Machine learning - Deep learning

Applications to BioImage analysis

SPAOM2018

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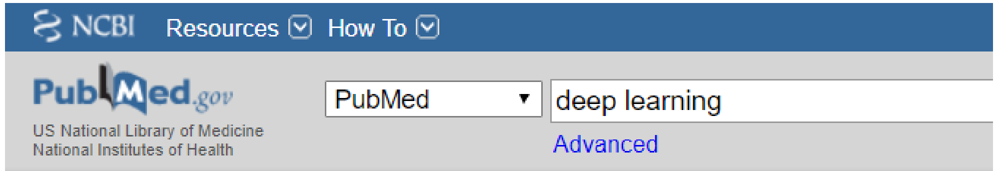
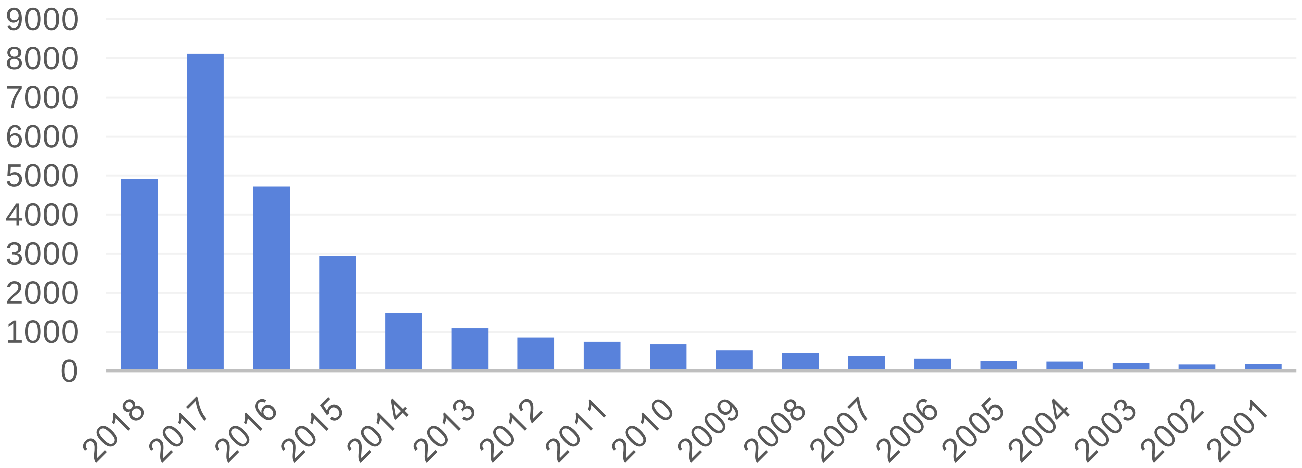
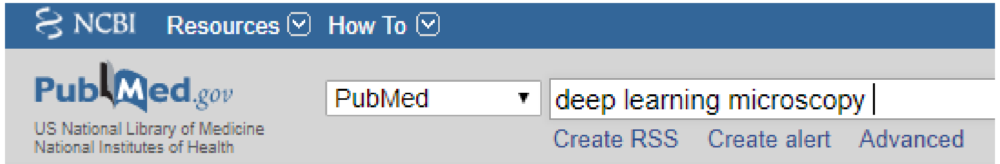
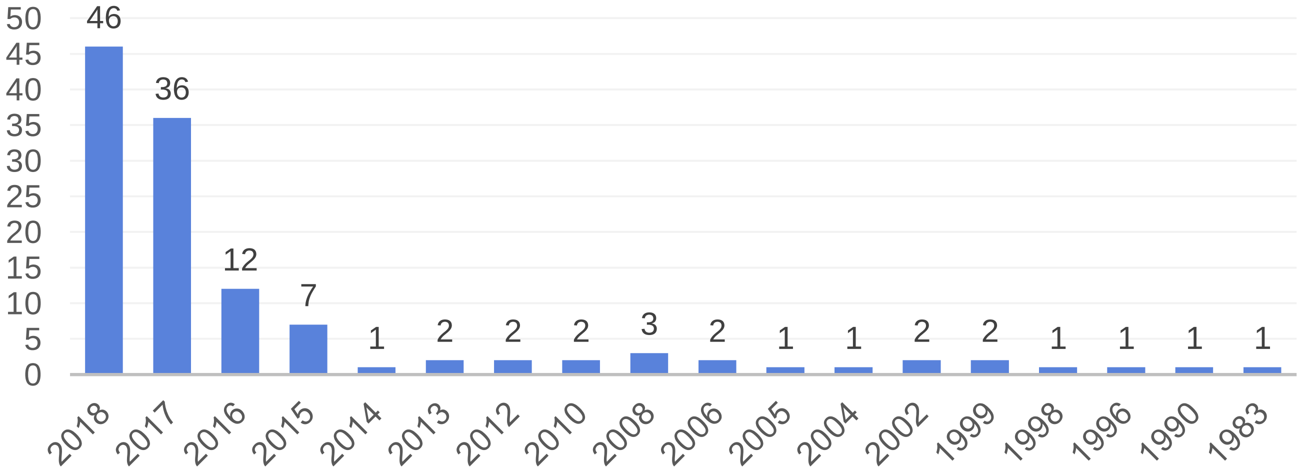
BIOMEDICAL IMAGING
AND INSTRUMENTATION GROUP

Current situation







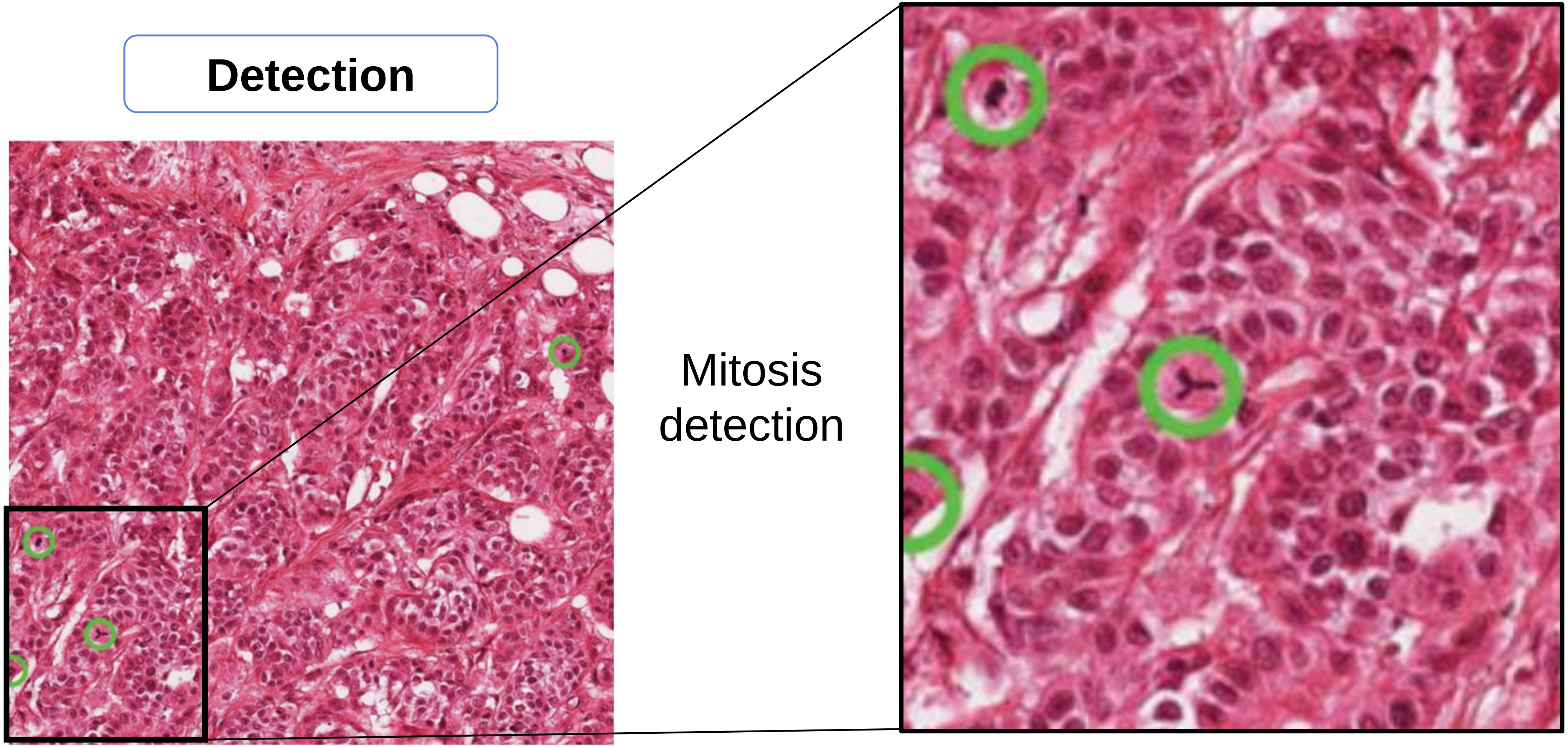


Breakdown of published papers in Medical Image Analysis until January 2017:

- G. Litjens *et al.*, *A survey on deep learning in medical image analysis*. Medical image analysis 2017
- F. Xing, *et al.*, *Deep Learning in Microscopy Image Analysis: A Survey*. IEEE Transactions on Neural Networks and Learning Systems 2017

Current situation

Detection

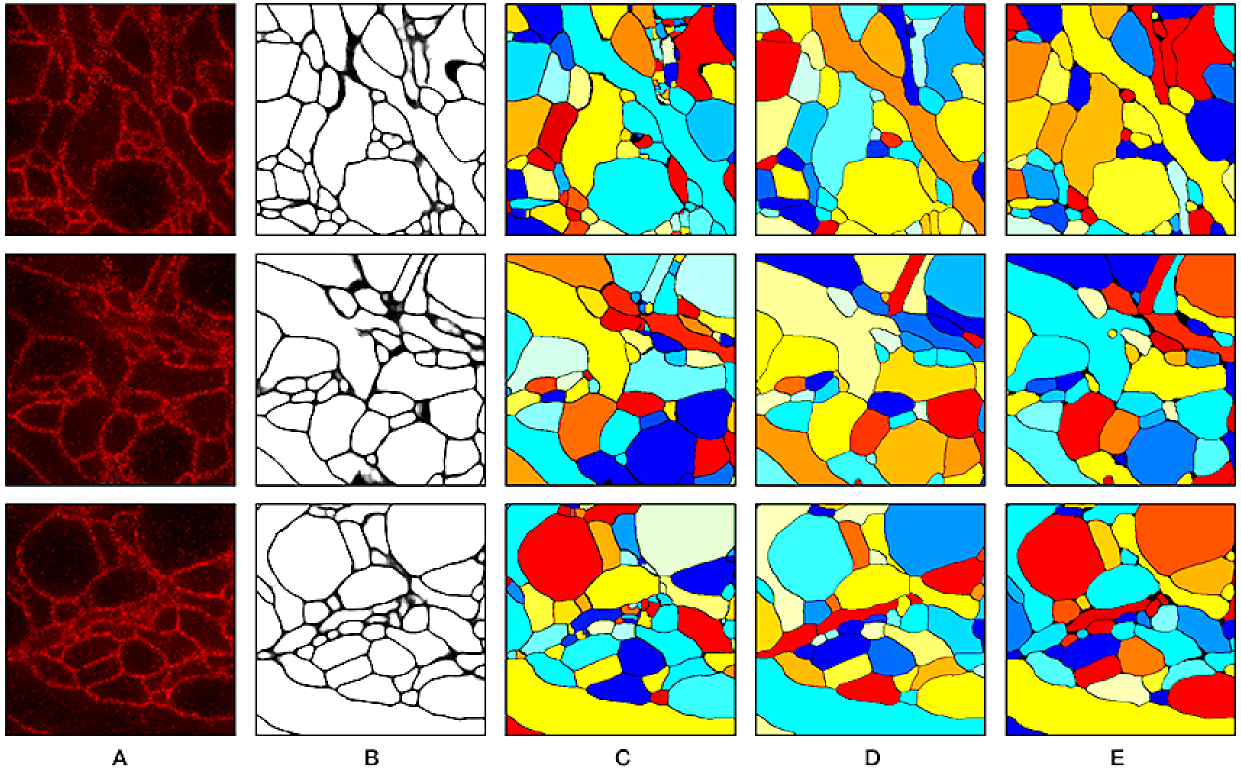


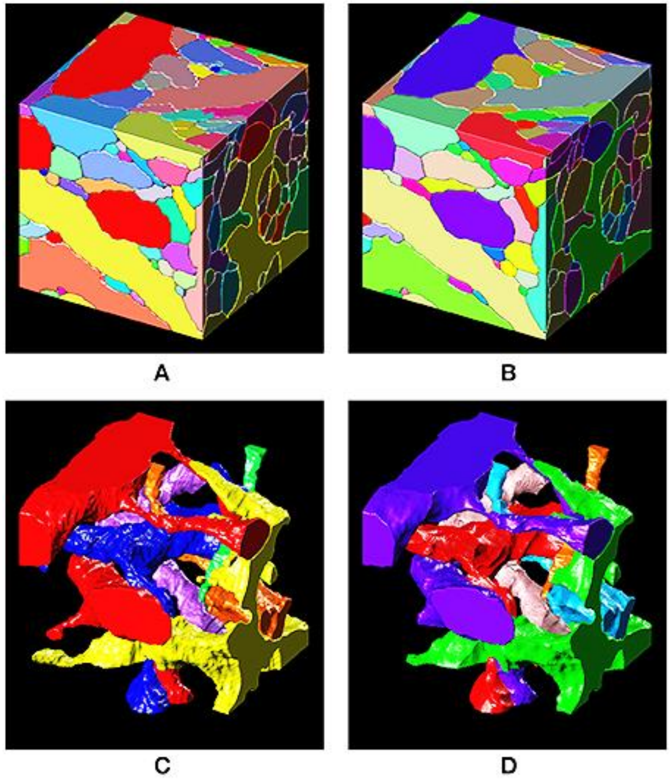
H. Chen, *et al.*, *Automated mitosis detection with deep regression networks*, ISBI 2016

Current situation

Segmentation in 3D

Boundary prediction in 3D anisotropic Expansion Microscopy image



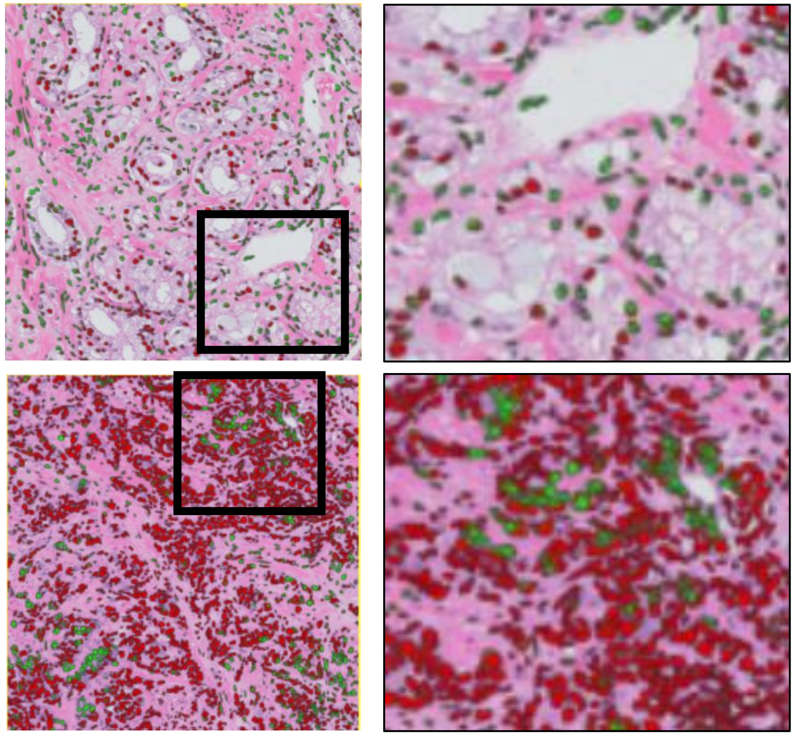


Y.G. Yoon, *et al.*, *Feasibility of 3D Reconstruction of Neural Morphology Using Expansion Microscopy and Barcode-Guided Agglomeration*, Frontiers in Computational Neuroscience 2017

Current situation

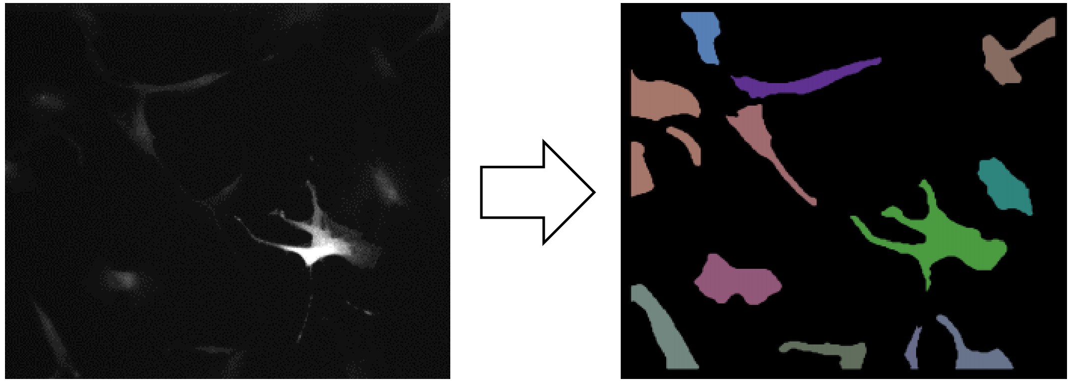
Classification

Nucleus level segmentation and classification



Multi-object segmentation

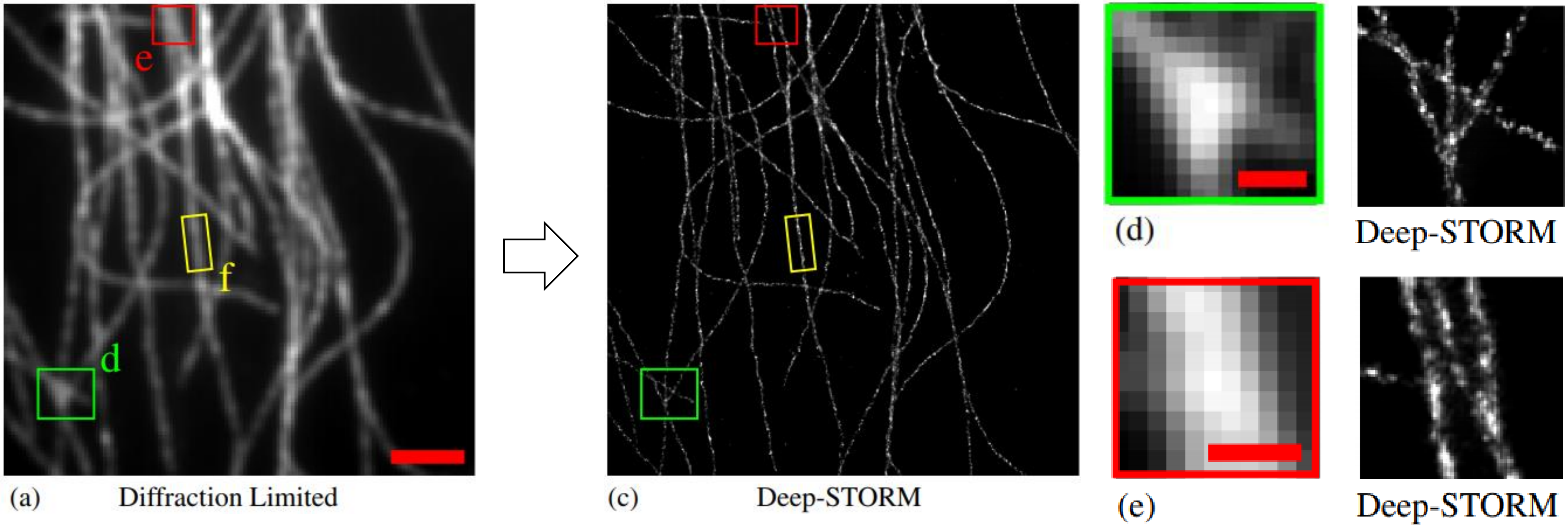
Cell instance segmentation
(Fluo-MSC)



A. Serag, *et al. A multi-level deep learning algorithm to estimate tumor content and cellularity of prostate cancer*, Openreview 2018
C. Payer, *et al.*, *Instance Segmentation and Tracking with Cosine Embeddings and Recurrent Hourglass Networks,* MICCAI 2018

Super-resolution imaging

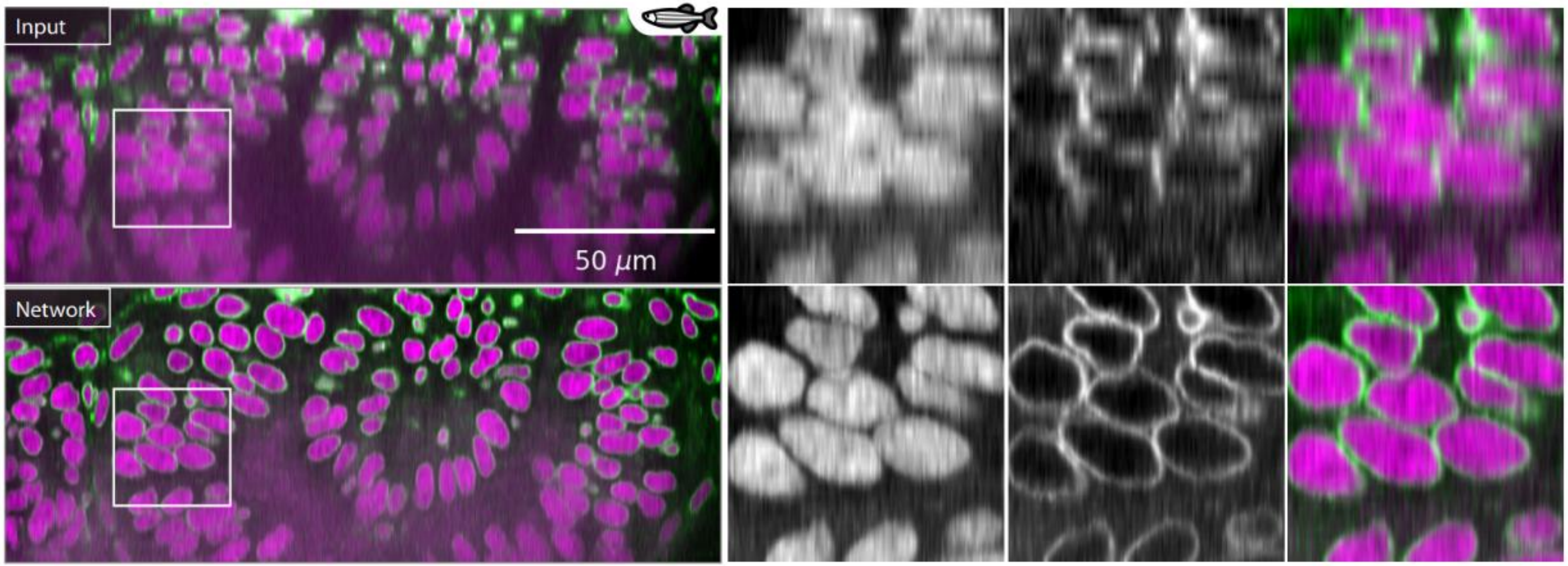
Experimentally measured microtubules.



E. Nehme, *et al.*, *Deep-STORM: super-resolution single-molecule microscopy by deep learning*, Optica 2018

Content-Aware image restoration

Zebrafish retina (nuclei and the nuclear envelope) in the anisotropic raw data (top row) and the isotropic restoration with deep learning.



M. Weiger *et al., Content-Aware Image Restoration: Pushing the Limits of Fluorescence Microscopy,* biorXiv 2018

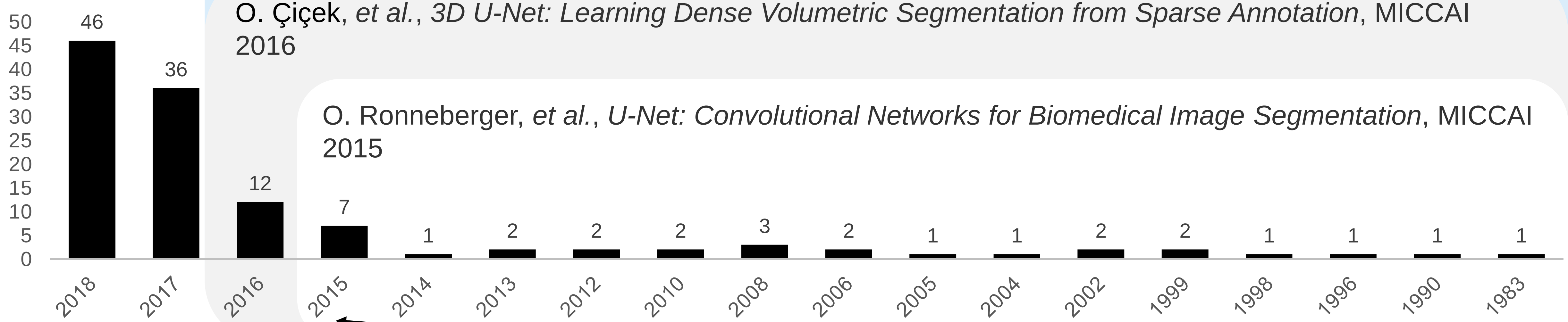
Why deep learning?

Why deep learning?

Effective deep learning architectures

O. Çiçek, *et al.*, *3D U-Net: Learning Dense Volumetric Segmentation from Sparse Annotation*, MICCAI 2016

O. Ronneberger, *et al.*, *U-Net: Convolutional Networks for Biomedical Image Segmentation*, MICCAI 2015



F. Chollet, *et al., https://keras.io* 2015

Deep Learning toolbox, Matlab 2015

User friendly libraries

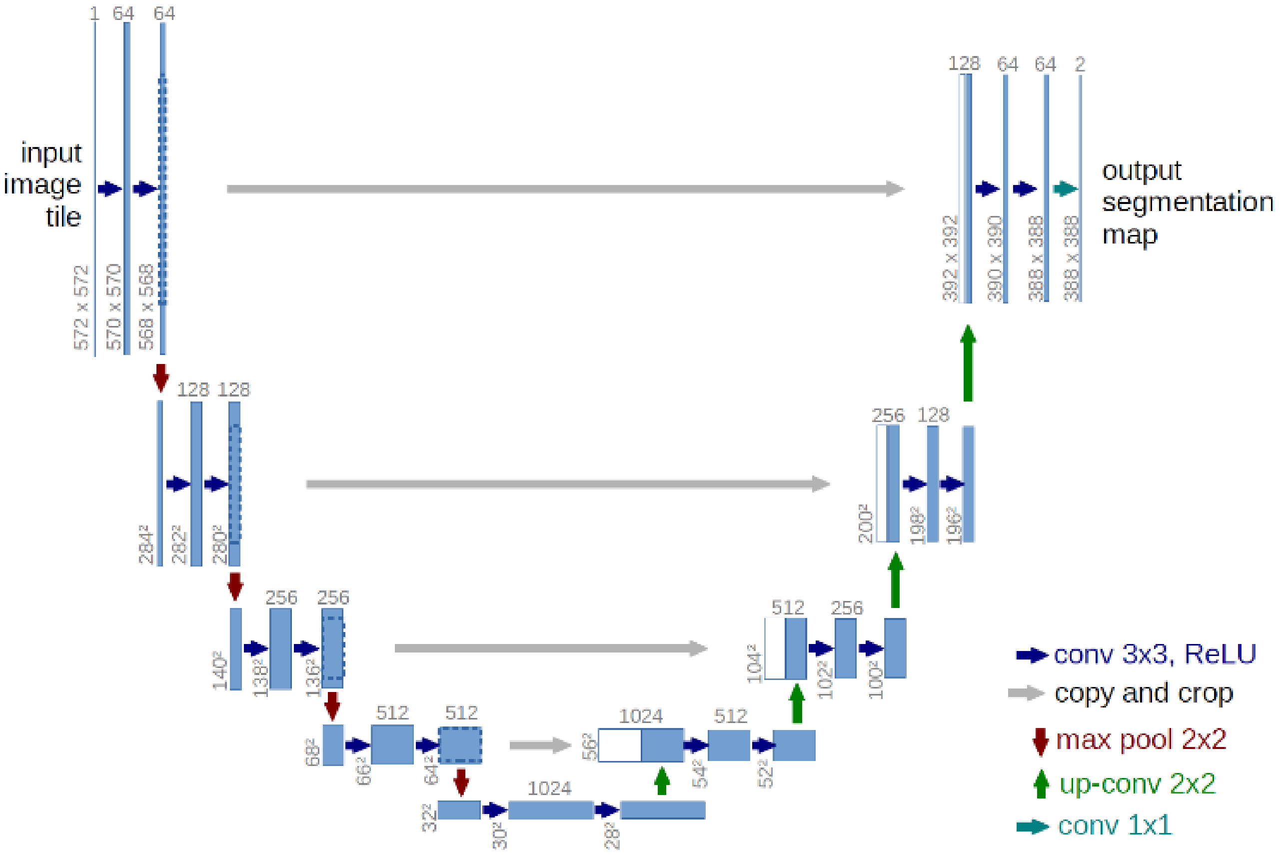
Why deep learning?

Advantages of U-net architecture

I. Image processing

- Contracting path extracts high dimension features → abstract analysis.
- Expanding path refines the processing.

II. Data augmentation applied to medical image processing.



O. Ronneberger, *et al.*, *U-Net: Convolutional Networks for Biomedical Image Segmentation*, MICCAI 2015

Why deep learning?

- DL methods fit thousands of parameters that allow to solve highly complex problems.
- Existence of sophisticated architectures ((3D)U-net, AlexNet, Mask R-CNN, Fast R-CNN, LSTM)
- Processing time after training is about seconds.
- Neural network architecture can be built easily with current software (Python, Matlab, C++, R).

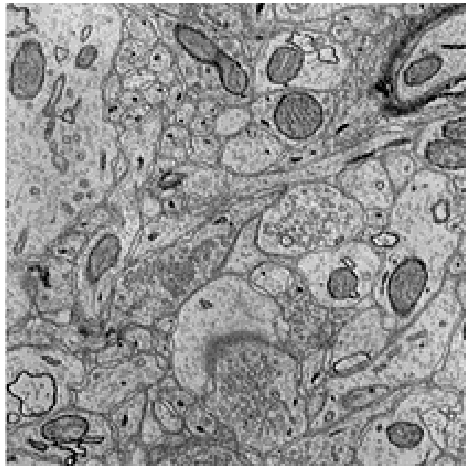
O. Ronneberger, *et al., U-Net: Convolutional Networks for Biomedical Image Segmentation*, MICCAI 2015
O. Çiçek, *et al., 3D U-Net: Learning Dense Volumetric Segmentation from Sparse Annotation*, MICCAI 2016
A. Krizhevsky, *et al., ImageNet Classification with Deep Convolutional Neural Networks.* NIPS 2012
K. He, *et al., Mask R-CNN* , arXiv 2018
R. Girshick, Fast R-CNN, IEEE ICCV 2018
S. Hochreiter, *et al., Long short-term memory,* Neural computation 1997

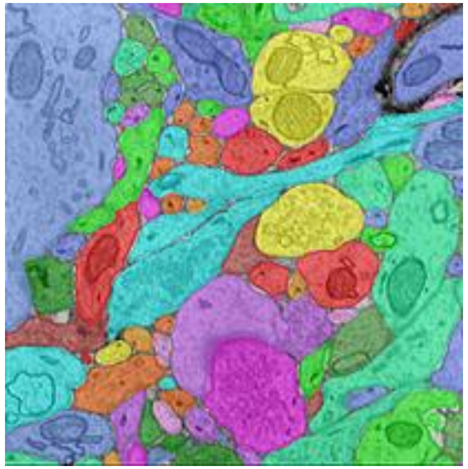
What do you need?

The problem to solve by machine learning techniques has to be well defined.

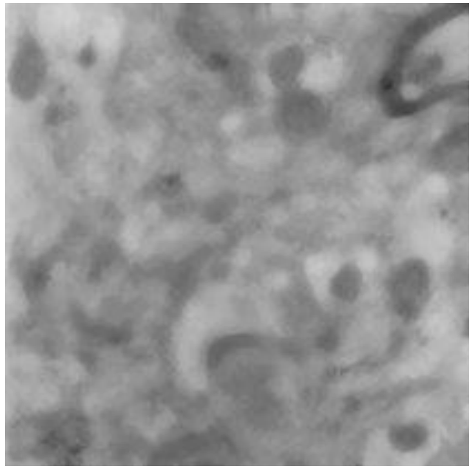
Classification: the number of classes has to be determined and their description cannot be ambiguous

Segmentation: The result of any manual annotation when performed twice by an expert, should always coincide.





High enough quality of data.



What do you need?

To train our own model

Technological infrastructure

- Graphics processing units (GPU)

NVIDIA Quadro P5000

- Cloud computing (Google, Amazon)

Data: Ground Truth (GT) → manual annotations supervised by experts

- GT has to represent the real scenario of the problem.
- Large enough to train the model and evaluate it.

What do you need?

Data augmentation

Patching

Geometrical transformations

Linear transformations (preserve shape)

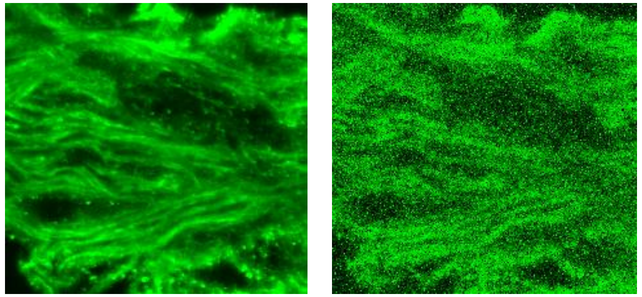
- Rotation
- Translation

Non-linear (elastic) transformations (shape changes)

- Zooming
- Shearing

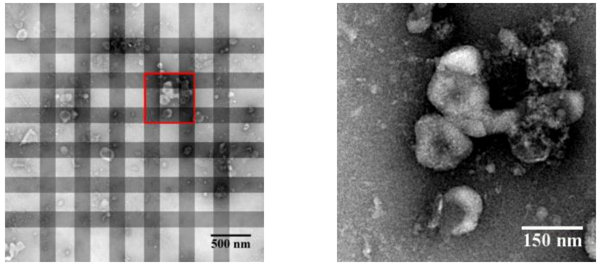
Add artifacts: noise

Noising

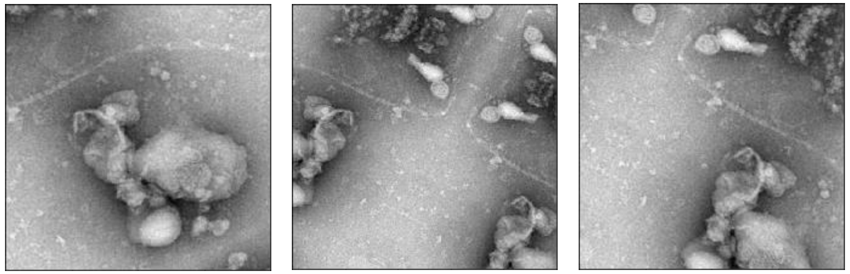


Original image | Noisy

Patching

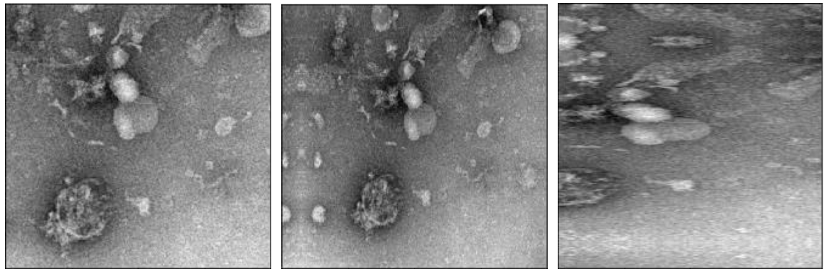


Linear transformations



Original patch | Rotation + Shift | Rotation

Non-linear transformations



Original patch | Zoom | Shearing

Evaluation of obtained results

- Quantitative evaluation
  - Computer science
    - Jaccard/Dice coefficients
    - Acyclic Oriented Graph
    - Precision – Recall – F1
  - Biological relevance
    - Complete tracks
    - Morphological measures (size, shape)
    - Branching correctness
    - Cell cycle accuracy
    - Fluorescence intensity
  - Practical usability
    - Number of tunable parameters
    - Generalizability

Resources

Data annotation

- Slicer
- Polygon-RNN++
- ImageJ

Data repositories

- Kaggle
- Cell tracking challenge

Deep learning software

- Python (Tensorflow, Keras)
- Matlab
- C++ (Caffe)

User friendly software

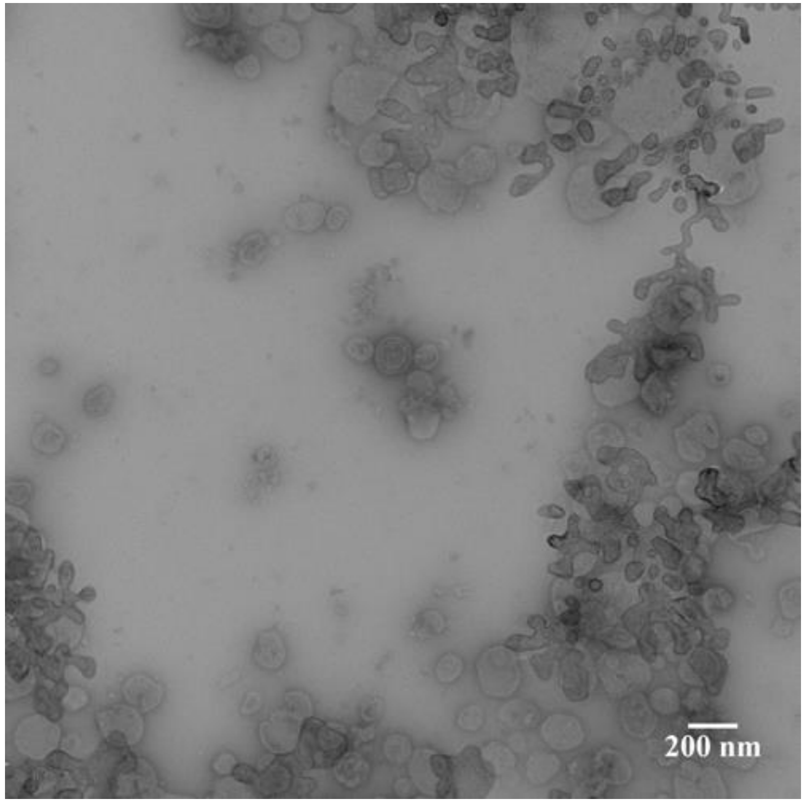
- ImageJ (U-net, CARE plugins)
- Cell profiler upcoming
- Ilastik upcoming

Example of deep learning usage

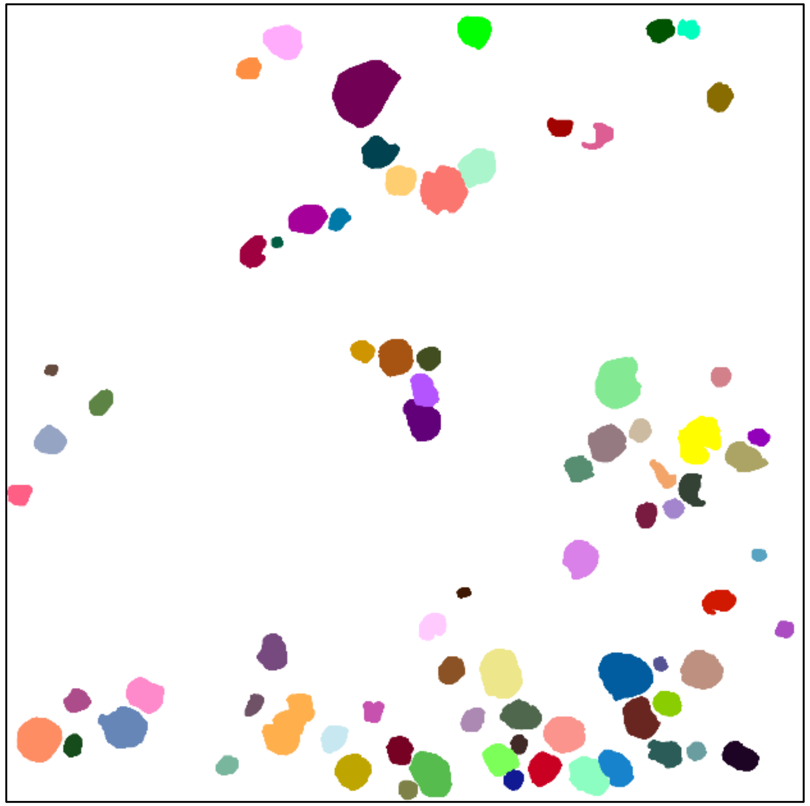
Example of deep learning usage

Automatic exosomes segmentation in transmission electron microscopy images

Original image



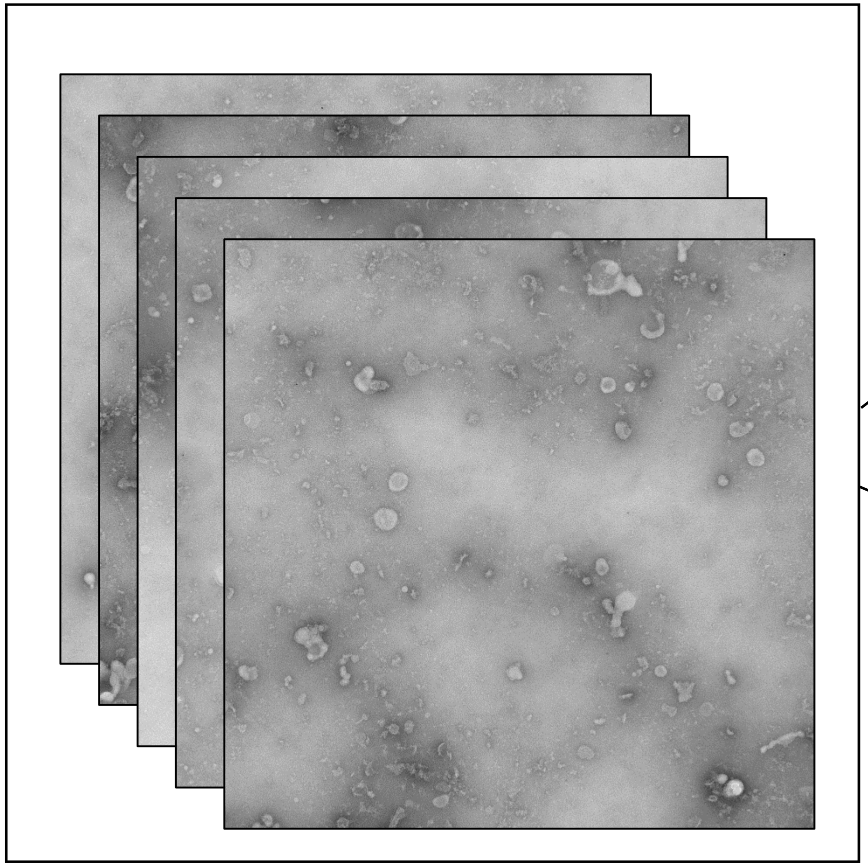
Ground truth



E. Gómez-de-Mariscal, M. Maška, *et al., FRU-net: Automatic segmentation of exosomes in transmission electron microscopy images.* (in prep.)

Example of deep learning usage

Split data into training and test INDEPENDENT datasets

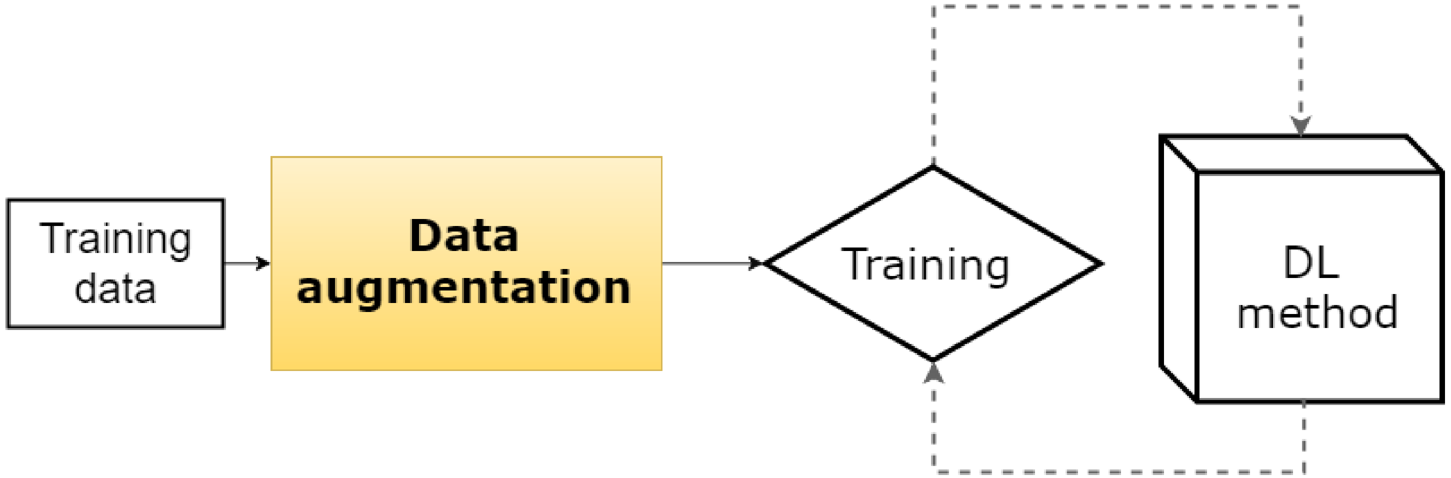


Training dataset
~ 80

Test dataset
~ 20

Example of deep learning usage

Increase training data and train your deep learning method

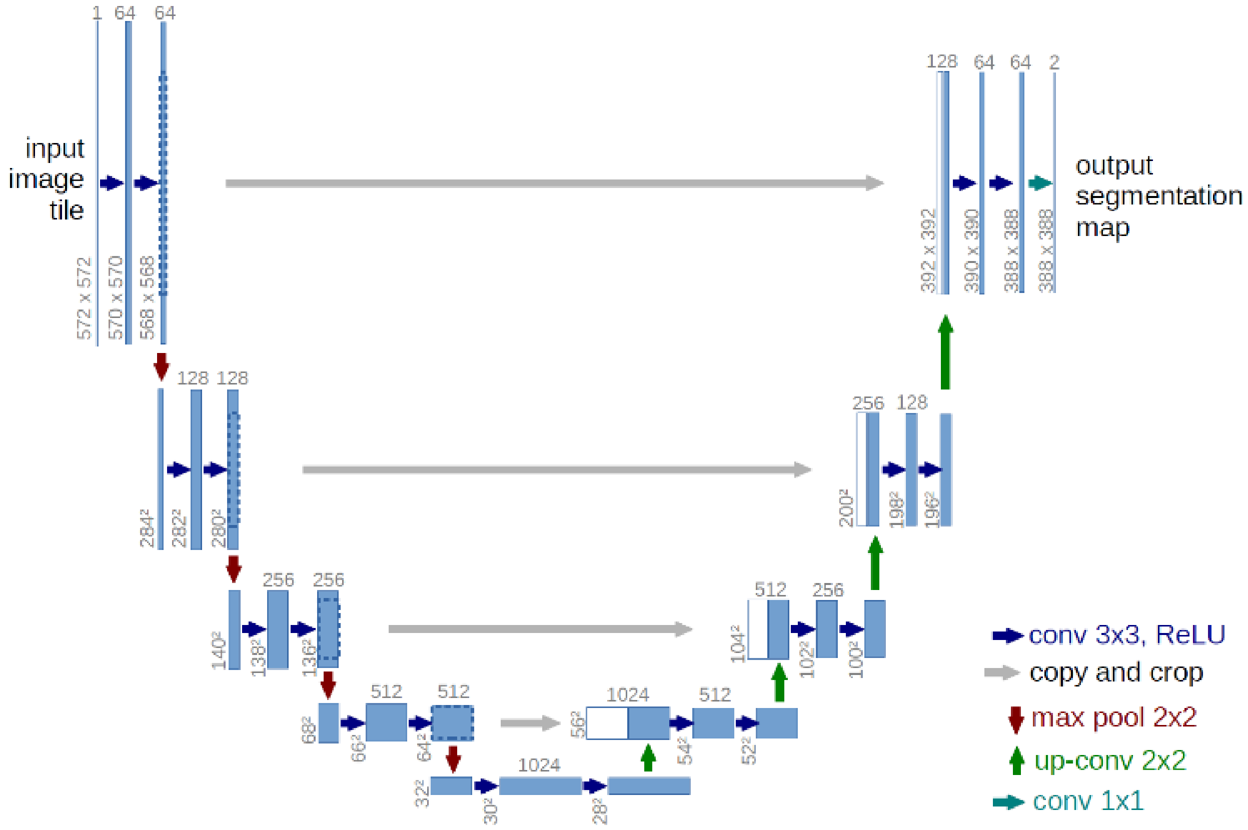


- DL architecture (number of layers and connections)
- Loss function
- Activation function
- Optimizer
- Batch size
- Batch normalization
- Drop out

Details of the method can be found in the manuscript

Example of deep learning usage

Architecture



U-net convolutional neural network

+

Residual layers

O. Ronnenberg, *et al.*, *U-Net: Convolutional Networks for Biomedical Image Segmentation*, MICCAI 2015

Residual layers

Convolutional layer fits a non linear function $G(x)$ to estimate the ground truth

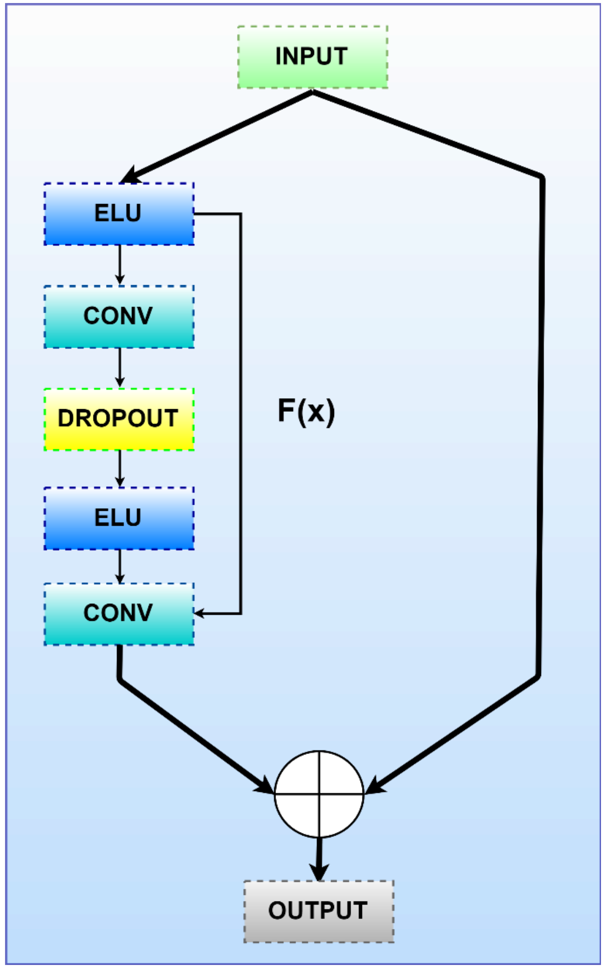
$$y = G(x)$$

Residual layer include x as a possible solution to the problem

$$y = x + F(x)$$

where $F(x)$ is estimated by the residual block.

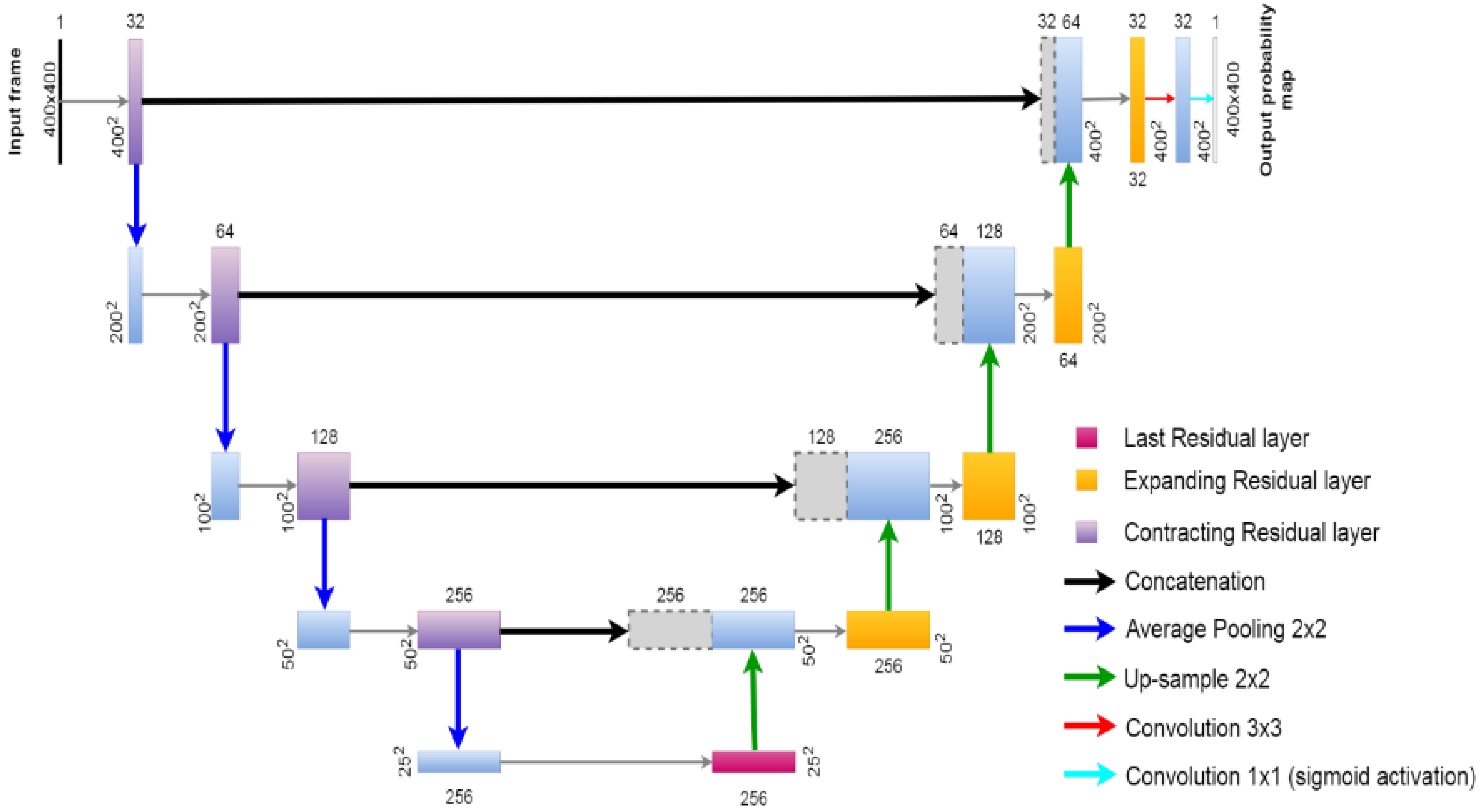
This approach limits the training error to x and prevents overfitting.

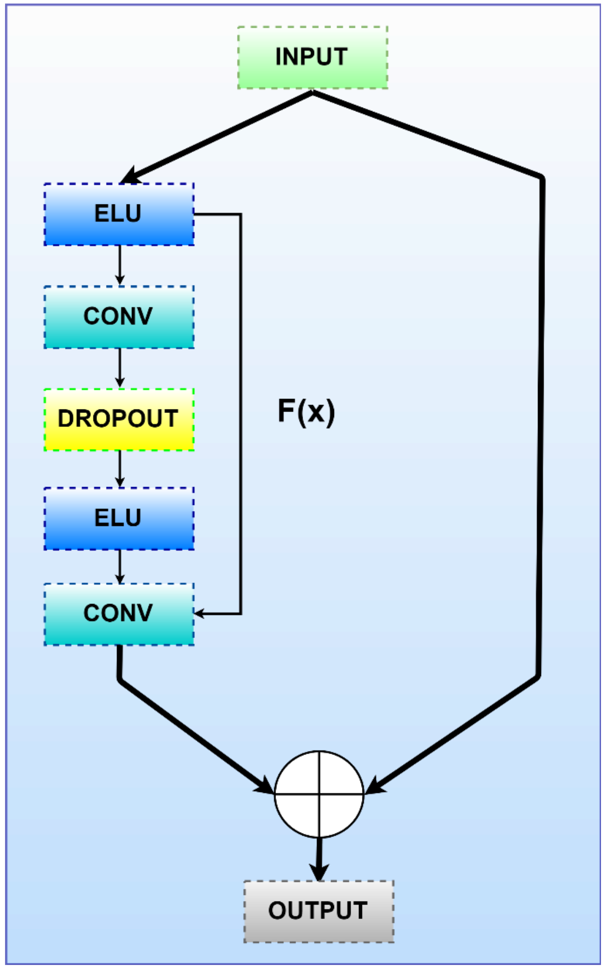


K. He, *et al., Deep residual learning for image recognition*, IEEE CVPR 2016

Example of deep learning usage

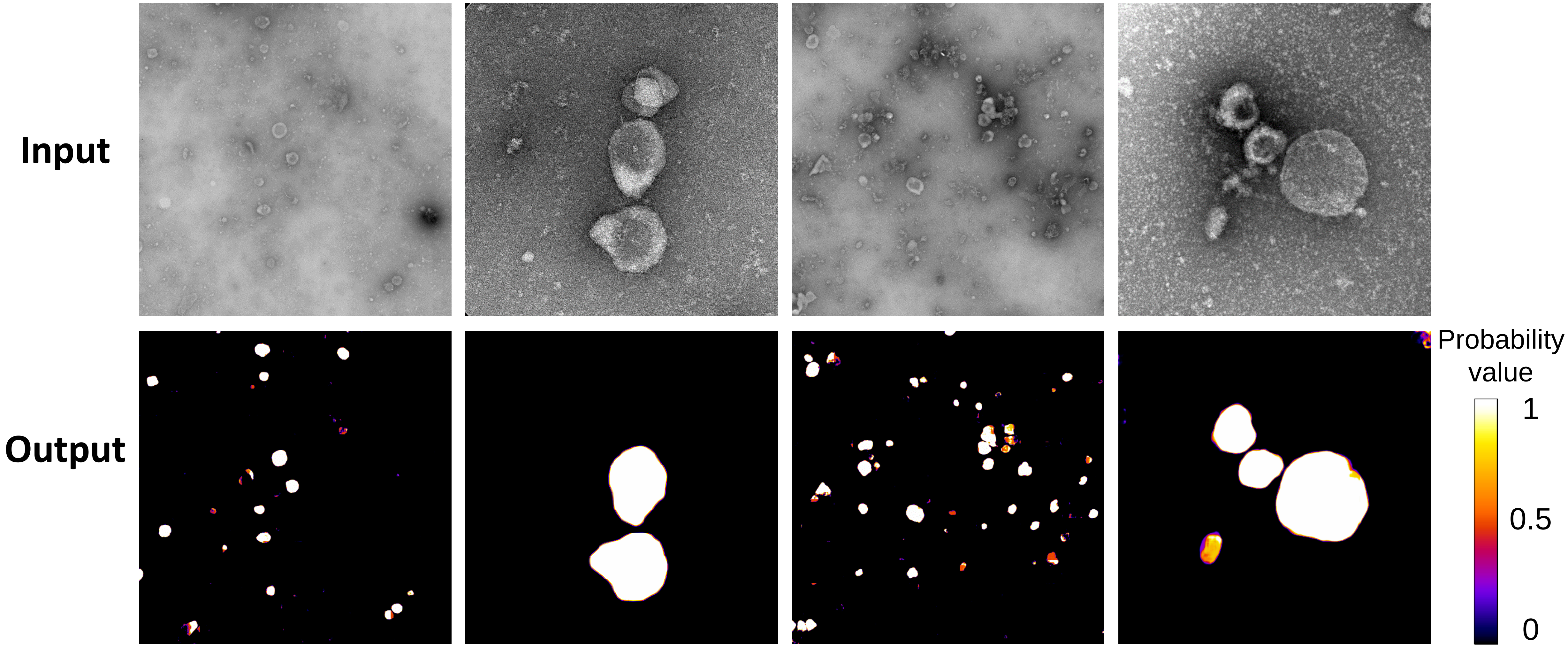
Proposed architecture: Fully Residual U-net





Example of deep learning usage

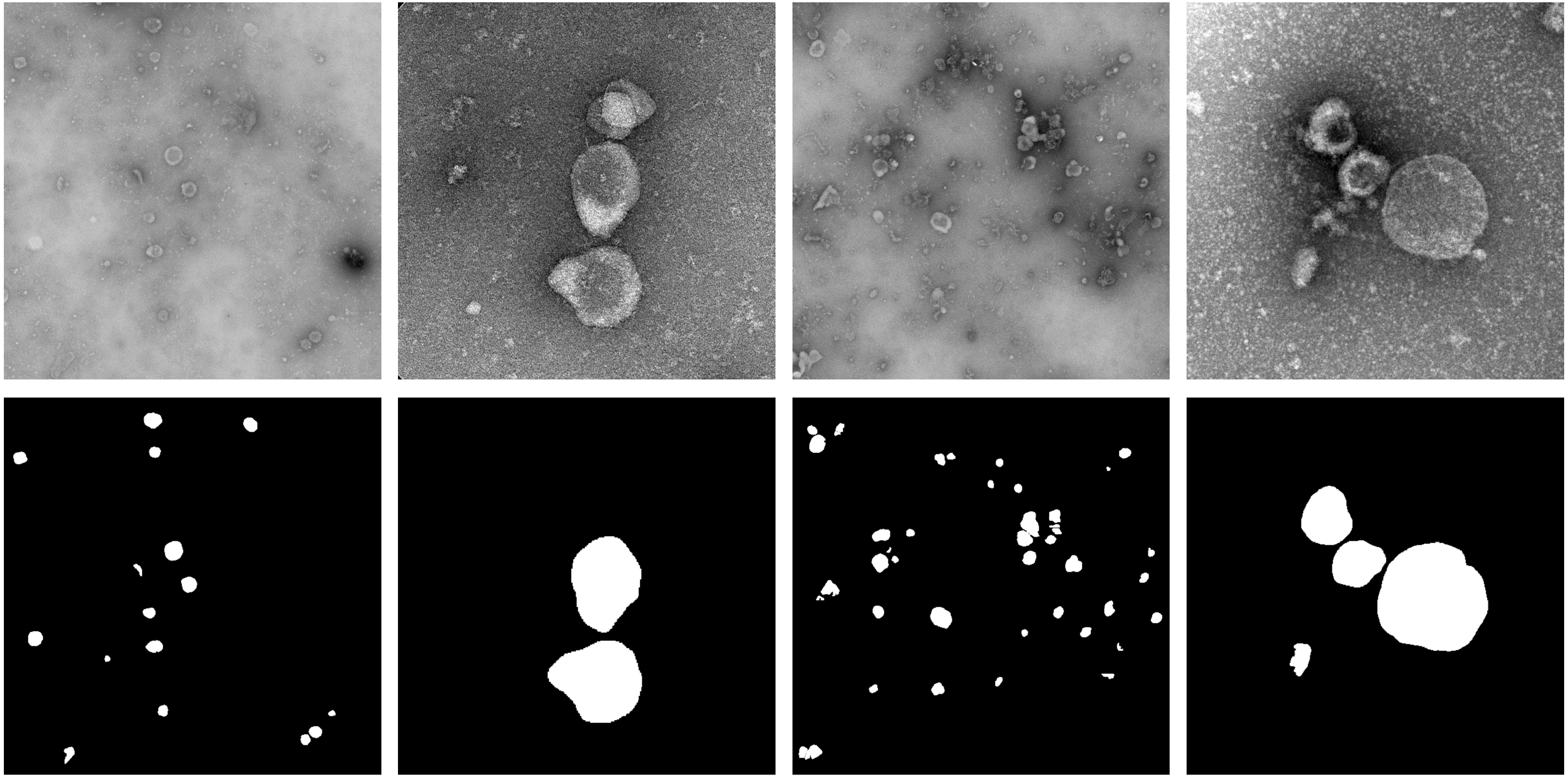
Output of a convolutional neural network (CNN): probability maps



Example of deep learning usage

Threshold probability maps to obtain binary masks

Input

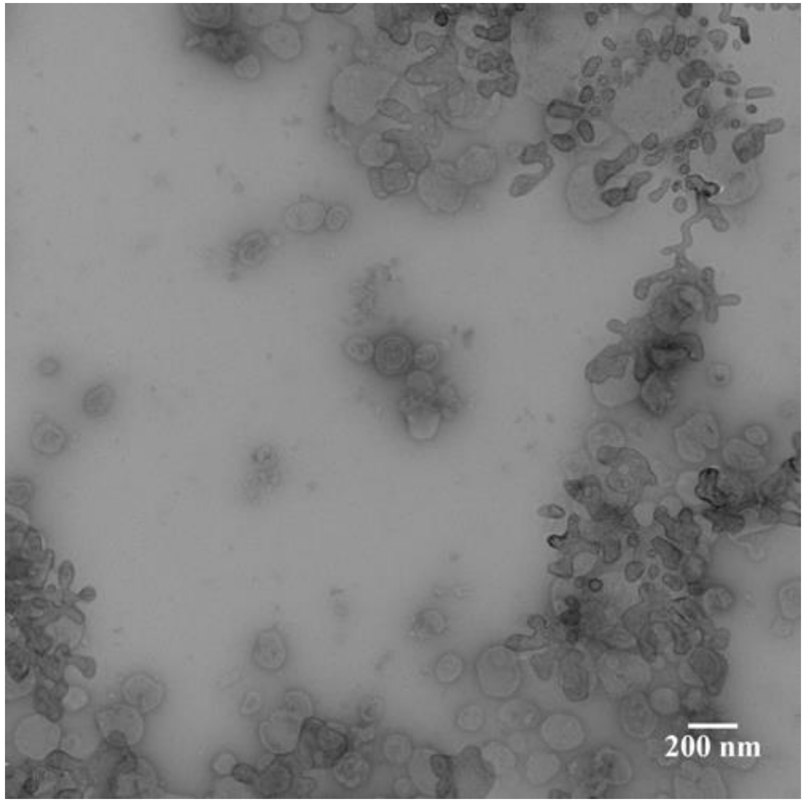


Binary mask

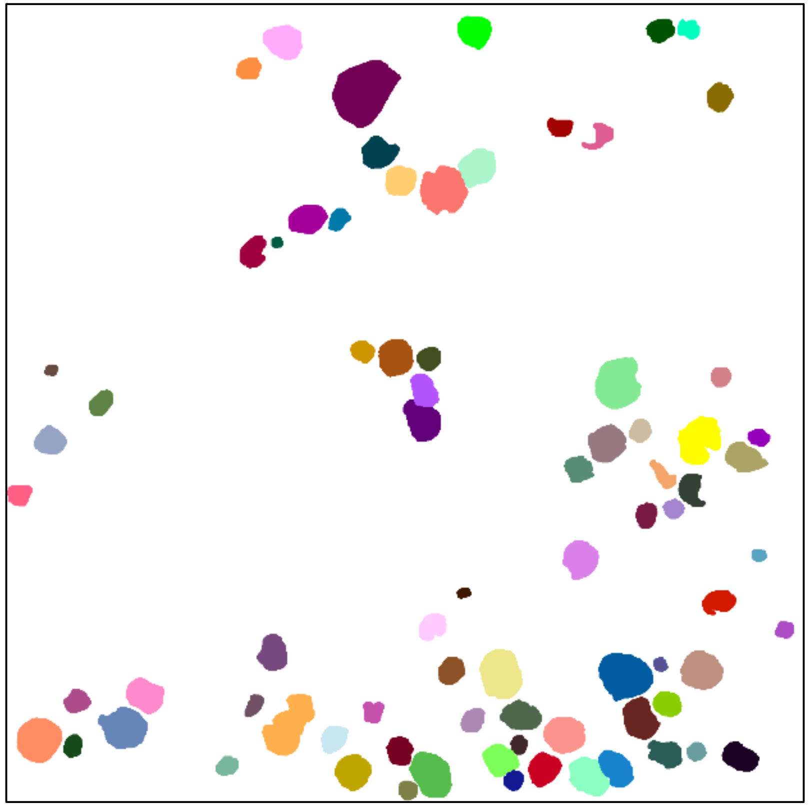
Example of deep learning usage

Automatic exosomes segmentation in transmission electron microscopy images

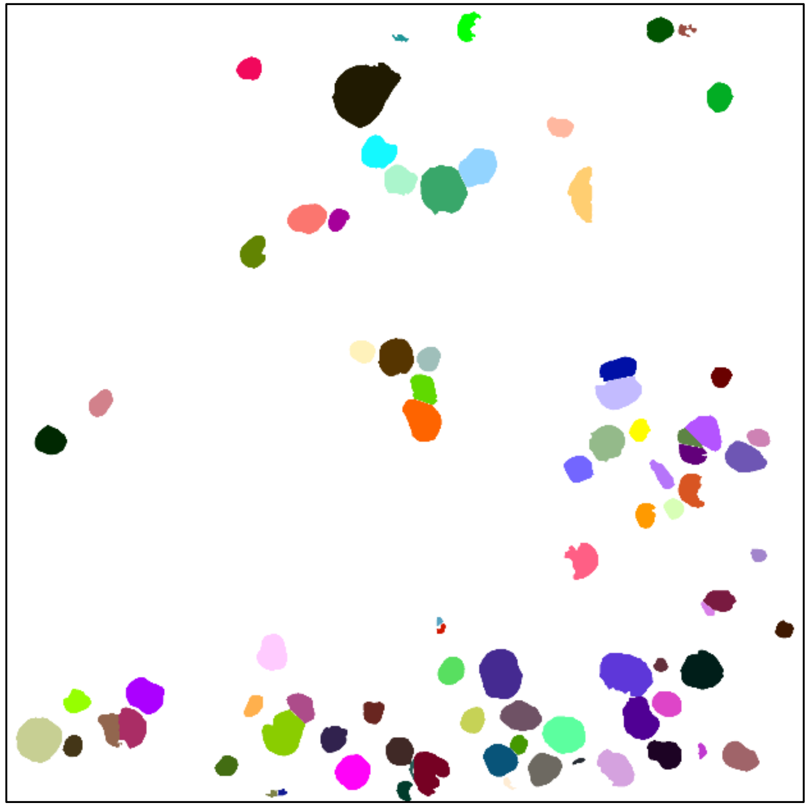
Original image



Ground truth



Fully Residual U-net result



E. Gómez-de-Mariscal, M. Maška, *et al., FRU-net: Automatic segmentation of exosomes in transmission electron microscopy images.* (in prep.)

Example of deep learning usage

Accuracy measures and comparison with state of the art methods

	Computer Science		Biological relevance	
	Segmentation	Detection	Diameter	Roundness
Method	Jaccard Coeff.	Acyclic Oriented Graphs	δ_d	δ_r
FRU-net	**0.74**	**0.81**	**0.09**	0.11
U-net	0.68	0.77	0.11	0.13
Exosome Analyzer	0.19	0.17	0.17	**0.07**

δ_d and δ_r: diameter and roundness similarity.

S_i and GT_i correspond to the segmentation and ground truth of the exosome *i.*

d and r to the diameter and roundness.

$$\delta_d = 1 - \frac{min(d_{Si}, d_{GTi})}{max(d_{Si}, d_{GTi})}$$

$$\delta_r = 1 - \frac{min(r_{Si}, r_{GTi})}{max(r_{Si}, r_{GTi})}$$

O. Ronnenberg, *et al.*, *U-Net: Convolutional Networks for Biomedical Image Segmentation*, MICCAI 2015
K. Štěpka, *et al.*, *Automatic Detection and Segmentation of Exosomes in Transmission Electron Microscopy*, ECCV 2016
P. Matula, *et al.*, *Cell tracking accuracy measurement based on comparison of acyclic oriented graphs*, PloS one 2015

Example of deep learning usage

P22

FULLY AUTOMATIC EXOSOMES SEGMENTATION IN TRANSMISSION ELECTRON MICROSCOPY IMAGES

Gómez-de-Mariscal E.[1], Maška M.[2], Kotrbová A.[3], Pospíchalová V.[3], Matula P.[2], Muñoz-Barrutia A.[1]

[1] Bioengineering and Aerospace Engineering Department, Universidad Carlos III de Madrid; Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain,
[2] Centre for Biomedical Image Analysis, Faculty of Informatics, Masaryk University, Brno, 602 00, Czech Republic and
[3] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, 611 37, Czech Republic

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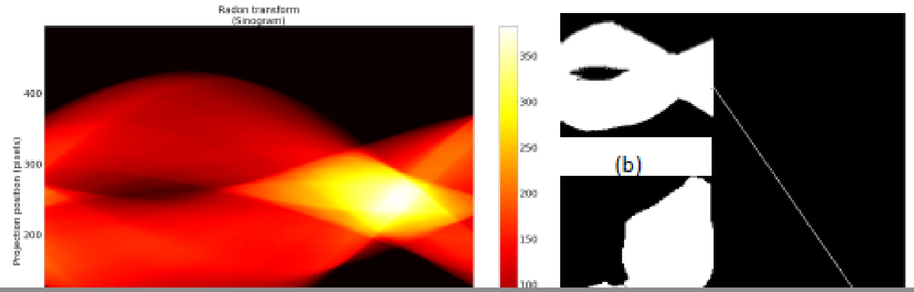
Web: http://image.hggm.es

ABSTRACT

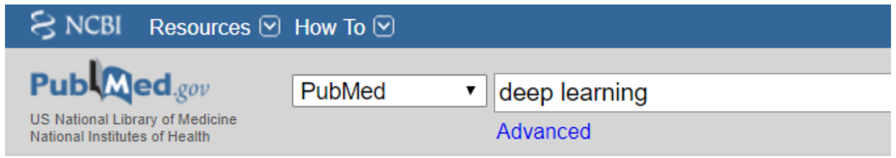
Exosomes are nano-scale cell-derived extracellular vesicles, involved in the intercellular communication. Exosomes quantification is currently done manually by biologists and its automation will help them to remarkably progress in their research. We present the Fully Residual Unet for the segmentation of exosomes in Transmission Electron Microscope images and the Radon transform properties to separate clusters. An accuracy over 80% and 2s processing time for 2048x2048 pixels image are achieved.

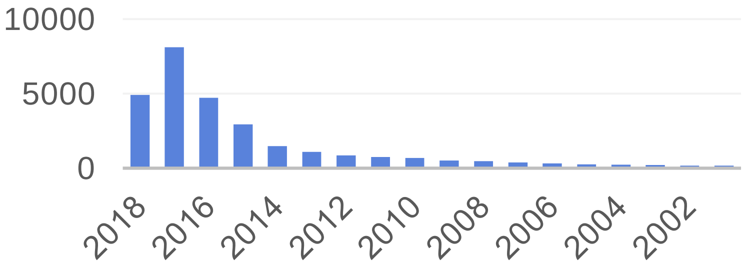
3. Look for Radon transform local minimums to split exosomes clusters.



Summary

- ✓ Current situation



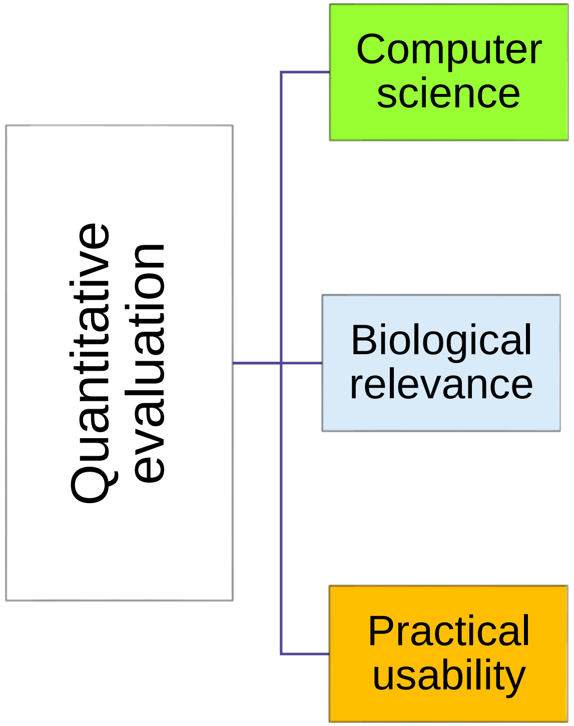


- ✓ Why deep learning?

- ✓ What do you need?

NVIDIA Quadro P5000

- ✓ Evaluation of obtained results



- ✓ Resources

- ✓ Example of deep learning usage

Thank you!

Machine learning - Deep learning

Applications to BioImage analysis

SPAOM2018

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Link to slides:

https://image.hggm.es/es/estibaliz-gomez

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