Deep Learning in Life Sciences

Intro to BioImage Analysis and Deep Learning Utilization

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About me

- Charles University
  - BioImage Analyst, under [Core Facility]
  - GloBIAS, CzechBIAS
- National Library of Technology
  - Data Stewardship & PIDs specialist
- University of Chemistry and Technology
  - Assistant Professor: Advanced signal and image processing
  - Institutional Data Steward

more at: www.schaetz.cz or
https://www.schaetz.cz/bia-overview/intro.html

Overview

(Super Quick) Intro to BioImage Analysis

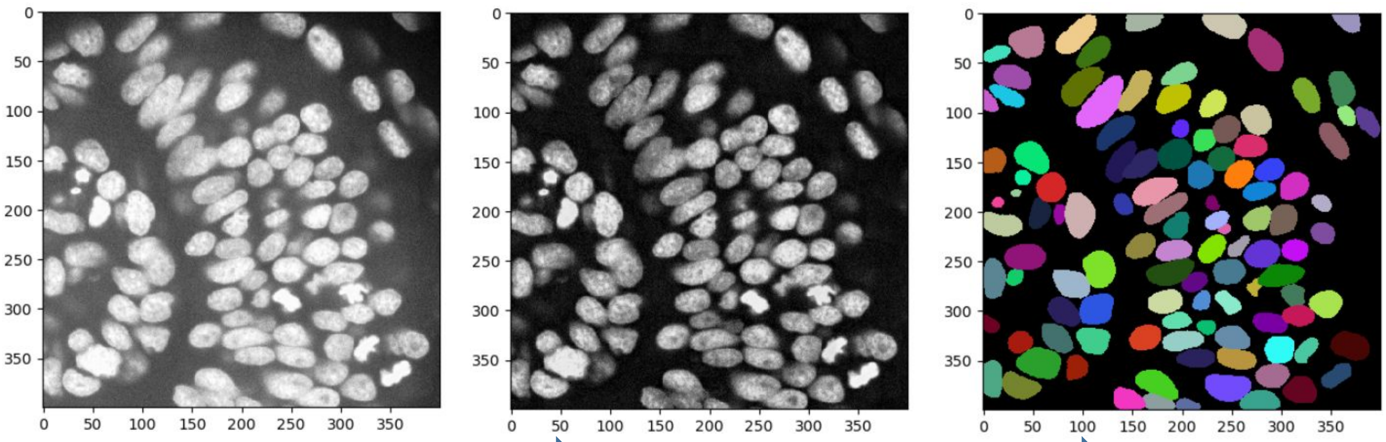
AI4LIFE

Noise2Void

StarDist

Bio-image Analysis

- Image Data Analysis workflows
- Goal: **Quantify observations, substantiate conclusions with numbers**



	area	mean_intensity	major_axis_length	minor_axis_length
0	594.0	40572.809764	28.611591	26.537947
1	645.0	43764.872868	33.511511	24.566916
2	1105.0	51970.561991	45.232031	31.456308
3	718.0	47015.487465	31.023274	29.520883
4	791.0	49132.515803	36.382253	27.718301
...	...	...	...	...
105	238.0	30477.126050	20.252197	15.276536
106	615.0	32886.154472	41.030760	19.874280
107	110.0	33042.445455	14.366347	9.945911
108	222.0	43304.180180	25.370549	11.637599
109	167.0	43378.808383	17.895110	13.015369

Image filtering → Image segmentation → Feature extraction

Haase, R. (2023). Introduction to bio-image analysis I. F1000 Research Limited.

Quantitative bio-image analysis

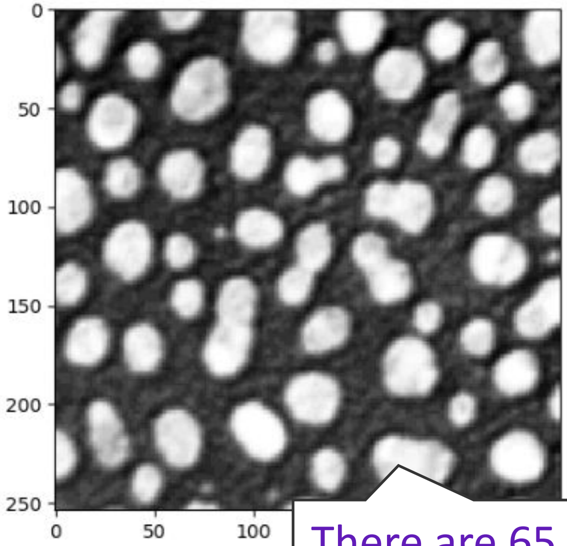
Deriving <u>quantitative information</u> from images of biological samples taken with microscopes

cat height = <u>1.5</u> x microscope height

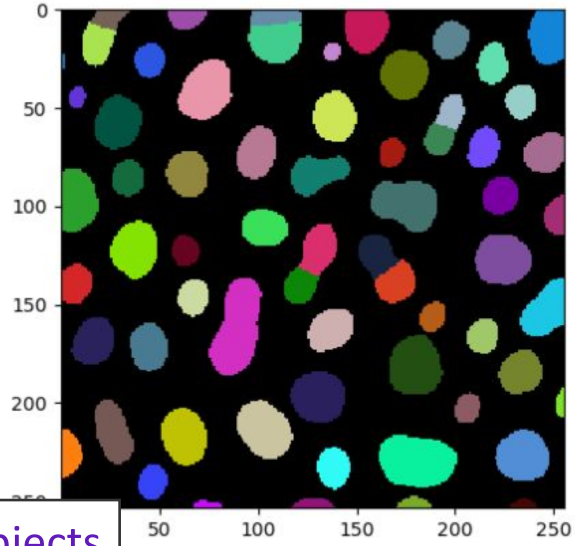
Reliable bio-image analysis

- Algorithms must be reliable (trustworthy).
- Visualization helps gaining trust in automated methods.

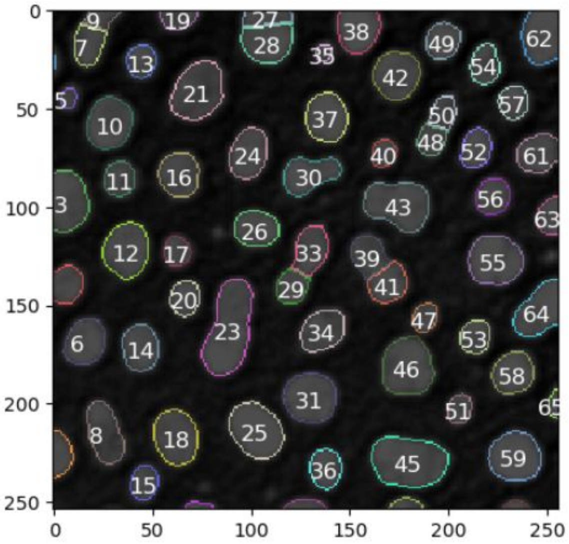
Original image



Label image



Overlay



There are 65 objects in this image.

Source: M. Zoccoler & R. Haase licensed CC-BY
https://haesleinhuepf.github.io/BioImageAnalysisNotebooks/60_data_visualization/overlay_text_on_image.html

Images and pixels

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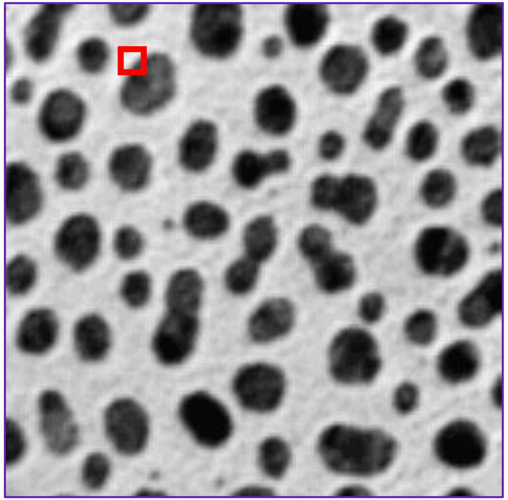
- An image is just a matrix of numbers
- Pixel: “picture element”
- The edges between pixels are an artefact of the imaging / digitization. They are not real!



48	48	48	40	40	32	32	24	24	24	24	24	24	24
48	48	40	32	32	24	24	16	16	16	24	24	24	24
48	48	40	32	24	24	16	16	16	16	24	24	32	40
40	40	32	24	24	16	16	8	16	16	24	24	40	48
32	32	32	24	24	16	24	24	32	48	56	64	72	88
24	24	24	16	16	16	24	32	56	72	88	96	112	120
24	16	16	16	24	32	48	64	96	120	128	144	152	152
16	8	16	16	32	40	72	96	128	160	176	184	184	184
16	8	16	24	48	72	104	136	160	176	184	192	192	184
16	8	24	32	72	104	136	168	184	192	200	200	192	184
24	24	48	64	104	136	160	184	184	192	192	192	184	184
32	40	64	88	128	168	184	192	192	184	184	176	176	176
40	56	88	120	152	192	192	192	192	184	184	176	176	176
48	64	104	144	176	208	200	184	184	184	184	176	176	168

Pixel size versus resolution

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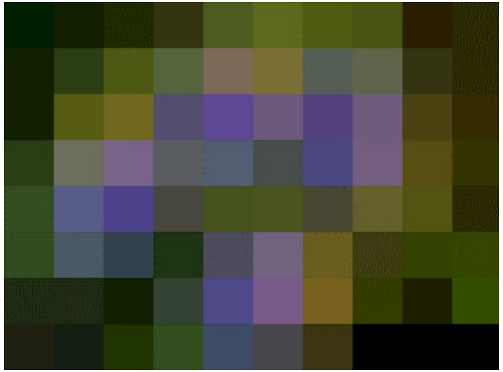
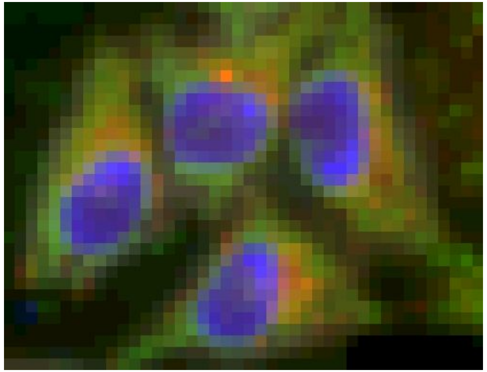
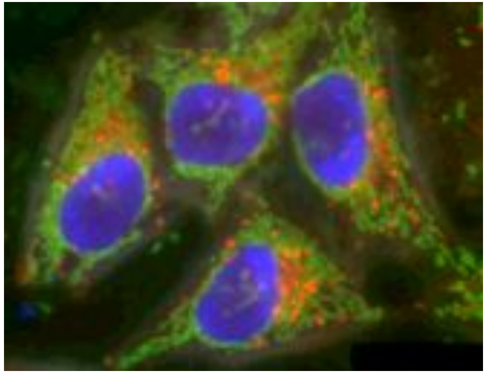
- Pixel size is a digital property of an image.
- You configure it during the imaging session at the microscope.



Pixel size: 3.3 µm



Pixel size: 0.8 µm



Pixel size: 0.05 µm

- We are not talking about resolution!

Pixel size versus resolution

- Resolution is a property of your imaging system.
- The measure of how close object can be in an image while still being differentiable, is called spatial resolution.

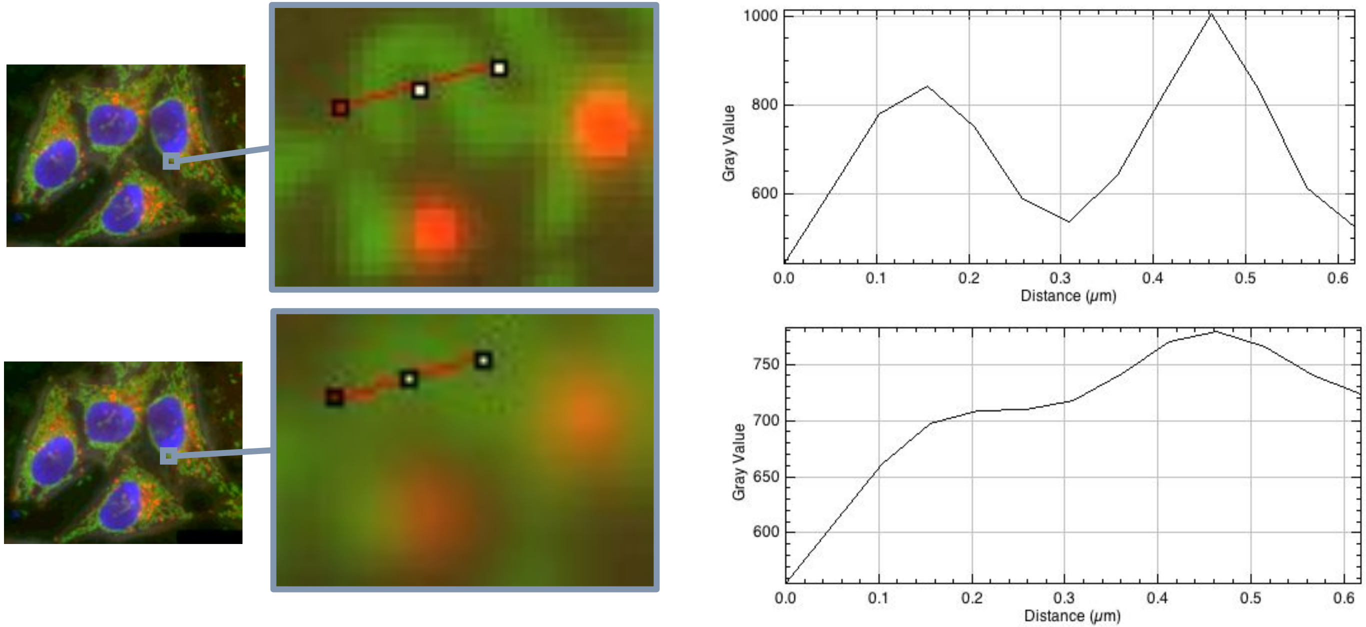
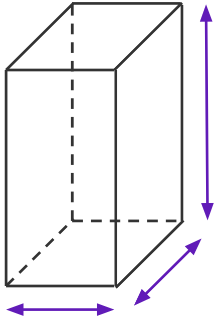
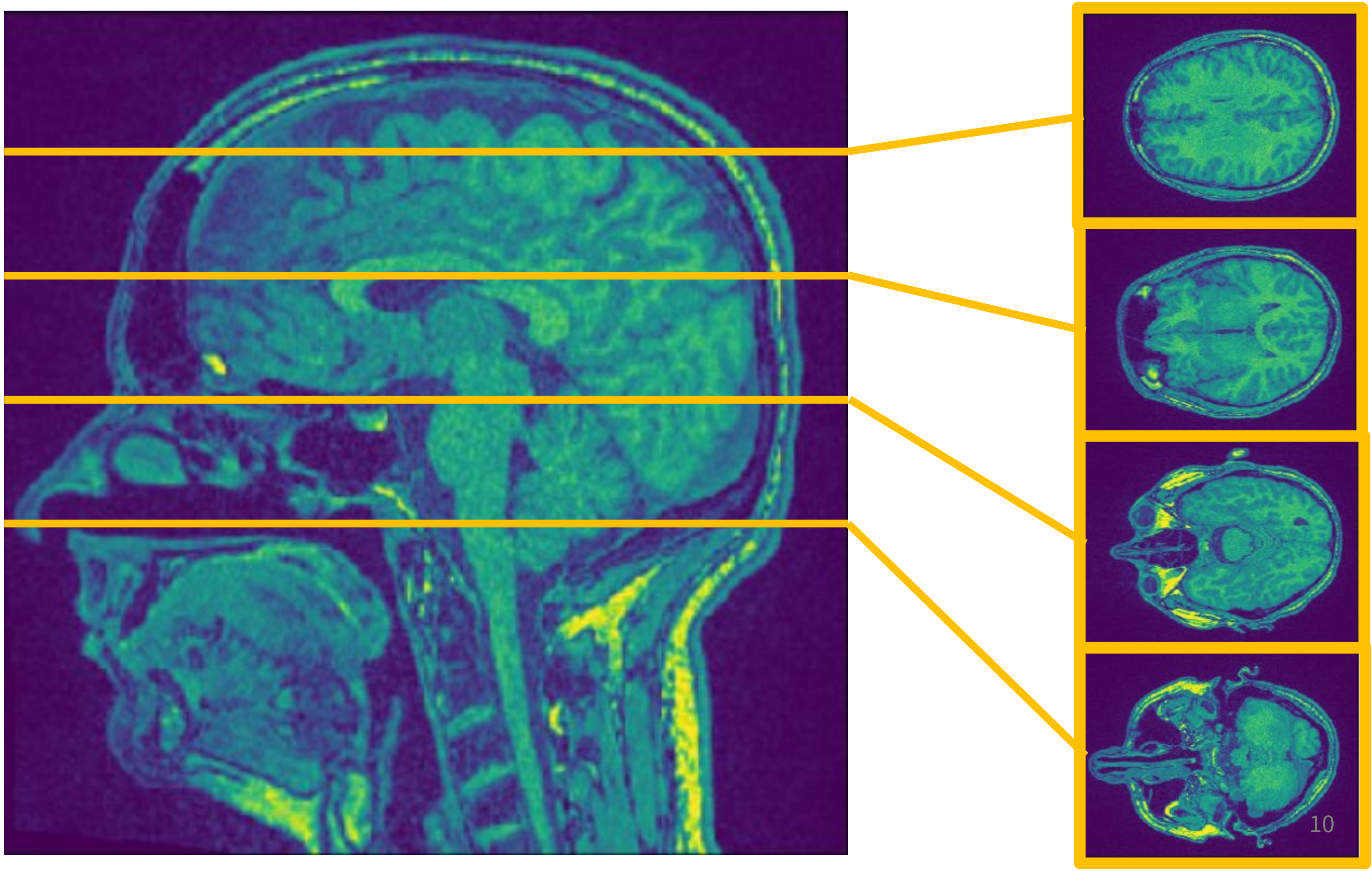


Image stacks and voxels

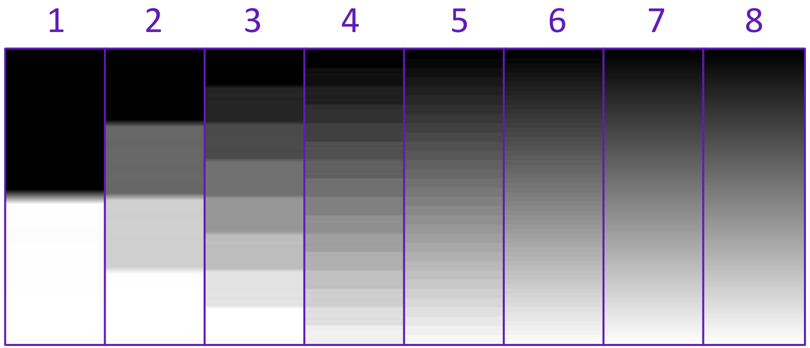
- 3-dimensional images consisting of voxels
- “Image stack”
- Often anisotropic (not equally large in all directions)

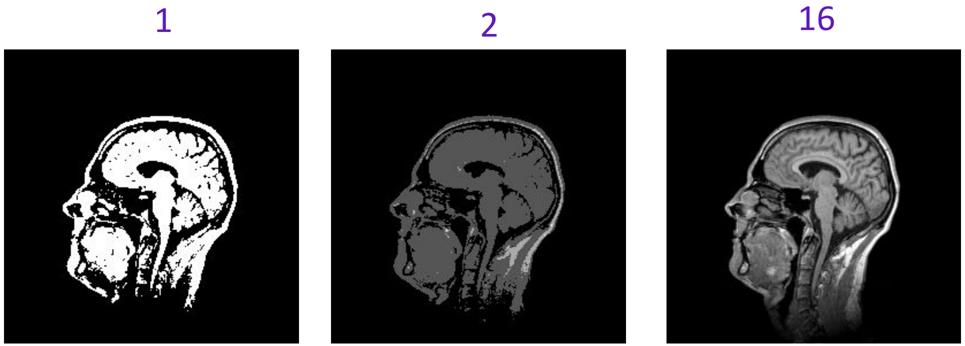




Bit-depth

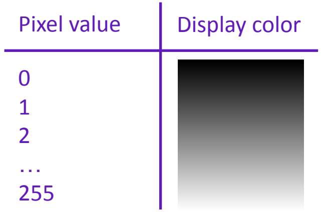
- A bits is the smallest memory unit in computers, *atomic data*.
- The bit-depth *n* enumerates how many different intensity values are present in an image:
  - 2^n grey values
- In microscopy, images are usually stored as 8, 12 or 16-bit images.

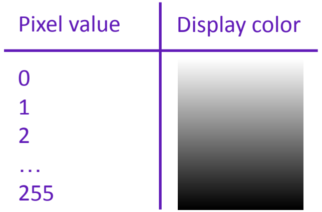


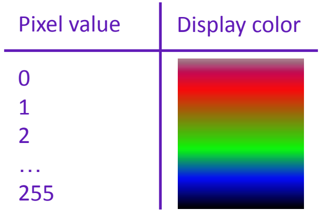


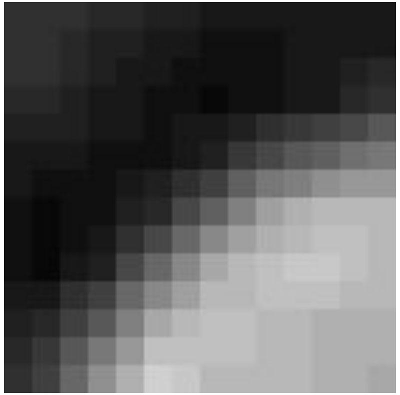
Colormaps / lookup tables

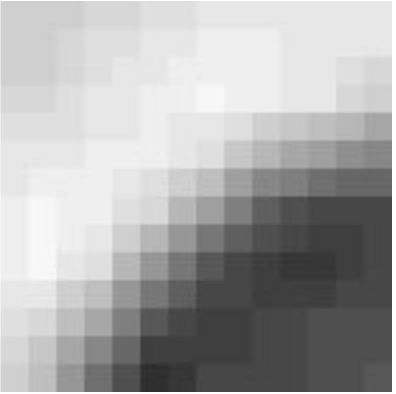
- The lookup table decides how the image is displayed on screen.
- Applying a different lookup table does not change the image. All pixel values stay the same, they just appear differently

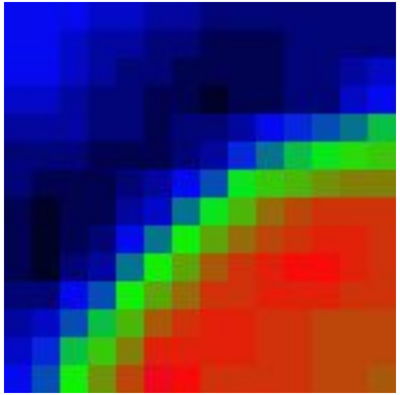






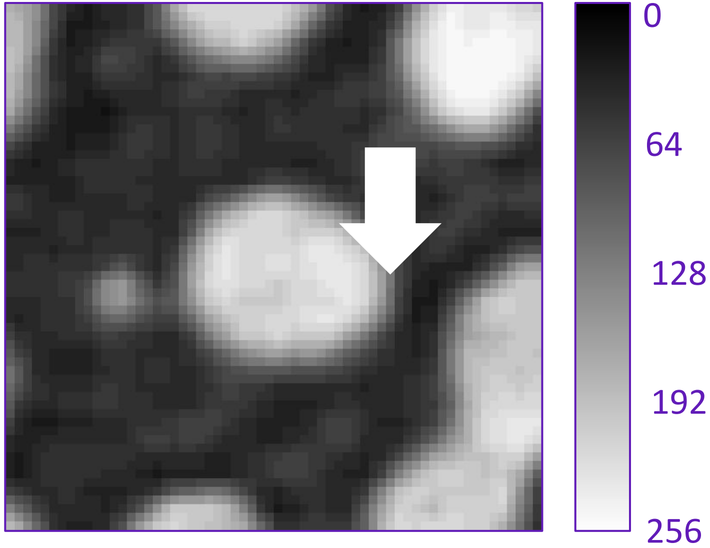


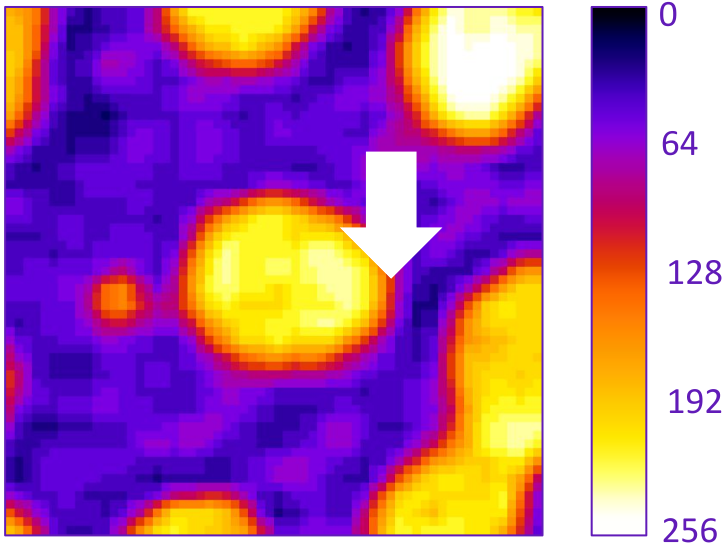




Colormaps / lookup tables

- Which intensity does the marked pixel have?





0	64	128	192

Colormaps / lookup tables

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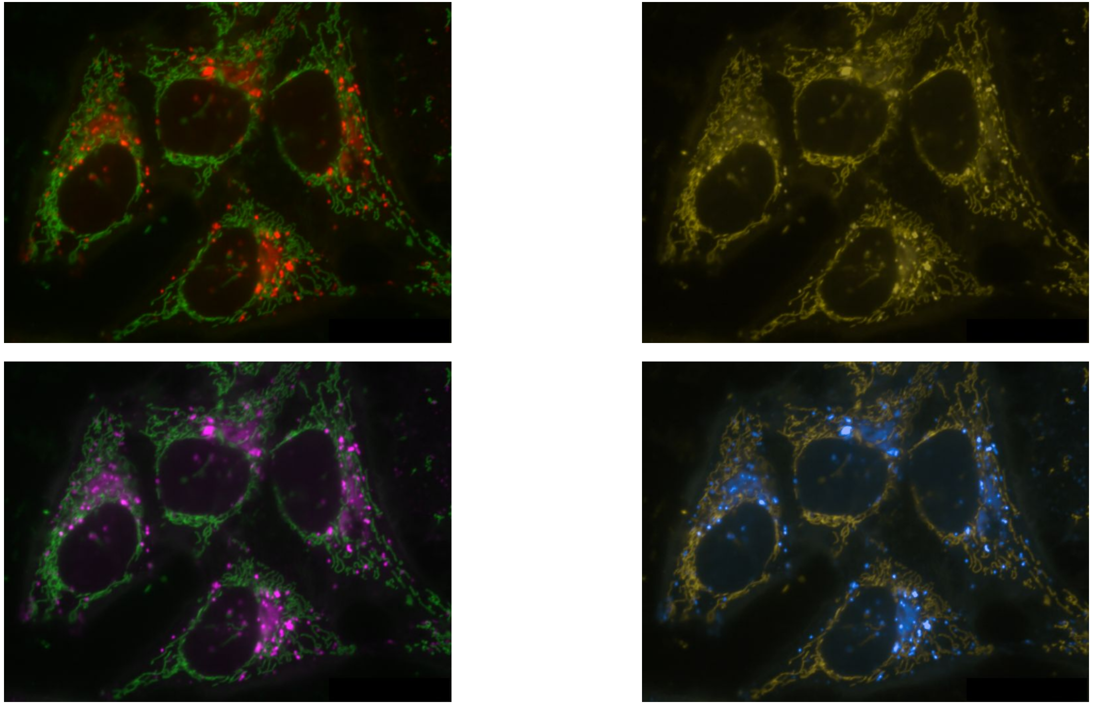
- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!

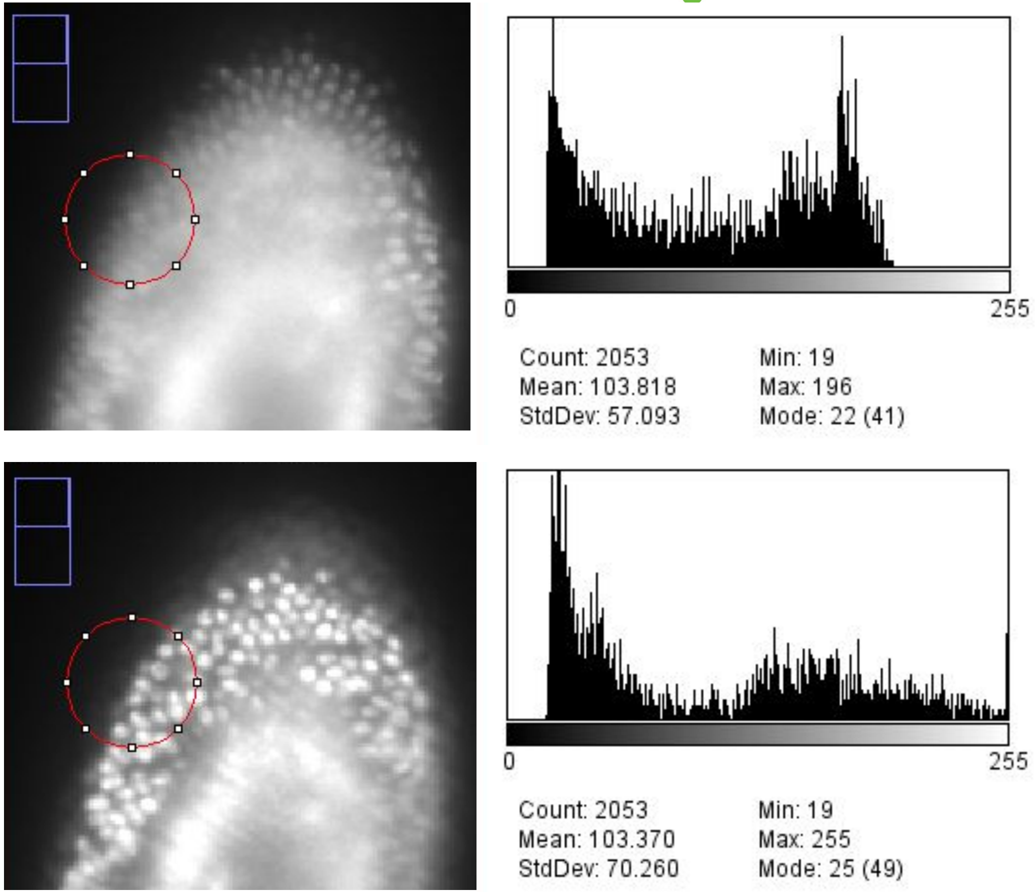
Common view | Red/green blind people may see it



Replace red with magenta!

Histograms

- Histograms are summaries of images
- Tell stories, e.g. about image quality



Filters

- An image processing filter is an operation on an image.
- It takes an image and produces a new image out of it.
- Filters change pixel values.

- There is no "best" filter. Which filter fits your needs, depends on the context.
- Filters do not do magic. They can not make things visible which are not in the image.

- Application examples
    - Noise-reduction
    - Artefact-removal
    - Contrast enhancement
    - Correct uneven illumination

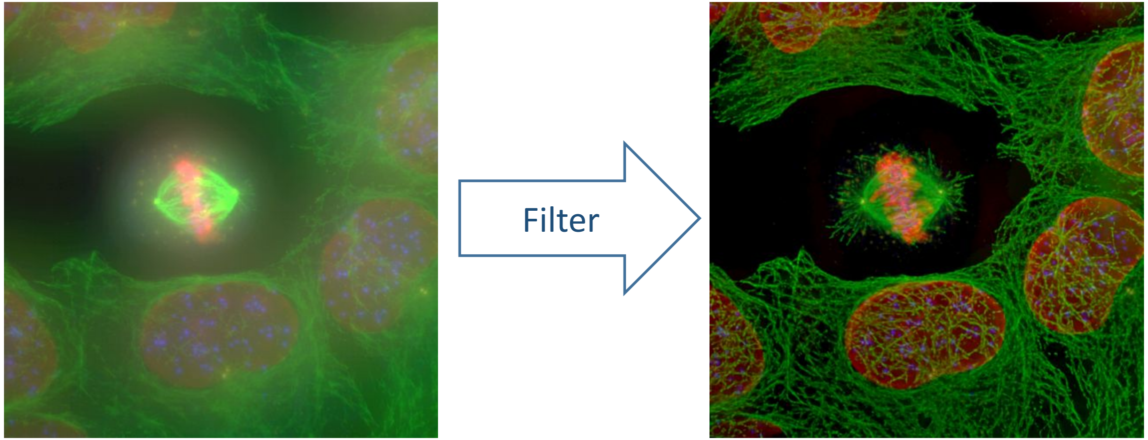
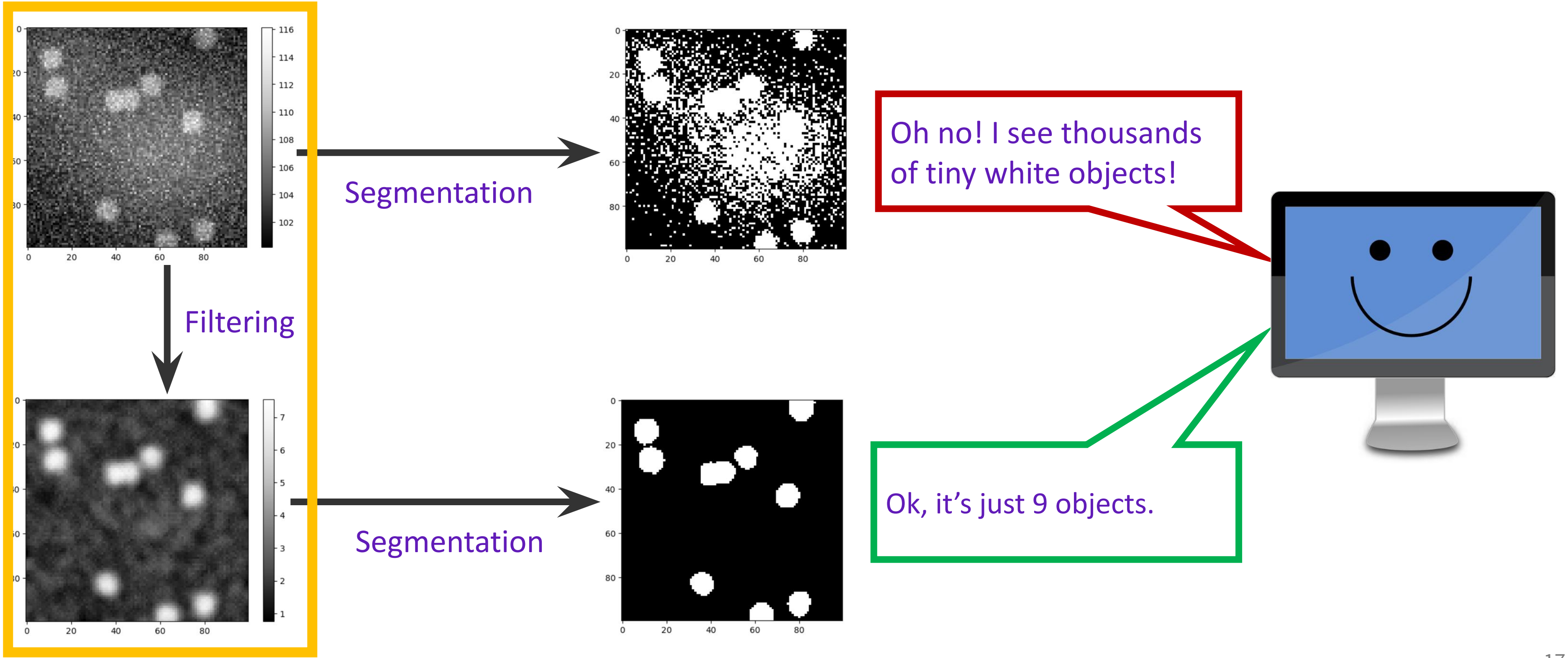


Image source: Alex Bird / Dan White MPI CBG

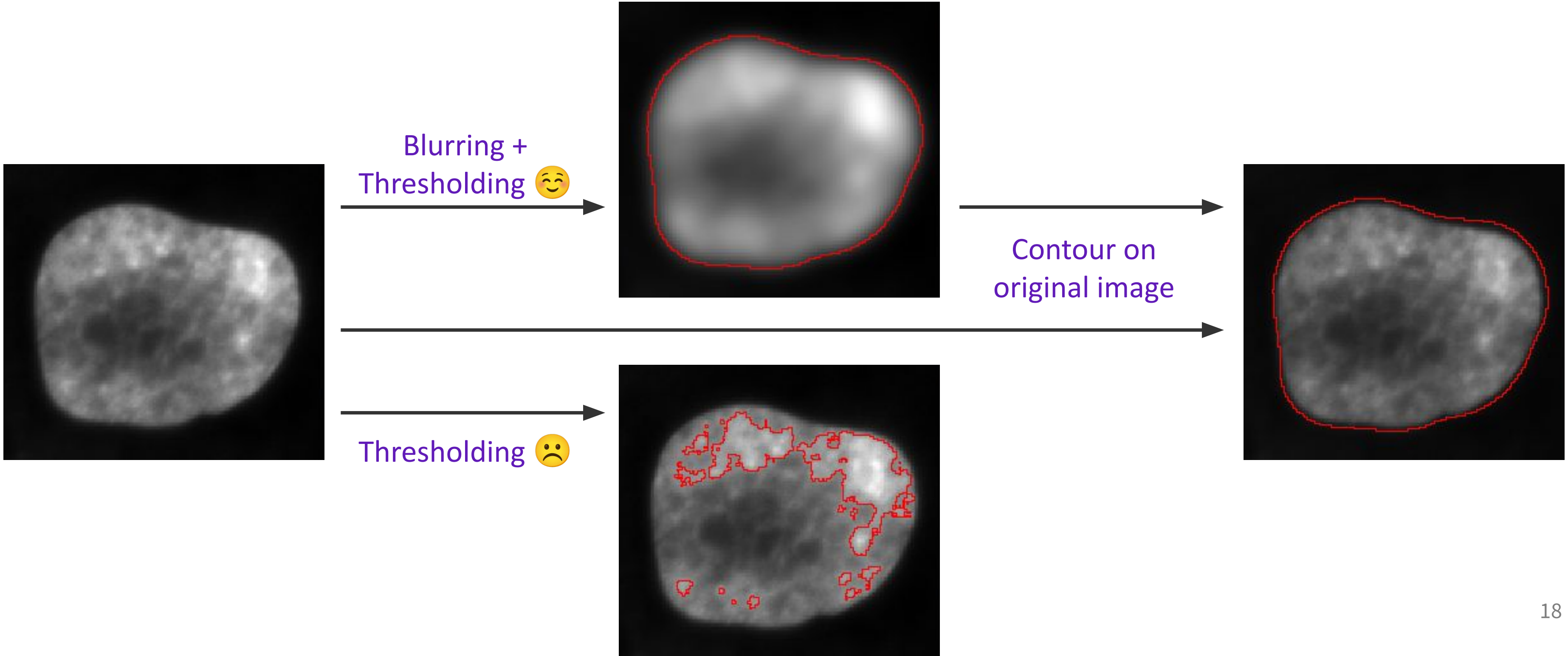
Image filtering

- We need to remove the noise to help the computer *interpreting* the image



Filtering for improving thresholding results

- In case thresholding algorithms outline the wrong structure, blurring in advance may help.
- However: **Do not** continue processing the blurred image, continue with the original!



Segmentation / binarization

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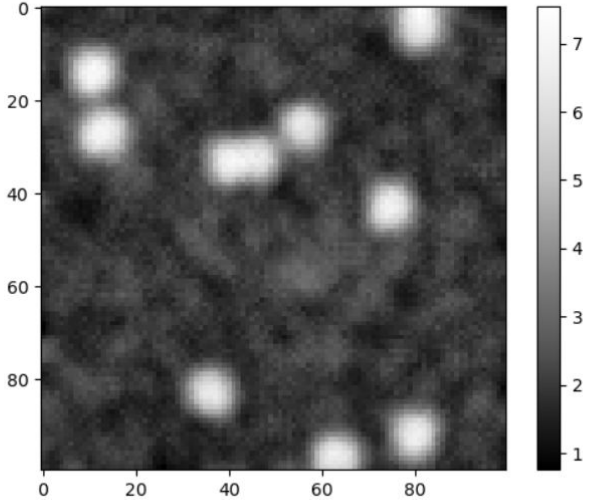
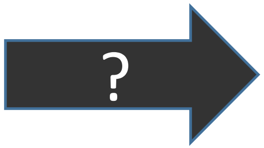
Thresholding

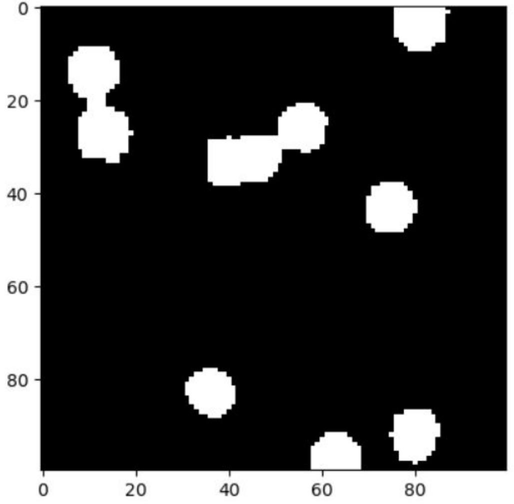
- Very basic and yet efficient segmentation technique
- Histogram based, to determine an intensity threshold
- Not state-of-the-art in many fields (anymore)

Intensity image



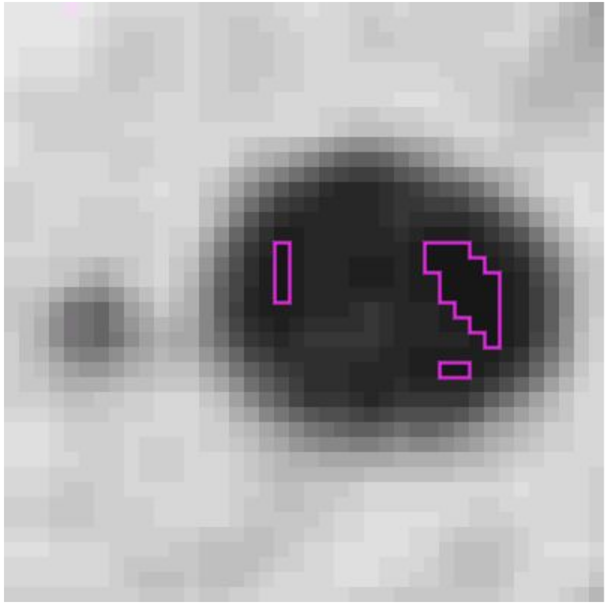


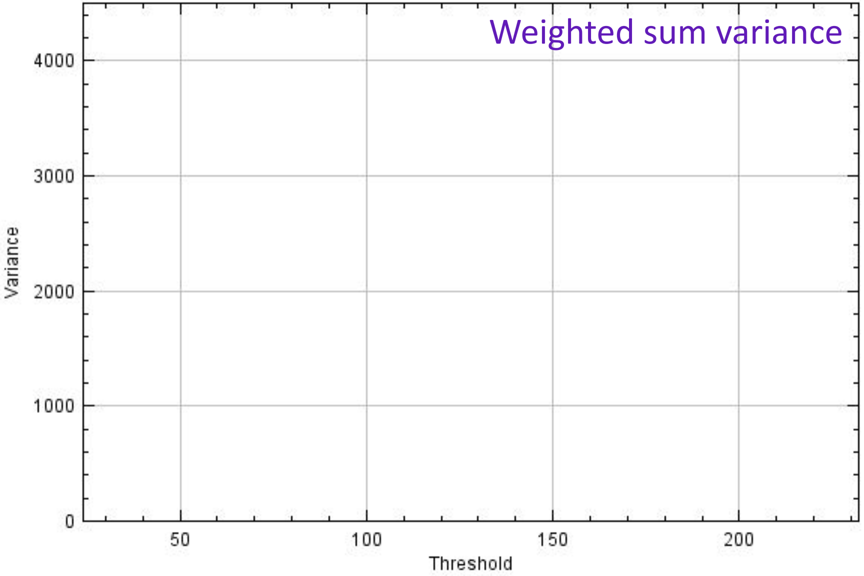
Binary image



Thresholding: Otsu's method

- Searching for a threshold where the variance in both classes (above/below threshold) becomes minimal.
- Weighted (!) sum variance





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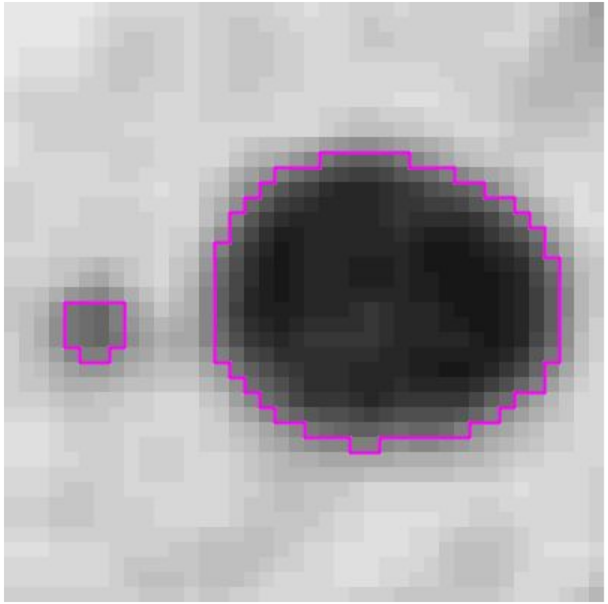
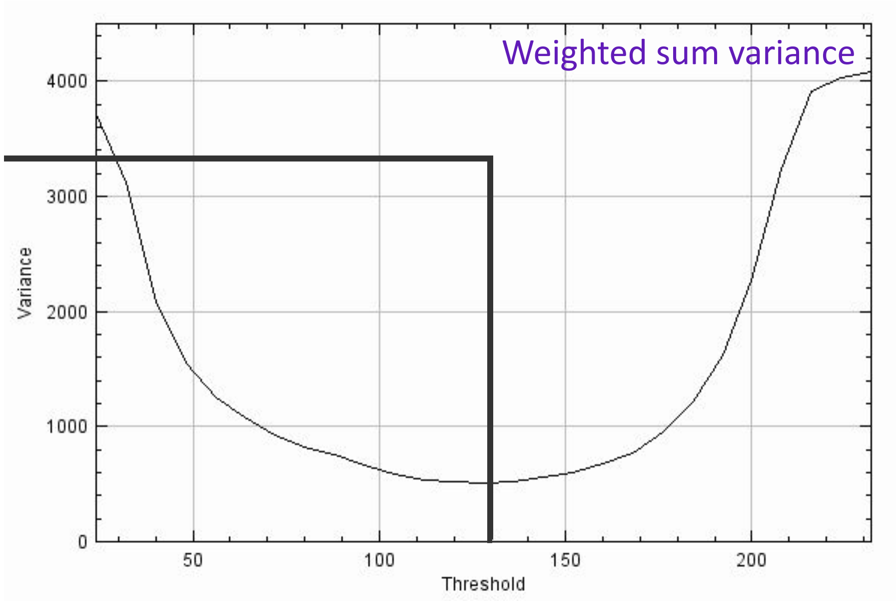
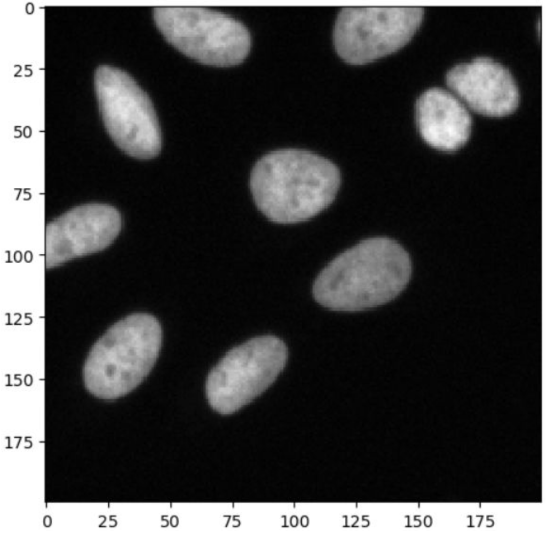




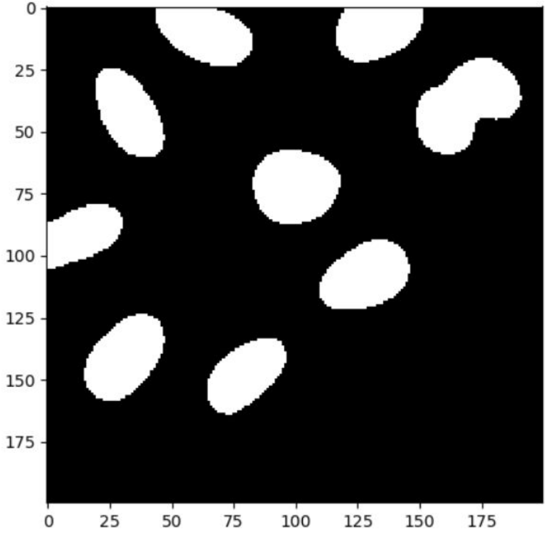
See also: http://www.labbookpages.co.uk/software/imgProc/otsuThreshold.html

Terminology

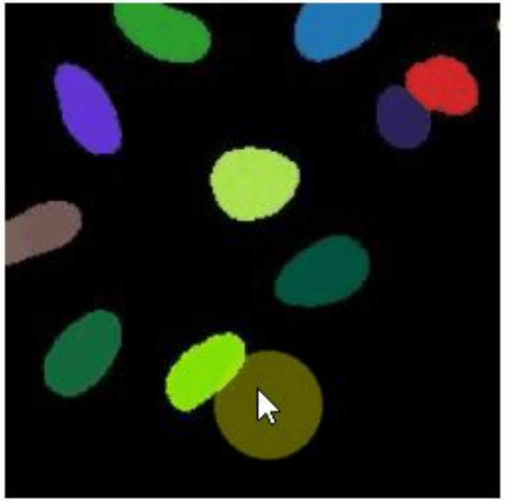
Intensity image



Binary image

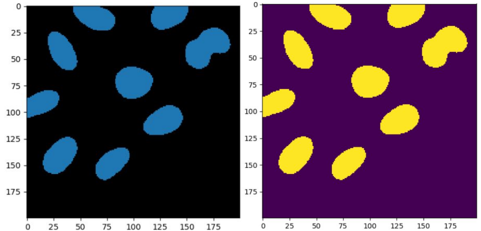


Label image



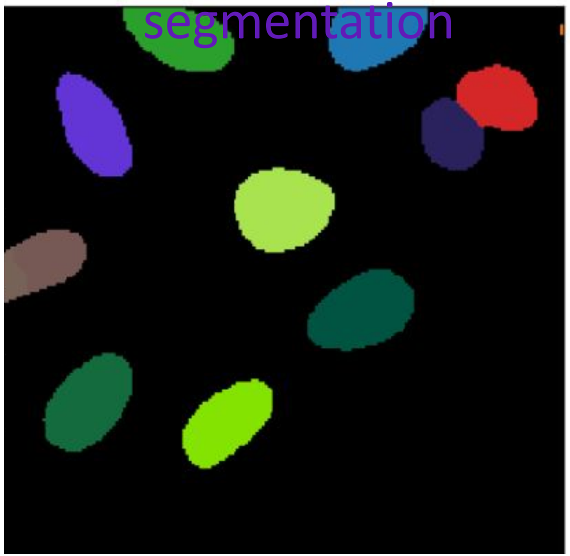
[y=152, x=92] = 0

No matter how they are displayed



Terminology

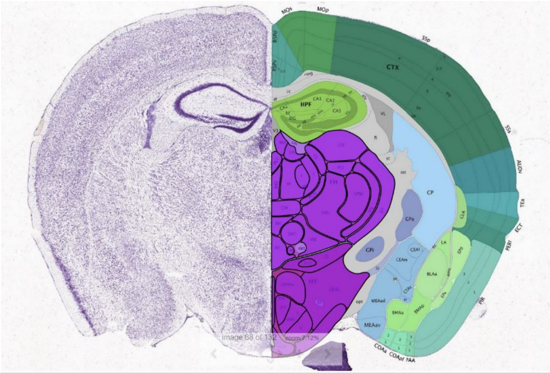
Instance segmentation

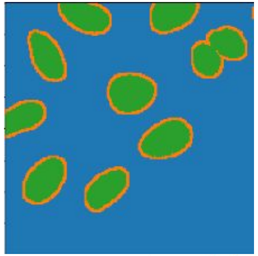


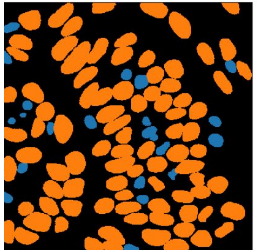
Instances:

- Cells, nuclei, cats, dogs, cars, trees

Semantic segmentation







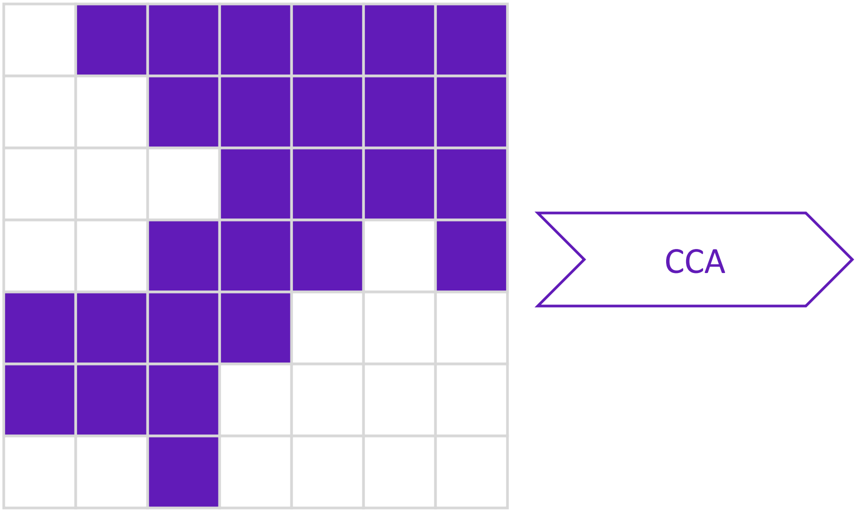
Regions:

- Anatomical, geographical
- All pixels belonging to the same type of object have the same value

Source: Allen Mouse Brain Atlas [dataset]. Available from mouse.brain-map.org. Allen Institute for Brain Science (2011).

Connected component labelling

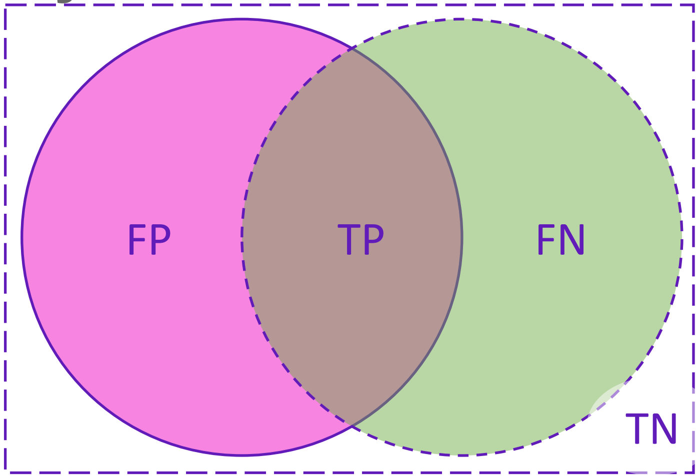
- In order to allow the computer differentiating objects, connected component analysis (CCA) is used to mark pixels belonging to different objects with different numbers
- Background pixels are marked with 0.
- The maximum intensity of a labelled map corresponds to the number of objects.



1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

Segmentation quality estimation

- In general
  - Define what's positive and what's negative.
  - Compare with a reference to figure out what was true and false
  - Welcome to the Theory of Sets



A — Prediction A

B — Reference B (ground truth)

ROI — Region of interest

TP — True-positive

FN — False-negative

FP — False-positive

TN — True-negative

Overlap (a.k.a. Jaccard index) $$\frac{TP}{TP + FN + FP}$$ How much do A and B overlap?

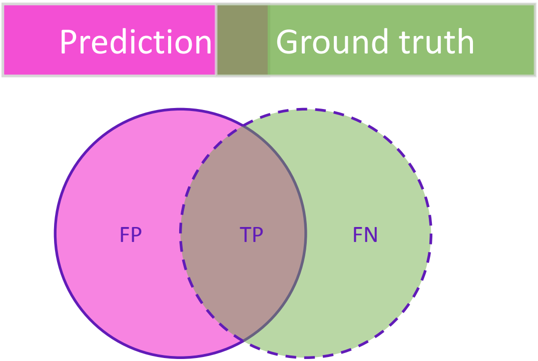
Precision $$\frac{TP}{TP + FP}$$ What fraction of points that were predicted as positives were really positive?

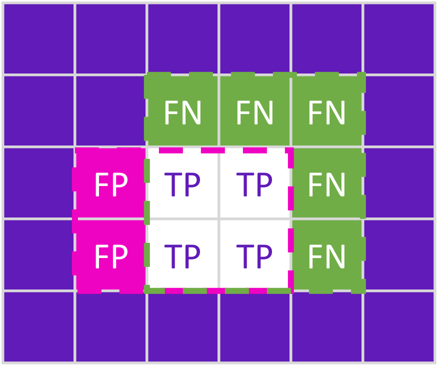
Recall (a.k.a. sensitivity) $$\frac{TP}{TP + FN}$$ What fraction of positives points were predicted as positives?

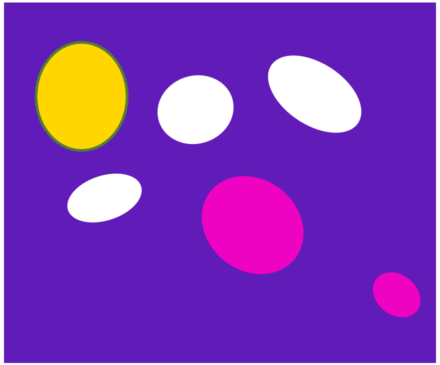
Pixel-wise versus Object-wise evaluation

- Object wise: Detection quality

- Pixel wise: Segmentation quality







Precisio
n

$$\frac{TP}{TP+FP}$$

Recall
(a.k.a.
sensitivity)

$$\frac{TP}{TP+FN}$$

True-positive: 4

False-negative: 5

False-positive: 2

Precision: 4/6 = 66%
Recall: 4/9 = 44%

True-positive: 3

False-negative: 1

False-positive: 2

Precision: 3/4 = 75%
Recall: 3/5 = 60%

- Voxel-wise Youden-Index

$$YI = p_{TP} + p_{TN} - 1$$

- Volume error

$$\Delta_V = V_A - V_B$$

$$\delta_V = \frac{\Delta_V}{V_B}$$

- Dice Index

$$DI(A,B) = \frac{2\,|A \cap B|}{|A| + |B|}$$

- Jaccard Index

$$JI(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{DI}{2 - DI}$$

- Contour distance

$$d_{e,min}(a,B) = \min(d_e(a,b) | b \in B)$$

$$\bar{d}_c(A,B) = \frac{\sum_{\forall a \in C(A)} d_{e,min}(a, C(B))}{|C(A)|}$$

$$\bar{d}_{bil,c}(A,B) = \frac{\bar{d}_c(A,B) + \bar{d}_c(B,A)}{2}$$

- Hausdorff distance

$$d_H(A,B) = \max(d_{e,min}(a,B) | a \in A)$$

$$d_{bil,H}(A,B) = \max(d_H(A,B), d_H(B,A))$$

- Simplified Hausdorff distance

$$d_H(A,B) = \max(d_{e,min}(a, C(B)) | a \in C(A))$$

- Volume standard deviation

$$\delta_{\bar{V}} = 2\frac{|V_A - V_B|}{|V_A + V_B|}$$

- Classification error

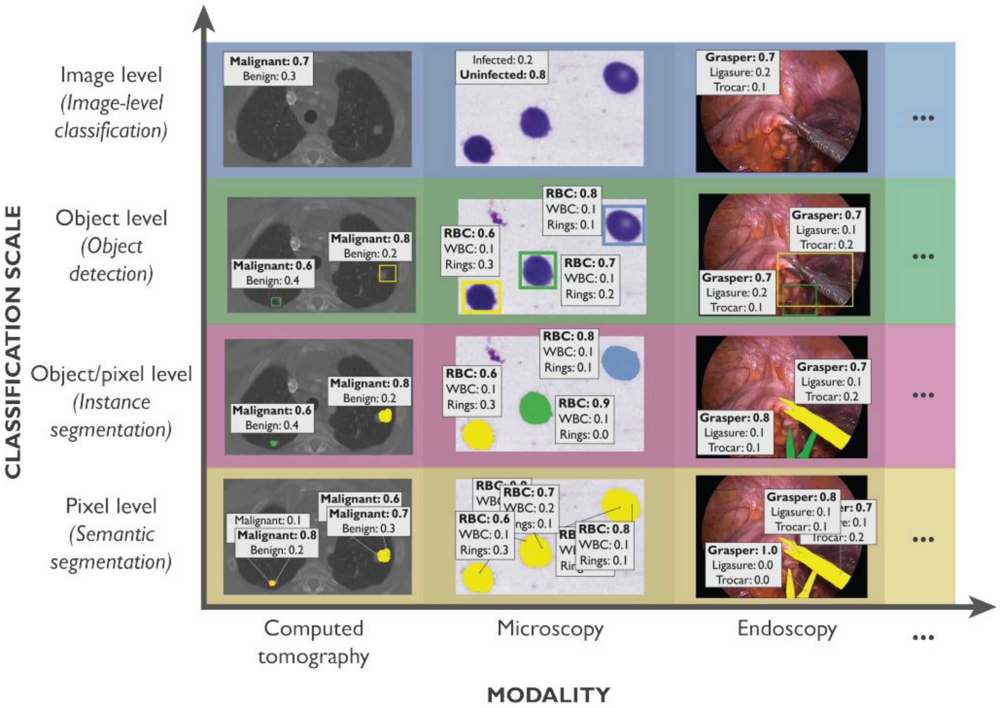
$$e_{Class} = \frac{H}{|TP| + |FN|}$$

- Hamming distance

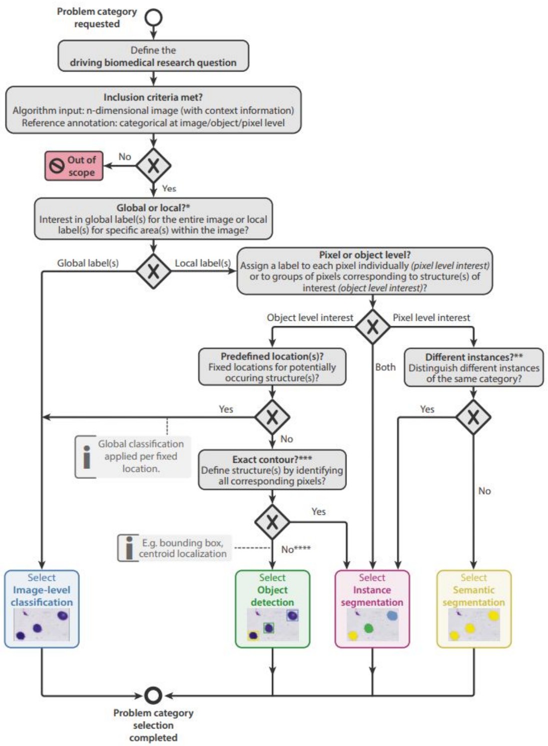
$$\begin{aligned} d_h &= |A \cup B| - |A \cap B| \\ &= |FP| + |FN| \end{aligned}$$

What metric to use when?

- "Metrics reloaded: Pitfalls and recommendations for image analysis validation"
  Maier-Hein, Reinke et al. https://arxiv.org/abs/2206.01653



+ S1

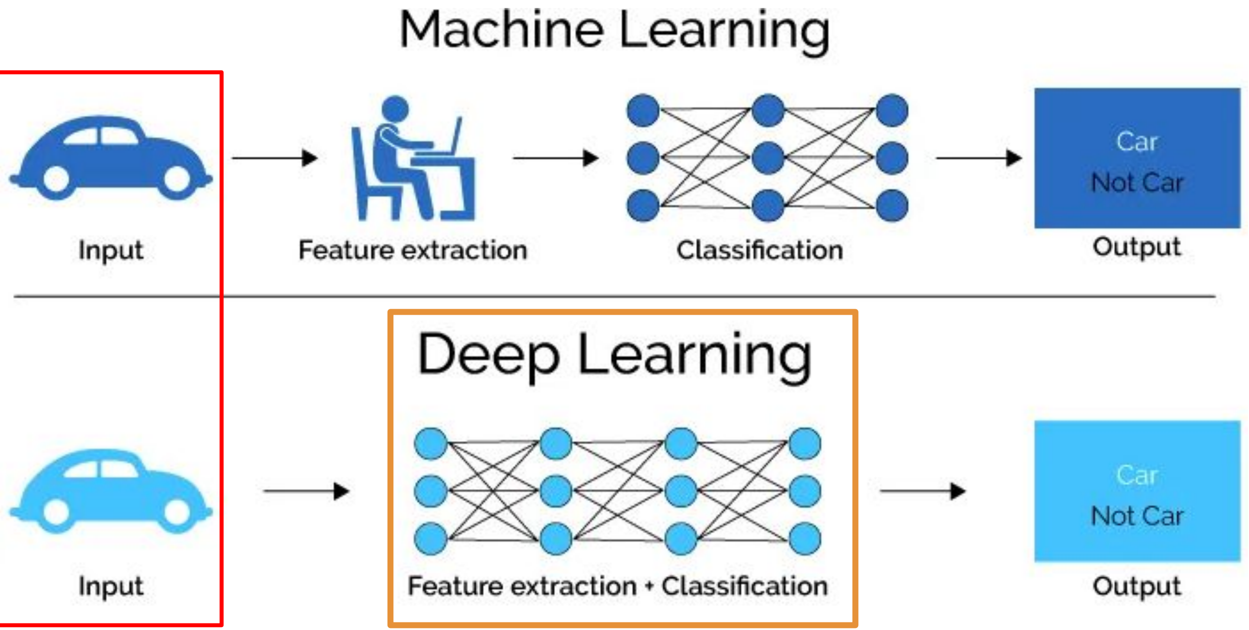


Deep Learning & BioImage Analysis

DeepLearning inLifeSciences

Training models from scratch is super expensive. We need hundreds to thousands of high quality annotated images/instances. So transfer learning, fine tuning or task specific models are used. Changing one parameter in your experiment might mean retraining part of your model!

High variability:
microcopy type
staining
acquisition sett.
batches
protocols
histogram var.
…



StarDist, N2V, Unet, Cellpose, …

Acknowledgements

PoL Physics of Life TU Dresden

BiAPoL team

- **Robert Haase**
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- Stefan Hahmann
- Somashekhar Kulkarni

Former lab members:

- Ryan George Savill
- Laura Zigutyte

Networks

BioDIP Biopolis Dresden Imaging Platform

CENTER FOR SYSTEMS BIOLOGY DRESDEN

NFDI4BIOIMAGE

neubias network of european bioimage analysts

image.sc

German BioImaging Gesellschaft für Mikroskopie und Bildanalyse

Funding

DFG public

Federal Ministry of Education and Research

Chan Zuckerberg Initiative

DeepLearning inLifeSciences

AI4LIFE & BioImage Model Zoo

AI4Life

BioImage.IO

Advanced AI models in one click

The BioImage Model Zoo and FAIR data principles are core facets of the AI4Life project.

OBJECTIVES

and our goals

1

Democratized availability of AI-based image analysis methods

2

Establish standards for the submission, storage and FAIR access

3

Simple model deployment, sharing, and dissemination through a new developer-facing service

4

Organize **Open Calls and Challenges** for image analysis problems

5

Empower **common image analysis** platforms with **AI integration**

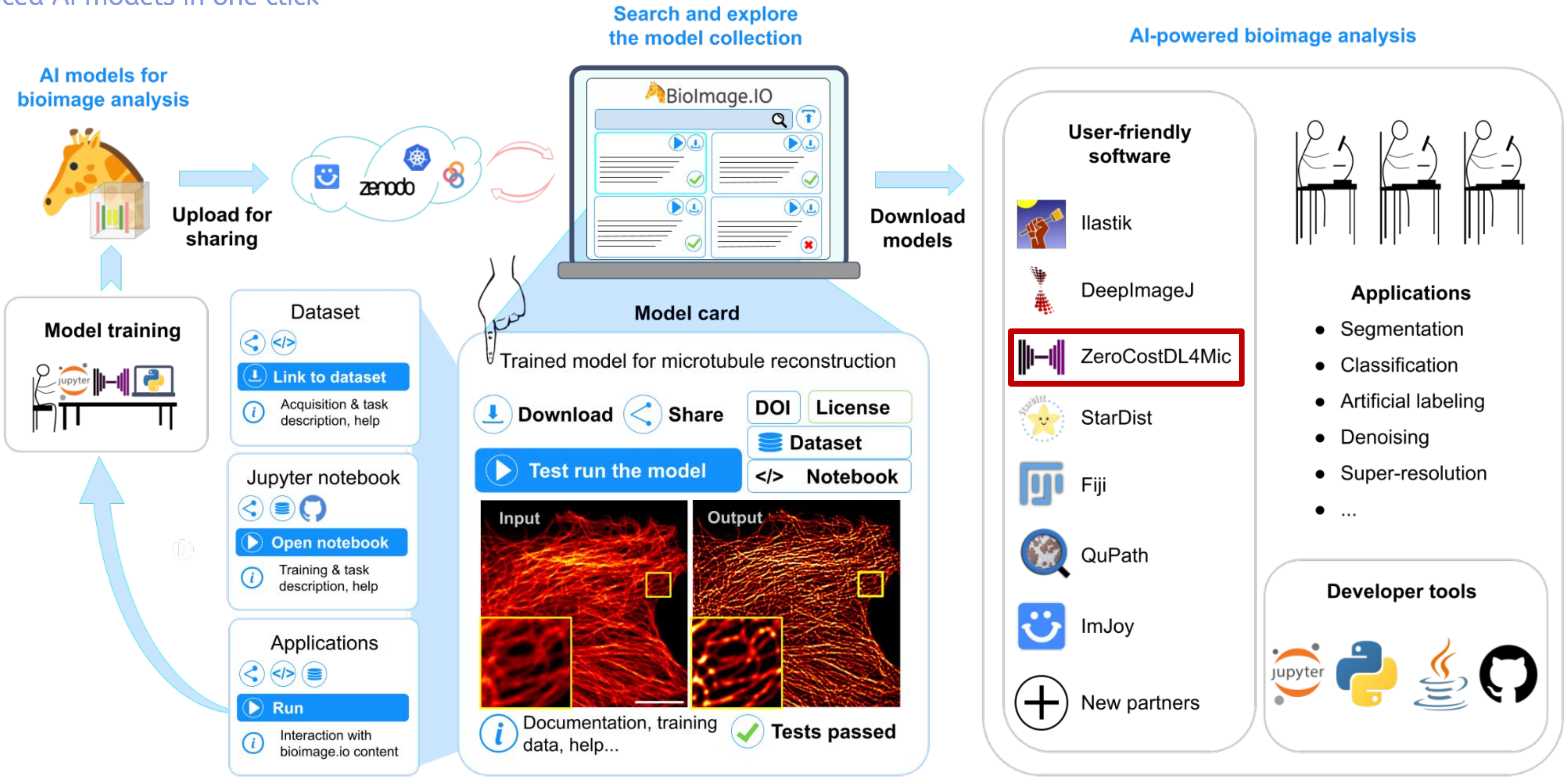
6

Organizing outreach and training events i.e. image analysis courses/workshops and participation in international conferences

BioImage.IO

Advanced AI models in one click

DeepLearning inLifeSciences



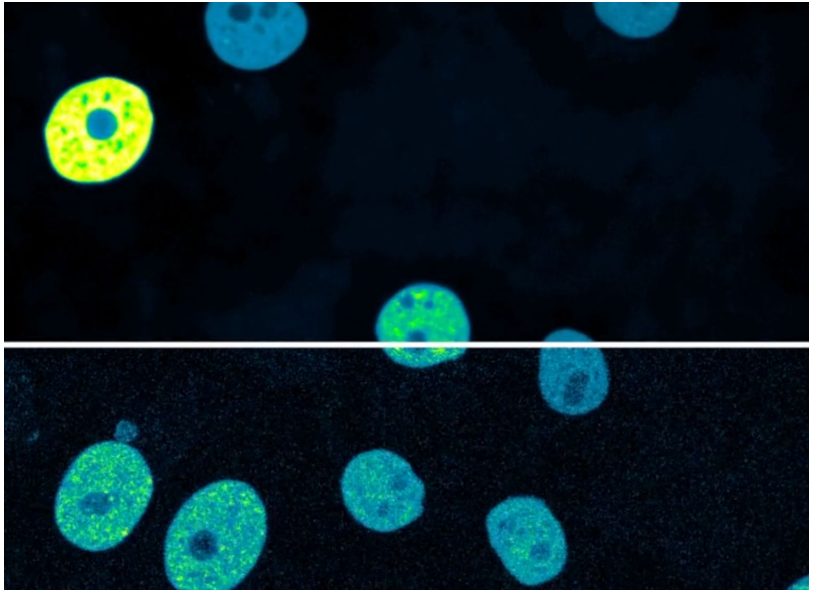
Smart image denoising with noise2void

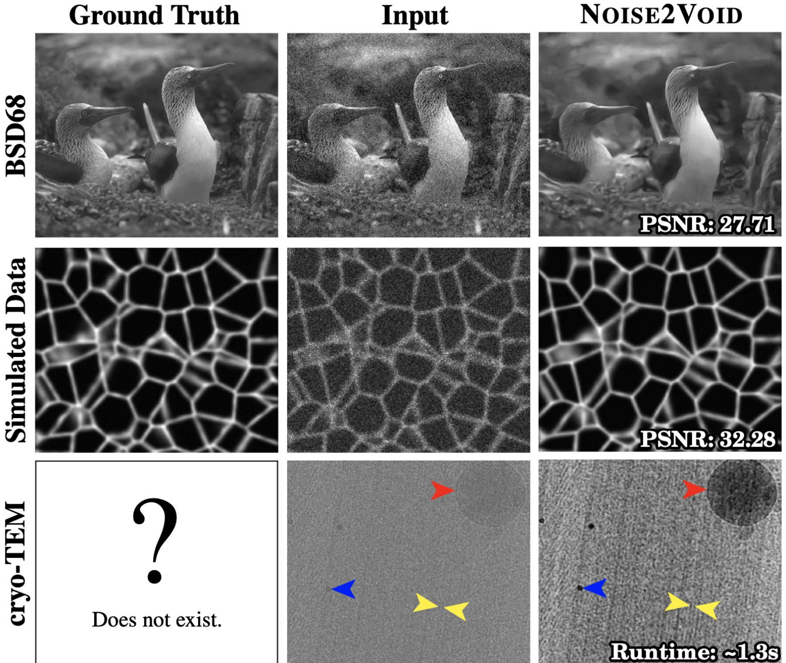
Alexander Krull, Tim-Oliver Buchholz, Florian Jug. *Noise2Void - Learning Denoising from Single Noisy Images*.
Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, 2019.

Alexander Krull, Tim-Oliver Buchholz, Florian Jug. *https://arxiv.org/abs/1811.10980*.
GitHub repository.

Noise2Void (N2V)

Machine learning model for denoising. Designed based on the search for statistical dependencies in the image.



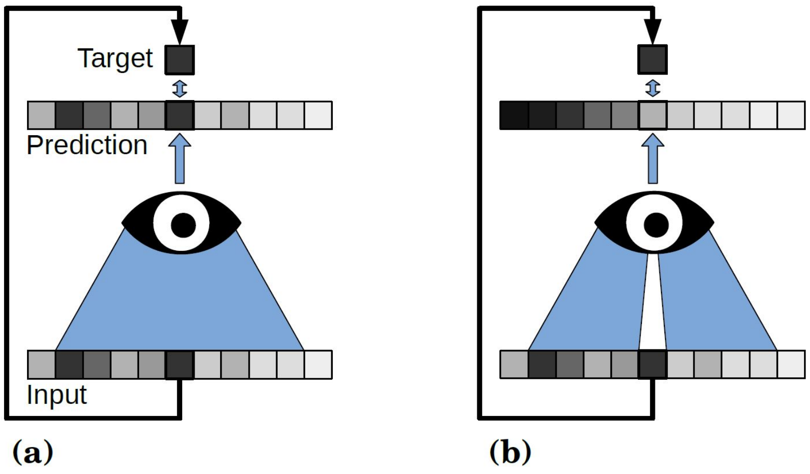


Motivation

- Smart and universal denoising of various image types for visualization.
- Ready-to-use tool in ImageJ, open source code on GitHub
- The original article also described the limitations of the method.
- Availability of test data from the article.
- Deep Learning model (black box)
    - with very well described dependencies
    - focused on re-usability
- Possibility to export BioImage Model Zoo package for publication.

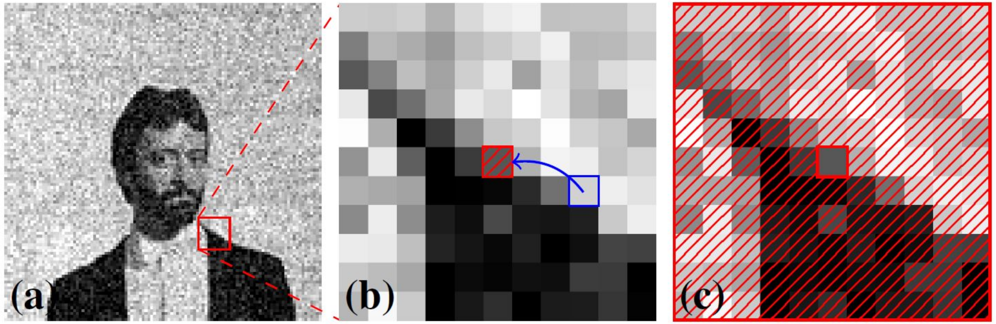
Implementation of Noise2Void

- Assumption: noise changes but image information remains.
    - The signal is statistically dependent on itself in the image.
    - Noise is statistically independent of the signal.
- Creating blind spots in the training image **(b)** will help the model recognize dependencies in the image.



Supervised Learning

A classical learning approach: the input (an image with noise) is compared against a target (ground through)



N2V innovation: input **(a)** is divided into subsections **(b)**. In training part b, a random pixel is copied to the center. Target **(c)** has no center pixel.

Using the N2V model

It is necessary to take into account:

- DL model is a "black box"
- It will only be as good as the data we use to train it (“copying the teacher”)

The following must be observed:

- Apply to same data as training (bit depth, luminance distribution)
- To share the model, it is good to keep the training data + description.

Practical use:

- Visualization, not the basis for segmentation. Not yet usable for brightness quantification!

Limitations

Structured noise
N2V assumes that the noise has no statistical dependence, and therefore does not remove structured noise (checker/mosquito noise, bad frequency filter).

Seemingly easy to use
DL methods are relatively new, very effective, but there is still no standardized description of their use (mainly for scientific data). It is very easy to misuse them.

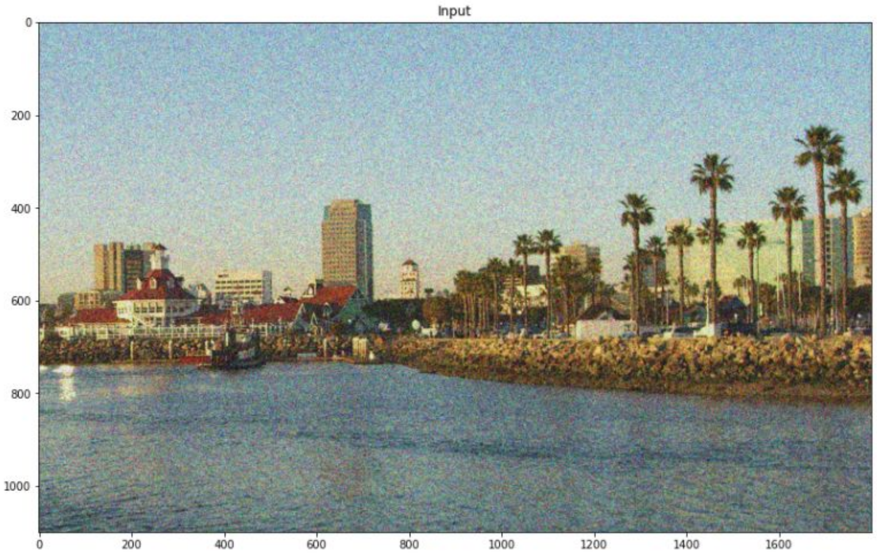
Ethical rules of image analysis
Due to the complexity of Deep Learning, it is necessary to be careful for now.

Cromey, D. W. (2010, June 22). **Avoiding Twisted Pixels: Ethical Guidelines for the Appropriate Use and Manipulation of Scientific Digital Images**. Science and Engineering Ethics. Springer Science and Business Media LLC. http://doi.org/10.1007/s11948-010-9201-y

DeepLearning inLifeSciences

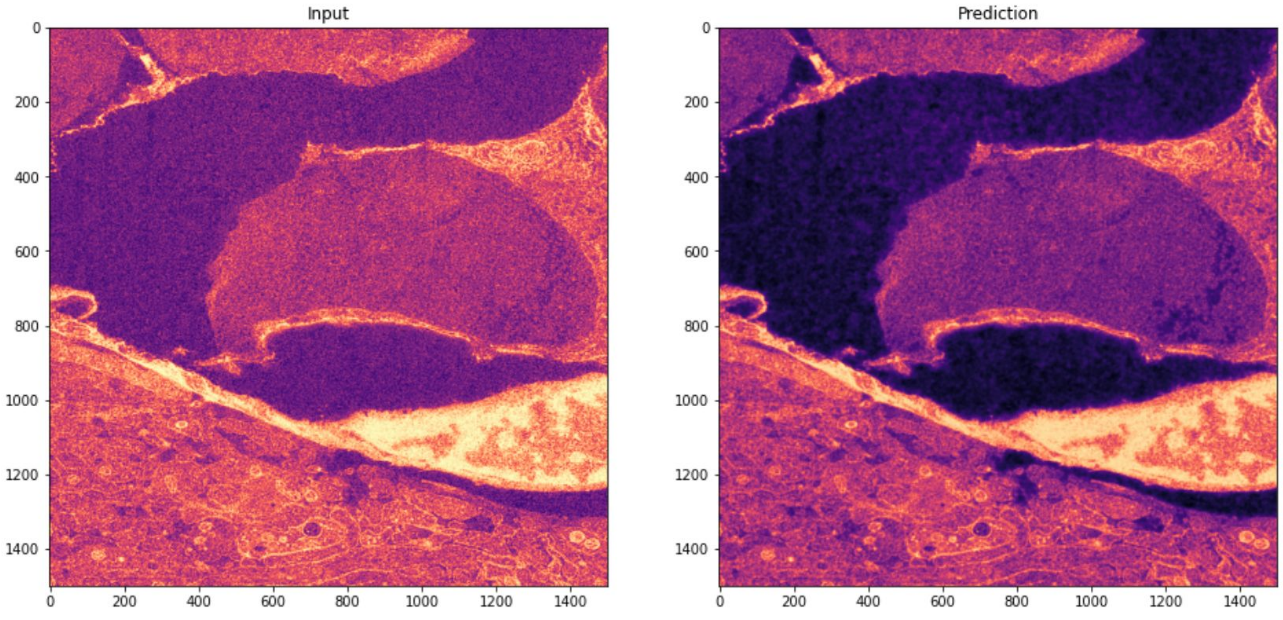
Python - Jupyter notebook

https://github.com/juglab/n2v/blob/master/examples/ 2D RGB



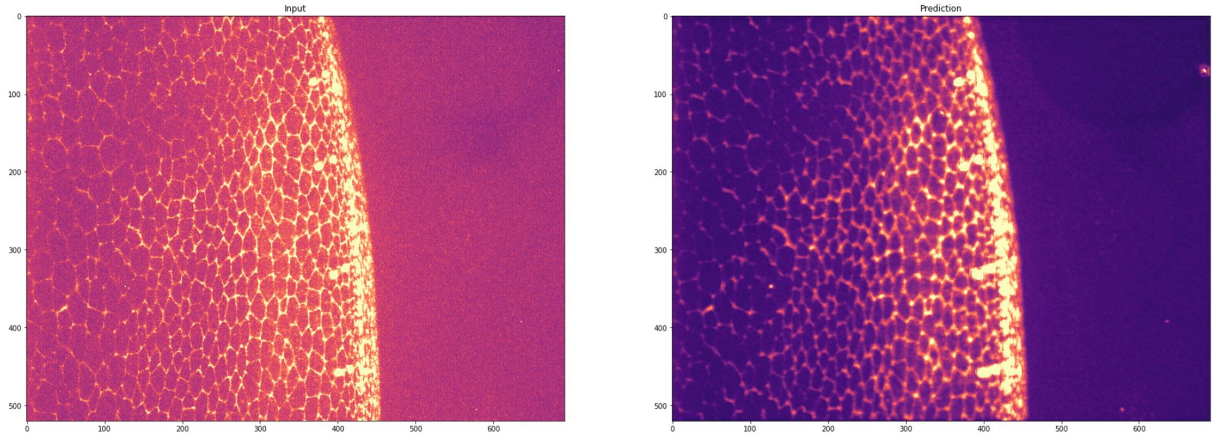
Python - Jupyter notebook

https://github.com/juglab/n2v/blob/master/examples/ 2D SEM



Python - Jupyter notebook

https://github.com/juglab/n2v/blob/master/examples/ 3D



Save results

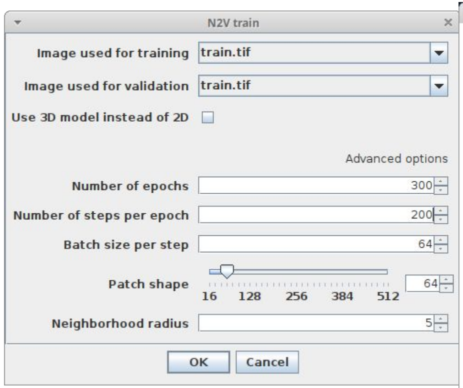
```
save_tiff_imagej_compatible('prediction.tif', pred, 'ZYX')
```

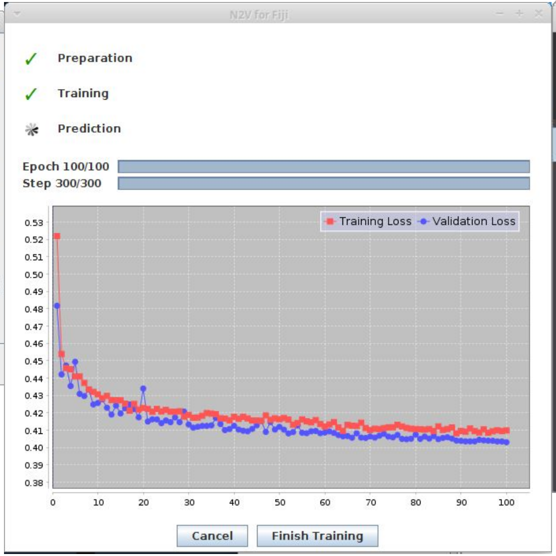
DeepLearning inLifeSciences

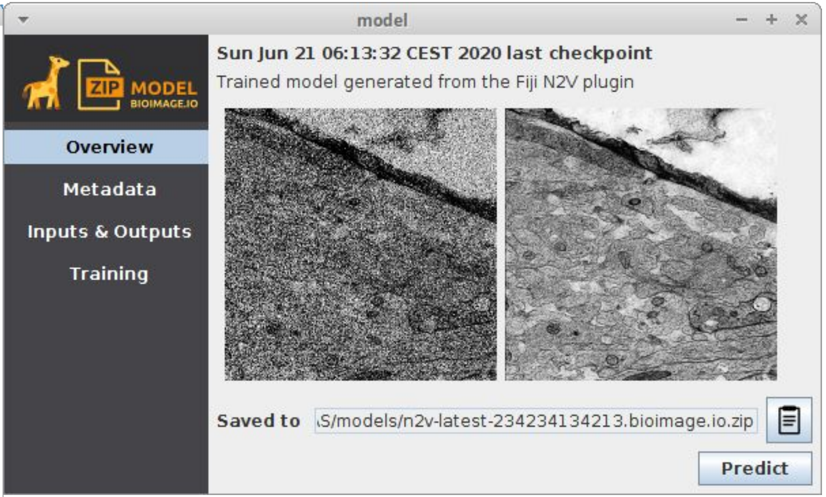
ImageJ plugin

N2V Fiji plugin - https://imagej.net/plugins/n2v

Dependent on CSBDeep







Summary

Ideal for:

- Visualization
- Preprocessing for segmentation
- Preprocessing for counting objects

Simple use through ImageJ - both training and application. Or use in other Python-enabled applications.

But:

- Black box
- May produce artifacts (statistically dependent noise)

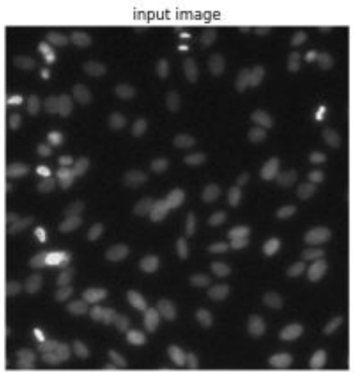
StarDist
Object Detection with Star-convex Shapes

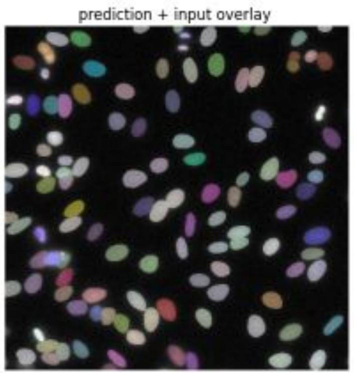
Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers.
Cell Detection with Star-convex Polygons.
International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), Granada, Spain, September 2018.

Martin Weigert, Uwe Schmidt, Robert Haase, Ko Sugawara, and Gene Myers.
Star-convex Polyhedra for 3D Object Detection and Segmentation in Microscopy.
The IEEE Winter Conference on Applications of Computer Vision (WACV), Snowmass Village, Colorado, March 2020

What is StarDist?

- Deep learning tool designed to localize convex objects (cell nuclei).
- Available as:
  - Package for training custom prediction model (Python).
  - Pretrained model ready to use.
  - Plugin(s) using pre-trained models.





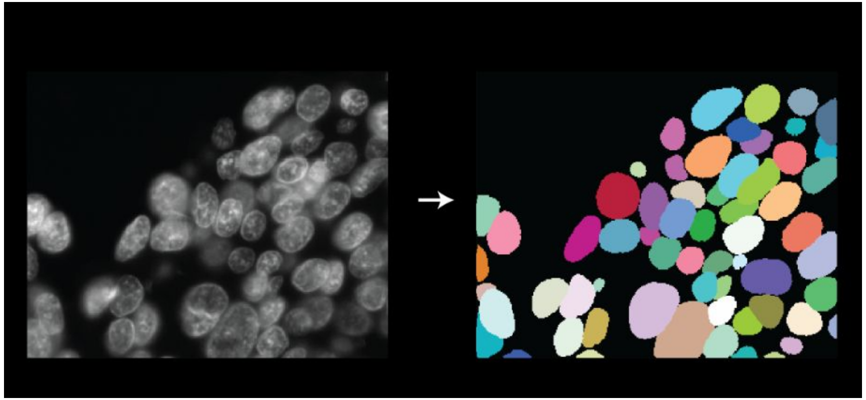
Motivation & what to learn

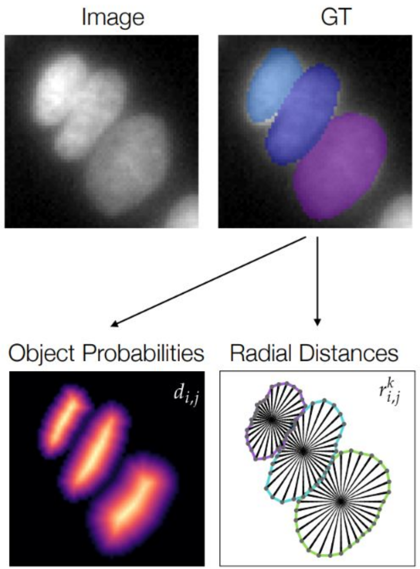
- Easy to use.
- Can detect overlapping objects.
- Robust to intensity changes.
- Usable for both 2D and 3D data.
- Available as plugin.

- What is StarDist?
- The main idea
- Why to use StarDist
- Example (in FIJI)
- Other software plugins

How it works

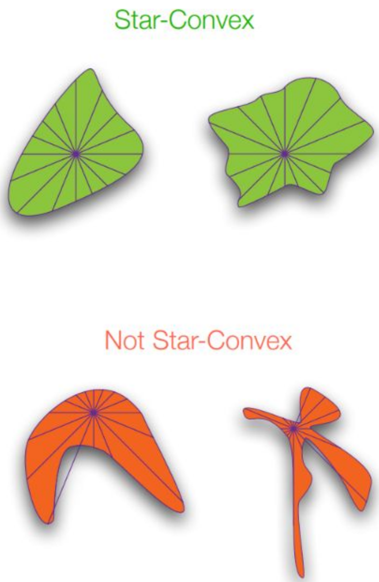
- Tool designed to localize cell nuclei via star-convex polygons.
- Similar to methods that directly predict shapes for each object of interest.

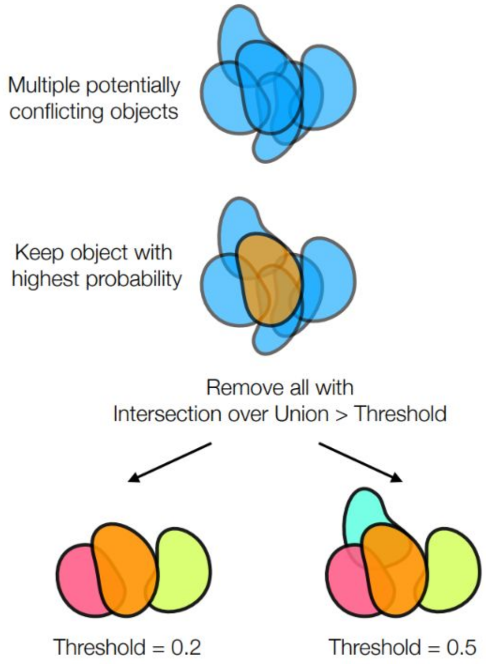




How it works

- Segmentation based on Star-Convex objects.
- Capability to handle intersection/overlapping objects.





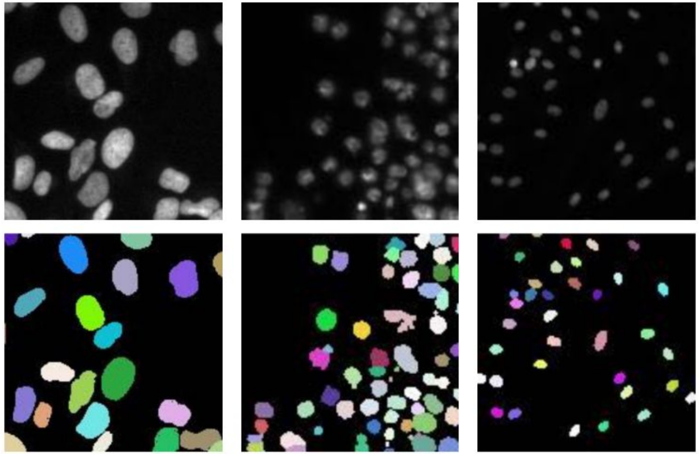
Why to use StarDist

- Easy to use.
- Can detect overlapping objects.
- Robust to intensity changes.
- Usable for both 2D and 3D data.
- Available as plugin.
- Available models are widely usable.
- Possibility to retrain model for specific data.

DeepLearning inLifeSciences

Pretrained models

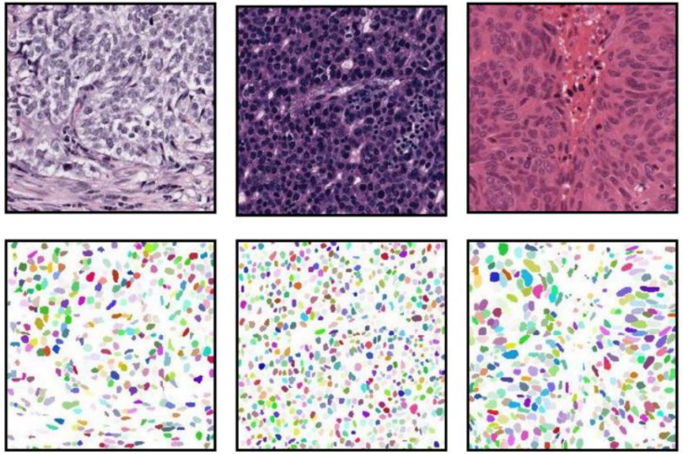
Fluorescence Microscopy
Single Channel



Data Science Bowl 2018
Caicedo et al. (2018)

~ 600 images (2D)
~ 20k annotations

Histopathology
RGB H&E



MoNuSeg
Kumar et al (2017)

~ 30 Images (2D)
~ 22k annotations

Examples

- What is in Imagej/FIJI.
  - 2 models
- Basic settings.
- DIfference of overlap settings (synthetic images).

DeepLearning inLifeSciences

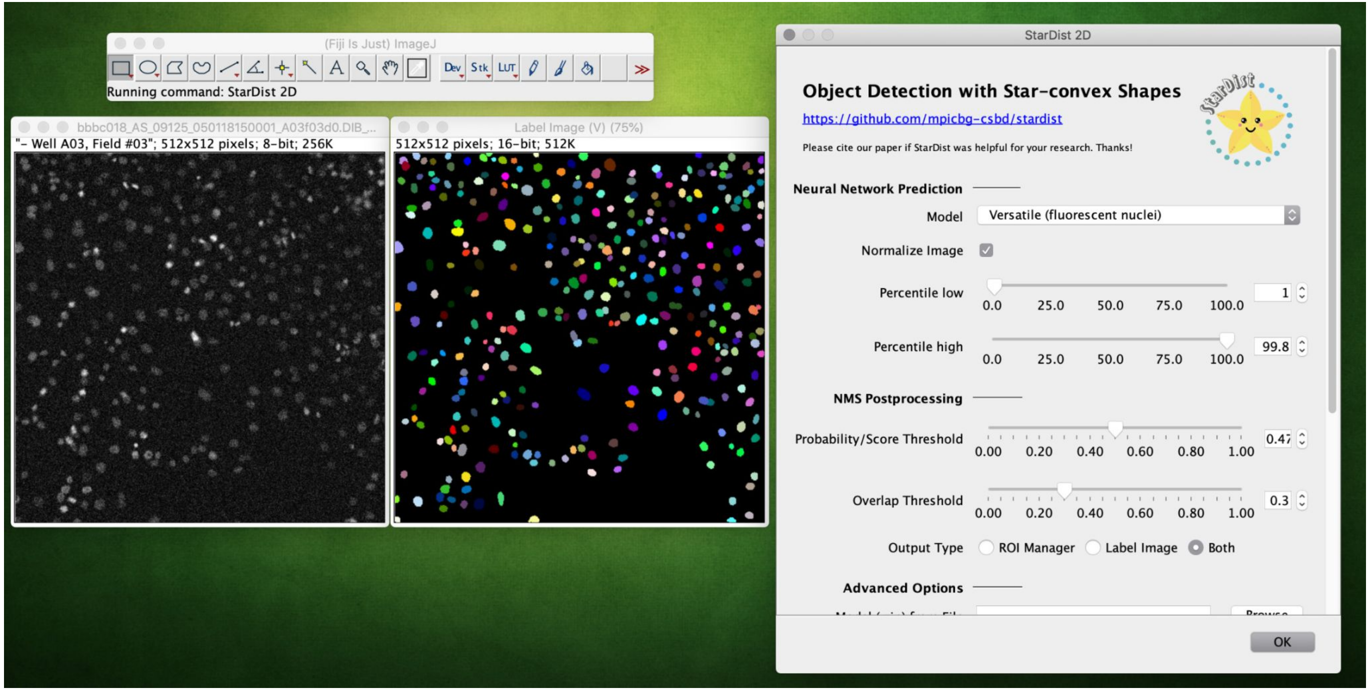
Examples - Overview

Plugin currently supports only 2D images.

2 models:

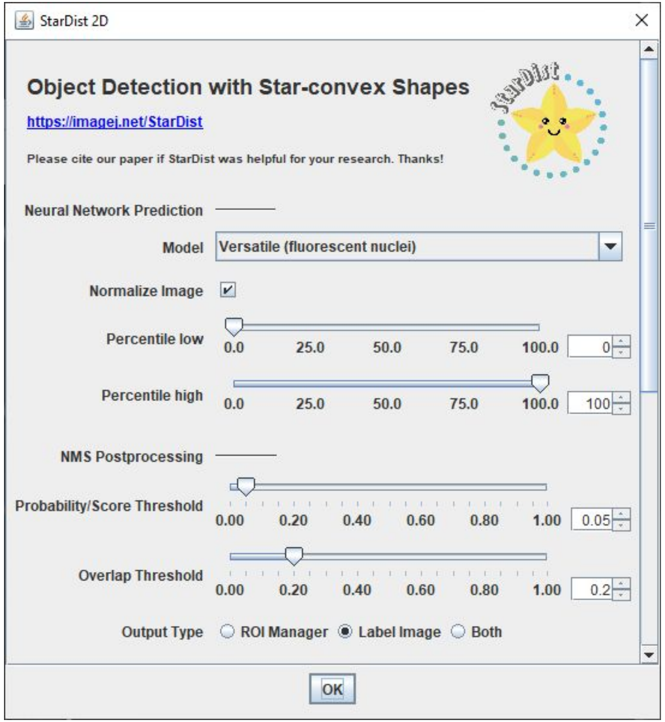
Versatile (fluorescent nuclei)

Versatile (H&E nuclei)



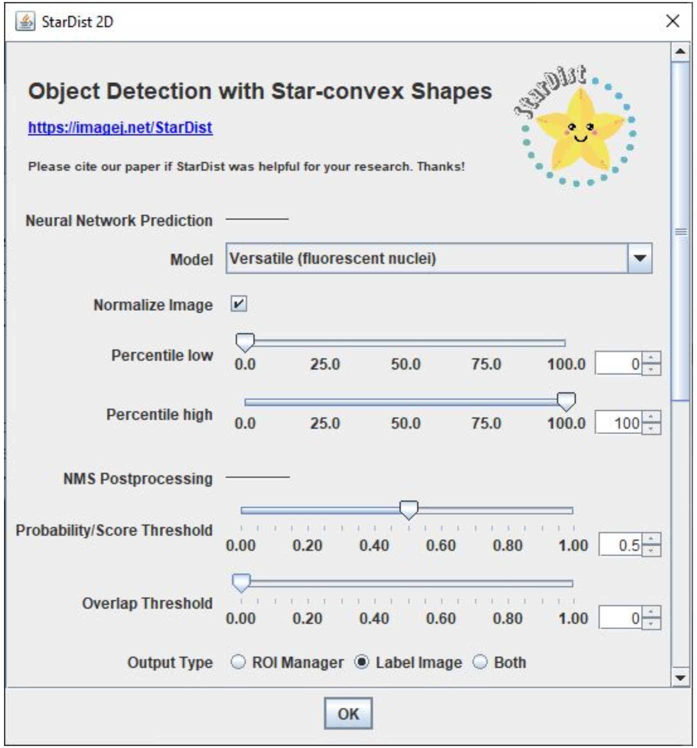
DeepLearning inLifeSciences

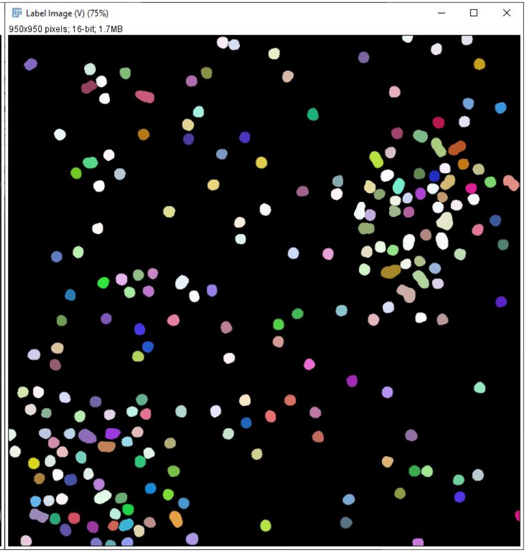
Examples - Settings



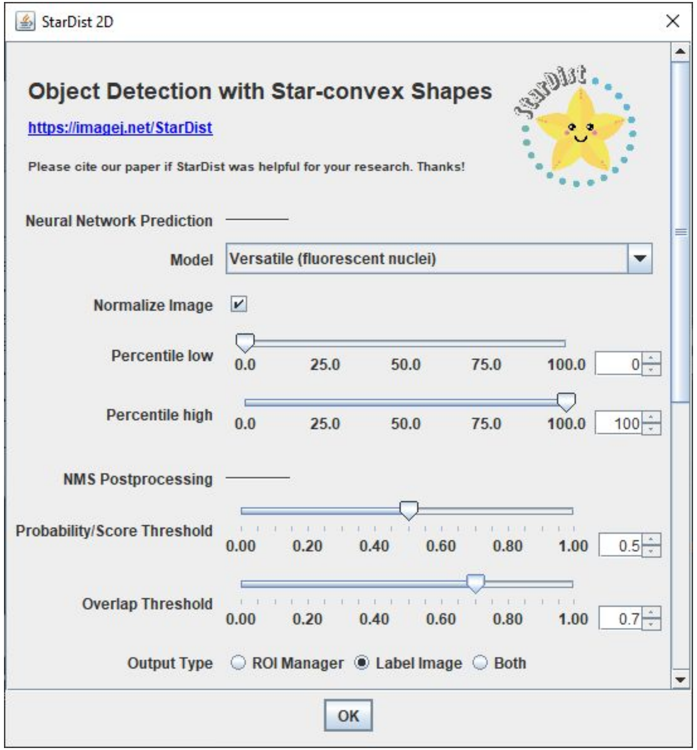
- Preprocessing settings
  - Normalization of image values.
  - Correction of “underexposure”.
  - Correction of “overexposure”.
- StarDist settings
  - Probability Threshold - how sure we want to be in detection of object.
  - Overlap Threshold - how much overlap we want to allow.

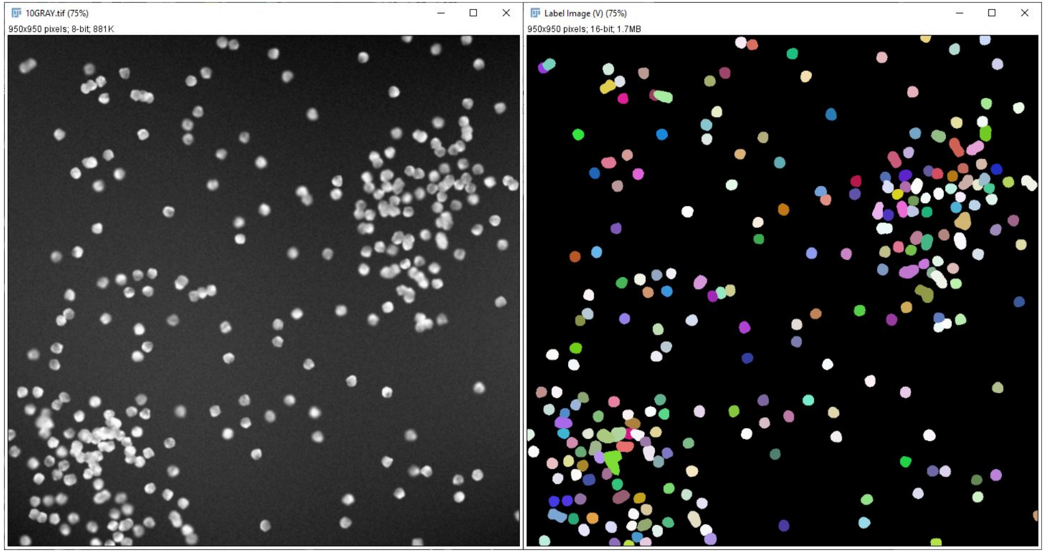
Examples - Overlap



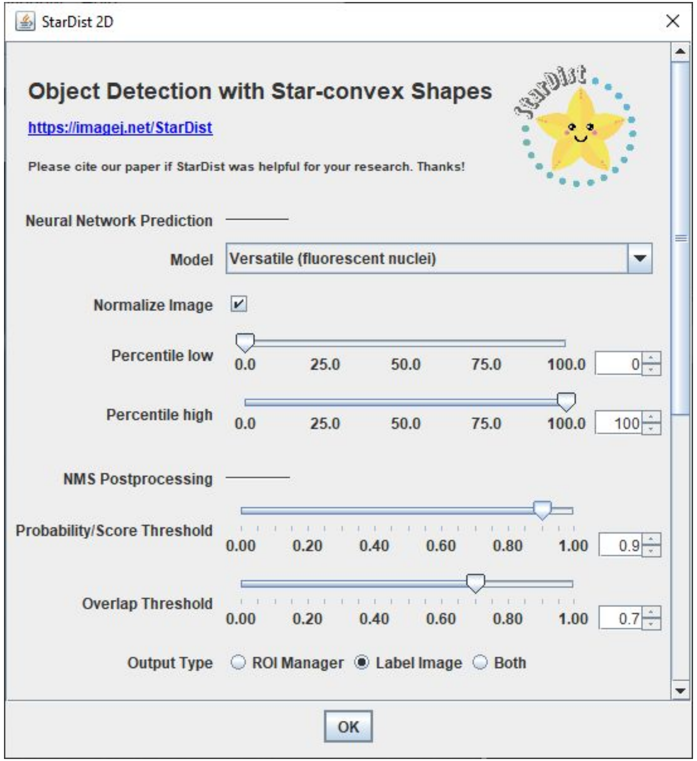


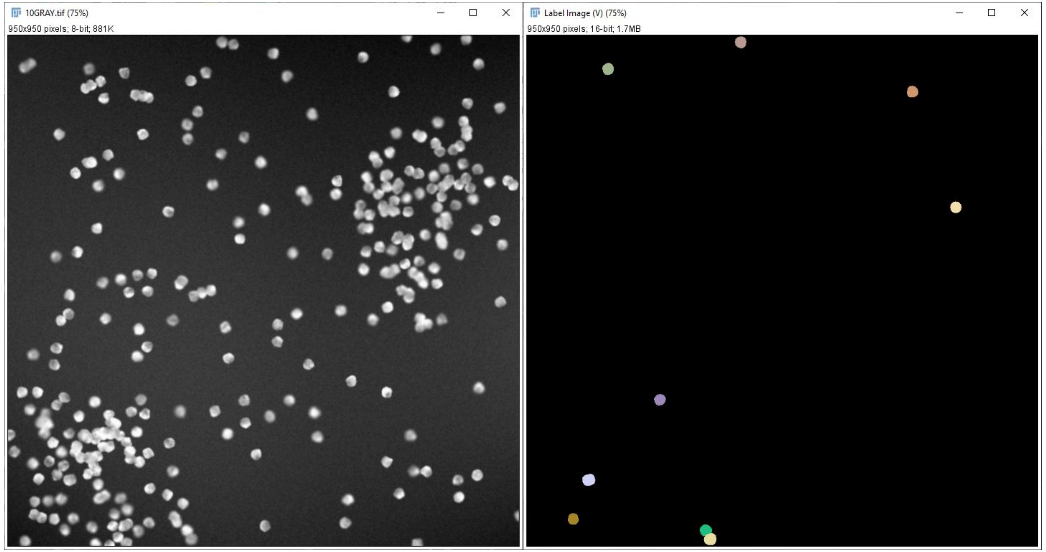
Examples - Overlap





Examples - Overlap + Probability





Plugins

ImageJ/Fiji
Scriptable ImageJ/Fiji plugin that can be used to run pretrained StarDist models on 2D or 2D+time images.

Napari
Plugin for the Python-based multi-dimensional image viewer napari. It directly uses the StarDist Python package and works for 2D and 3D images.

QuPath
Inspired by the Fiji plugin, Pete Bankhead made a custom implementation of StarDist 2D for QuPath to use pretrained models.

Icy
Based on the Fiji plugin, Deborah Schmidt made a StarDist 2D plugin for Icy to use pretrained models.

KNIME
Stefan Helfrich has modified the Fiji plugin to be compatible with KNIME.

Resources

- Haase, R. (2023). Introduction to bio-image analysis I. F1000 Research Limited. http://doi.org/10.7490/F1000RESEARCH.1119427.1
- Haase, R. BioImage Analysis Lectures, https://www.youtube.com/playlist?list=PL5ESQNfM5lc7SAMstEu082ivW4BDMvd0U
- Haase, R. BioImage Analysis Notebooks, https://haesleinhuepf.github.io/BioImageAnalysisNotebooks/intro.html
- NEUBIAS Academy Lectures and Tools: https://www.youtube.com/c/NEUBIAS/videos
- Image to Knowledge Conference workshops: https://www.youtube.com/@I2KConference
- Schätz, M., Azevedo, M., & Sampaio, P. (2023). Internal ALM BioImage Analysis workshop 2023. Zenodo. https://doi.org/10.5281/zenodo.10205578

Exercises

Exercises

Building and training new models is time consuming, so focus will be on **exploring**, **understanding** and **reusing**.

Two models to tackle:

- Noise2Void - denoising single channel images
  - Train and apply denoising on provided images with high and low noise.
- StarDist - training and evaluating custom model
  - Explore num. of training epochs and vs **Quality Control**
  - Test it on high overlap data (0.6 probability): https://bbbc.broadinstitute.org/BBBC004

Both are heavily GPU dependent! CPU will take even 80-100x more time.

References

- Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers.
  Cell Detection with Star-convex Polygons.
  International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), Granada, Spain, September 2018.
- Martin Weigert, Uwe Schmidt, Robert Haase, Ko Sugawara, and Gene Myers.
  Star-convex Polyhedra for 3D Object Detection and Segmentation in Microscopy.
  The IEEE Winter Conference on Applications of Computer Vision (WACV), Snowmass Village, Colorado, March 2020
- ImageJ/Fiji plugin for StarDist: https://imagej.net/plugins/stardist