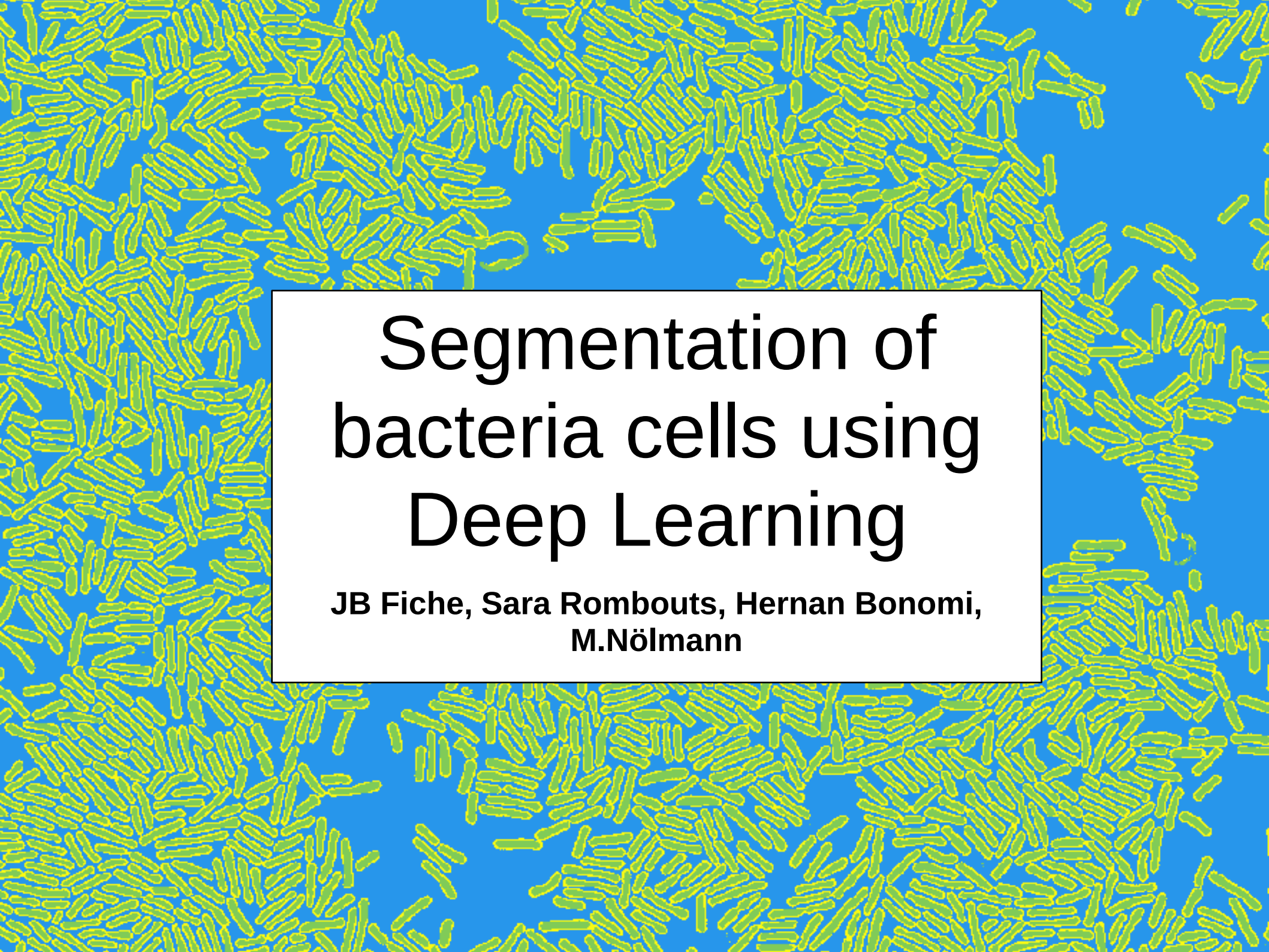

Segmentation of bacteria cells using Deep Learning

JB Fiche, Sara Rombouts, Hernan Bonomi, M.Nölmann

Outline

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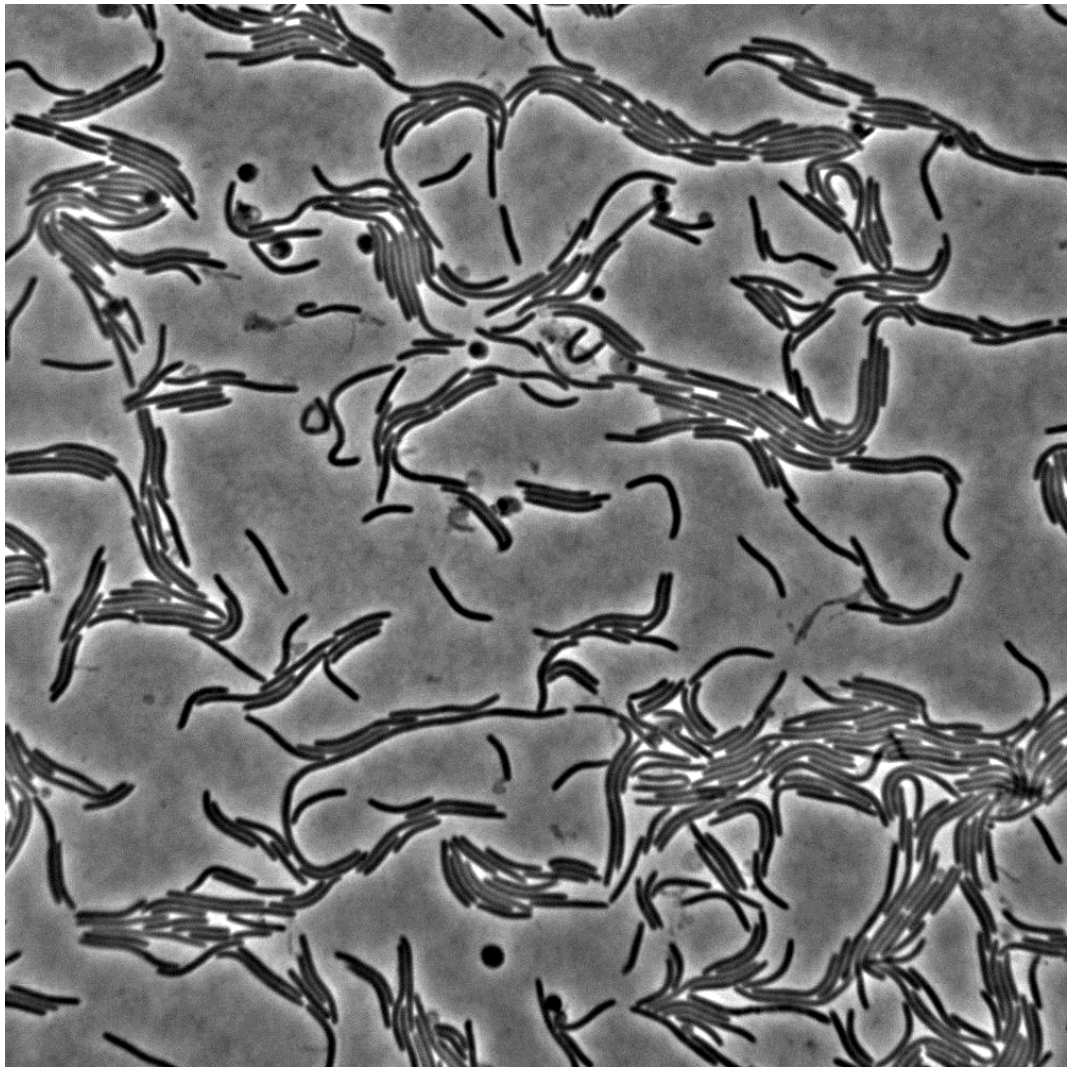
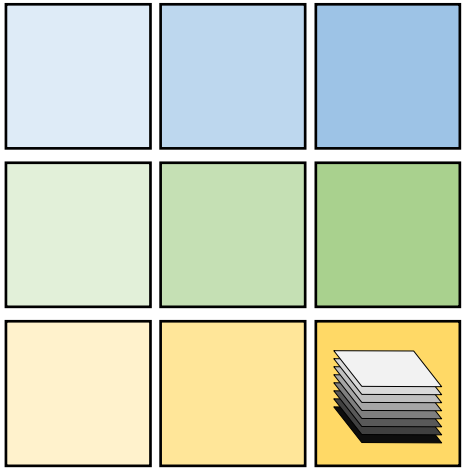
Tracking *Myxococcus xanthus* cells on an agar pad



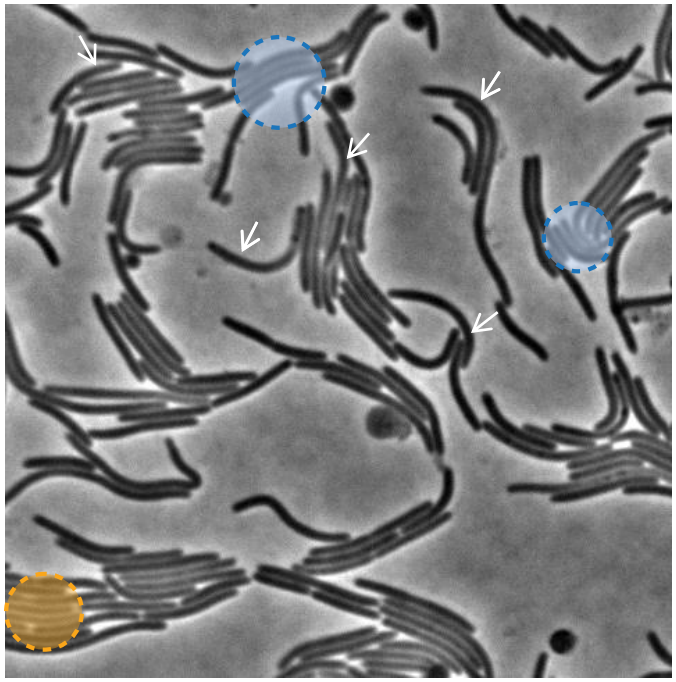


3x3 ROI for a total of ~600x600µm
Stack of 7 images
~40-50s to complete a cycle

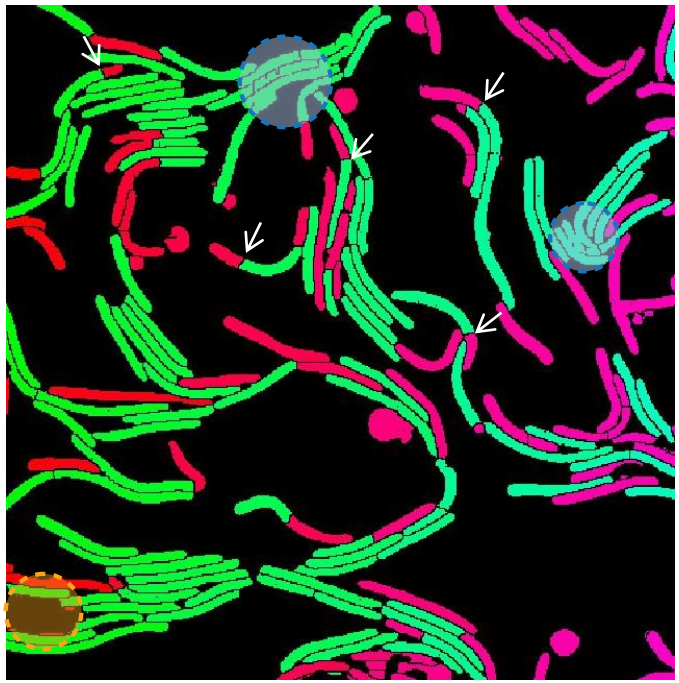
Myxococcus xanthus gliding assay on a 1,5% agar pad. We need a reliable method to segment the cells and track them over time :

- *predation behavior*
- *cell division*
- *etc.*

Segmentation of *Myxococcus xanthus* cells using super-segger



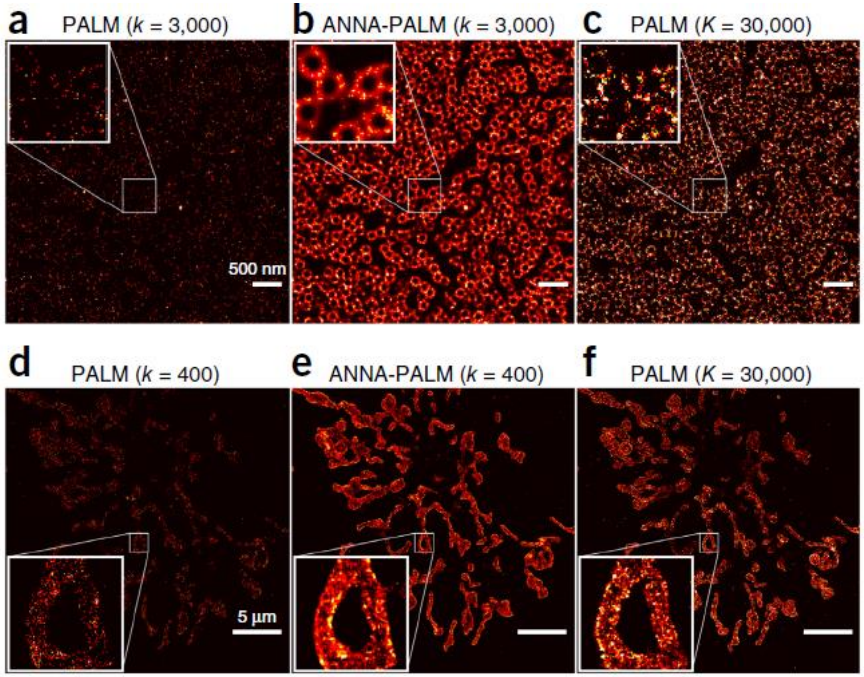
Super-segger



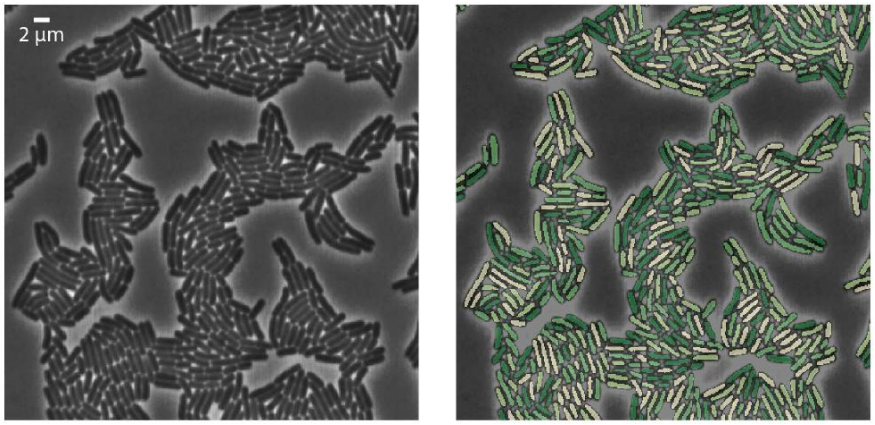
Even on a good quality phase-contrast image, >5% of the cells are not properly segmented using SuperSegger.

Stylianidou et al. 2016. Mol. Microbiol.

Deep-Learning applications for microscopy



Weigert et al. 2017. BioRxiv - Ouyang et al. 2018. Nat. Biotech.



Van Valen et al. 2016. PLOS Computational Biol.

Deep learning is a type of machine learning that has been applied to microscopy in order to :

- Improve super-resolution PALM/STORM image reconstruction
- Segment cells
- Perform filtering on low-signal images
- etc.

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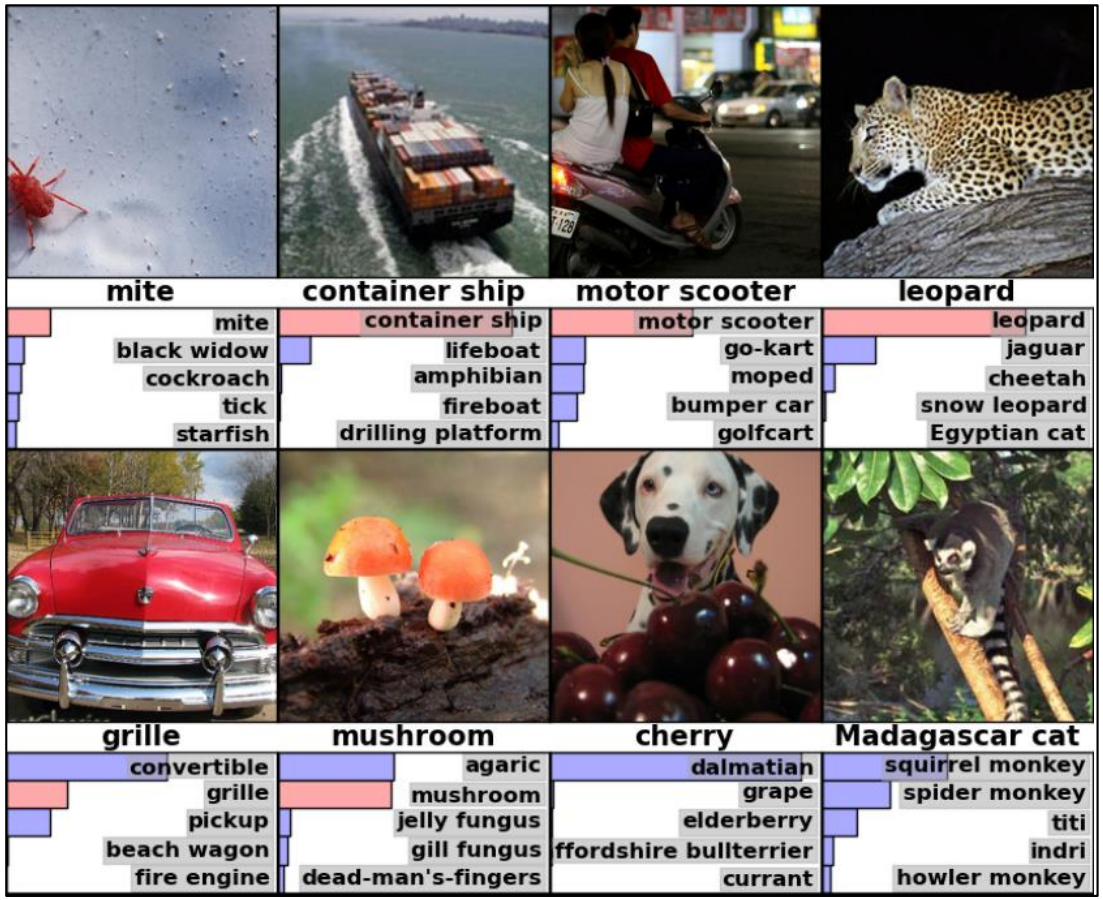
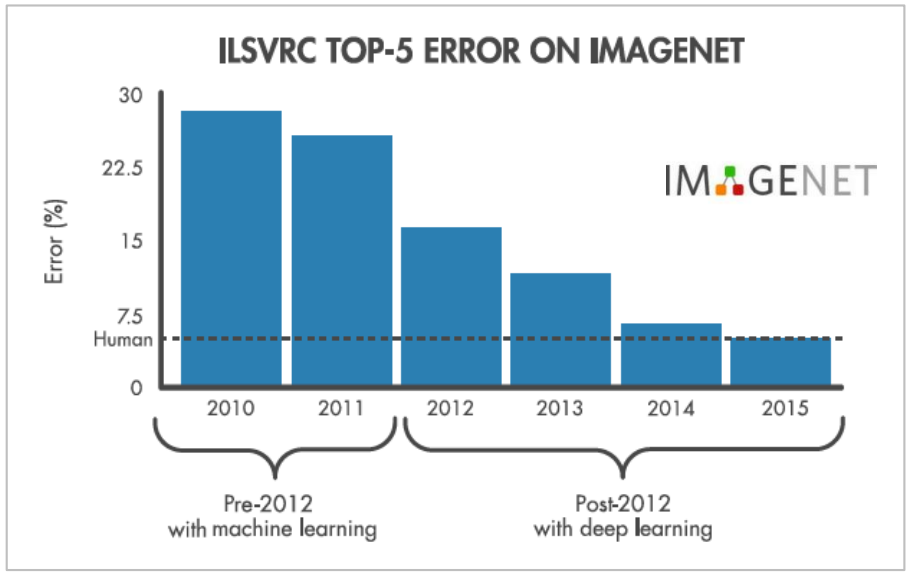
Image classification using Deep Learning

Since 2012, several deep learning algorithms based on CNN have been designed for image classification (AlexNet, GoogleNet, VGGNet, etc.) and features extraction.

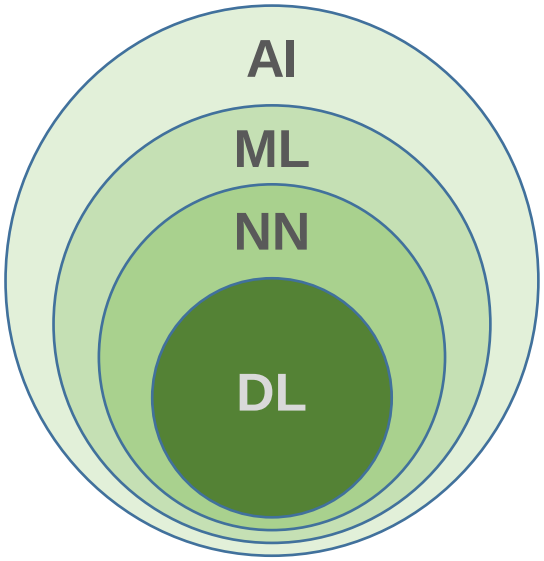




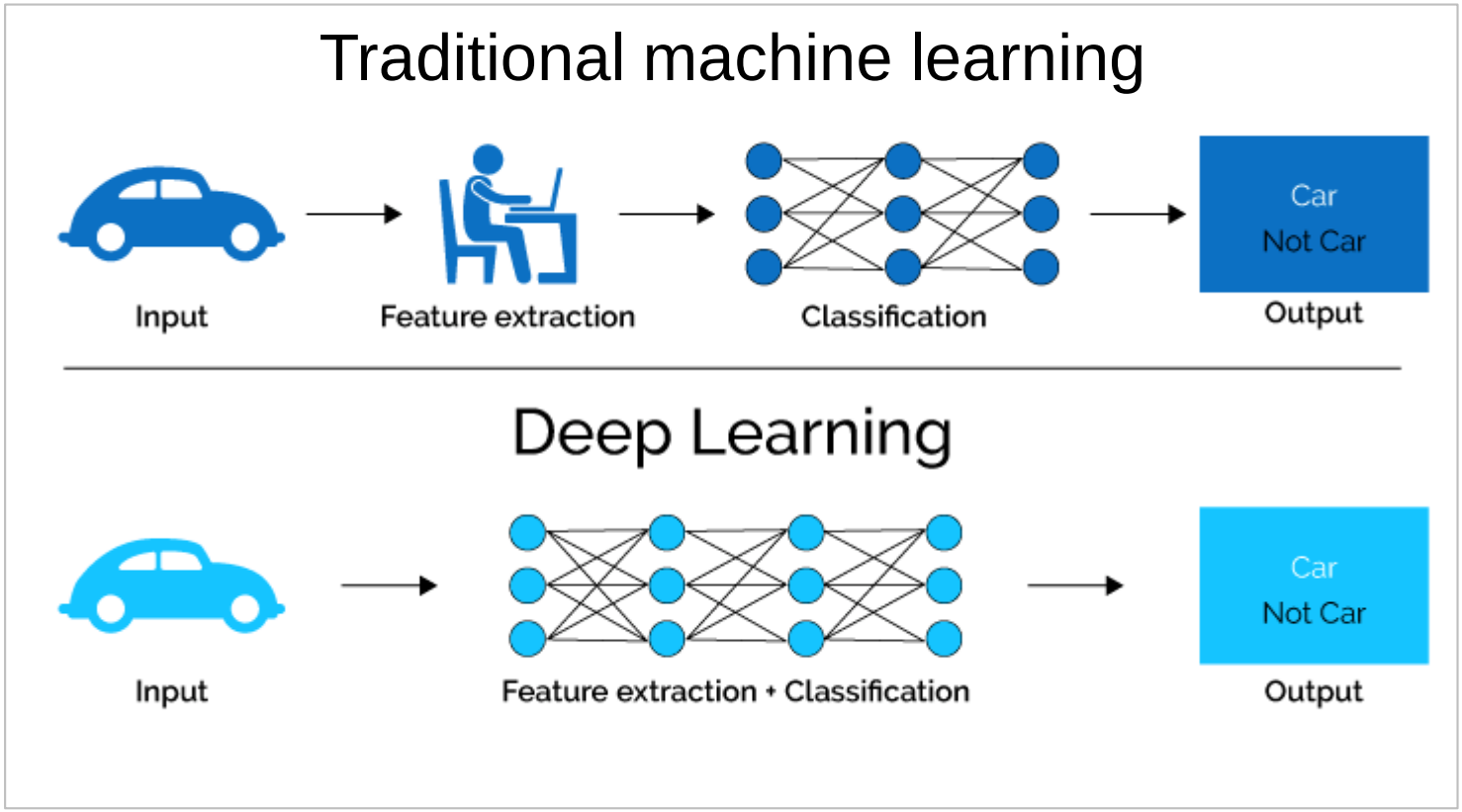
http://image-net.org/index

Krizhevsky – 2012 ImageNet classification with deep convolution neural networks – NIPS 2012

Deep Learning vs. Machine Learning



AI = artificial intelligence
ML = machine learning
NN = neural network
DL = deep learning



For image classification, objects recognition and localization, most algorithms use a Convolutional Neural Network (CNN).

Convolutional Neural Network

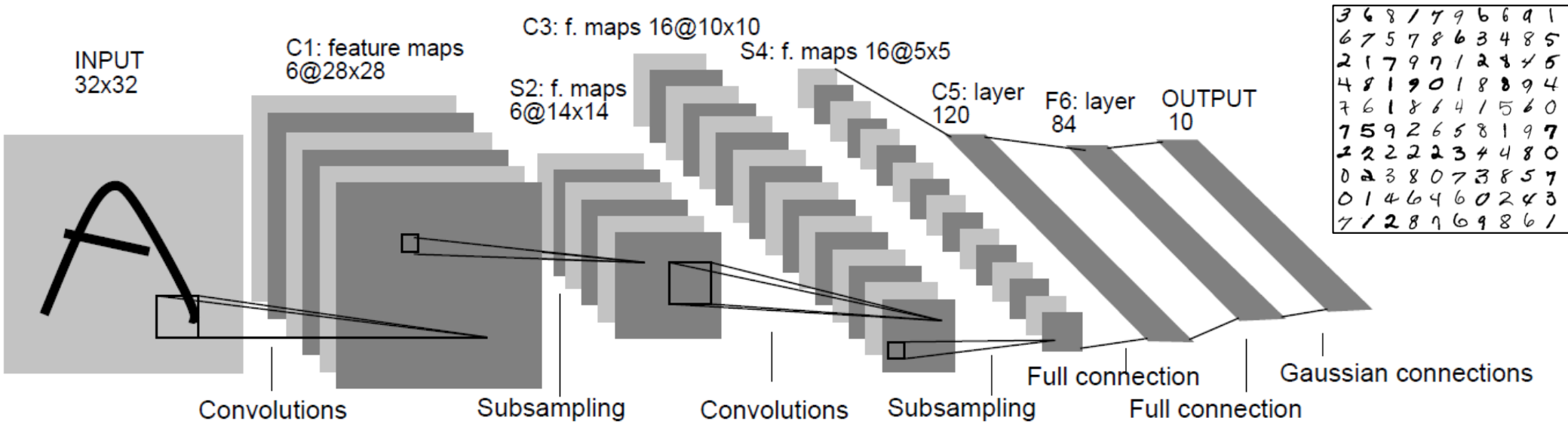
1943 – First description of Artificial Neural Network

1985 – Description of the back-propagation algorithm for training multi-layers NN

1988 – First description of the Convolutional NN

1998 – Application of CNN for digit recognition on ZIP codes

2012 – CNN AlexNet wins the 2012 ImageNet contest



LeCun – 1998 Proc of the IEEE

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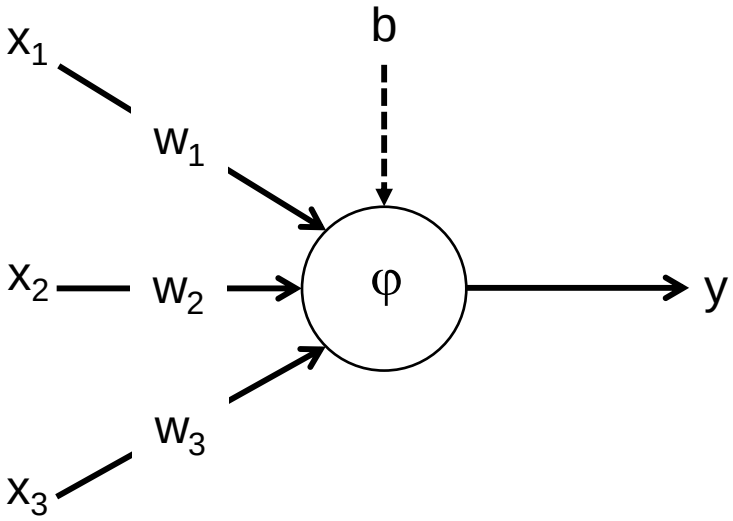
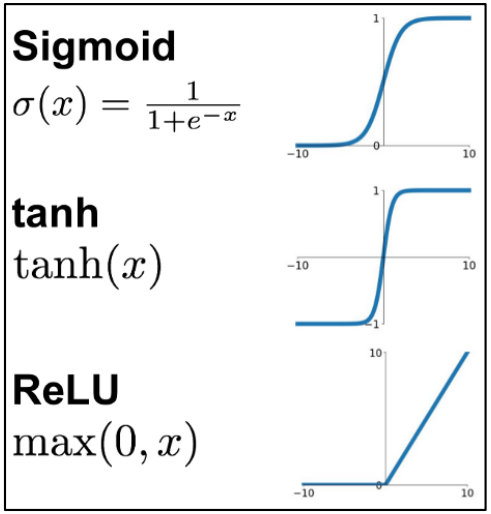
Definition of a neuron

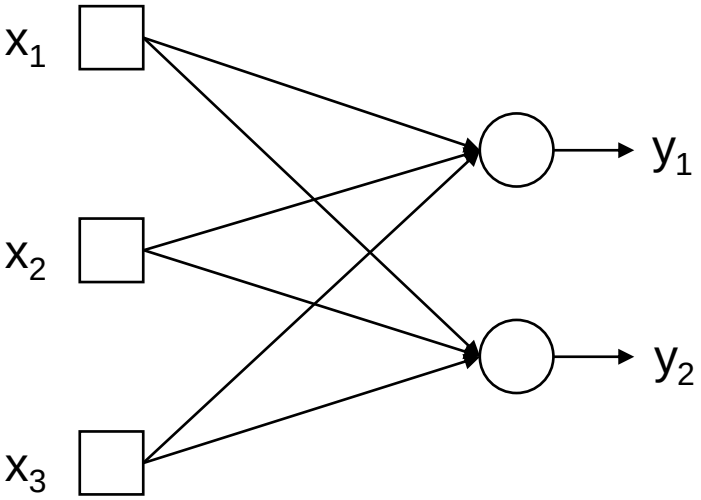


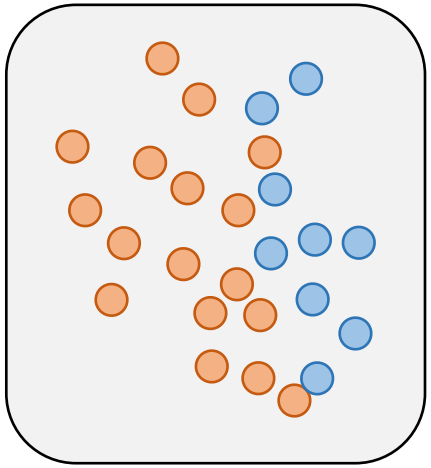
$$v = w_1 * x_1 + w_2 * x_2 + w_3 * x_3$$

$$y = \varphi(v)$$

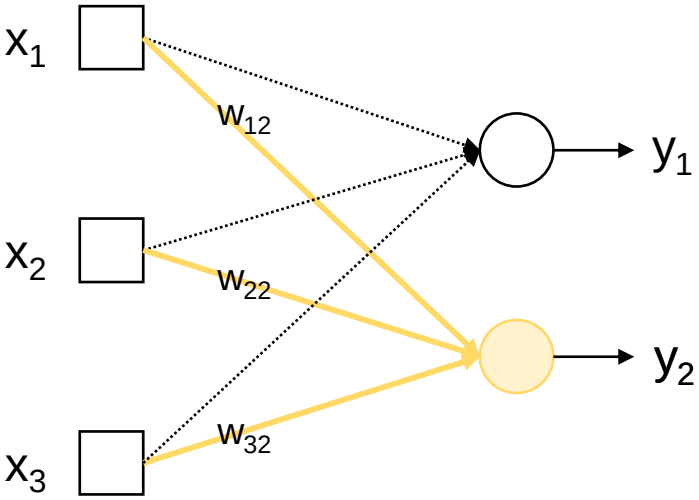


The simplest neural network : 1 layer

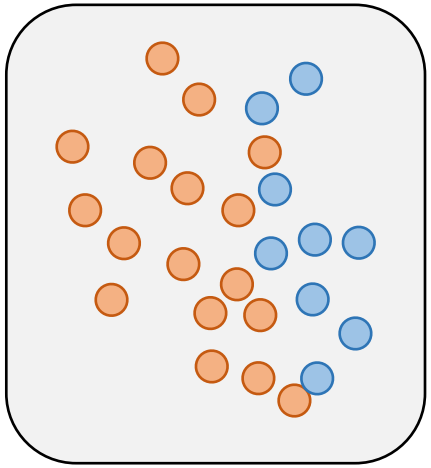




Training of the shallow neural network



$$\Rightarrow \quad e_i = t_i - y_i \qquad \Rightarrow \begin{cases} w_{ij} \leftarrow w_{ij} + \alpha \delta_i x_j \\ \delta_i = \varphi'(v_i) e_i \end{cases}$$



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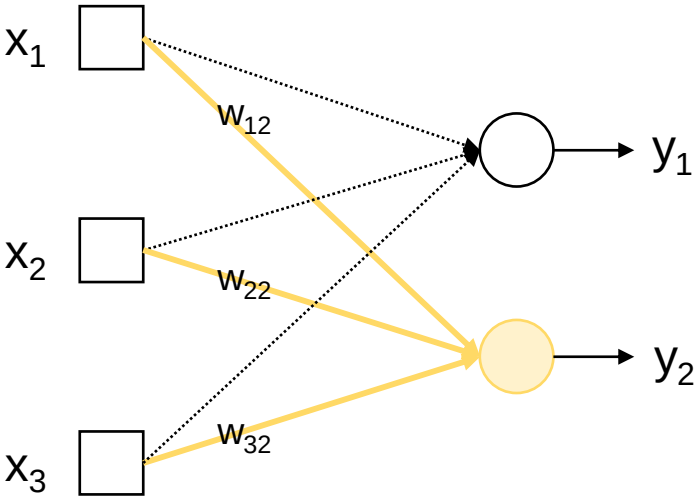

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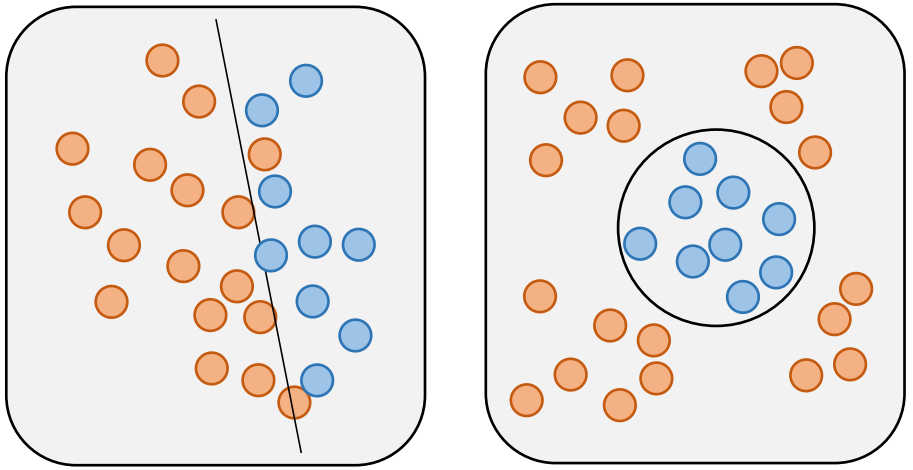
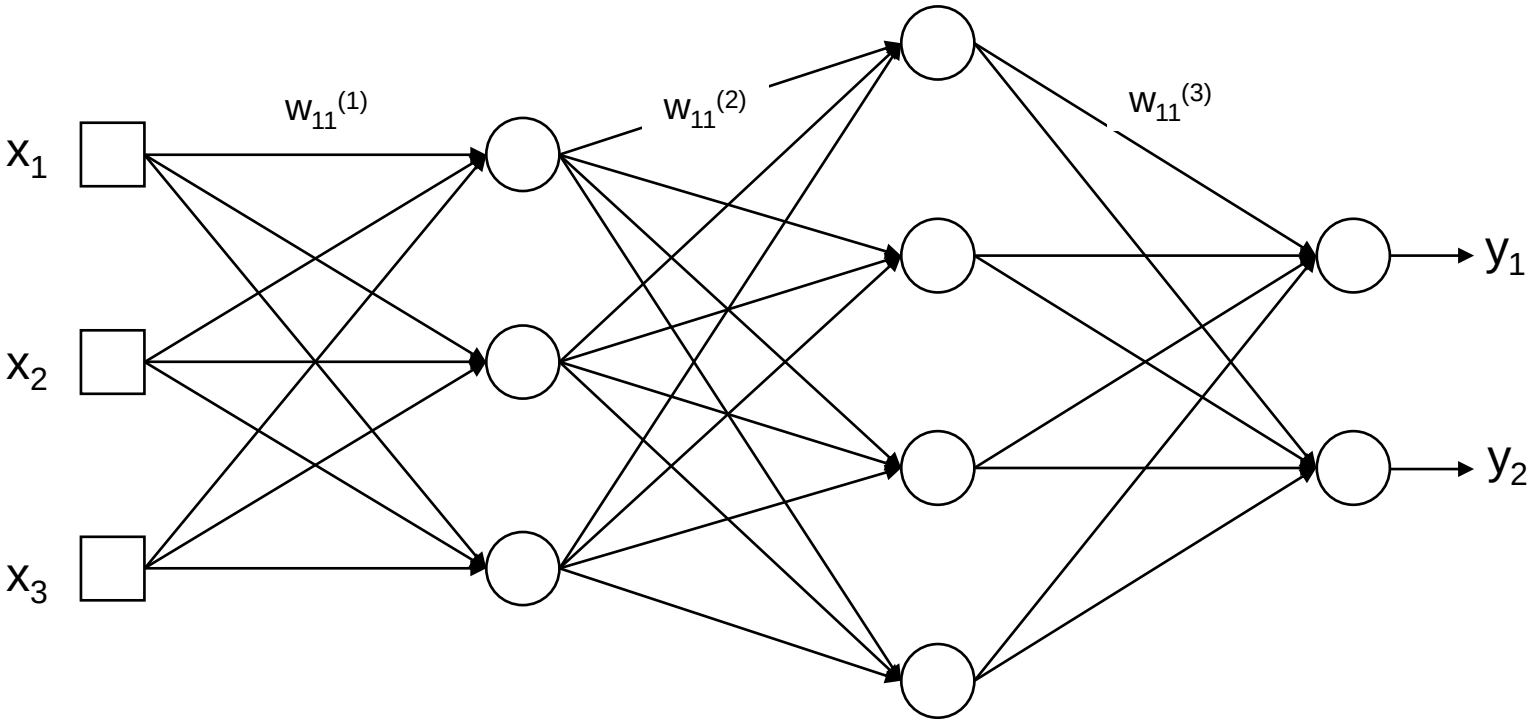


Linearly separable problem

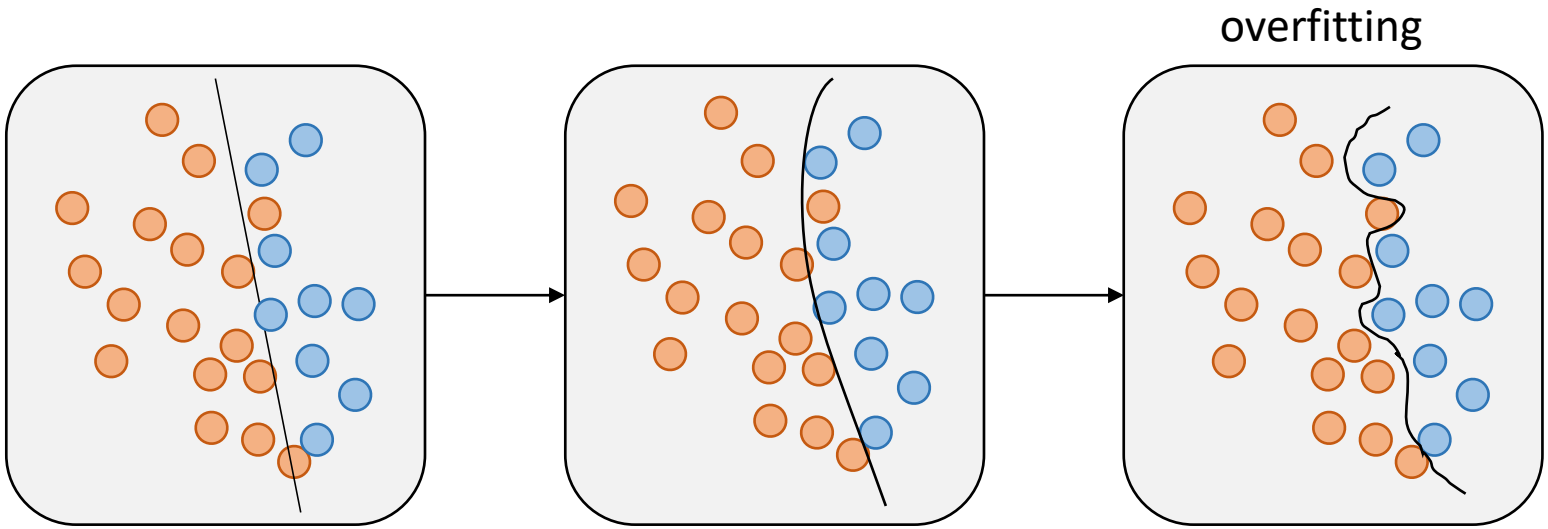
Deep neural network



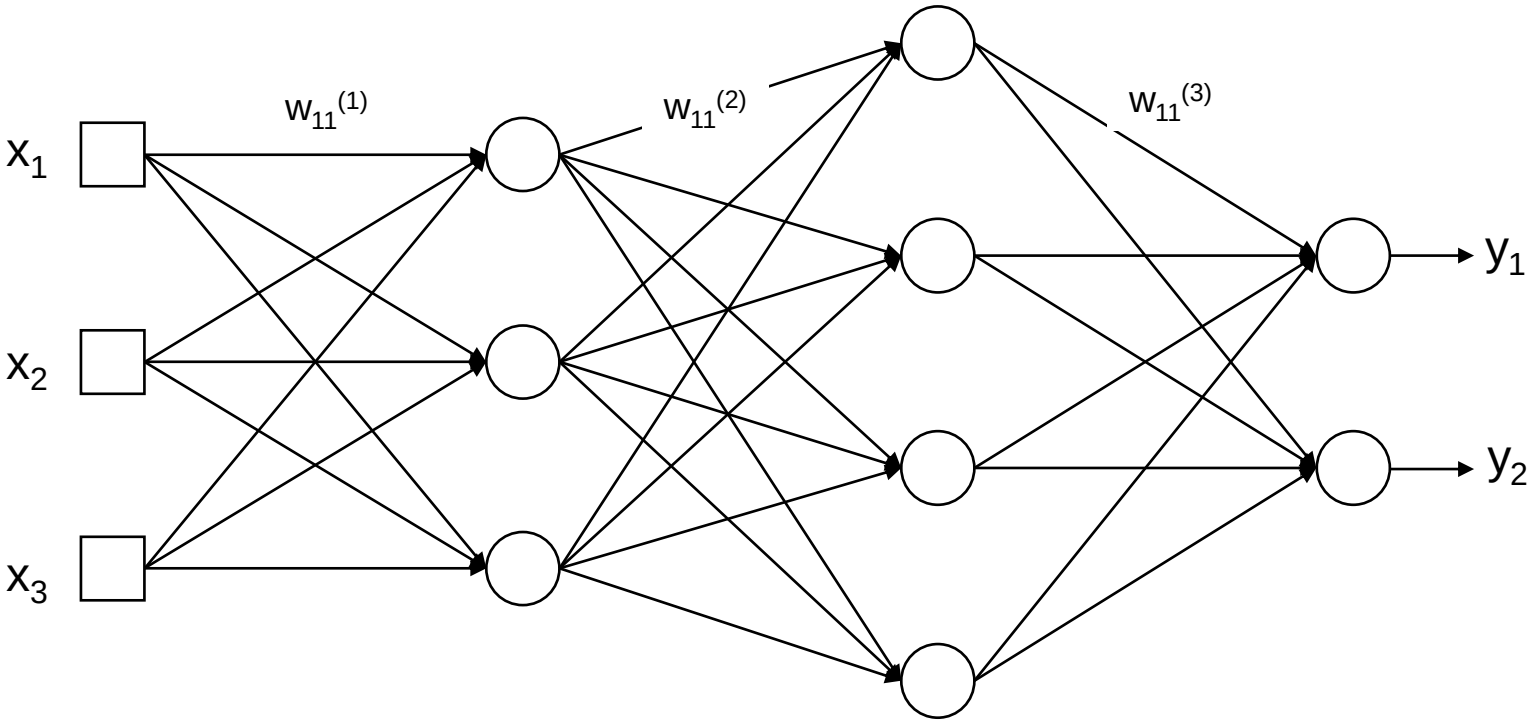
$$e^{(k)} = W^T \delta^{(k+1)}$$

$$\delta^{(k)} = \varphi'\left(v^{(k)}\right) e^{(k)}$$

Error back-propagation



Quick introduction on how neural network works?



$$e^{(k)} = W^T \delta^{(k+1)}$$

$$\delta^{(k)} = \varphi'\left(v^{(k)}\right) e^{(k)}$$

Error back-propagation

Sigmoid

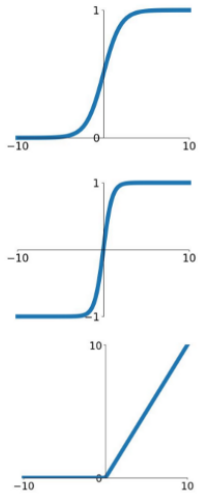
$\sigma(x) = \frac{1}{1+e^{-x}}$

tanh

$\tanh(x)$

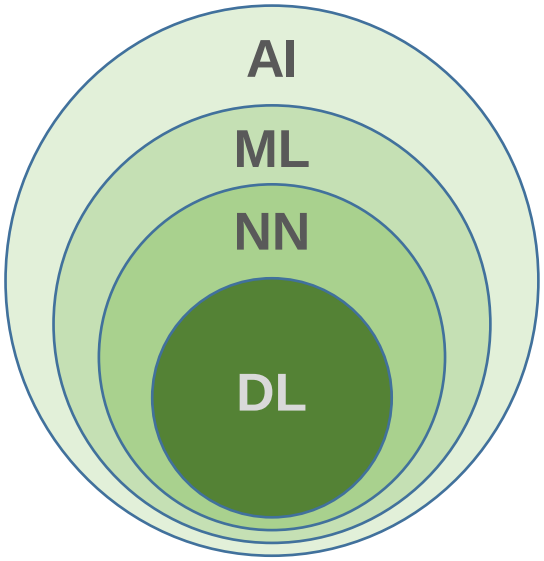
ReLU

$\max(0, x)$



The ReLU activation function is used in most of the recent NN as it helps prevent the **vanishing gradient** issue.

Deep Learning vs. Machine Learning



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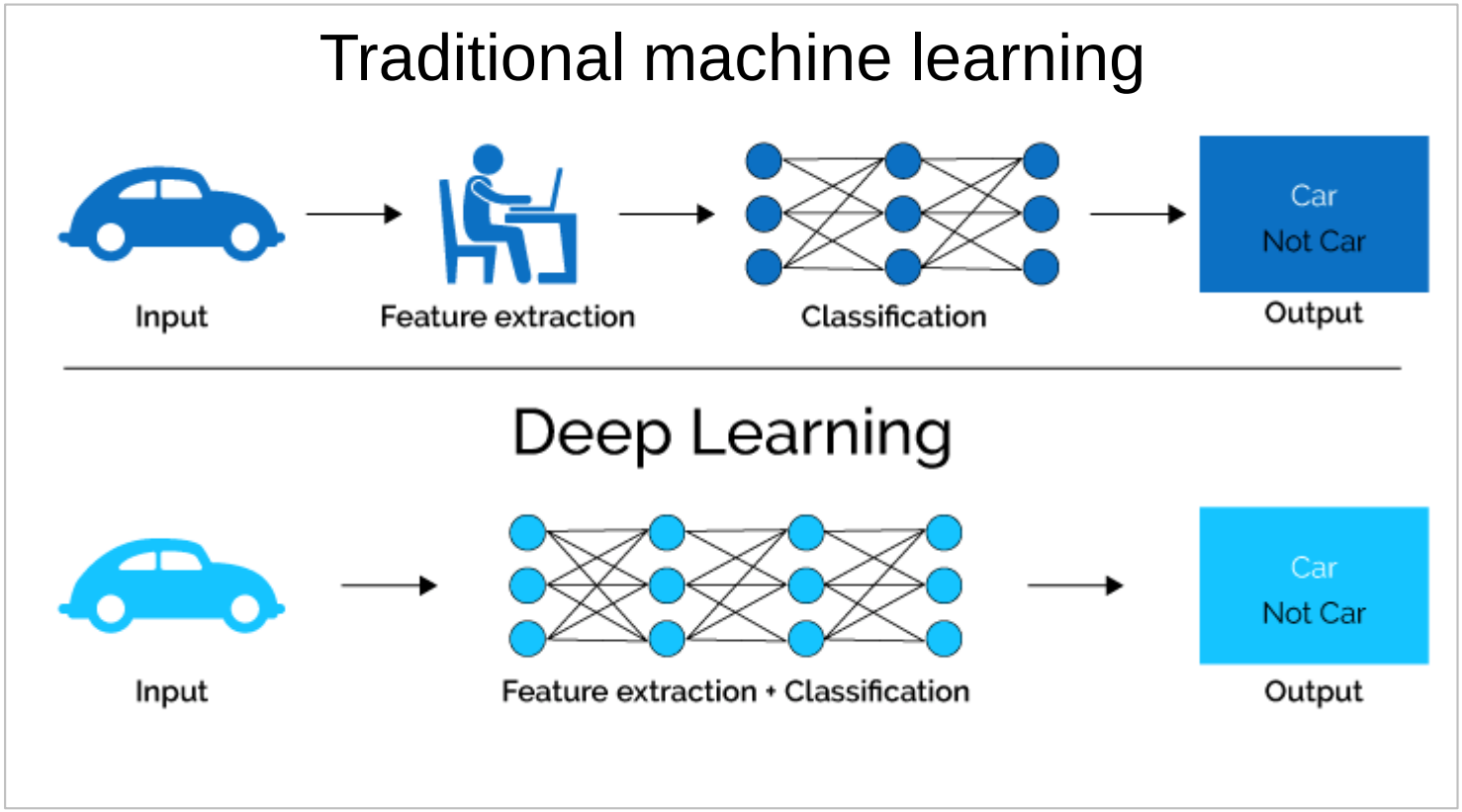

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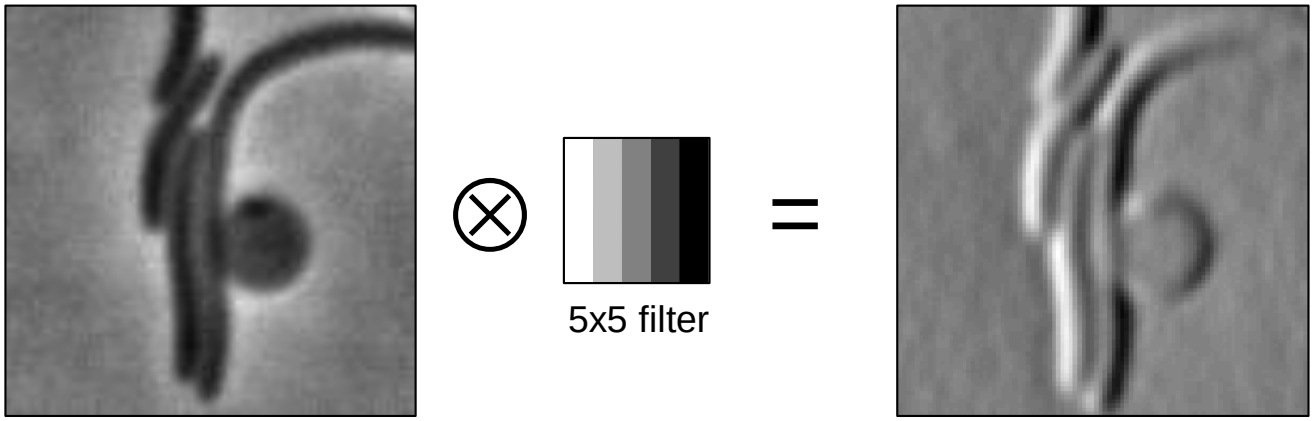
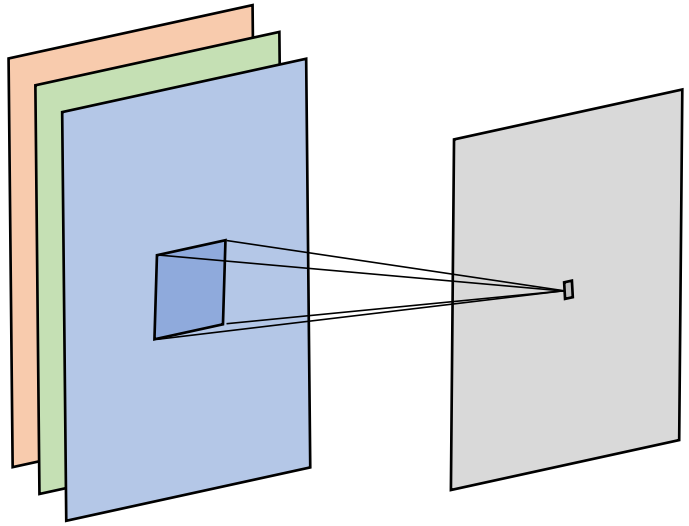
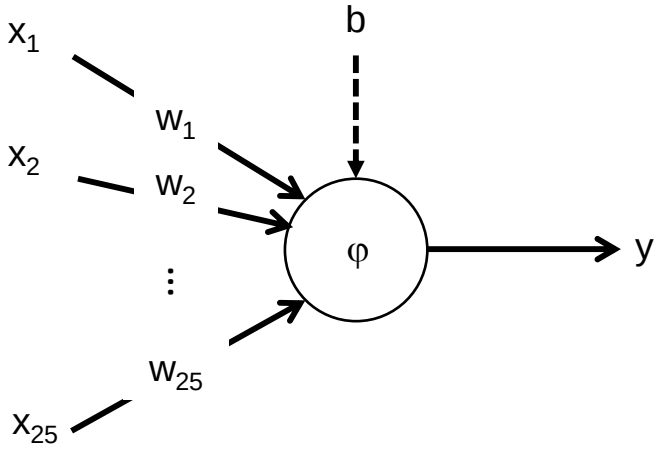
Convolutional neural network

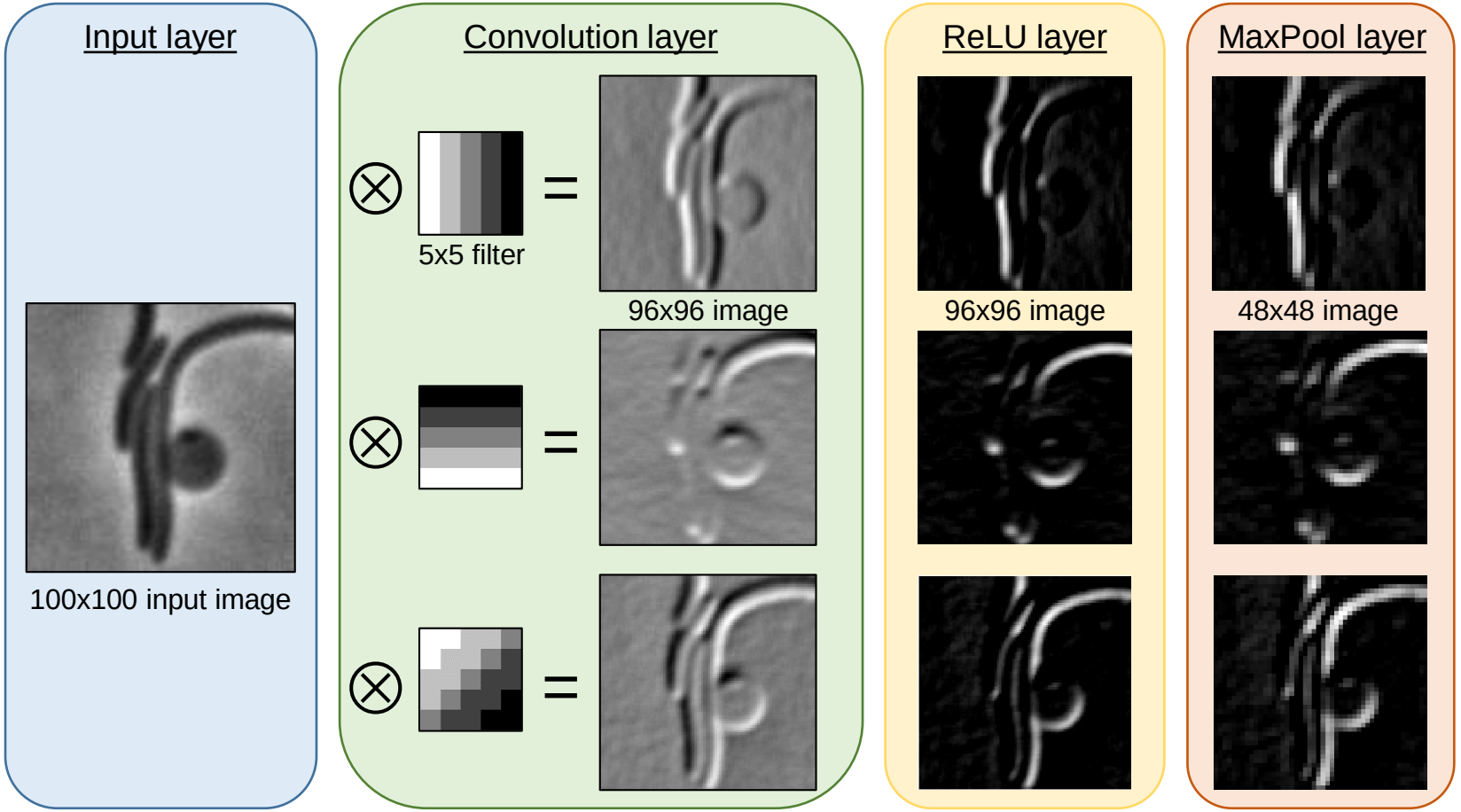


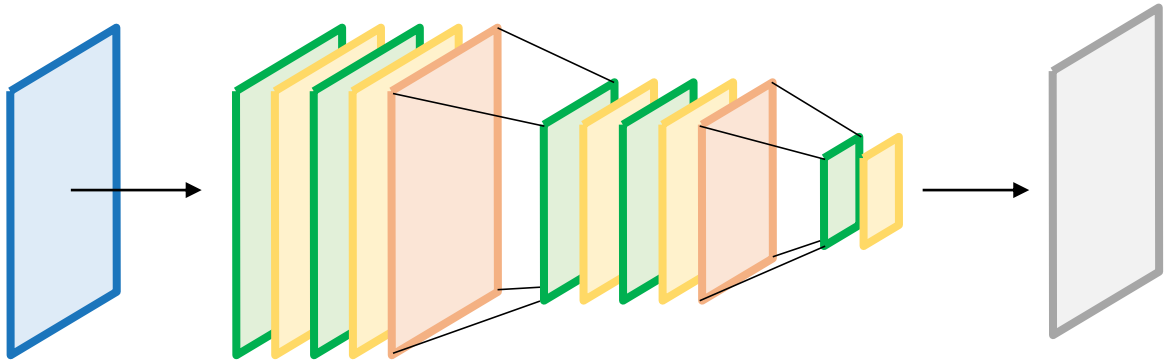




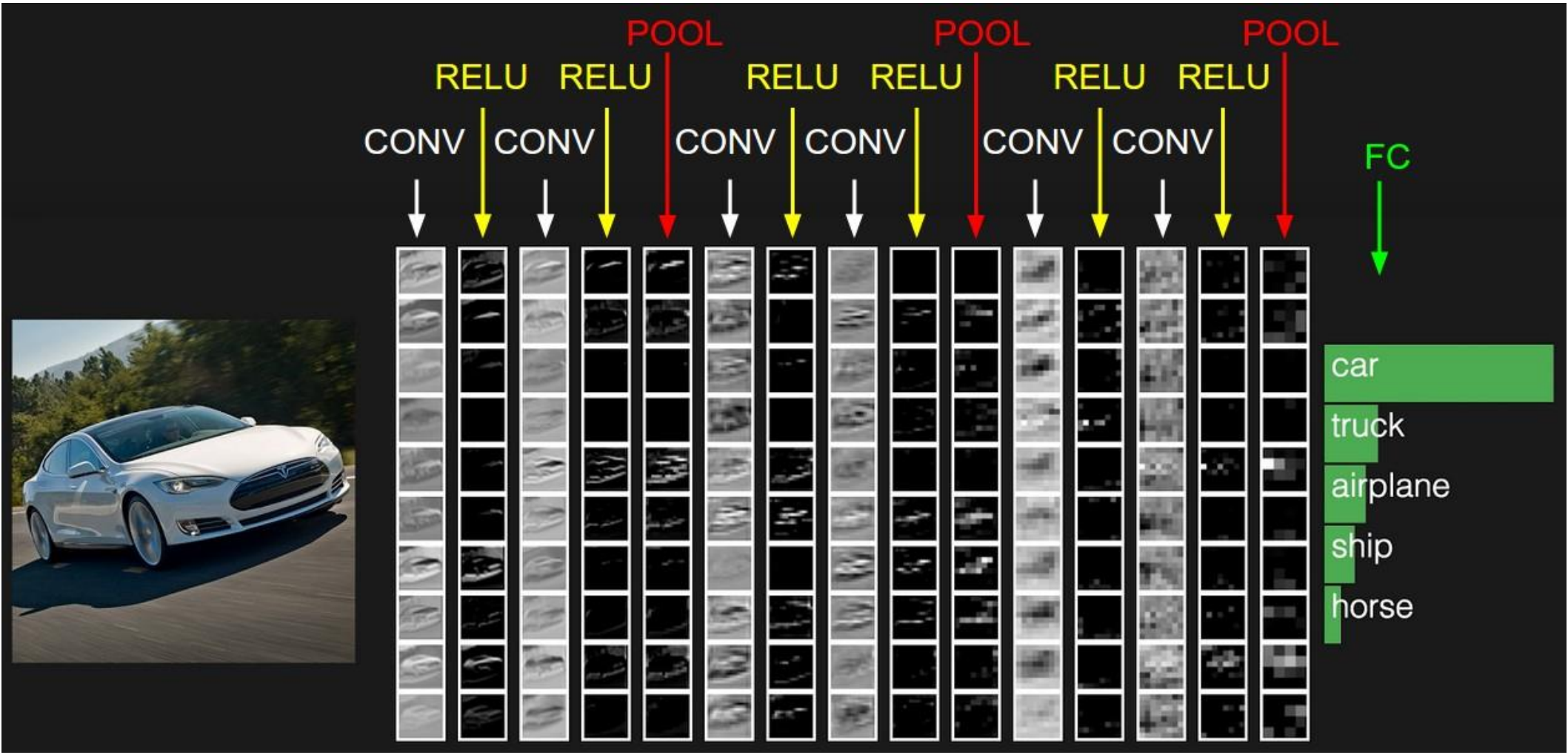
$$v = w_1 * x_1 + w_2 * x_2 + \ ... + \ w_{25} * x_{25}$$
$$y = \ \varphi(v)$$

Convolutional neural network



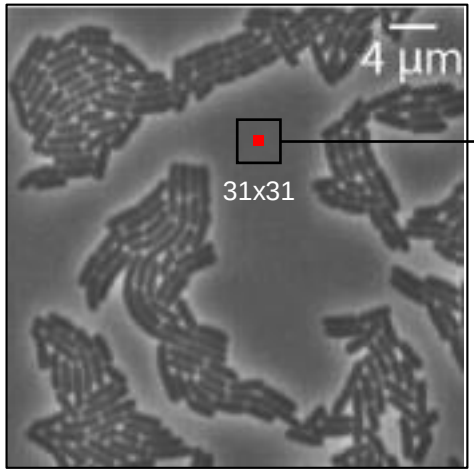


Convolutional neural network



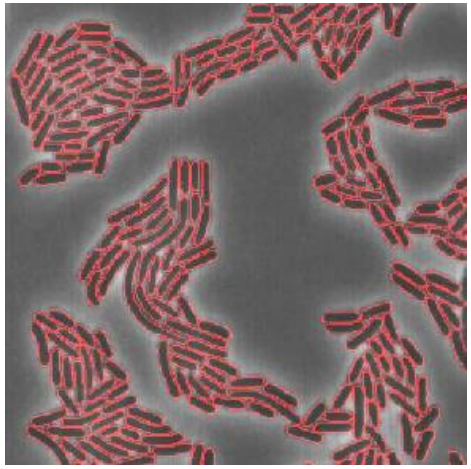
http://cs231n.github.io/convolutional-networks/

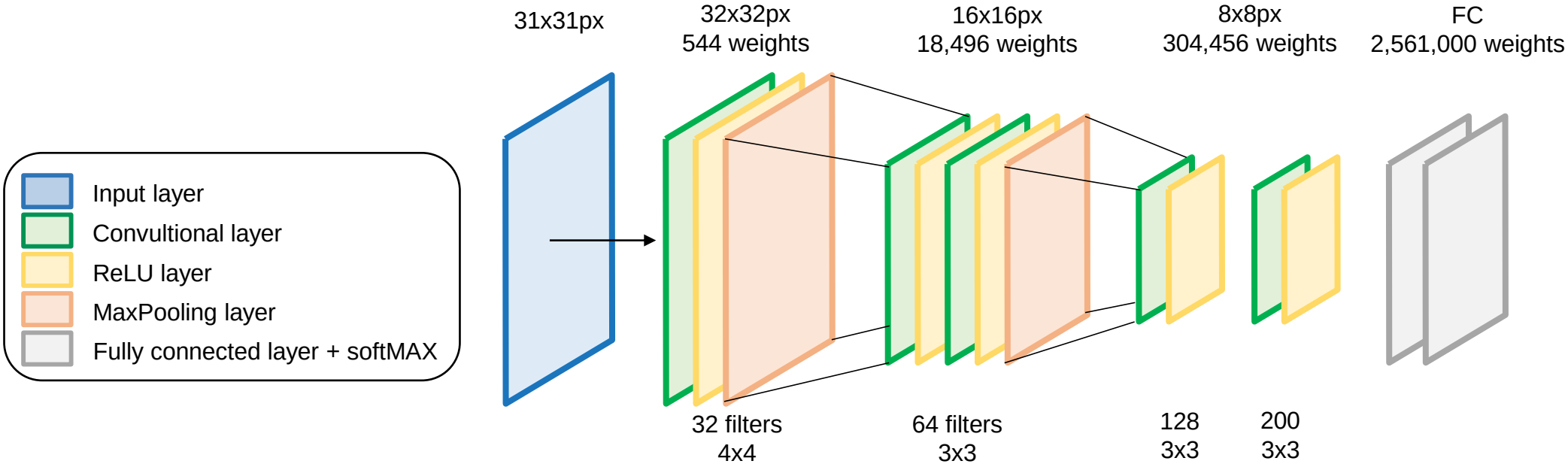
Convolutional neural network applied to cell segmentation



CNN

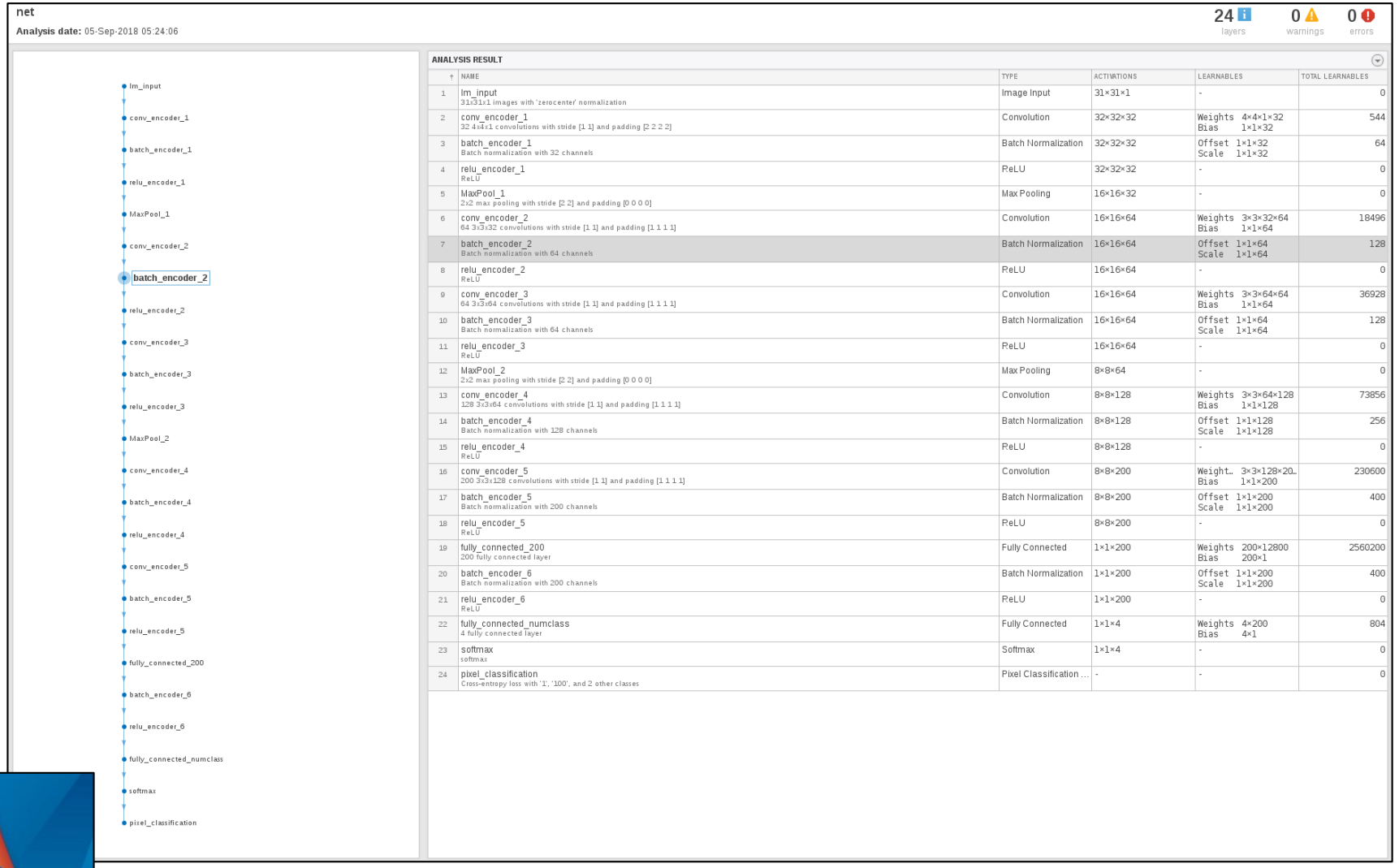
- Cell boundary
- Cell interior
- Background





Van Valen et al. 2016. Plos Computational Biology

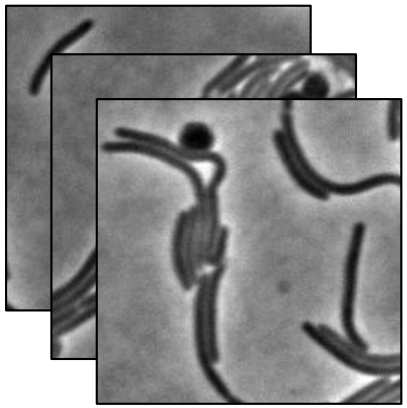
Van-Valen semantic CNN with Matlab



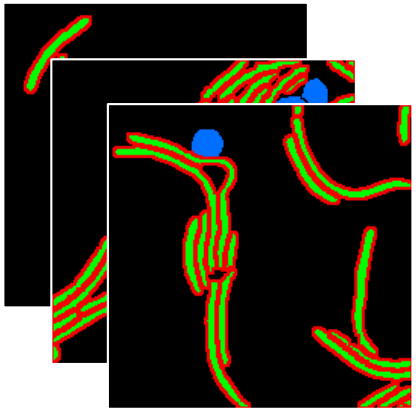
	NAME	TYPE	ACTIVATIONS	LEARNABLES	TOTAL LEARNABLES
1	Im_input 31×31×1 images with 'zerocenter' normalization	Image Input	31×31×1	-	0
2	conv_encoder_1 32 4×4×1 convolutions with stride [1 1] and padding [2 2 2 2]	Convolution	32×32×32	Weights 4×4×1×32 Bias 1×1×32	544
3	batch_encoder_1 Batch normalization with 32 channels	Batch Normalization	32×32×32	Offset 1×1×32 Scale 1×1×32	64
4	relu_encoder_1 ReLU	ReLU	32×32×32	-	0
5	MaxPool_1 2x2 max pooling with stride [2 2] and padding [0 0 0 0]	Max Pooling	16×16×32	-	0
6	conv_encoder_2 64 3×3×32 convolutions with stride [1 1] and padding [1 1 1 1]	Convolution	16×16×64	Weights 3×3×32×64 Bias 1×1×64	18496
7	batch_encoder_2 Batch normalization with 64 channels	Batch Normalization	16×16×64	Offset 1×1×64 Scale 1×1×64	128
8	relu_encoder_2 ReLU	ReLU	16×16×64	-	0
9	conv_encoder_3 64 3×3×64 convolutions with stride [1 1] and padding [1 1 1 1]	Convolution	16×16×64	Weights 3×3×64×64 Bias 1×1×64	36928
10	batch_encoder_3 Batch normalization with 64 channels	Batch Normalization	16×16×64	Offset 1×1×64 Scale 1×1×64	128
11	relu_encoder_3 ReLU	ReLU	16×16×64	-	0
12	MaxPool_2 2x2 max pooling with stride [2 2] and padding [0 0 0 0]	Max Pooling	8×8×64	-	0
13	conv_encoder_4 128 3×3×64 convolutions with stride [1 1] and padding [1 1 1 1]	Convolution	8×8×128	Weights 3×3×64×128 Bias 1×1×128	73856
14	batch_encoder_4 Batch normalization with 128 channels	Batch Normalization	8×8×128	Offset 1×1×128 Scale 1×1×128	256
15	relu_encoder_4 ReLU	ReLU	8×8×128	-	0
16	conv_encoder_5 200 3×3×128 convolutions with stride [1 1] and padding [1 1 1 1]	Convolution	8×8×200	Weight… 3×3×128×20… Bias 1×1×200	230600
17	batch_encoder_5 Batch normalization with 200 channels	Batch Normalization	8×8×200	Offset 1×1×200 Scale 1×1×200	400
18	relu_encoder_5 ReLU	ReLU	8×8×200	-	0
19	fully_connected_200 200 fully connected layer	Fully Connected	1×1×200	Weights 200×12800 Bias 200×1	2560200
20	batch_encoder_6 Batch normalization with 200 channels	Batch Normalization	1×1×200	Offset 1×1×200 Scale 1×1×200	400
21	relu_encoder_6 ReLU	ReLU	1×1×200	-	0
22	fully_connected_numclass 4 fully connected layer	Fully Connected	1×1×4	Weights 4×200 Bias 4×1	804
23	softmax softmax	Softmax	1×1×4	-	0
24	pixel_classification Cross-entropy loss with '1', '100', and 2 other classes	Pixel Classification …	-	-	0

How do we train this supervised deep learning network?

Test images



200x200px

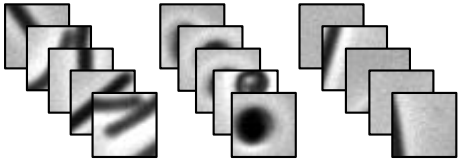


Ground truth

Step 1 : Manual labelling

100 images from 3 different experiments
On each image the four classes are observed

Create the training/testing set



31x31

Step 2 : Training & Testing sets

Training/testing set of 31x31 images, with the same number of images for each class.

- Training set : ~50,000 images/class
- Testing set : ~10,000

Training rules

- Background
- Cell outline
- Cell body
- Dirt / Dead cells

Step 3 : Training (2,5h)

After defining the training rules, the training start with the following parameters :

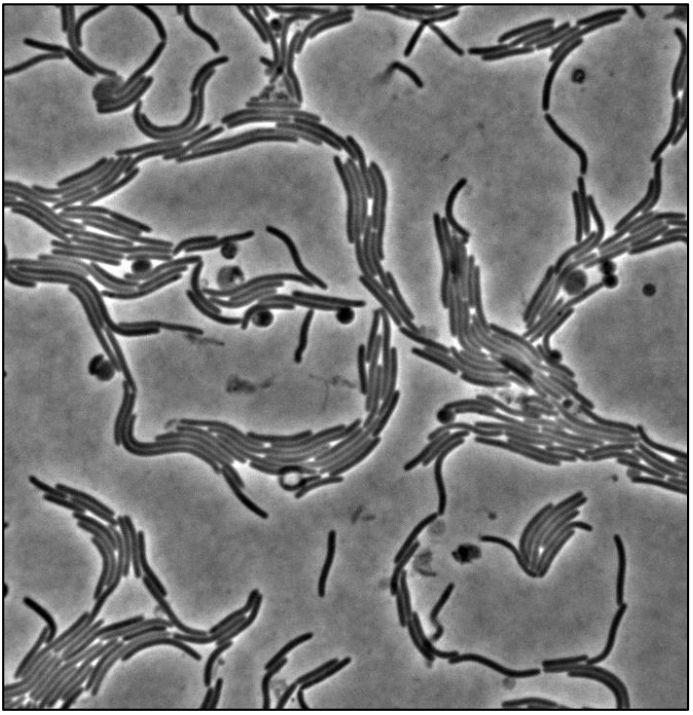
- Stochastic gradient descent method
- Minibatch = 2500
- Epoc = 21

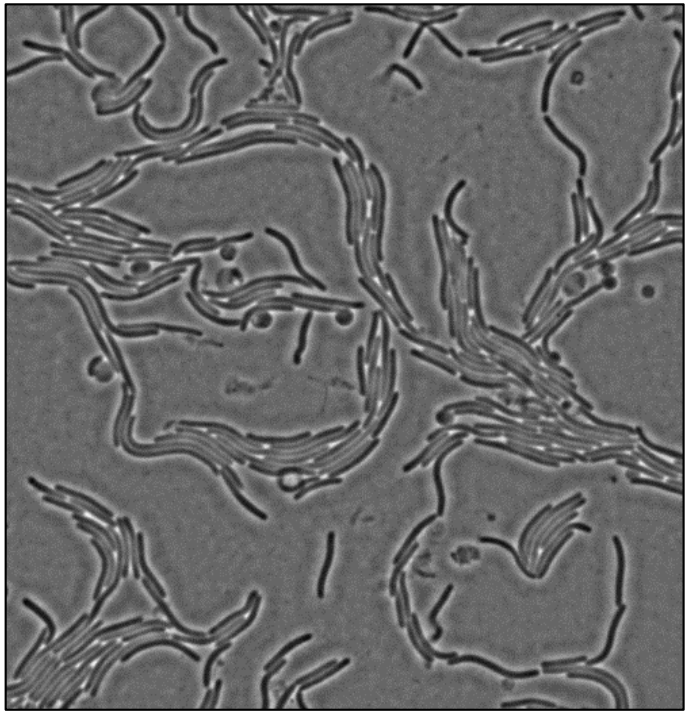
Deep Neural Network

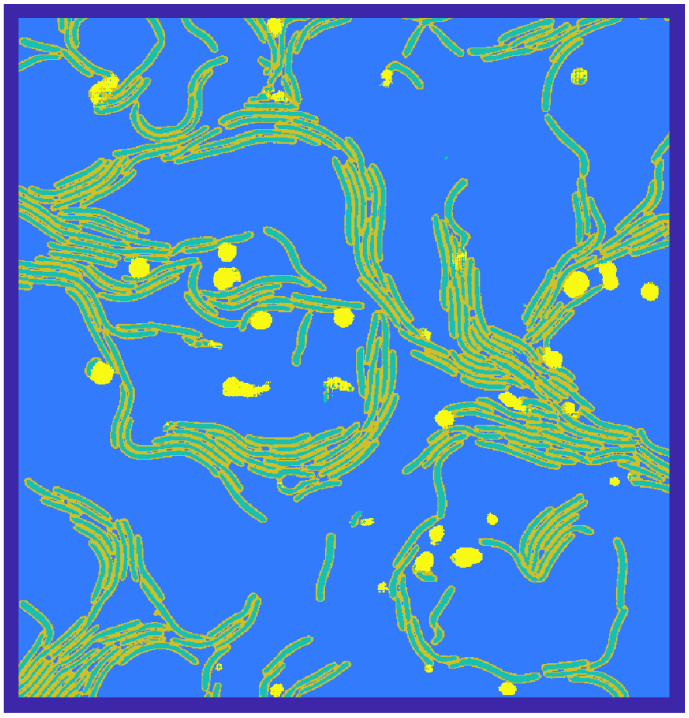
Segmentation of *Myxococcus xanthus* cells

Image normalization

Deep learning network







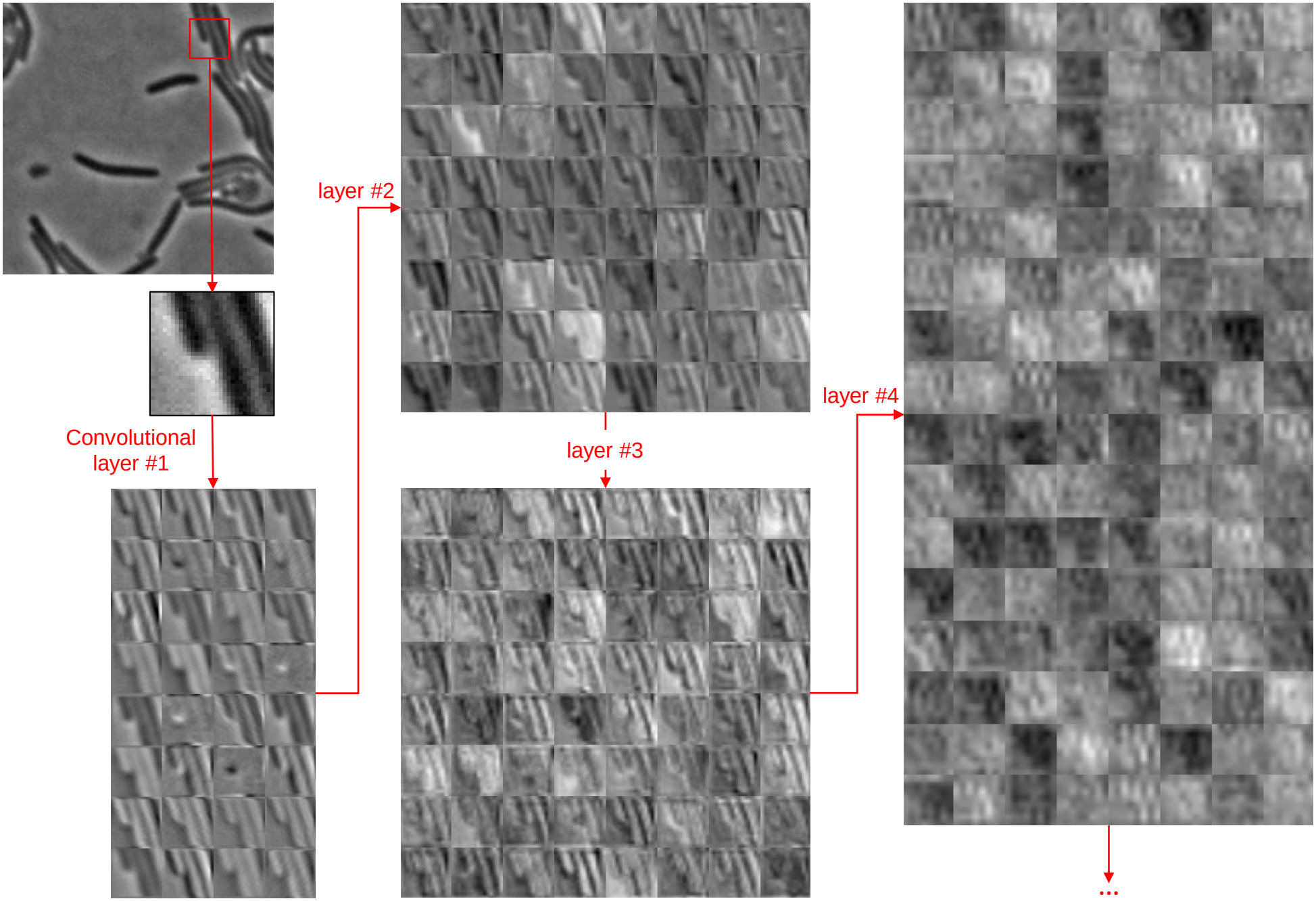
Time-lapse - ΔPilA AglZ-YFP
1000x1000 Phase contrast image

Computation time with 15 cores parallel computing : 4,5min

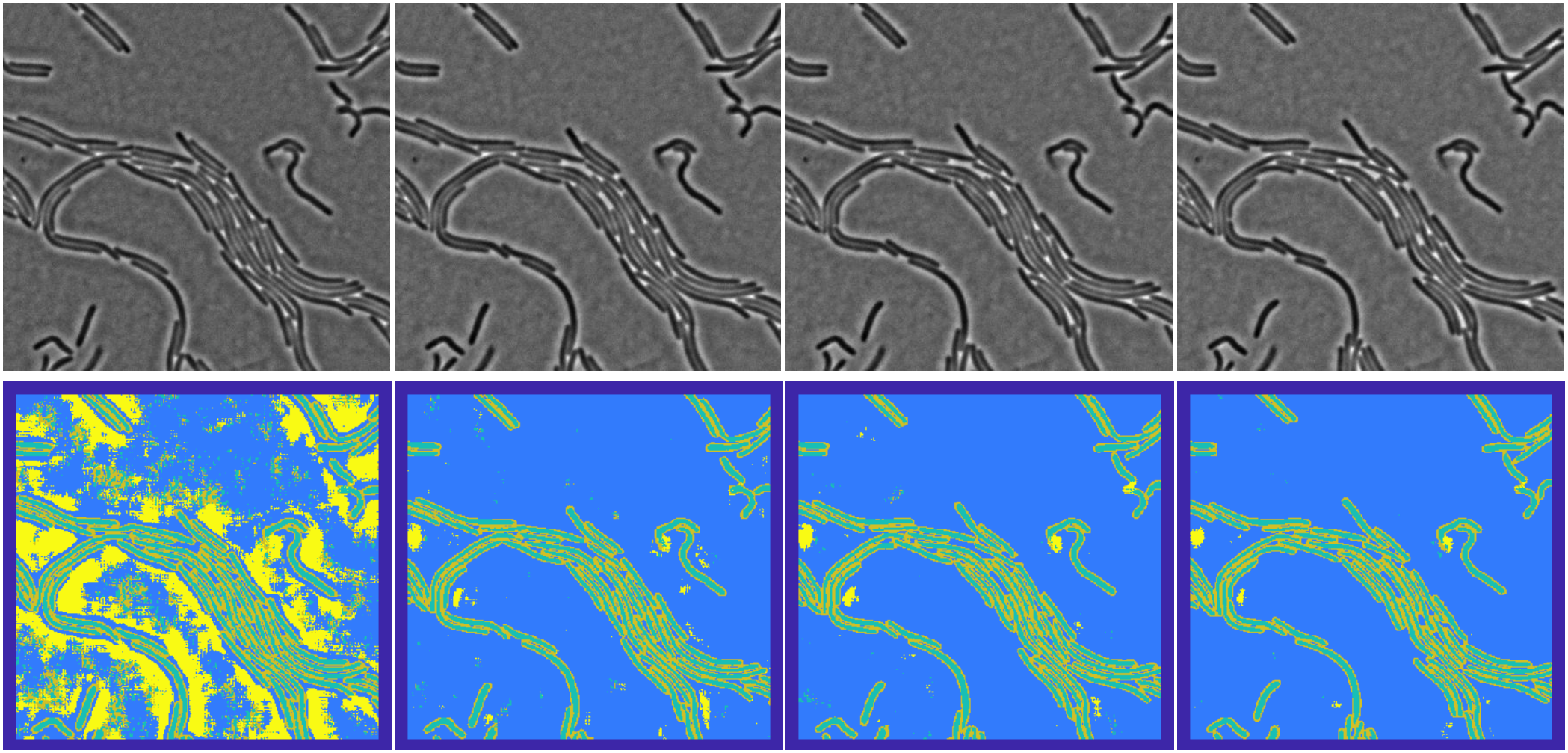
Accuracy for the testing set :

- global accuracy : 93.8 %
- accuracy for « background » : 94.1 %
- accuracy for « dirt » : 99.1 %
- accuracy for « cell inside » : 91 %
- accuracy for « cell contour »:91 %

A glimpse of what is going on « inside »



Limitations of the method

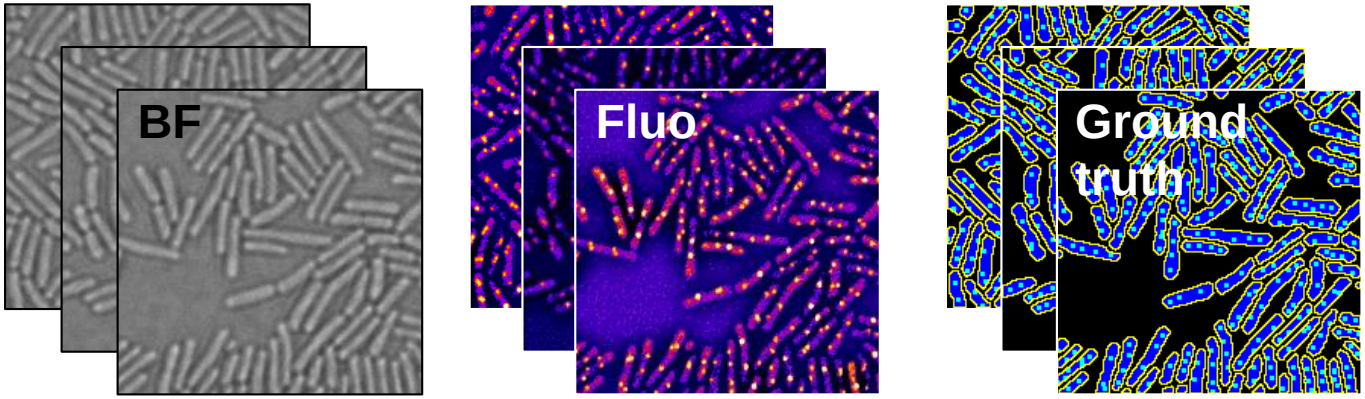


- Class 1 : Background
- Class 2 : Cell outline
- Class 3 : Cell body
- Class 4 : Dirt / Dead cells

For certain images, the classes got mixed up (particularly between classes 1 & 4). The classification might be sensitive to variation in the experimental conditions (intensity, …) → **the experimental conditions needs to be well controlled.**

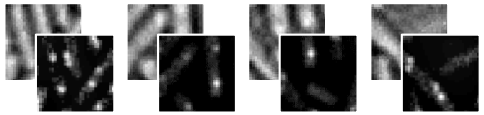
Segmentation of *B.subtilis* : Brightfield + Fluorescence

B.Subtilis - Channel 1 : BF - Channel 2 : Fros Ori-CFP



200x200px

Create the training/testing set



31x31

Training rules

- Background
- Cell outline
- Cell body
- Fluorescent spot

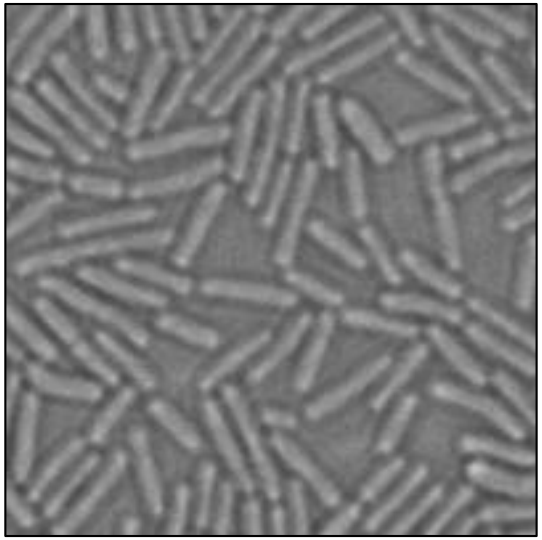
Deep Neural Network

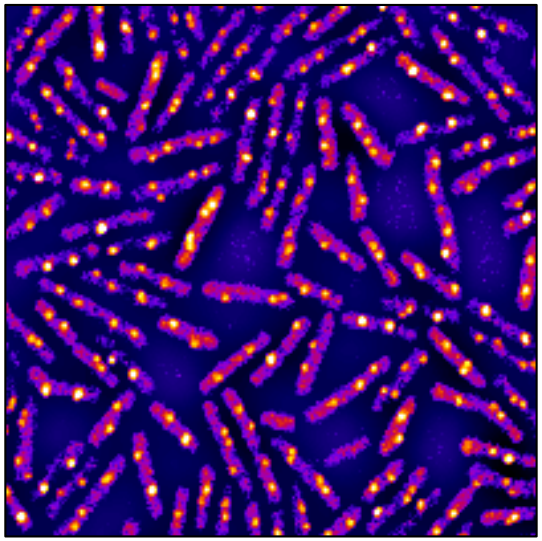
Training & Testing sets

Training/testing set of 31x31 images, with the same number of images for each class.

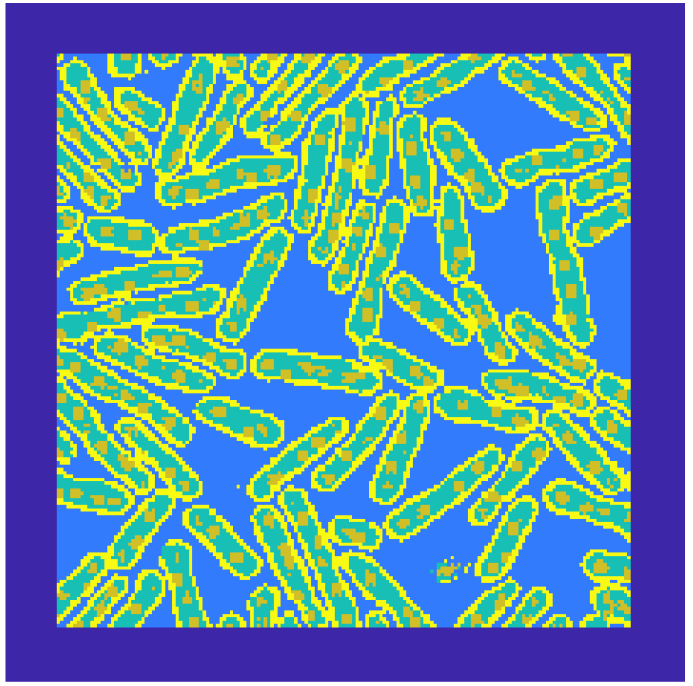
- Training set : ~9,500 images/class
- Testing set : ~2,500 images/class

Segmentation of *B.subtilis* : Brightfield + Fluorescence





CNN



Accuracy for the testing set :

- **Global accuracy : 87.6 %**
- Global accuracy for class1 : 91.6 %
- Global accuracy for class2 : 82.6 %
- Global accuracy for class3 : 91.6 %
- Global accuracy for class4 : 84.5 %

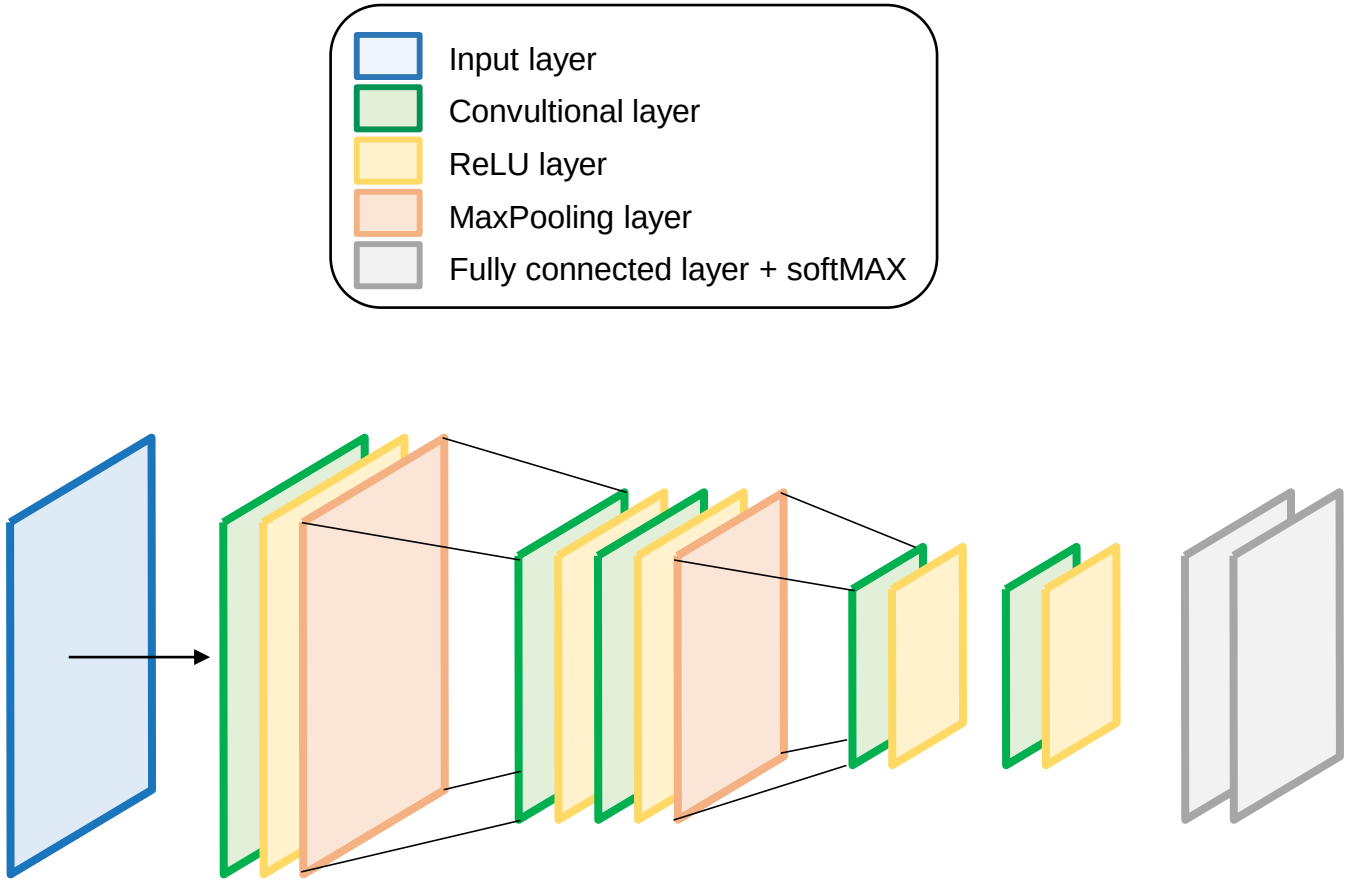
Conclusion and perspectives

- So far, deep learning look like a promising tool for segmentation :
  - ✓ *Myxococcus xanthus* cells on phase contrast images
  - ✓ Bacillus subtilis cells taking into account brightfield and fluorescence images
- The accuracy of segmentation is substancially better as compared to previous results obtained with SuperSegger

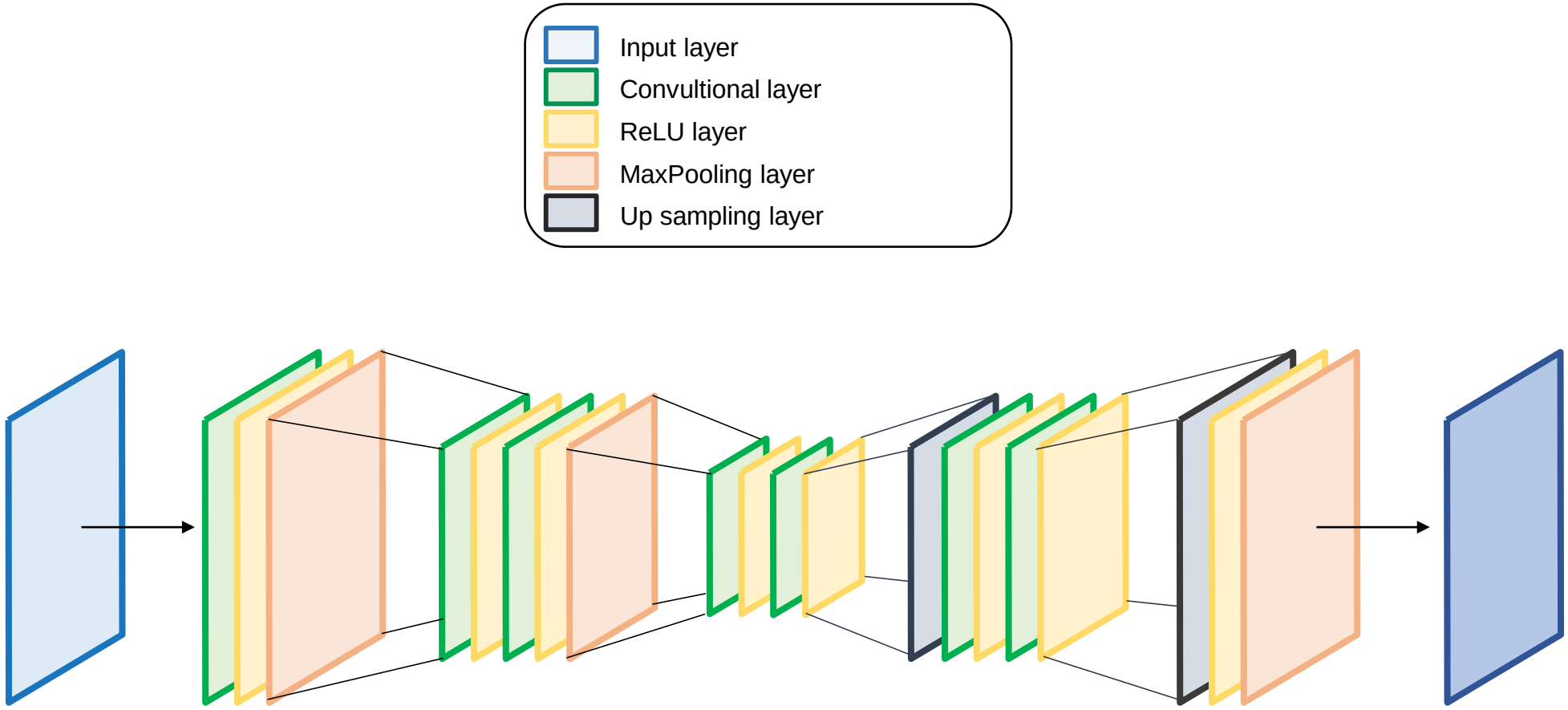
- Improve the computation time – for a 2000x2000 images it takes >15min

  → Fully convolutional network
- How to make the network less susceptible to experimental conditions?

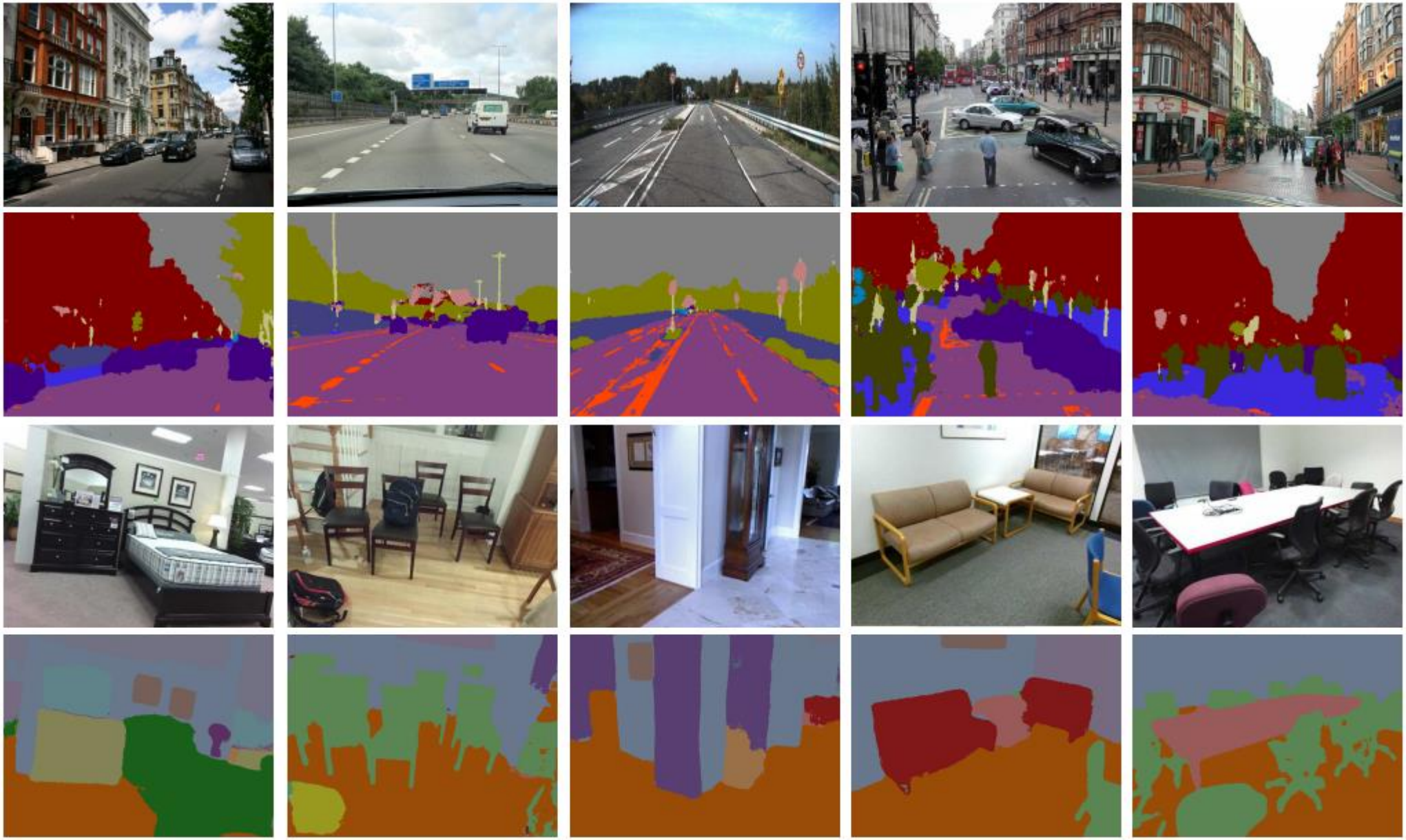
Fully convolutional network



Fully convolutional network

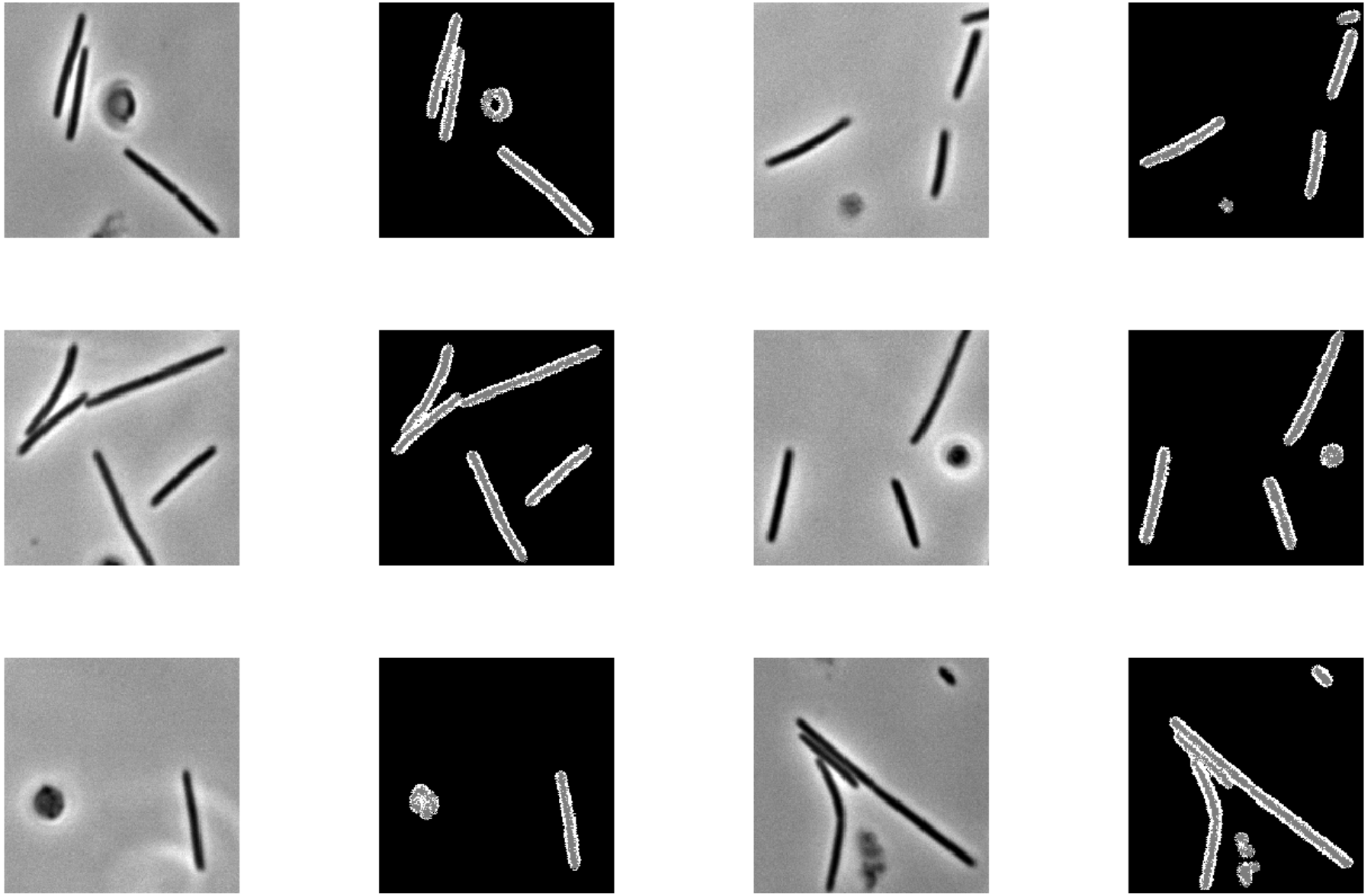


Fully convolutional network



Badrinarayanan et al. 2016. ArXiv

First test with the built-in network on Matlab (SegNet)



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Christophe Houbron
Diego Cattoni
Fanny Berard

Standford Lectures on neural network and deep Learning

https://www.youtube.com/playlist?list=PLC1qU-LWwrF64f4QKQT-Vg5Wr4qEE1Zxk