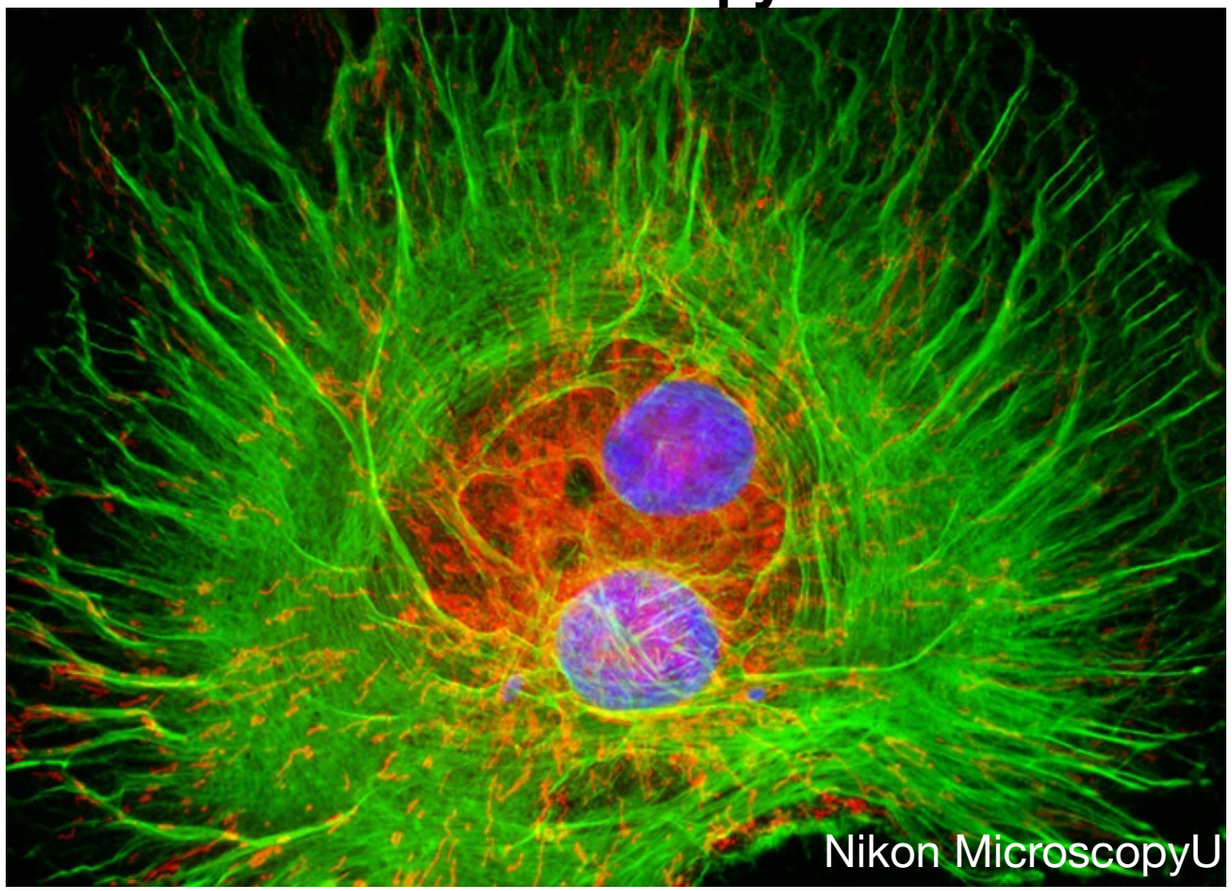
Image analysis
From algorithms to artificial intelligence

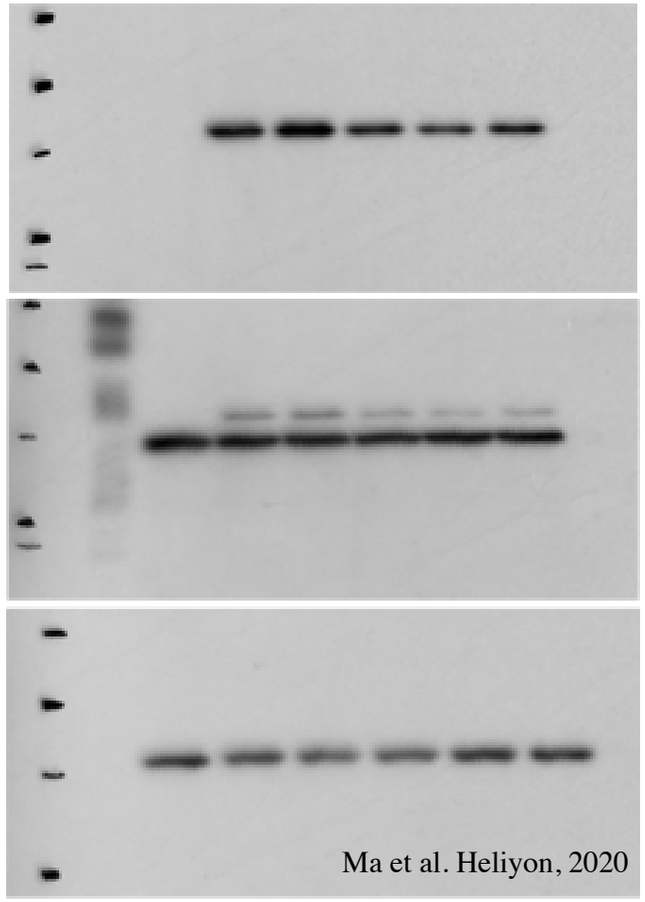
Reviews in Computational Biology

Serge Pelet, DMF

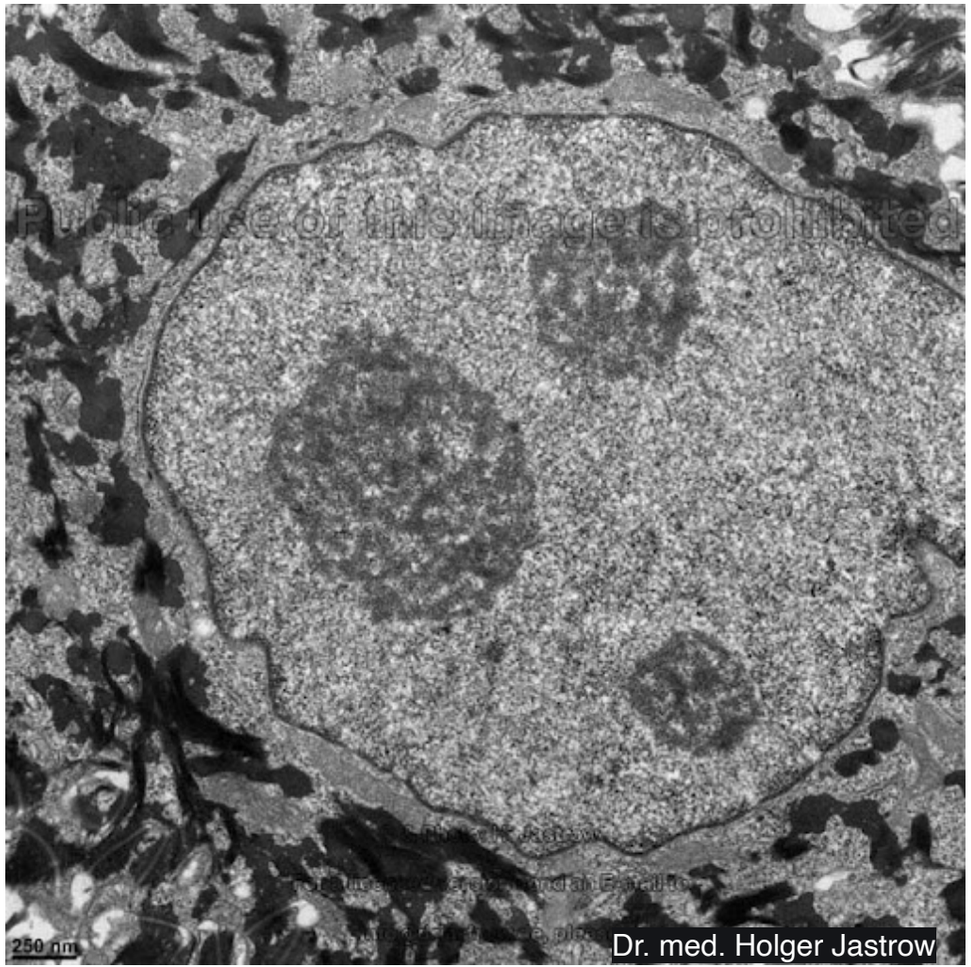
Fluorescence microscopy



Western blot



EM



MRI

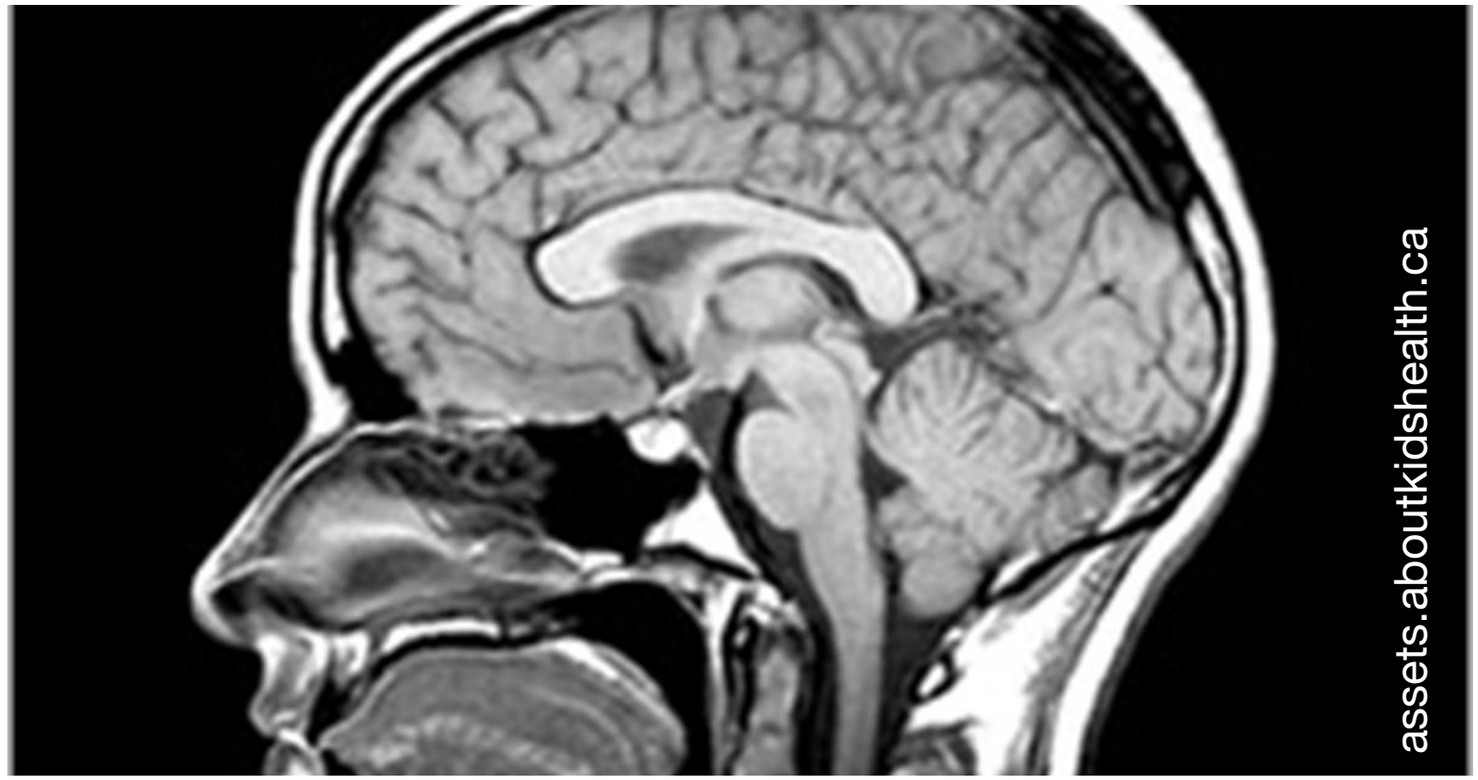
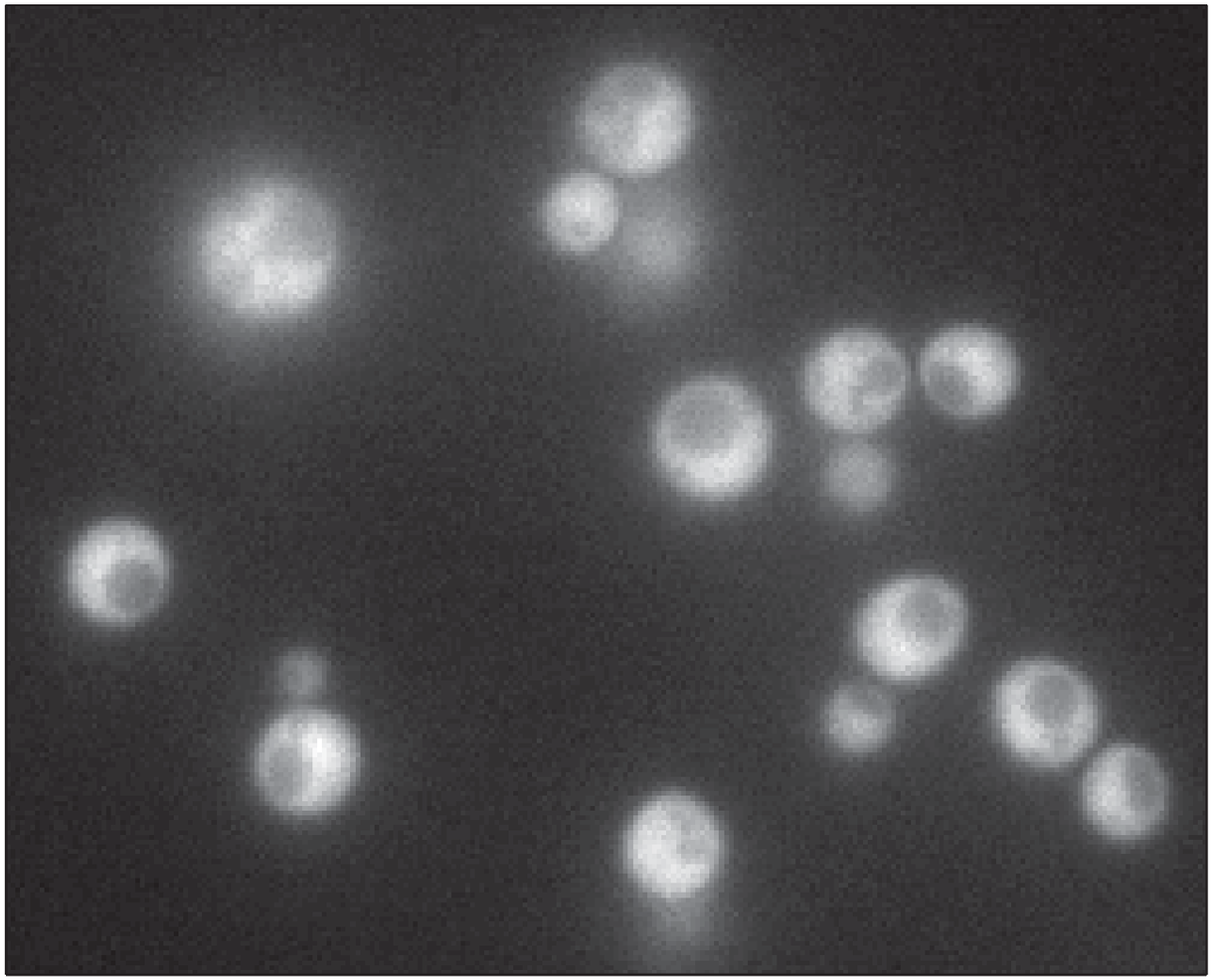
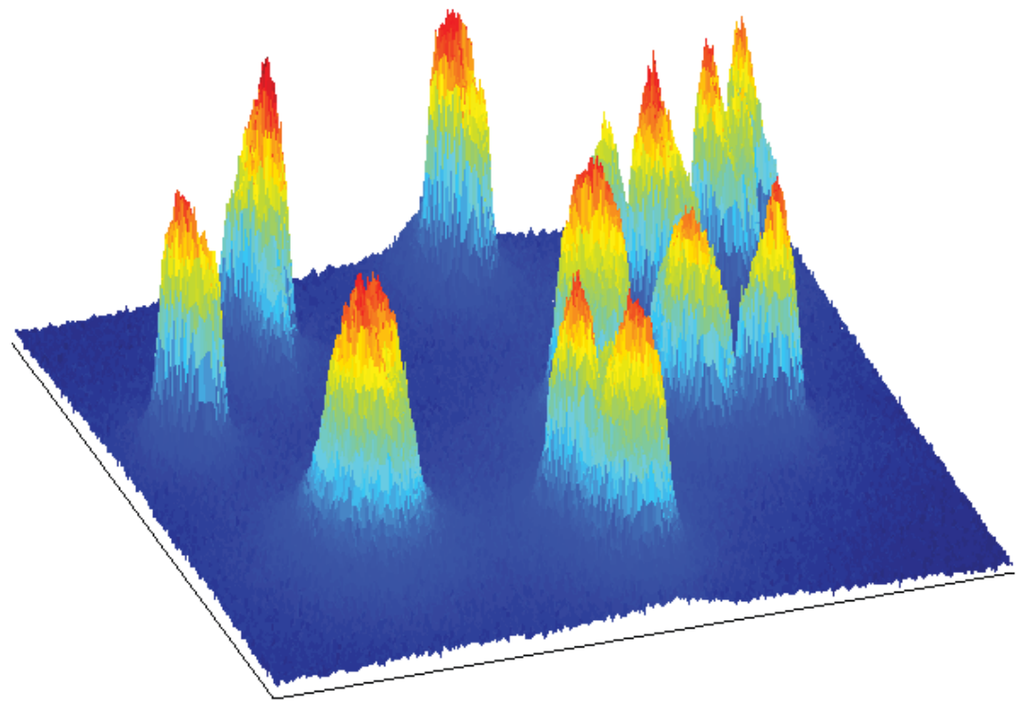


Image: 2D intensity map

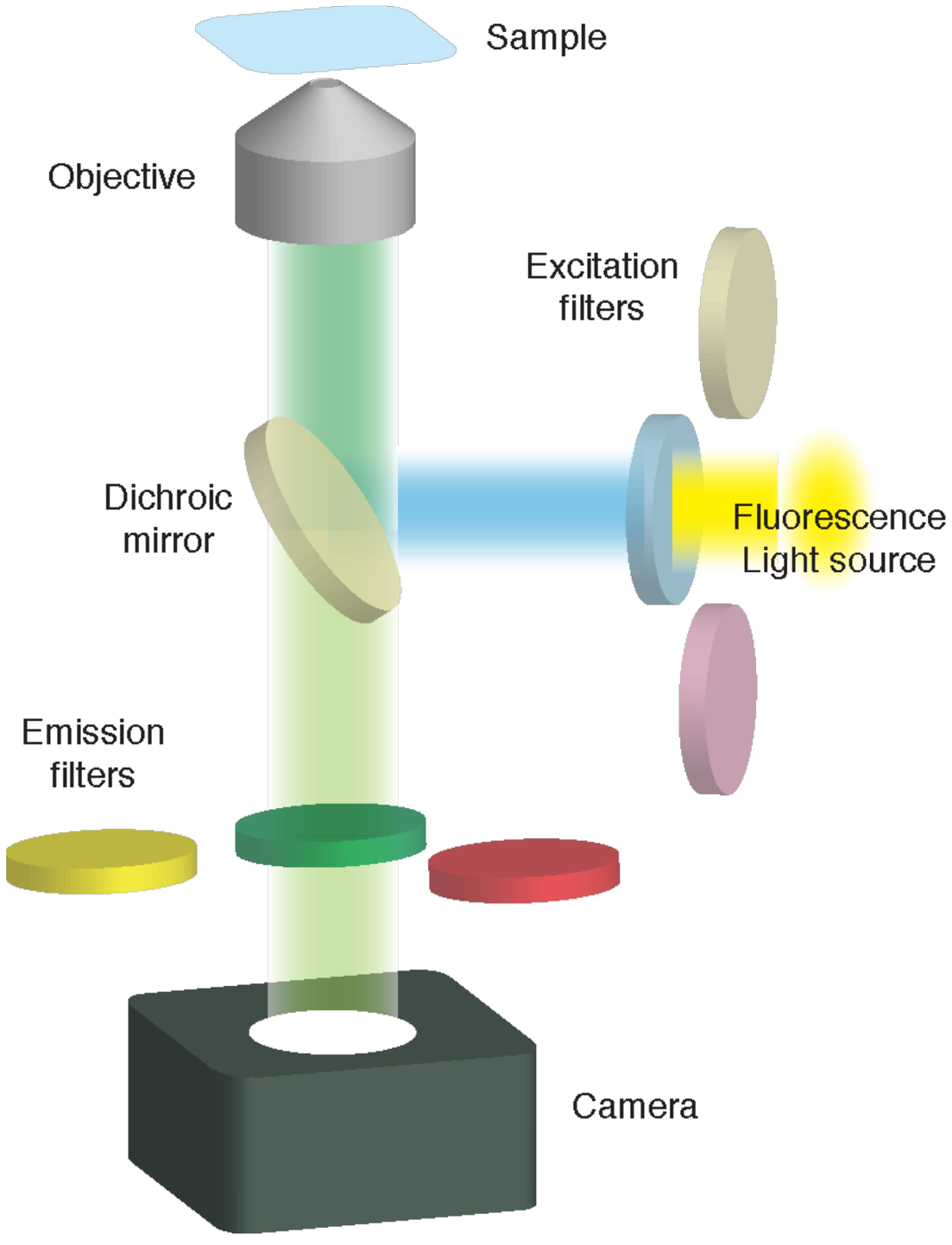
Image: 2D representation

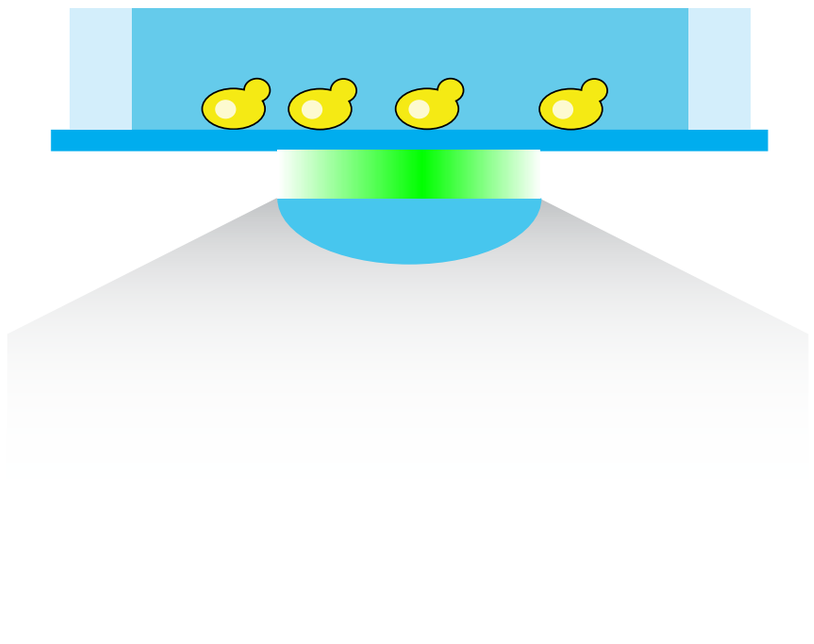


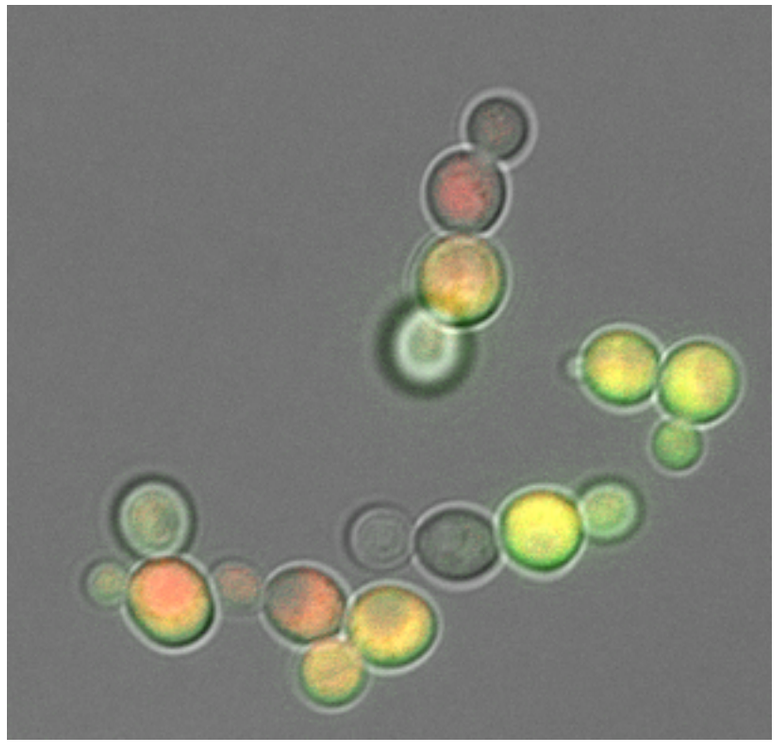
3D-representation



Fluorescence Microscopy

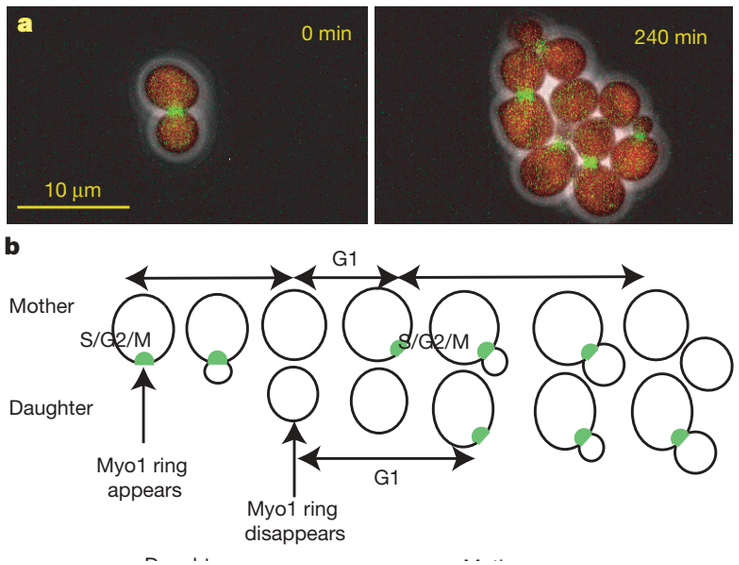


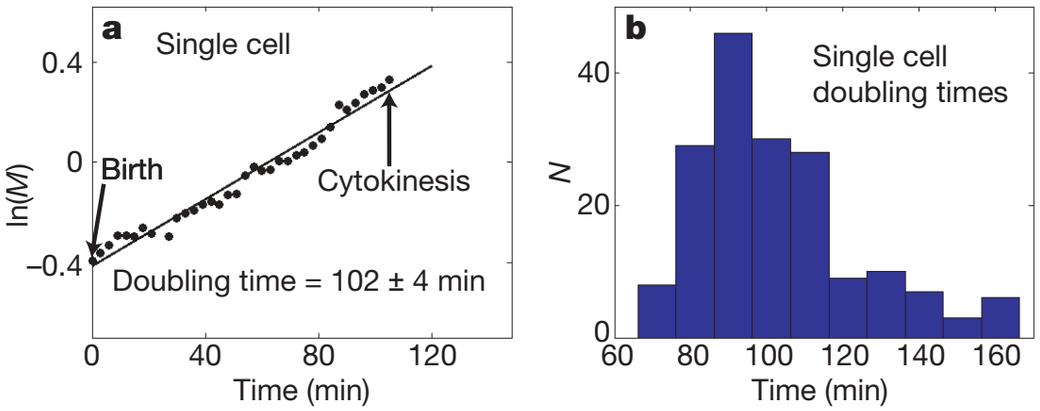




What to analyze?

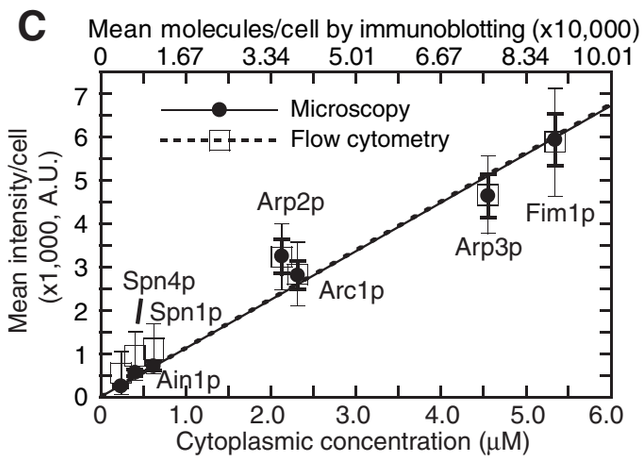
Cell size





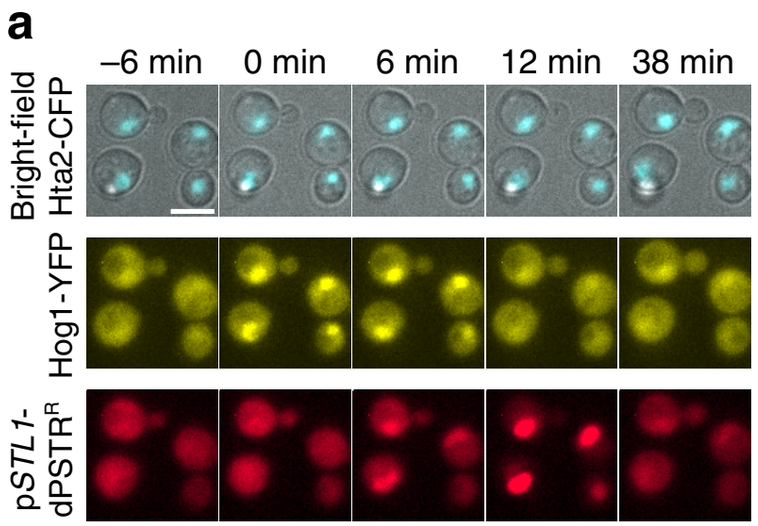
Di Talia et al. Nature, 2007

Expression level



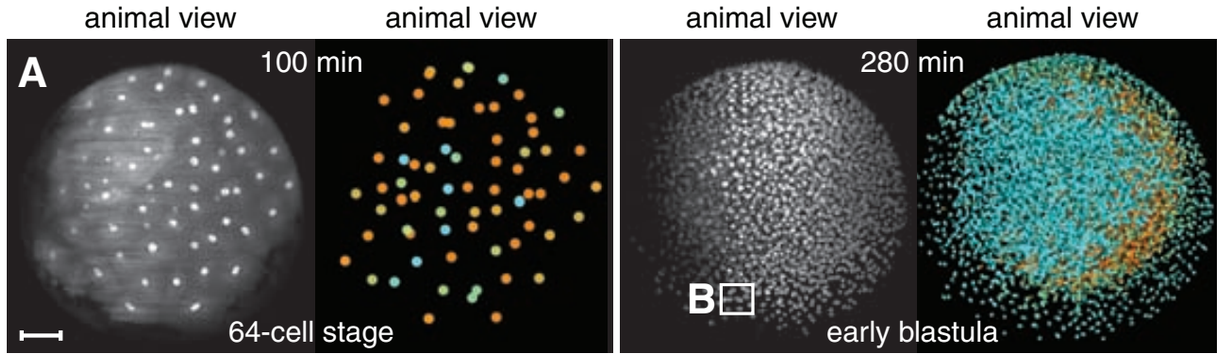
Wu et al. Science, 2005

Localization



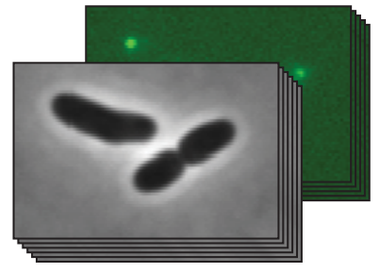
Aymoz et al. Nature Communications, 2016

Cell-cell interactions

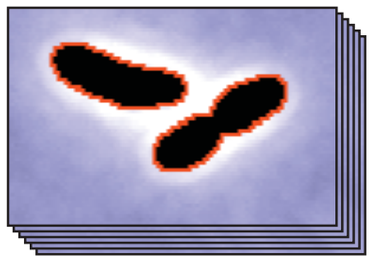


Keller et al. Science, 2008

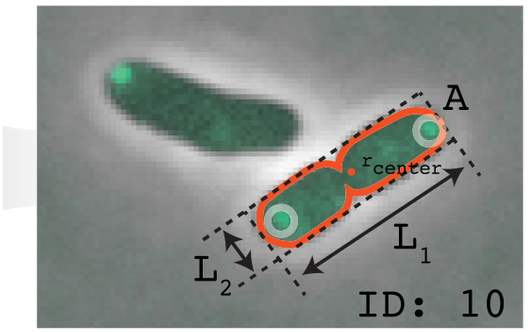
Analysis steps



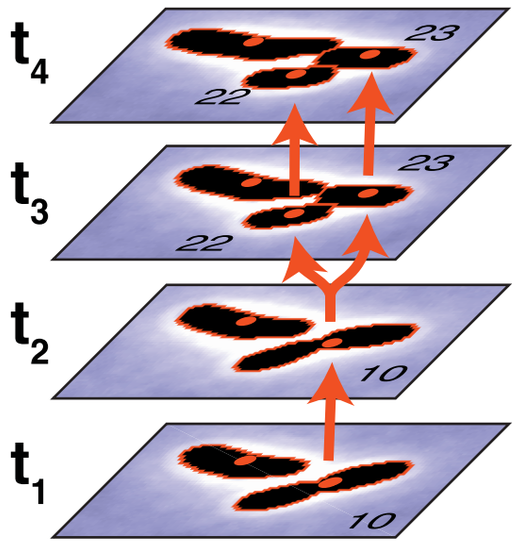
Object recognition (segmentation)



Feature Measurements

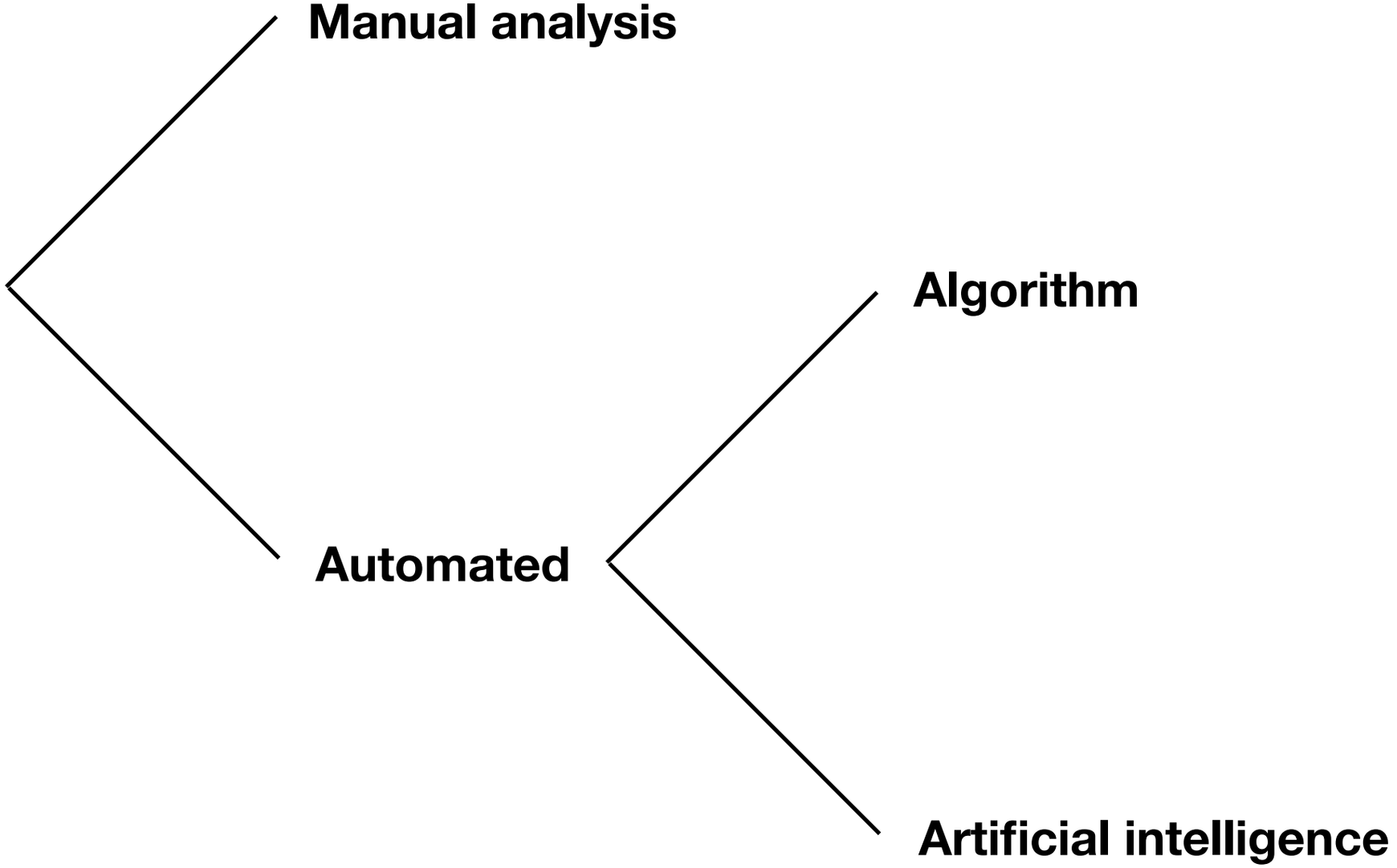


Tracking



Adapted from Stylianidou et al. Molecular Microbiology, 2016

How to analyze?



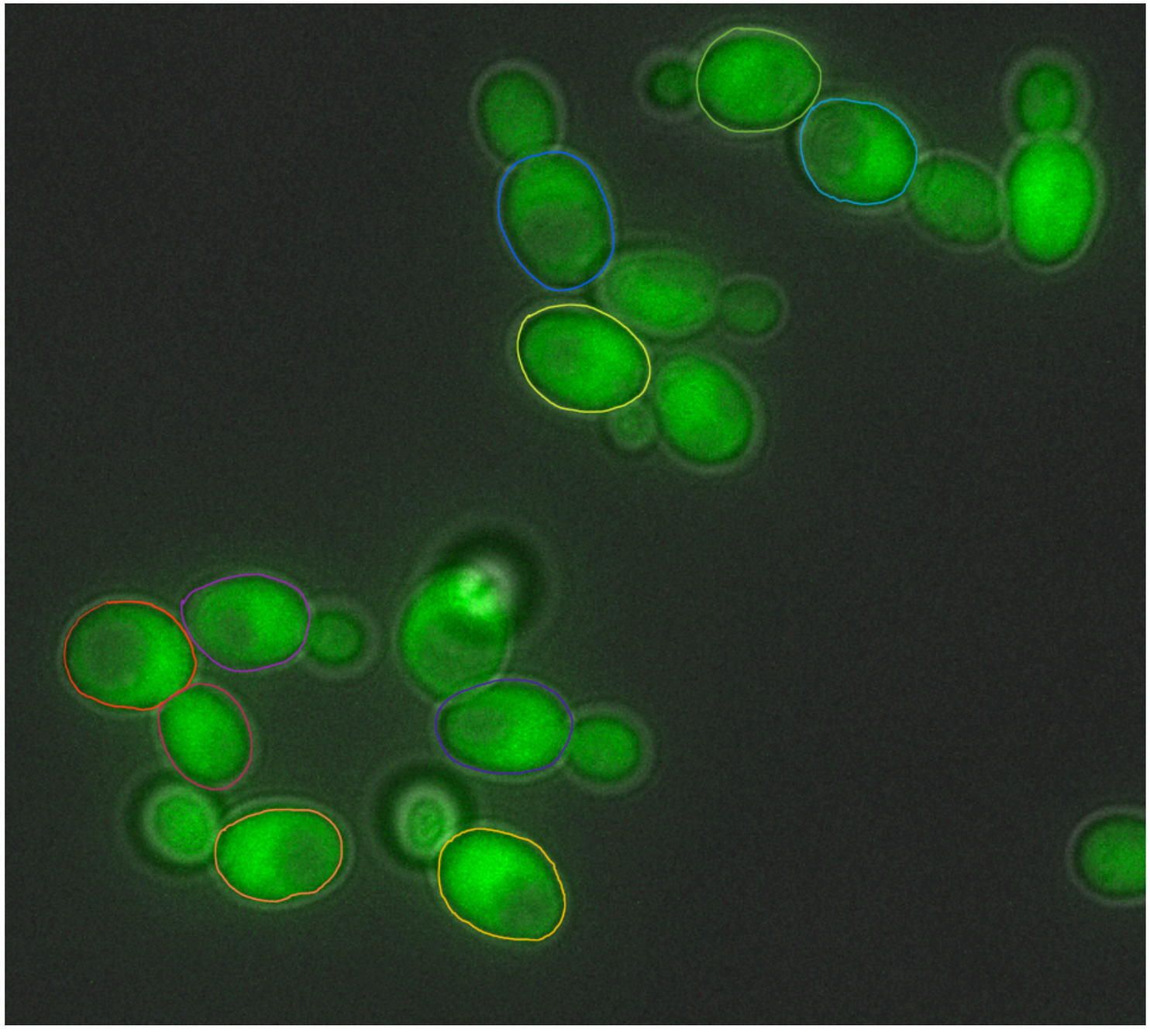
Manual analysis

Advantages

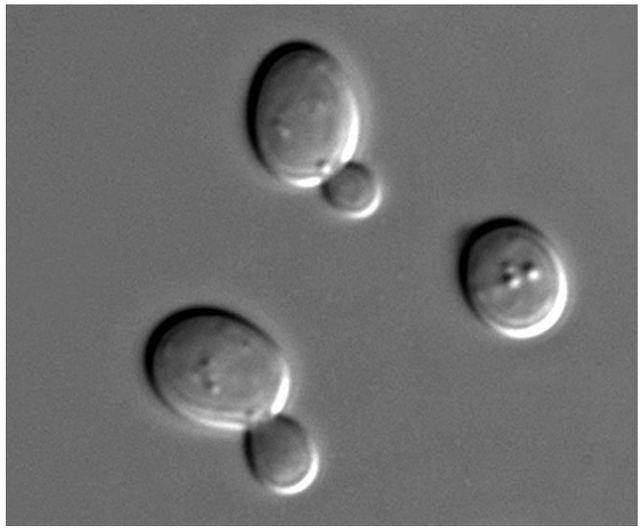
+ High precision
+ Great flexibility
+ Easy access
+ Semi-automation possible

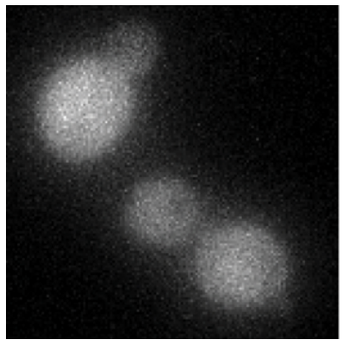
Disadvantages

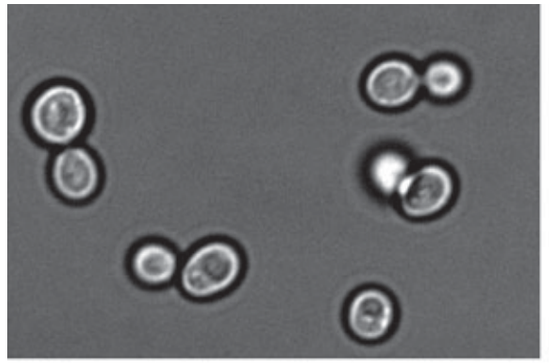
- Low throughput
  - Time consuming
  - Low number of cells
- Bias in cell selection

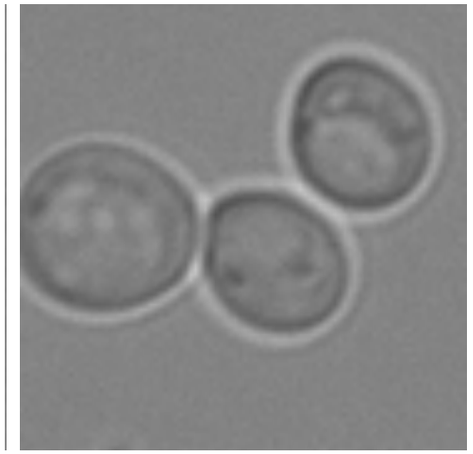


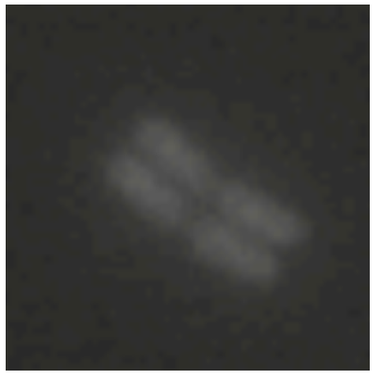
What is a yeast cell?

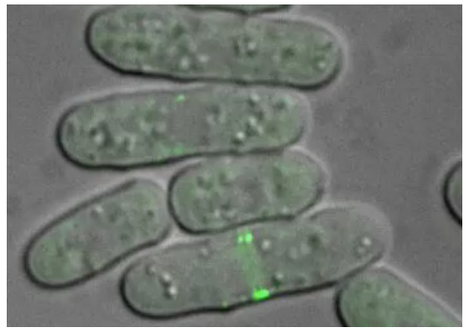


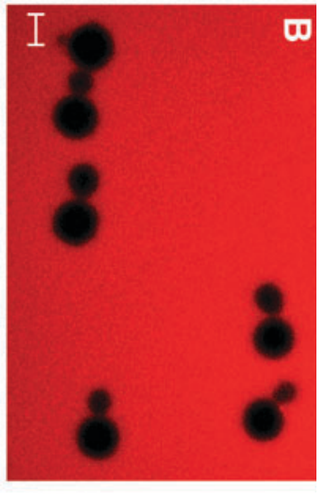


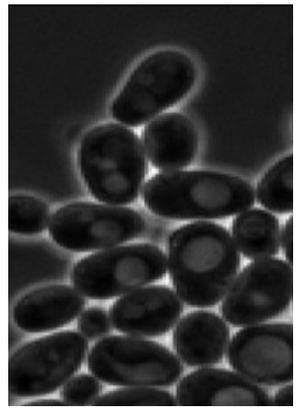


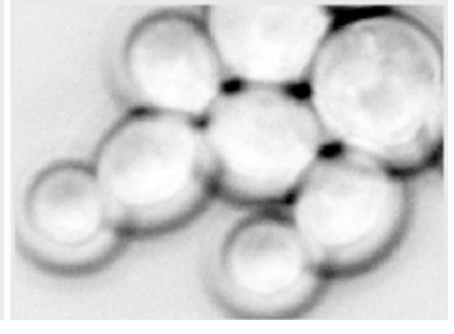


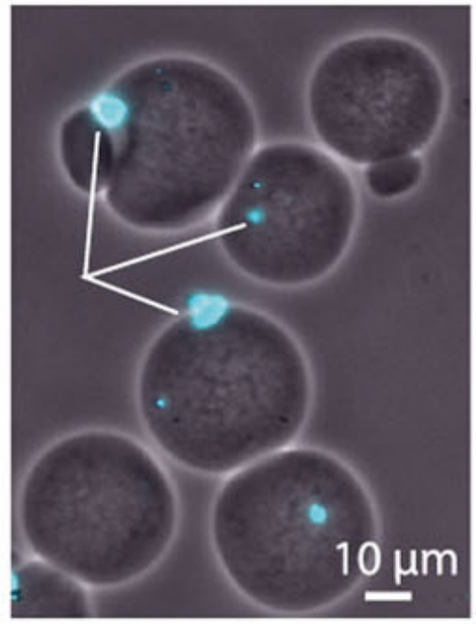




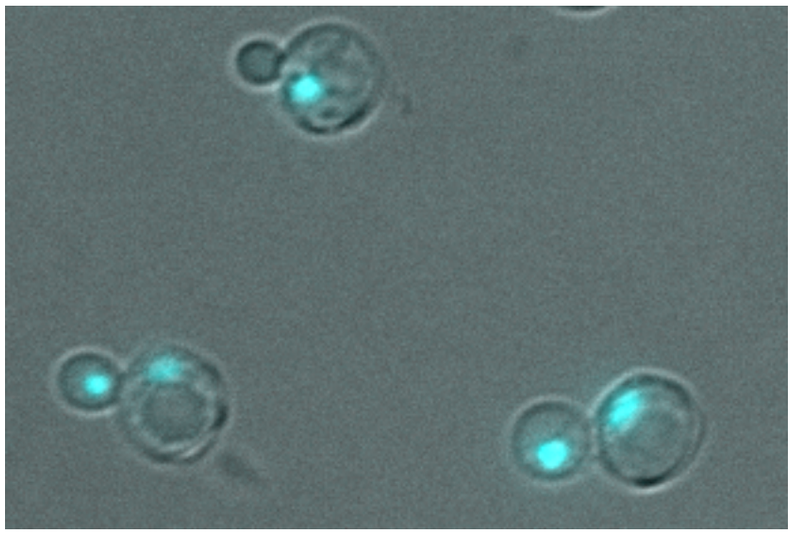


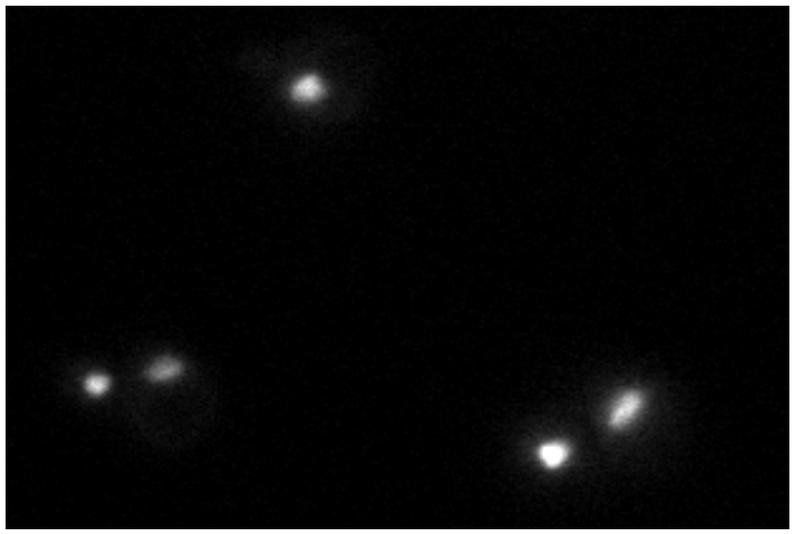




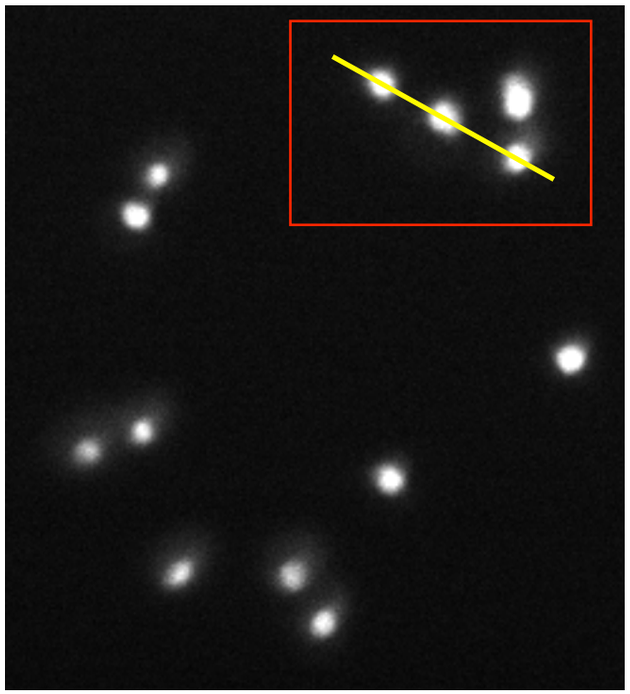


Contest: human vs machine

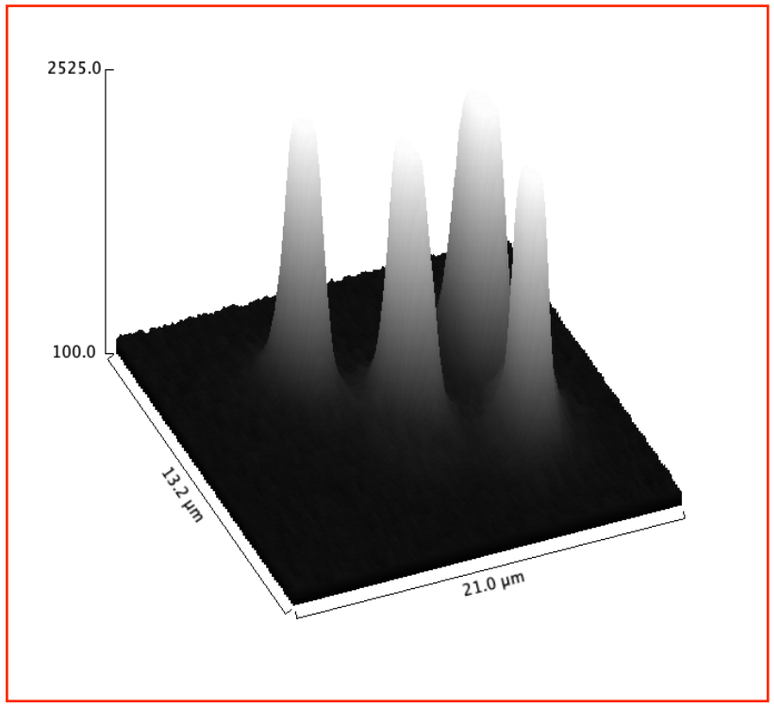




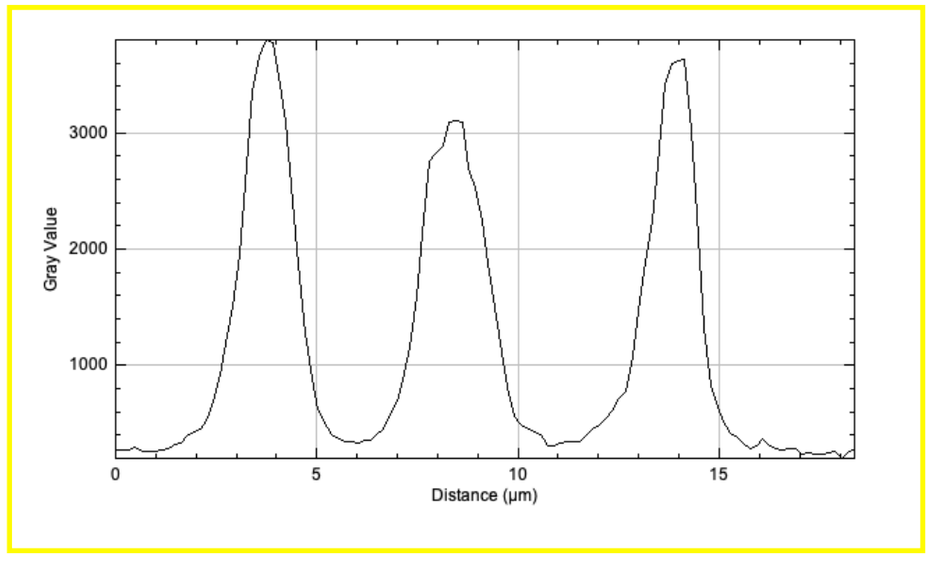
Thresholding

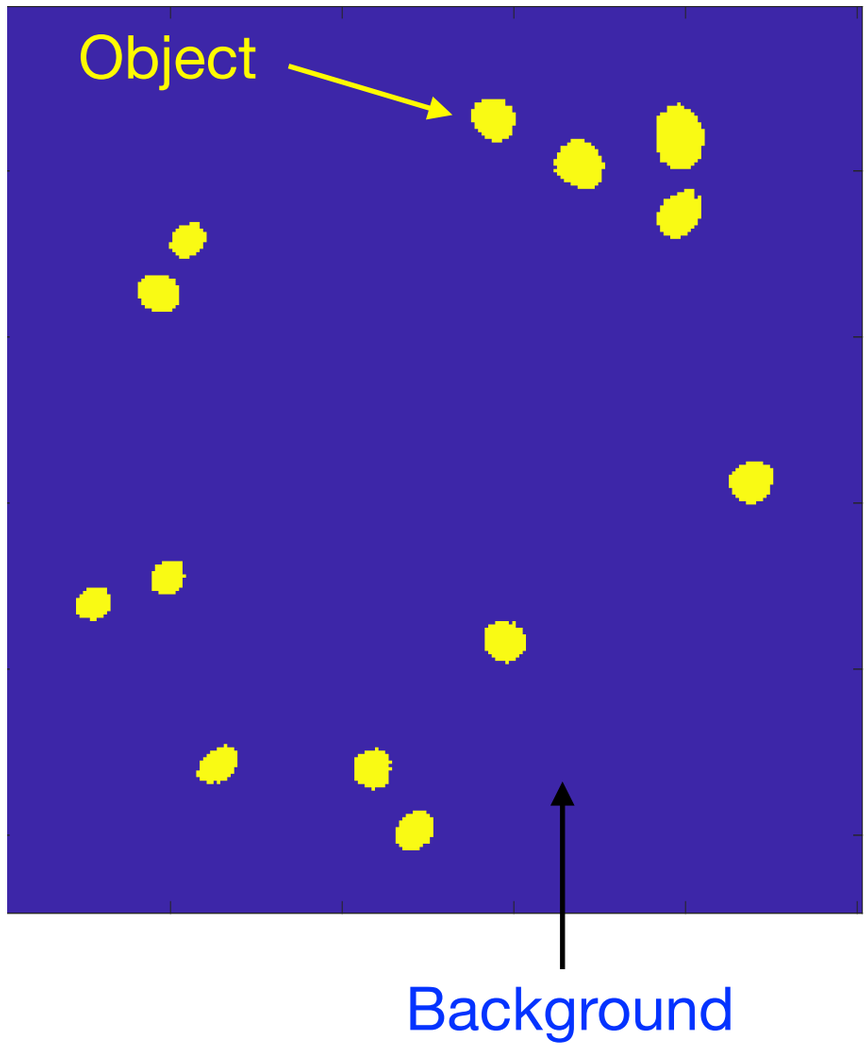


3D map



line scan

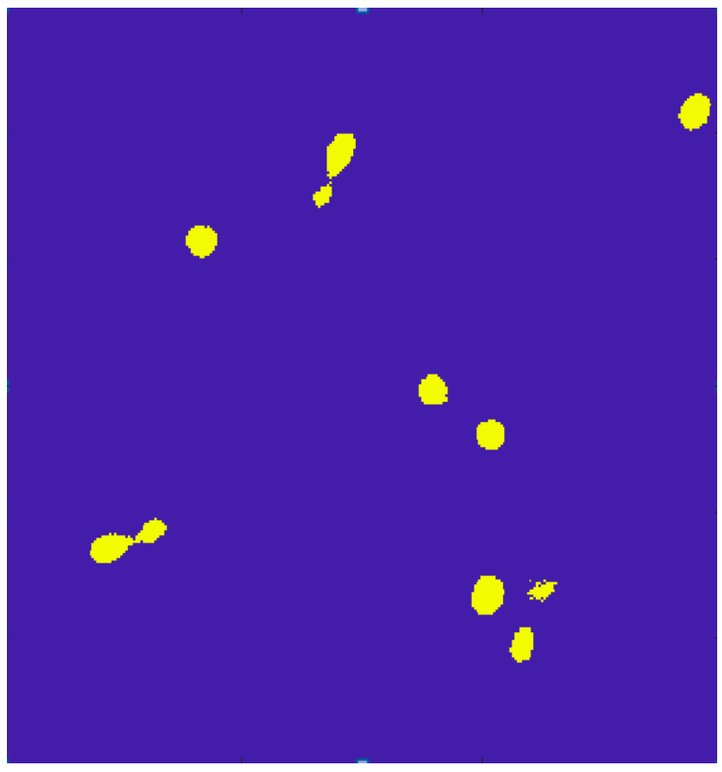




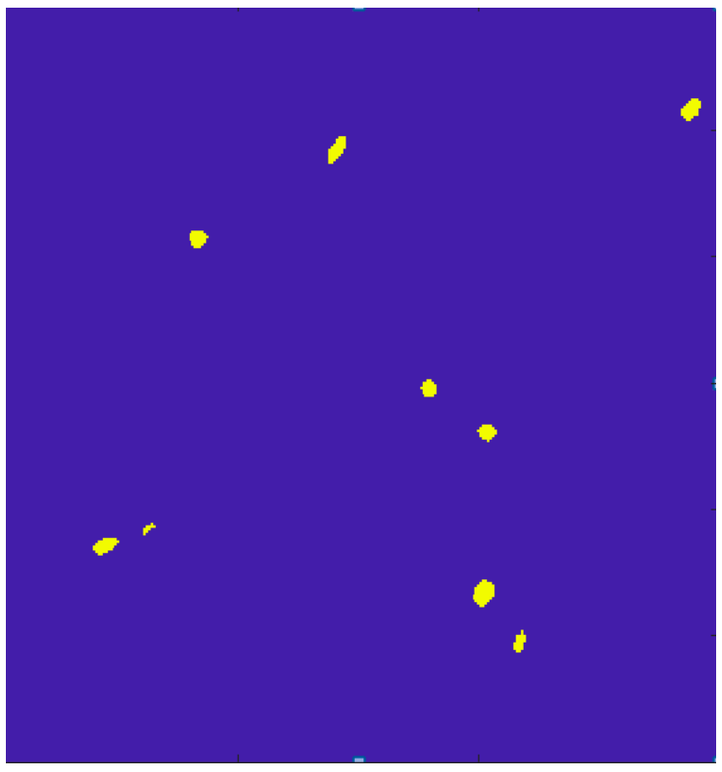
Next steps

Morphological operations

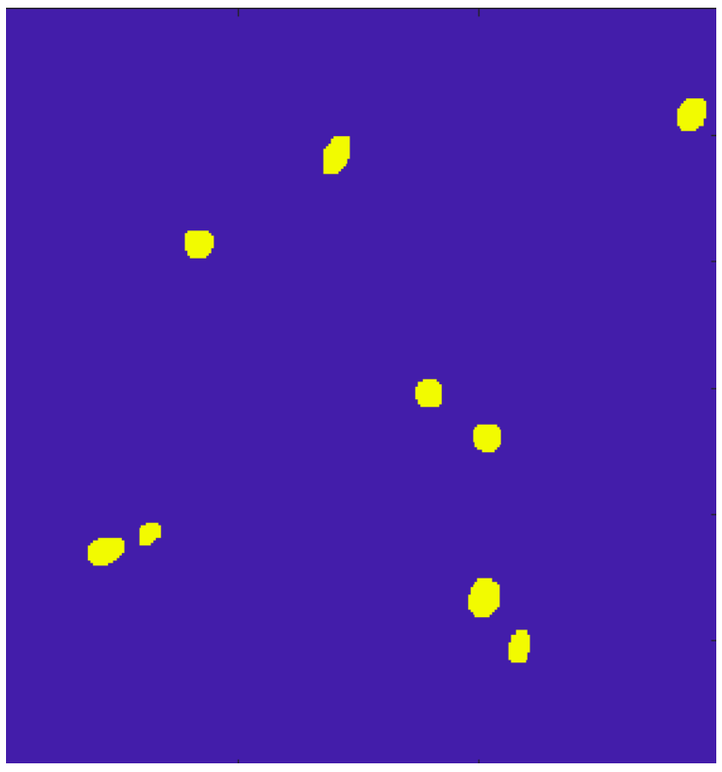
input



erosion

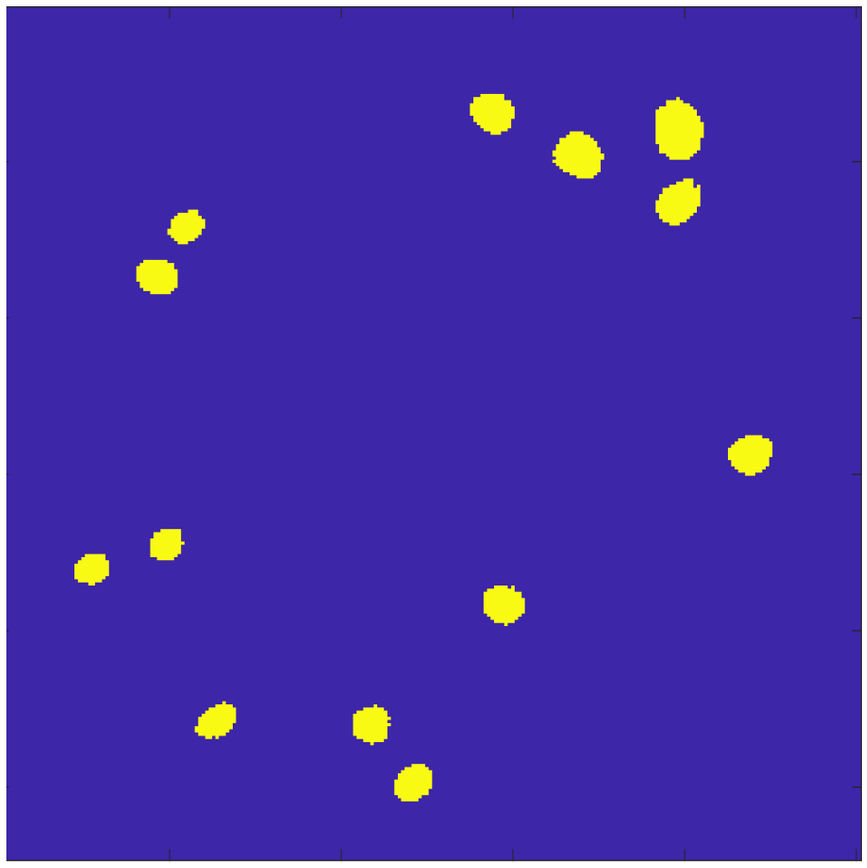


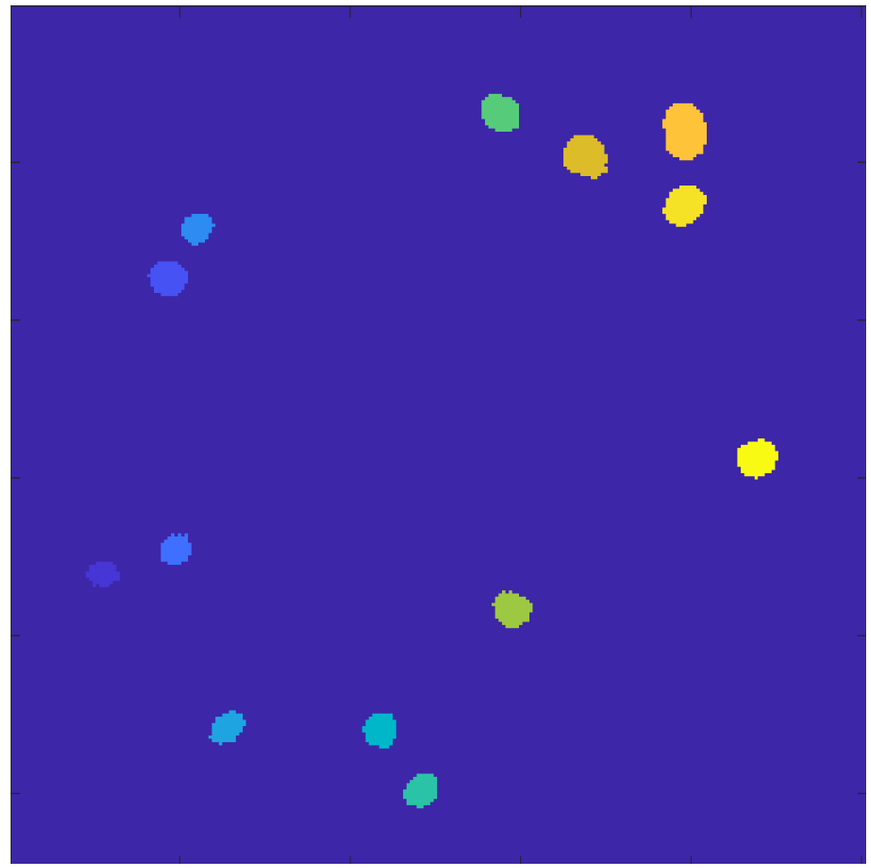
dilation



Next steps

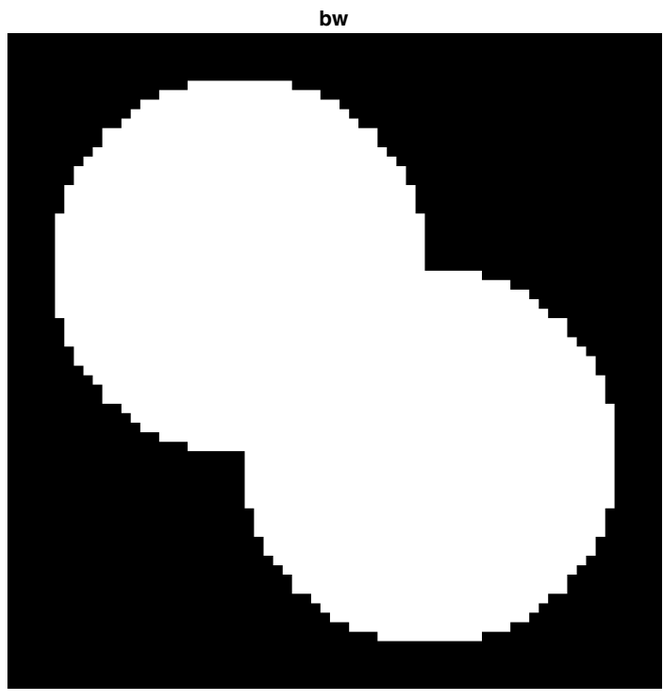
Identify individual objects

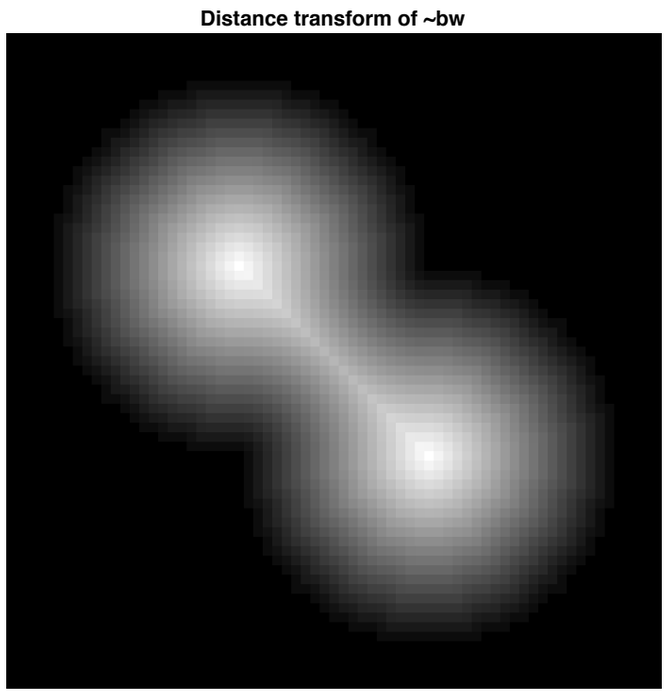


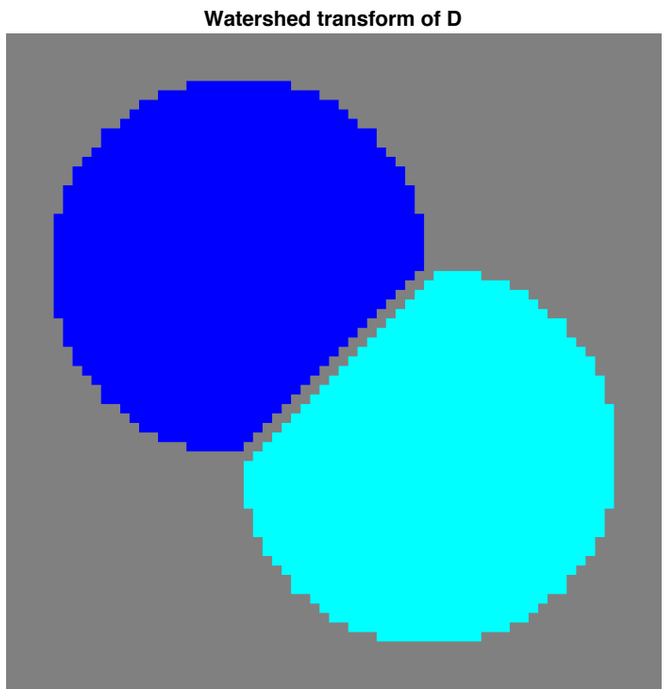


Next steps

Pb touching objects… watershed transforms



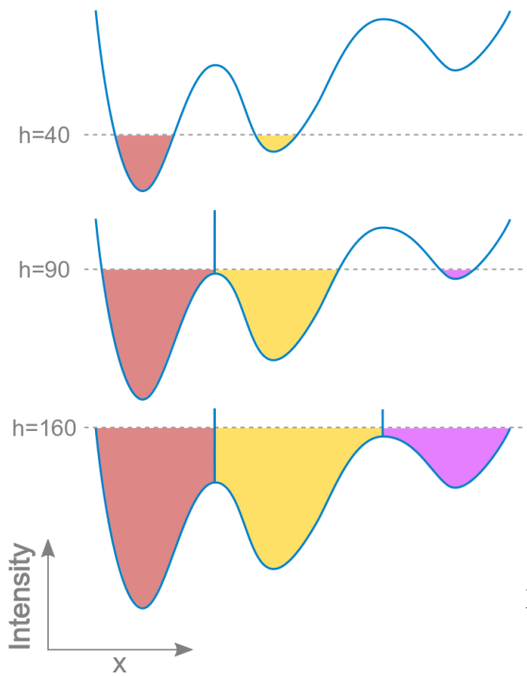




The Mathworks

Distance transform

Watershed



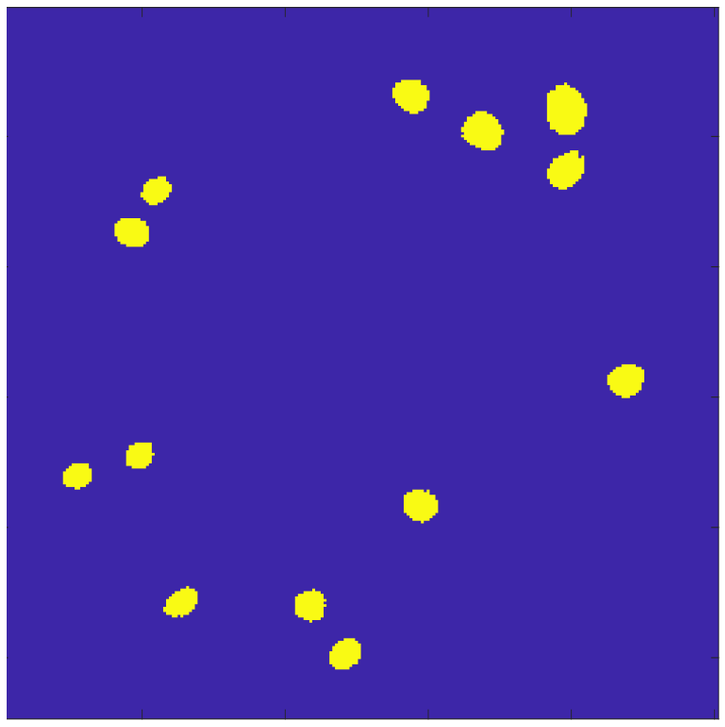
imagej.net/

Real world example…

Feature Measurements

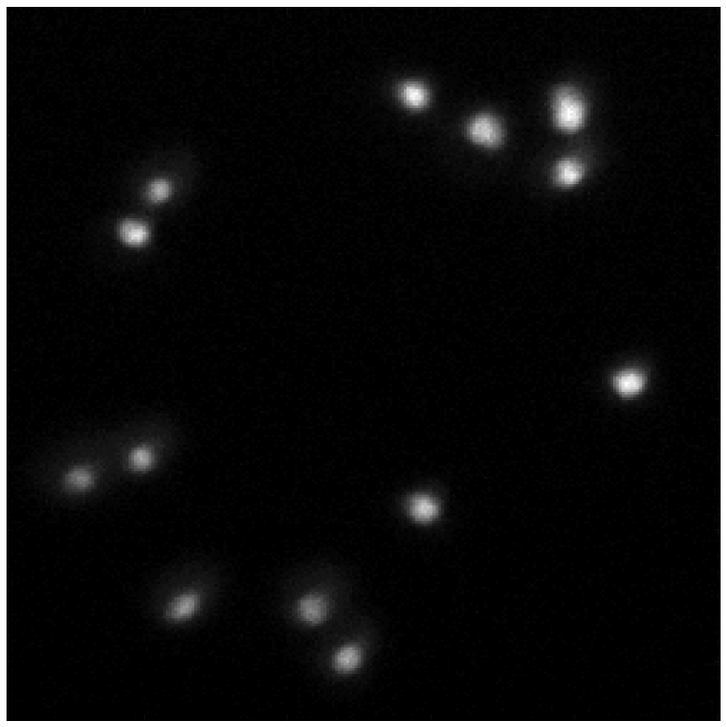
Cell geometry

- Area
- Major / Minor axis
- Angle
- Perimeter
- Solidity
- …



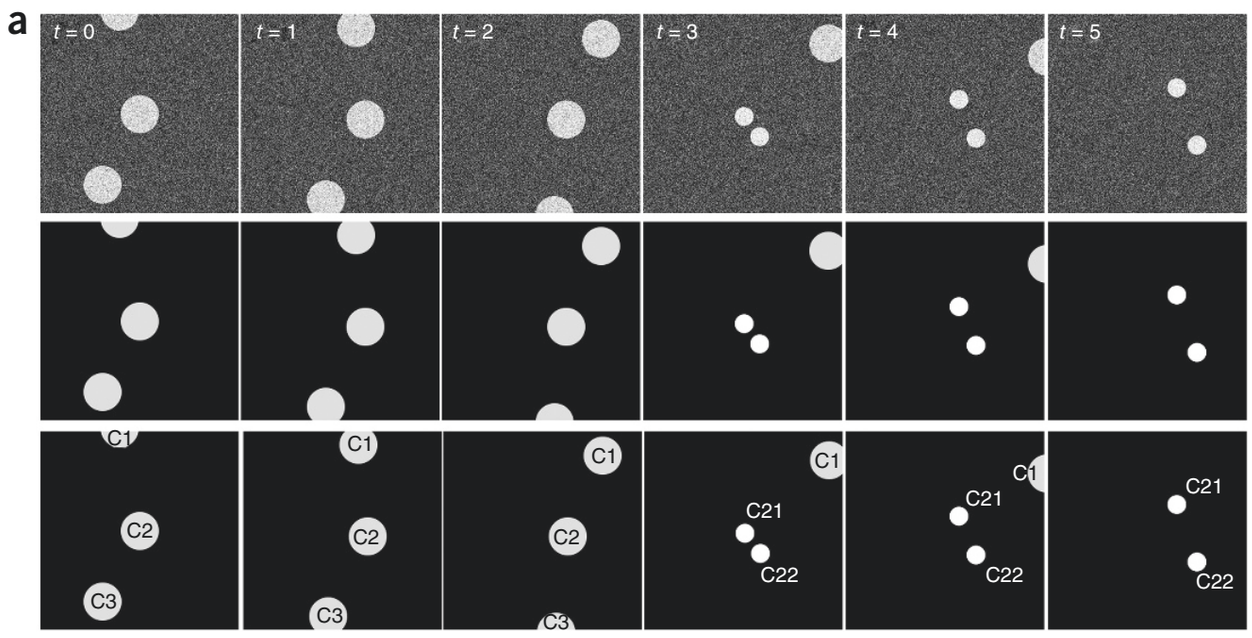
Cell Intensity

- Mean intensity
- Max / Min intensity
- Standard Deviation
- Texture
- Entropy
- …



- Multiple channels
- Cellular regions: Cytoplasm vs nucleus
- …

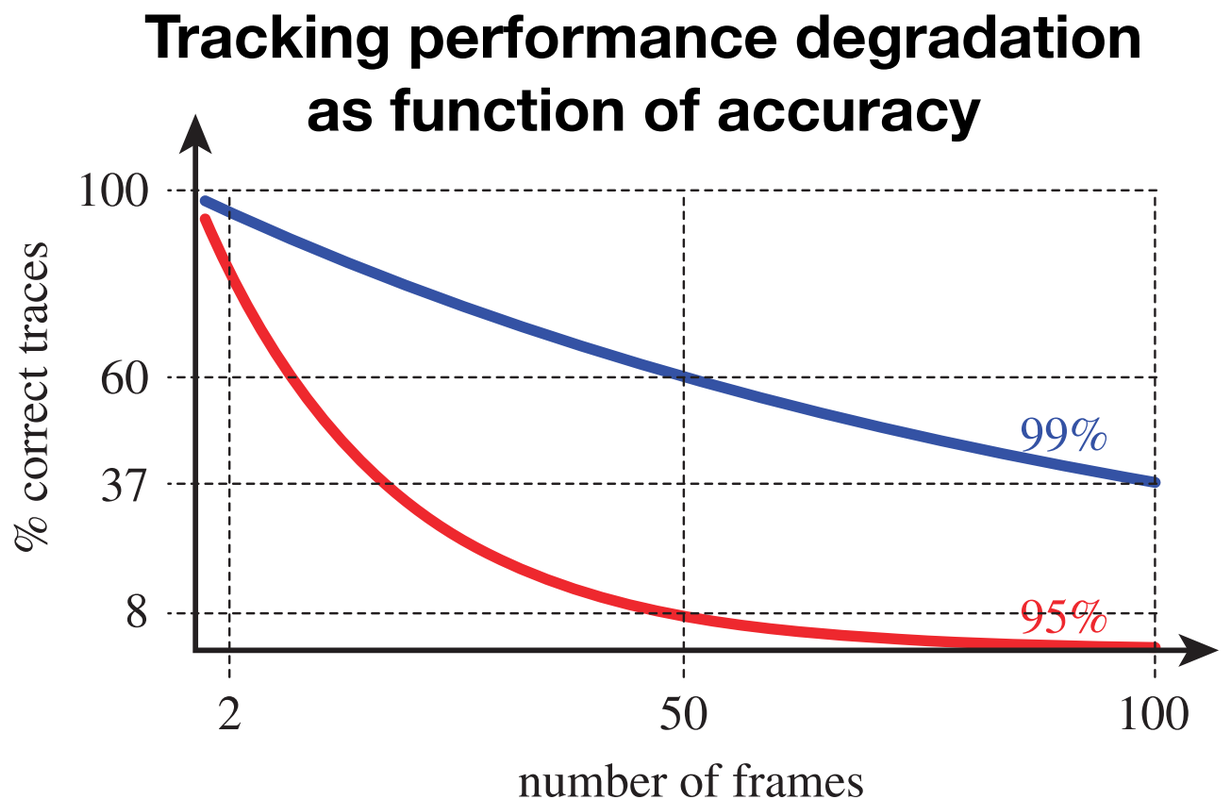
Tracking



Ulman et al. Nature Methods, 2017

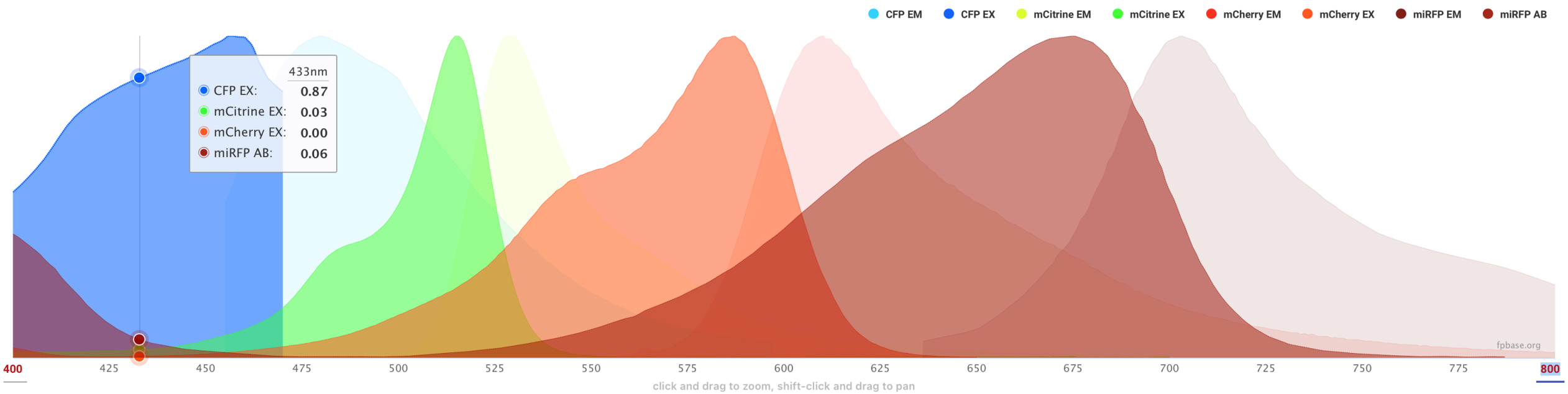
Tracking: following objects from one frame to the next

- Movement
- Appearance
- Disappearance
- Division
- Fusion
- Genealogy
- …



Versari et al. Journal of The Royal Society Interface, 2017

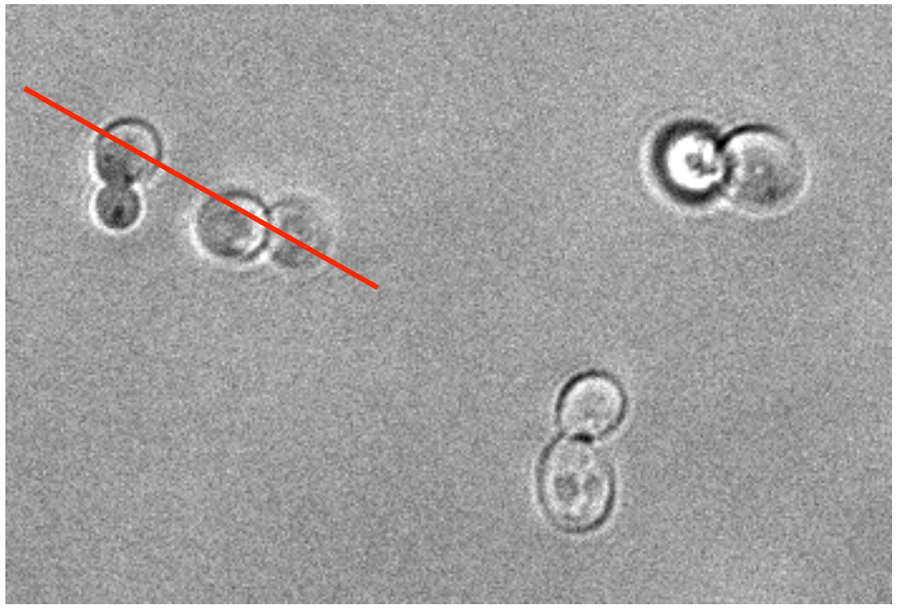
Fluorescent proteins

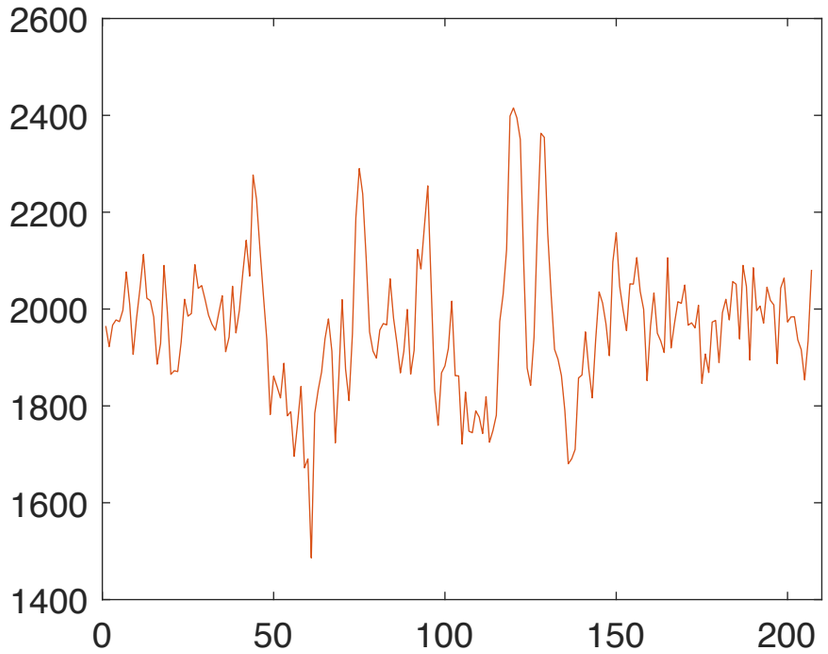


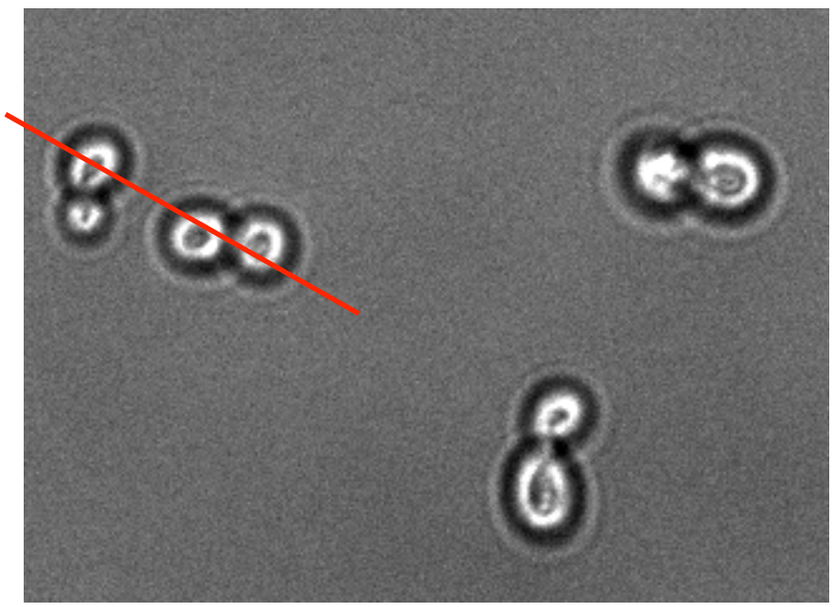
fpbase.org

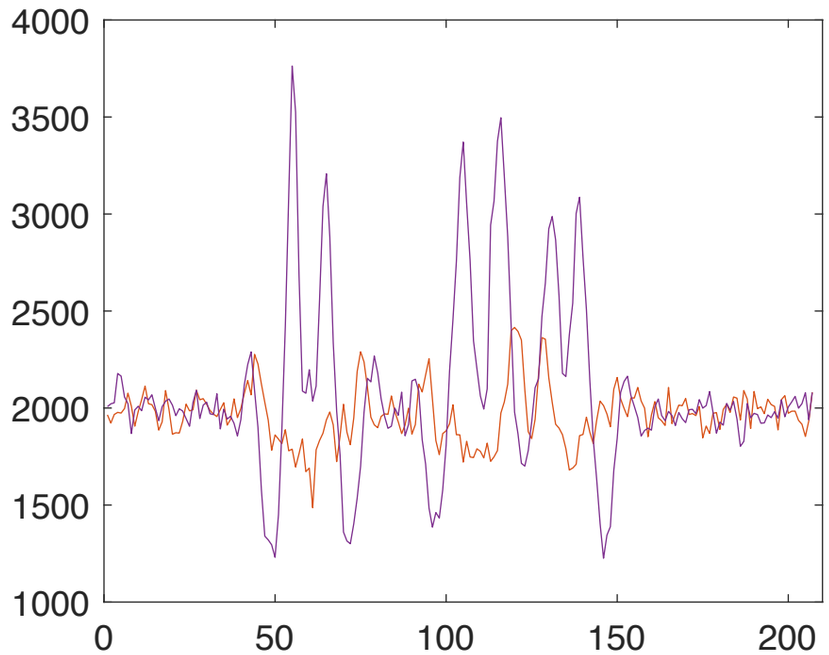
Fluorescent proteins have very wide spectra
=> difficult to combine more than 4 in the same experiment
=> Avoid using fluorescence for segmentation

Transmission Image







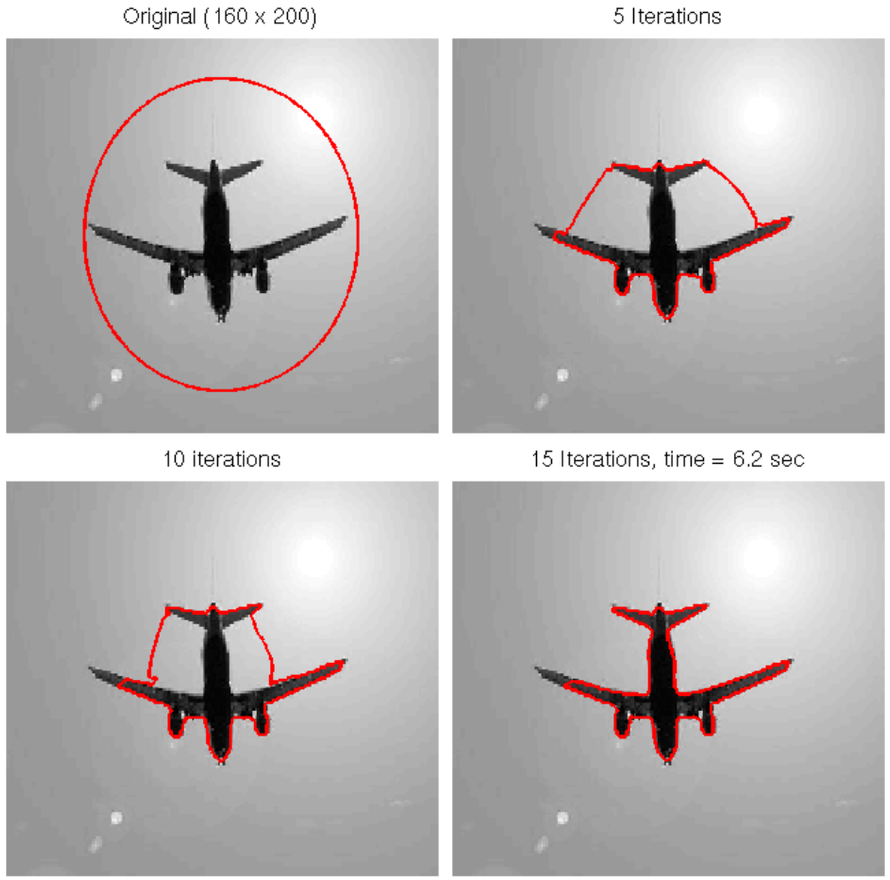


Small demo…

Other approaches

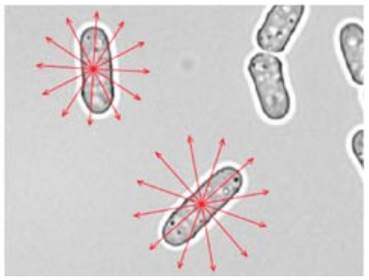
Snakes
Active contours

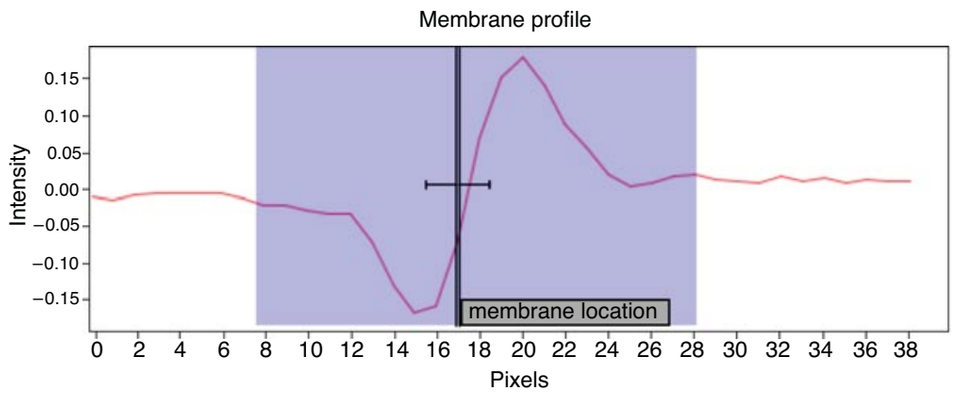
Figure 6: Segmentation of a test image using GAC/snakes. Results are shown at 4 different stages of the segmentation.



Goldstein, J Sci Comput (2010)

Graph cuts





Mayer et al. Current Protocols in Molecular Biology 2001

Artificial Intelligence

ImageNet 80’000 “words” 500-1000 images per word

mammal ⟶ placental ⟶ carnivore ⟶ canine ⟶ dog ⟶ working dog ⟶ husky

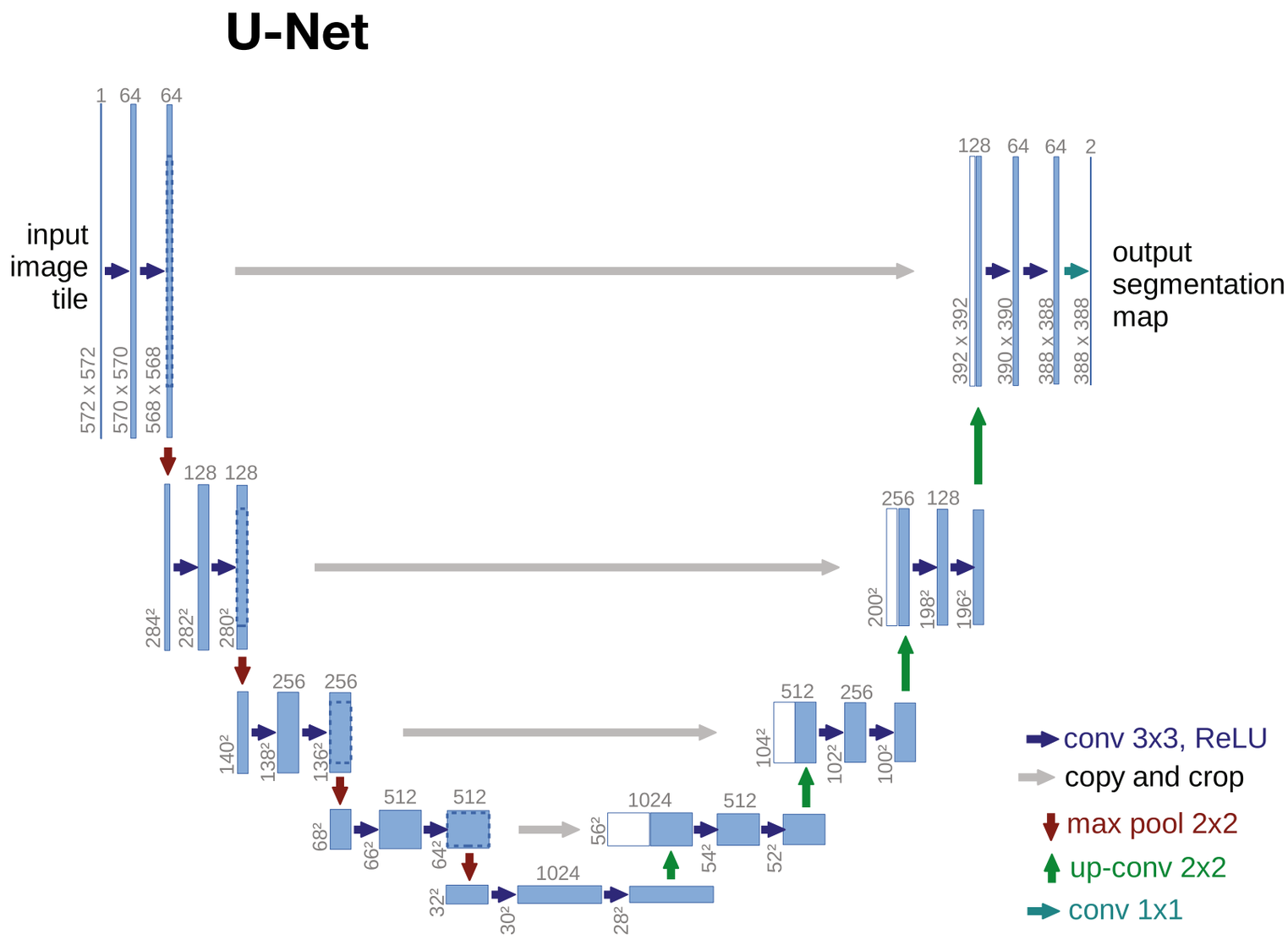
J. Deng et al. IEEE Computer Vision and Pattern Recognition, 2009

from 30% errors to 3% error in 6 years

- Large diversity in images improves training
- Great increase in accuracy from Neural Networks

Testing GoogLeNet…

Neural network

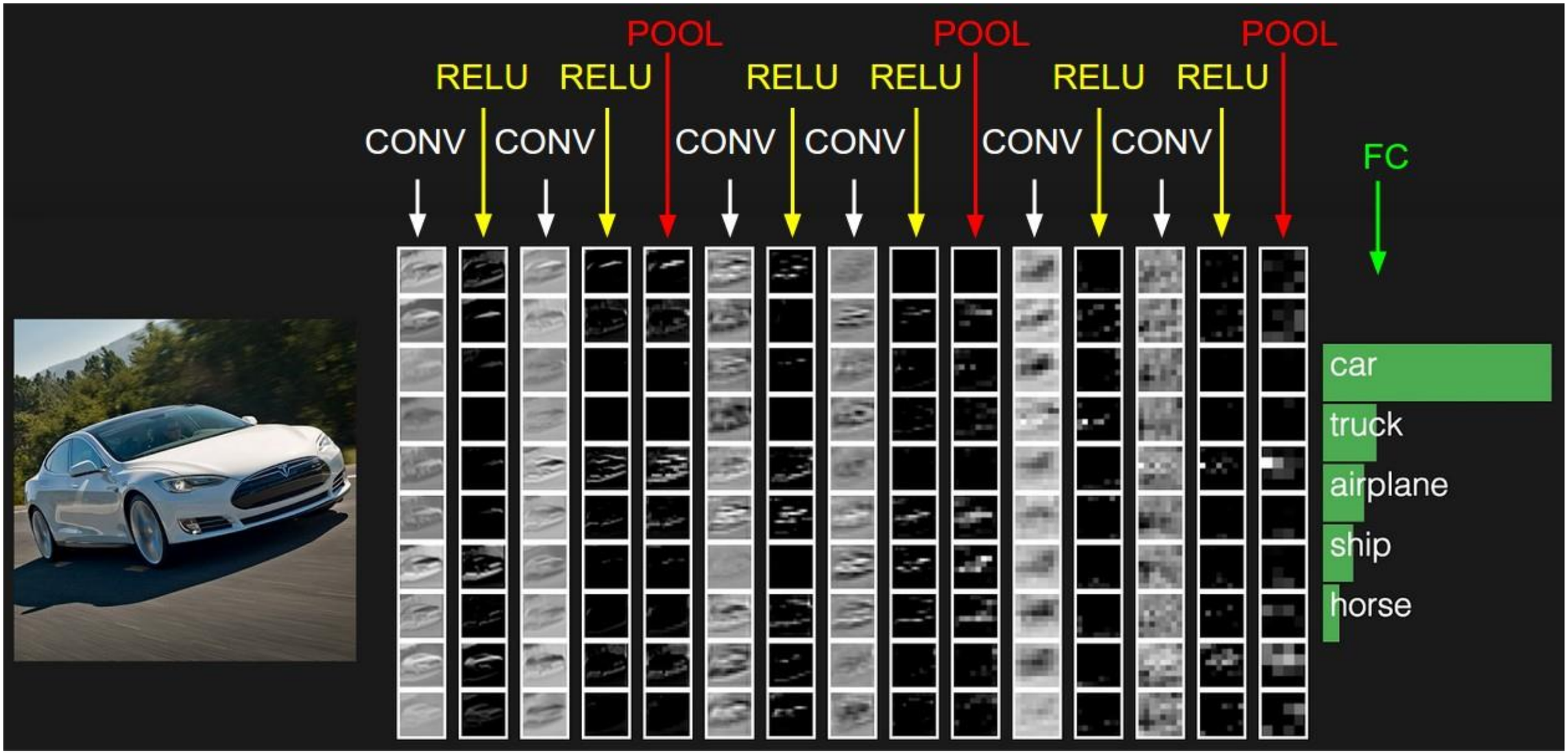


Ronneberger et al. Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015, 2015

23 Layers:

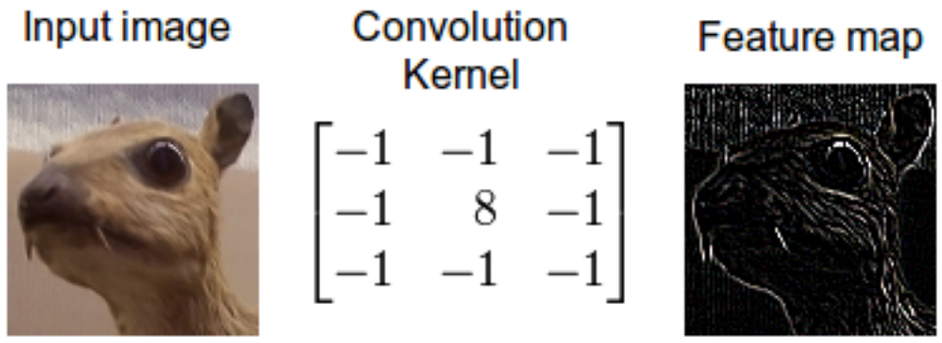
- convolution
- pooling
- Rectified Linear Unit (ReLU)

Neural network



courses.cs.washington.edu/

- convolution
- pooling
- Rectified Linear Unit (ReLU)



developer.nvidia.com

Neural network

Manual training: example

ImageNet:

Amazon mechanical turk:

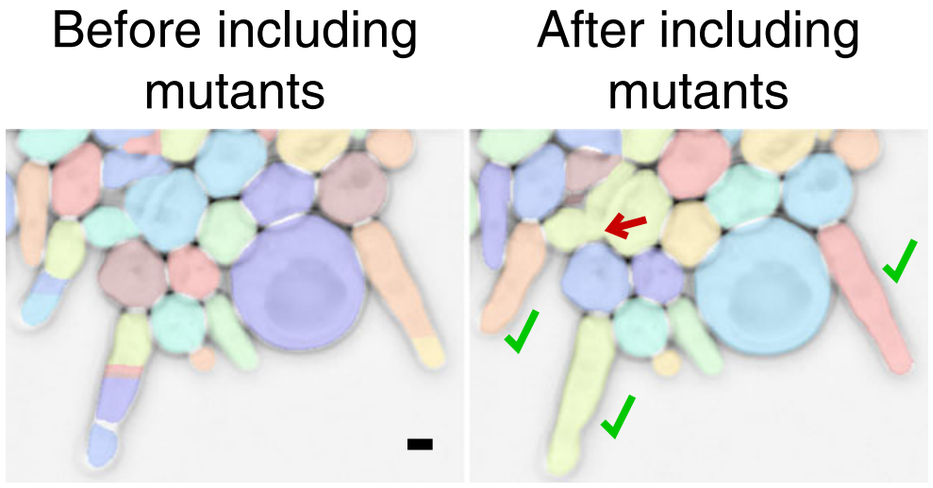
49’000 people during 3 years

YeaZ:

8’500 segmented yeast cells

Including mutants

Trained on phase contrast images

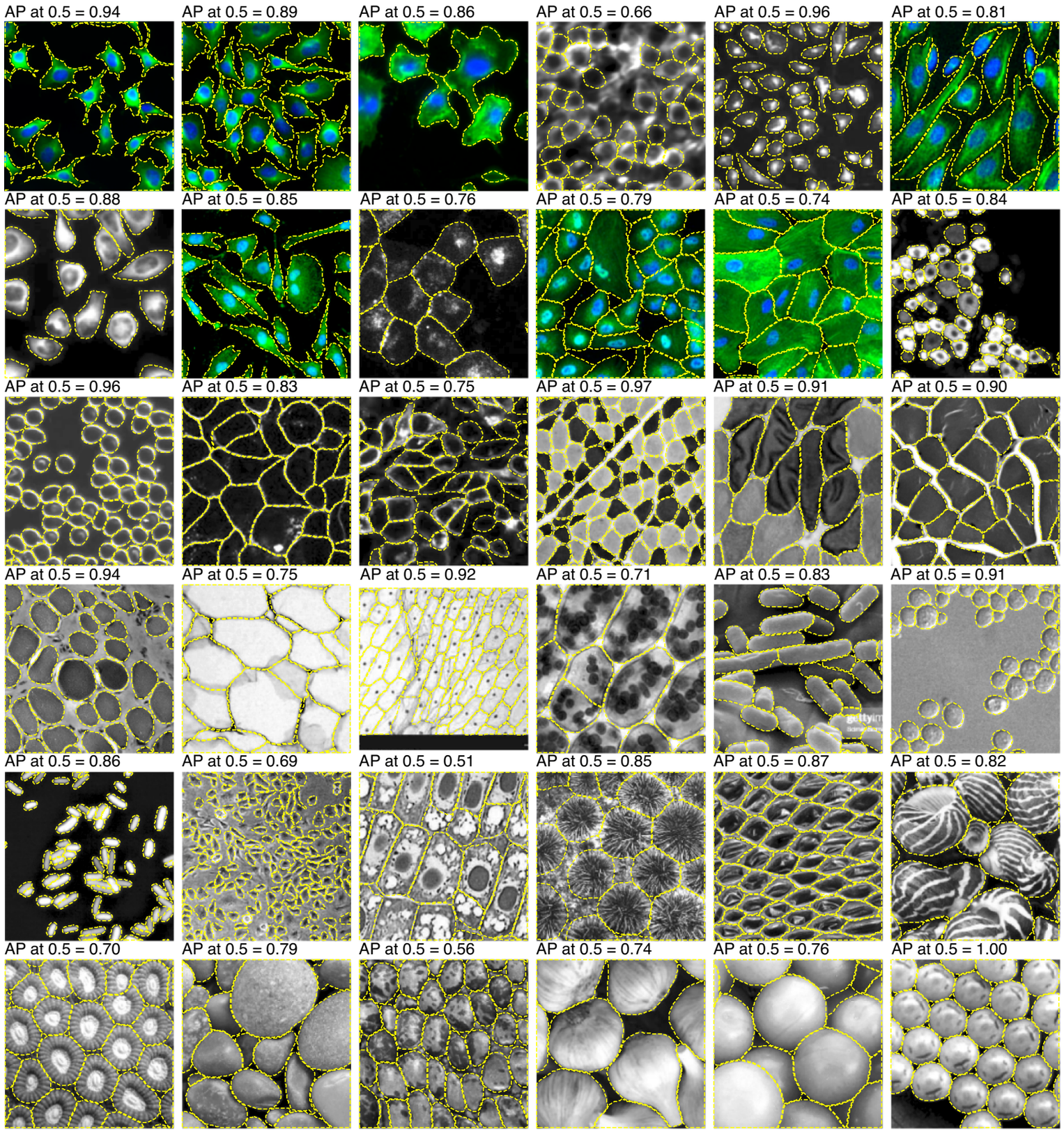


Dietler et al. Nature Communications, 2020

Data augmention

https://medium.com/analytics-vidhya/data-augmentation-in-deep-learning-3d7a539f7a28

CellPose



- 600 image
- Microscopy
  - Transmission
  - Fluorescence
    - Nucleus
    - Membrane
- non-microscopy

Stringer et al. Nature Methods, 2021

Conclusions

Manual segmentation
- Precision but low throughput

Algorithmic segmentation
- High throughput
- No perfect algorithm
- Lacks flexibility

AI-based segmentation
- High throughput
- Impressive performance
- Manual training!

Other uses of AI in image analysis:
- data improvements
- classification

Reviews: “AI segmentation”

von Chamier, L., Laine, R. F. & Henriques, R. Artificial intelligence for microscopy: what you should know. *Biochemical Society Transactions* **47**, 1029–1040 (2019).

Meijering, E. A bird’s-eye view of deep learning in bioimage analysis. *Computational and Structural Biotechnology Journal* **18**, 2312–2325 (2020).

Moen, E. *et al*. Deep learning for cellular image analysis. *Nat Methods* **16**, 1233–1246 (2019).

Example: “AI segmentation”

Dietler, N. *et al*. A convolutional neural network segments yeast microscopy images with high accuracy. *Nature Communications* **11**, 5723 (2020).

Stringer, C., Wang, T., Michaelos, M. & Pachitariu, M. Cellpose: a generalist algorithm for cellular segmentation. *Nat Methods* **18**, 100–106 (2021).

Stylianidou, S., Brennan, C., Nissen, S. B., Kuwada, N. J. & Wiggins, P. A. *SuperSegger*: robust image segmentation, analysis and lineage tracking of bacterial cells: Robust segmentation and analysis of bacteria. *Molecular Microbiology* **102**, 690–700 (2016).

Van Valen, D. A. *et al*. Deep Learning Automates the Quantitative Analysis of Individual Cells in Live-Cell Imaging Experiments. *PLoS Comput. Biol*. **12**, e1005177-24 (2016).

Reviews: “algorithmic segmentation”

Meijering, E. Cell Segmentation: 50 Years Down the Road [Life Sciences]. *IEEE Signal Processing Magazine* **29**, 140–145 (2012).

Wiesmann, V. et al. Review of free software tools for image analysis of fluorescence cell micrographs: REVIEW OF FREE SOFTWARE TOOLS FOR IMAGE ANALYSIS OF FLUORESCENCE CELL MICROGRAPHS. Journal of Microscopy 257, 39–53 (2015).

Example: “algorithmic segmentation”

Carpenter, A. E. *et al*. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* **7**, R100 (2006).

Chernomoretz, A., Bush, A., Yu, R., Gordon, A. & Colman-Lerner, A. Using Cell-ID 1.4 with R for microscope-based cytometry. *Curr Protoc Mol Biol* **Chapter 14**, Unit 14.18 (2008).

Wood, N. E. & Doncic, A. A fully-automated, robust, and versatile algorithm for long-term budding yeast segmentation and tracking. *PLoS ONE* **14**, e0206395 (2019).