Assessing cell-activity in time-lapse microscopy

Creative Collaboration Center

Stefan Baar

室蘭工業大学

Overview

- Introduction
- Motivation
- Experiments
- Cell activity

- Image processing
- Deep learning
- Results
- Annotations
- Summary

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Website: https://github.com/StefanBaar/stefanbaar.github.io

About me

- Born in Germany

2013 University of Jena (Germany)
- Master of Science: Astrophysics

2016 Muroran Institute of Technology
- Ph.D Superconductor Physics

2020 University of Hyogo
- Post doc Nishi-Harima Astronomical observatory

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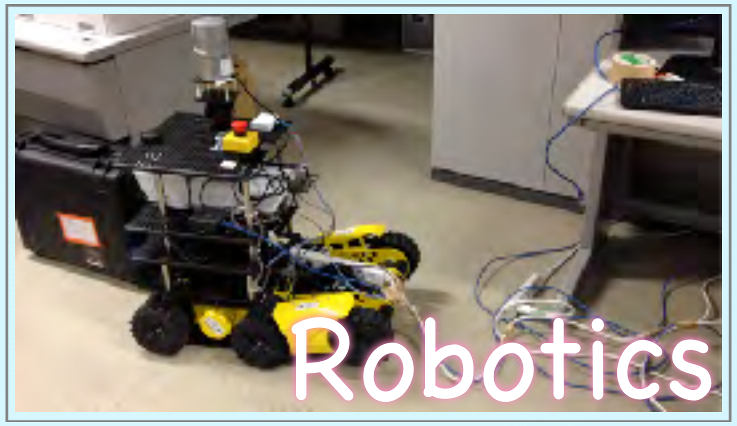

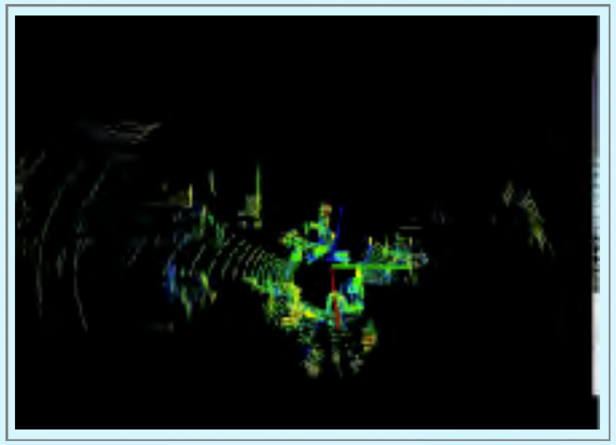

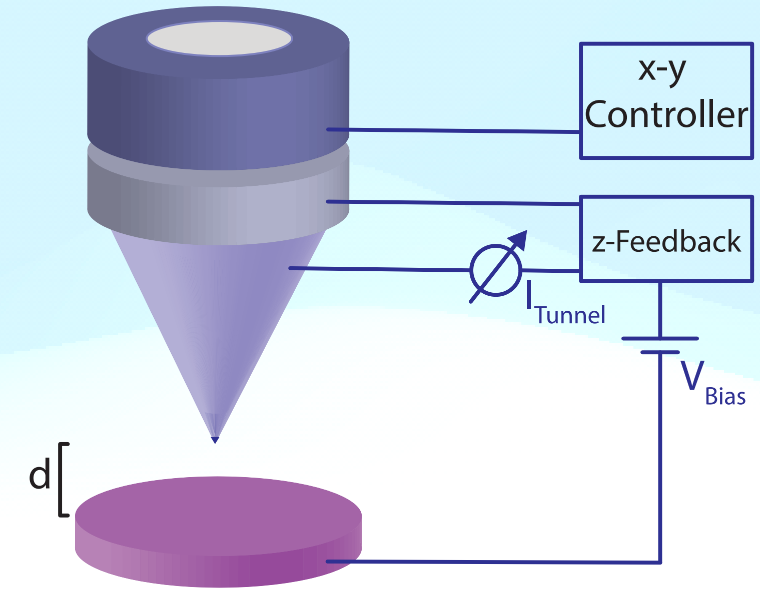

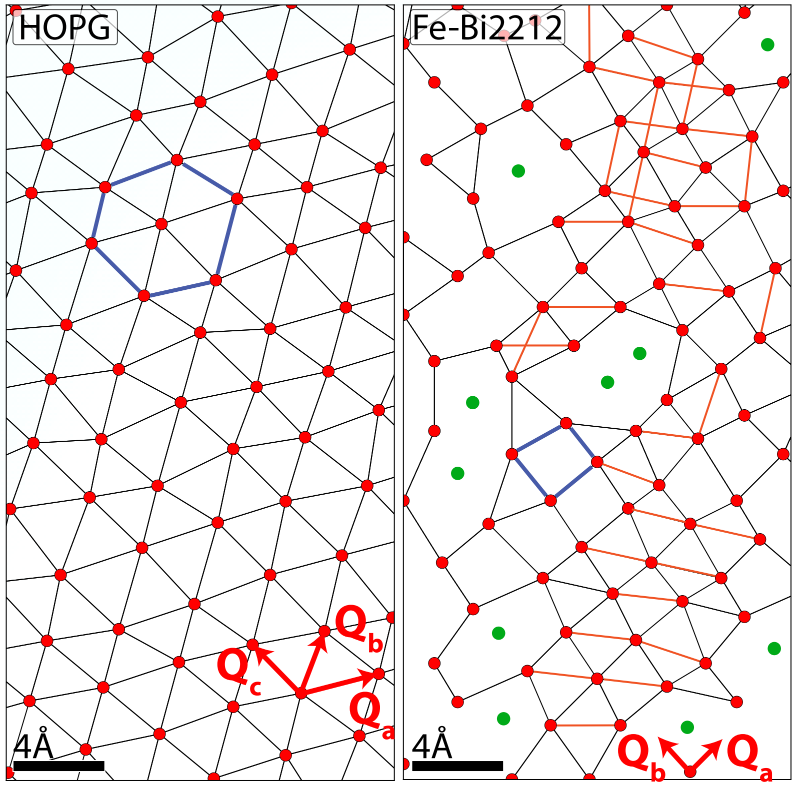

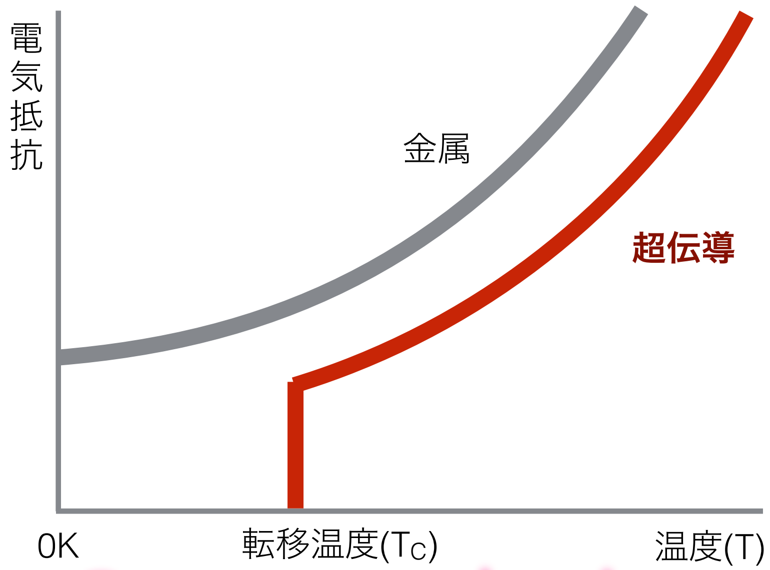

Superconductors

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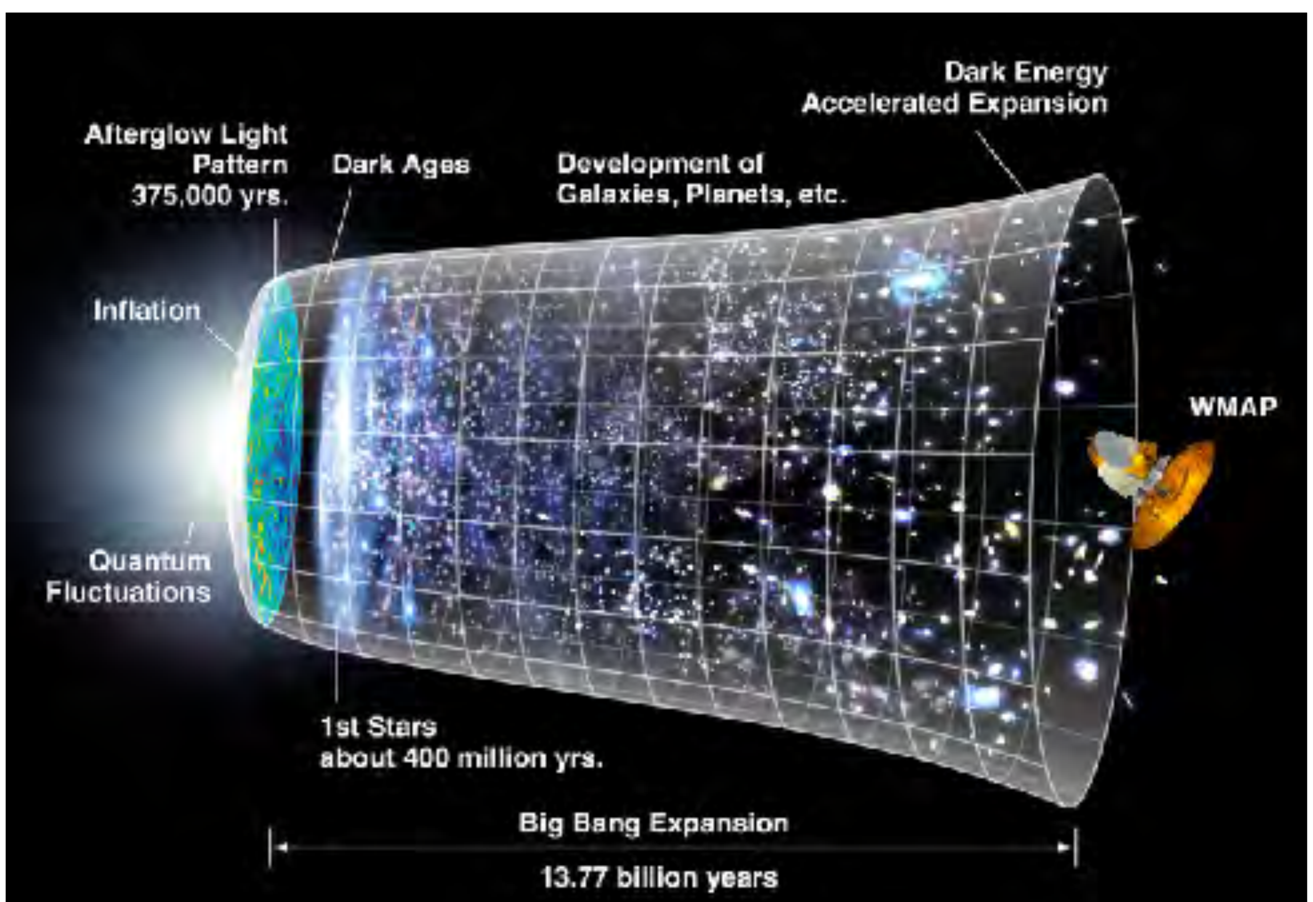
- Post doc Nishi-Harima Astronomical observatory

Interests

Website: https://stefanbaar.github.io/

GitHub: https://github.com/StefanBaar

- Cosmology
- Photography
- Programming
- Deeplearning
- Robotics



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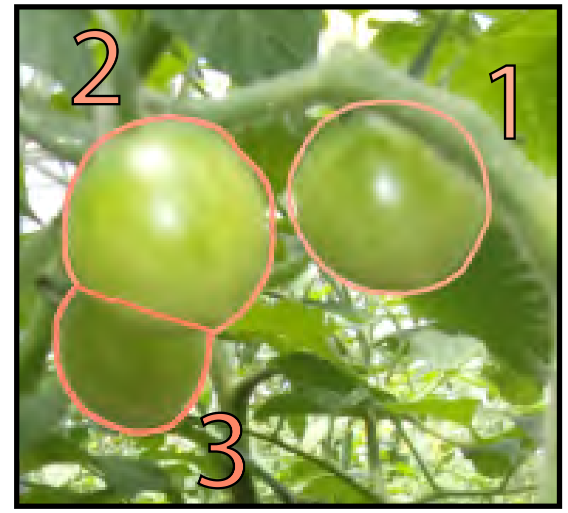
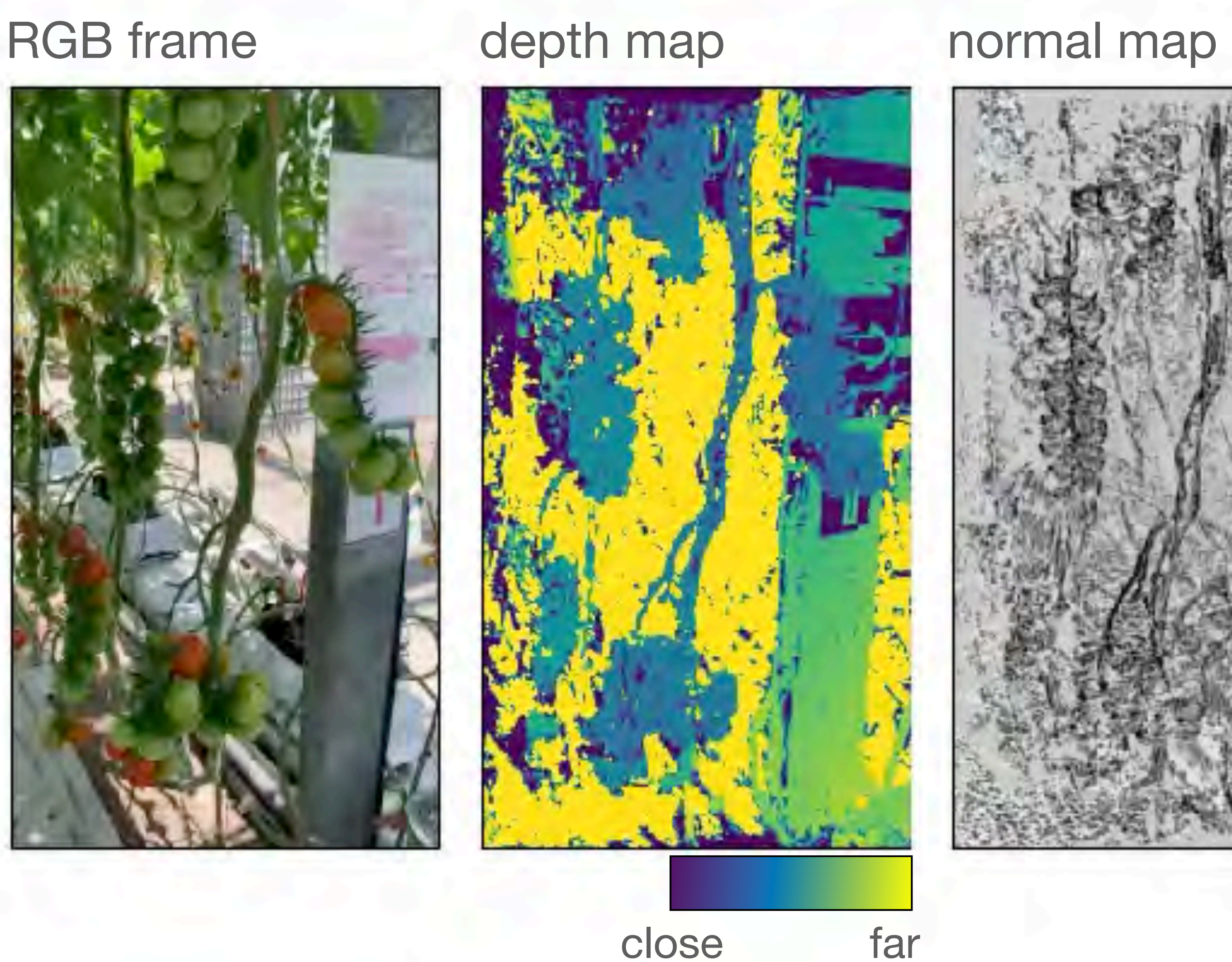
- **IOT (Internet of Things)**
- **Automation in Agriculture**
- **Drones**
- **Structure from motion**





Computer vision based cell activity estimation

Motivation

Automated (large scale) drug screening / causal research

- -Alzheimer's disease
- -Parkinson's disease
- -Cancer

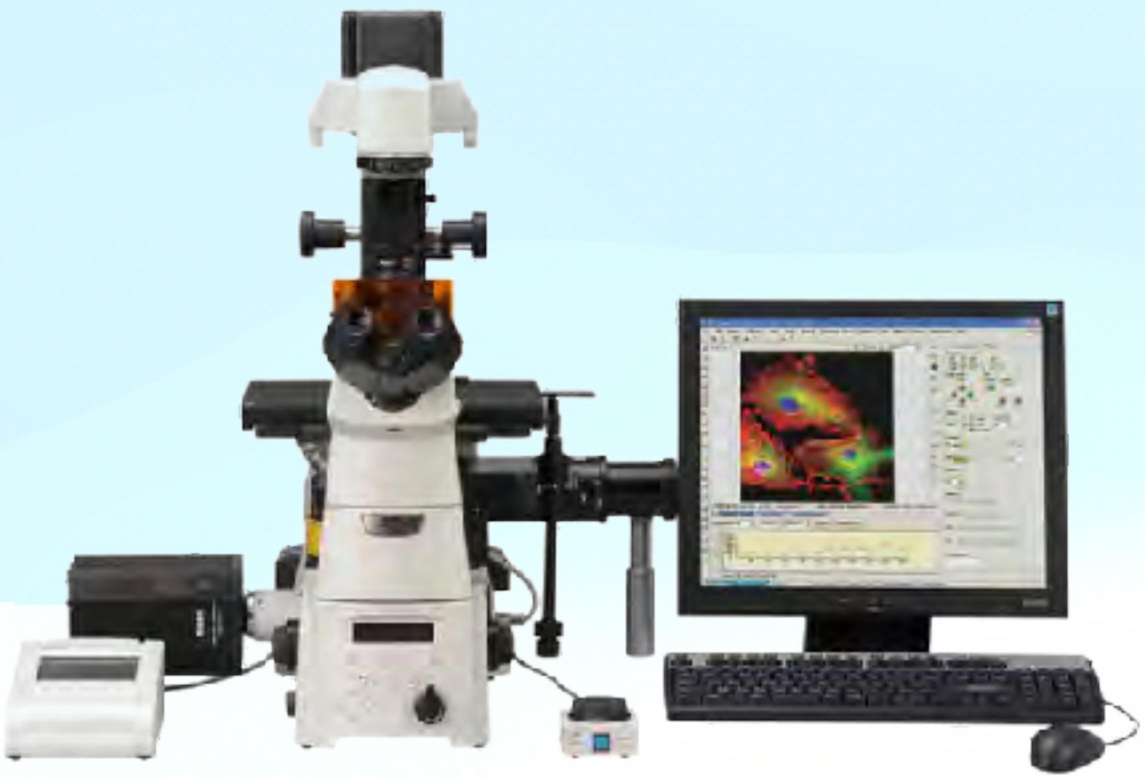
Influence of inhibitors and pathogens on cell cultures

Cell activity (inhibited?)

Computer vision based data analysis system

Experiments

Nikon ECLIPSE Ti2 Series



- FOV: 640μm×640μm -> 1608 pixel × 1608 pixel
- bright-field

Incucyte® SX1

- FOV: 640μm×480μm -> 1608 pixel × 1206 pixel
- bright-field / Fluorescence

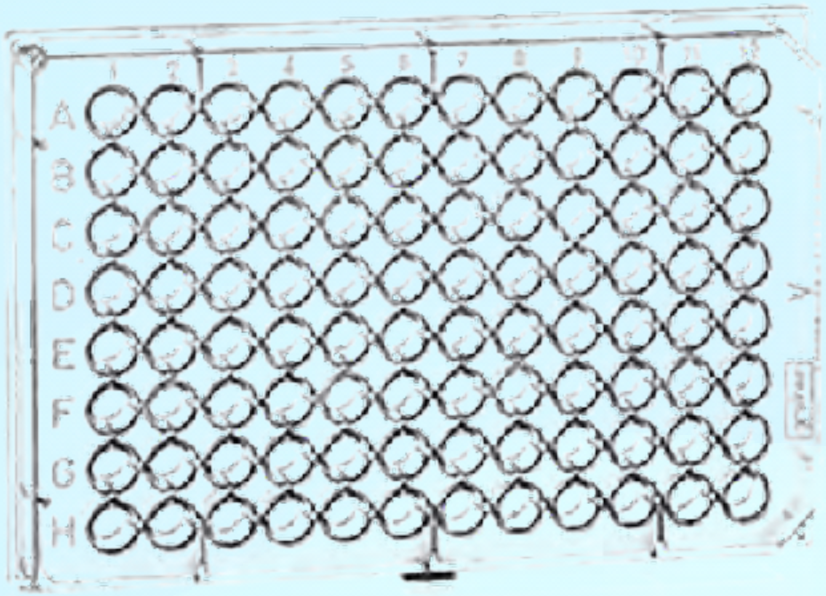
Used in Tokuraku sensei’s lab

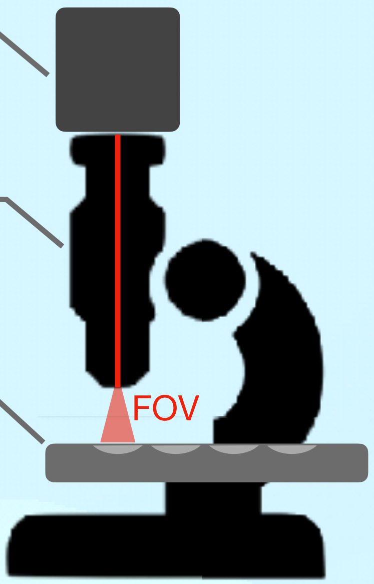
https://www.sartorius.com/en/products/live-cell-imaging-analysis/live-cell-analysis-instruments/sx1-live-cell-analysis-instrument

https://www.microscope.healthcare.nikon.com/products/inverted-microscopes/eclipse-ti-series

Data apprehension

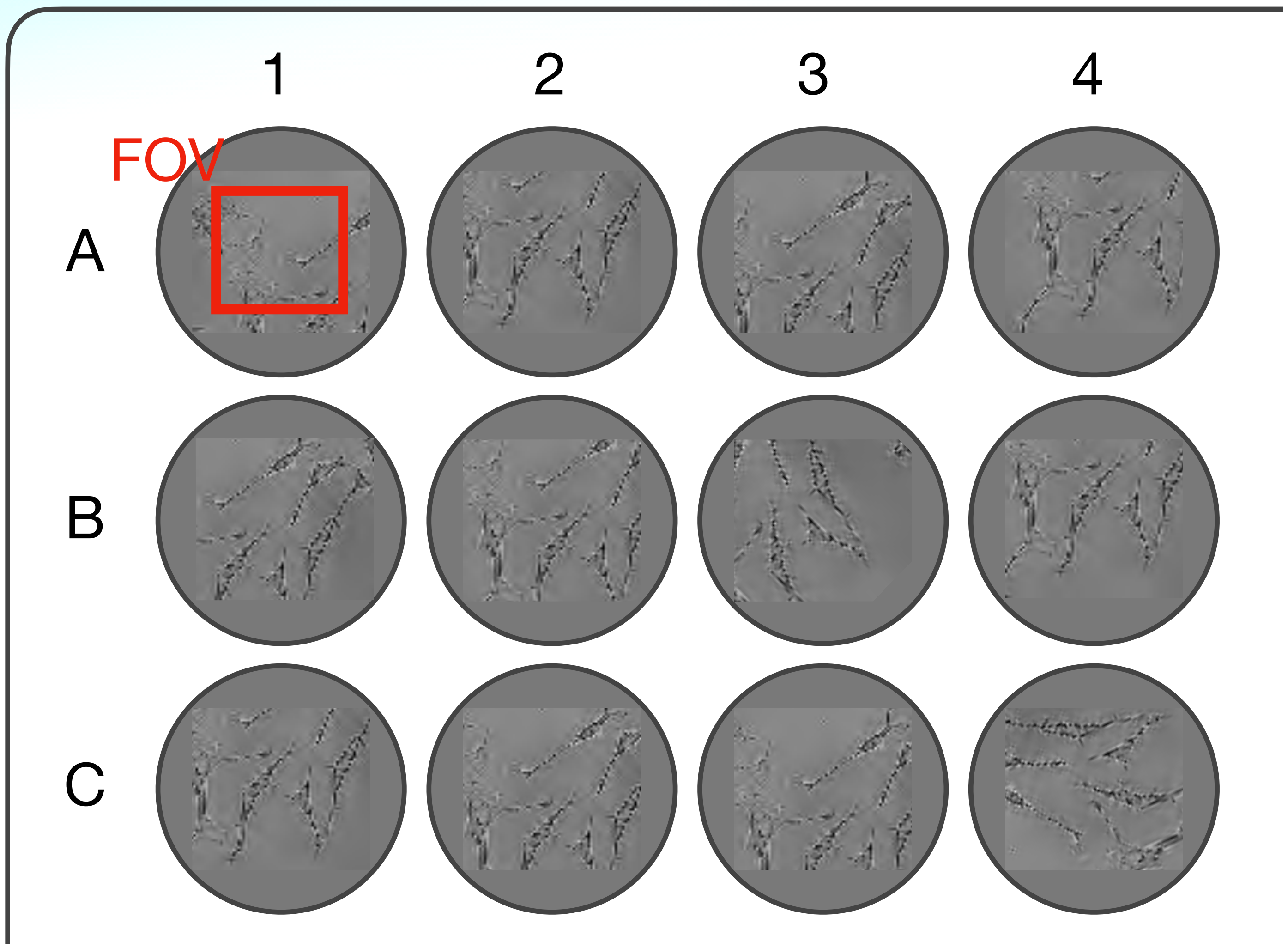
96-well microplate





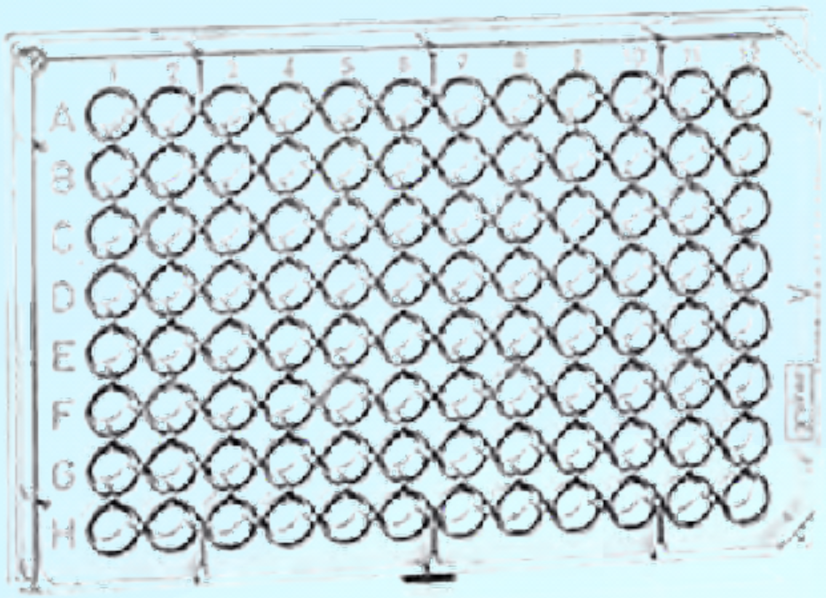
Simultaneous time-lapse observation

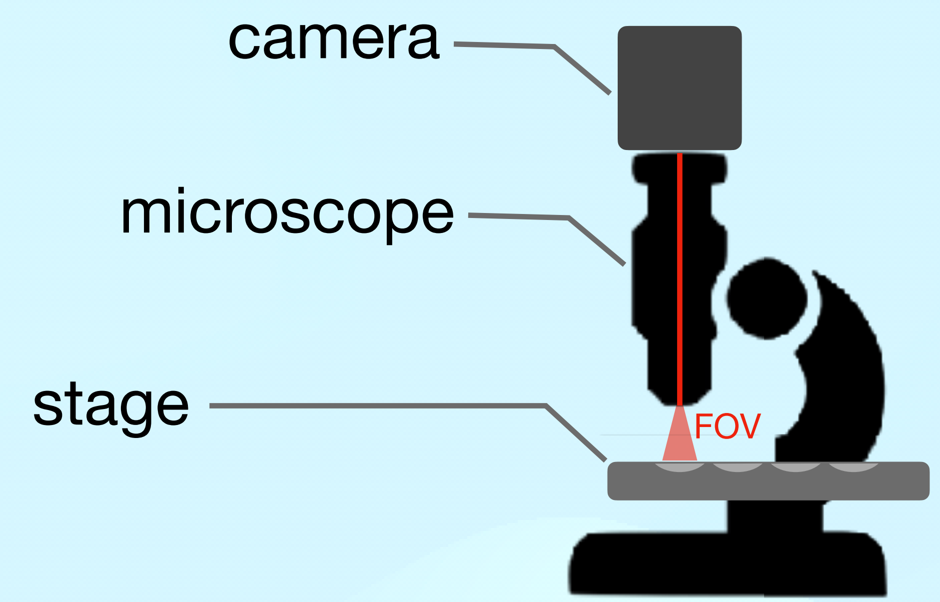
- 96 samples
- **various inhibitors**
  - **Taxol**
  - **CytochalasinD**
  - **etc.**
- **various concentrations**
- 1 frame per minute —> 300 min
- 4 frames per hour —> 36 hours



Data apprehension

96-well microplate





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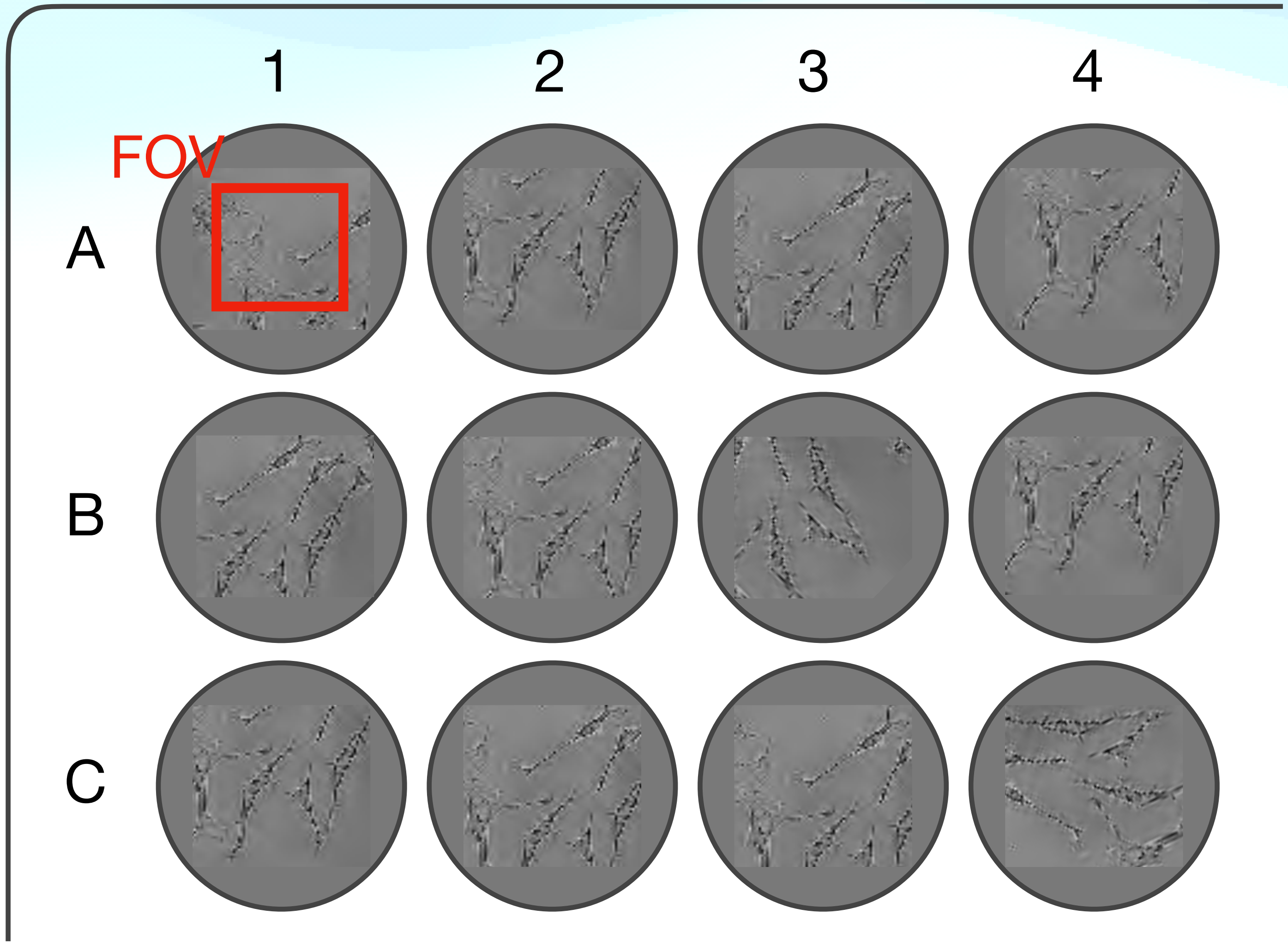
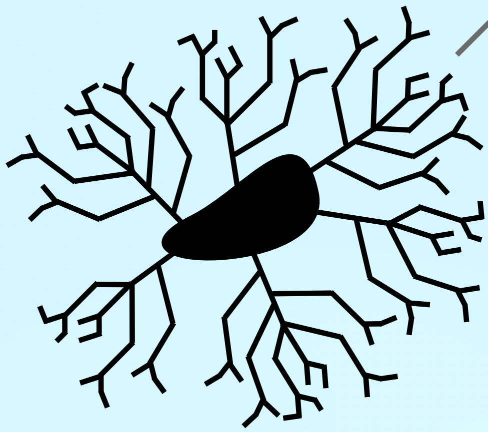


Cells



Astrocyte (astroglia)

- biochemical control of endothelial cells (blood-brain barrier)

SH-SY5Y **human blastoma cells**

しんけい め さいぼうしゅ
人の神経芽細胞種

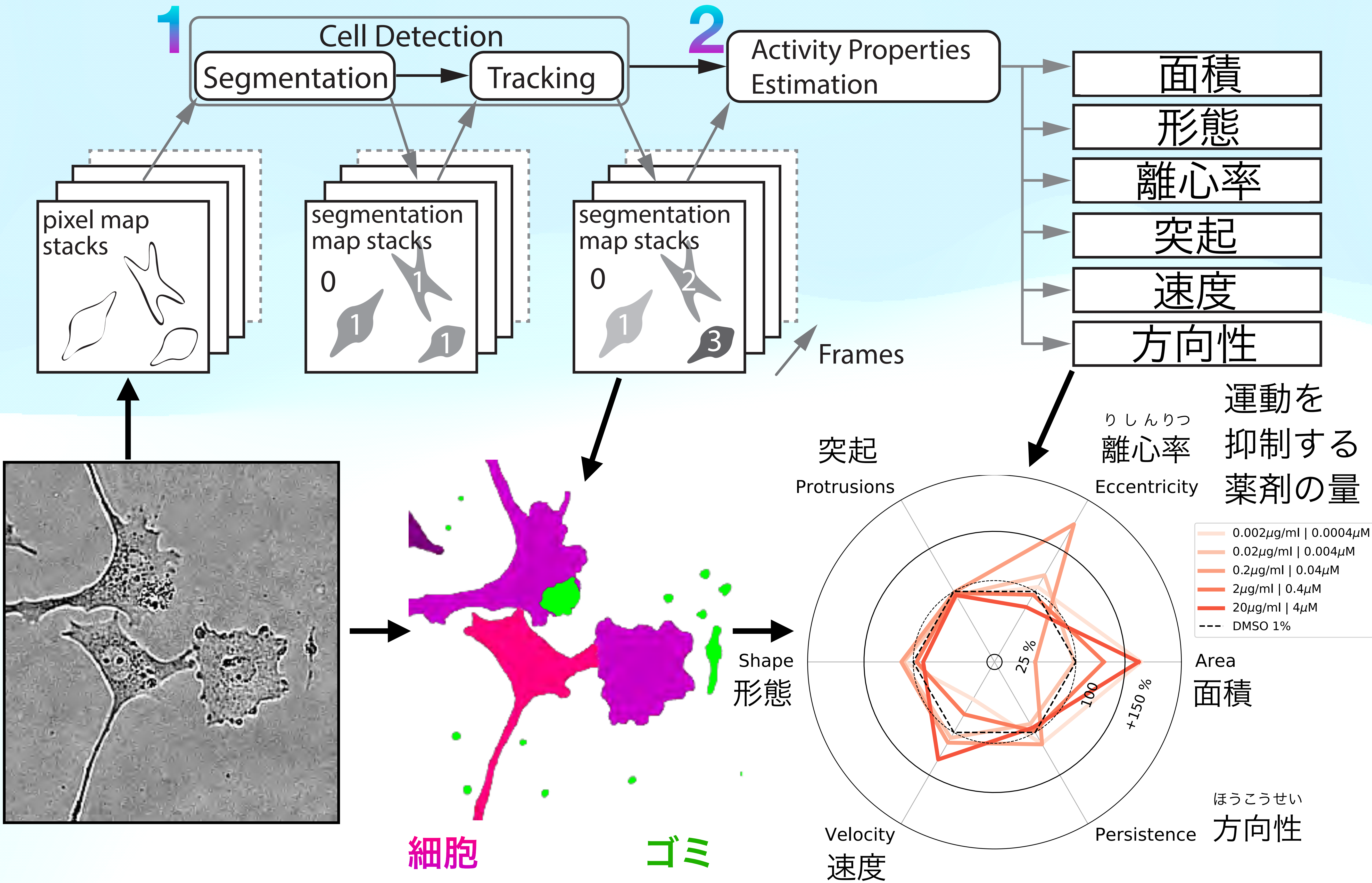
- Derived from **SK-N-SH** cell
- 1970 from metastatic cells found in the **bone marrow** aspirate of a four-year-old female

Widely used in:

- analysis of neuronal differentiation
- metabolism
- function related to neurodegenerative processes
- Neurotoxicity
- neuroprotection

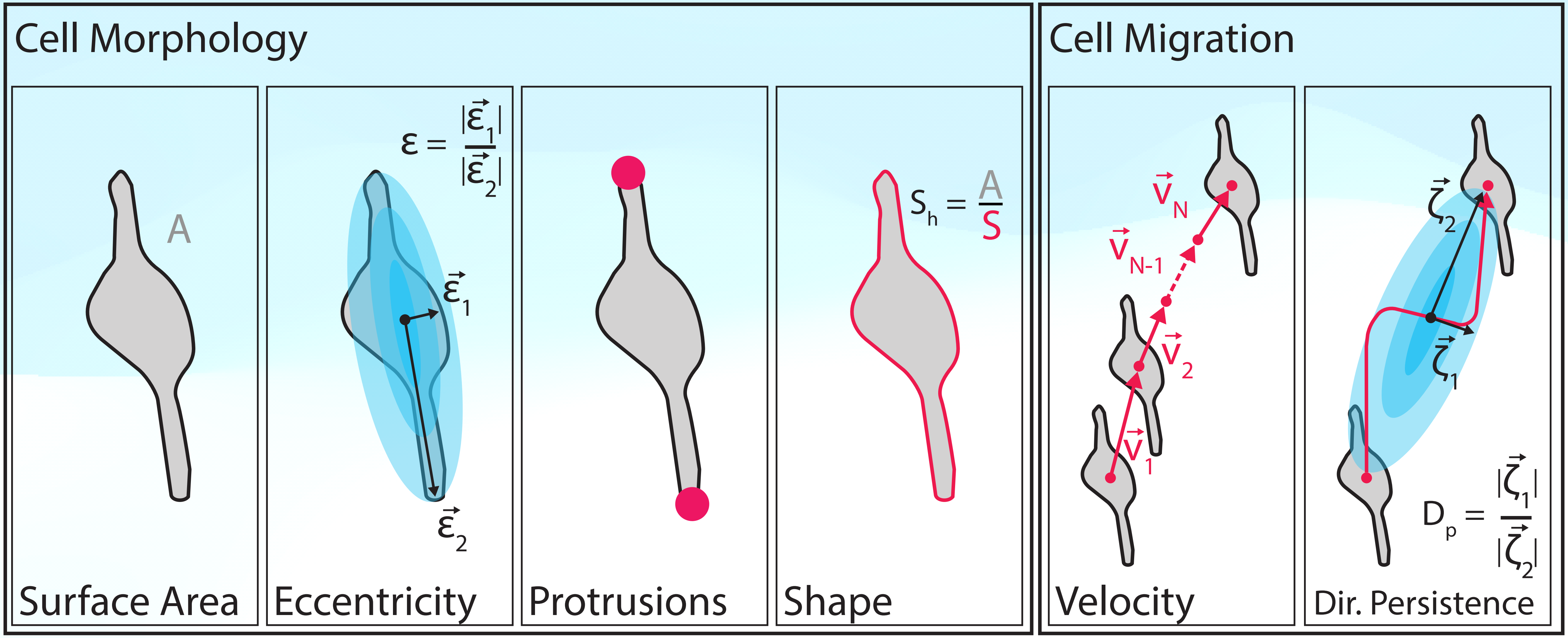
Processing

Baar et. al 2022:
Scientific reports:
Towards a comprehensive approach for characterizing cell activity in bright-field microscopic images
https://assets.researchsquare.com/files/rs-1460651/v1_covered.pdf?c=1648148502



さいぼうかっせい ど とくせい
細胞活性度特性

When adding compounds (chemicals) How does the cell activity change?



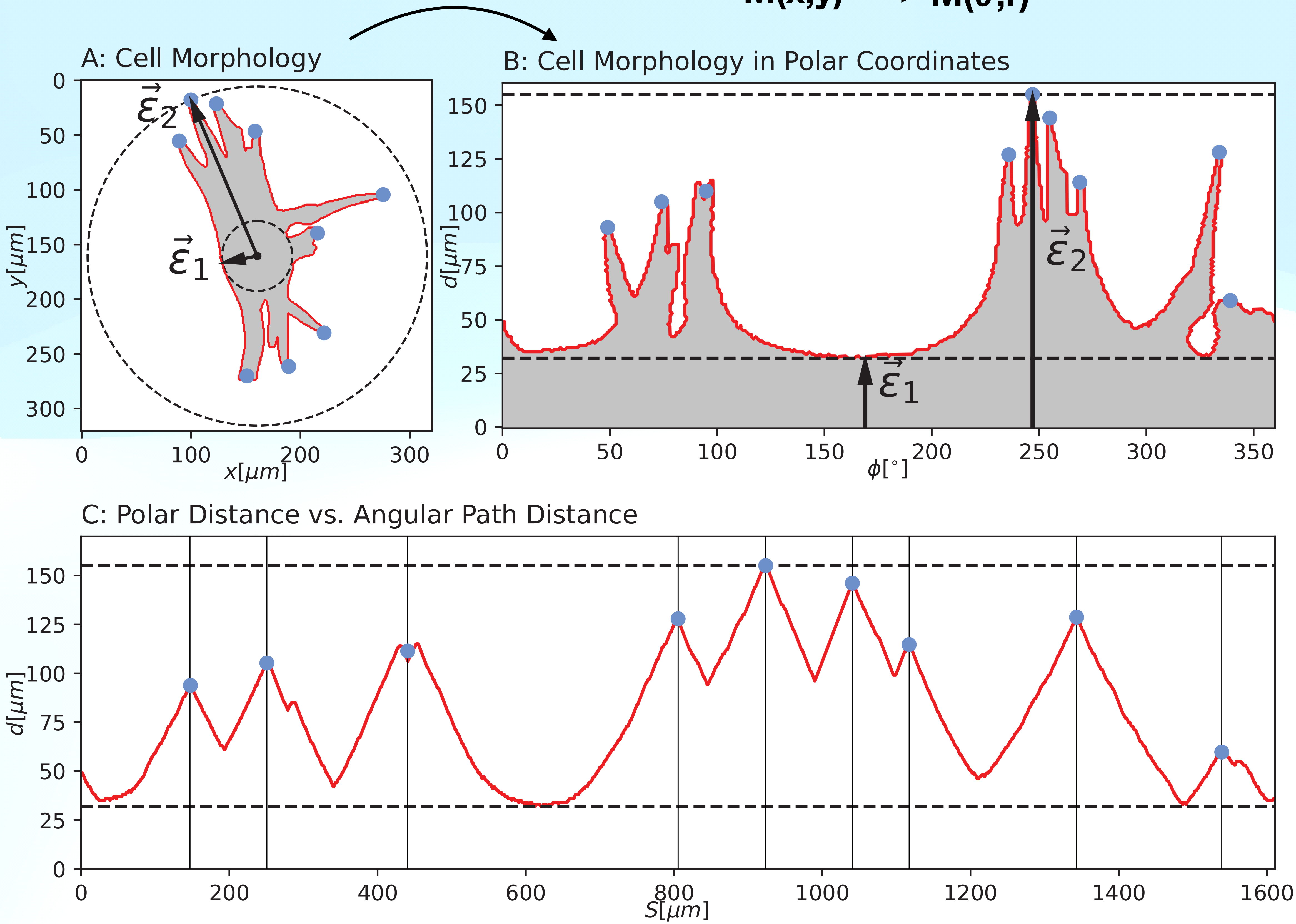
Baar et. al 2022: **Towards a comprehensive approach for characterizing cell activity in bright-field microscopic images**

Scientific reports:
https://assets.researchsquare.com/files/rs-1460651/v1_covered.pdf?c=1648148502

突起検出と離心率

極座標変換（ごくざひょうへんかん）

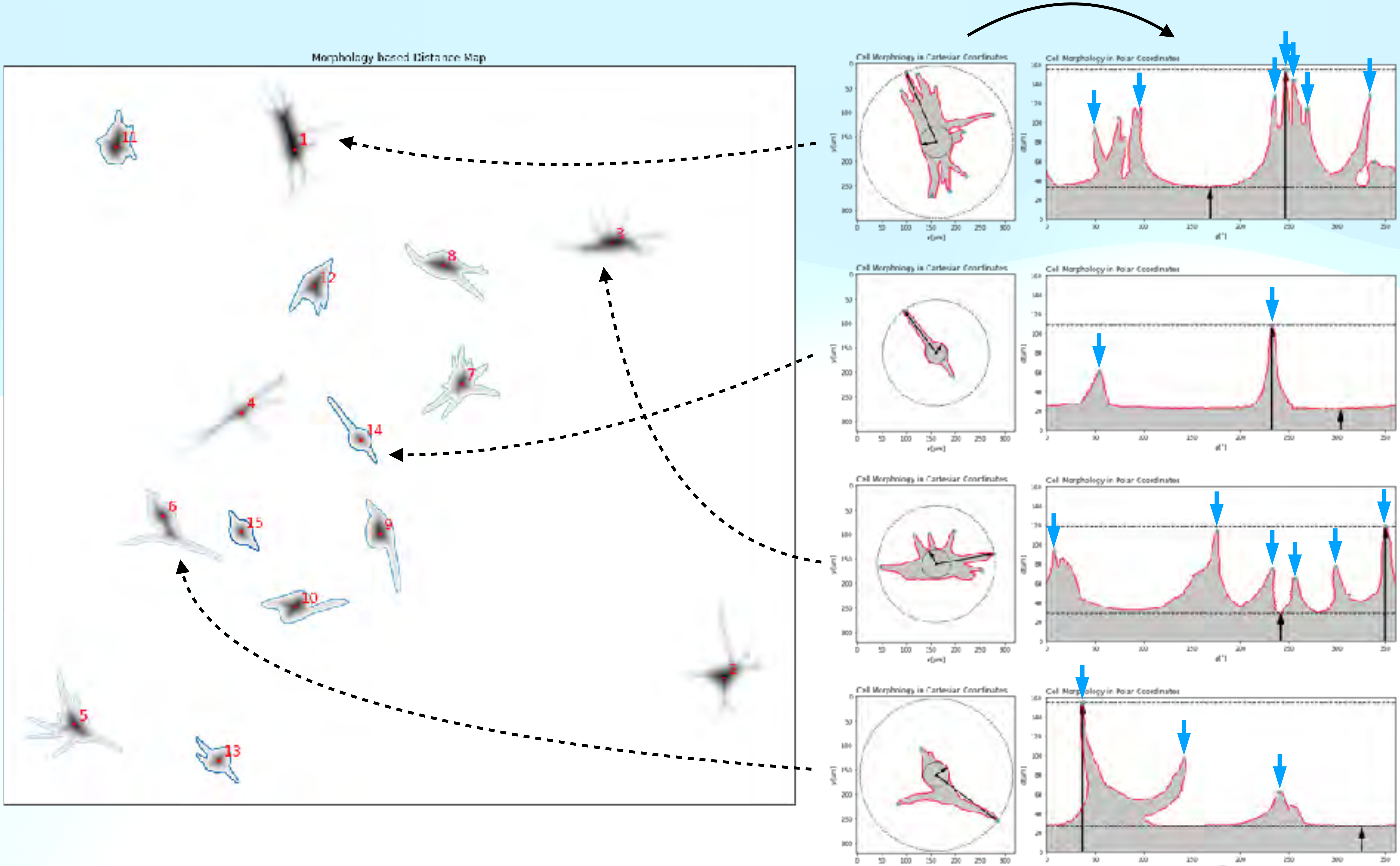
M(x,y) ⟶ M(ϑ,r)



突起検出

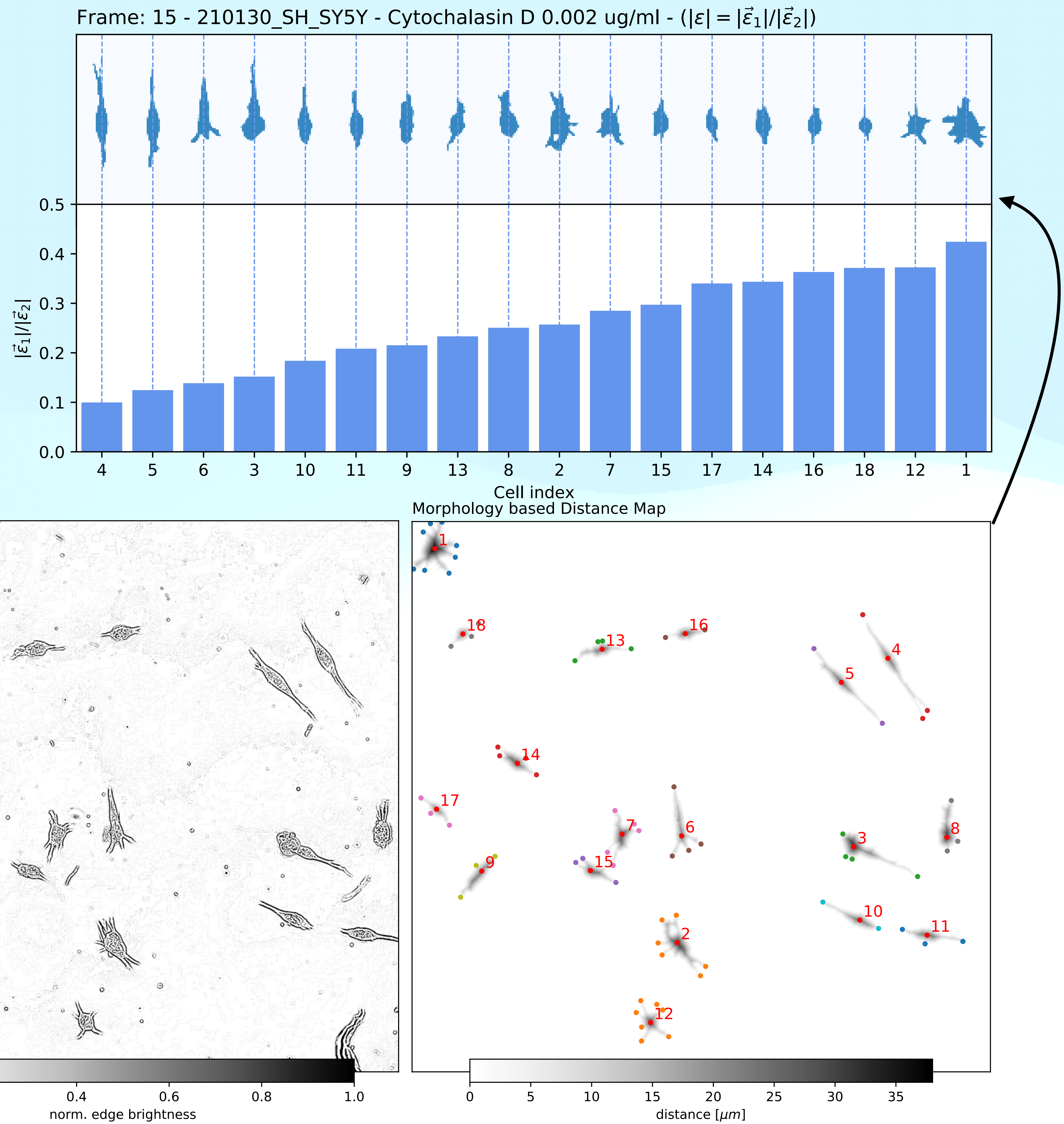
M(x,y) → M(ϑ,r)

個々の細胞を抽出する（ちゅうしゅつ） → 極座標変換（ごくざひょうへんかん）



りしんりつ

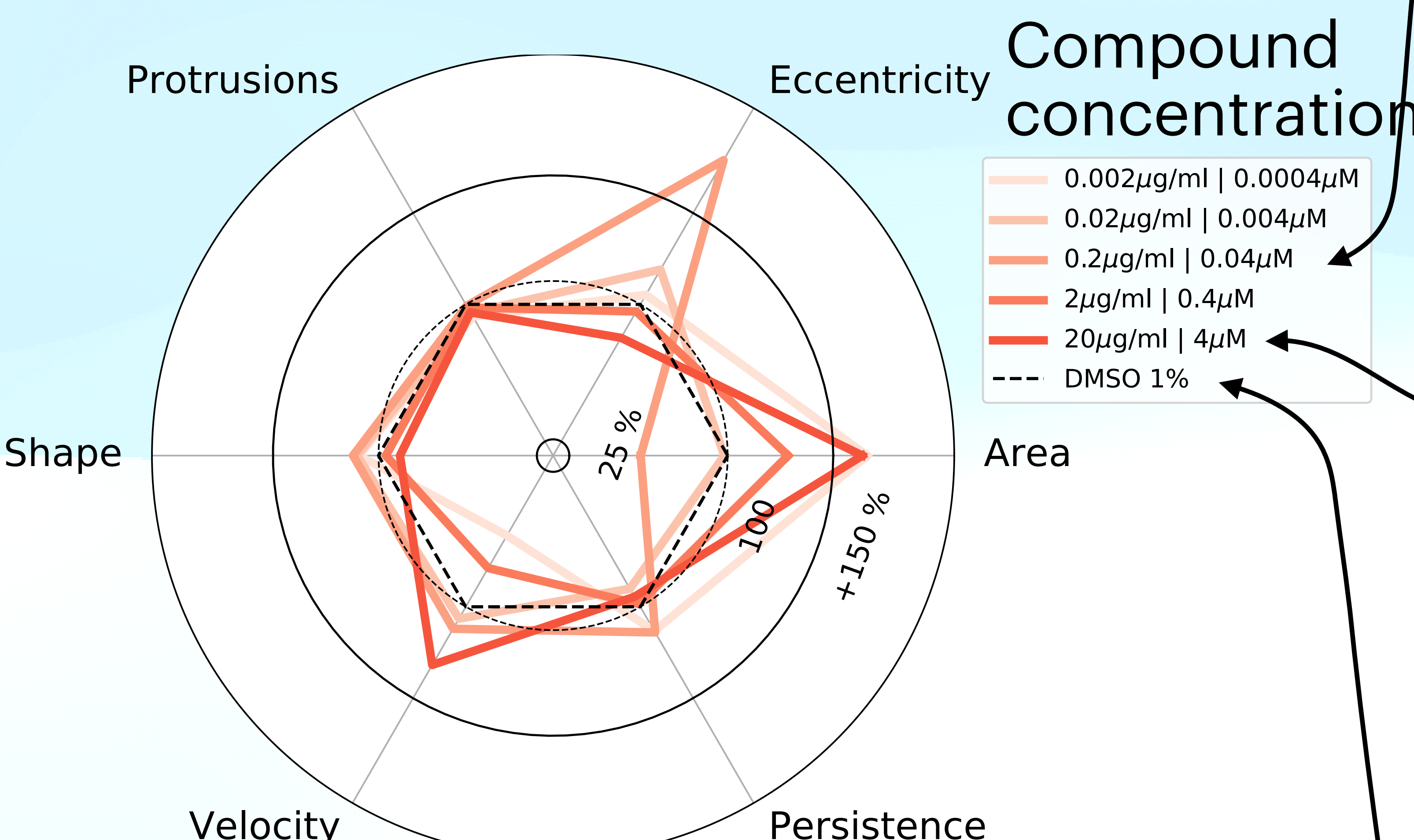
離心率



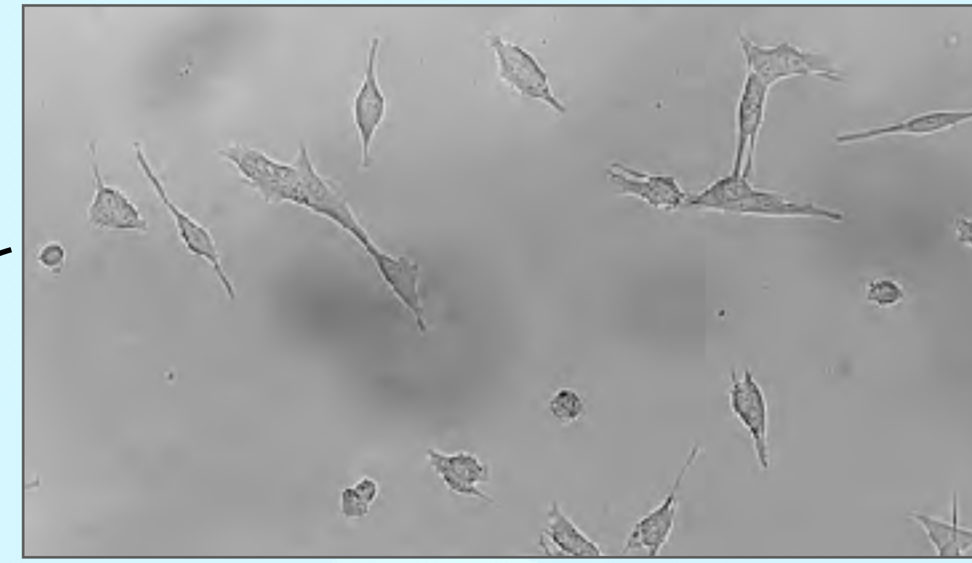
Frame: 15 - 210130_SH_SY5Y - Cytochalasin D 0.002 ug/ml - ($|\varepsilon| = |\vec{\varepsilon}_1|/|\vec{\varepsilon}_2|$)
$|\vec{\varepsilon}_1|/|\vec{\varepsilon}_2|$
0.0
0.1
0.2
0.3
0.4
0.5
4 5 6 3 10 11 9 13 8 2 7 15 17 14 16 18 12 1
Cell index
Low-pass image
Morphology based Distance Map
0.0 0.2 0.4 0.6 0.8 1.0
norm. edge brightness
0 5 10 15 20 25 30 35
distance [μm]

Controll sample （DMSO 1%）

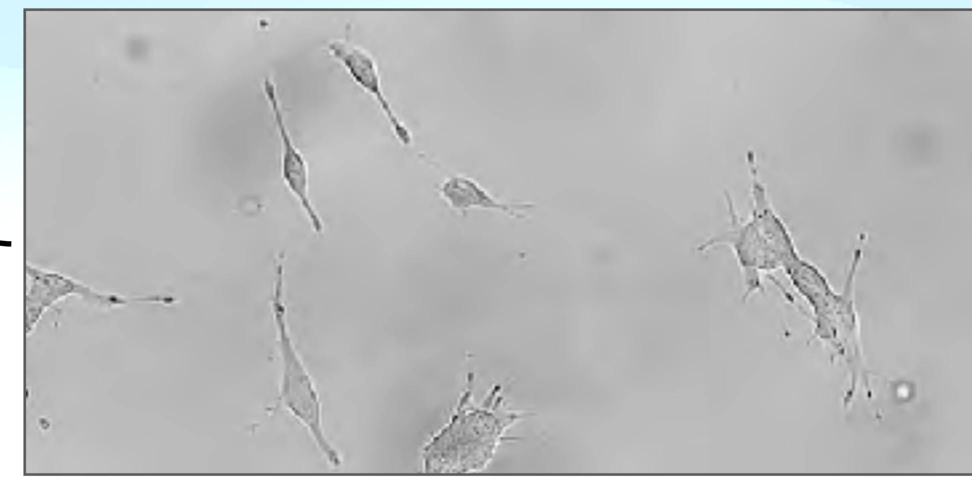
CytocahlasinD + Taxol



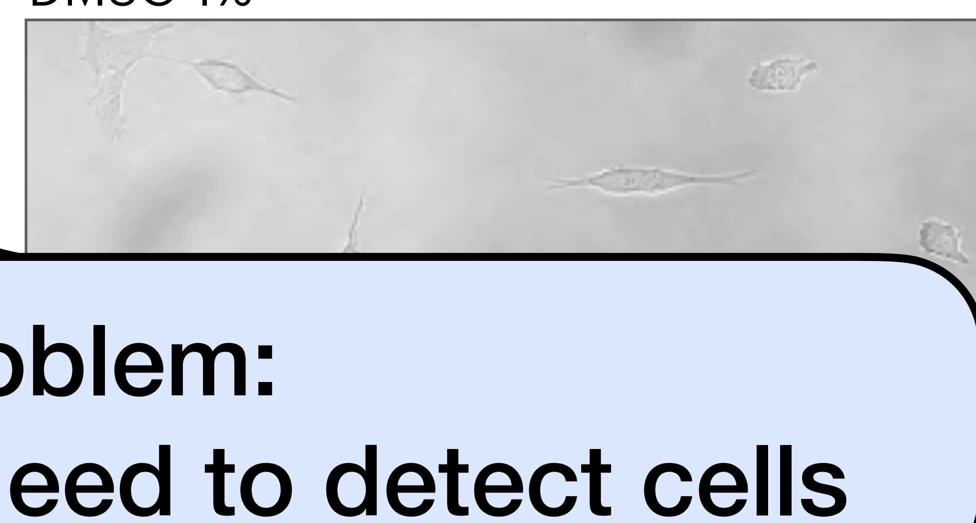
CytoD: 0.02μg/ml +Taxol: 0.04 μM



CytoD: 20 μg/ml +Taxol: 4μM



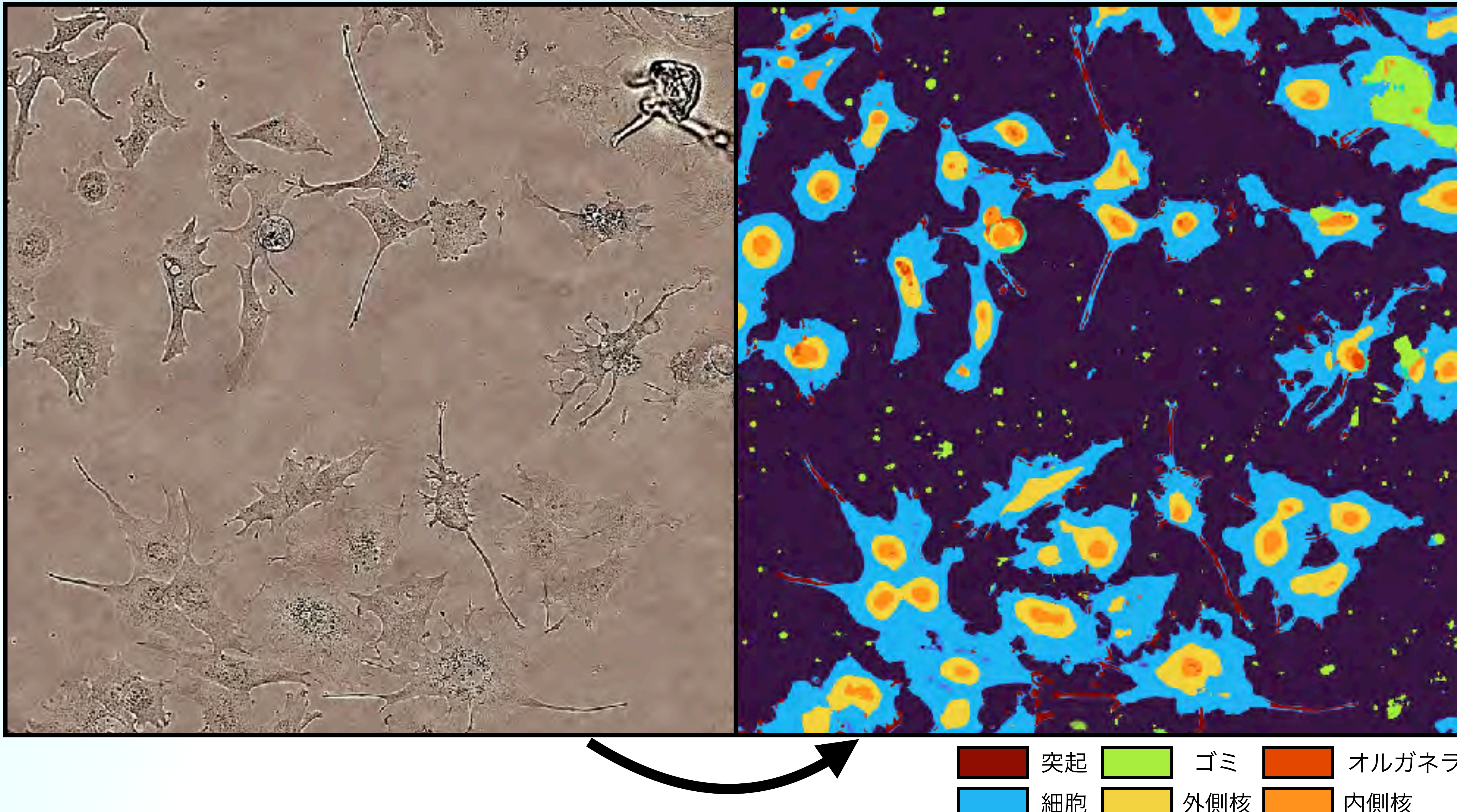
DMSO 1%



Goal: Measure Cell activity For various chemical complounds

Problem:
- Need to detect cells

Deep learning based cell segmentation



Locate cells and their morphology

Cell Finding Methods

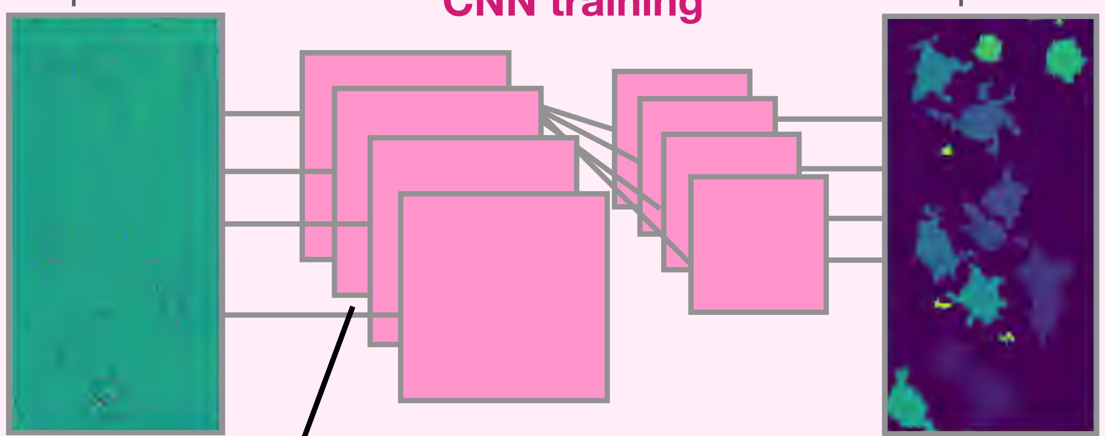
Deep Learning

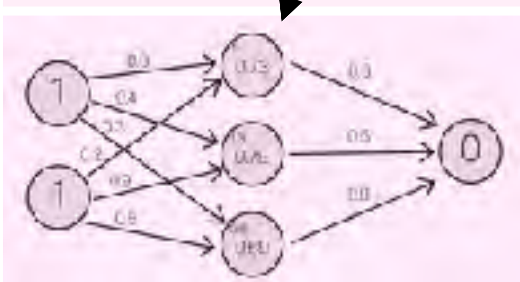
Neural Networks

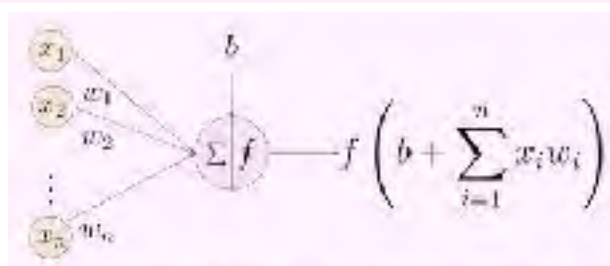
Input A

CNN training

Input B



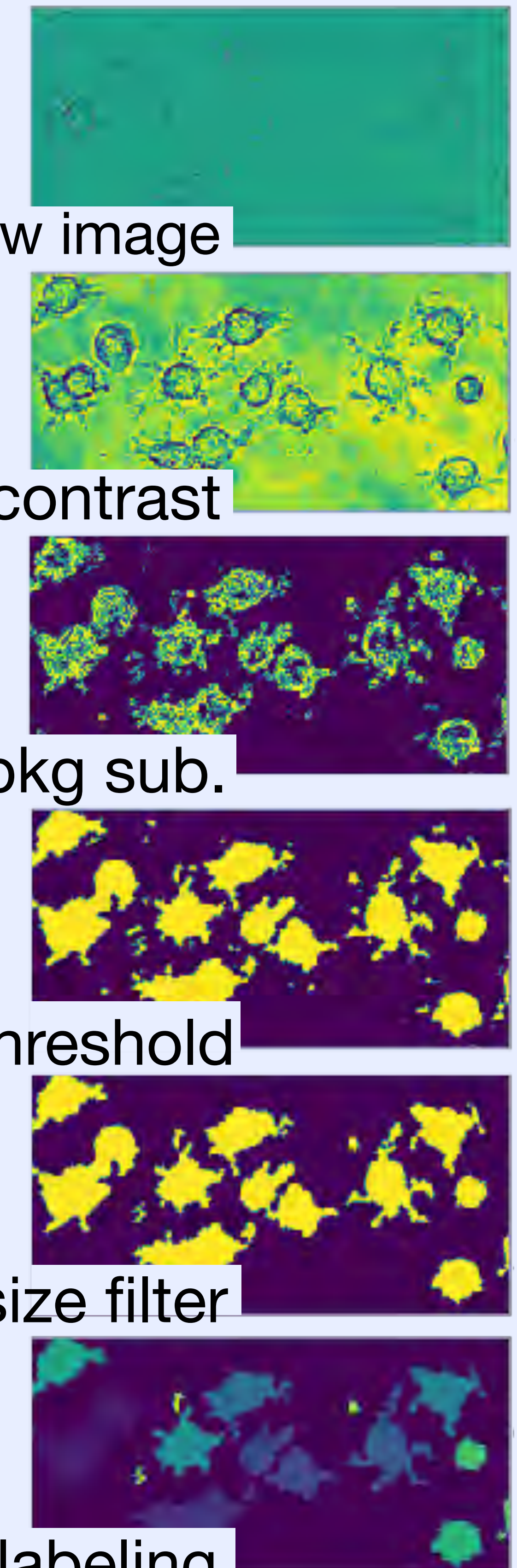




Rule Based

- Filters
- Morphology
- Logic
- Unsupervised ML
  (Regression, Cascades, DR, Clustering, NN, etc.)

Results from Rule Based Computer Vision Approach are screened. Good Results are used to train NN.



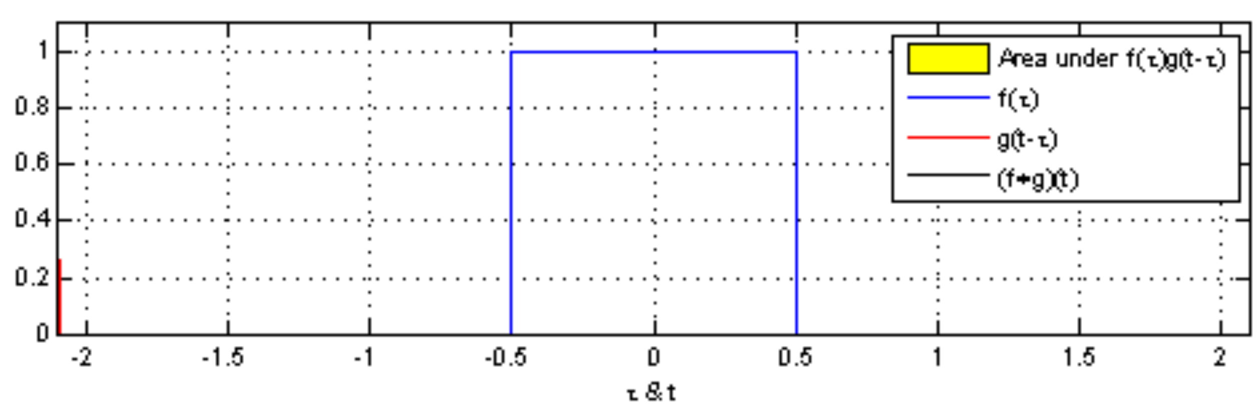
The main difference between Deep Learning and Rule Based CV approaches lies in a tradeoff between precision/ accuracy and comprehensibility.

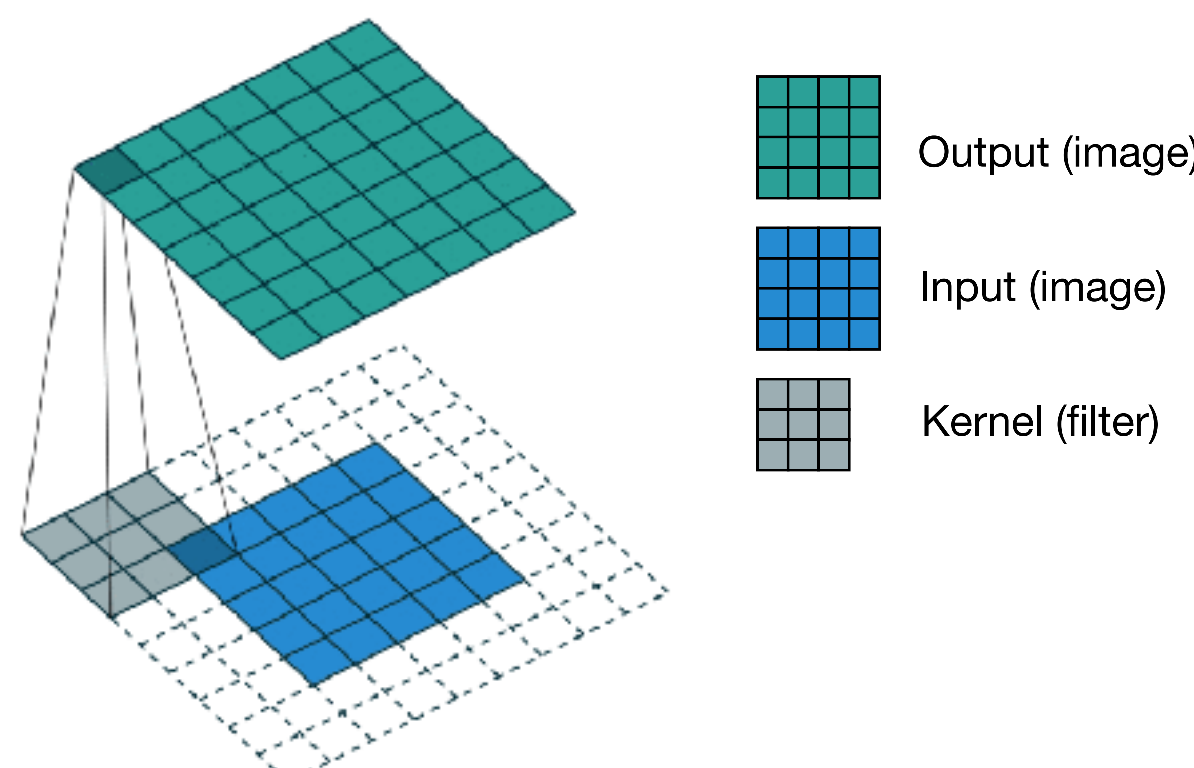
Deep Learning	Rule Based
Blackbox	Manual Feature Extr.
Labelled Datasets	Limited Flexibility
Flexible	
High Accuracy ⟷	Comprehensible

Simple Kernels / Filters

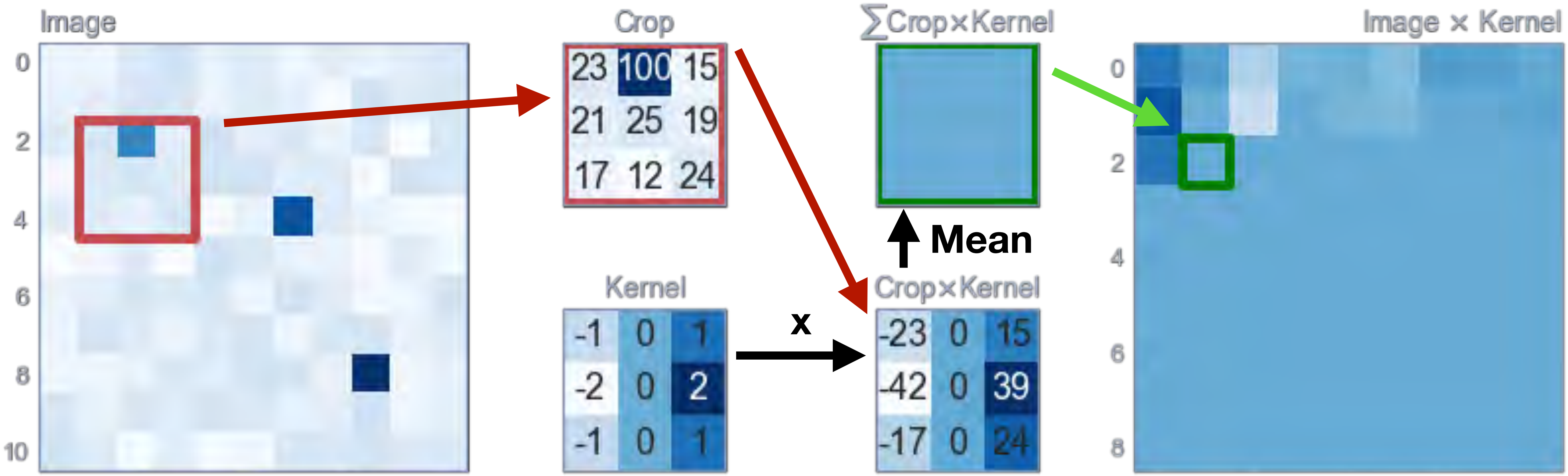
Operation	Filter	Convolved Image
Identity	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	
Edge detection	$\begin{bmatrix} 1 & 0 & -1 \\ 0 & 0 & 0 \\ -1 & 0 & 1 \end{bmatrix}$	
	$\begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}$	
	$\begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix}$	

Operation	Filter	Convolved Image
Sharpen	$\begin{bmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{bmatrix}$	
Box blur (normalized)	$\frac{1}{9}\begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix}$	
Gaussian blur (approximation)	$\frac{1}{16}\begin{bmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{bmatrix}$	

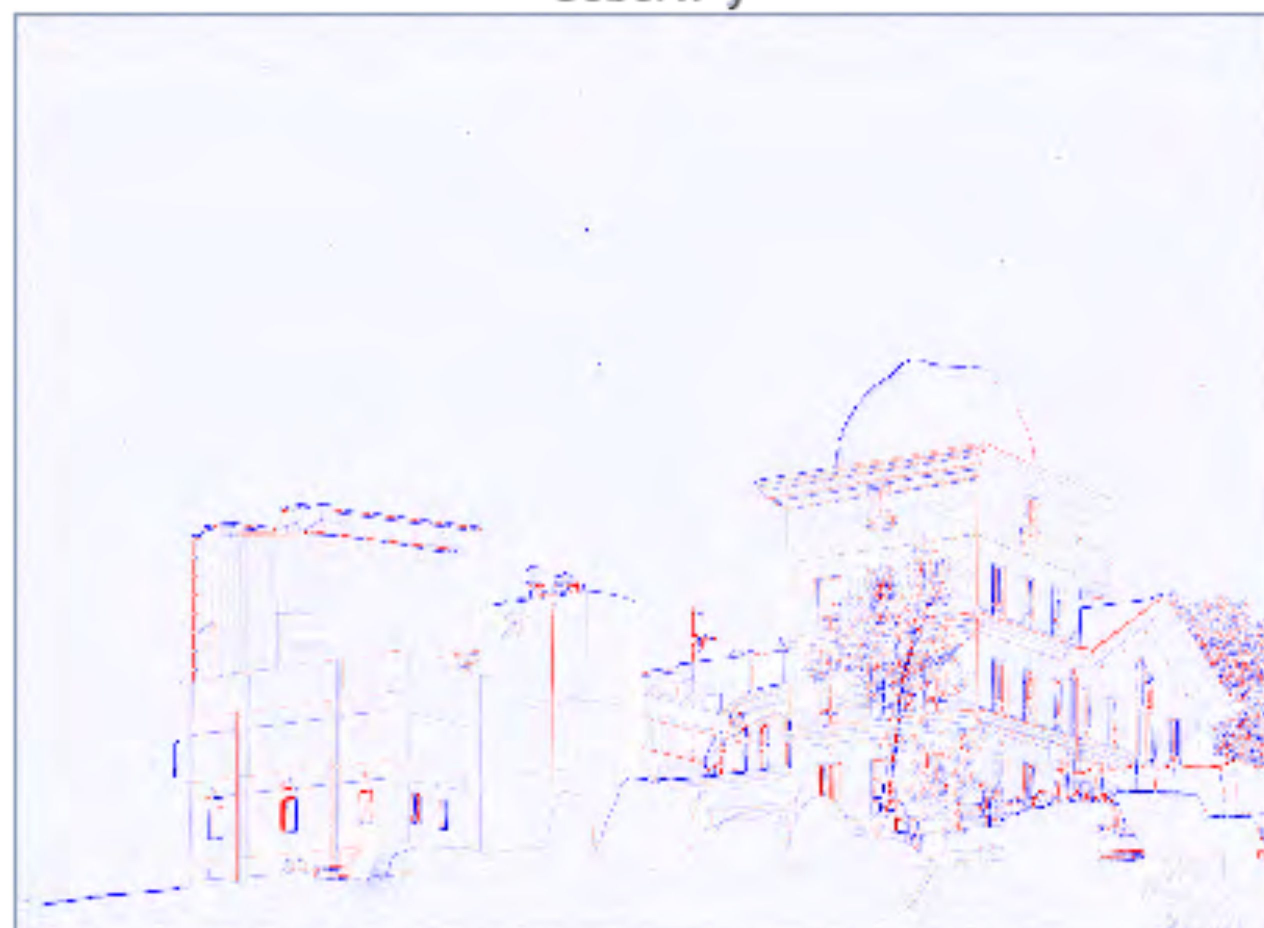




Convolution

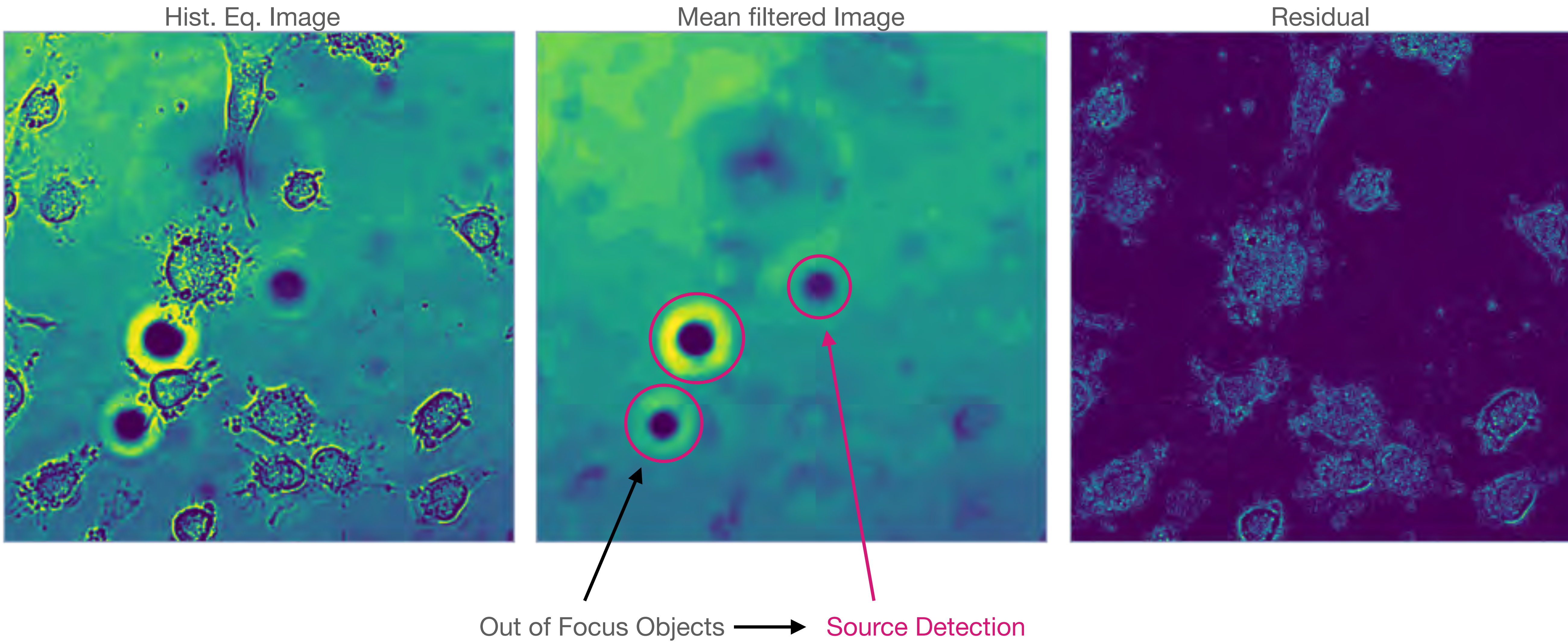


Sobel x+y

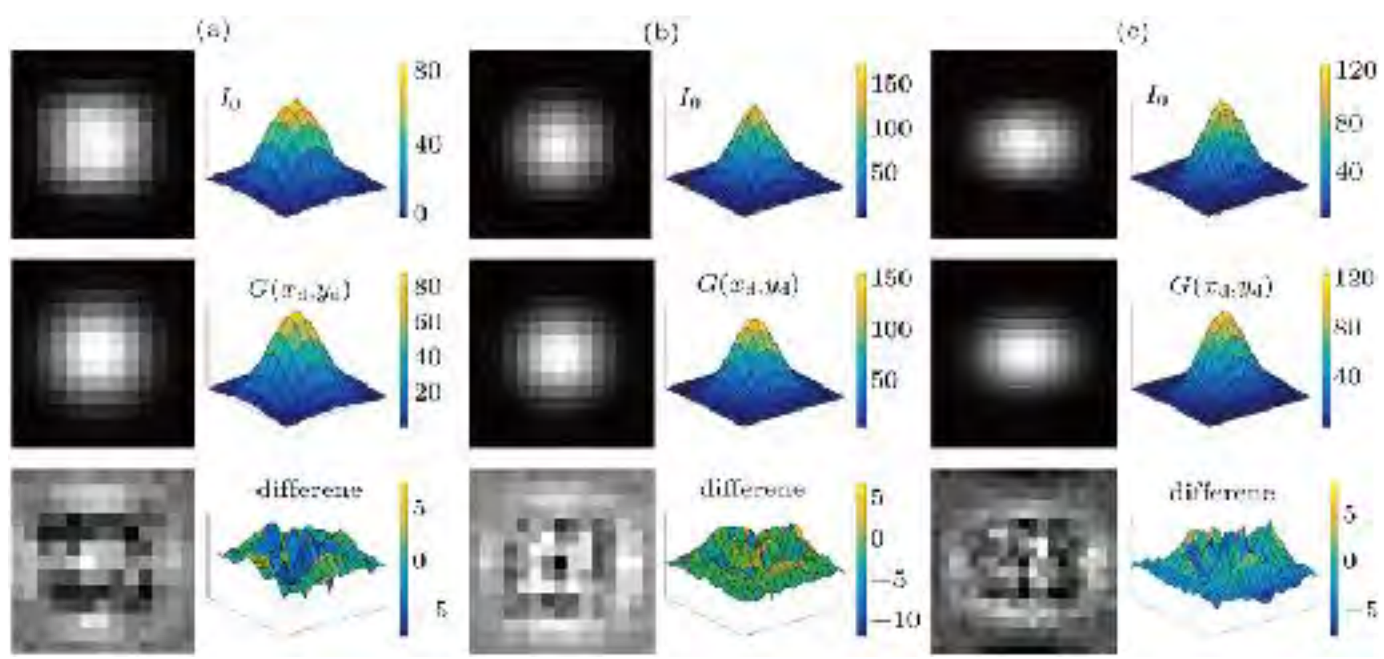


https://stefanbaar.github.io/seminar/convolution.html

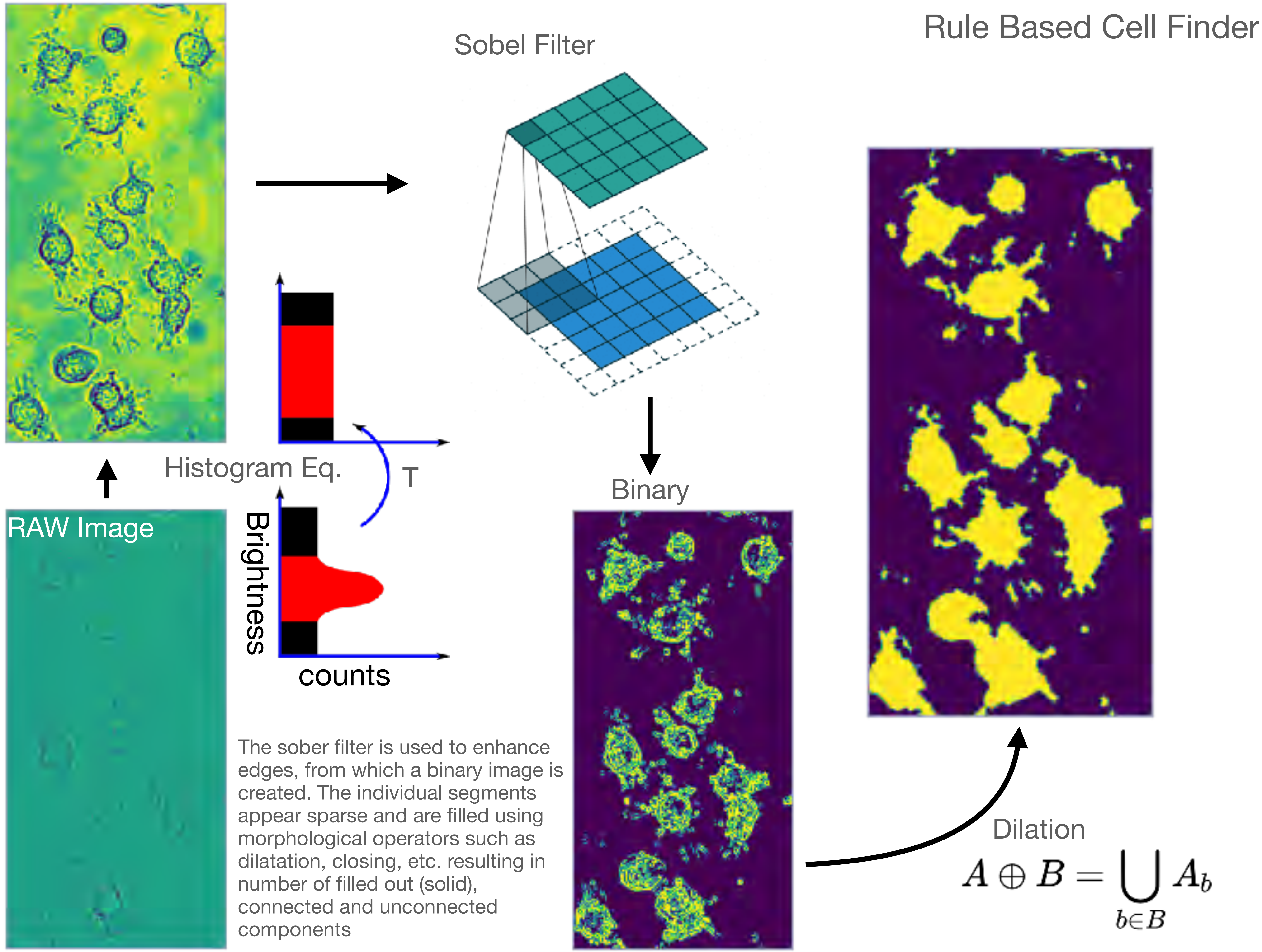
Background removal



Background is uneven (complicated R^2 structure embedded in R^3) and contains defocused contaminations, which need to be subtracted to optimize cell boundary tracking.

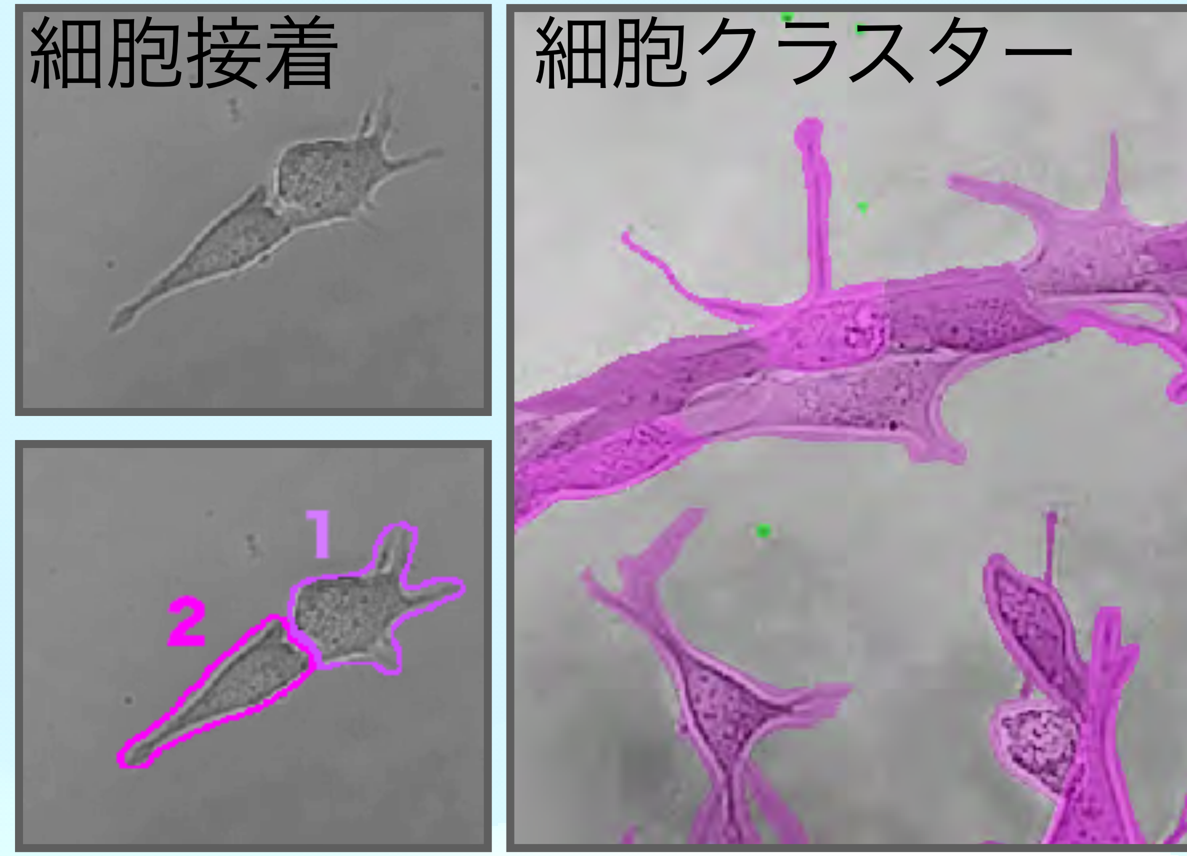


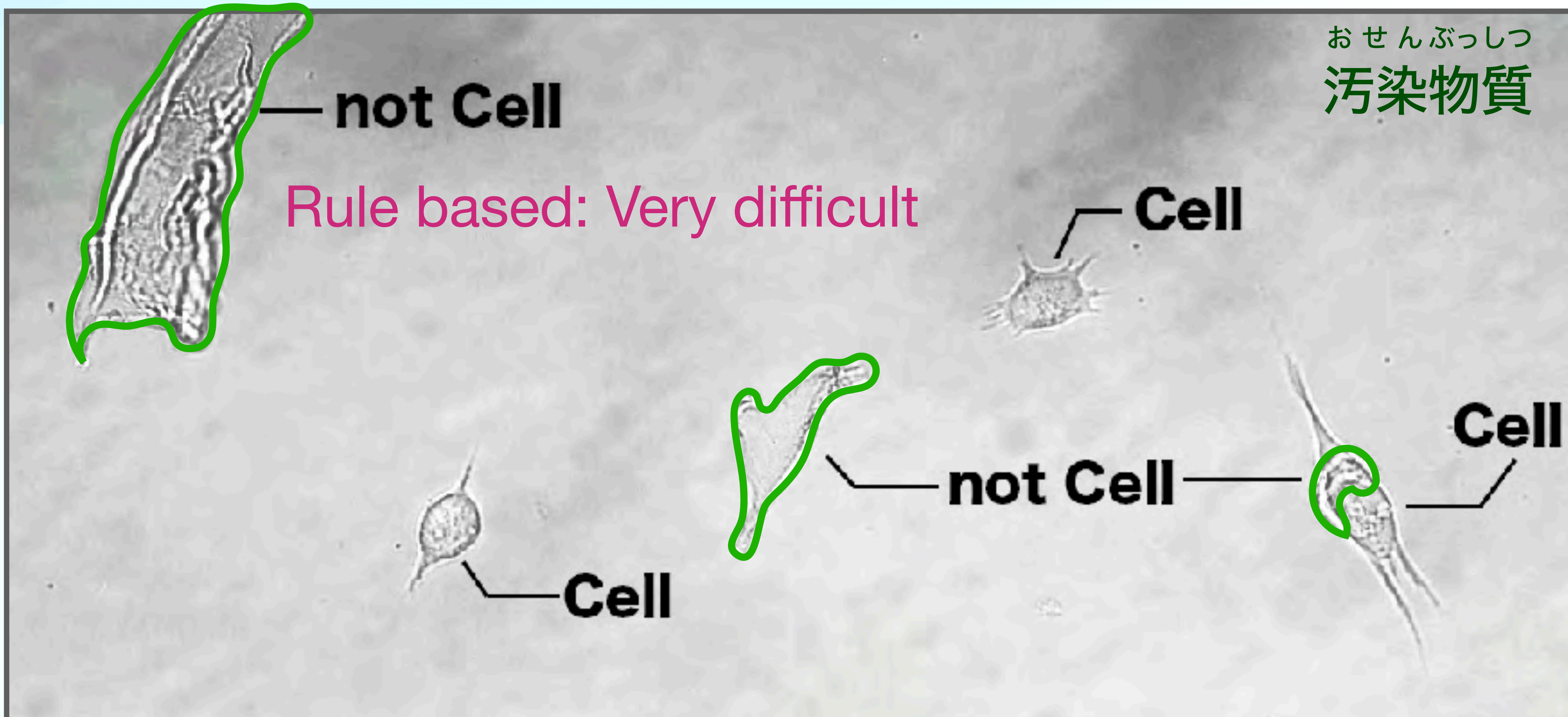
- Gauss/Bessel Fit
- Subtraction


Rule Based Cell Finder
Sobel Filter
RAW Image
Histogram Eq.
T
Brightness
counts
Binary
Dilation
$A \oplus B = \bigcup_{b \in B} A_b$
The sober filter is used to enhance edges, from which a binary image is created. The individual segments appear sparse and are filled using morphological operators such as dilatation, closing, etc. resulting in number of filled out (solid), connected and unconnected components

かだい
課題

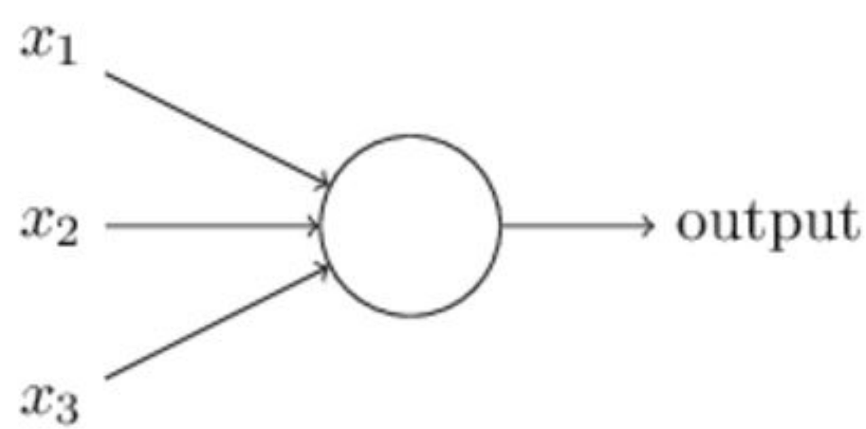
さいぼう ま せっちゃく
- 細胞間 接着
- 細胞クラスター
- 汚染物質(培地中のゴミ)





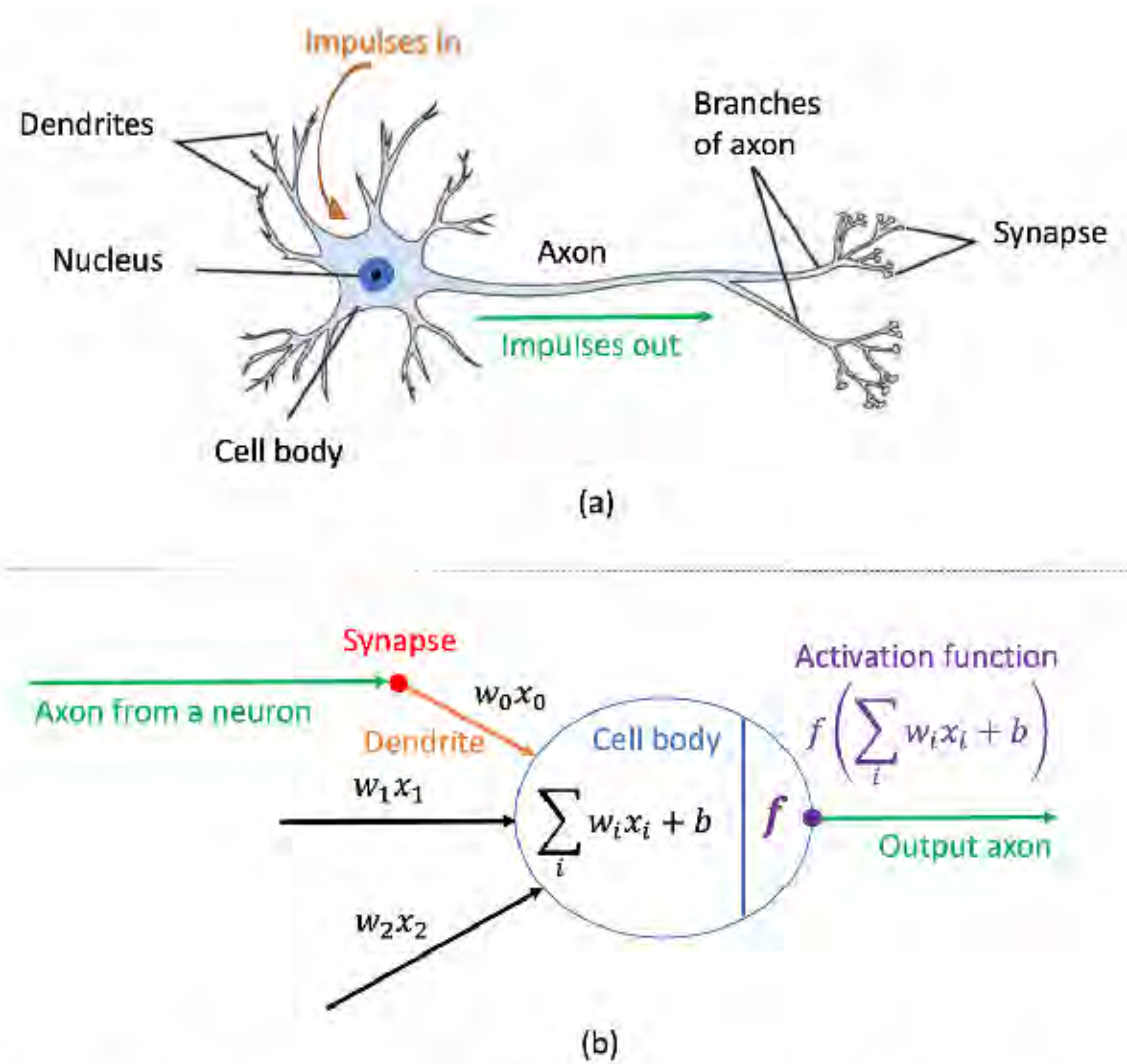
Artificial Neural Networks

Perceptron



$$\text{output} = \begin{cases} 0 & \text{if } \sum_j w_j x_j \leq \text{ threshold} \\ 1 & \text{if } \sum_j w_j x_j > \text{ threshold} \end{cases}$$

Rosenblatt, 1950

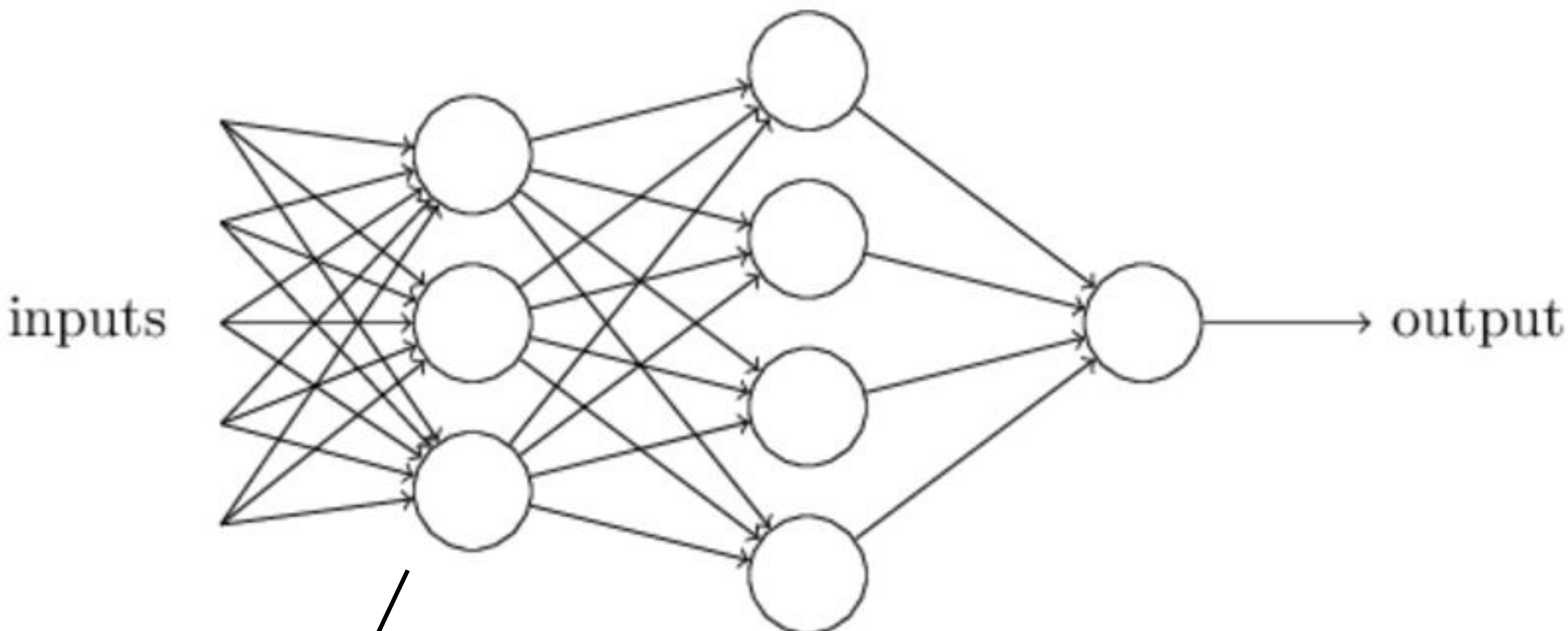


Only analogy!!

Figure 3-1: A comparison between a human neuron and an ANN neuron. (a) Shows an illustration of a human neuron; (**b**) reports the associated artificial neuron, where synapses are modeled by the set of inputs $x_{1:n}$. The cell body is modeled by the biological counterpart functionality, that is in collecting together weighted inputs and filter them throughout an activation function.

Roffo et al. 2017

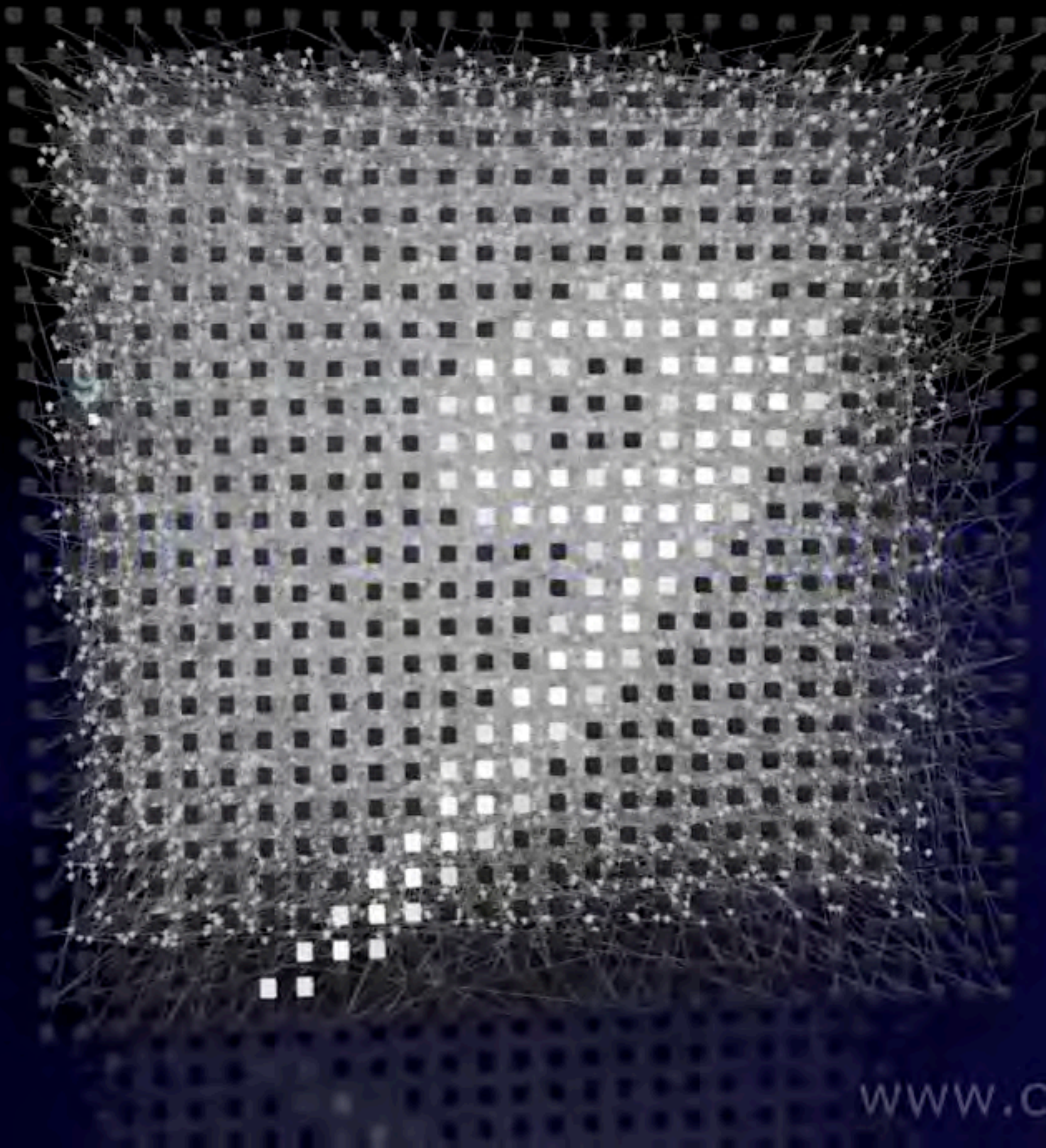
Multi-layer perceptron



Michael Nielsen, 2016

Detect handwriting in late 1980s (LeCun et al. 1989)

http://yann.lecun.com/exdb/publis/pdf/lecun-90c.pdf



Convolutional Neural Networks (CNNs)

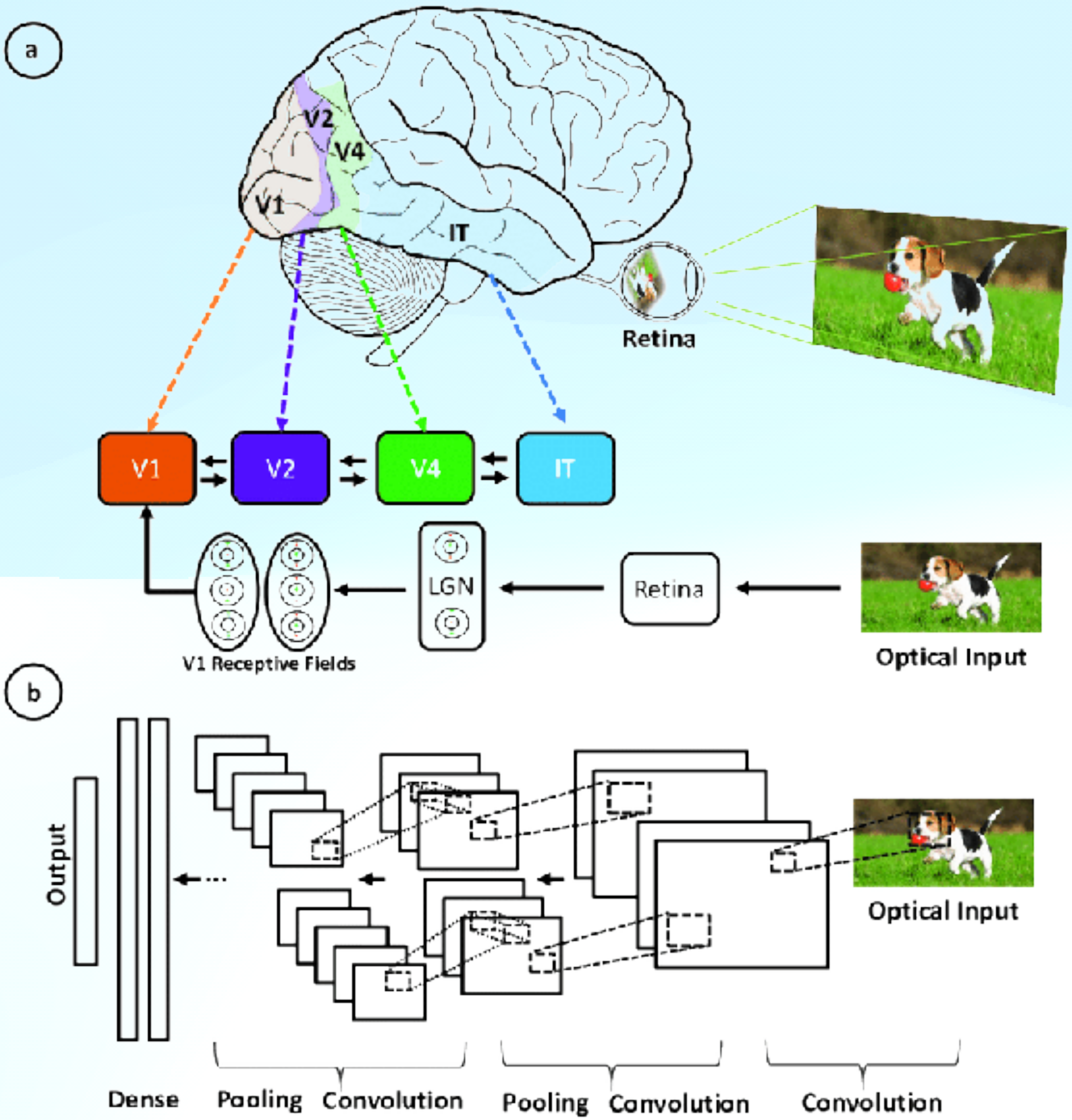


Illustration of the correspondence between the areas associated with the primary **visual cortex** and the layers in a convolutional neural network.

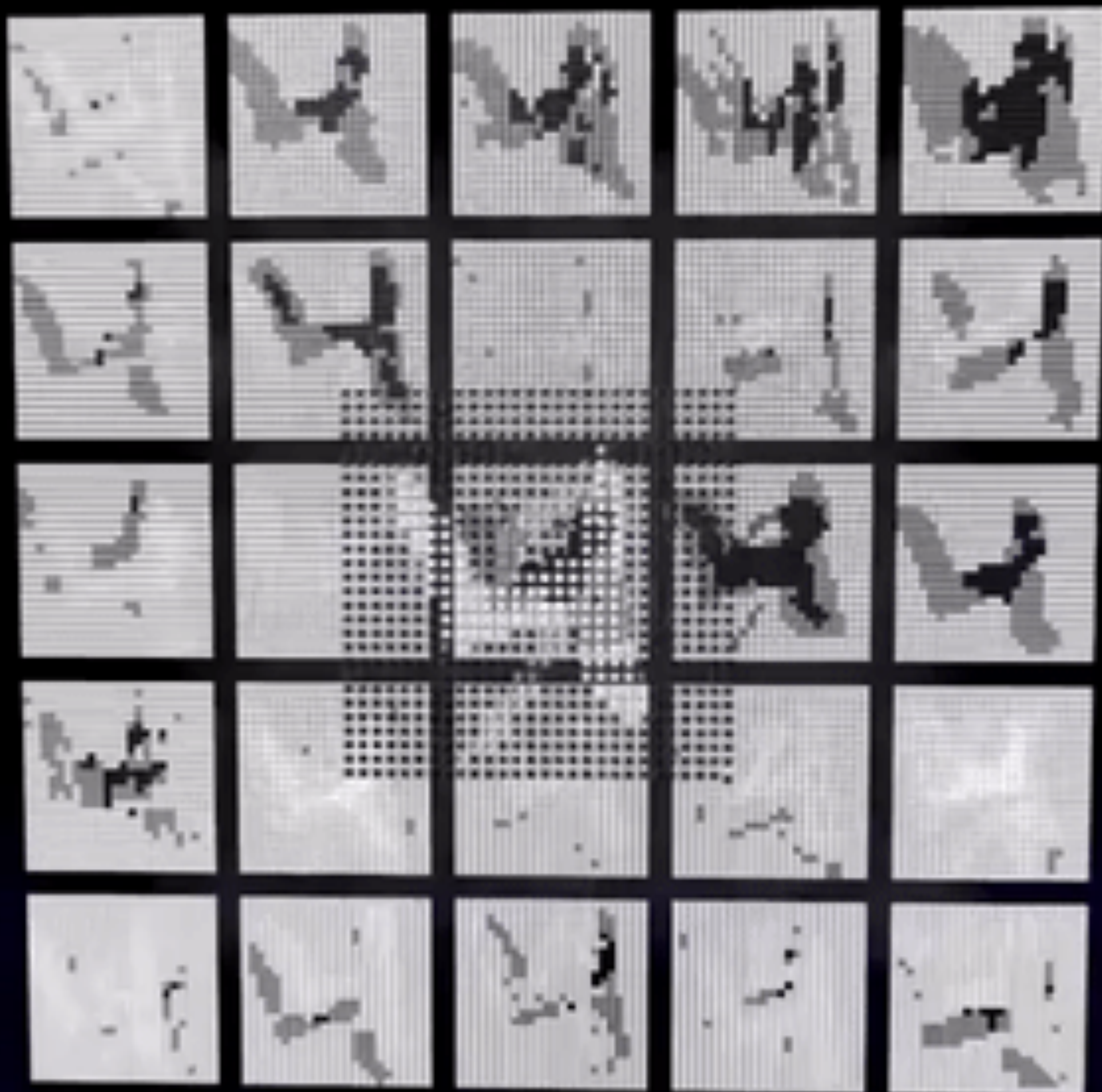
(a) Four **Brodmann areas** associated with the ventral visual stream. The figure reports also a block diagram showing just a few of the many forward and backward projections between these areas.

(b) The sketch of the **AlexNet** convolutional neural network in which pairs of convolution operator followed by a max pooling layer are **roughly analogous to the hierarchy of the biological visual system**.

Only analogy!!

Roffo et al. 2017

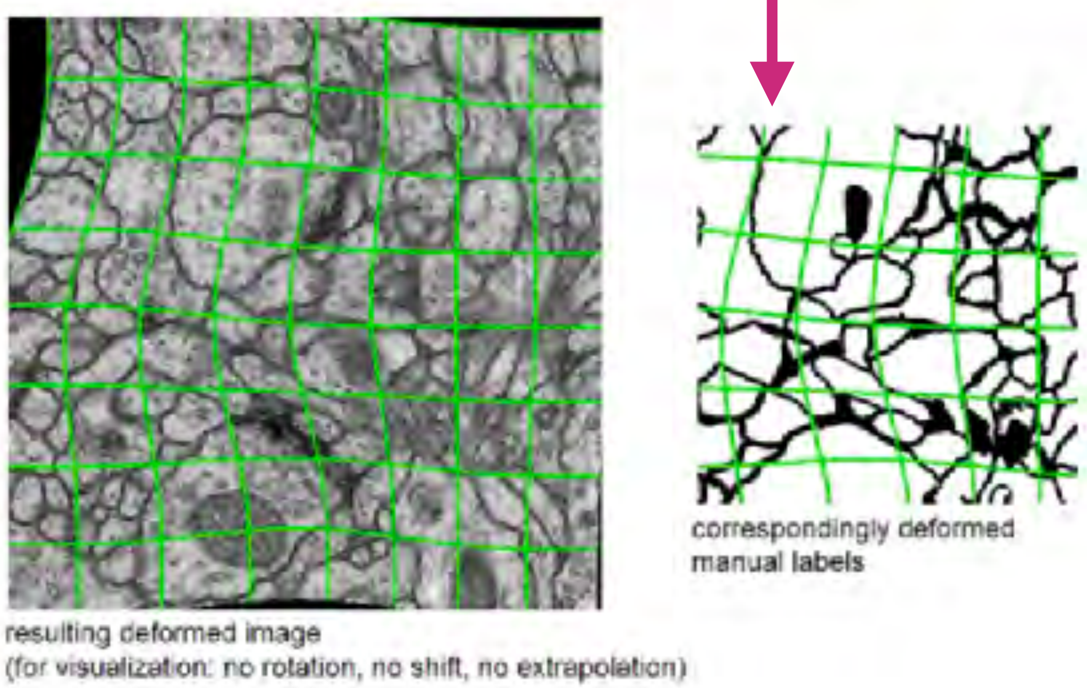
LGN: Lateral geniculate nucleus



Related research

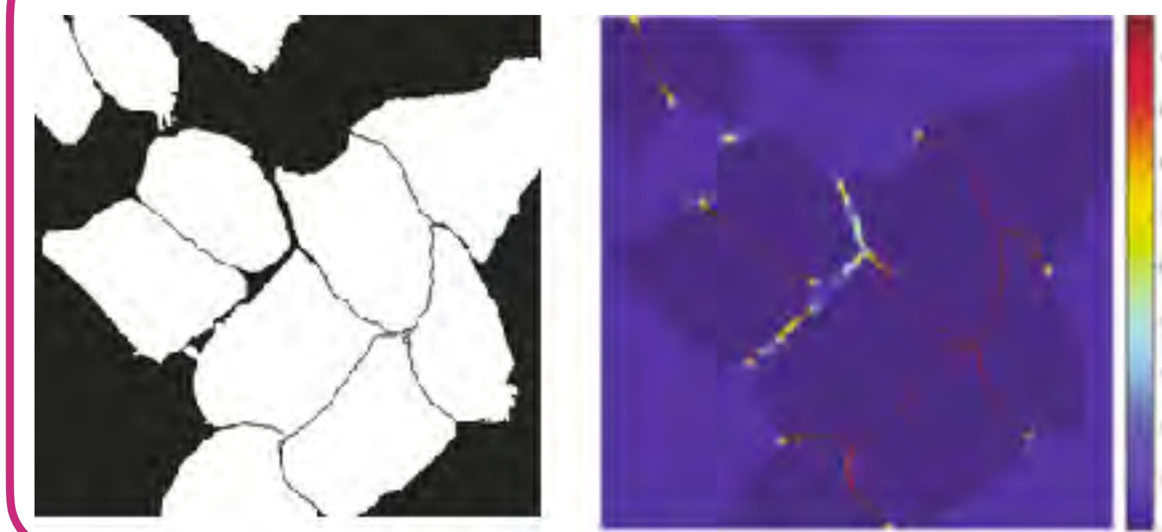
- Cell adhesion

- **Weight function penalization**
- **UNet使う**
- **歪み（ゆがみ） augmentation**



Elastic Deformation for Data Augmentation

Separation of Touching Objects



Weight Map

To compute the weight map as above, d1(x) is the distance to the nearest cell border at position x, d2(x) is the distance to the second nearest cell border. Thus, at the border, weight is much higher as in the figure.

$$w(\mathbf{x}) \quad \ldots \quad (d_1(\mathbf{x}) + d_2(\mathbf{x}))^2$$

Thus, t
by the
the sm

Problem:
-uses Java
-Not maintained
-Outdated

2018 Falk et al.
U-Net: deep learning for cell counting, detection, and morphometry

2016 Ronneberger et al.:
U-Net: Convolutional Networks for Biomedical Image Segmentation

関連研究

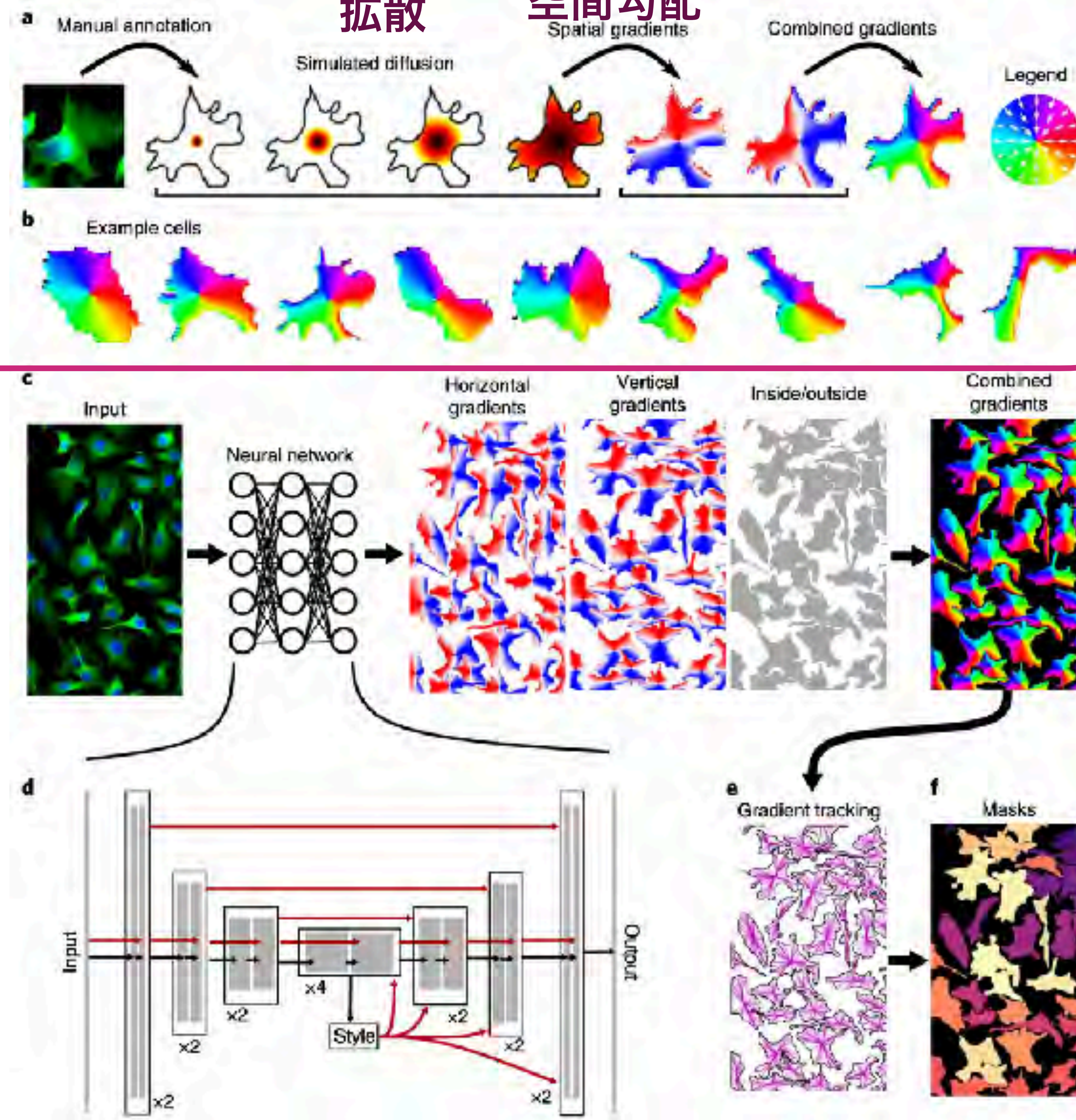
2020 Stringer et al.
Cellpose: a generalist algorithm for cellular segmentation

細胞接着の分裂

Compute:
- spacial gradients　空間勾配（くうかんこうばい）
- Simulated diffusion
  シミュレートされた拡散（かくさん）

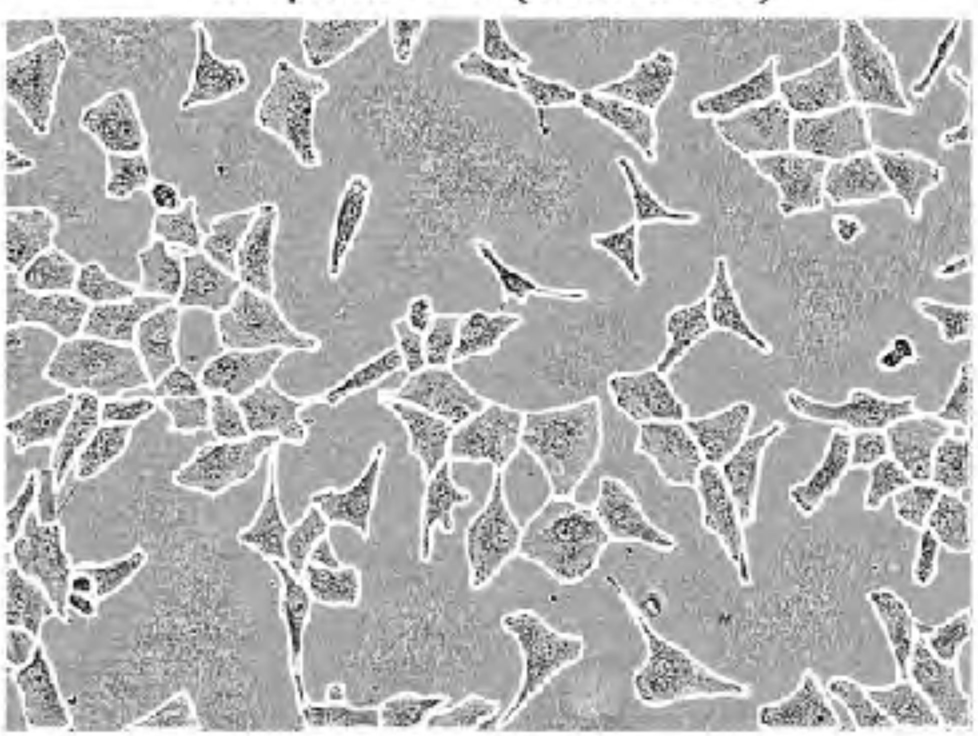
from annotation data to train NN

拡散　空間勾配



- 注釈ソフト (re-training)
- Pre-trained network
- パラメータの最適化を必要

Cellpose 2.0 (finetuned)



Cellpose 2.0: how to train your own model

https://www.biorxiv.org/content/10.1101/2022.04.01.486764v1.full.pdf

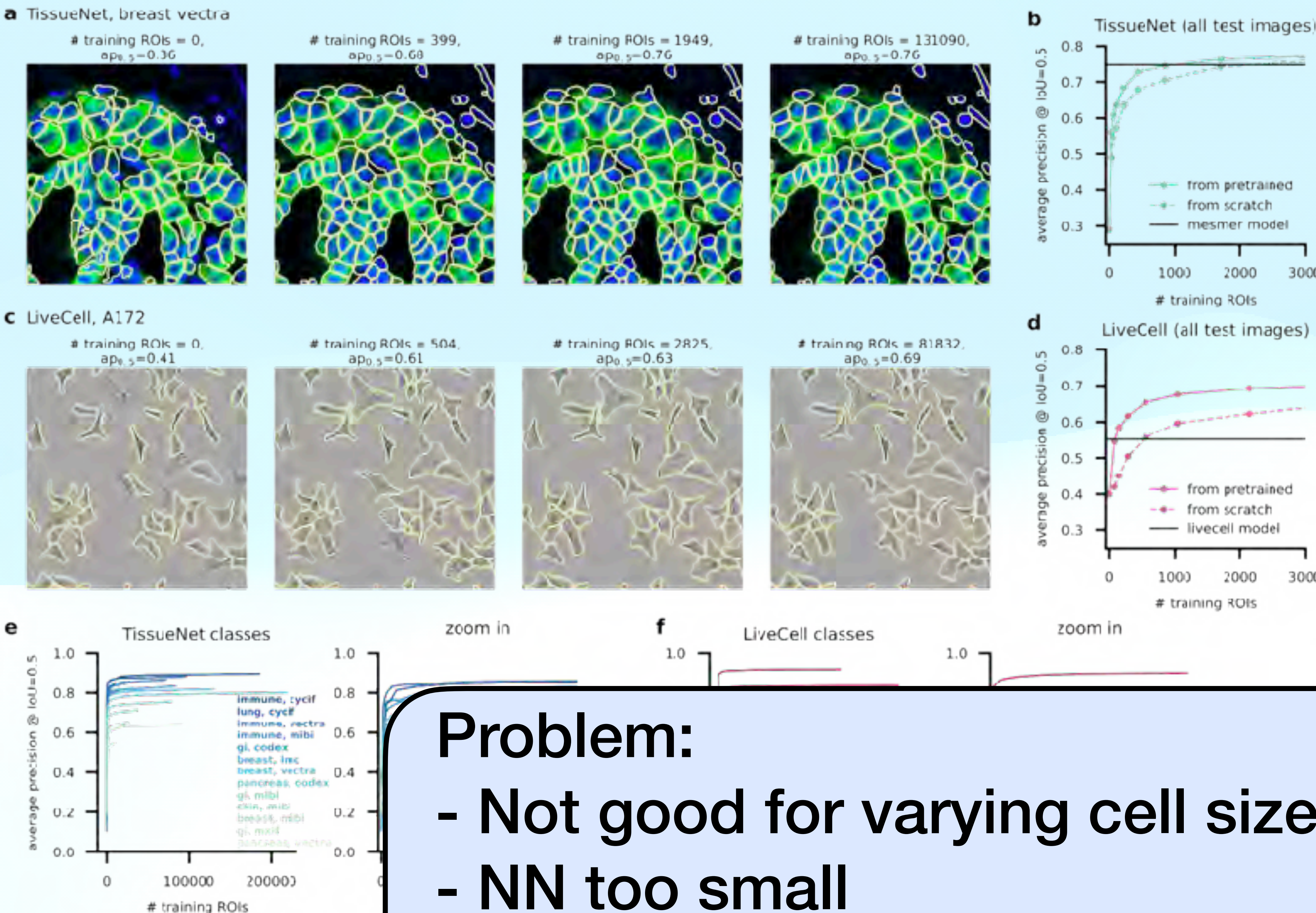
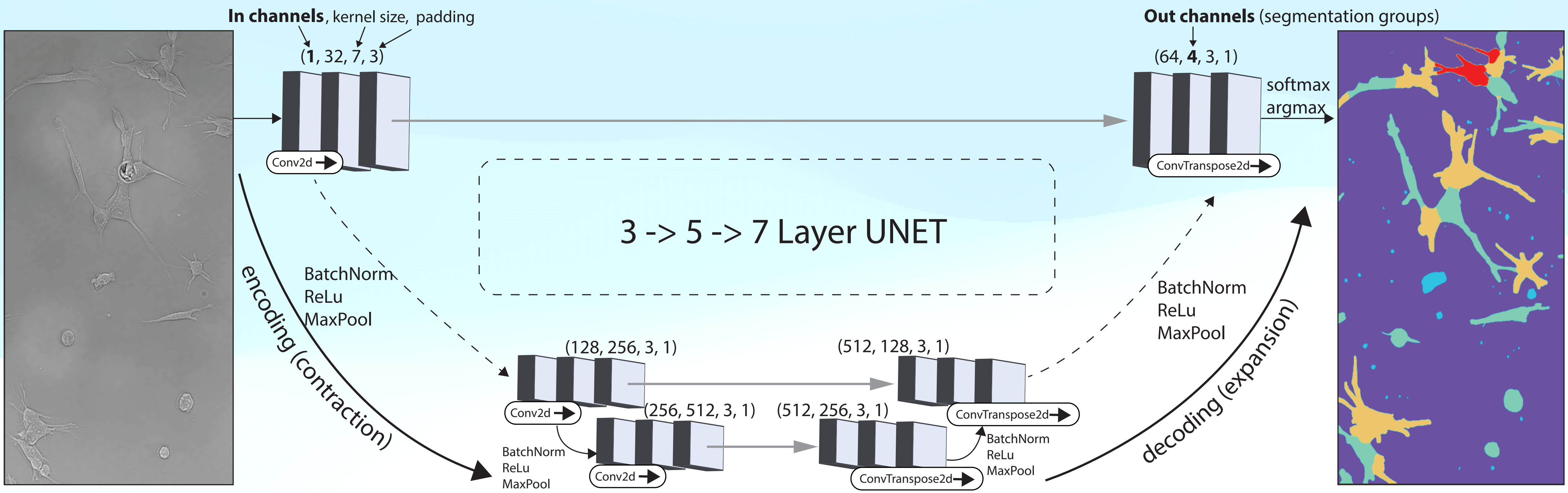


Figure 3: State-of-the-art cellular segm
incrementally more images and initialized
dataset. **b,** Average precision of the mod
from the Cellpose parameters or initialize
Tissuenet dataset. **cd,** Same as (a,b) for
the caveat that this model was trained to r
the TissueNet dataset. (right) Zoom-in for
curves shown in (e,f), as well as the average

Problem:
- Not good for varying cell sizes
- NN too small
- LiveCell annotation not good enough
  (high quantity, but low quality)
- No contaminants

Encoder-decoder NN

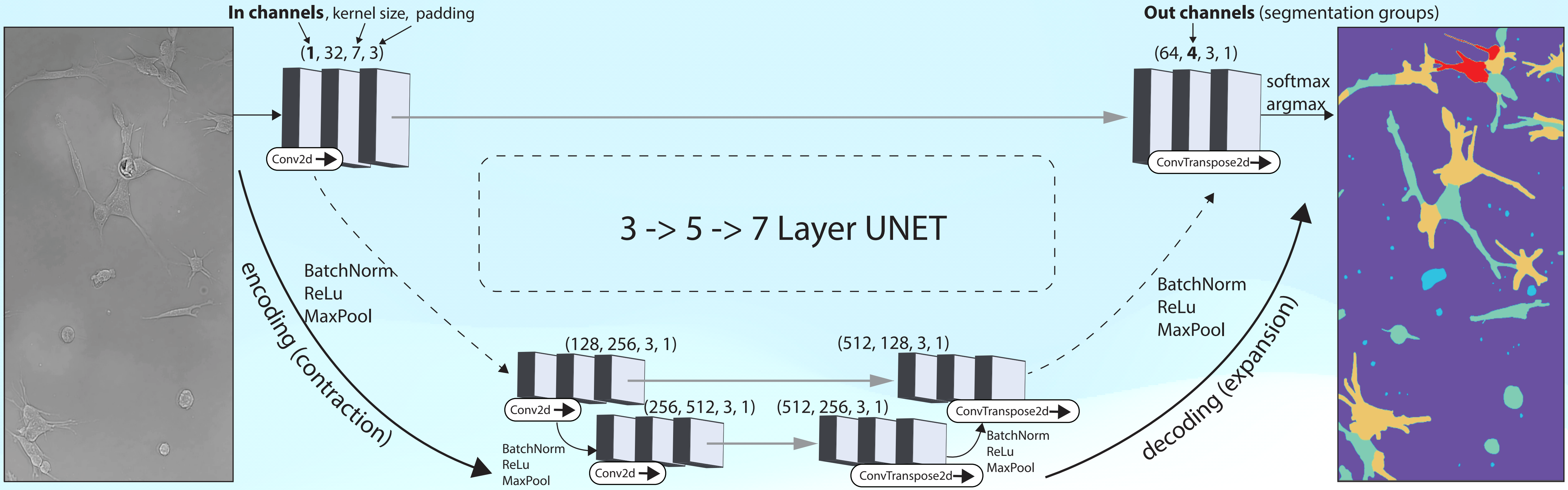


画像アノテーションの例: 上：生画像、下：汚染物質を含む画像注釈汚染物質を含む画像注釈、個々の細胞、その内容物（核、突起物など）を含む。

Forward pass → Inference

https://github.com/StefanBaar/cell_activity

Training - optimization



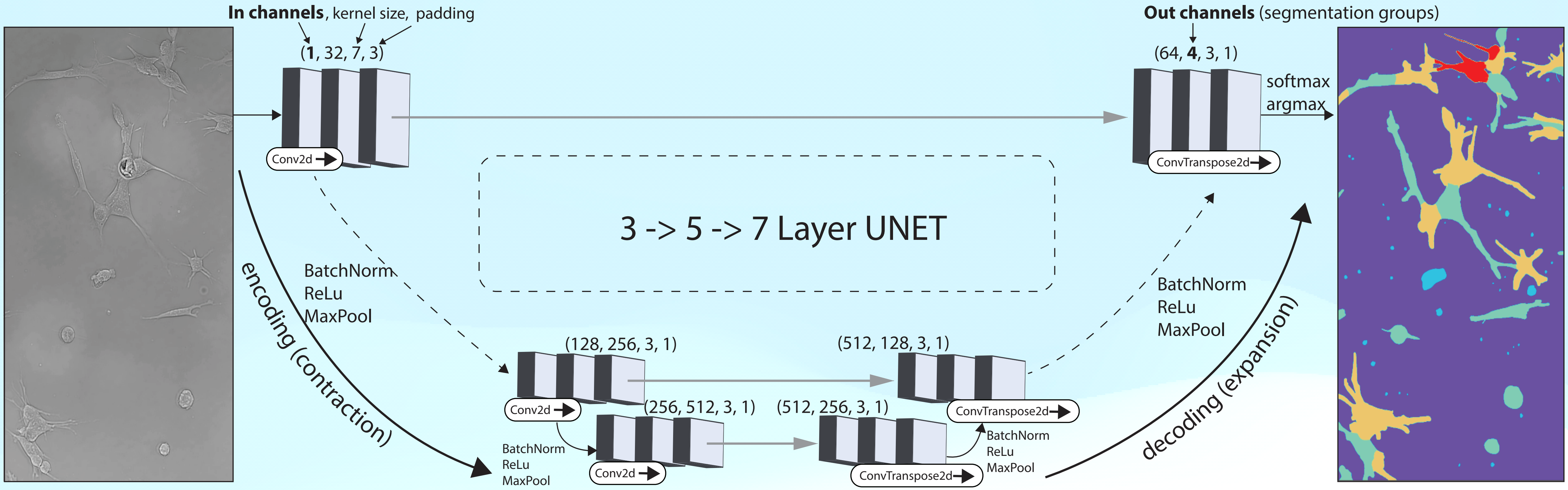
Input A (Image) → **Adjust parameters** ← Input B (annotations)

Input A (Image) → Output B (masks)

Compare (Input B <-> Output B) —> **Loss: [0,….,1]**

Backpropagation

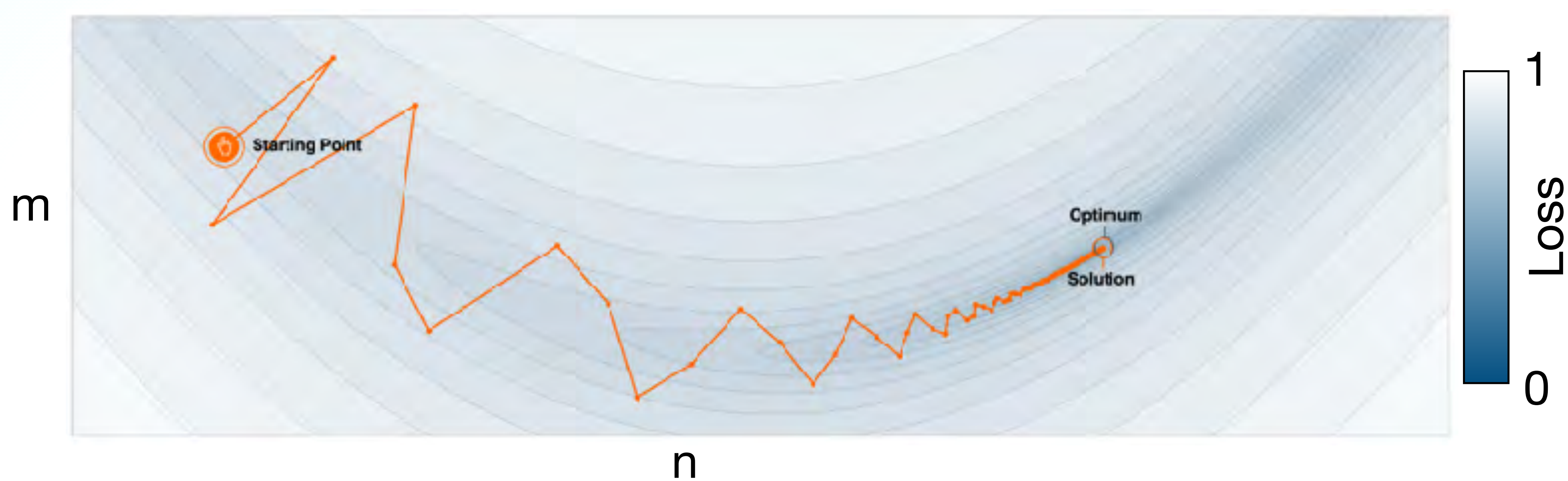
Training - optimization



Input A (Image) **→** Adjust parameters **←** Input B (annotations)

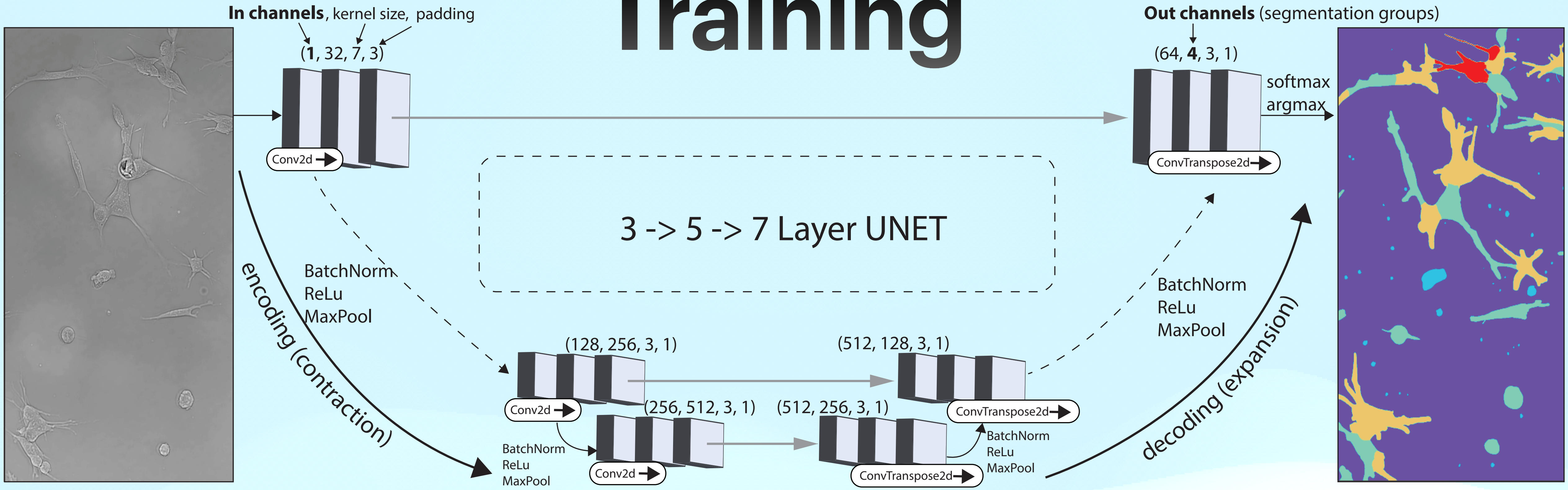
Parameter space (m,n)

Backpropagation



https://m2dsupsdlclass.github.io/lectures-labs/slides/02_backprop/index.html#68

Training



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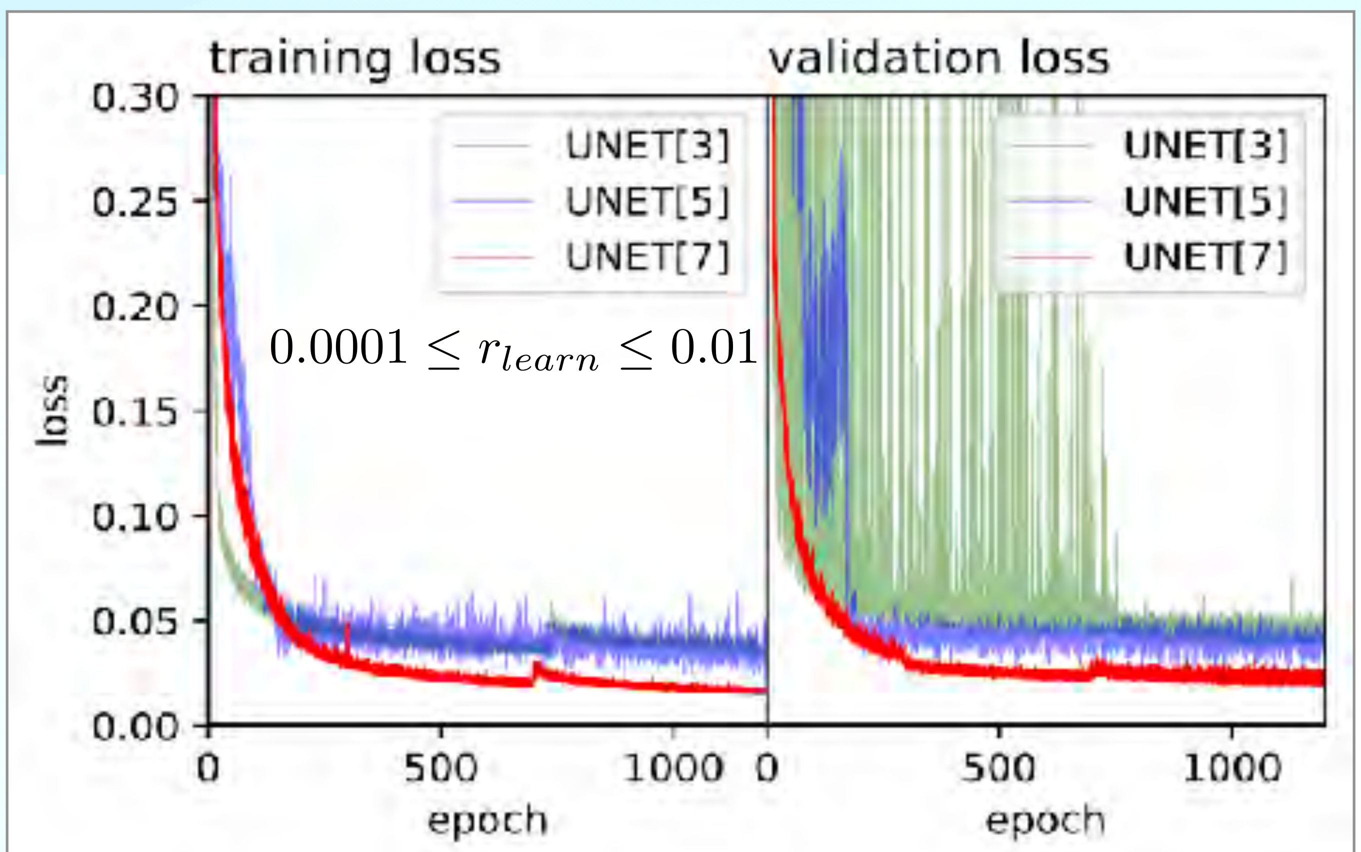


クロスエントロピーの損失進化: ３つUNET深さレベルのため、学習データ (左) 検証データ (右) depths.

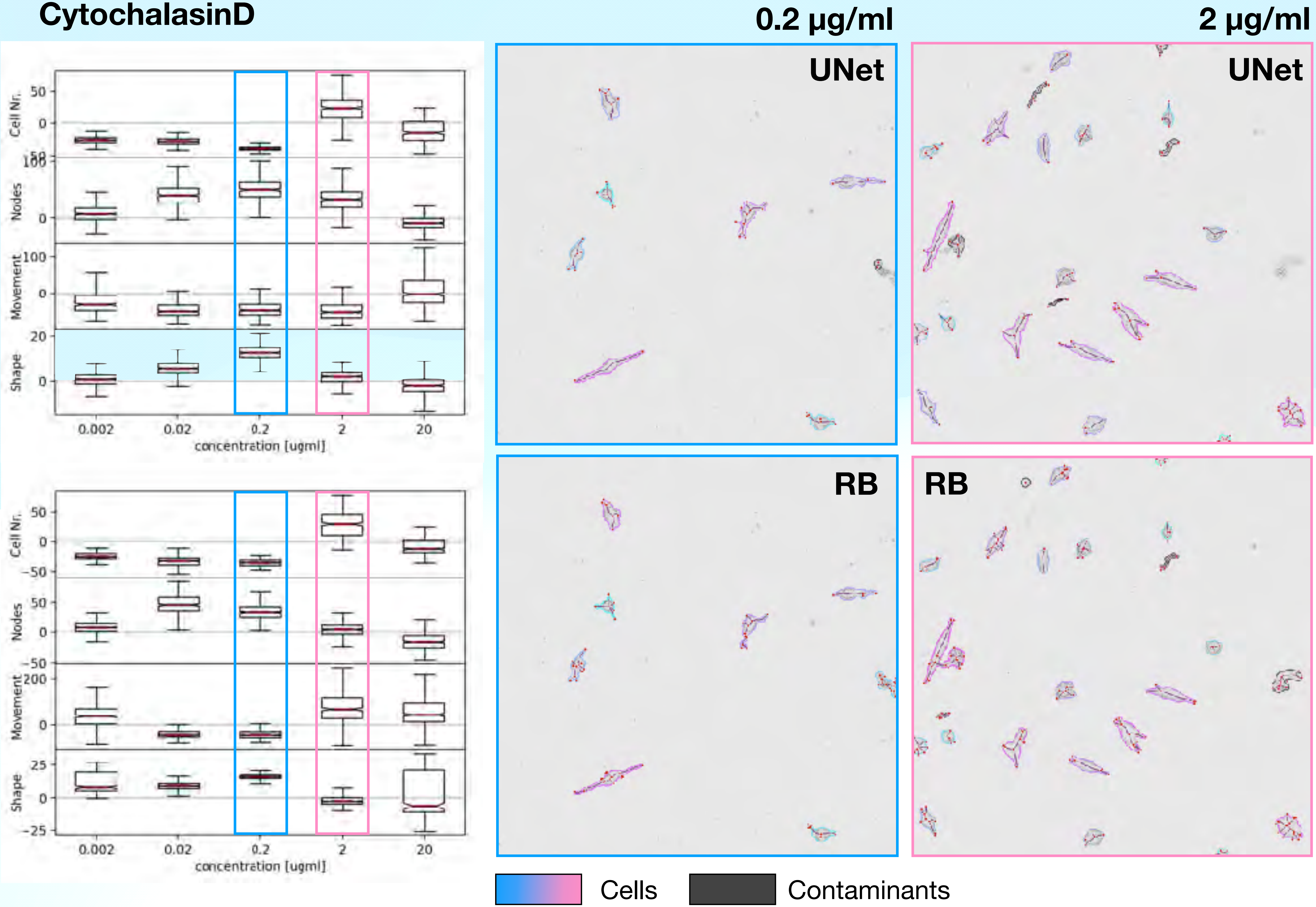
Segmentation Accuracy

model	mAP	IOU
UNET[3]	0.822	0.46
UNET[5]	0.911	0.79
UNET[7]	0.982	0.81

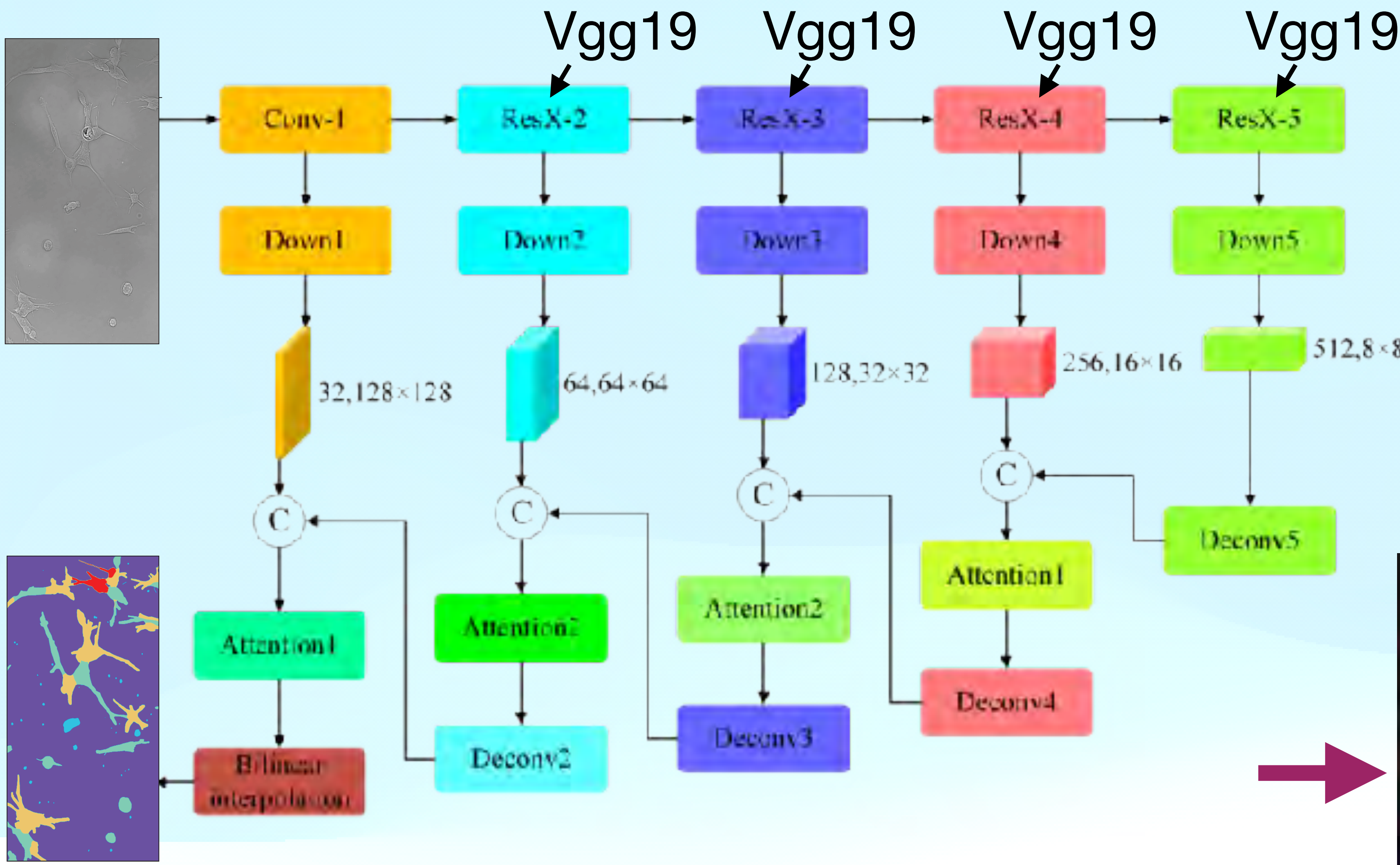
完璧ではないが、十分に良い

Multiple UNET++, deeplab

CytochalasinD



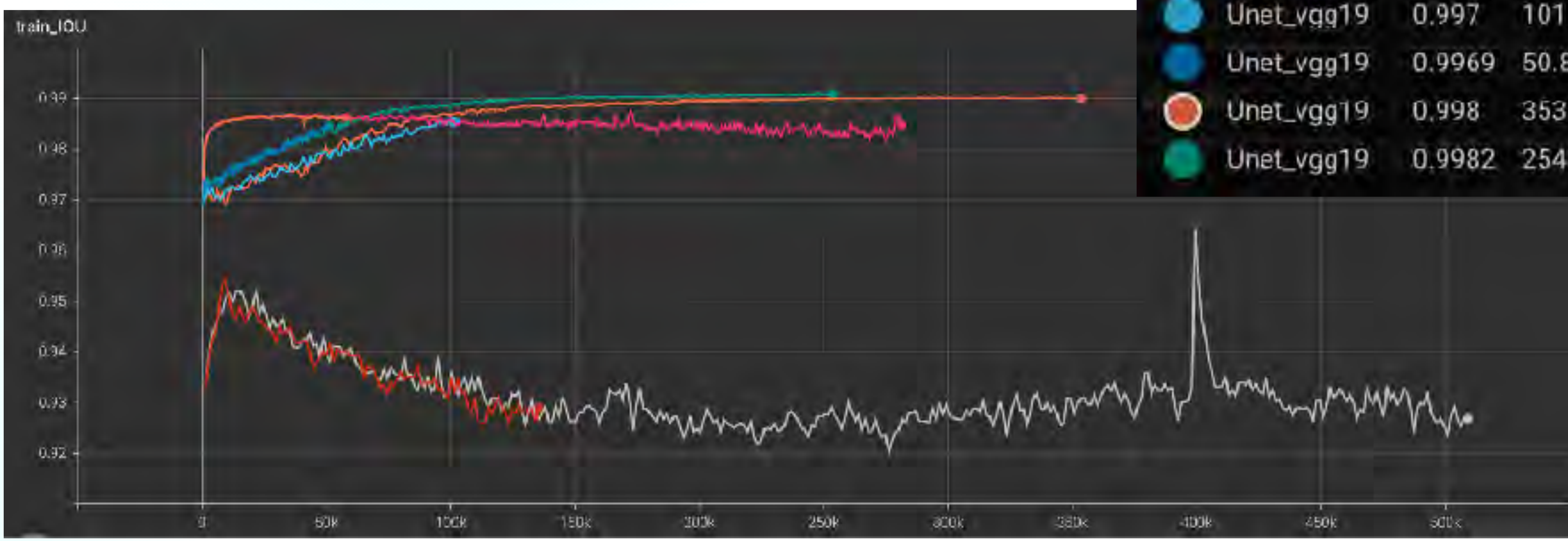
MAnet



Training

Best results:
multi attention neural nets (MAnet)
Li et. al. 2020

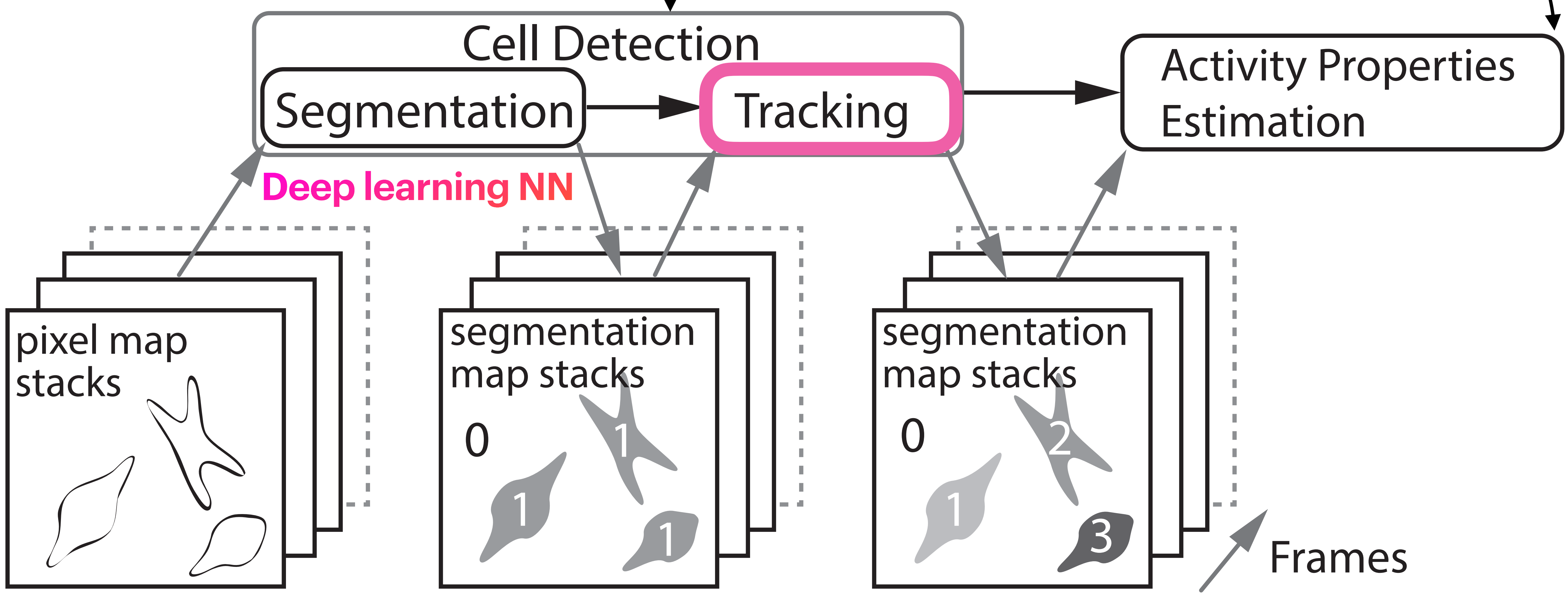
Name	Value	Step	Relative
Linknet_vgg19	0.9835	135.4k	1d 12h 36m 38s
Linknet_vgg19	0.9841	354.7k	4d 2h 31m 15s
MAnet_vgg19	0.9972	58k	1d 4h 35m 37s
MAnet_vgg19	0.9969	281.3k	5d 21h 4m 17s
Unet_vgg19	0.997	101.5k	1d 15h 35m 45s
Unet_vgg19	0.9969	50.8k	1d 2h 32m 34s
Unet_vgg19	0.998	353.2k	5d 20h 59m 56s
Unet_vgg19	0.9982	254k	5d 14h 26m 11s



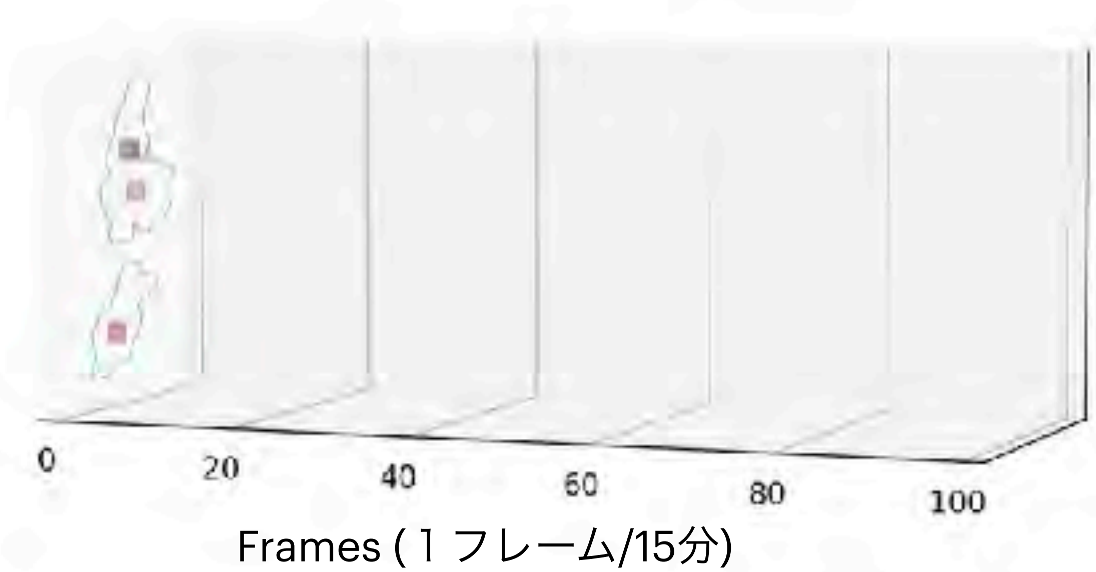
Also:
-YOLO
-DeepLab

https://arxiv.org/abs/2009.02130

1細胞検出　2細胞活性

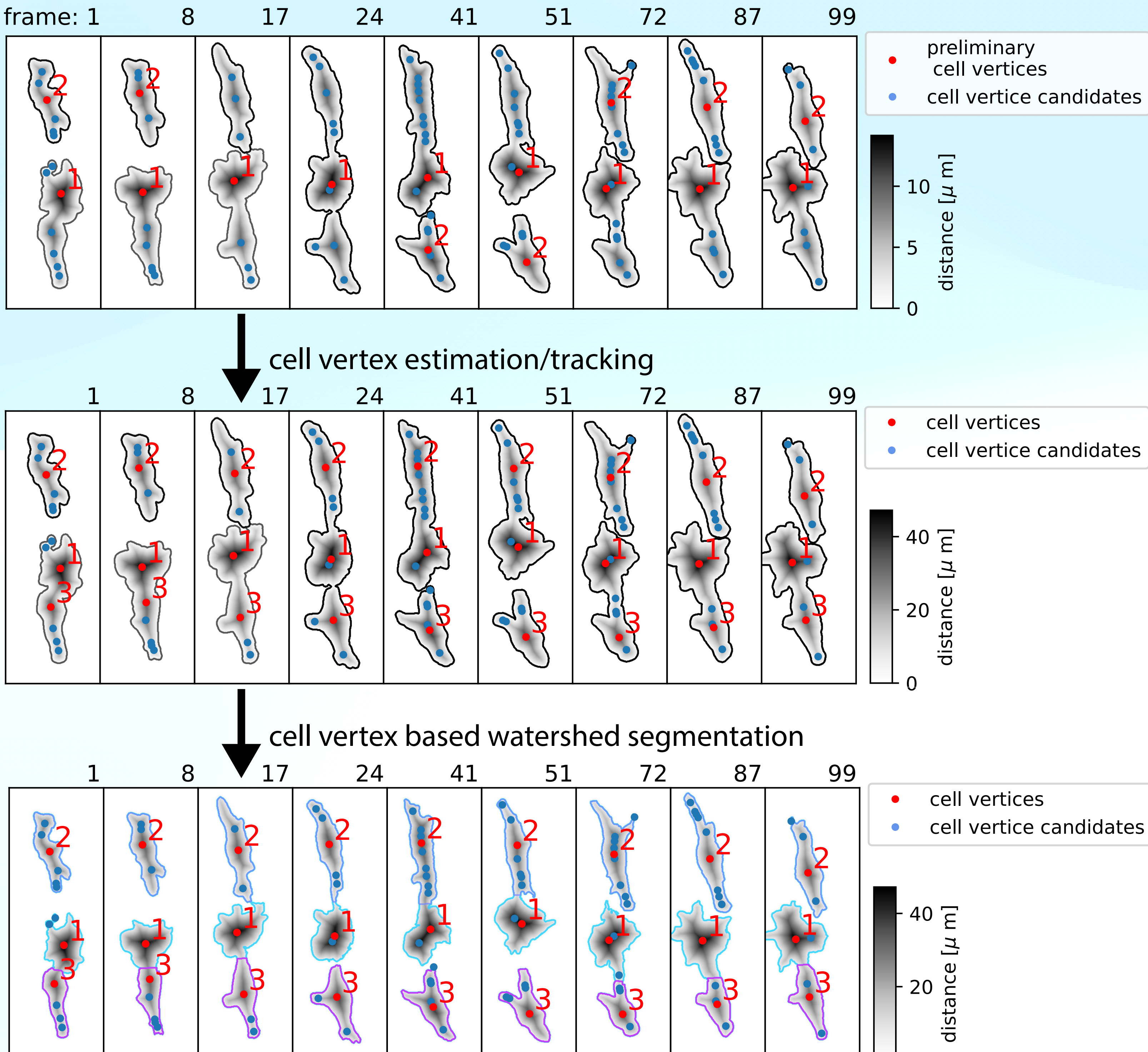


Worst case: flawed detection ==> bad tracking result



Cell Tracking

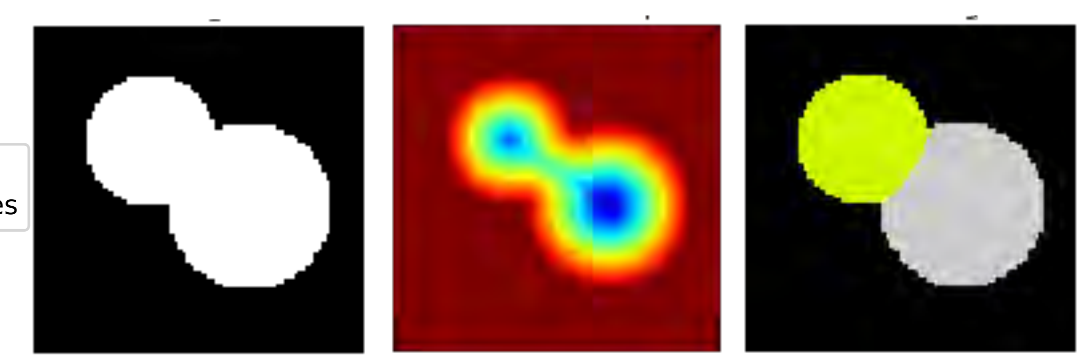
Kd-tree based overlap estimation



Tou ... ted

Problem:
- Clusters
- Adhesion

Co... ...es from local maxima of the distance map



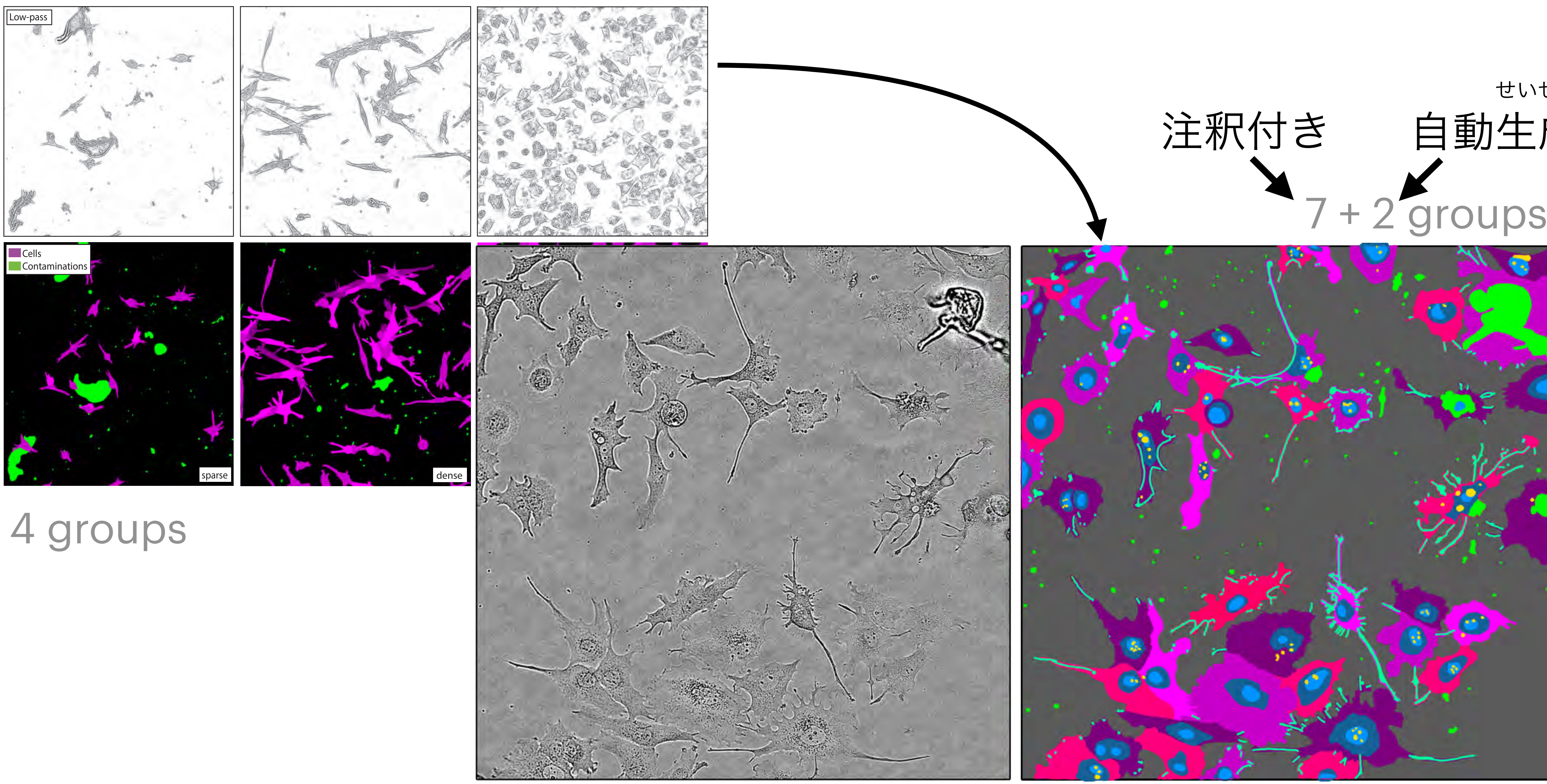
時間分解最近傍探索追跡

-> **watershed segmentation**

Multi-label annotations

今までの取り組み：21 images (1608x1608) approx. 300 cells

今の取り組み：　1 image (1608x1608) approx. 46 cells



4 groups

注釈付き　自動生成（せいせい）

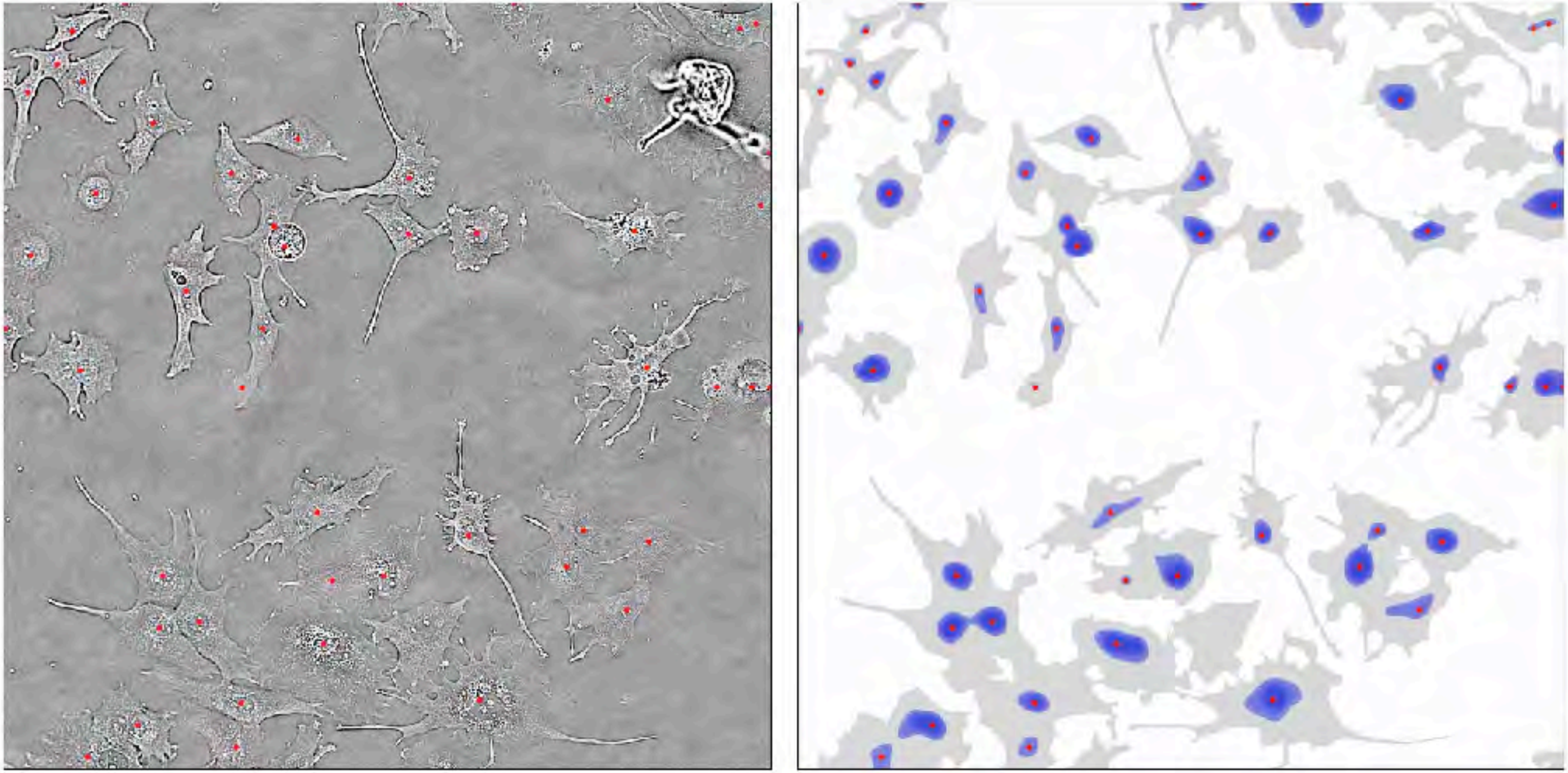
7 + 2 groups

Self-produced annotations on tablet

https://github.com/StefanBaar/cell_annotations

cells
nucleus (outer)
nucleus (inner)
contaminants
cell inners
protrusions (ridge)

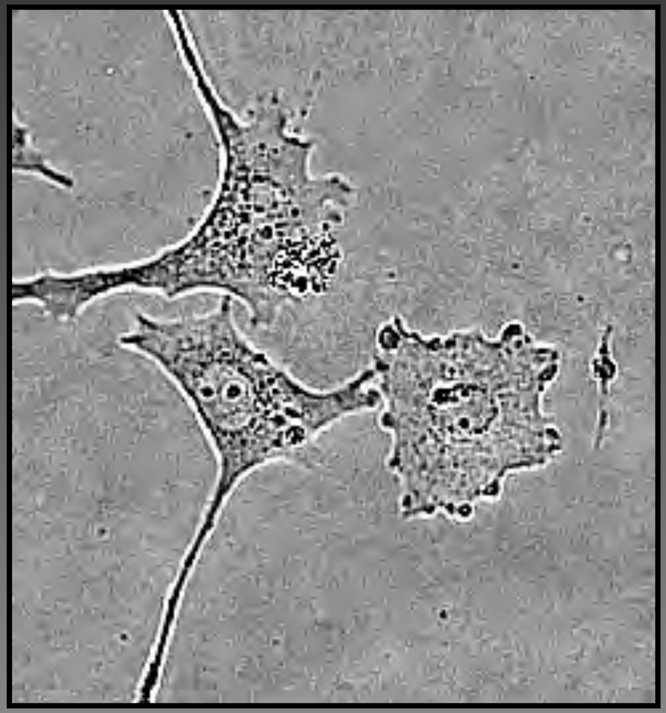
結果



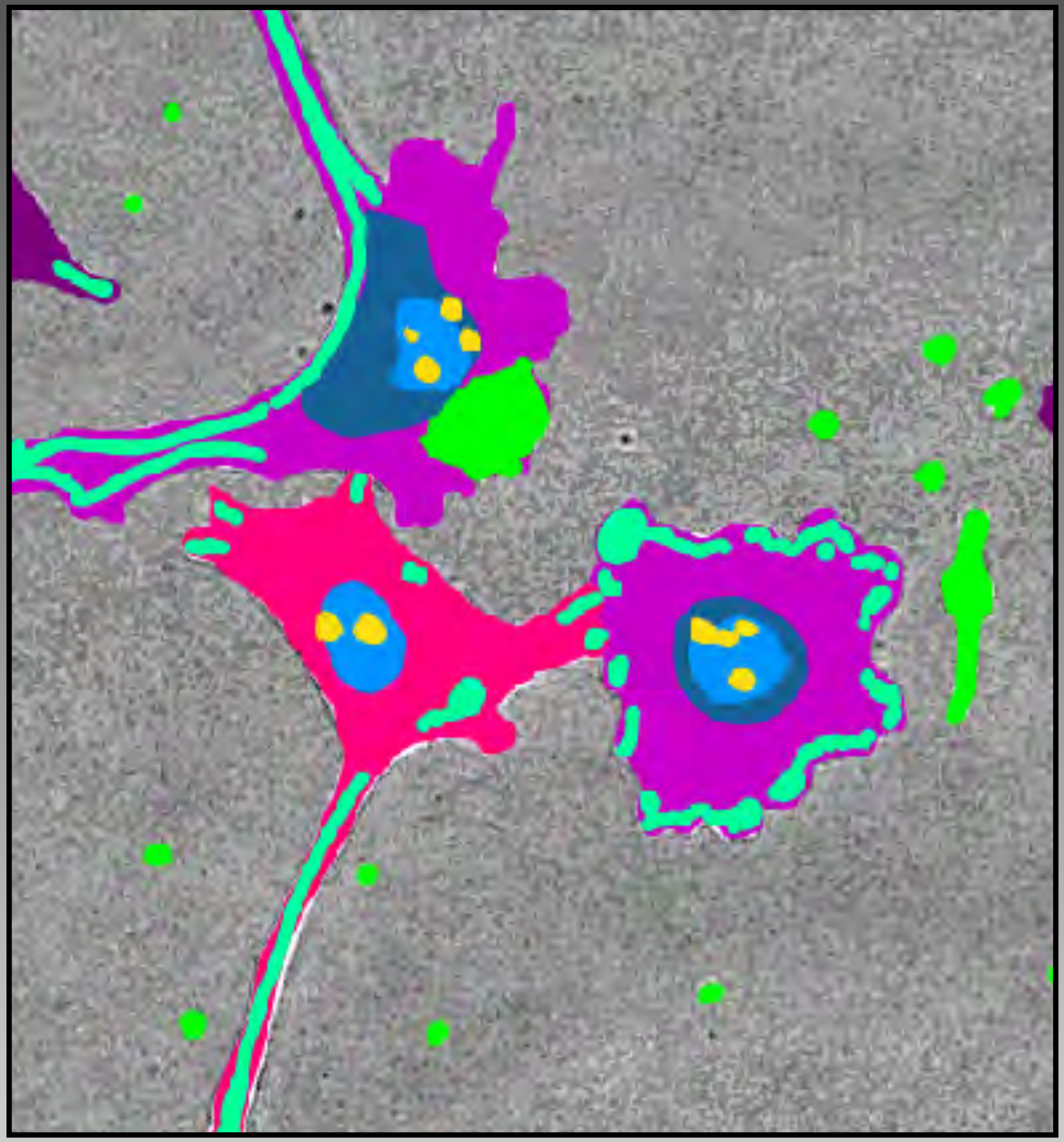
Evaluation coming soon ...

レイヤーベースのファイル

細胞画像

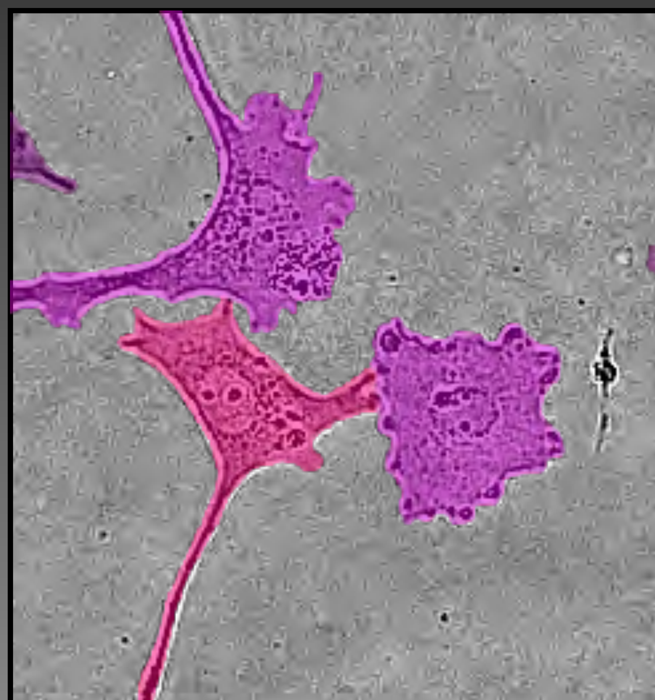


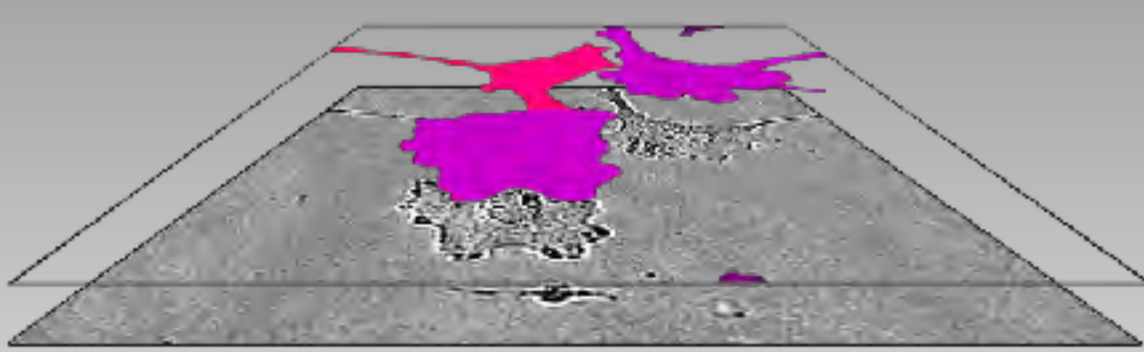
細胞の形態だけじゃなくて



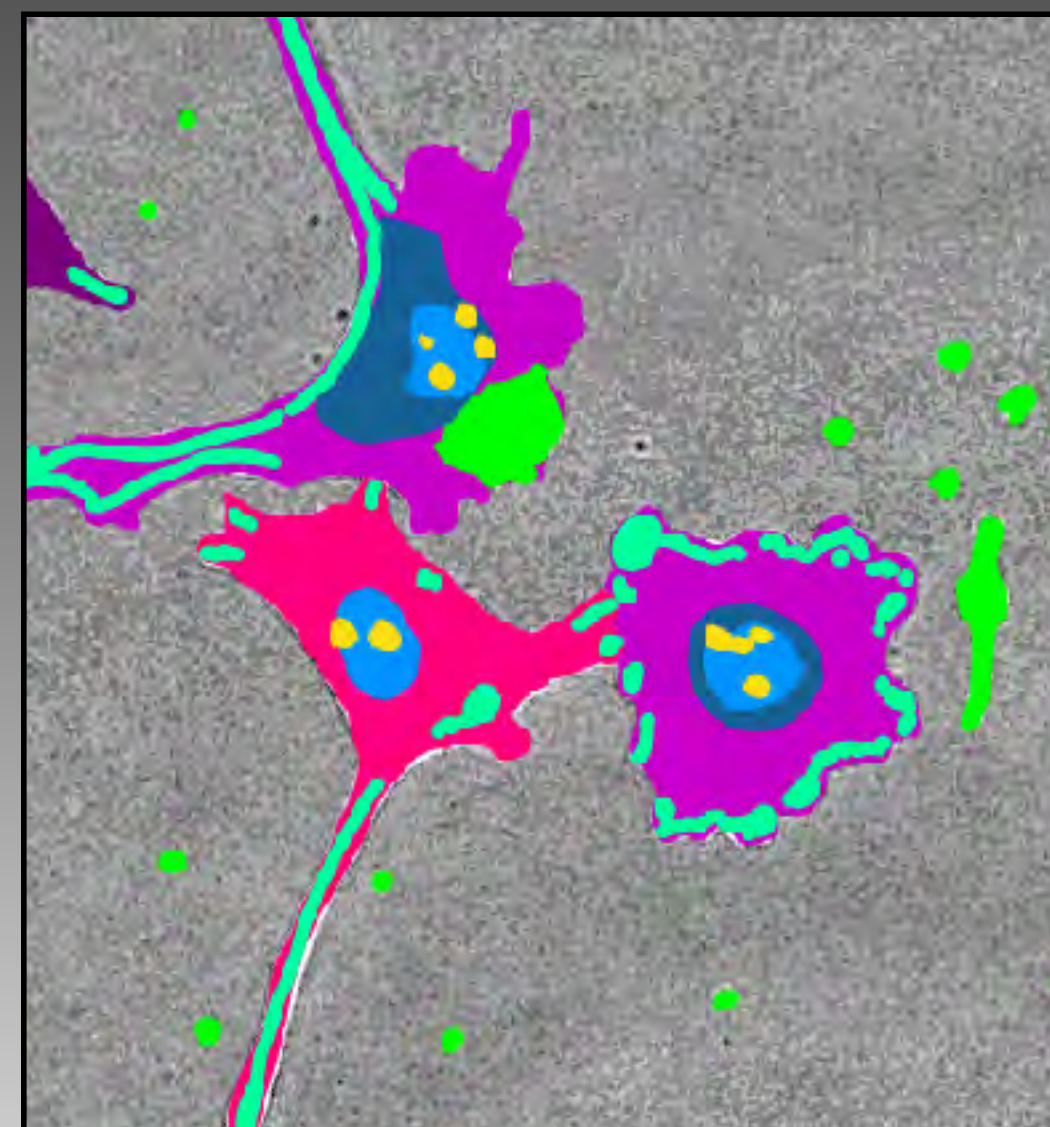
レイヤーベースのファイル

Cell



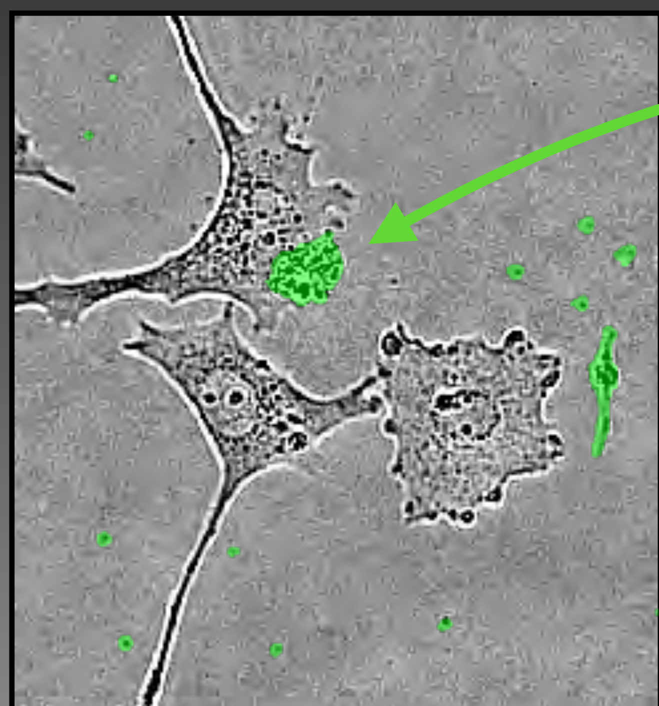


Vector annotations:
Converted to pixel annotations

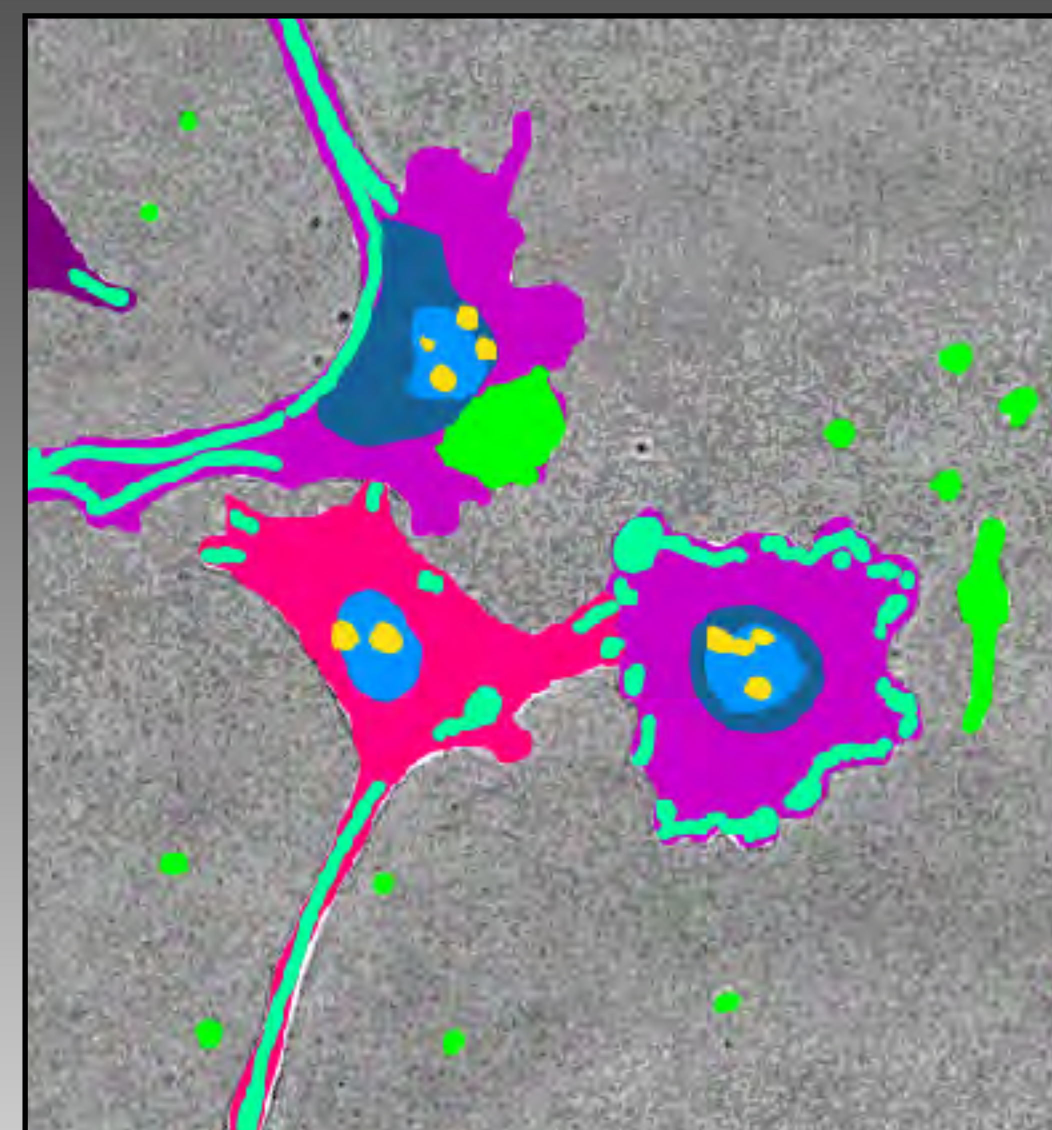


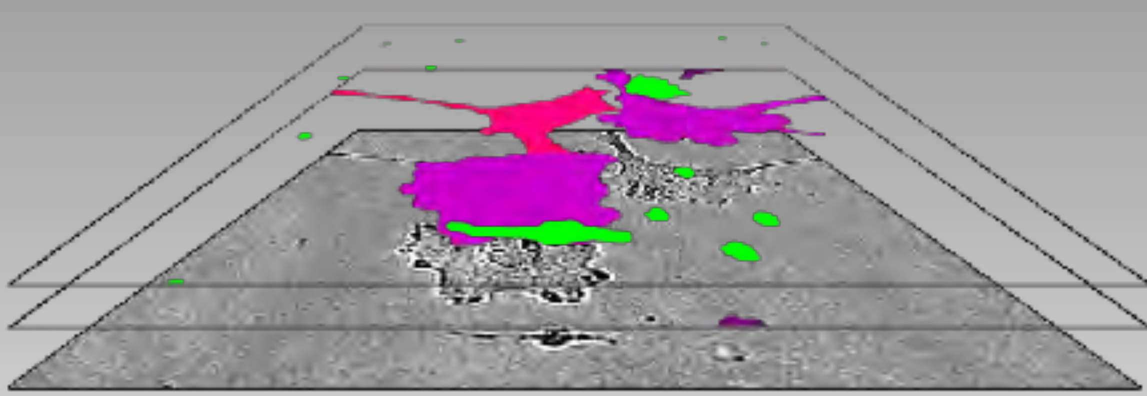
レイヤーベースのファイル

Contaminants



contaminants



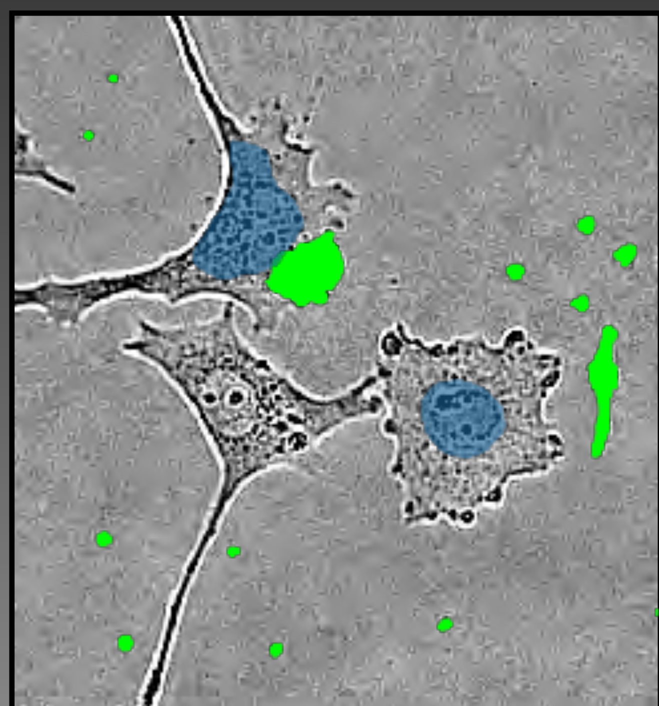


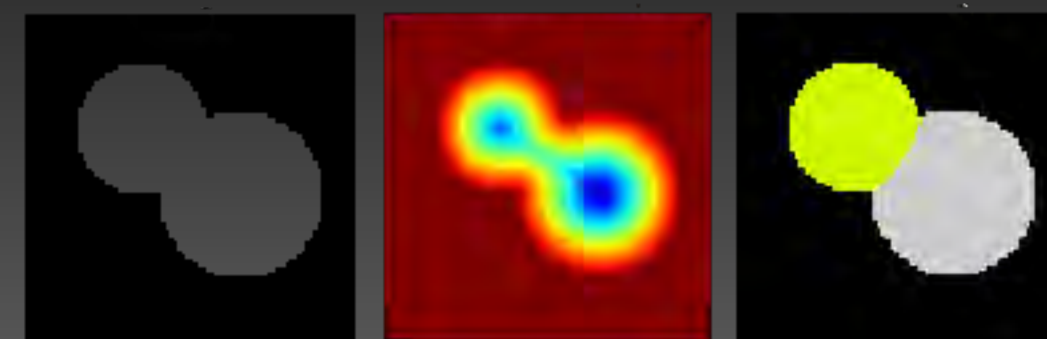
レイヤーベースのファイル

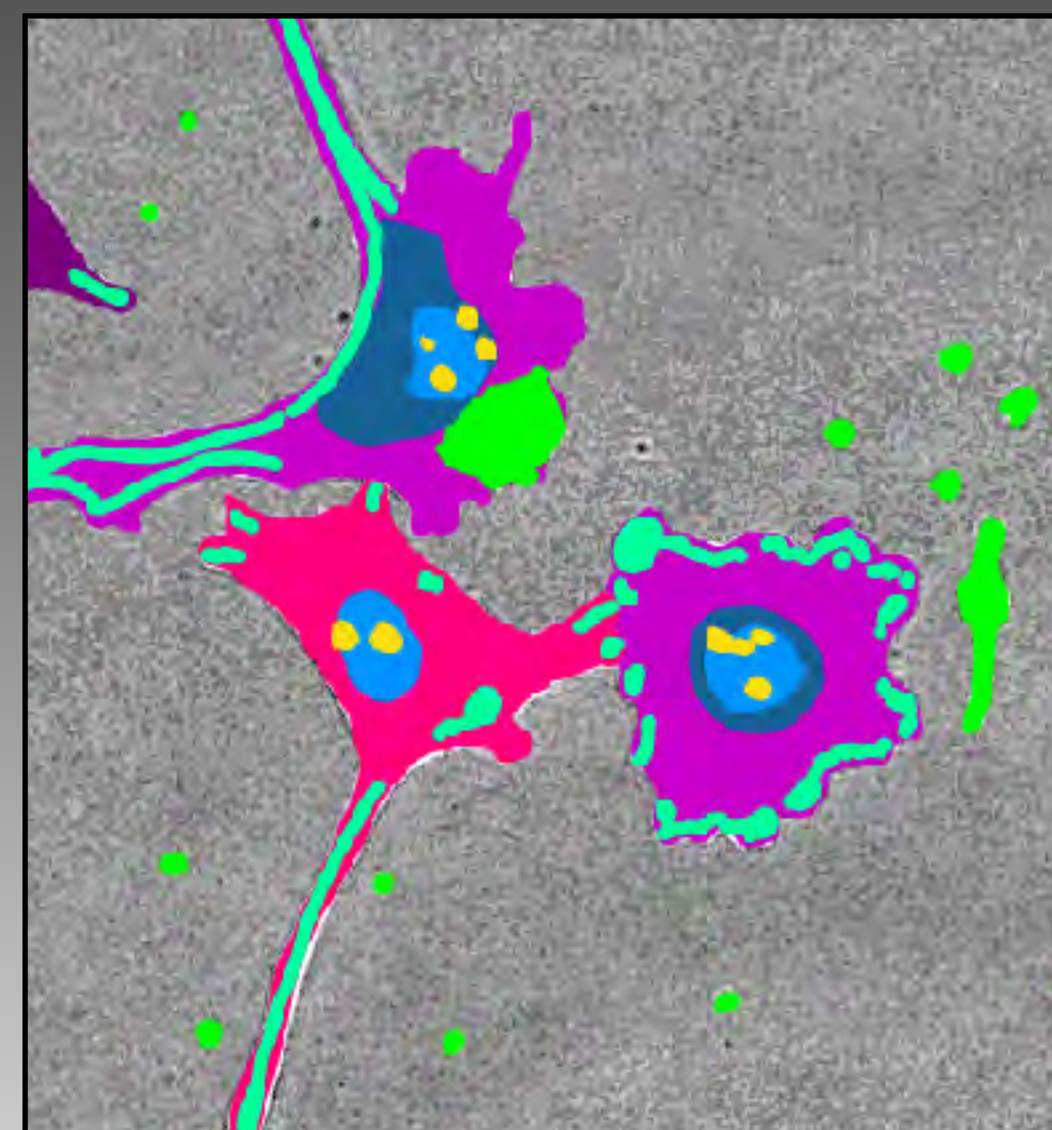
細胞分裂を強化するための細胞核の検出

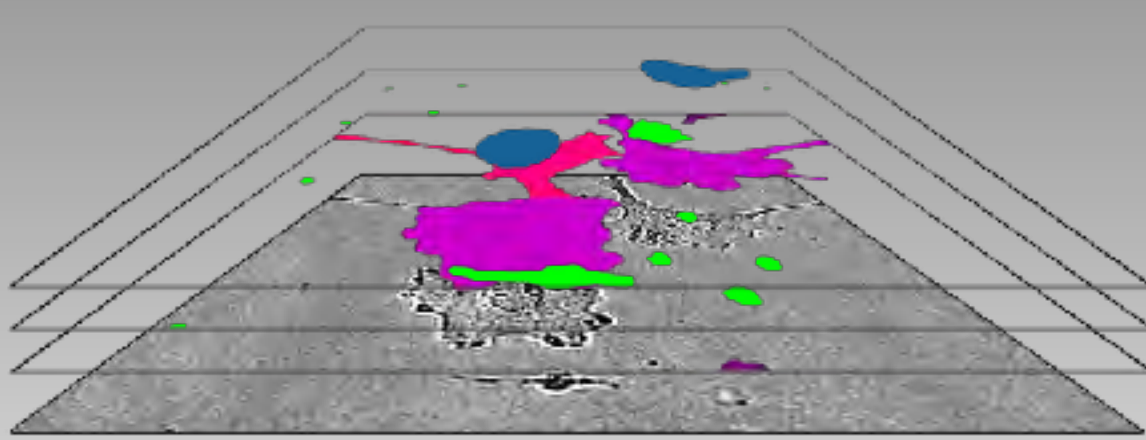
Via watershed/random walk segmentation

Cell nucleus



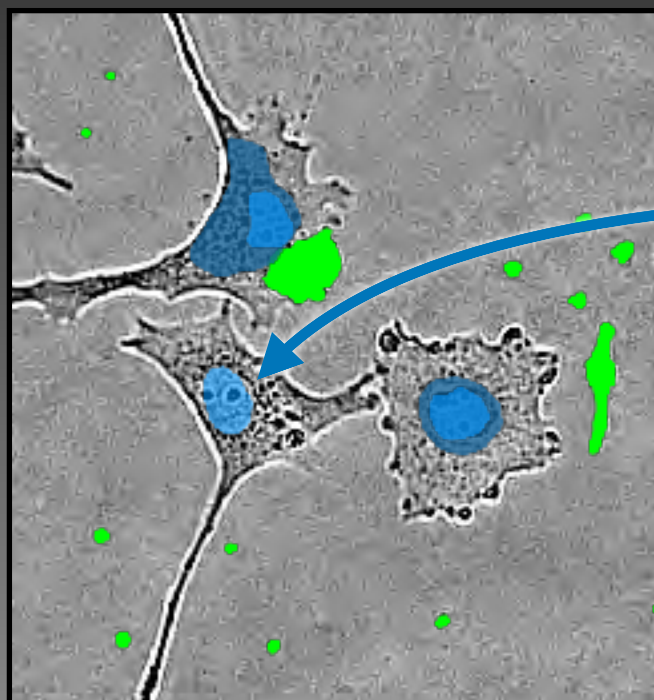




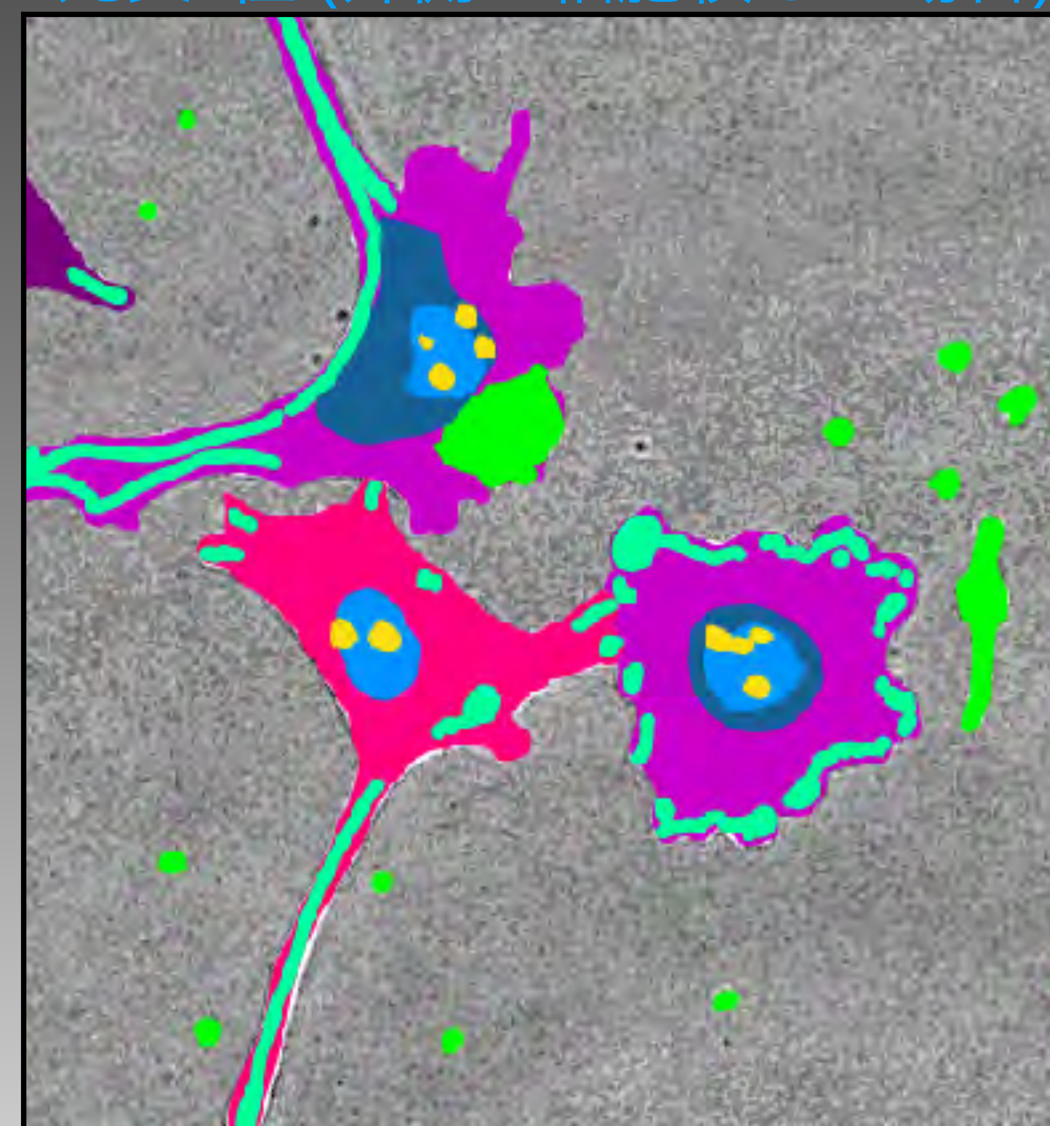


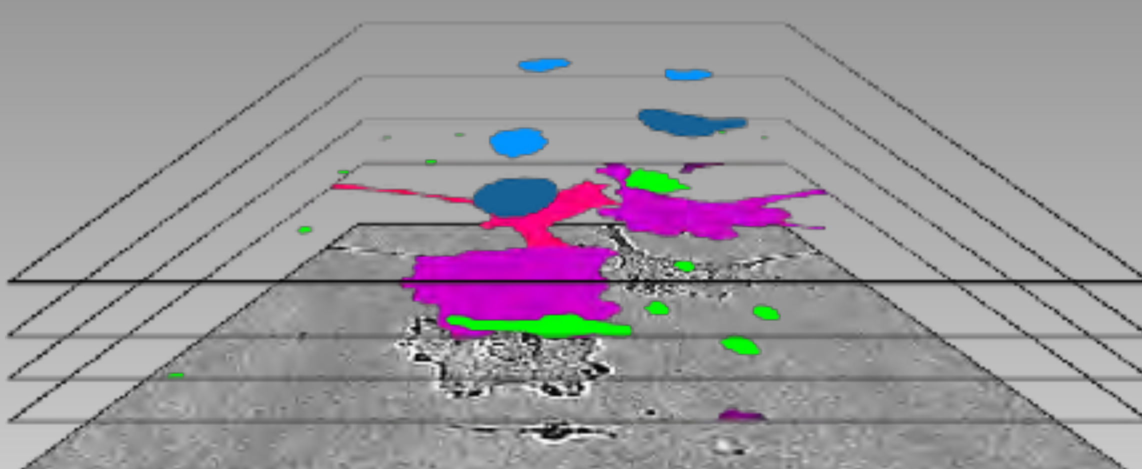
レイヤーベースのファイル

Inner nucleus



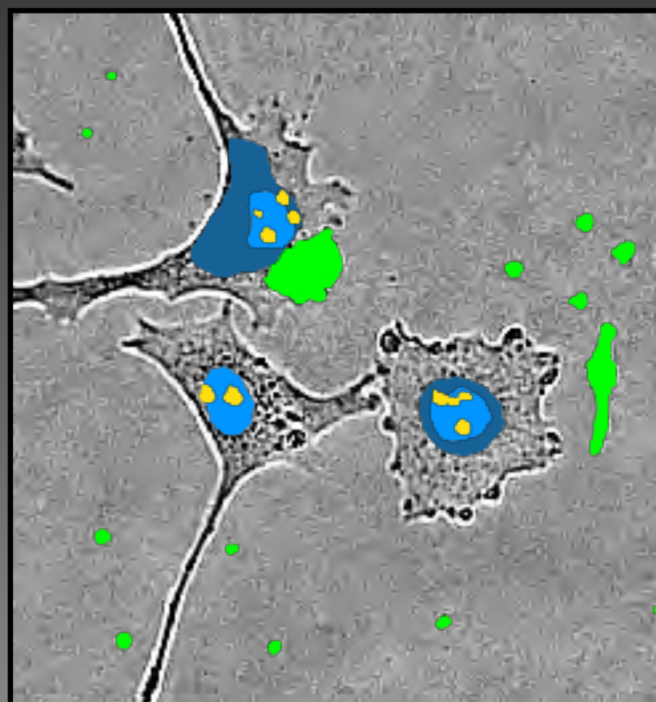
じょうちょうせい
冗長 性 (外側の細胞核ない場合)

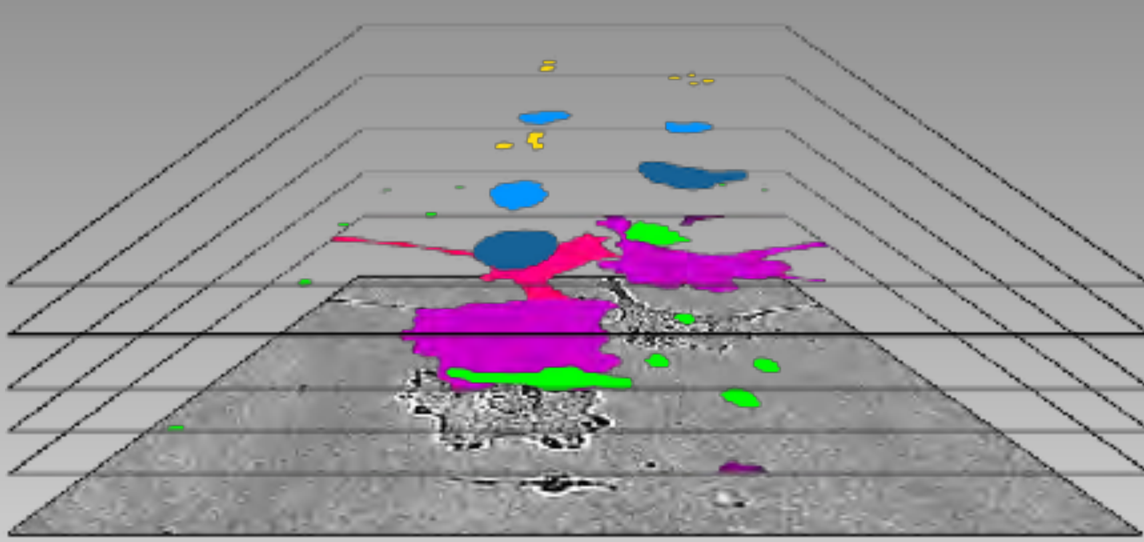




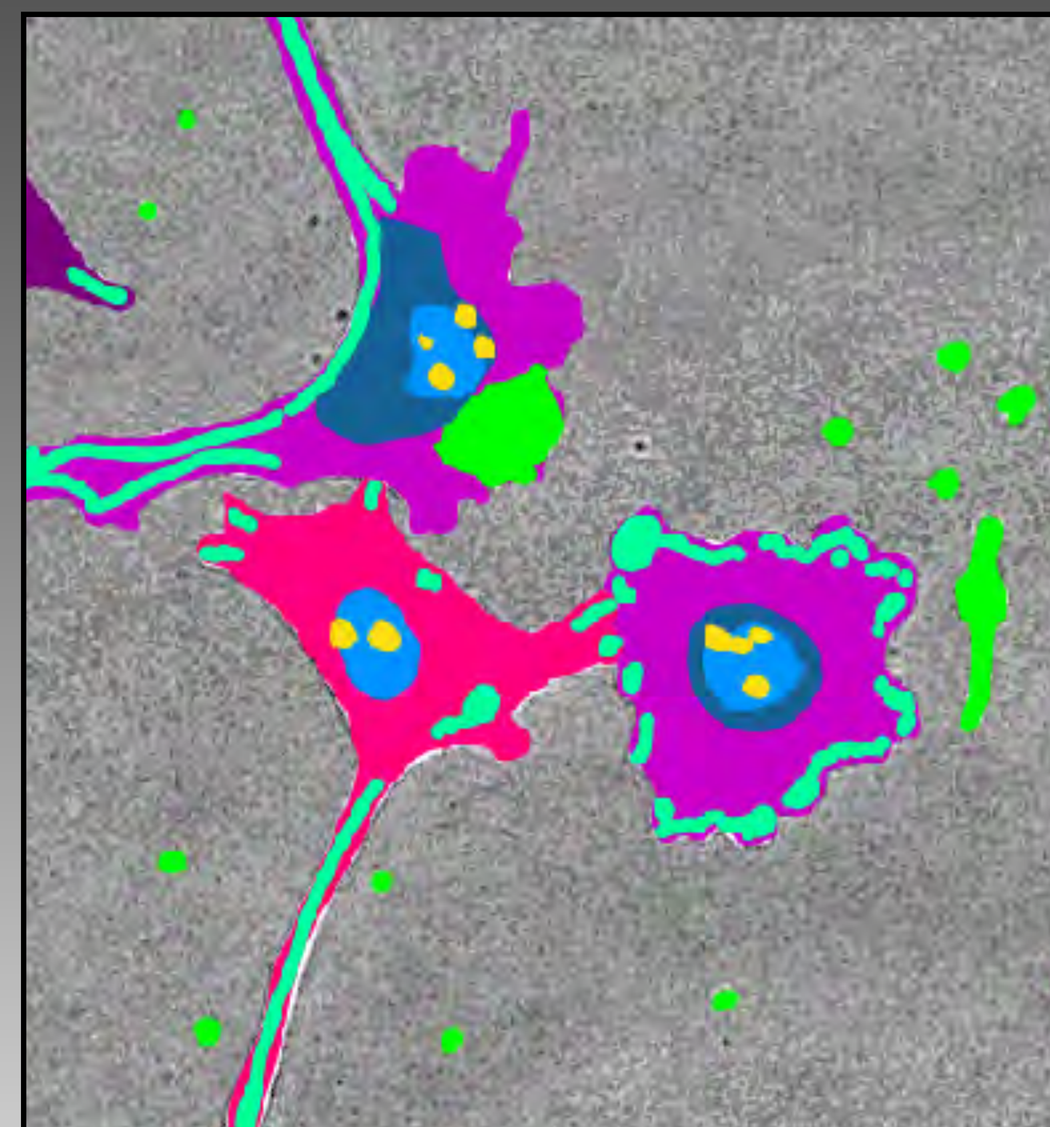
レイヤーベースのファイル

Cell organelle



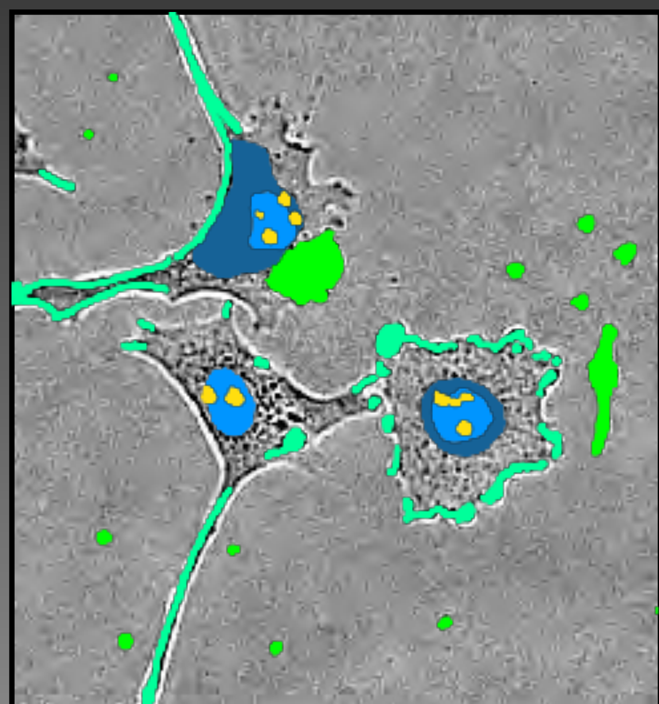


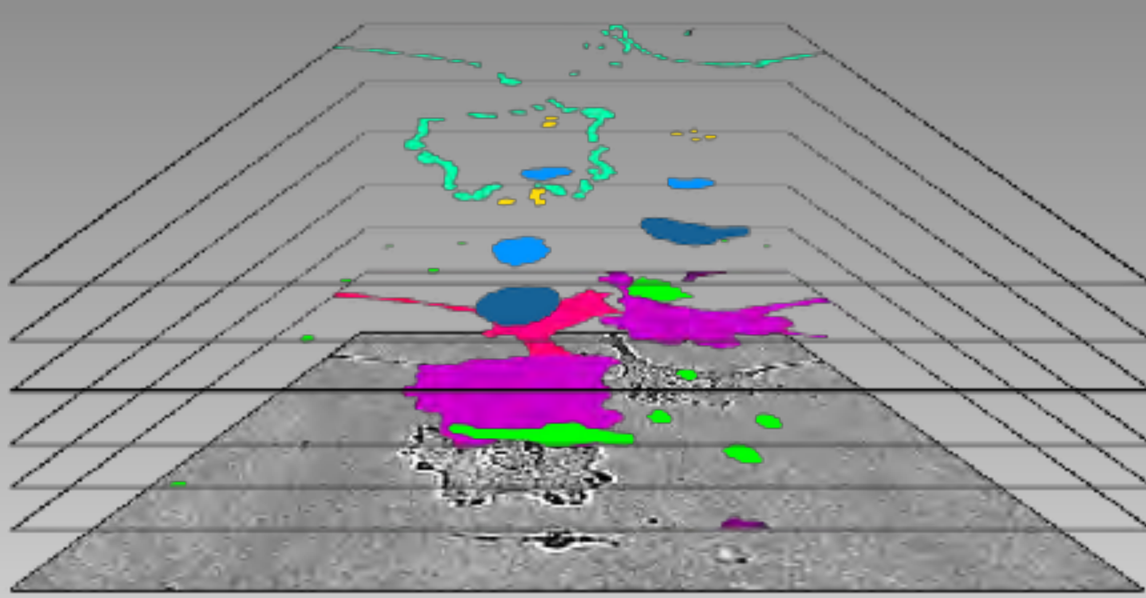
かつどう
細胞活動推定のため（今後の研究）



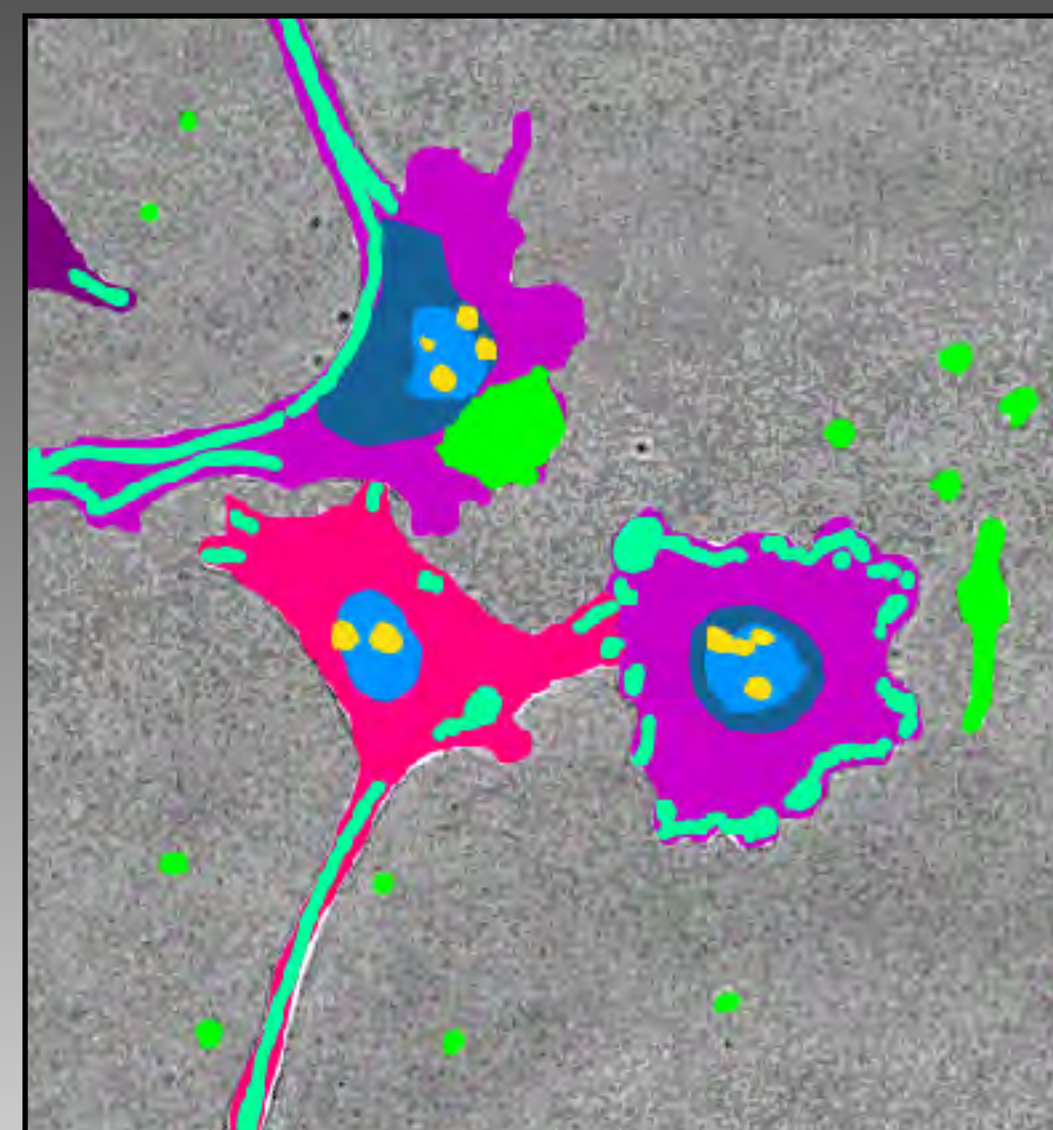
レイヤーベースのファイル

protrusion ridges

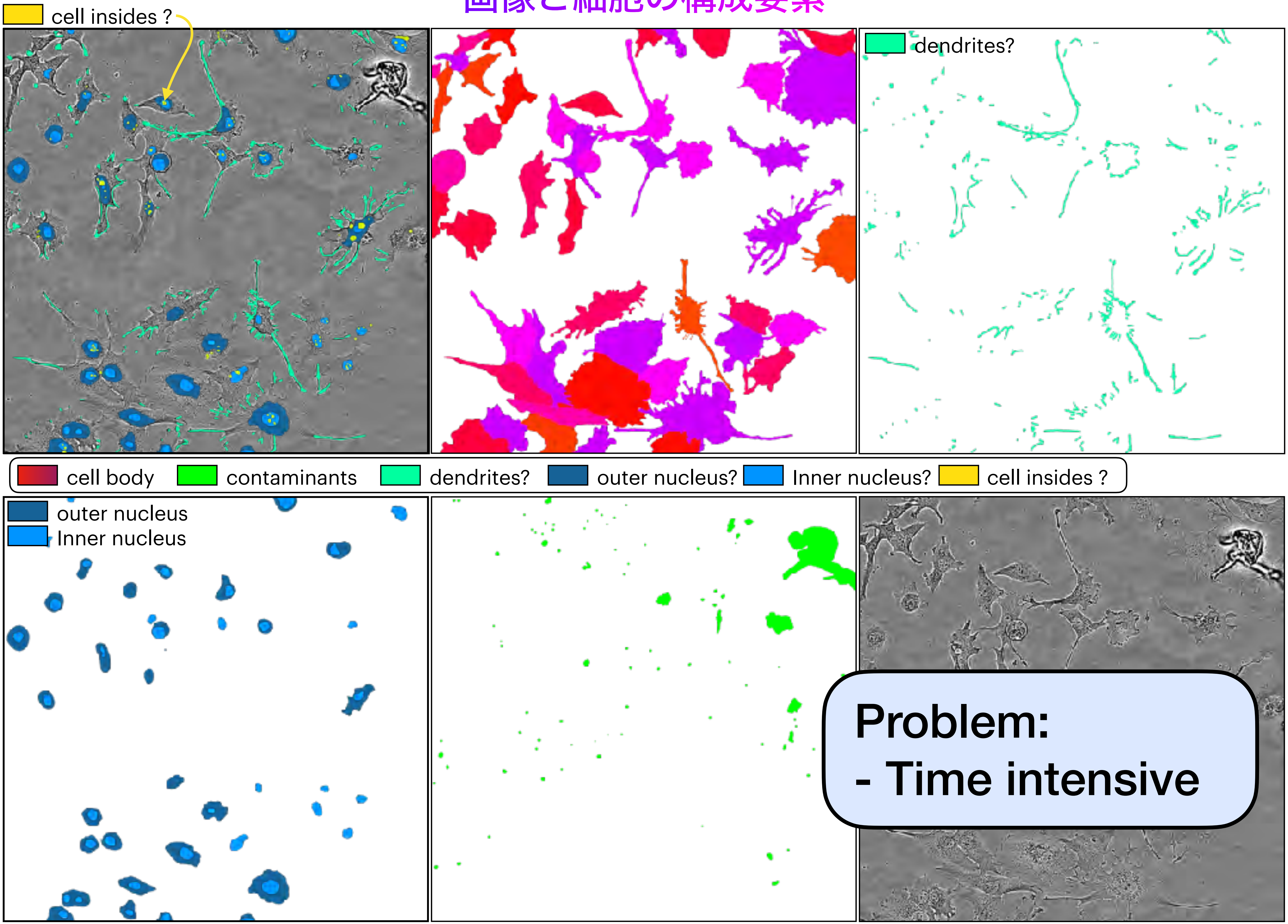




かつどう
細胞活動推定のため



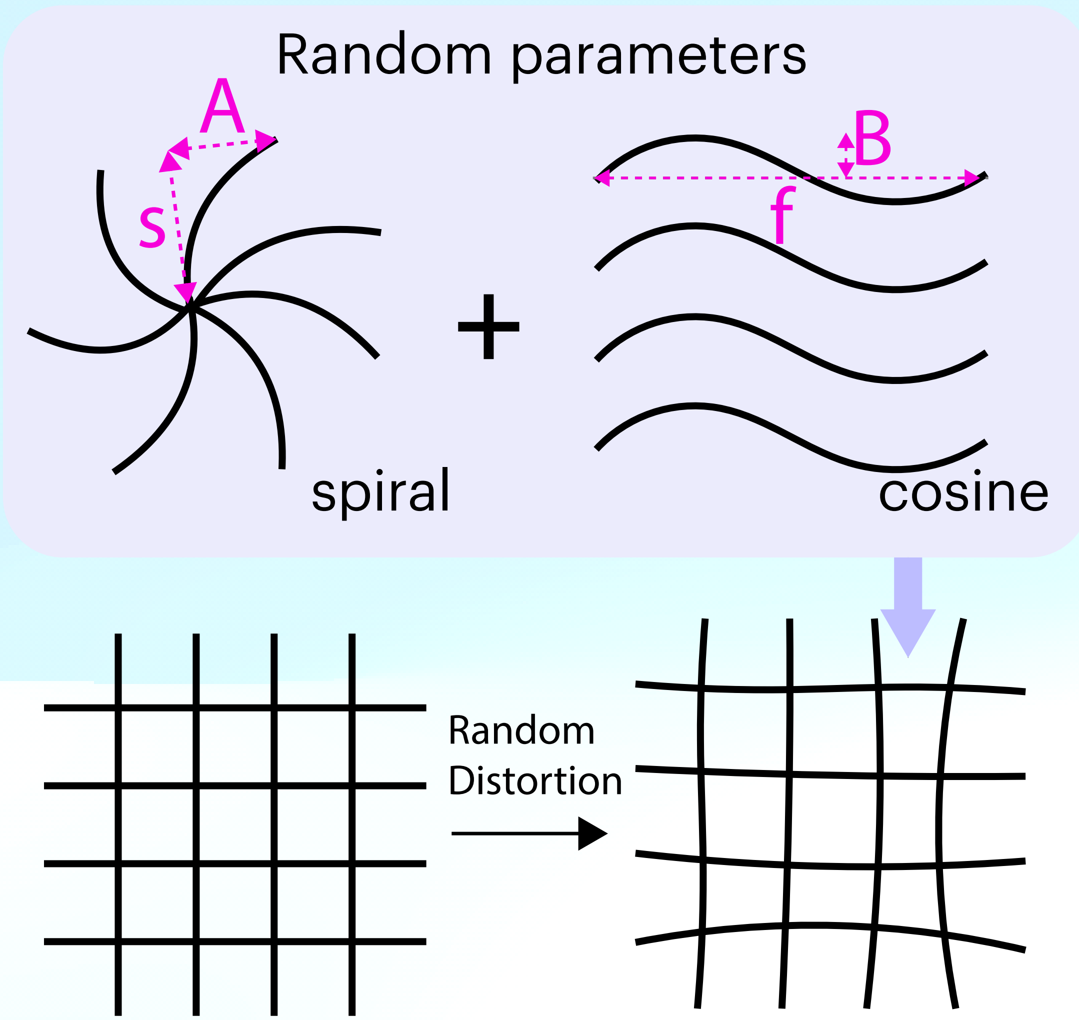
画像と細胞の構成要素



Problem:
- Time intensive

Augmentations

ランダムな幾何学(きかがく)的歪(ゆが)み



極座標系(きょくざひょうけい) : (θ, d)

Swirl distortion

$$S = \phi + A \times \exp^{-d\times(ln(2)s)^{-1}} + \theta$$

$$\theta = \arctan\left(\frac{y - y_0}{x - x_0}\right),$$

$$d = \sqrt{(y - y_0)^2 + (x - x_0)^2}$$

Wave distortion

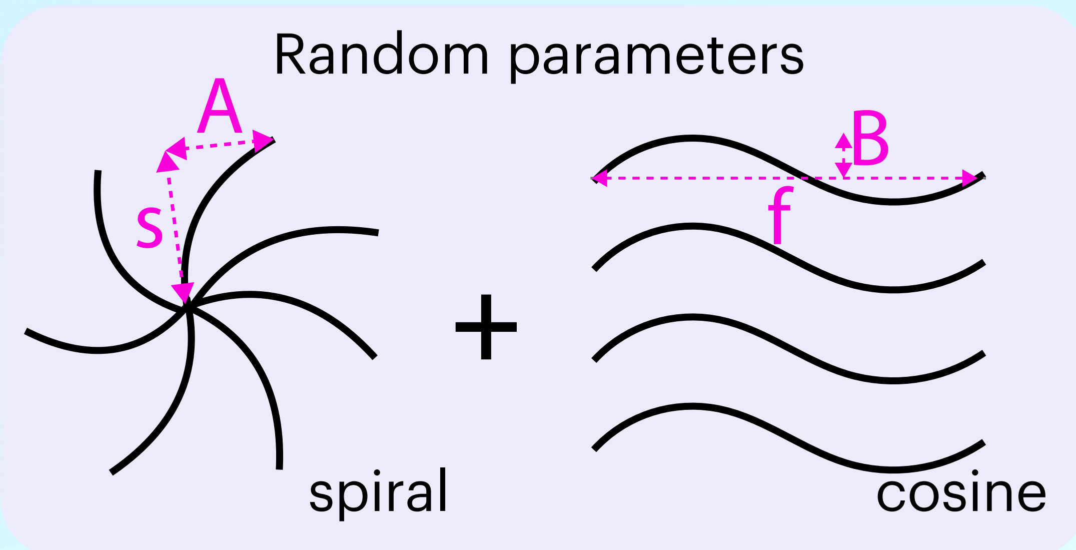
$$p'(x, y) = \begin{pmatrix} B & -\cos(f) \\ -\sin(f) & B \end{pmatrix} \times p(x, y)$$

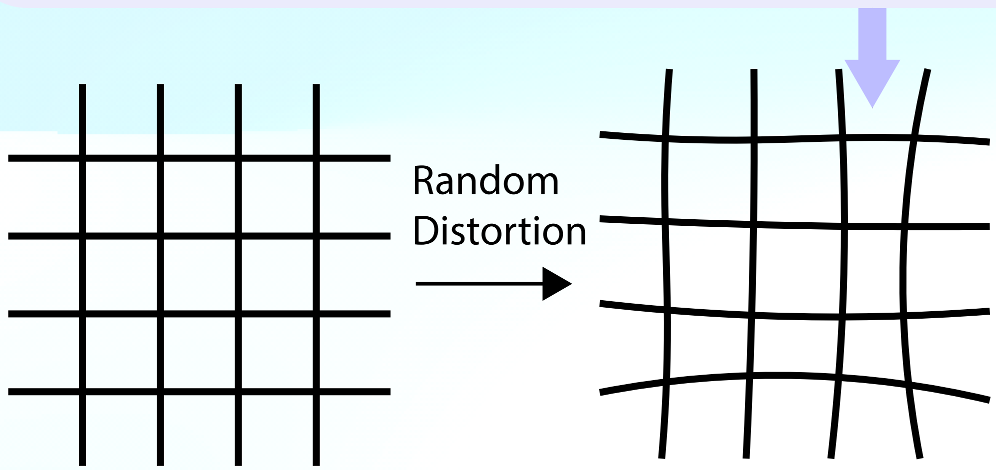
with

swirlamplitude :	$(h/4 \leq A \leq h/2) \in \mathbb{R}$
swirlextend :	$(0 \leq s \leq 0.1) \in \mathbb{R}$
waveamplitude :	$(B, 0 \leq B \leq h/10) \in \mathbb{R}$
phase :	$(0 \leq f \leq 2\pi) \in \mathbb{R}$

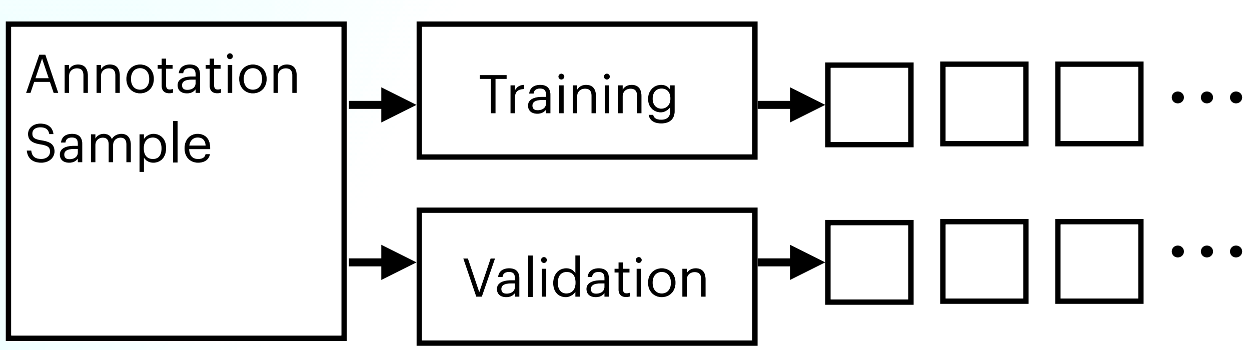
Augmentations

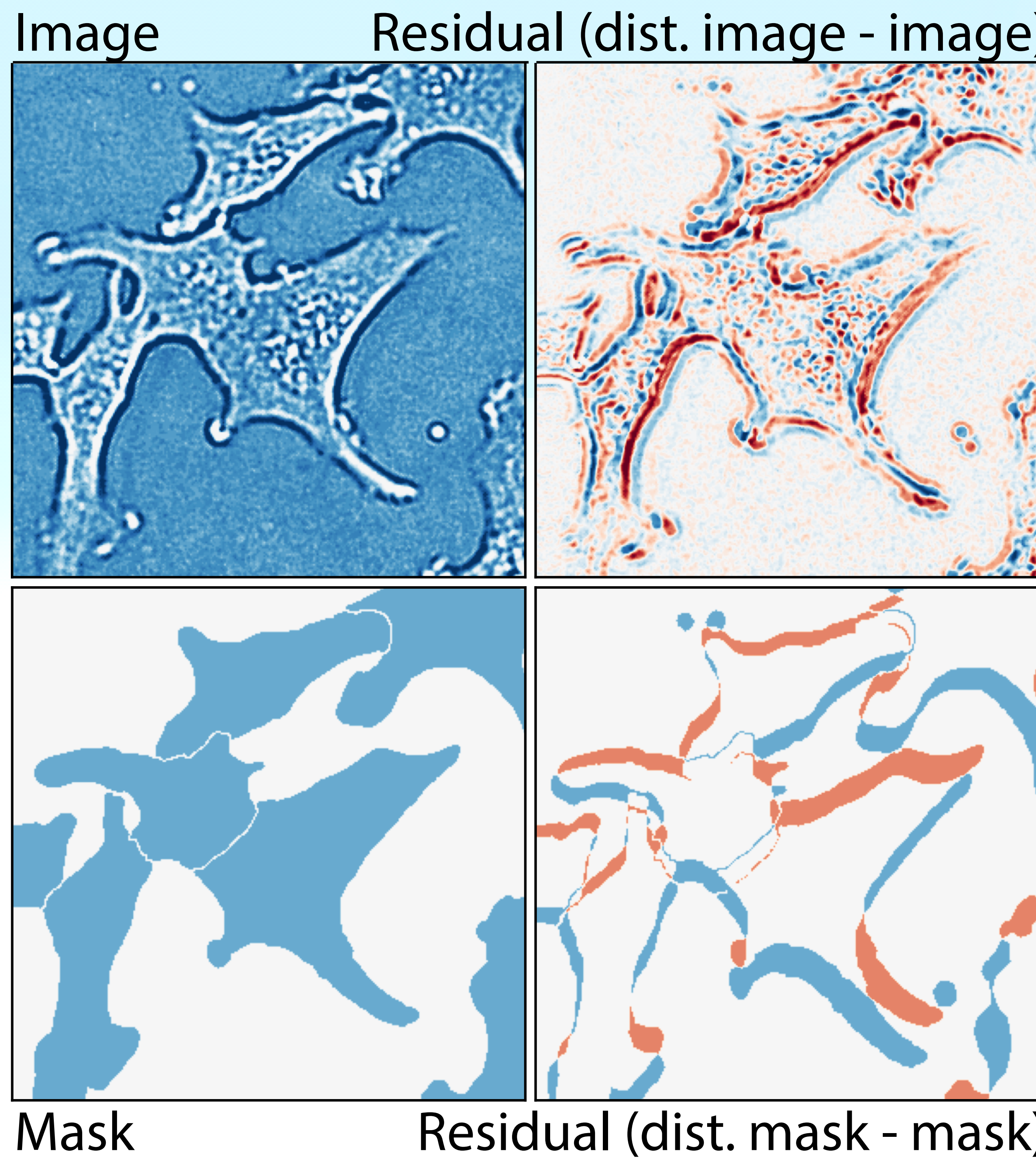
ランダムな幾何学的歪み



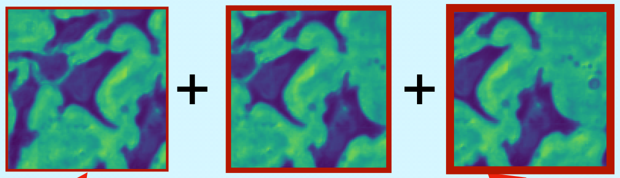


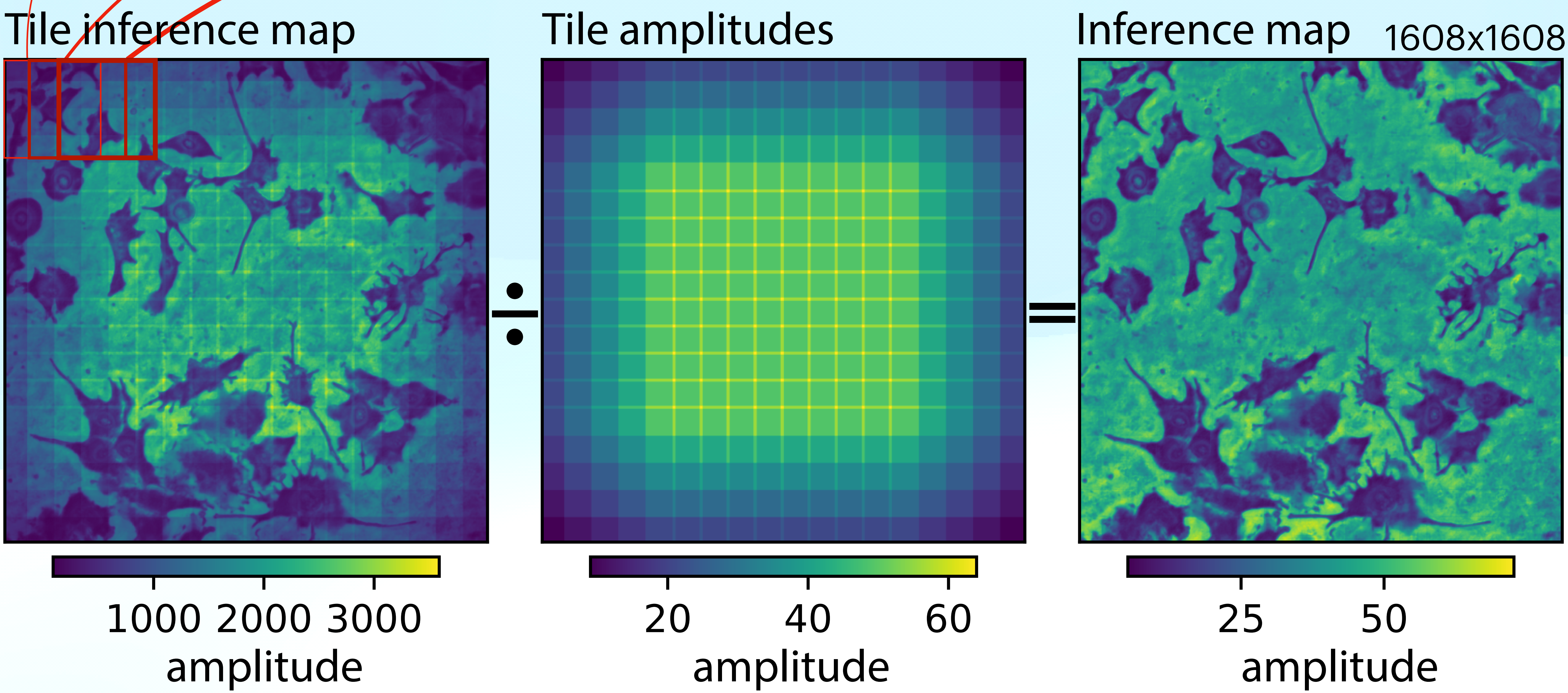
10万(320x320)training/evaluation サンプルを作る





Inference





注釈グループ多いから —> 小さな画像ウィンドウが重なり合う

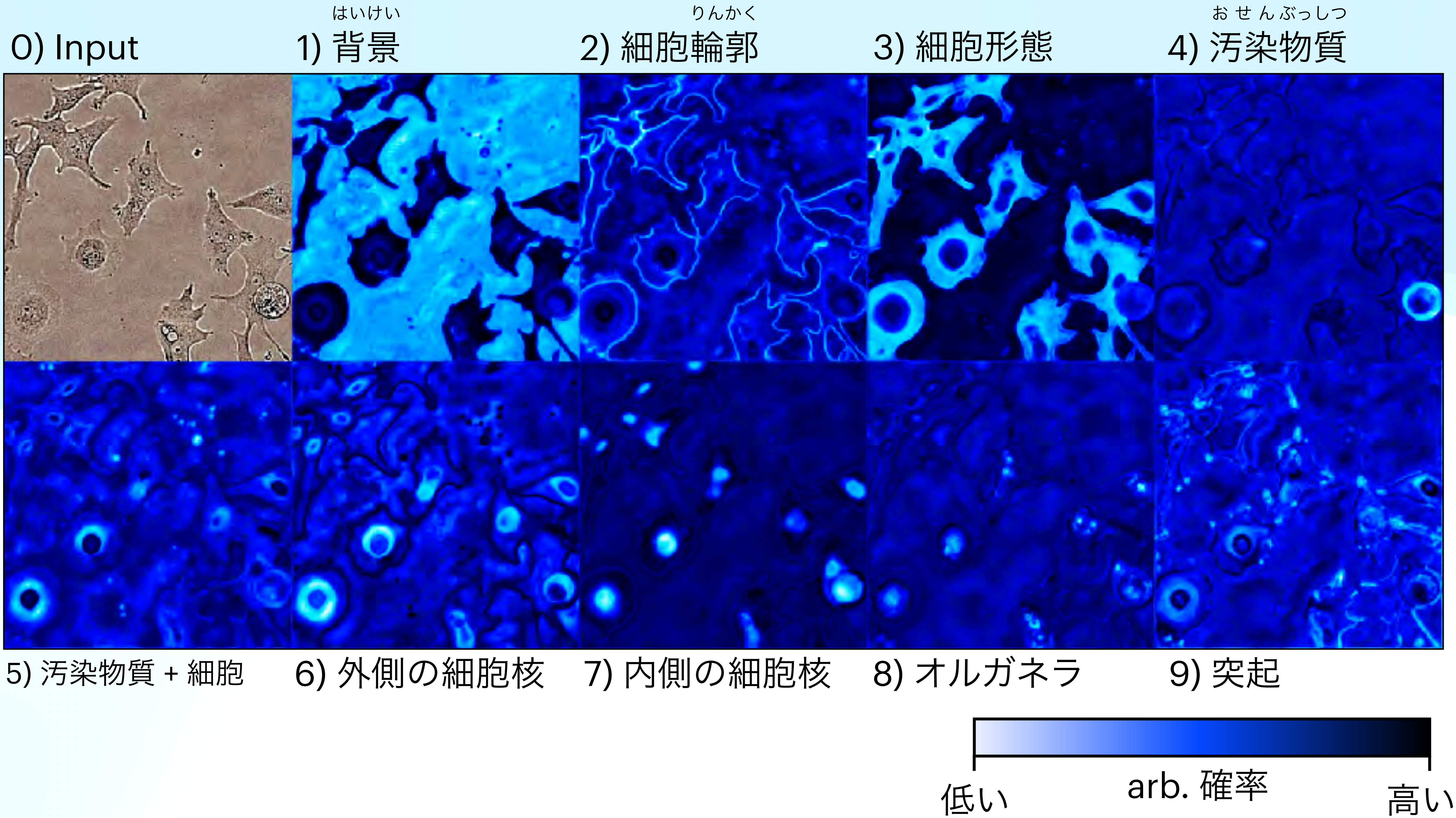
Tiled inference:

- produce overlapping windows 320x320
- Produce multiple inference maps
- 重複するinferenceマップの平均化する

- 画像エッジの信頼性（しんらいせい）を高める
- 確率の精度（せいど）を上げる

Inference results

分裂グループ確率

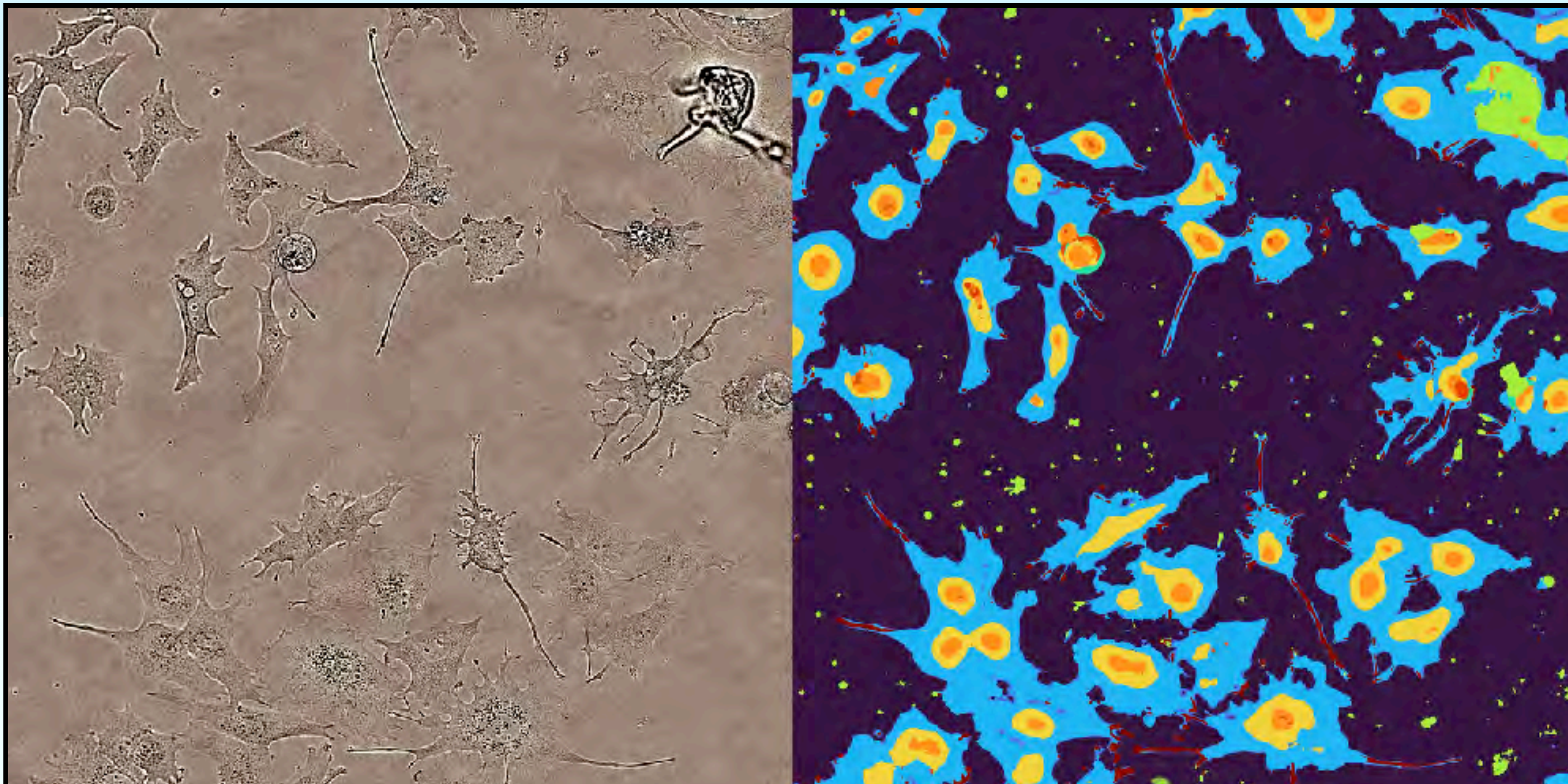


分裂結果

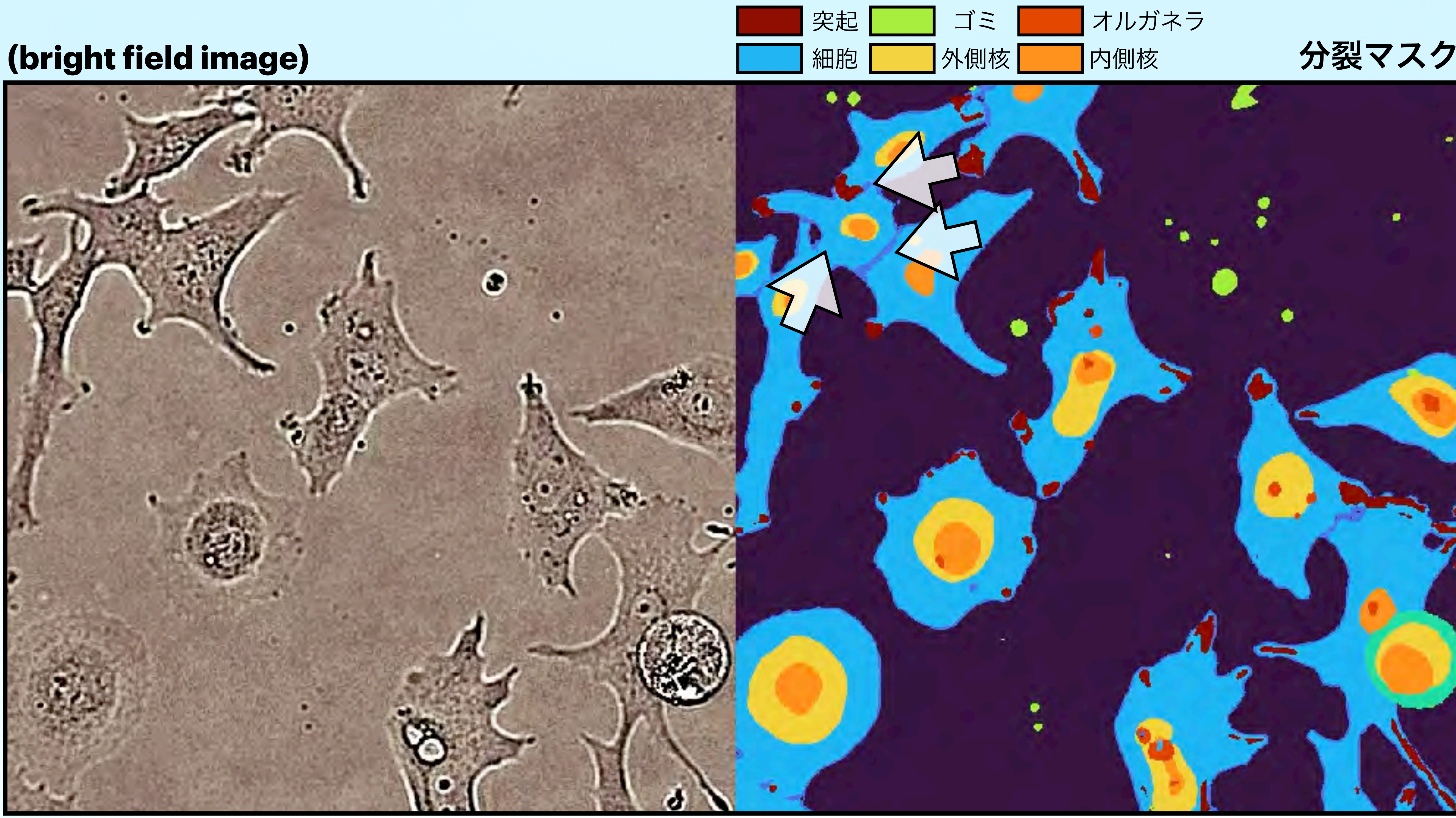
(bright field image)

突起　ゴミ　オルガネラ
細胞　外側核　内側核

分裂マスク



分裂結果



まとめ

Many established approaches focus on:

- Cell counting (Not precise morphology detection)
- Detection in wide variety of data

Costume approach for:

- **Precise cell Morphology detection (& tracking)**
- **Inner cell component detection (& tracking)**

Quality of annotations are important! (Not quantity)

Deep Learning	Rule Based
Blackbox	Manual Feature Extr.
Labelled Datasets	Limited Flexibility
Flexible	Parameters
High Accuracy	Comprehensible
Sometimes unpredictable	Robust on stable data