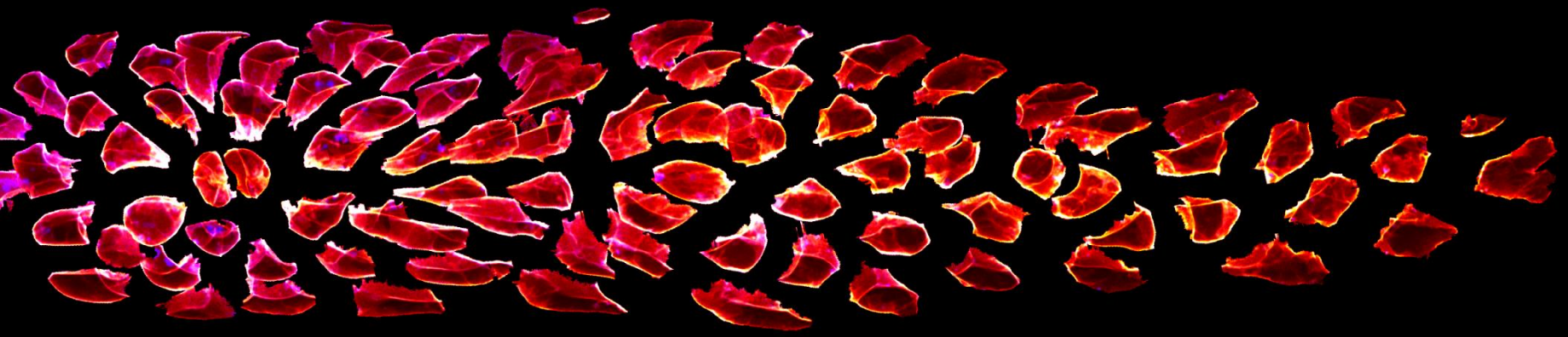
Bonus Lecture! Yay!

Jonas Hartmann



EMBL Bio-IT Course: *Image Processing with Python*

8. – 10. May 2017

EMBL Heidelberg

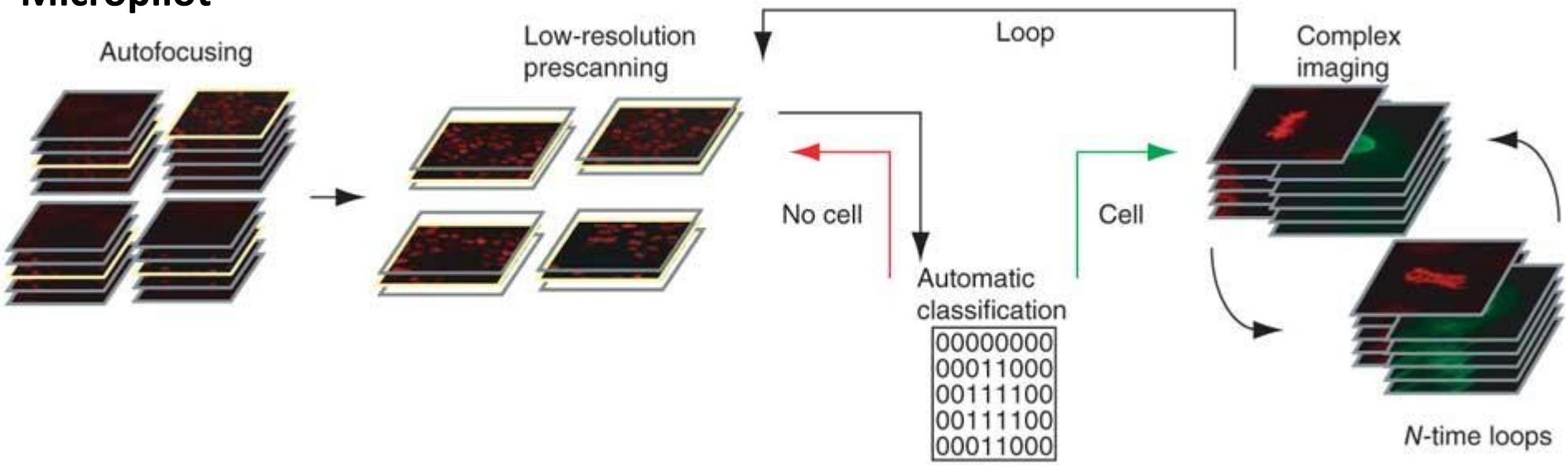
Agenda

- Smart microscopy
- Tracking objects
- Machine learning: pixel prediction
- Single-cell data analysis
- Machine learning: deep learning

Smart Microscopy

- Direct coupling of microscopy and image analysis
- Example 1: Region of interest auto-detection
  - Identify object of interest in low-res high-FOV image
  - Instruct microscope to image object of interest in high-res

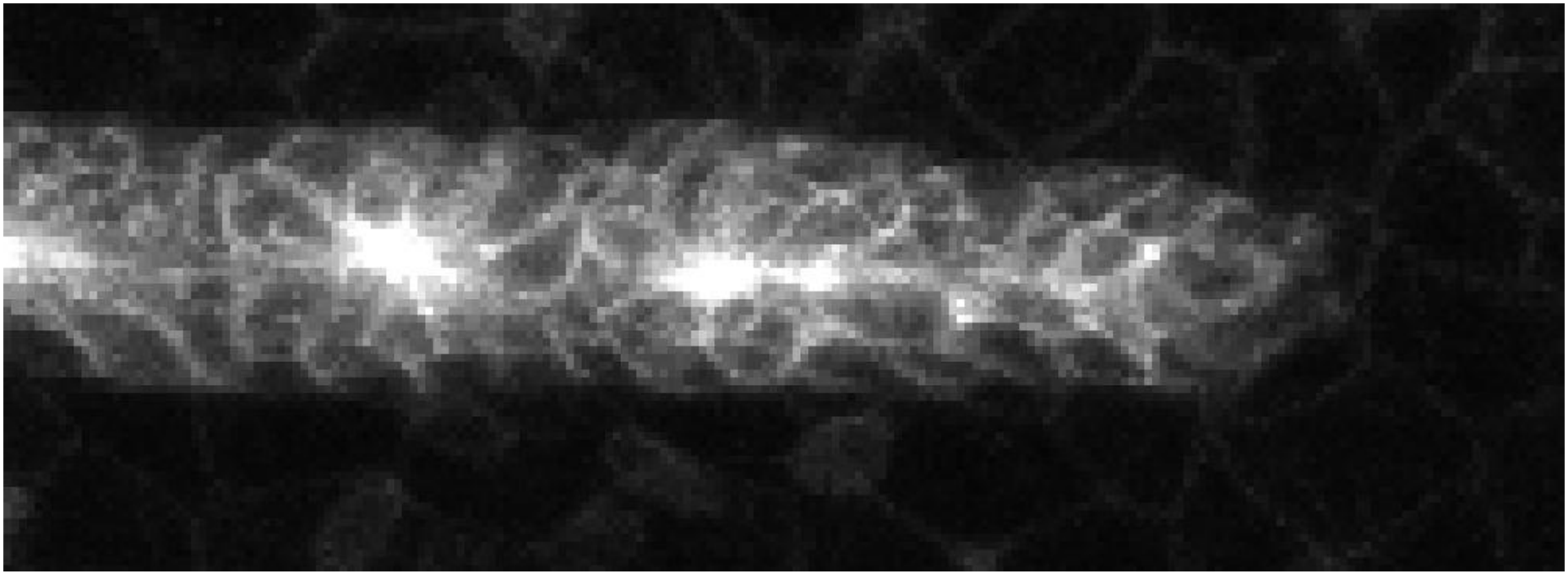
Micropilot



Published 2011 in Nature Methods by Ellenberg & Pepperkok groups

Smart Microscopy

- Direct coupling of microscopy and image analysis
- Example 2: Live tracking
  - Identify leading edge of migrating cells in low-res high-speed image
  - Instruct microscope to follow the leading edge to take high-res slow images



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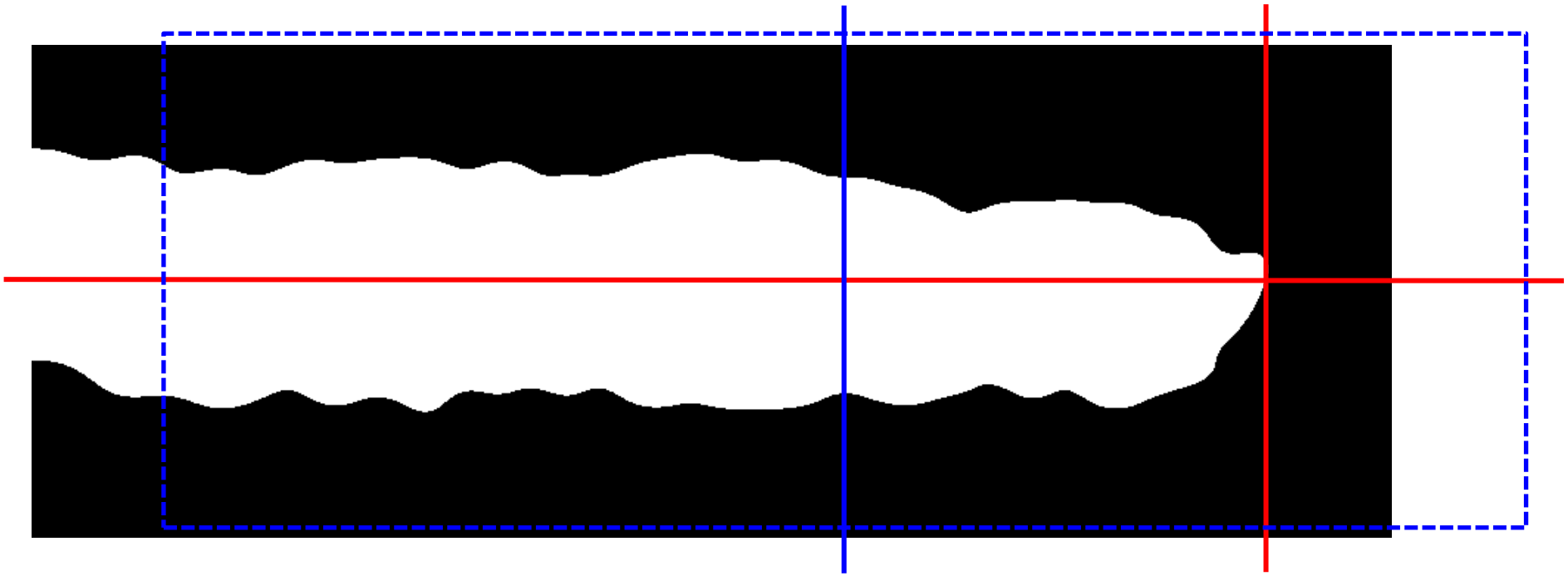

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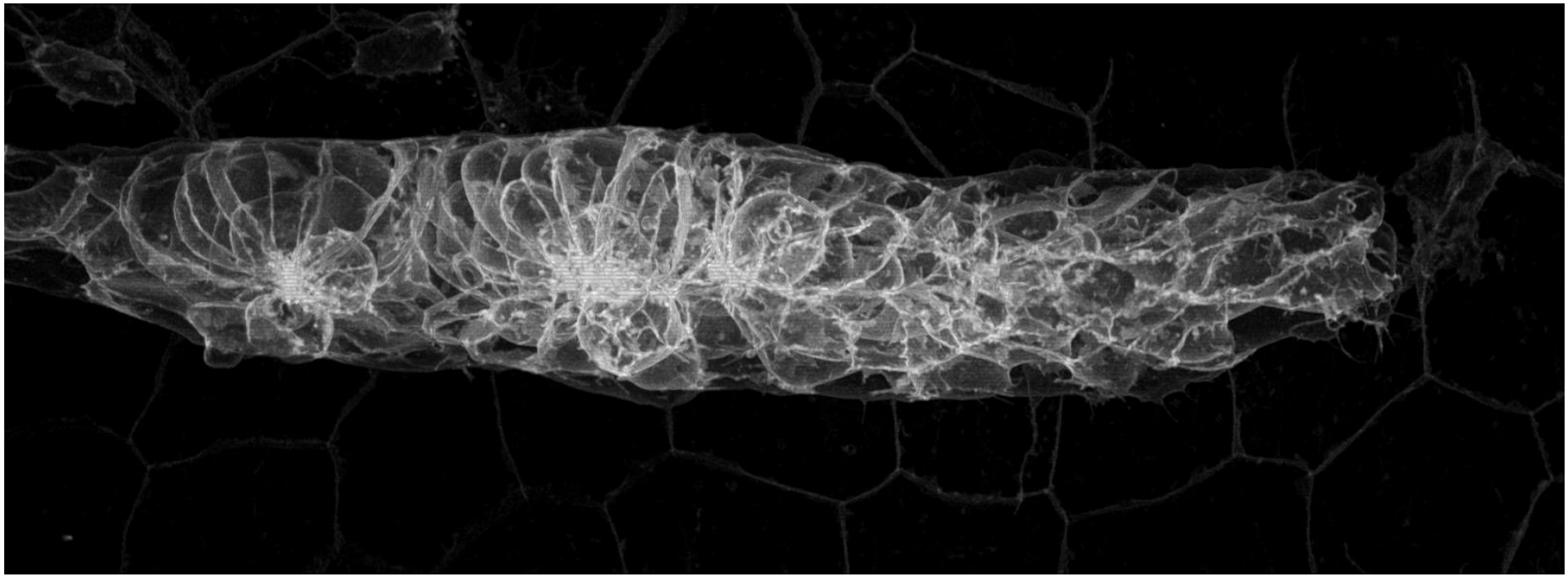

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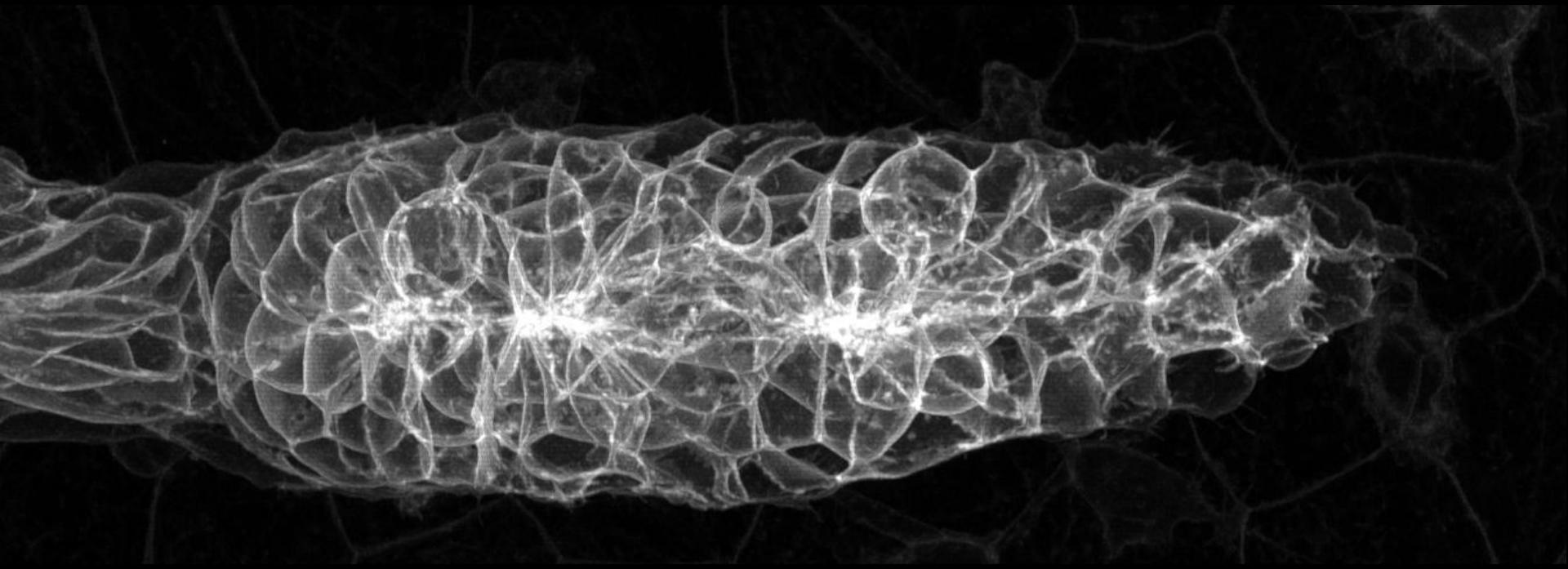
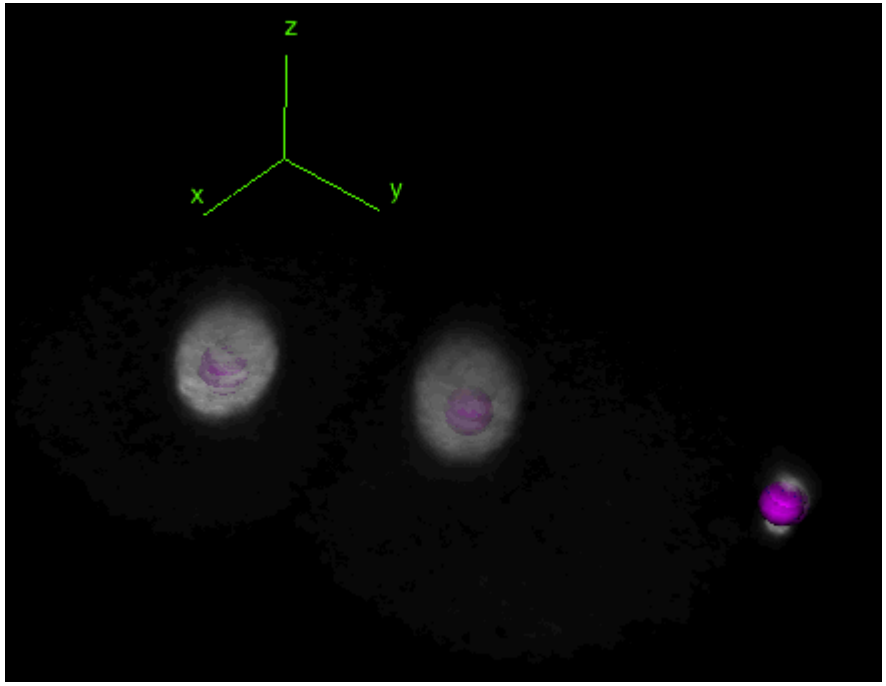
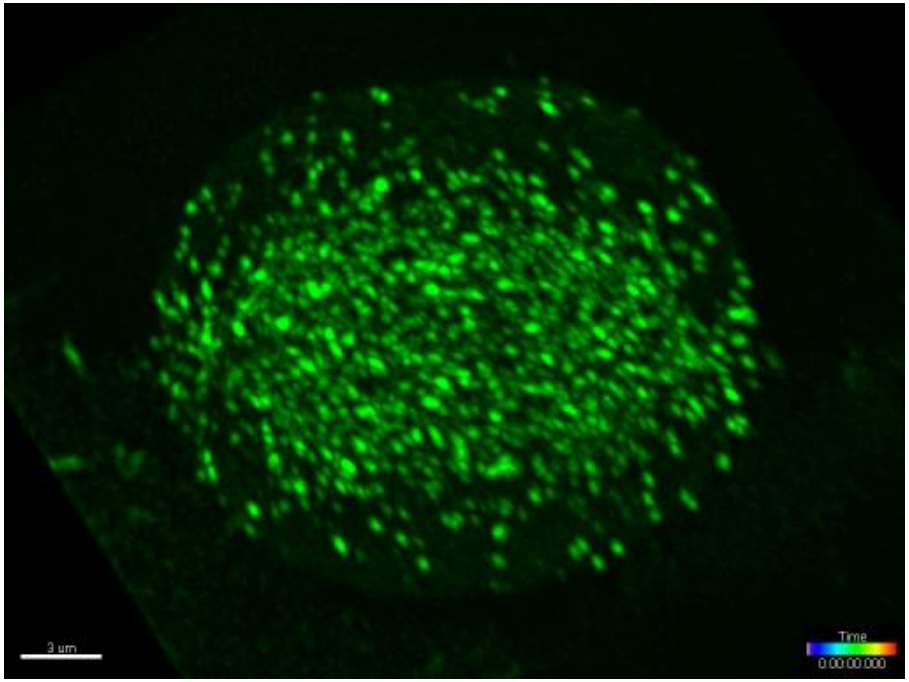


Tracking

- **Goal: assign each labeled object at time *t0* a corresponding object at *t1***
  - **Approach: segment independently, then link objects (by optimization)**
  - **Linking based on: space, feature space, object physics**
  - **Easier for small *δt***
  - **Challenges: poor segmentation, overlapping objects, dividing/merging objects**



From Fiji's TrackMate plugin

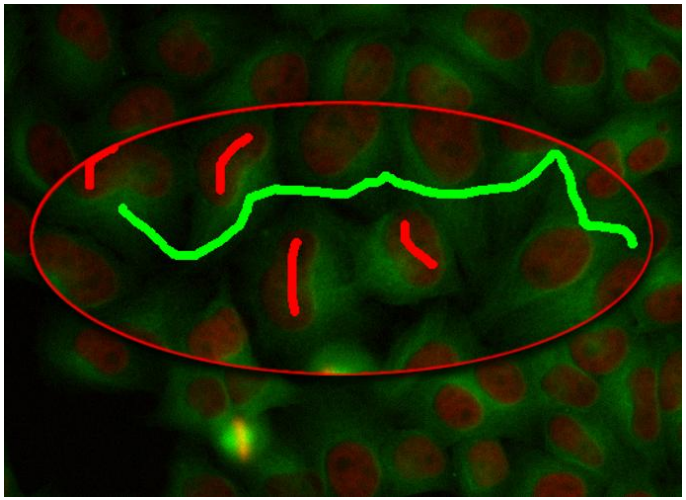


From Eric Betzig's group

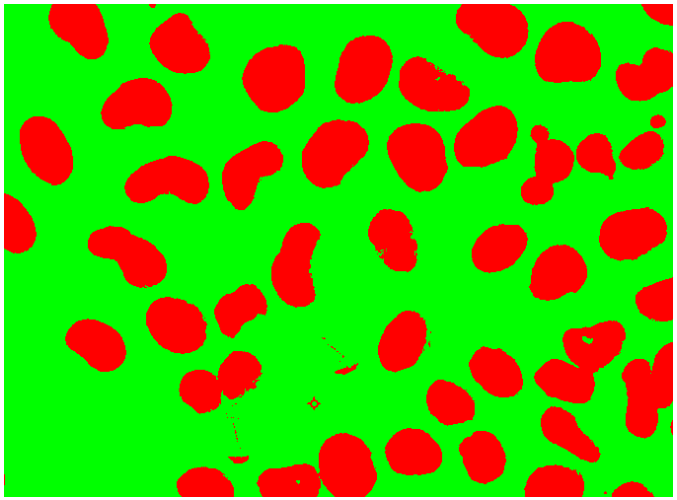
Machine Learning: Pixel Prediction

- **Goal: foreground-background detection by machine learning**
  - **This is a classification task: classify pixels into groups**
  - **Approach: supervised learning**
    - **Manually label example pixels**
    - **Extract features (raw intensity, filtered intensity, neighborhood)**
    - **Train classifier (e.g. random forest)**
    - **Make prediction for all other pixels**

Ilastik



From the Ilastik website



Outcome quality differs...

Also useful as `preprocessing` (use probabilities)!

Single-Cell Data Analysis

- **What to do with single cell data?**
  - **Correlate and quantify features at a per-cell level**
  - **Explore cell-cell variability (dimensionality reduction)**
  - **Identify groups, subgroups (clustering, classification)**
  - **Identify relationships, trajectories, population structures (graphs)**
  - **Infer models predicting cell variability and behavior (machine learning)**

Single-Cell Data Analysis

- **What to do with single cell data?**

from segmentation

Feature Extraction

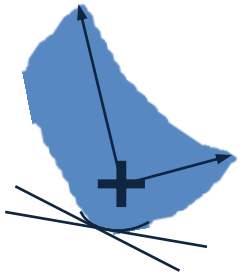
Standardization

Dimensionality Reduction

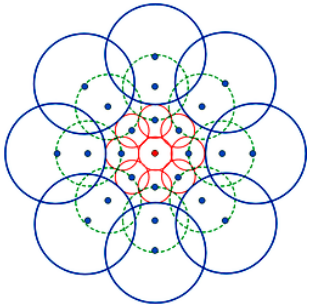
Clustering, etc…

to interpretation

Measures



Descriptors



Features

Single cells	Shape	Intensity	Neighborhood			
	$a_{1,1}$	$a_{1,2}$	…	…	…	$a_{1,n}$
	$a_{2,1}$	$a_{2,2}$	…	…	…	$a_{2,n}$
	…	…	…	…	…	…
	…	…	…	$a_{i,j}$	…	…
	…	…	…	…	…	…
	$a_{m,1}$	$a_{m,2}$	…	…	…	$a_{m,n}$

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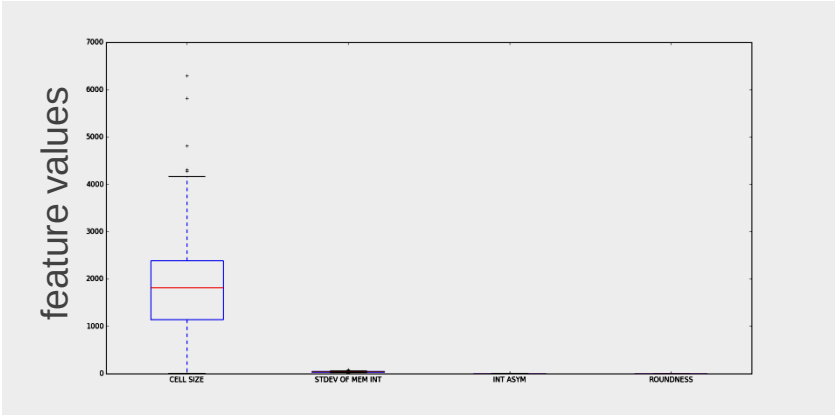
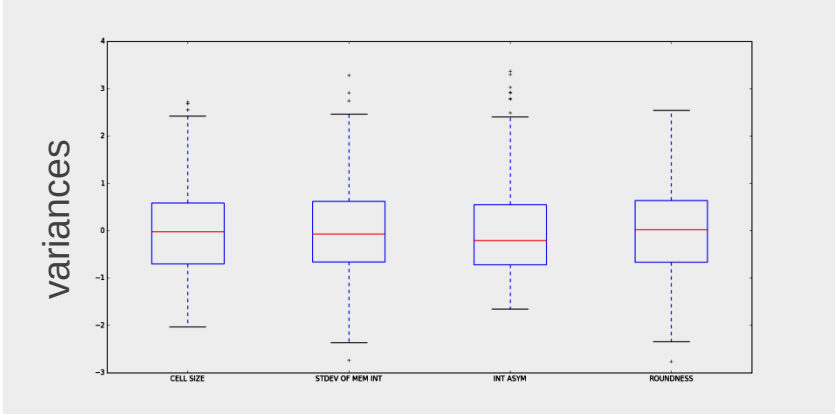


$$X' = \frac{X - \bar{X}}{\sigma}$$



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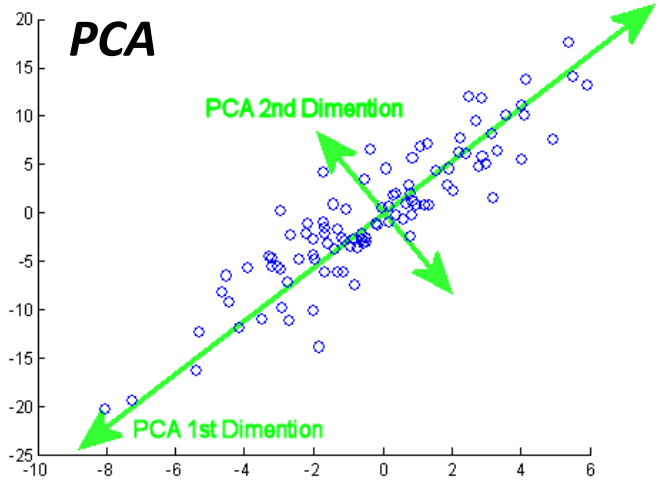

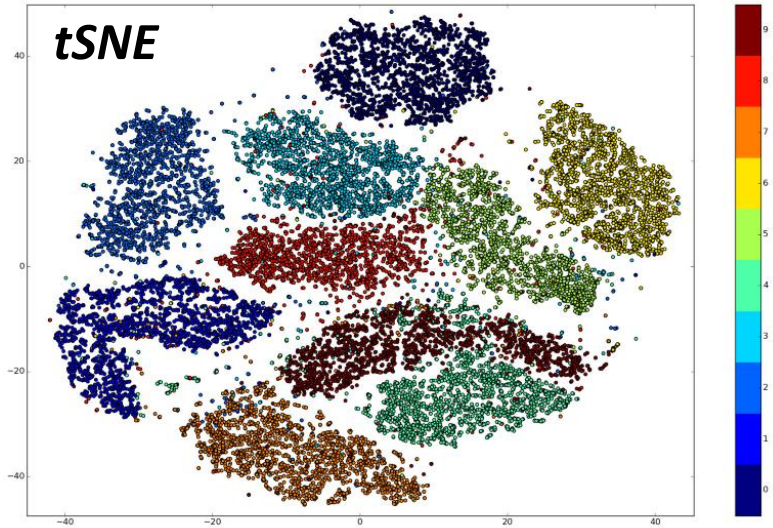

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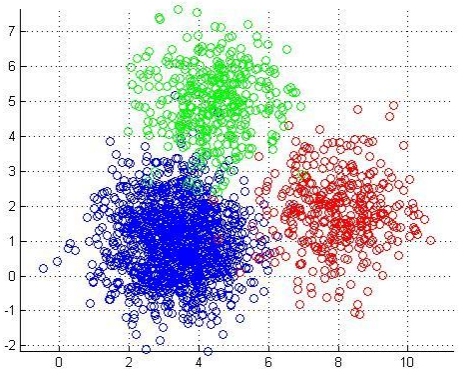
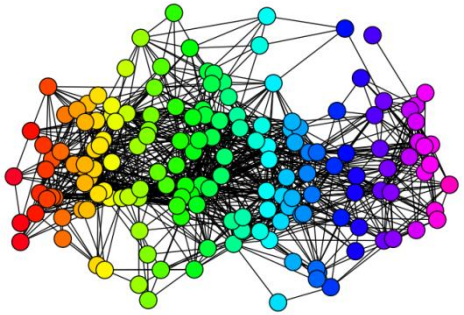


Clustering (k-means)

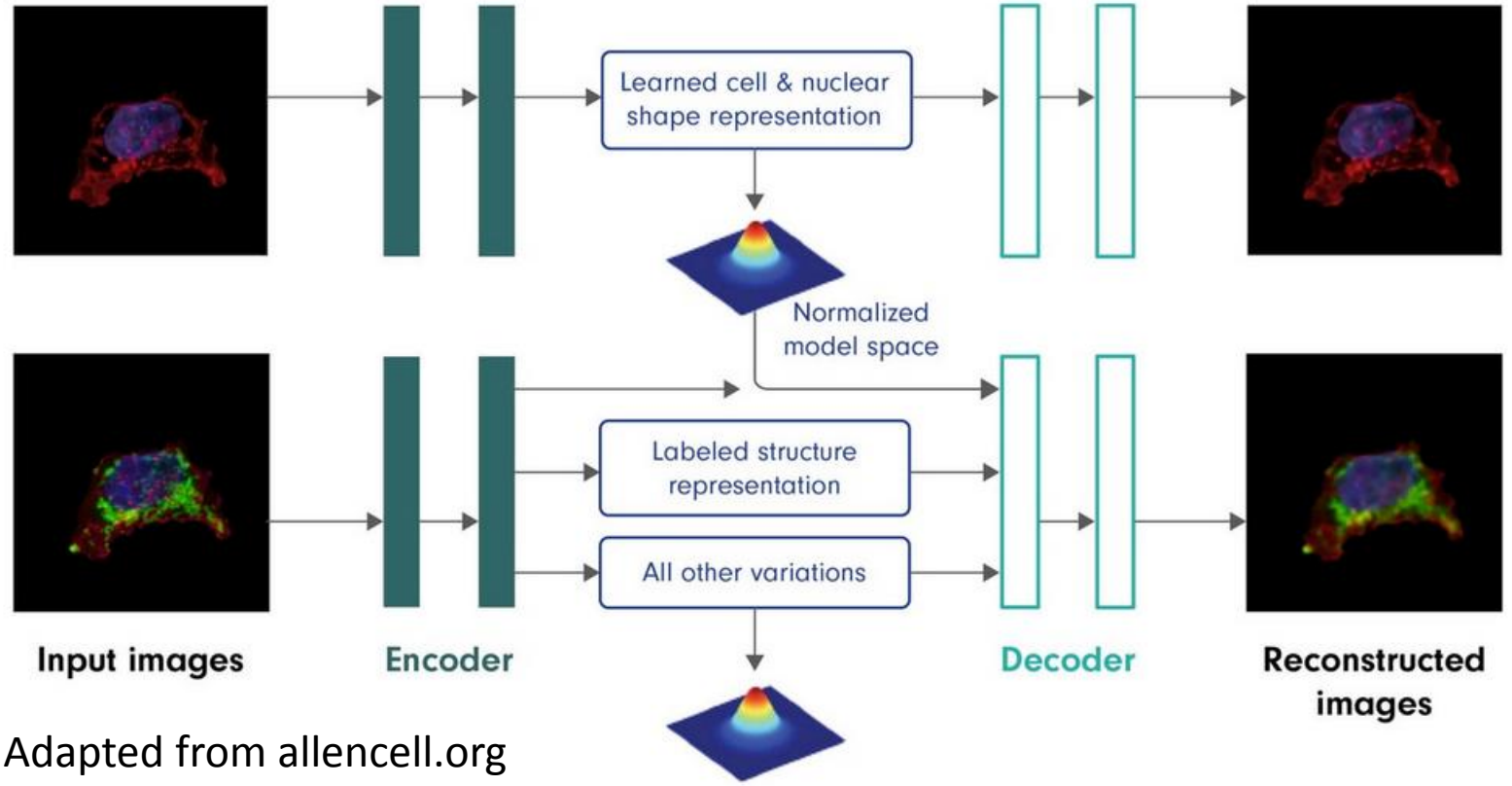


Graph (similarity)

Deep Learning

- `Deep Learning` skips feature extraction (by also learning that)
- Some very recent interesting stuff: www.allencell.org

Autoencoder (deep neural network)



Adapted from allencell.org

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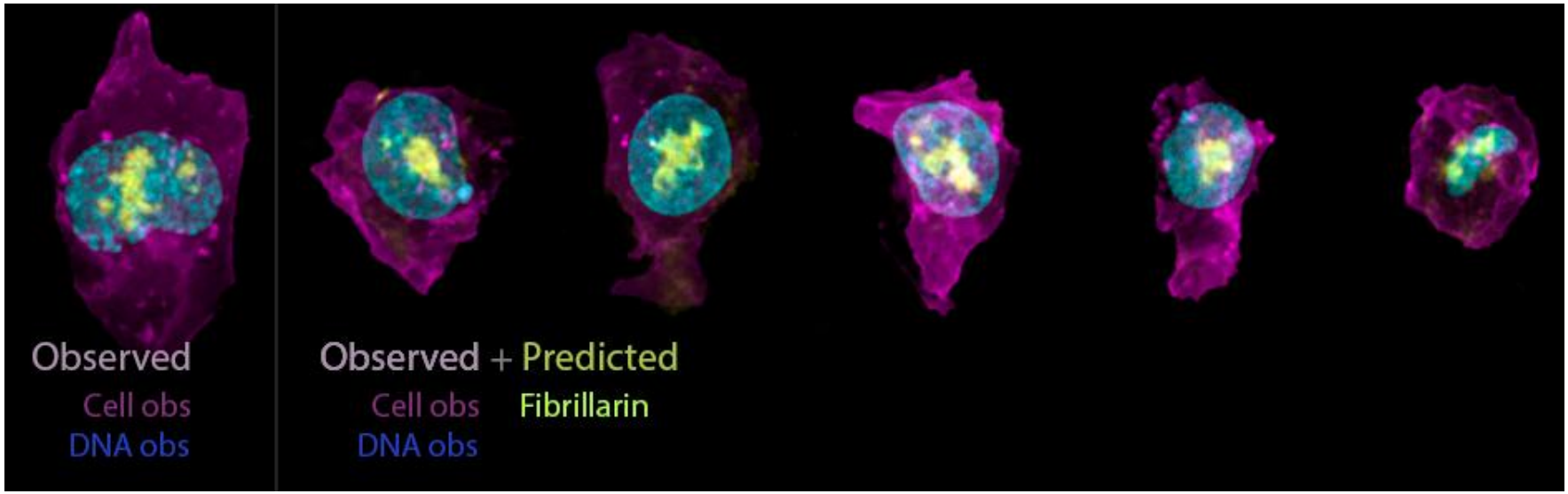

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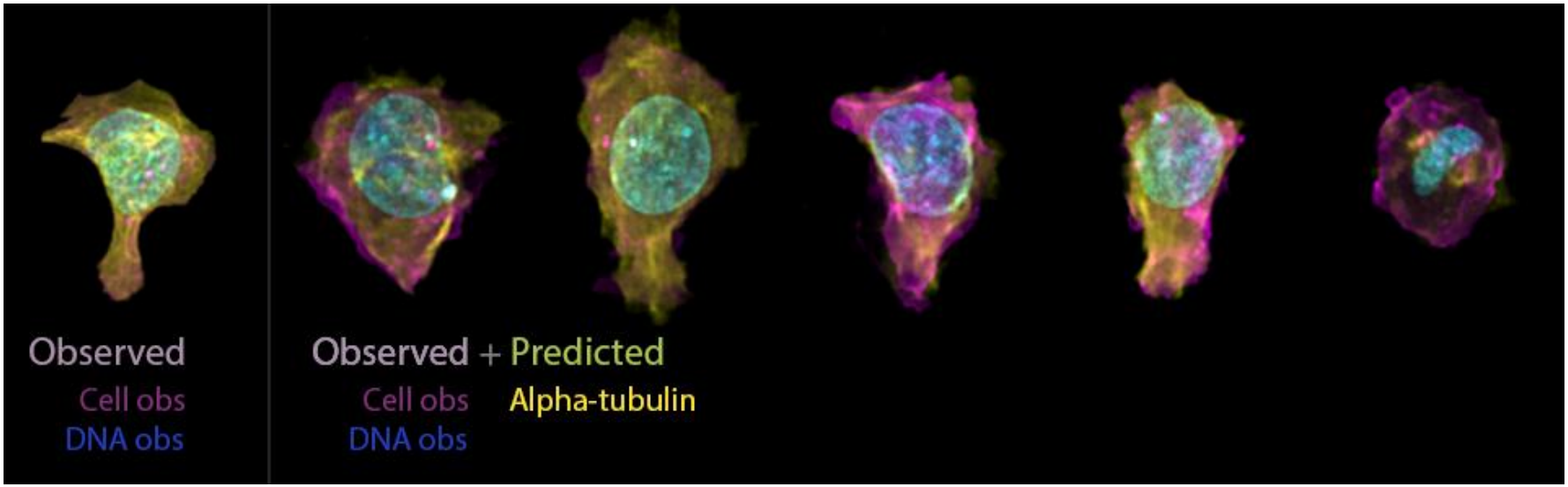

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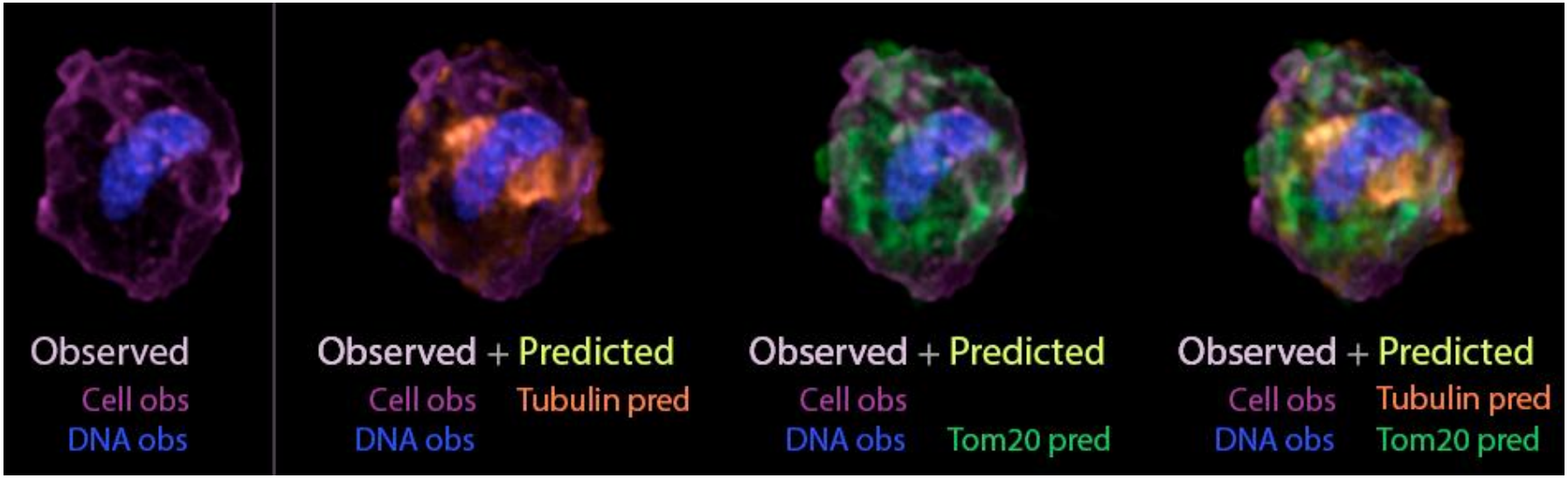

Adapted from allencell.org

- Advantages: really cool, allows construction of ‘atlases’, …
- Disadvantages: encoded model is a ‘black box’

That‘s all!