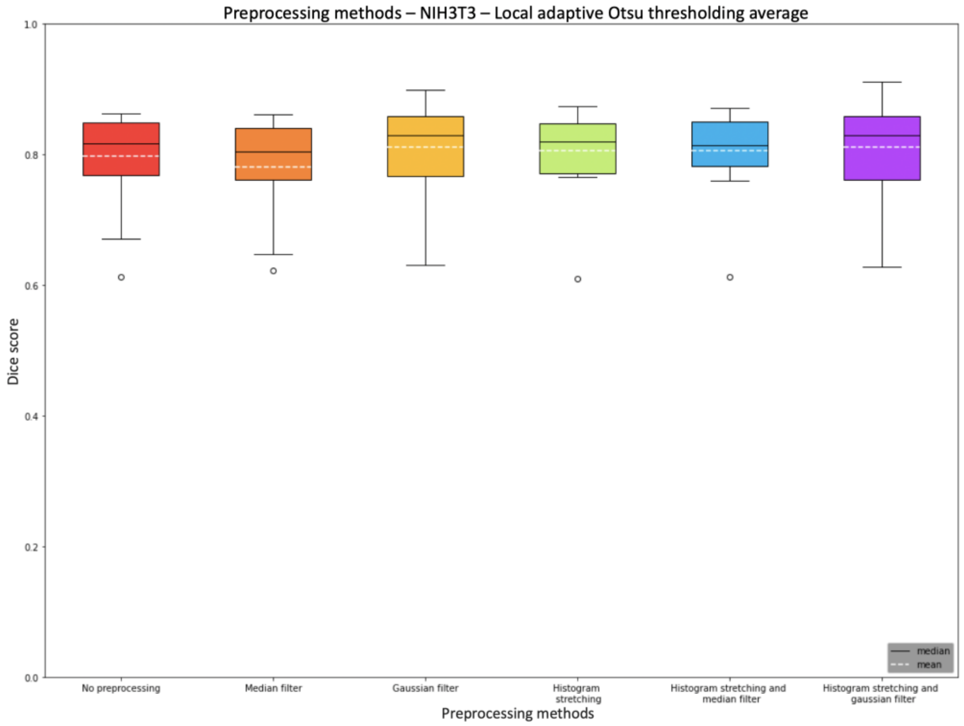




Local adaptive Otsu thresholding

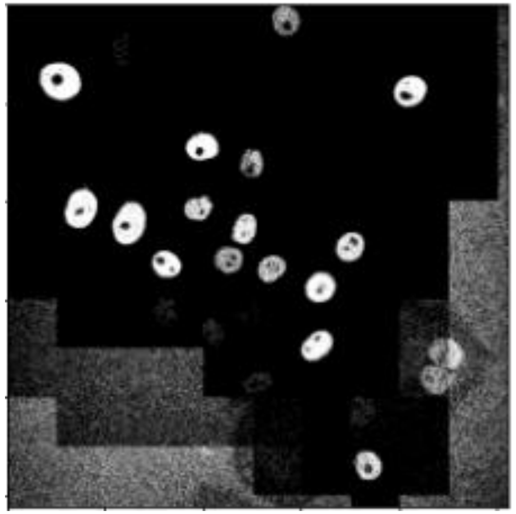
Preprocessing methods N2DL-HeLa and NIH3T3





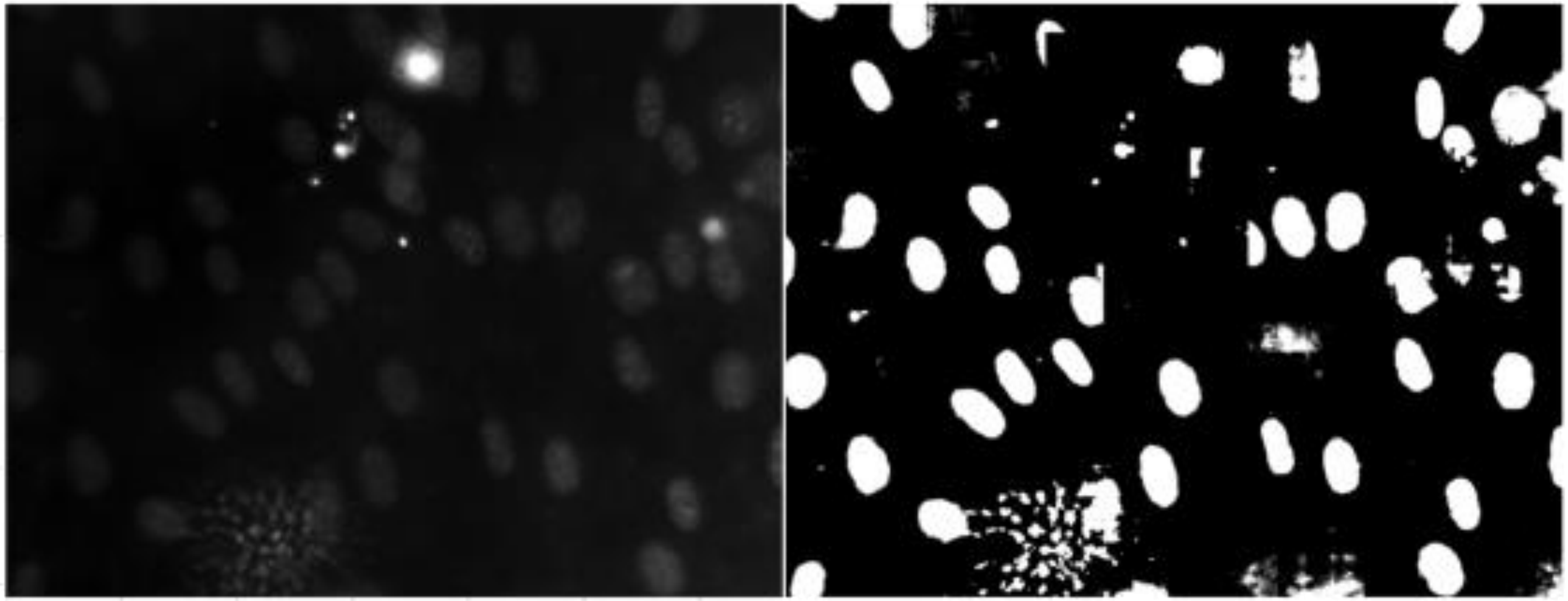
Challenges

RANDOM NOISE



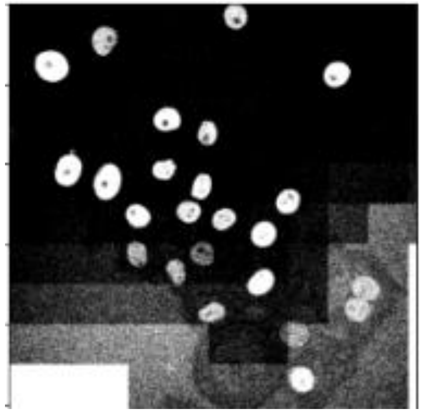
UNSEGMENTED EDGES

NIH3T3: Effects of local adaptive Otsu thresholding

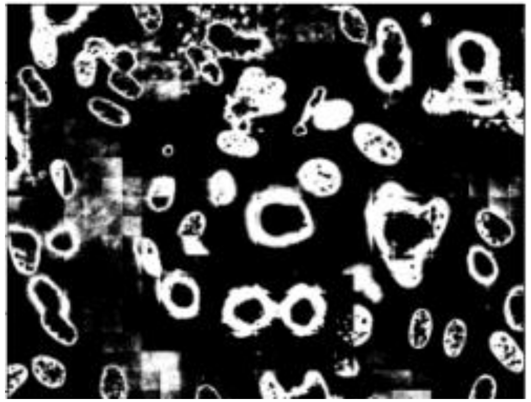


Local adaptive two-level Otsu thresholding

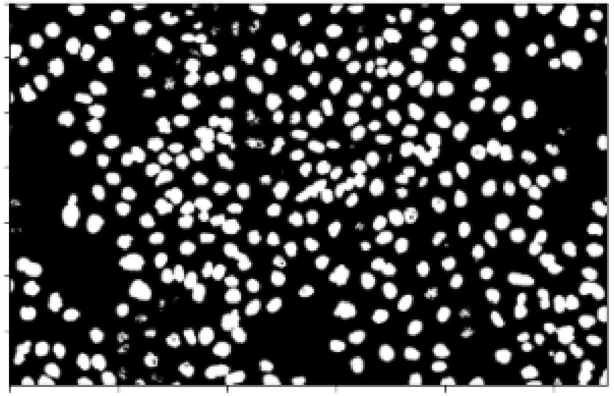
Local Two-level Otsu thresholding average - N2DH-GOWT1 - t31



Local Two-level Otsu thresholding clip average - NIH3T3 - dna32



Local Two-level Otsu thresholding average - N2DL-HeLa - t75



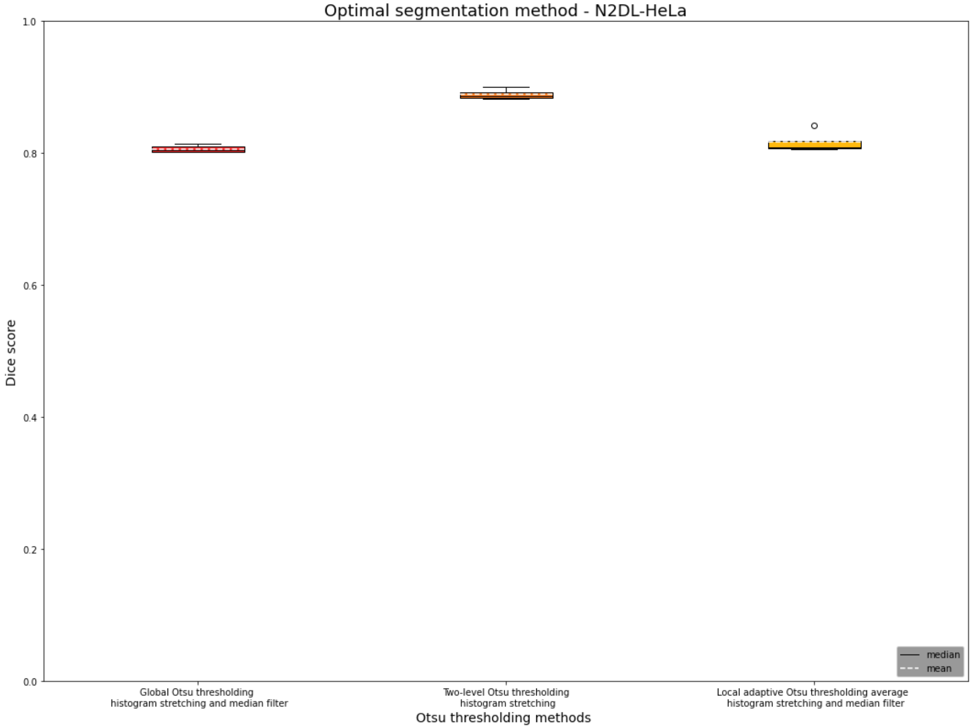
Challenges

Challenges

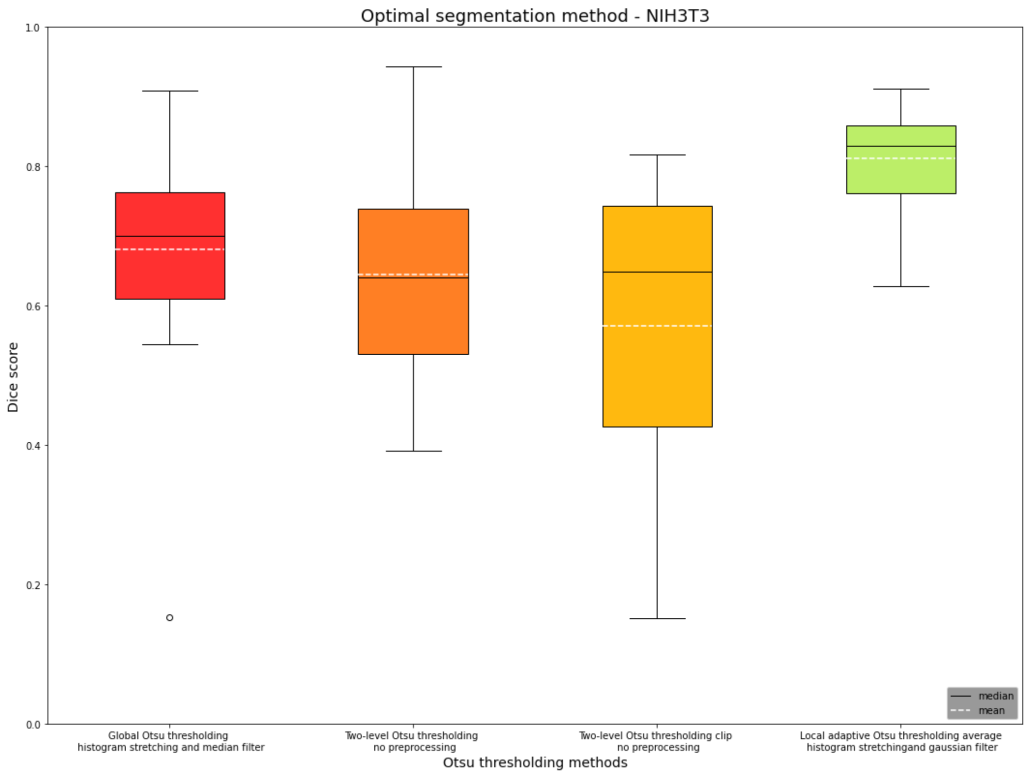
- Runtime
- Optimal bin size
- Optimal filter size
- Edge issue
- Local adaptive Otsu thresholding count vs. average

All challenges solved?

Optimal segmentation method for N2DH-GOWT1 and N2DL-HeLa



Optimal segmentation method NIH3T3

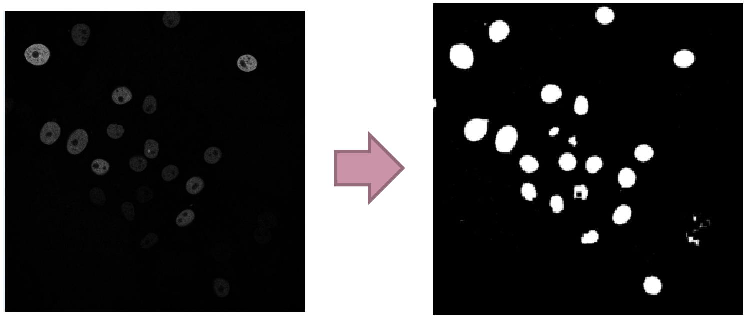


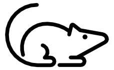
		Global Otsu Thresholding	Two-level Otsu thresholding	Two-level Otsu Clip	Local adaptive thresholding average
N2DH-GOWT1	Preprocessing method	Histogram stretching	Median filter		
	Dice Score	**0.89**	0.85		
N2DL-HeLa	Preprocessing method	Histogram stretching and median	Histogram stretching		Median filter and histogram stretching
	Dice Score	0.80	**0.89**		0.81
NIH3T3	Preprocessing method	Histogram stretching and median	No preprocessing	No preprocessing	Gaussian filter and histogram stretching
	Dice Score	0.70	0.64	0.65	**0.83**

Conclusion

The perfect recipe

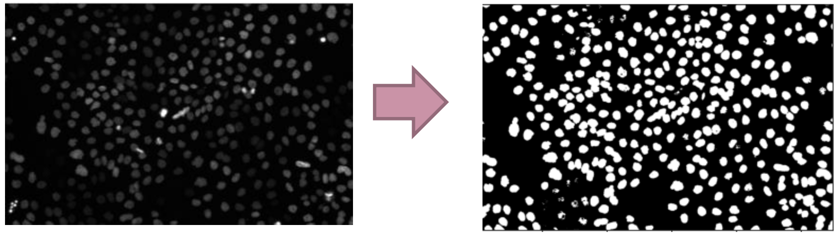
N2DH-GOWT1
(first dataset)

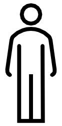




- **Preprocessing:** histogram stretching
- **Postprocessing:** hole filling
- **Otsu:** global Otsu thresholding
- **Final median Dice Score:** 0.8864

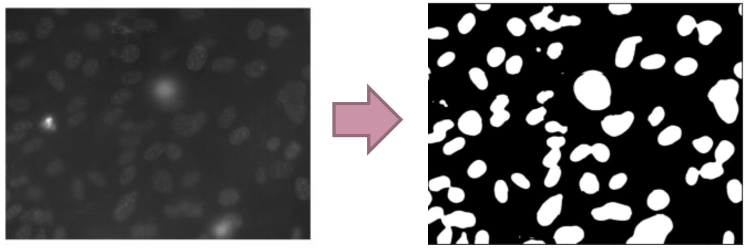
N2DL-HeLa
(second dataset)





- **Preprocessing:** histogram stretching
- **Otsu:** two-level Otsu thresholding
- **Final median Dice Score:** 0.8866

NIH3T3
(third dataset)



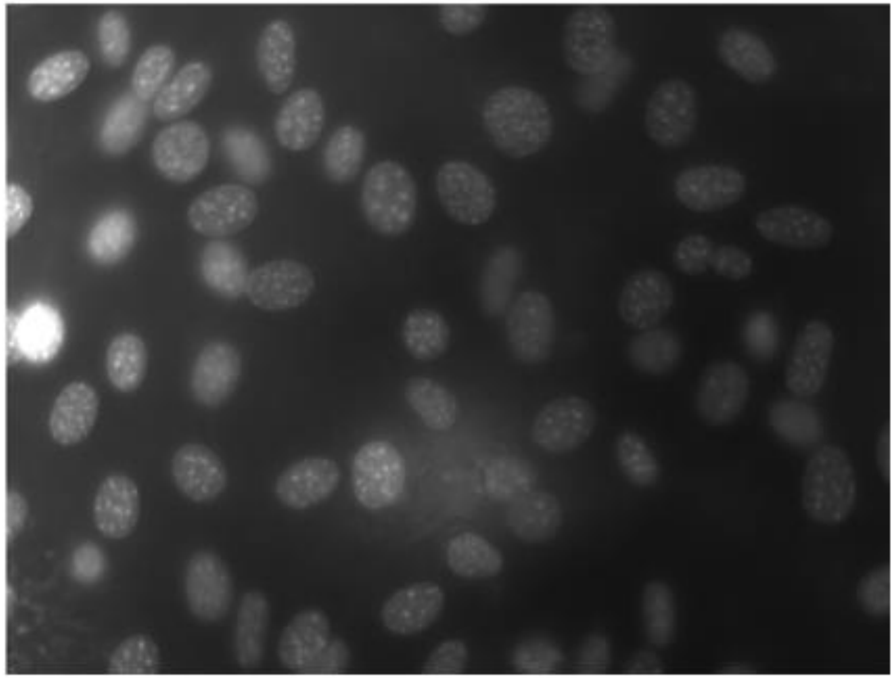
- **Preprocessing:** Gaussian filter + histogram stretching
- **Otsu:** local adaptive Otsu thresholding
- **Final median Dice Score:** 0.8293

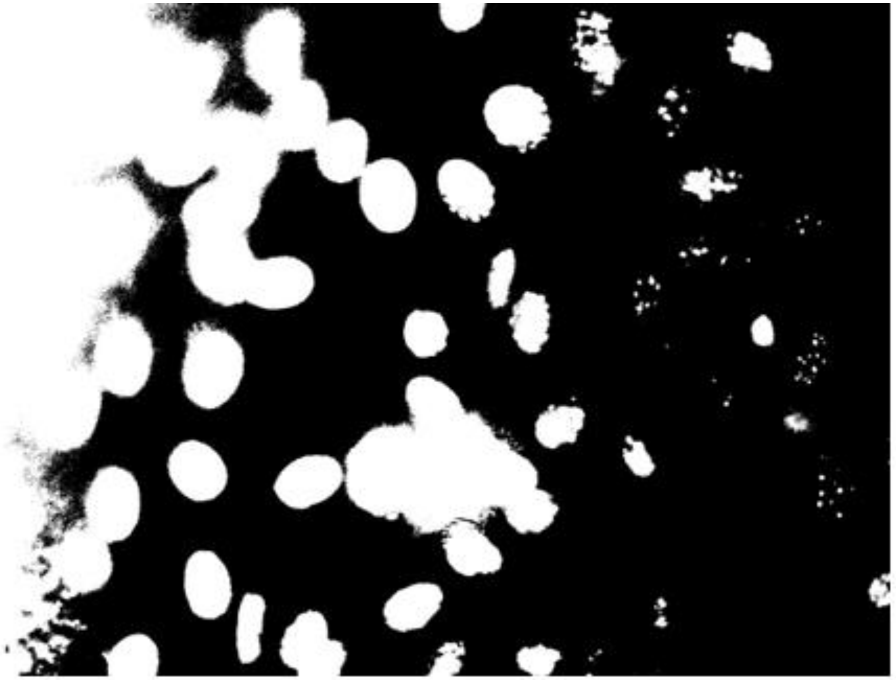
N: nuclear 2D: two-dimensional H: high resolution L: low resolution

Thank you for your attention!

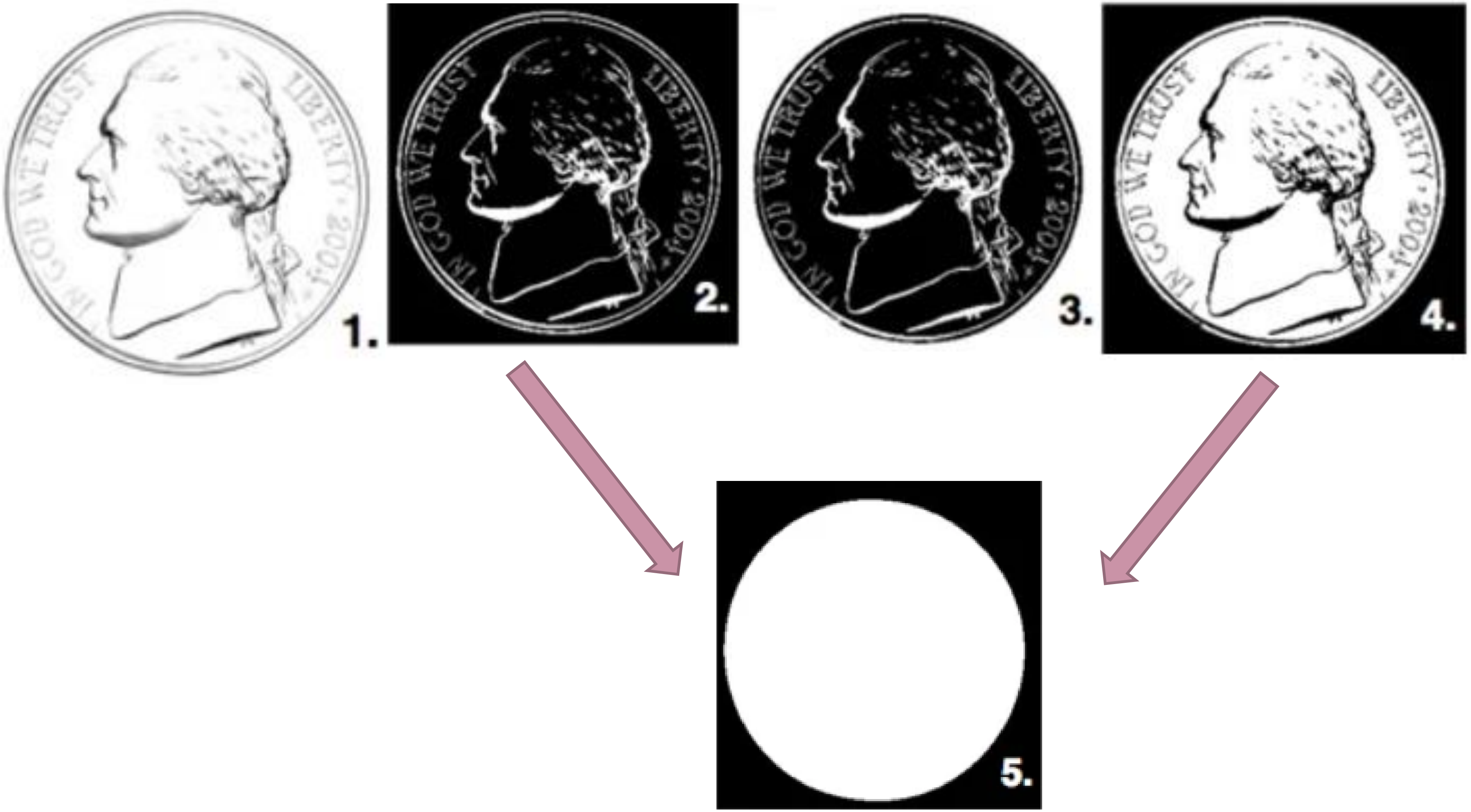
Extra slides

Why local thresholding?





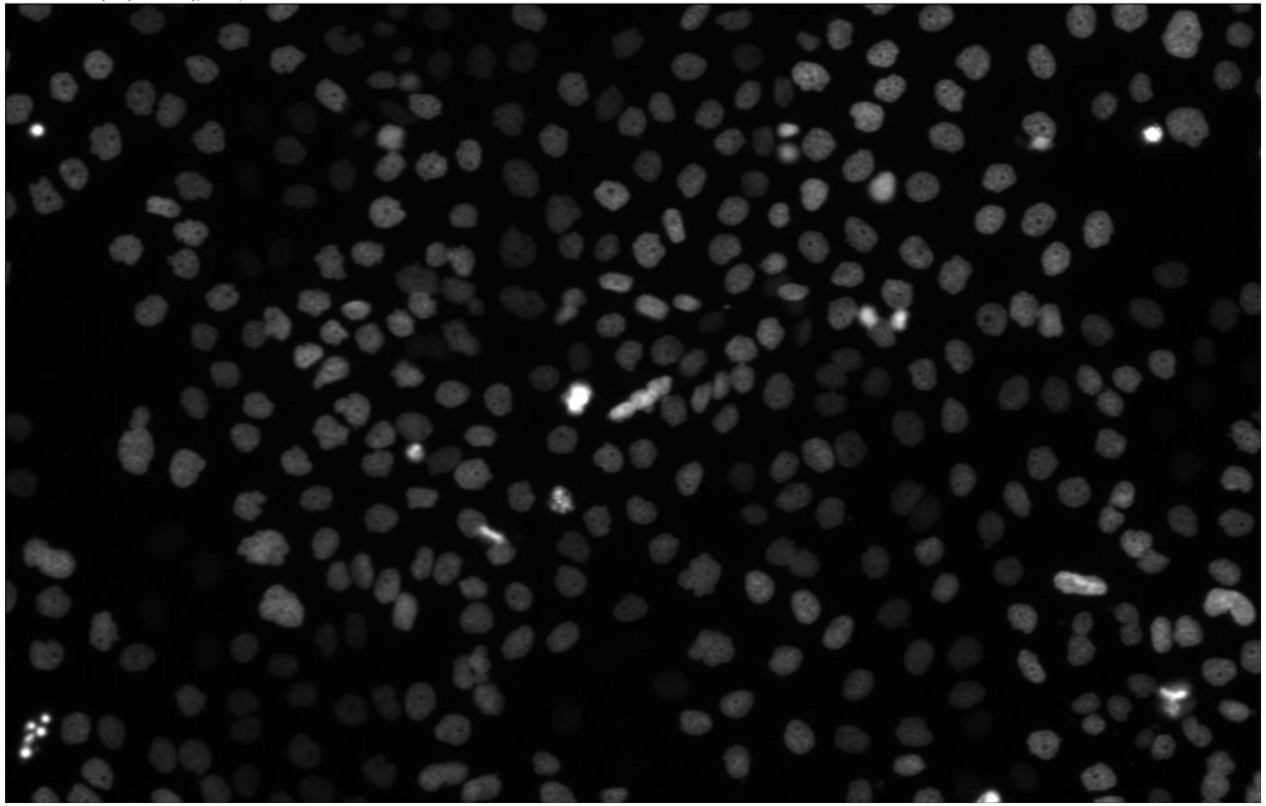
Hole filling



Solution Ideas

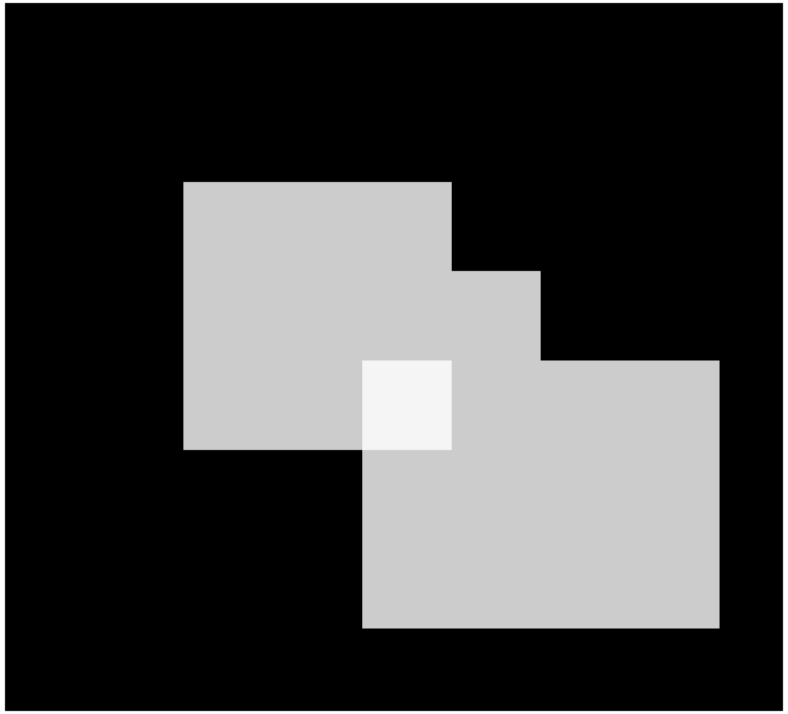
Challenge	Solution
Random noise	Gaussian filter, median filter
Holes	Median filter
Low contrast	Histogram stretching
Reflections	Intensity clipping, multithresholding
Brightness	Local thresholding

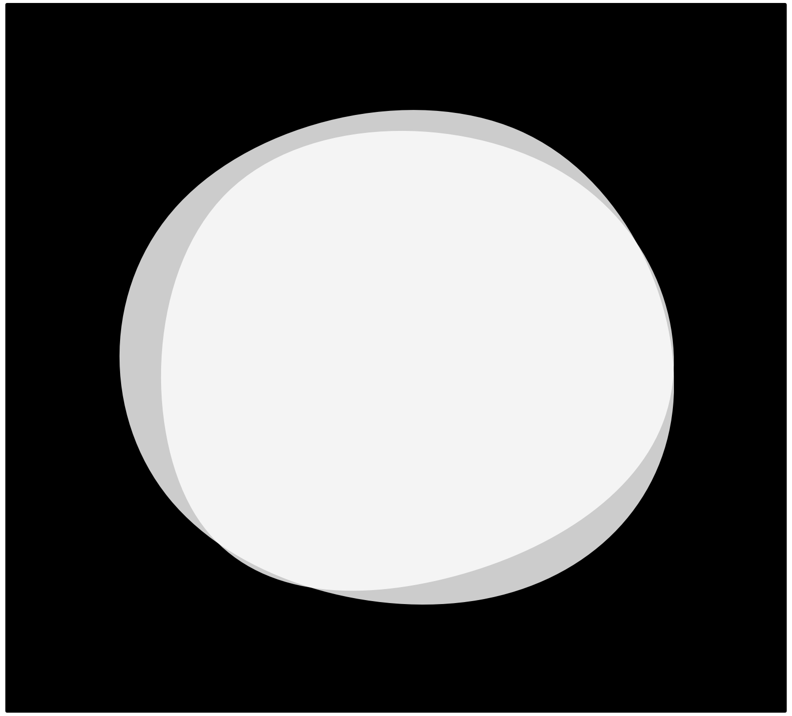
Challenges: N2DL-HeLa



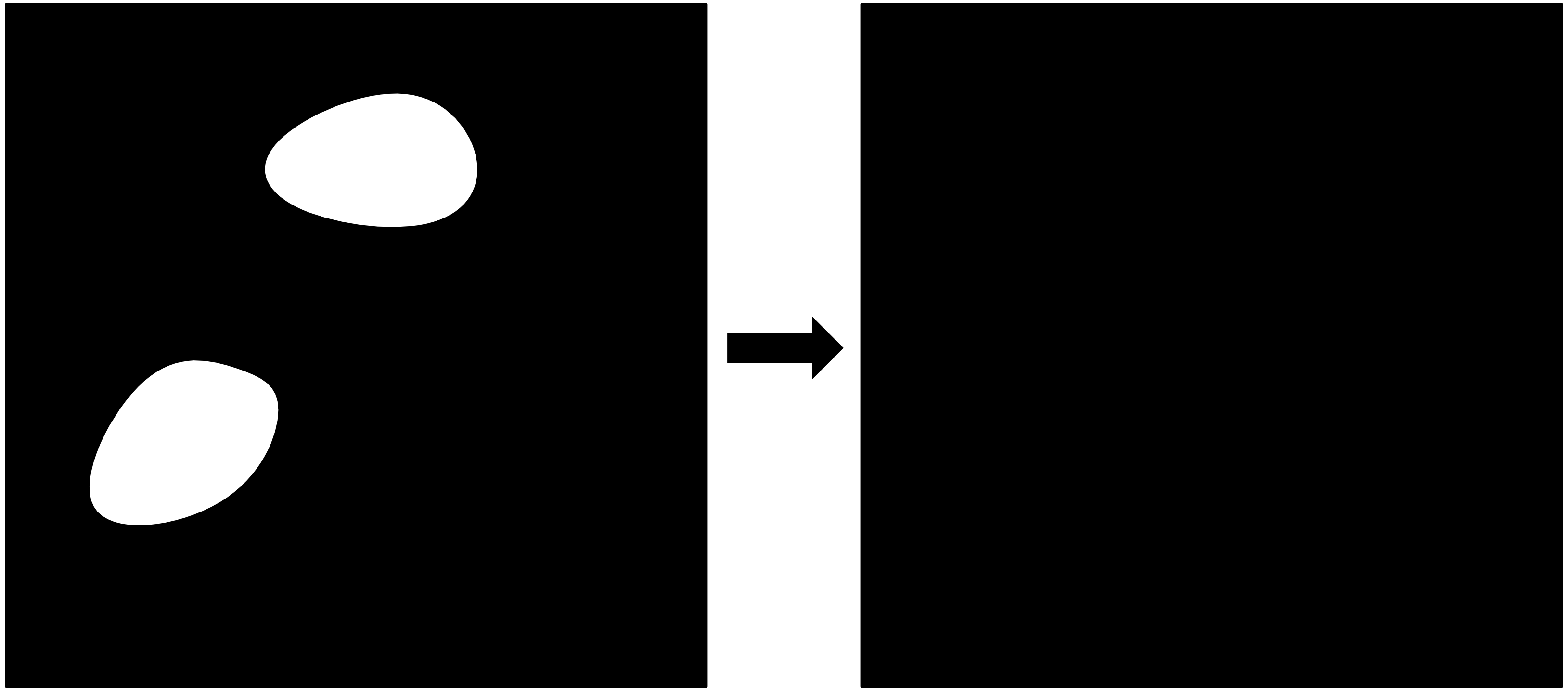
Reflections

Dice score - Issues





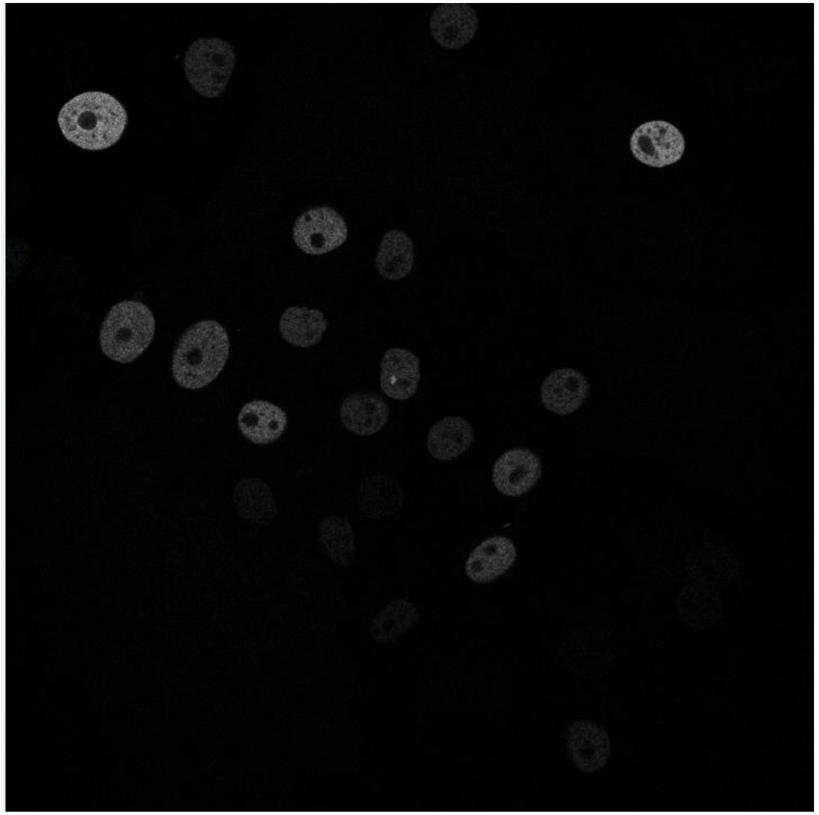
Evaluation algorithm – Dice score



~ 80% Accuracy

N2DH-GOWT1

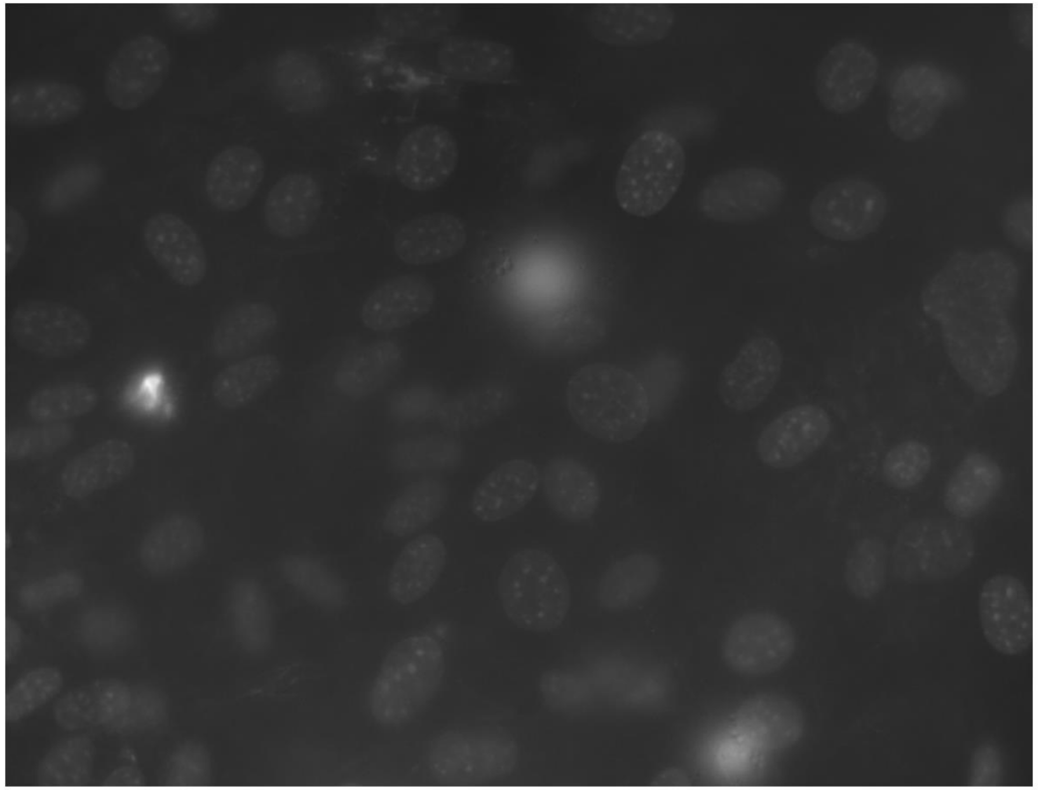
Cell type: embryonic stem cells | Organism: Mouse (Mus musculus)



- Express Oct4-GFP
- Leica TGS SP5 (confocal microscope)
- 6 images total
- 1024x1024 pixels
- 10-20 nulei per image

NIH3T3

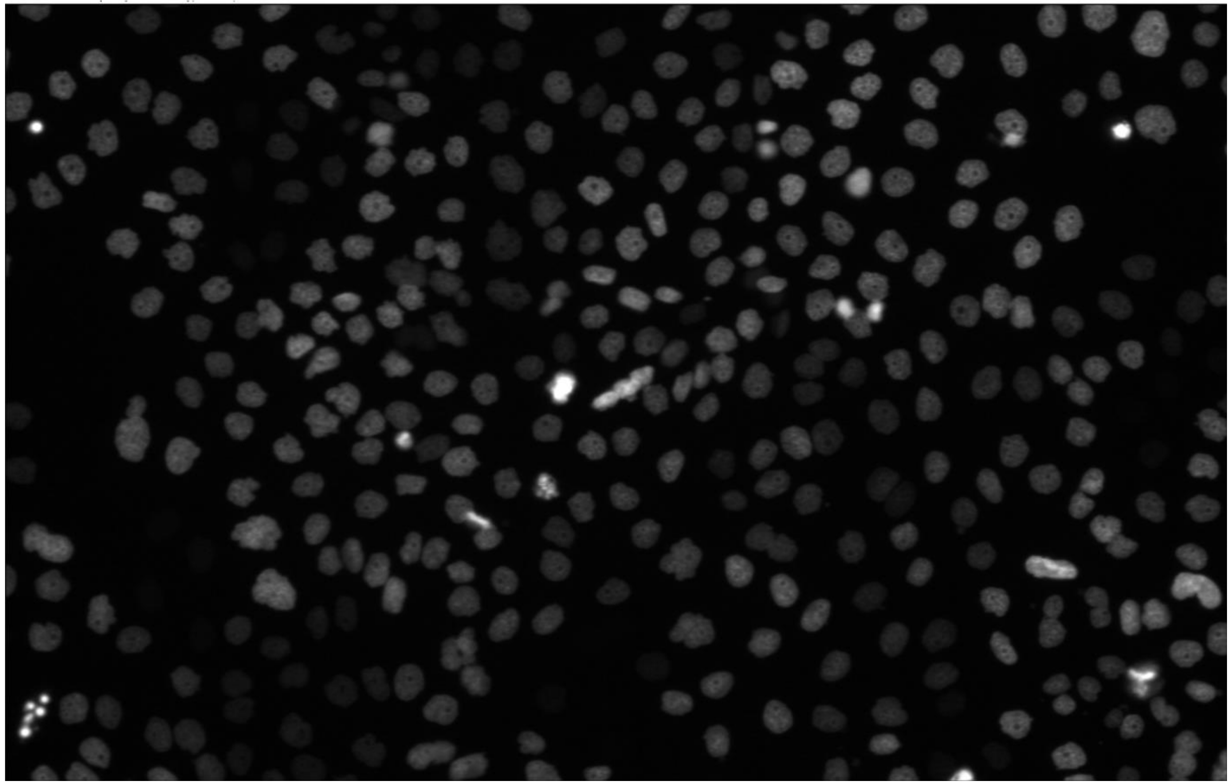
Cell type: Embryonic fibroblast cells | Organism: Mouse (Mus musculus)



- Stained with Hoechst (fluorescent dye)
- Fluorescence microscope
- 18 images total
- Size: 1344x1024 pixels
- about 60 nuclei per image

N2DL-HeLa

Cell type: cervical adenocarcinoma cells | organism: human (*Homo sapiens*)



- Express H2B-GFP
- Olympus IX81 Confocal Microscope
- 4 images total
- Size: 1100x 700 pixels
- 30-50 nuclei per image

Otus Thresholding usage

- Locate cell boundaries
- Cell type classification
  - Cell counting
  - Cell phenotype analysis (size, shape,..)
- **But not used for very complex images !**

Otsu Thresholding

Class probabilities ($\omega_{0,1}$):

$$\omega_0 = \Pr(C_0) = \sum_{i=1}^{k} p_i = \omega(k)$$

$$\omega_1 = \Pr(C_1) = \sum_{i=k+1}^{L} p_i = 1 - \omega(k)$$

Class mean levels ($\mu_{0,1}$) and total mean level (μ_T):

$$\mu_0 = \sum_{i=1}^{k} i \Pr(i|C_0) = \sum_{i=1}^{k} ip_i/\omega_0 = \mu(k)/\omega(k)$$

$$\mu_1 = \sum_{i=k+1}^{L} i \Pr(i|C_1) = \sum_{i=k+1}^{L} ip_i/\omega_1 = \frac{\mu_T - \mu(k)}{1 - \omega(k)}$$

$$\mu_T = \mu(L) = \sum_{i=1}^{L} ip_i$$

Class variances ($\sigma^2_{0,1}$) and total variance (σ^2_T):

$$\sigma_0^2 = \sum_{i=1}^{k} (i - \mu_0)^2 \Pr(i|C_0) = \sum_{i=1}^{k} (i - \mu_0)^2 p_i/\omega_0$$

$$\sigma_1^2 = \sum_{i=k+1}^{L} (i - \mu_1)^2 \Pr(i|C_1) = \sum_{i=k+1}^{L} (i - \mu_1)^2 p_i/\omega_1$$

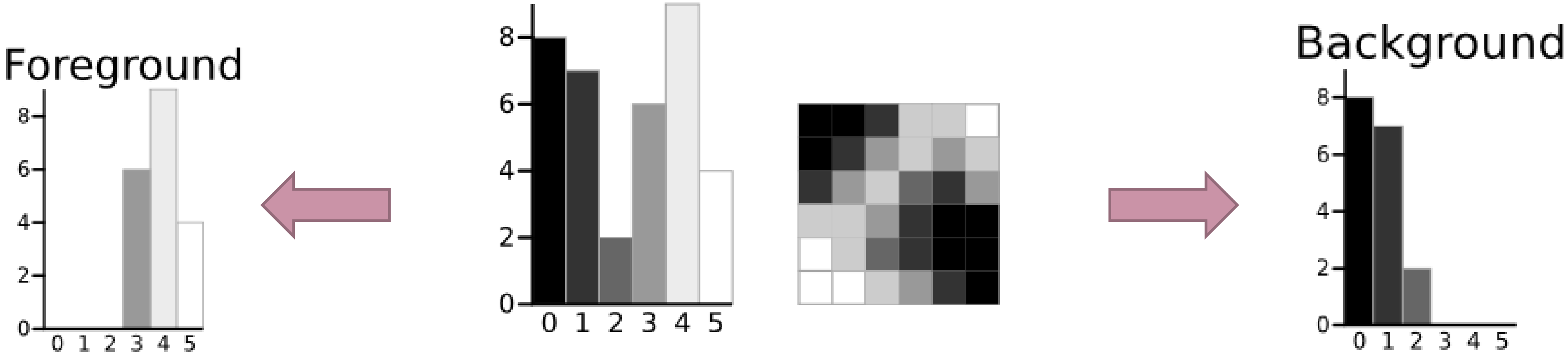
$$\sigma_T^2 = \sum_{i=1}^{L} (i - \mu_T)^2 p_i$$

L = Number of gray levels in the picture
n_i = Number of pixels at level i
N = Total number of pixels
p_i = Probability of level i
k = Threshold intensity

$p_i = n_i/N$

Otsu, N. "A threshold selection method from gray-level histograms." IEEE Transactions on Systems, Man, and Cybernetics 9:1 (1979), pp 62-66.

Otsu Thresholding



$$\text{Weight} \quad W_f = \frac{6+9+4}{36} = 0.5278$$

$$\text{Mean} \quad \mu_f = \frac{(3\times 6)+(4\times 9)+(5\times 4)}{19} = 3.8947$$

$$\begin{aligned}\text{Variance} \quad \sigma_f^2 &= \frac{((3-3.8947)^2\times 6)+((4-3.8947)^2\times 9)+((5-3.8947)^2\times 4)}{19} \\ &= \frac{(4.8033\times 6)+(0.0997\times 9)+(4.8864\times 4)}{19} \\ &= 0.5152\end{aligned}$$

$$\text{Weight} \quad W_b = \frac{8+7+2}{36} = 0.4722$$

$$\text{Mean} \quad \mu_b = \frac{(0\times 8)+(1\times 7)+(2\times 2)}{17} = 0.6471$$

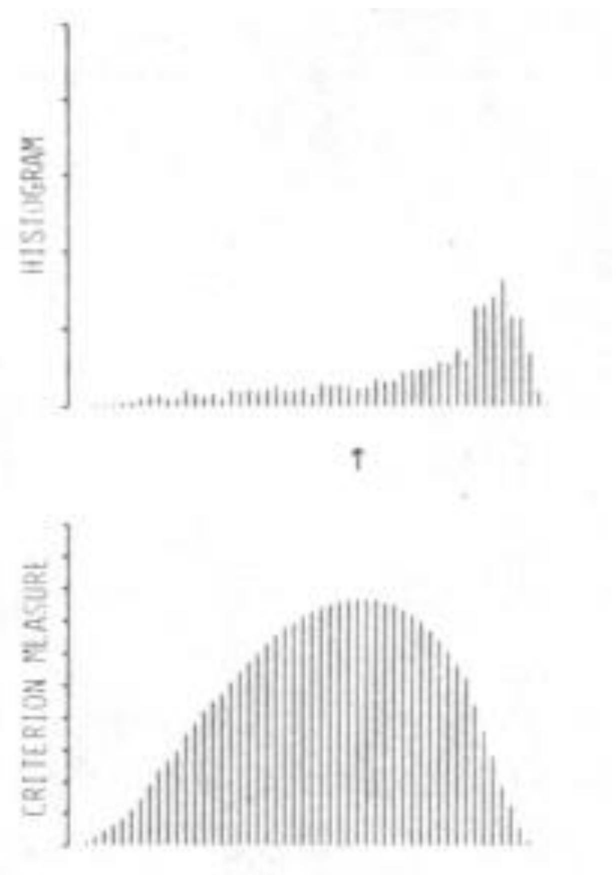
$$\begin{aligned}\text{Variance} \quad \sigma_b^2 &= \frac{((0-0.6471)^2\times 8)+((1-0.6471)^2\times 7)+((2-0.6471)^2\times 2)}{17} \\ &= \frac{(0.4187\times 8)+(0.1246\times 7)+(1.8304\times 2)}{17} \\ &= 0.4637\end{aligned}$$

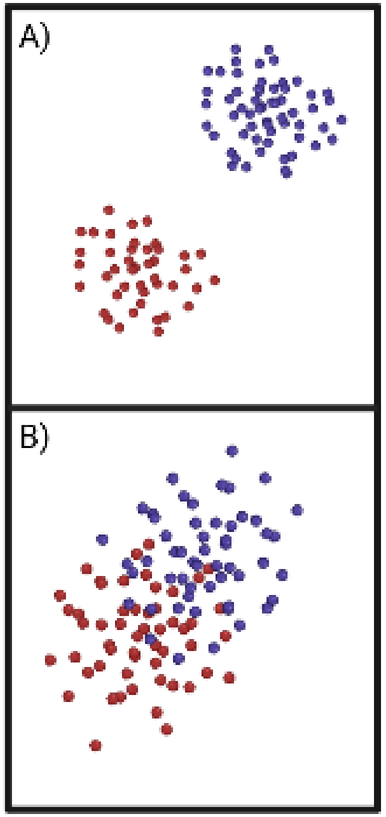
Discriminant Criterion Measure

<u>Criterion measure</u>

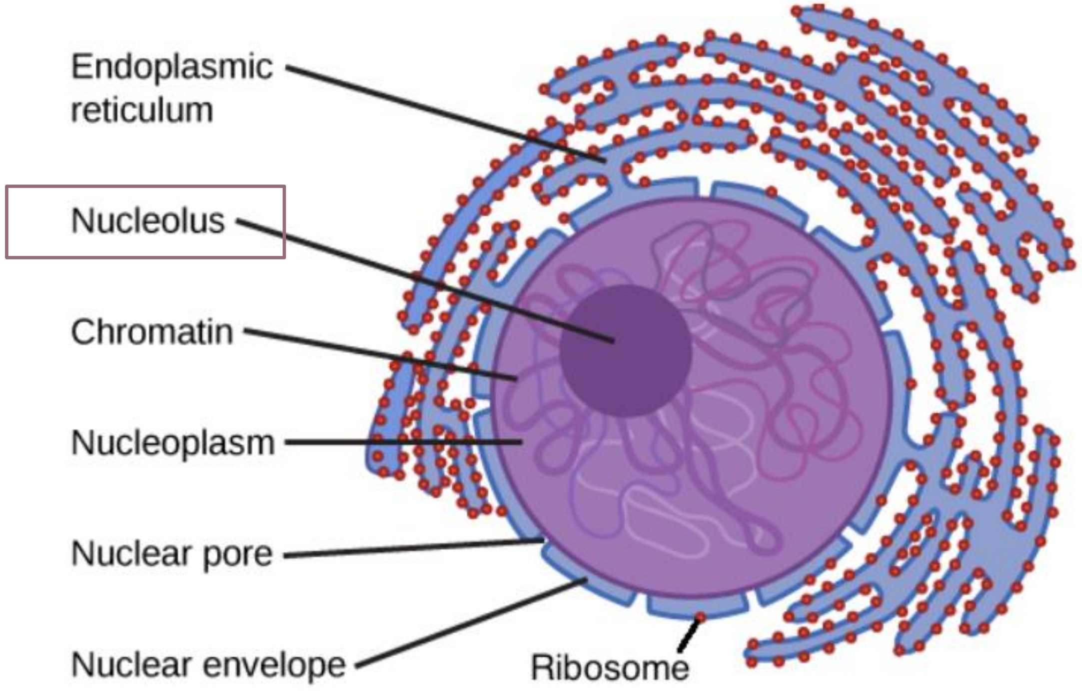
$$\eta(T) = \sigma_B^2(T)/\ \sigma_T^2$$

σ_B^2= Between-class variance
σ_T^2 = Total variance
$0 \leq \eta^* \leq 1$





Holes



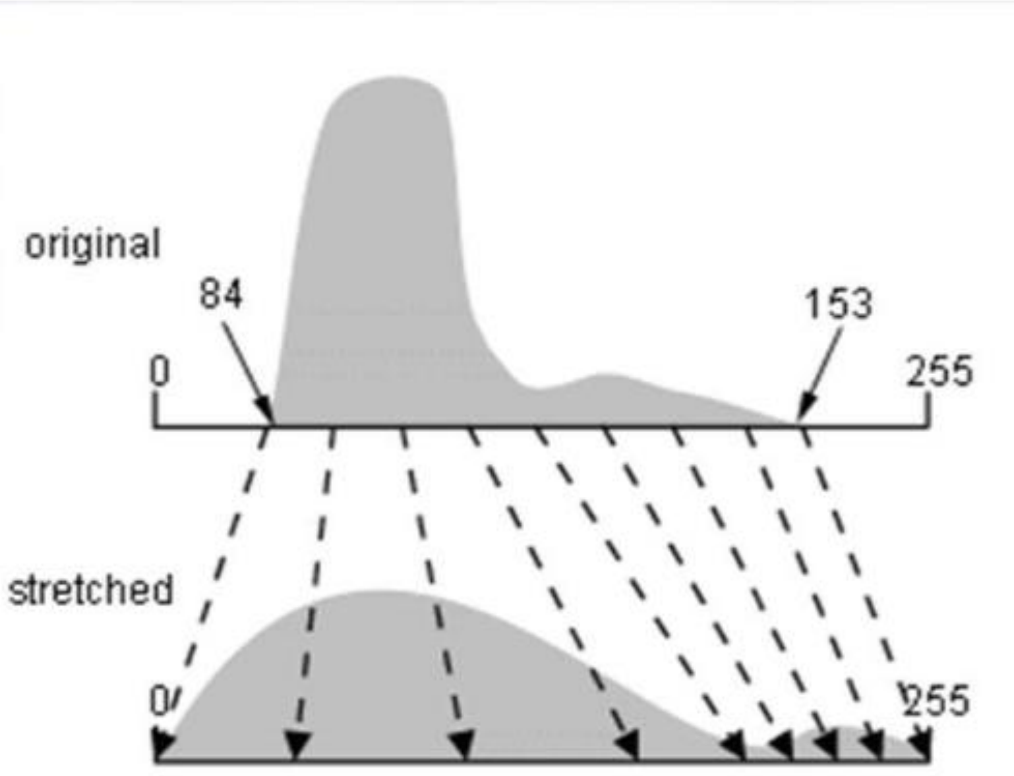
Bildquelle: OpenStax Biology.

Histogram stretching

- stretching range of intensity values to achieve a higher contrast -> linear scaling

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

Example Histogram stretching



Two-level Otsu Thresholding

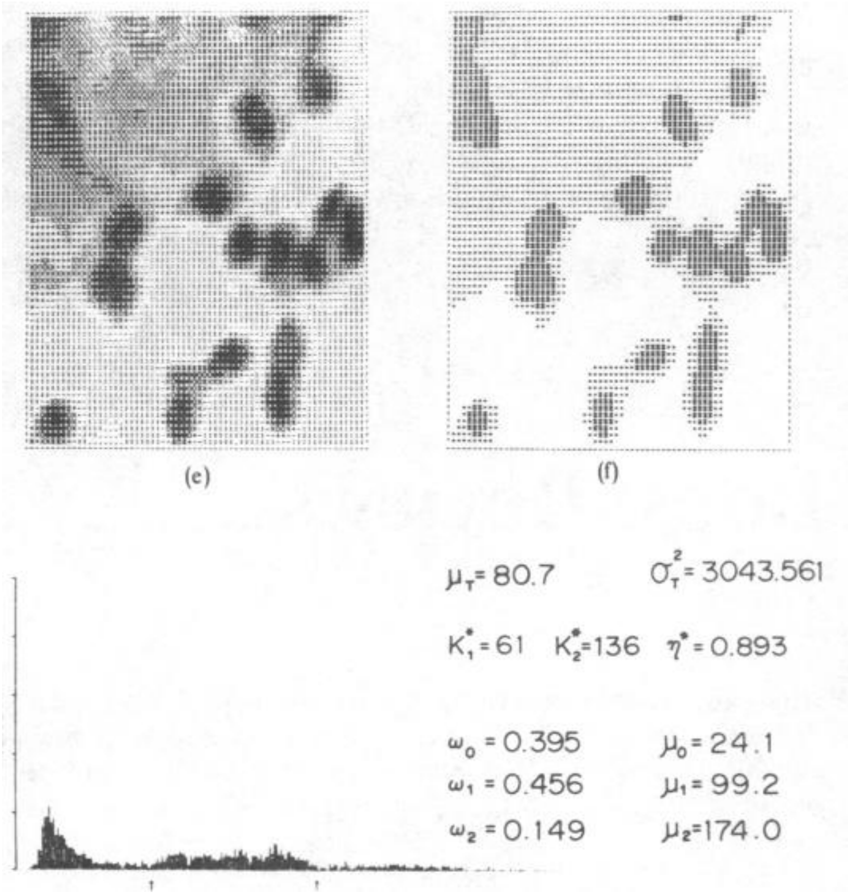
Two-level Otsu Thresholding

$$\sigma_B^2 = \omega_1(\mu_1 - \mu_T)^2 - \omega_2(\mu_2 - \mu_T)^2 - \omega_3(\mu_3 - \mu_T)^2$$

$\omega_{1,2,3}$ = Probabilities of class occurrence

$\mu_{1,2,3}$ = Class mean levels

μ_T = Total mean level



Otsu, N. "A threshold selection method from gray-level histograms." IEEE Transactions on Systems, Man, and Cybernetics 9:1 (1979), pp 62-66.

Application of Filter

- Choose Filtermask
- Convolution
  - Flipping filter
  - Multiplication of filter values with image intensity values
  - Summation of the multiplication results