

Image Processing III-IV



2025
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Innovative Biotechnological Approaches for Biofilm

Curso Binacional Uruguay – Chile 2025

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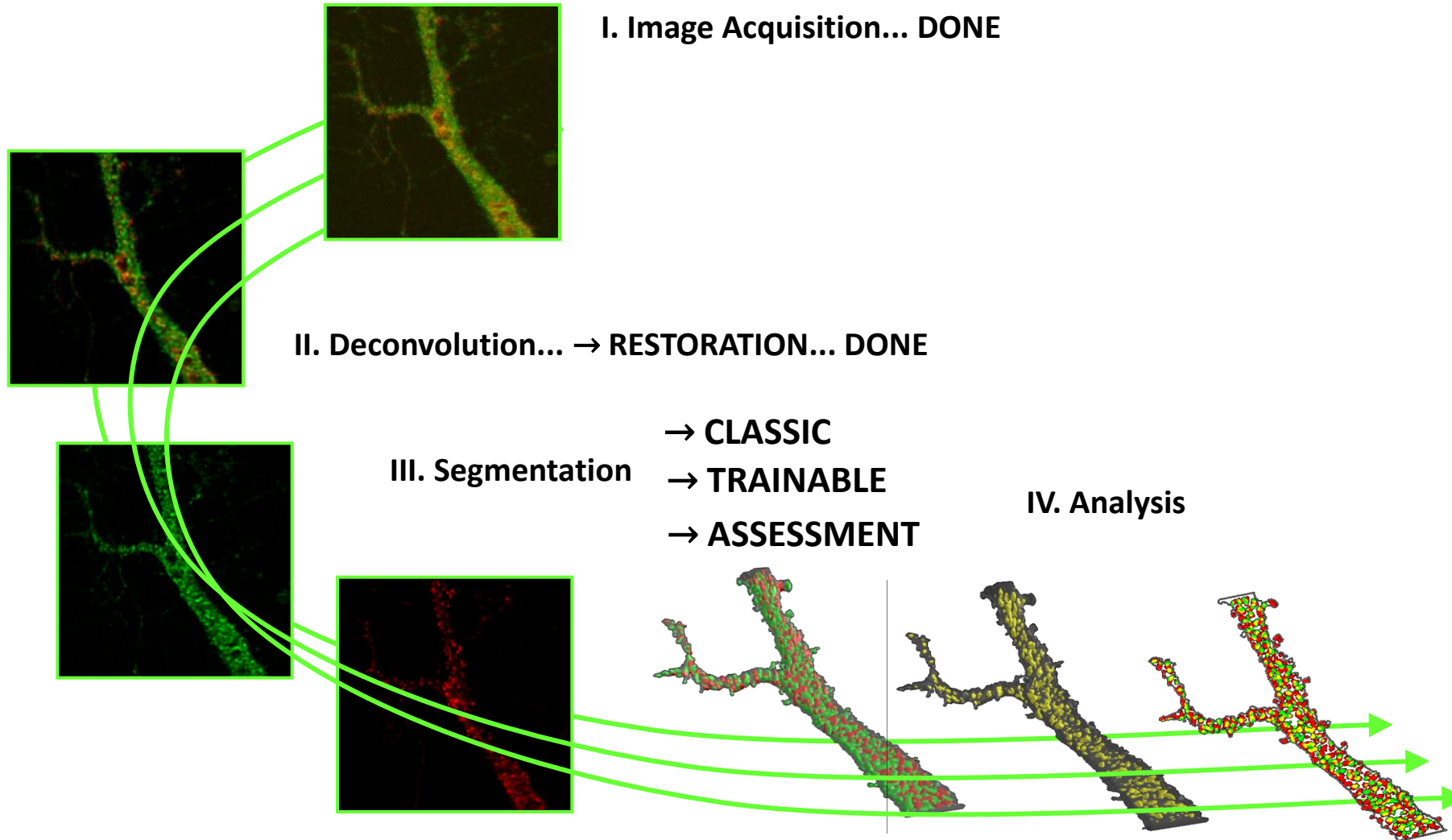
Laboratory for Scientific Image Analysis (SCIAN-Lab)
Centro de Informática Médica y Telemedicina (CIMT)
Biomedical Neuroscience Institute (BNI)
Instituto de Ciencias Biomédicas (ICBM)
www.scian.cl / www.cimt.cl



Thanks / Bedankt



Outline



Outline

- I. Similarity Descriptors, Quality Indices... and Checklists!
- II. Processing
 - Trainable Segmentation
- III. Software Tools

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About: Indicators (Metrics) & Checklists

[nature](#) > [nature methods](#) > [perspectives](#) > article

Perspective | Published: 14 September 2023

Community-developed checklists for publishing images and image analyses

[Christopher Schmied](#) , [Michael S. Nelson](#), [Sergiy Avilov](#), [Gert-Jan Bakker](#), [Cristina Bertocchi](#), [Johanna Bischof](#), [Ulrike Boehm](#), [Jan Brocher](#), [Mariana T. Carvalho](#), [Catalin Chiritescu](#), [Jana Christopher](#), [Beth A. Cimini](#), [Eduardo Conde-Sousa](#), [Michael Ebner](#), [Rupert Ecker](#), [Kevin Eliceiri](#), [Julia Fernandez-Rodriguez](#), [Nathalie Gaudreault](#), [Laurent Gelman](#), [David Grunwald](#), [Tingting Gu](#), [Nadia Halidi](#), [Mathias Hammer](#), [Matthew Hartley](#), ... [Helena Klara Jambor](#)  [+ Show authors](#)

[Nature Methods](#) **21**, 170–181 (2024) | [Cite this article](#)

33k Accesses | **19** Citations | **456** Altmetric | [Metrics](#)

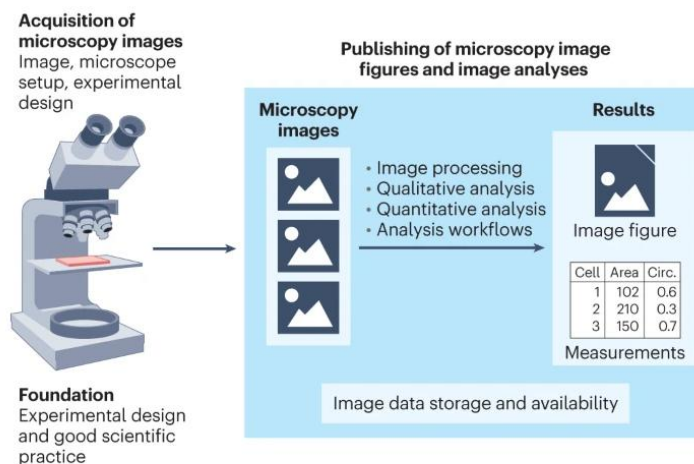
Abstract

Images document scientific discoveries and are prevalent in modern biomedical research. Microscopy imaging in particular is currently undergoing rapid technological advancements. However, for scientists wishing to publish obtained images and image-analysis results, there are currently no unified guidelines for best practices. Consequently, microscopy images and image data in publications may be unclear or difficult to interpret. Here, we present community-developed checklists for preparing light microscopy images and describing image analyses for publications. These checklists offer authors, readers and publishers key recommendations for image formatting and annotation, color selection, data availability and reporting image-analysis workflows. The goal of our guidelines is to increase the clarity and reproducibility of image figures and thereby to heighten the quality and explanatory power of microscopy data.

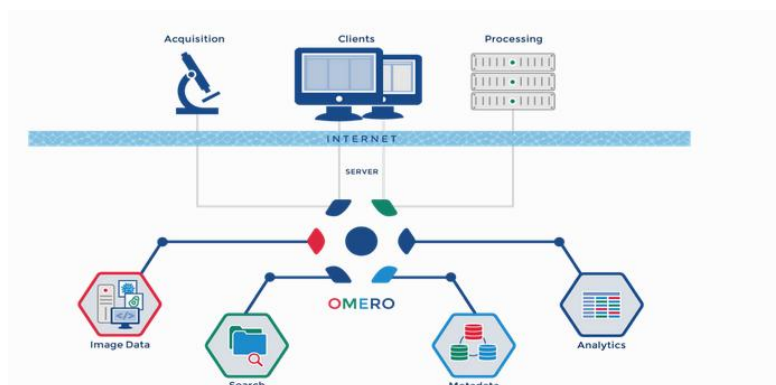
- <https://doi.org/10.1038/s41592-023-01987-9>

Fig. 1: Scope of the checklists.

Community-developed checklists for publishing images and image analyses



The checklists present easy-to-use guidelines for publishing microscopy image figures and image-analysis workflows. The results may include images or measurements of images (for example cell area or cell circularity (circ.)).



- <https://omero.readthedocs.io/en/stable/users/index.html>

[nature](#) > [nature methods](#) > [perspectives](#) > article

Perspective | Published: 12 February 2024

Metrics reloaded: recommendations for image analysis validation

[Lena Maier-Hein](#) , [Annika Reinke](#) , [Patrick Godau](#), [Minu D. Tizabi](#), [Florian Buettner](#), [Evangelia Christodoulou](#), [Ben Glocker](#), [Fabian Isensee](#), [Jens Kleesiek](#), [Michal Kozubek](#), [Mauricio Reyes](#), [Michael A. Riegler](#), [Manuel Wiesenfarth](#), [A. Emre Kavur](#), [Carole H. Sudre](#), [Michael Baumgartner](#), [Matthias Eisenmann](#), [Doreen Heckmann-Nötzel](#), [Tim Radsch](#), [Laura Acion](#), [Michela Antonelli](#), [Tal Arbel](#), [Spyridon Bakas](#), [Arriel Benis](#), ... [Paul F. Jäger](#)  [+ Show authors](#)

[Nature Methods](#) **21**, 195–212 (2024) | [Cite this article](#)

12k Accesses | **227** Altmetric | [Metrics](#)

Abstract

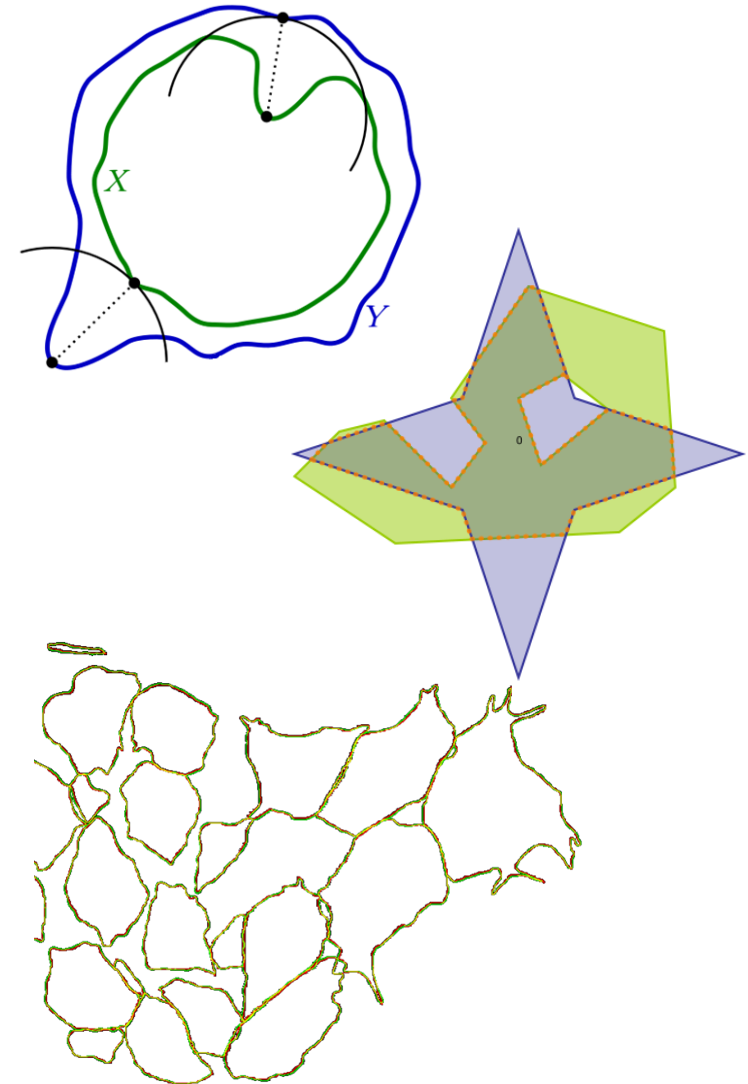
Increasing evidence shows that flaws in machine learning (ML) algorithm validation are an underestimated global problem. In biomedical image analysis, chosen performance metrics often do not reflect the domain interest, and thus fail to adequately measure scientific progress and hinder translation of ML techniques into practice. To overcome this, we created Metrics Reloaded, a comprehensive framework guiding researchers in the problem-aware selection of metrics. Developed by a large international consortium in a multistage Delphi process, it is based on the novel concept of a problem fingerprint—a structured representation of the given problem that captures all aspects that are relevant for metric selection, from the domain interest to the properties of the target structure(s), dataset and algorithm output. On the basis of the problem fingerprint, users are guided through the process of choosing and applying appropriate validation metrics while being made aware of potential pitfalls. Metrics Reloaded targets image analysis problems that can be interpreted as classification tasks at image, object or pixel level, namely image-level classification, object detection, semantic segmentation and instance segmentation tasks. To improve the user experience, we implemented the framework in the Metrics Reloaded online tool. Following the convergence of ML methodology across application domains, Metrics Reloaded fosters the convergence of validation methodology. Its applicability is demonstrated for various biomedical use cases.

- <https://doi.org/10.1038/s41592-023-02151-z> *
- <https://www.nature.com/articles/s41592-023-02151-z>
- <https://europepmc.org/article/MED/38347141>

> Similarity Descriptors

Main categories

- **ROI-ROI distance**
(Hausdorff distance, chamfer distance, mean border distance, etc...)
- **ROI-ROI intersection**
(sensitivity/specificity, Dice/Jaccard coeffs.)
- **ROI features**
(number, shape, feature points/parts, color/texture based like textures or entropy)



> Segmentation Assessment Indicators

- Hausdorff distance (**metric**)

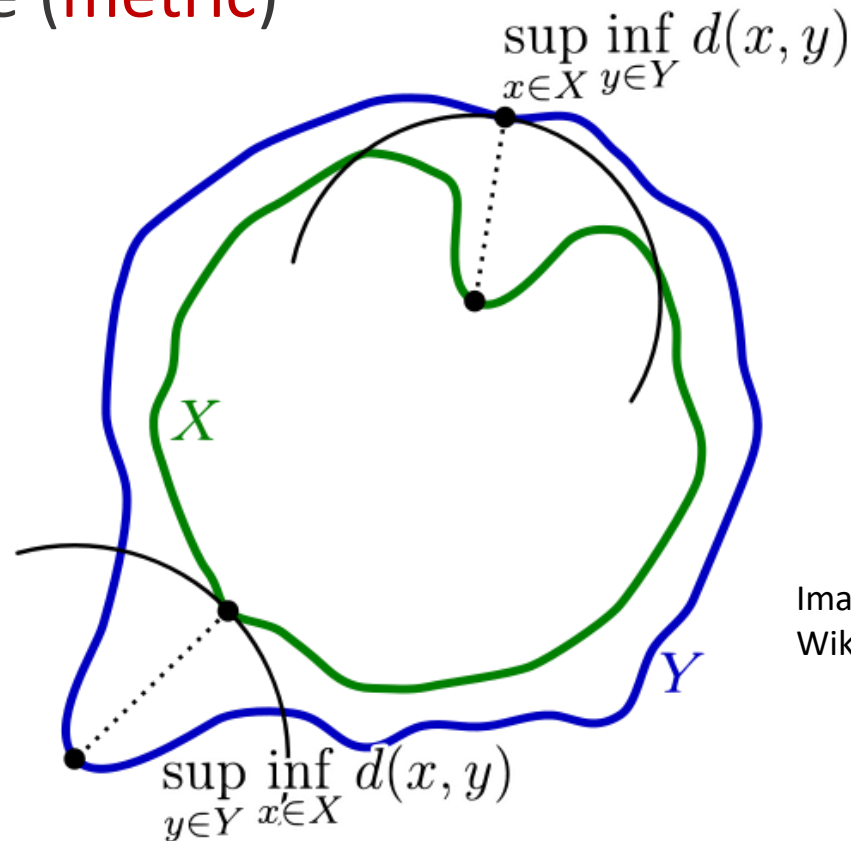
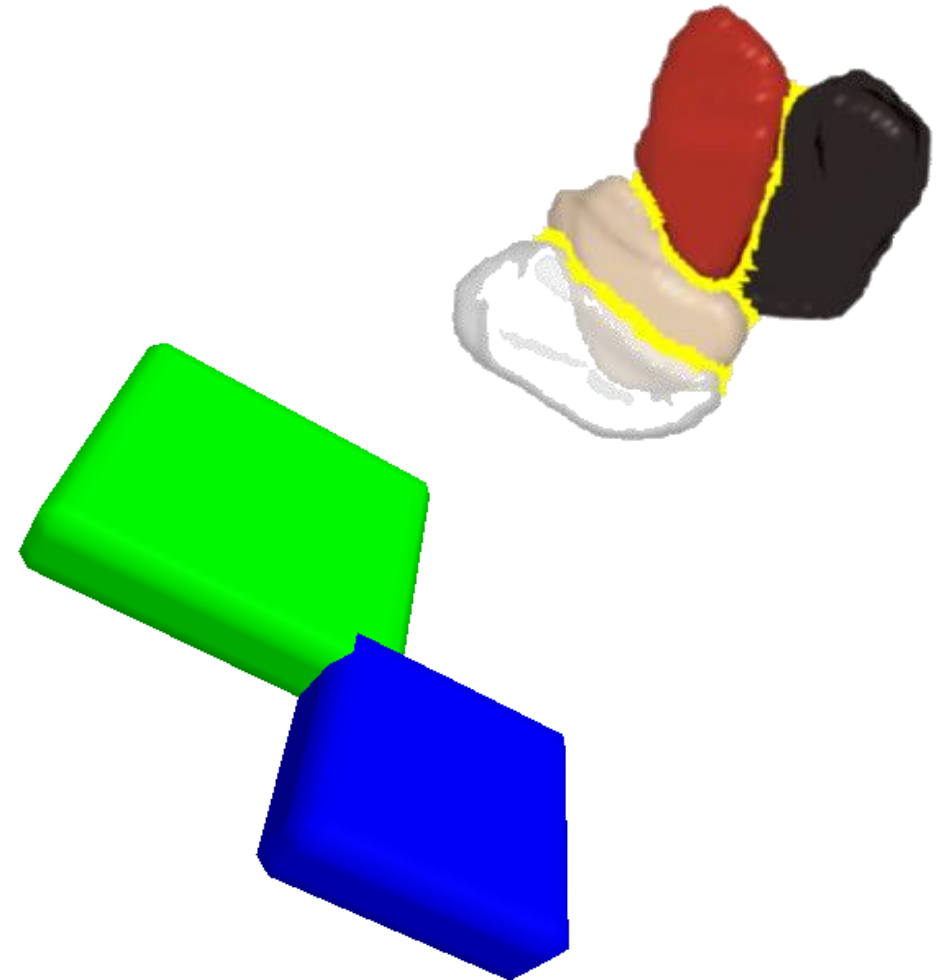


Image source:
Wikimedia commons

$$d_H(X, Y) = d_H(Y, X) = \max \left\{ \sup_{x \in X} \inf_{y \in Y} d(x, y), \sup_{y \in Y} \inf_{x \in X} d(x, y) \right\}$$

> Segmentation Assessment Indicators

- ROI-ROI intersections
 - Computed between the initial user drawings and the segmented objects
 - Pixel(voxel) or polygon(mesh) approaches for computation



> Segmentation Assessment Indicators

- Pixel (voxel) based ROI-ROI intersection
- Quantifiable by boolean AND operation



A (over), B



B (over), A



A **AND** B
(intersection)



A **OR** B
(union)



A **XOR** B

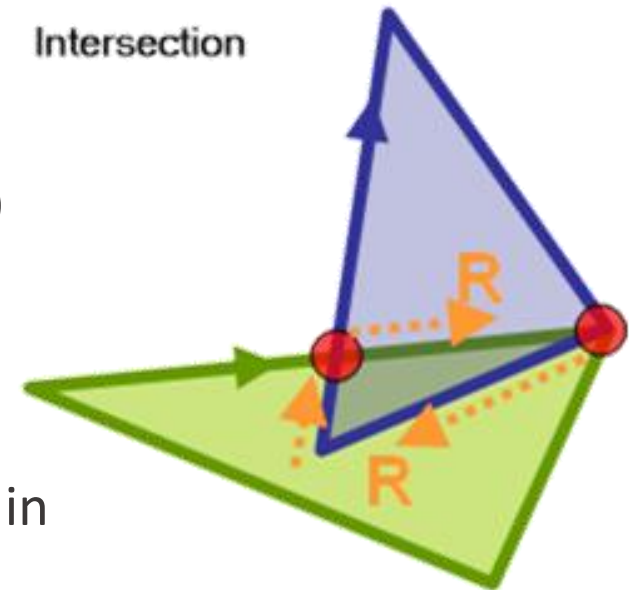
P	Q	P AND Q
0	0	0
0	1 (255)	0
1 (255)	0	0
1 (255)	1(255)	1 (255)

P	Q	P OR Q
0	0	0
0	1 (255)	1 (255)
1 (255)	0	1 (255)
1 (255)	1(255)	1 (255)

P	Q	P XOR Q
0	0	0
0	1 (255)	1 (255)
1 (255)	0	1 (255)
1 (255)	1(255)	0

> Segmentation Assessment Indicators

- 2D polygon ROI-ROI intersection
 - Available algorithms:
 - Weiler-Atherton clipping algorithm (BOOST C++ library)
 - Vatti clipping algorithm (PolyClipper C++/C#, Delphi library)
 - Core:
 - segment-segment intersection
 - polygons represented by a list of consecutive vertices in clockwise/counterclockwise order
 - handling of special cases (intersection is a point, an edge, etc.)



Weiler K & Atherton P. "Hidden Surface Removal using Polygon Area Sorting". Computer Graphics 11(2):214-222, 1977.

http://www.boost.org/doc/libs/1_51_0/libs/geometry/doc/html/geometry/reference/algorithms/intersection.html

<http://barendgehrels.blogspot.com/2010/12/intersections-1.html>

Vatti B. "A generic solution to polygon clipping", Communications of the ACM 35(7): 56-63, 1992.

<https://sourceforge.net/projects/polyclipping/>

> Segmentation Assessment Indicators

Let...

- GT : a ground truth region of interest
- S : the corresponding segmented region
- TP : # of true object pixels *true positives*
- TN : # of true background pixels *true negatives*
- FP : # of false object pixels *false positives*
- FN : # of false background pixels *false negatives*

Assuming that a ground truth segmentation exists,
which is not always feasible (perfect/ideal ROIs)...

> Segmentation Assessment Indicators

Sensitivity (**metric**)

- Fraction of ROI elements (pixels, voxels) correctly detected

$$\text{Sensitivity}(S, GT) = \frac{TP}{TP + FN}$$

Few false negatives →
high sensitivity (near 1)

Do we find all the ROI pixels/voxels?

Specificity (**metric**)

- Fraction of background elements correctly detected

$$\text{Specificity}(S, GT) = \frac{TN}{TN + FP}$$

Few false positives →
high specificity (near 1)

Do we exclude all the non-ROI pixels/voxels?

https://en.wikipedia.org/wiki/Accuracy_and_precision#In_binary_classification

https://en.wikipedia.org/wiki/Precision_and_recall

> Segmentation Assessment Indicators

Jaccard similarity index /
Tanimoto coefficient (**metric**)

Dice coefficient (**metric**)


$$J(S, GT) = \frac{|S \cap GT|}{|S \cup GT|}$$
$$= \frac{TP}{TP + FP + FN}$$


$$DC(S, GT) = \frac{2 \cdot |S \cap GT|}{|S| + |GT|}$$
$$= \frac{2TP}{(FP + TP) + (TP + FN)}$$
$$= \frac{2TP}{2TP + FP + FN}$$



Perfect match (overlap) → Jaccard index = 1, Dice coeff. = 1

No match (overlap) → Jaccard index = 0, Dice coeff. = 0

> Segmentation Assessment Indicators

- NSD: normalized sum of distances (**NOT a metric**)
Accounts for the accumulated distances from non-overlapping pixels/voxels enclosed by contours S and GT .
- Values range within 0 and 1 (from no overlap to perfect match).
- D_k : distance between ROI elements (e.g. polygon segments)

$$\text{NSD}(S, GT) = \frac{\sum_k [S \neq GT] * D_k}{\sum_k D_k}$$

References

- Dice/Jaccard coefficients
 - Dice, Lee R. (1945) Measures of the amount of ecologic association between species. *Ecology* **26**, 297–302.
 - Sørensen, T. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Kong. Danske Vidensk. Selskab* **5**, 1–34.
- Hausdorff distance
 - Atallah, M. J. (1983) A linear time algorithm for the Hausdorff distance between convex polygons. *Info. Process. Lett.* **17**, 207–209.
- NSD
 - Coelho, L. P., Shariff, A., & Murphy, R. F. (2009) Nuclear segmentation in microscope cell images: a hand-segmented dataset and comparison of algorithms. In *Proceedings of the 6th IEEE International Symposium on Biomedical Imaging: From Nano to Macro, ISBI*. Boston, MA, USA. pp. 518–521.

Outline

- I. Similarity Descriptors, Quality Indices... and Checklists!
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- Image processing: parasites

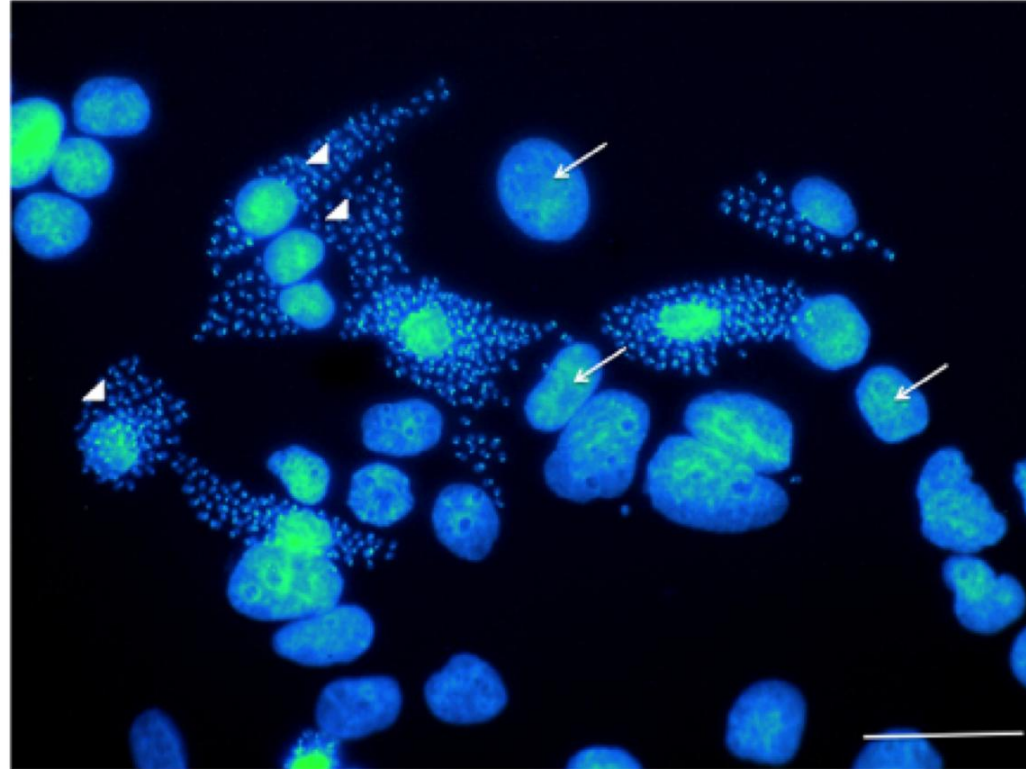
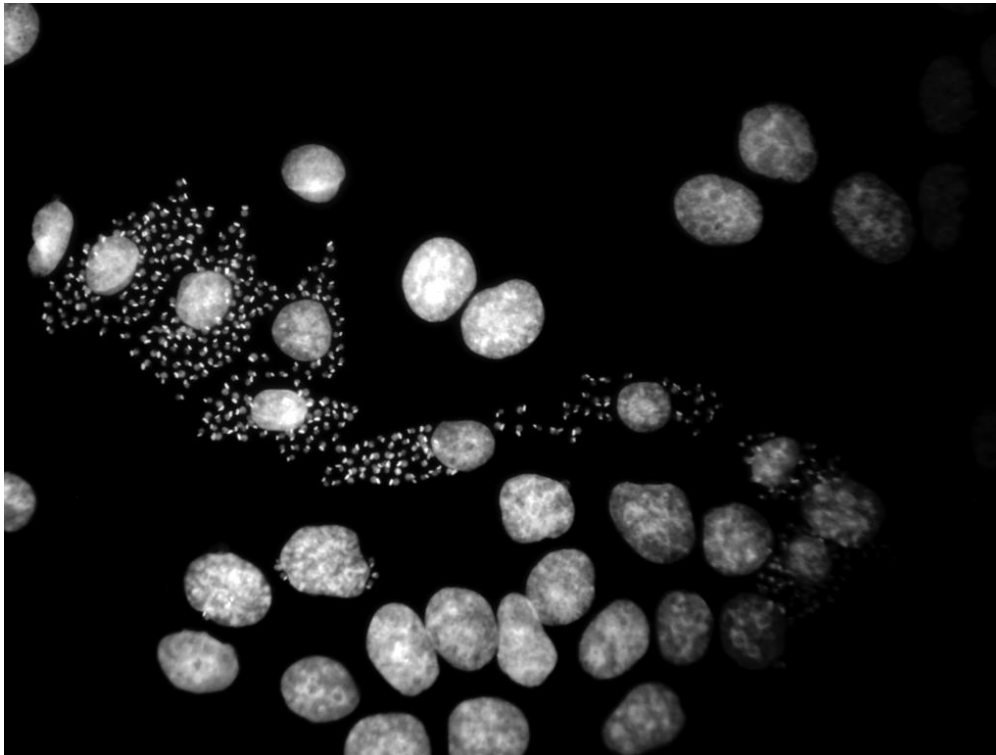


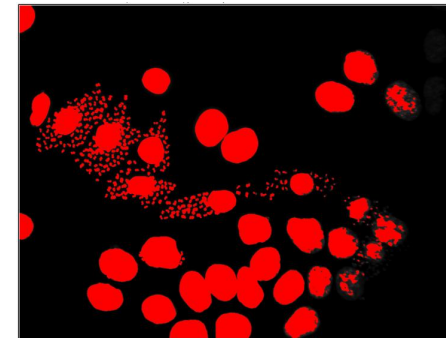
Fig. 1. Infection of BeWo cells with *T. cruzi* amastigotes. BeWo cells were challenged with *T. cruzi* Ypsilon strain trypomastigotes at a parasite:cell ratio of 1:1 for 24 h and were processed for DAPI staining after 48 h. The arrows show BeWo cell nuclei, and the arrowheads show intracellular amastigotes. Scale bar: 10 μm .

- image Segmentation

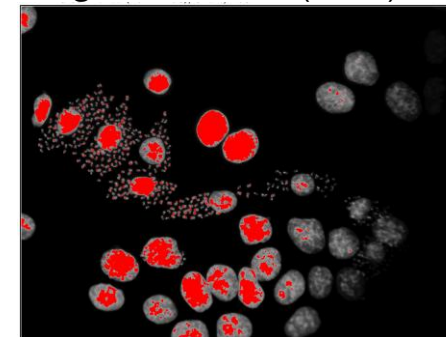
- The simplest segmentation... a manual global threshold



raw image



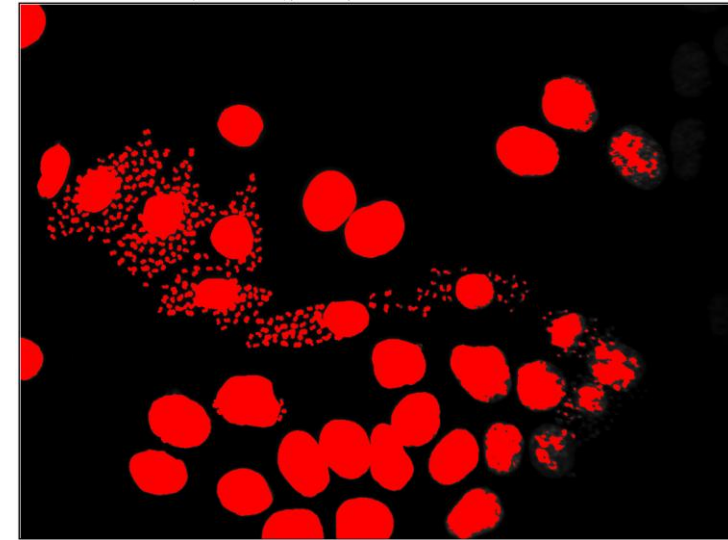
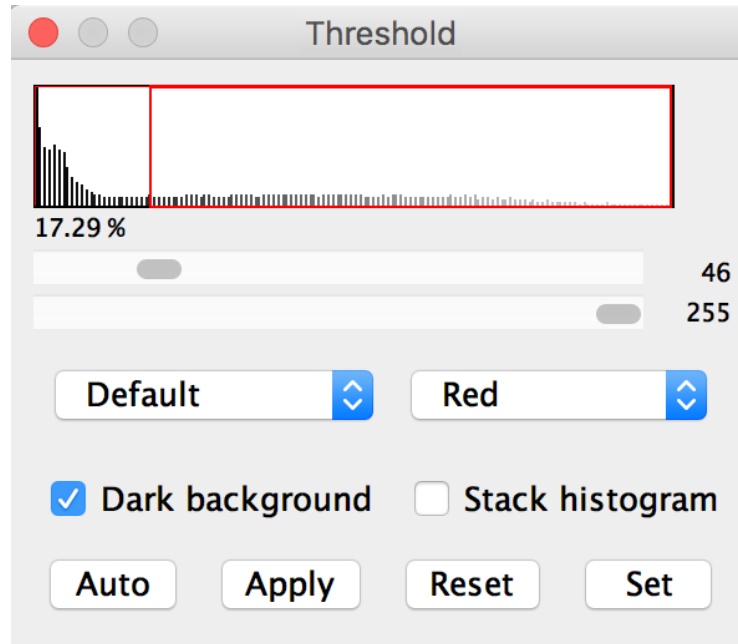
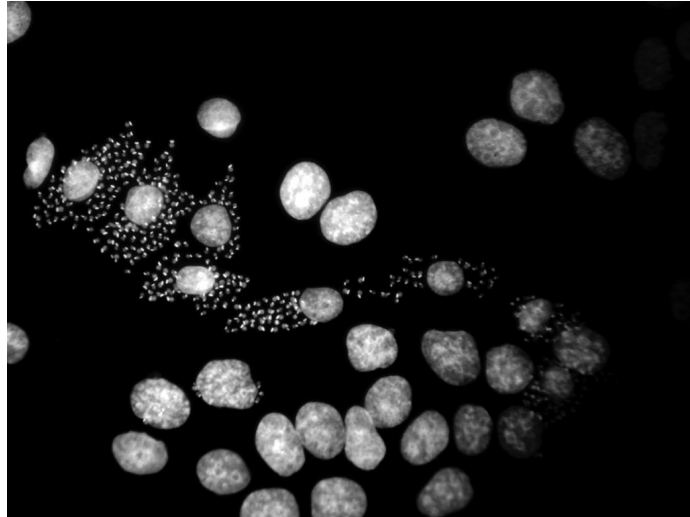
segmentation (>46)



segmentation (>158)

Segmentation

- How to define the threshold ? ...

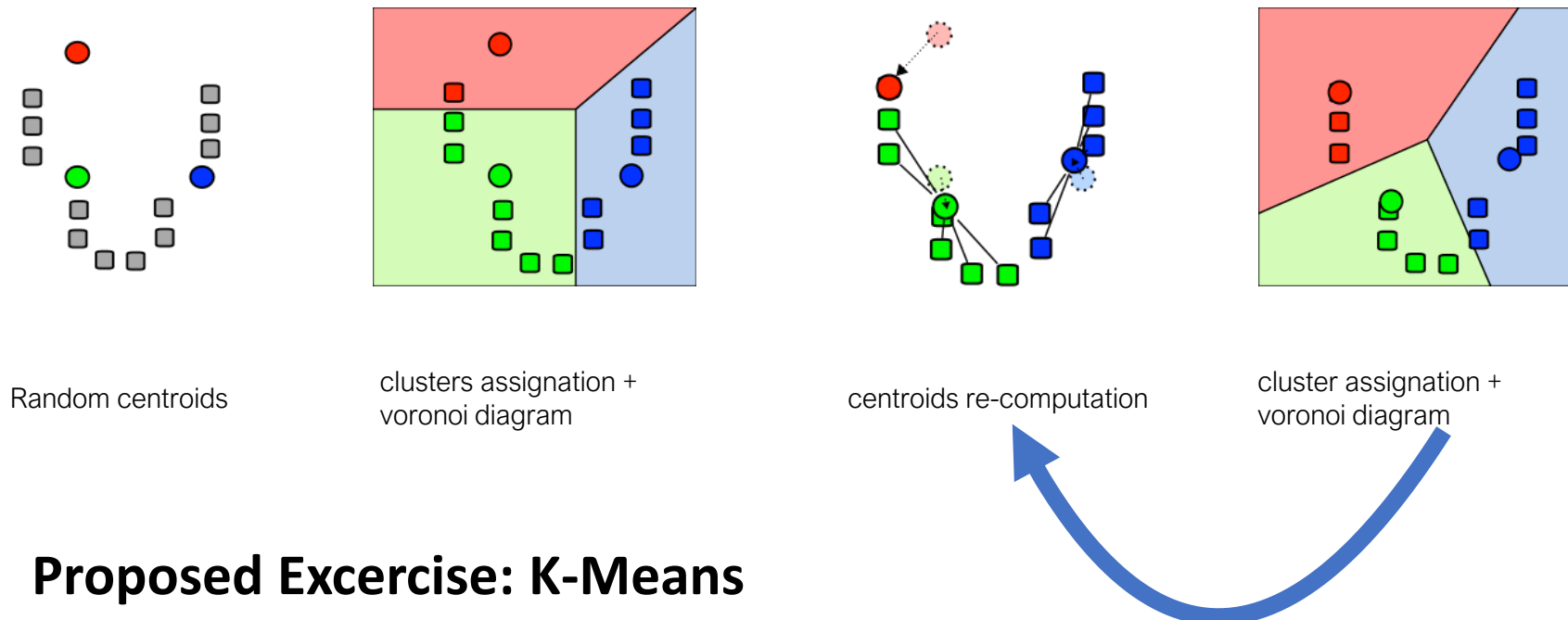


Supervised Segmentation

- We don't have examples (!)
- We know there are two groups (or three?): cells, and background.
- This is another kind of learning problem:
 - Supervised: regression, classification
 - Unsupervised: **clustering**

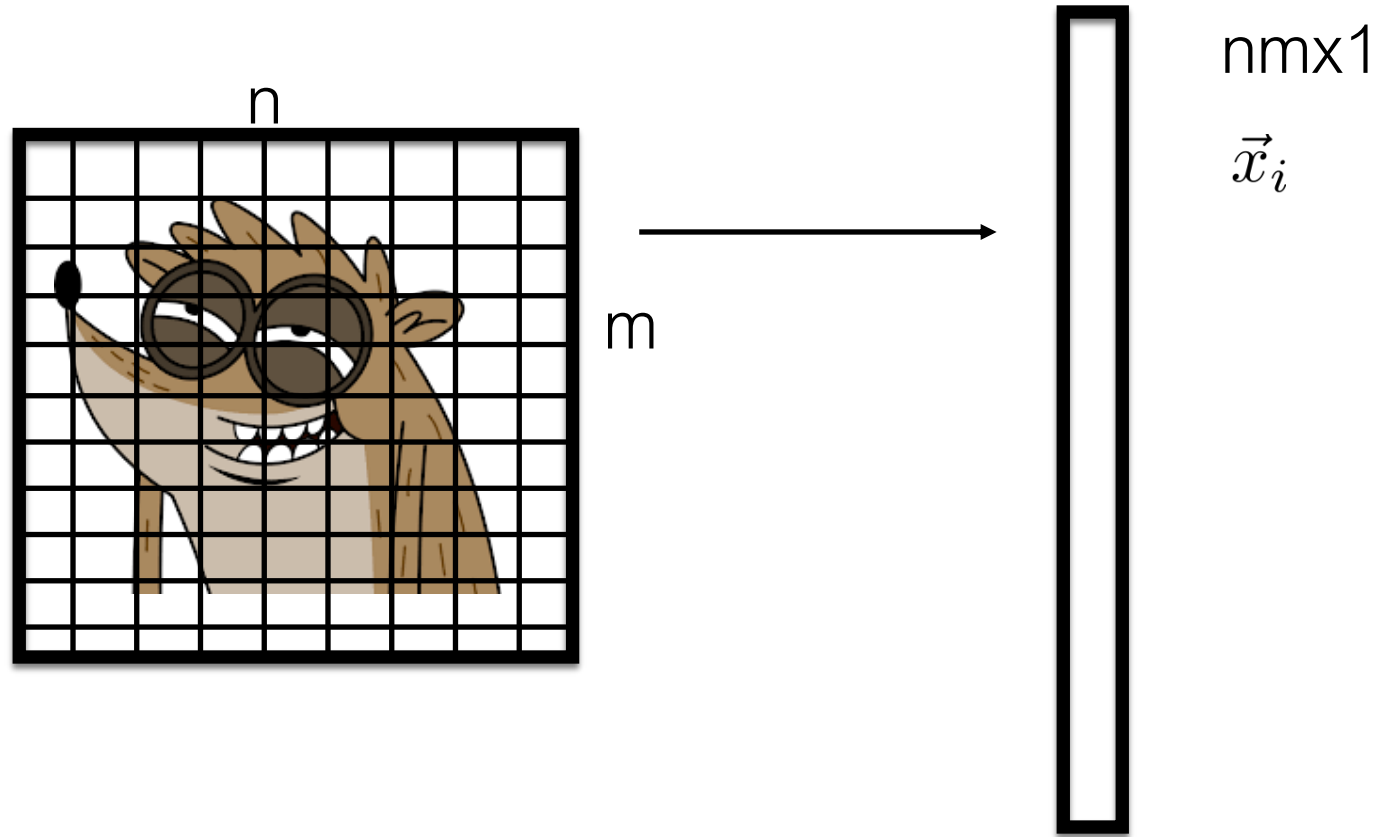
Supervised Segmentation

- image Segmentation: unsupervised approach
 - We can model it as how to discover the best k groups or clusters at a pixel level.
 - K-means clustering ($k=3$):



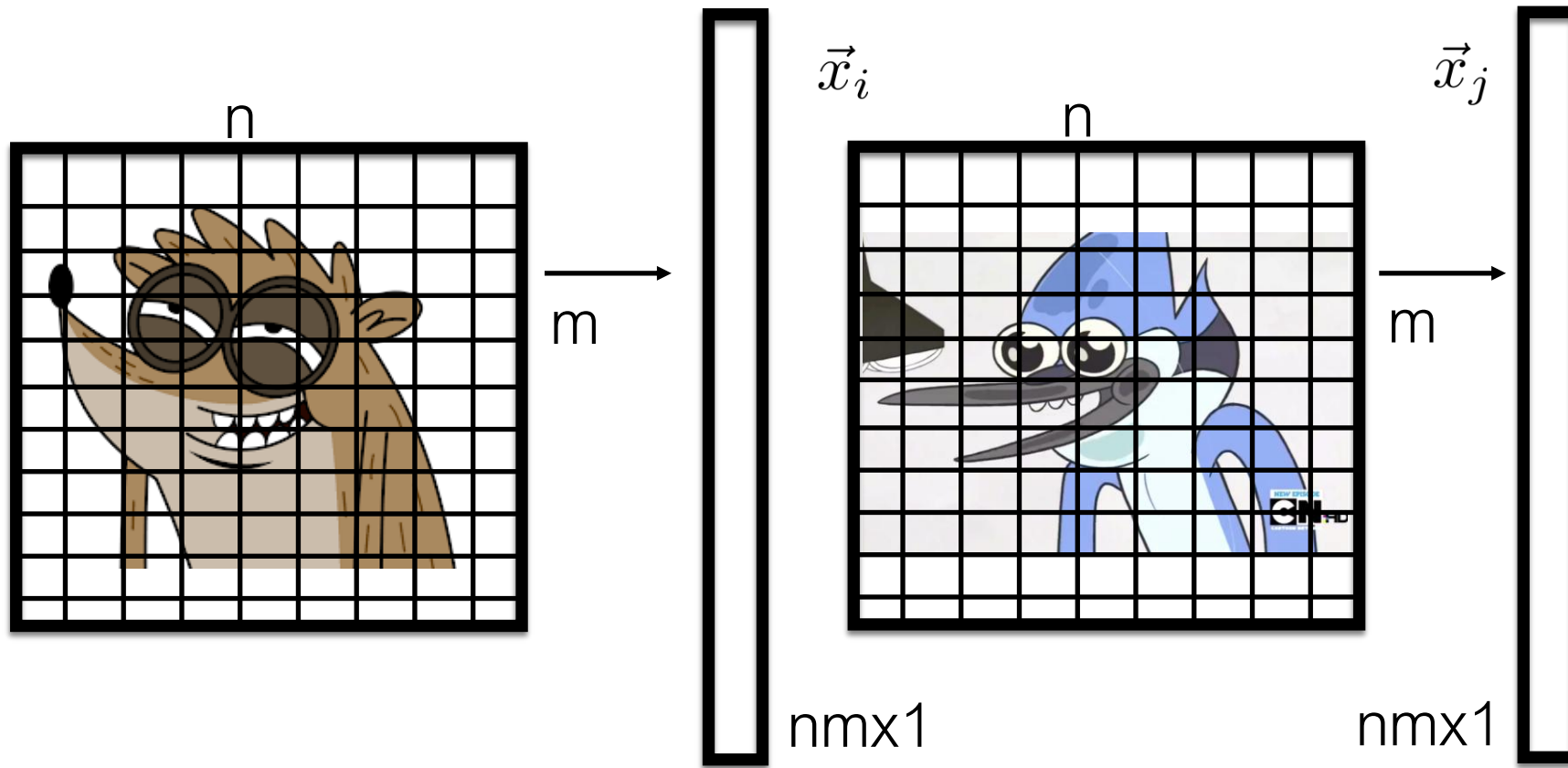
Supervised Segmentation

- SHAPE DESCRIPTION: High dimension?
- How to understand an image in high dimension?



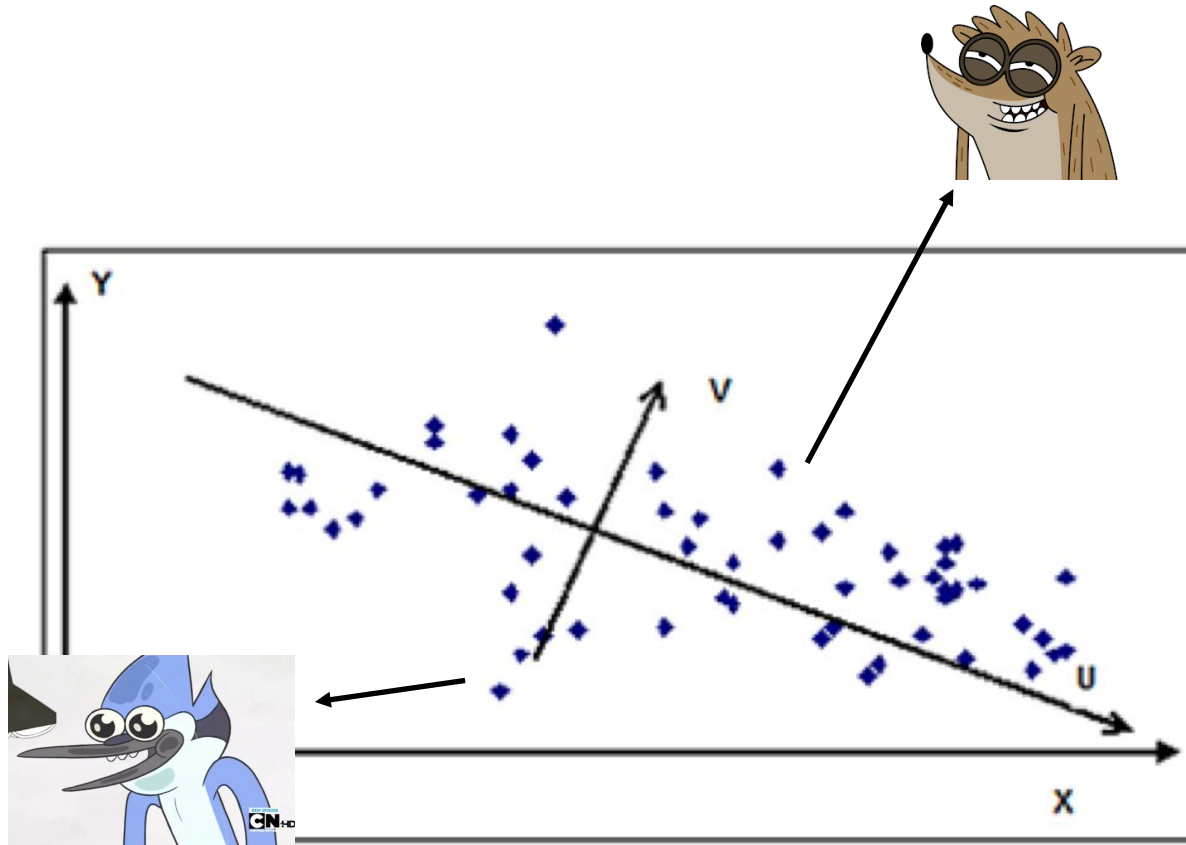
Supervised Segmentation

- SHAPE DESCRIPTION: High dimension?
- For 2D images, we now have a nm size vector per image



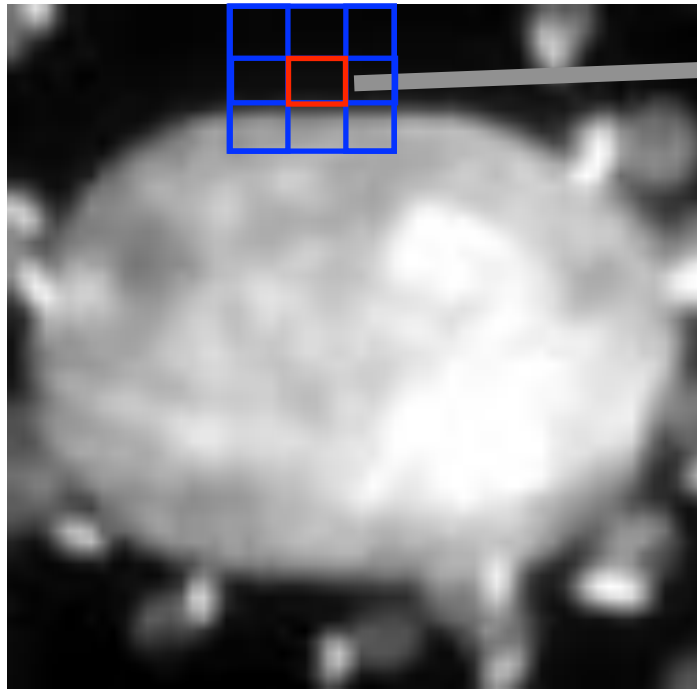
Supervised Segmentation

- SHAPE DESCRIPTION: High dimension?
- Now, each image is a point in your feature space.



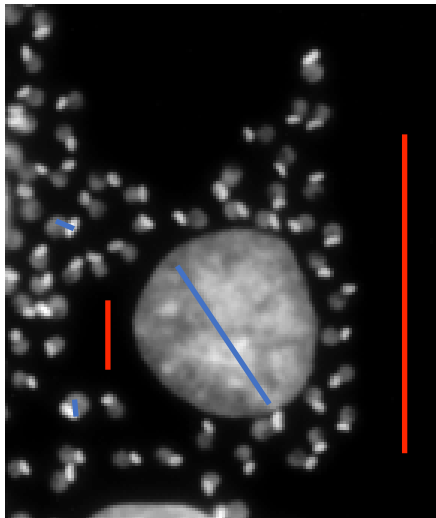
Supervised Segmentation

- But, we can understand images in **HIGHER** dimensions.



- ▶ Intensity (0)
- ▶ Variance 3x3
- ▶ Mean 3x3
- ▶ Sobel 3x3
- ▶ (any convolution)
- ▶ Others

- We can quickly build examples.
- Switch from unsupervised to supervised problem.



- ▶ Class A (background)
- ▶ Class B (objects)

- ▶ **Proposed Exercise: Use Weka (Fiji) and/or Ilastik to train random forest approaches to segment nuclei and parasites.**