

# Intelligent Analysis of Biomedical Images

Presenter: Mohammad H. Rohban, Ph.D.

Fall 2023

Courtesy: Slides are adopted from the Beth Cimini's presentation (March 27, 2020)

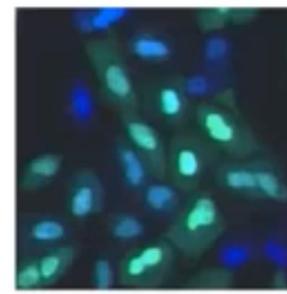
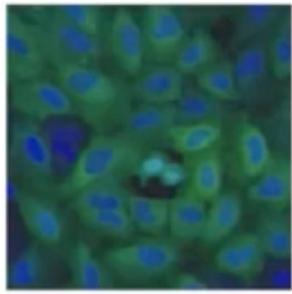
# Images contain a wealth of information



**complex model systems  
single-cell resolution  
quantitative  
multiplexed**

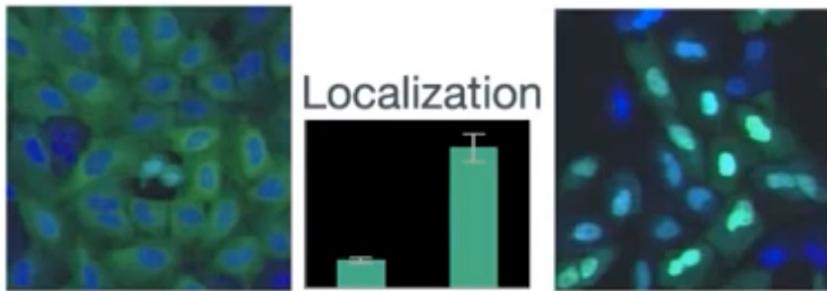
# Visual appearance can be quantified

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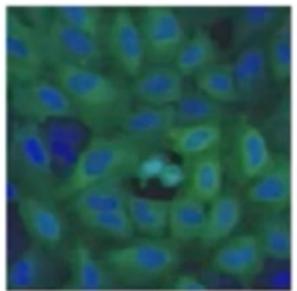
# Visual appearance can be quantified

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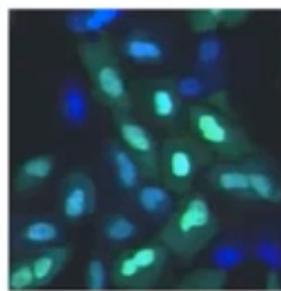
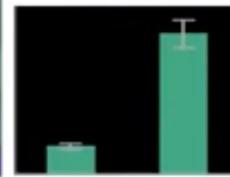


# Visual appearance can be quantified

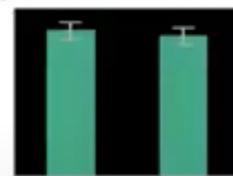
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Localization

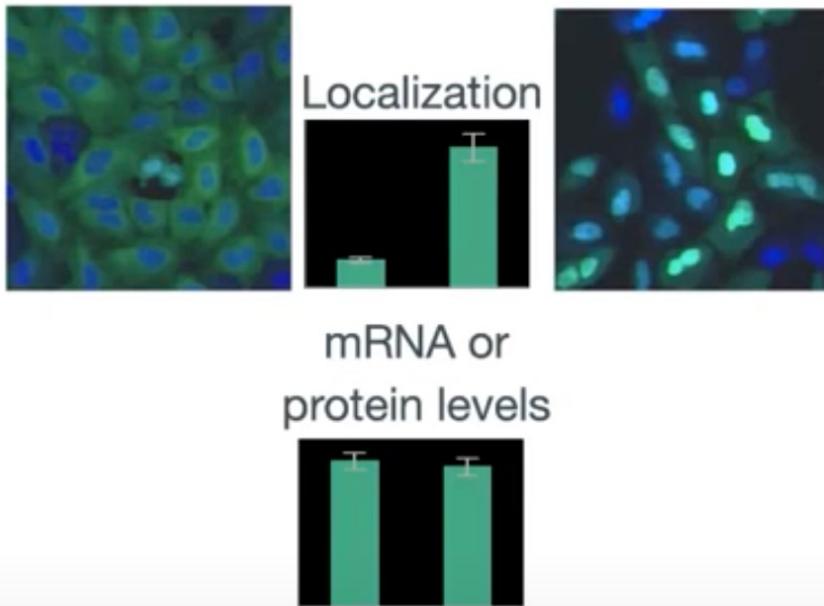


mRNA or  
protein levels



# Visual appearance can be quantified

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- Automatic image analysis is
  - Objective
  - Quantitative, with statistics
  - Can measure multiple properties at once
  - Can quantify heterogeneity (single-cell measurements)
  - Distinguishes subtle changes, even those undetectable by eye
  - Faster, less tedious

# Human brains are not built for quantification

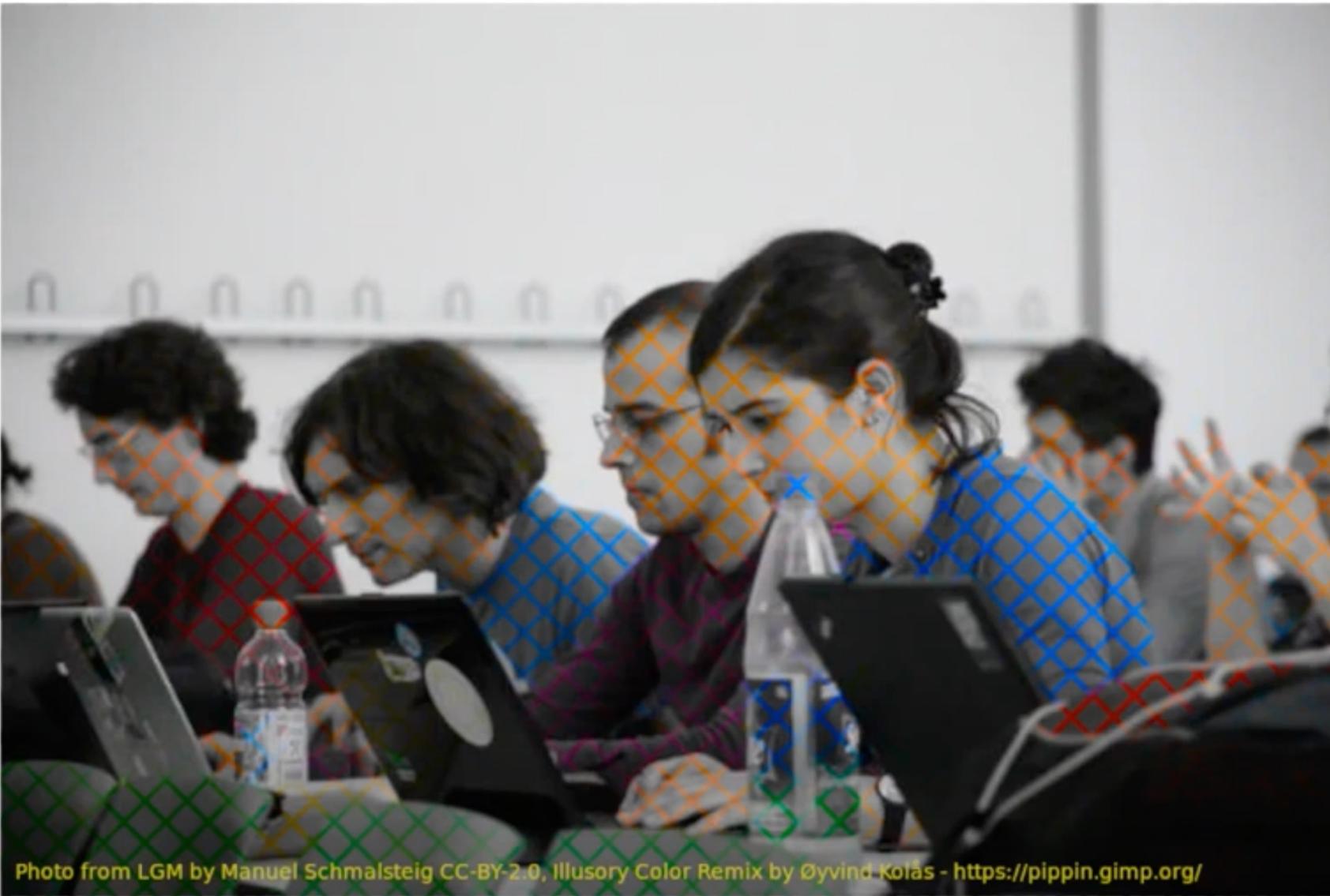
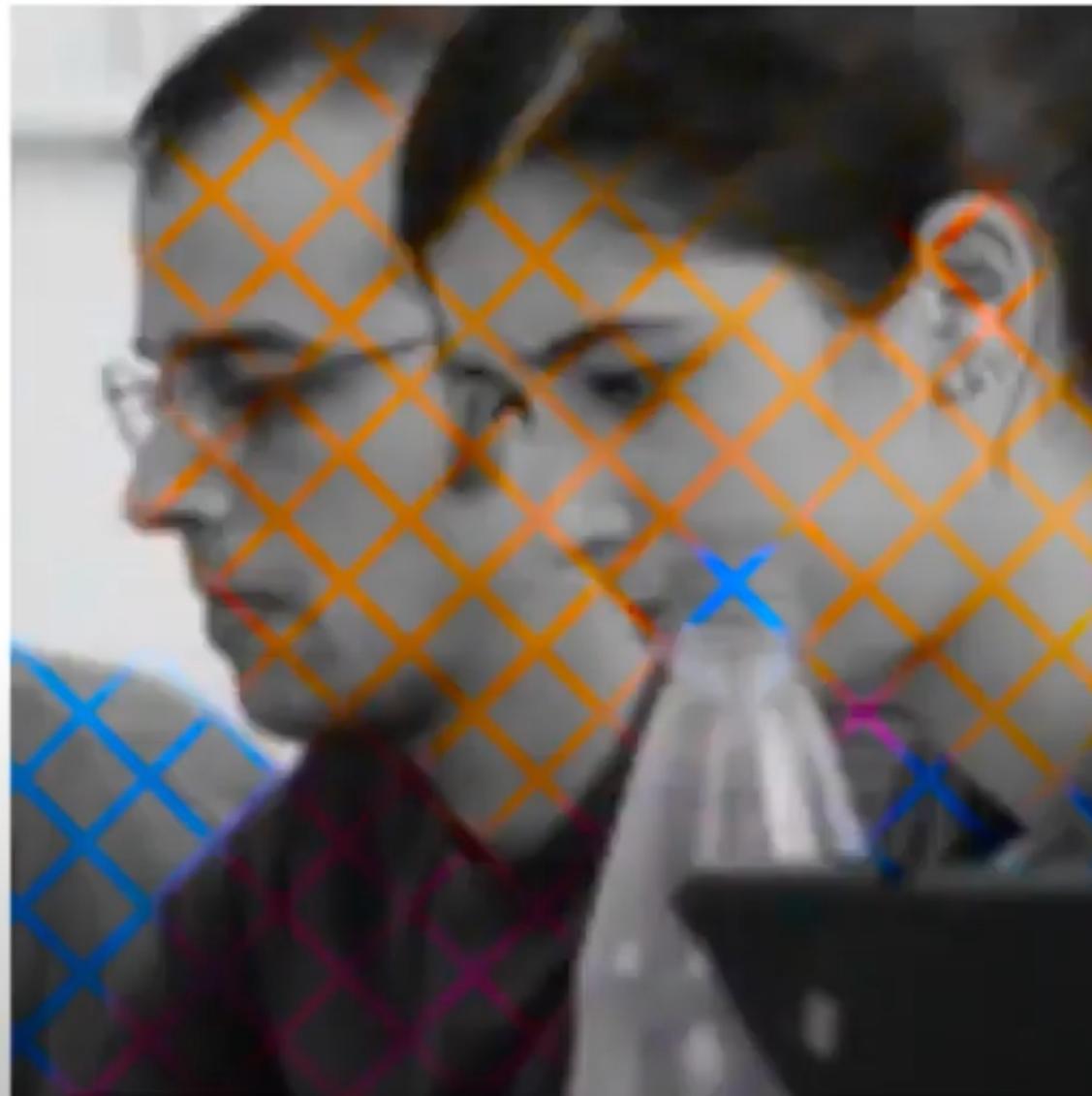


Photo from LGM by Manuel Schmalsteig CC-BY-2.0, Illusory Color Remix by Øyvind Kolås - <https://pippin.gimp.org/>

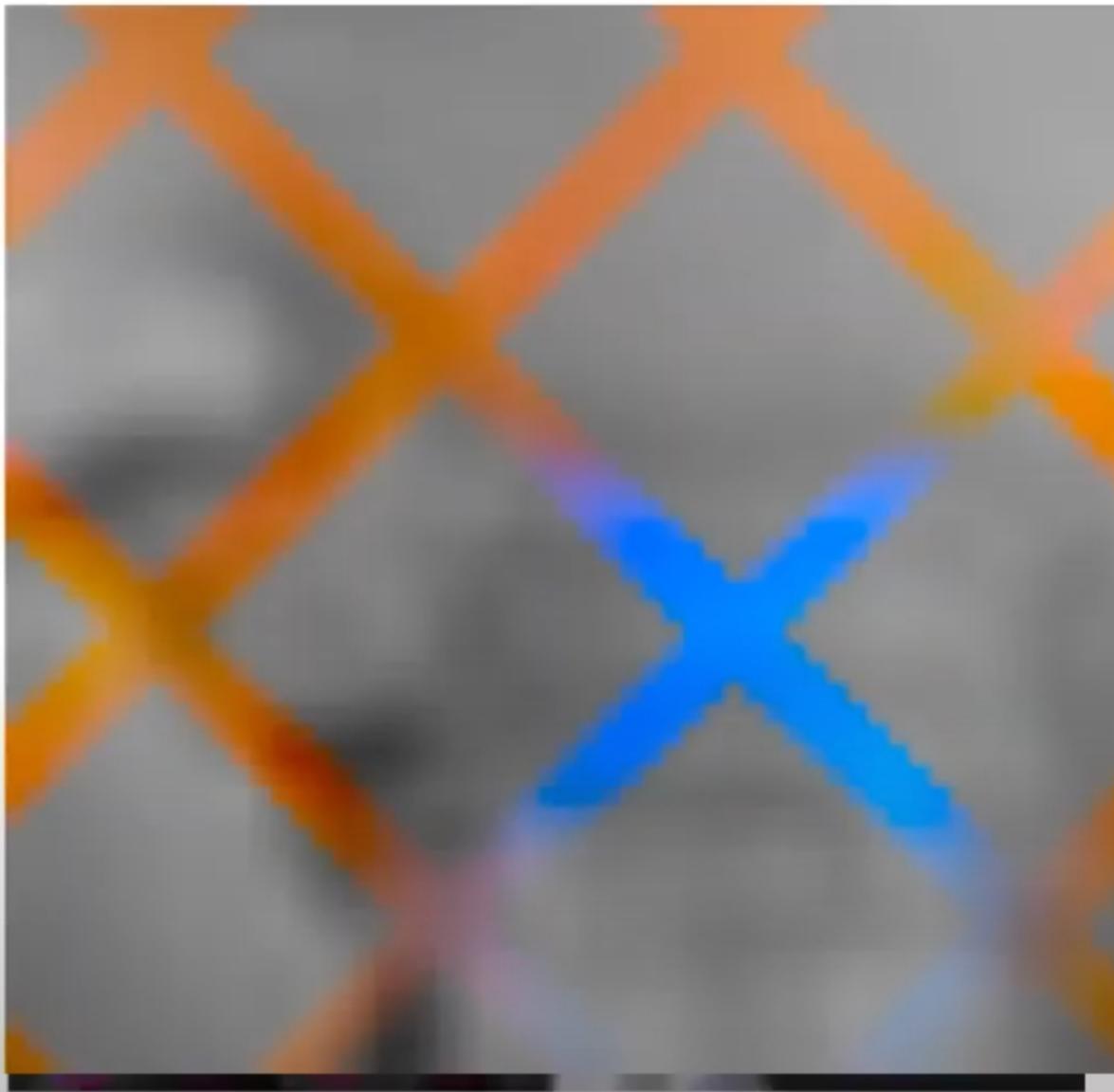
Øyvind Kolås

# Human brains are not built for quantification



Øyvind Kolås

# Human brains are not built for quantification



Øyvind Kolås

# Tools for quantitative image analysis- ImageJ

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Image Processing and Analysis in Java

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Fiji is an image processing package—a "batteries-included" distribution of [ImageJ](#), bundling a lot of plugins which facilitate scientific image analysis.

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**Advantages-** very fast image processing, lots of plugins for measurement, image processing, even gel analysis

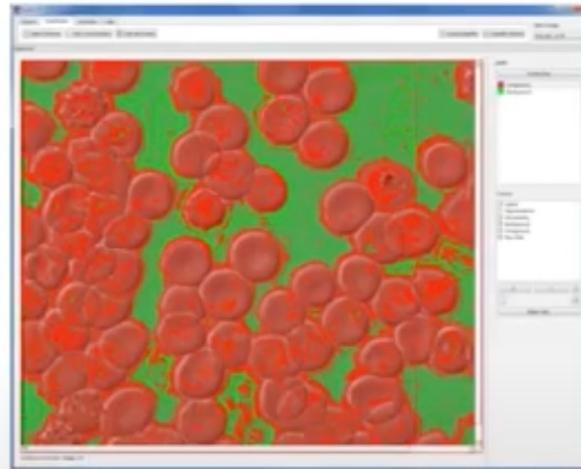
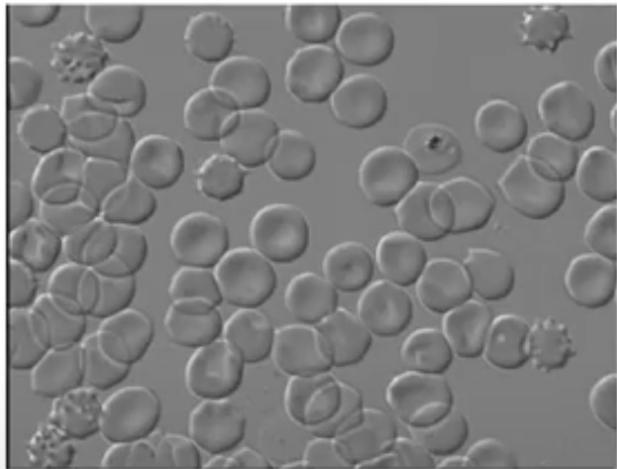
**Disadvantages-** need to be able to write scripts and/or macros in order to apply the same analysis to many images

# Tools for quantitative image analysis- ilastik



ilastik

the interactive learning and segmentation  
toolkit



**Advantages-** easy to use, can identify foreground from background in brightfield, tissue, or other difficult types of images

**Disadvantages-** can really only tell you where your objects of interest are, not much about their properties

# Software overview

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**CellProfiler™**  
cell image analysis software

**Image analysis &  
quantification**



**CellProfiler Analyst**

Interactive data exploration, analysis, and classification of large  
biological image sets

**Image-centric  
data analysis &  
machine learning**

- Available from [www.cellprofiler.org](http://www.cellprofiler.org) for Windows, Mac and Linux

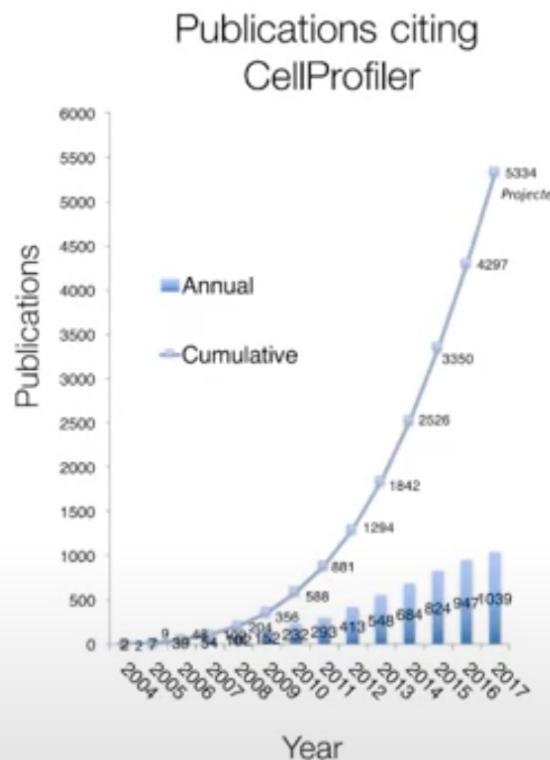
# CellProfiler

## Open-source software for image analysis



CellProfiler™  
cell image analysis software

- Free and open-source; Windows, Mac, Linux
- Cited in **1,000+** papers per year
- Used in **7/10** top pharma companies
- In the **Top 10** most popular papers in Genome Biology
- Ranked **most flexible** and **usable** in independent analysis (*Wiesmann et al.*)



# Object identification

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- Once the images are loaded, how do you find objects of interest?

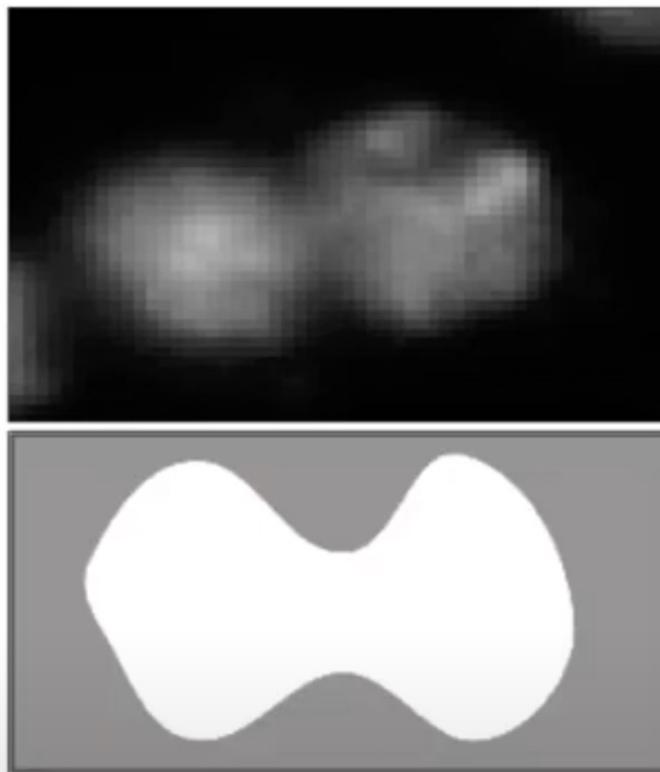


# Object identification

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- Once the images are loaded, how do you find objects of interest?

**Step 1:** Distinguish the foreground from the background (thresholding)

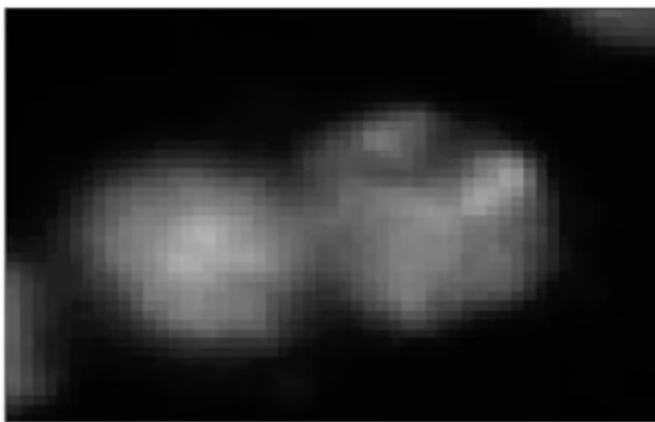


# Object identification

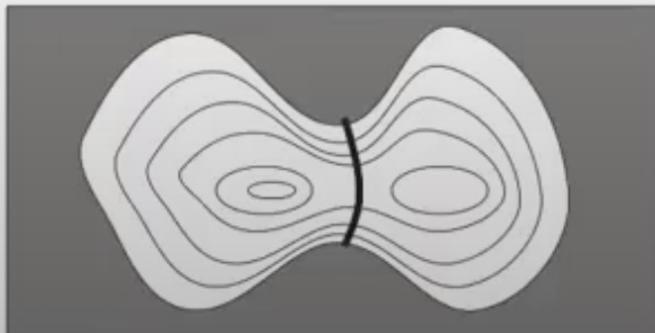
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- Once the images are loaded, how do you find objects of interest?

**Step 1:** Distinguish the foreground from the background (thresholding)



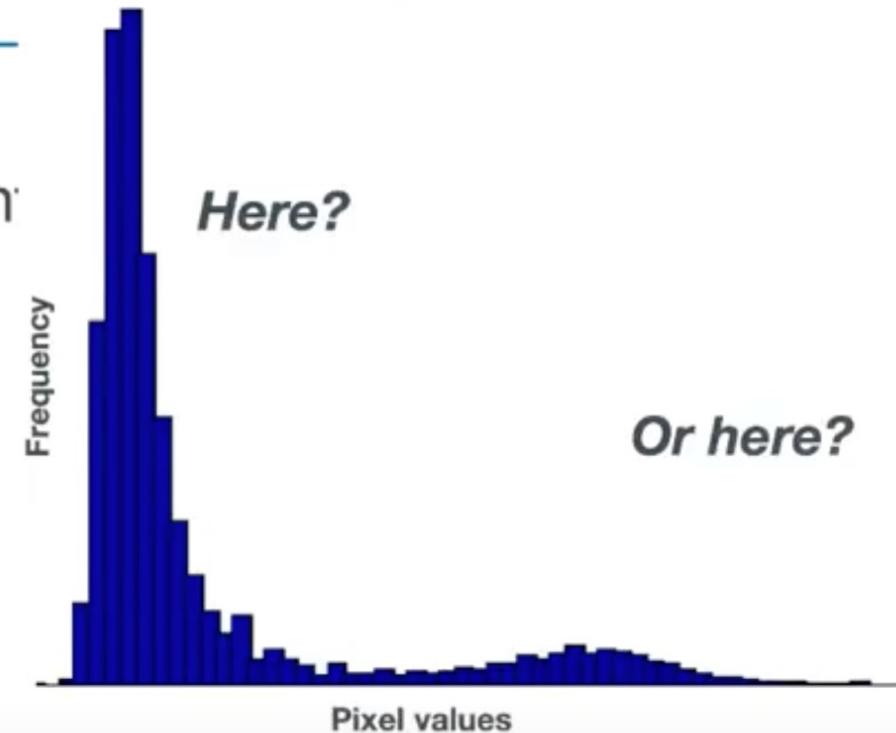
**Step 2:** Split/merge “objects” properly



# Image thresholding

- **Definition:** Division of the image into

*What is the best threshold value for dividing the intensity into foreground and background pixels?*



# Separating touching objects

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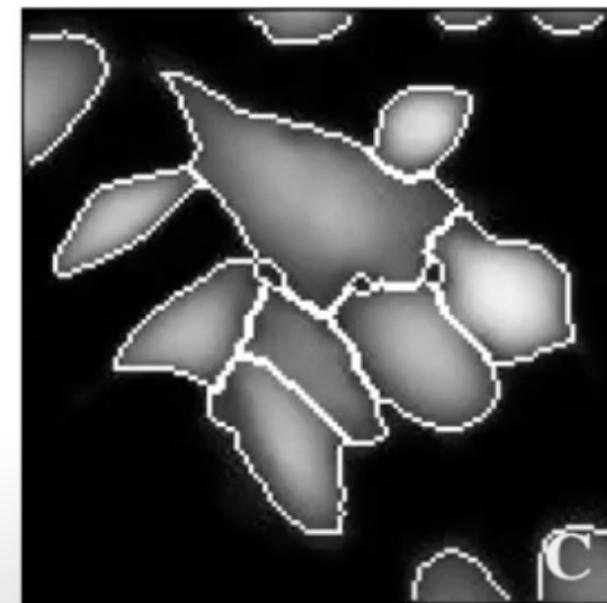
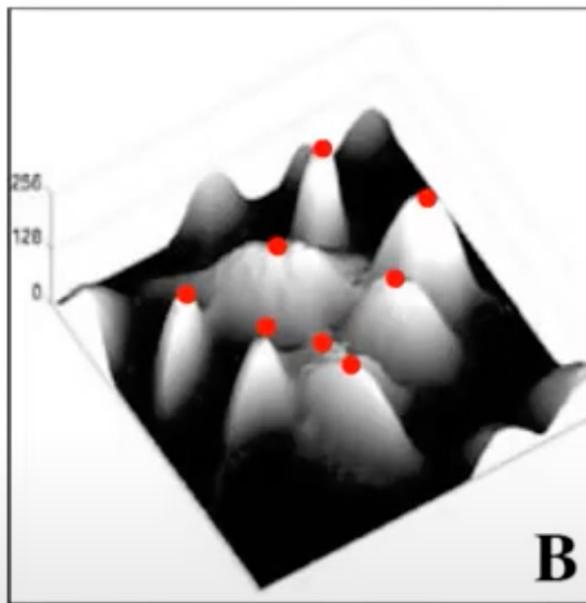
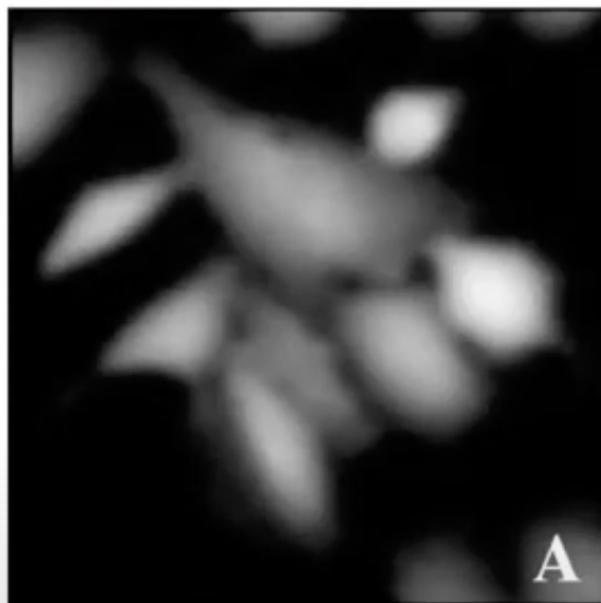
How many objects are in this central clump?  
How do you know?



Images from Carolina Wahlby

# Separating touching objects

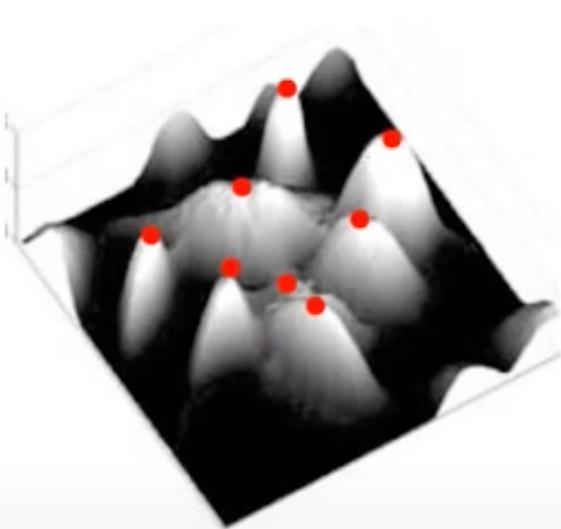
Once the foreground objects have been identified, what next?



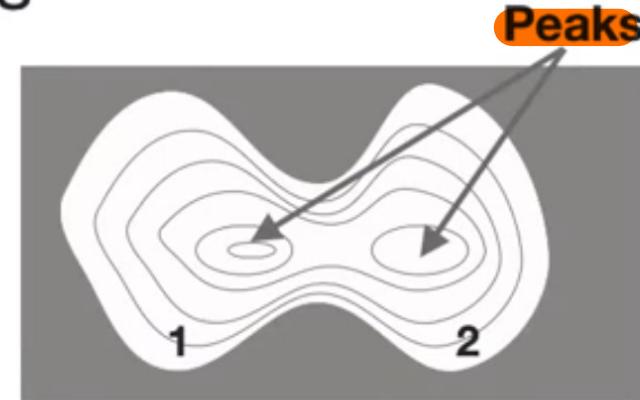
- We need to distinguish multiple objects contained in the same “clump”

# Separating touching objects

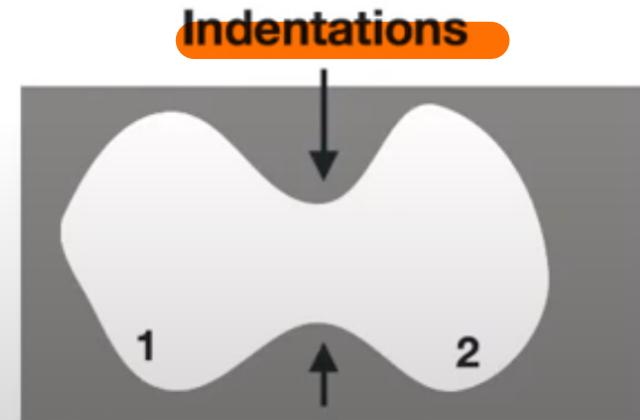
Clump identification: Two options



- Intensity: Works best if objects are brighter at center, dimmer at edges

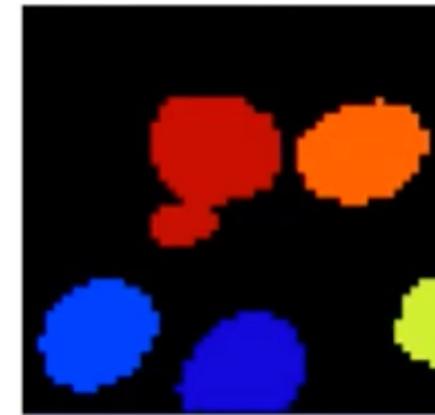
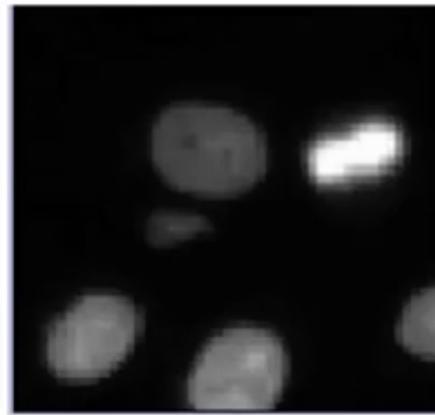


- Shape: Works best if objects have indentations where clumps touch (esp. if objects are round)



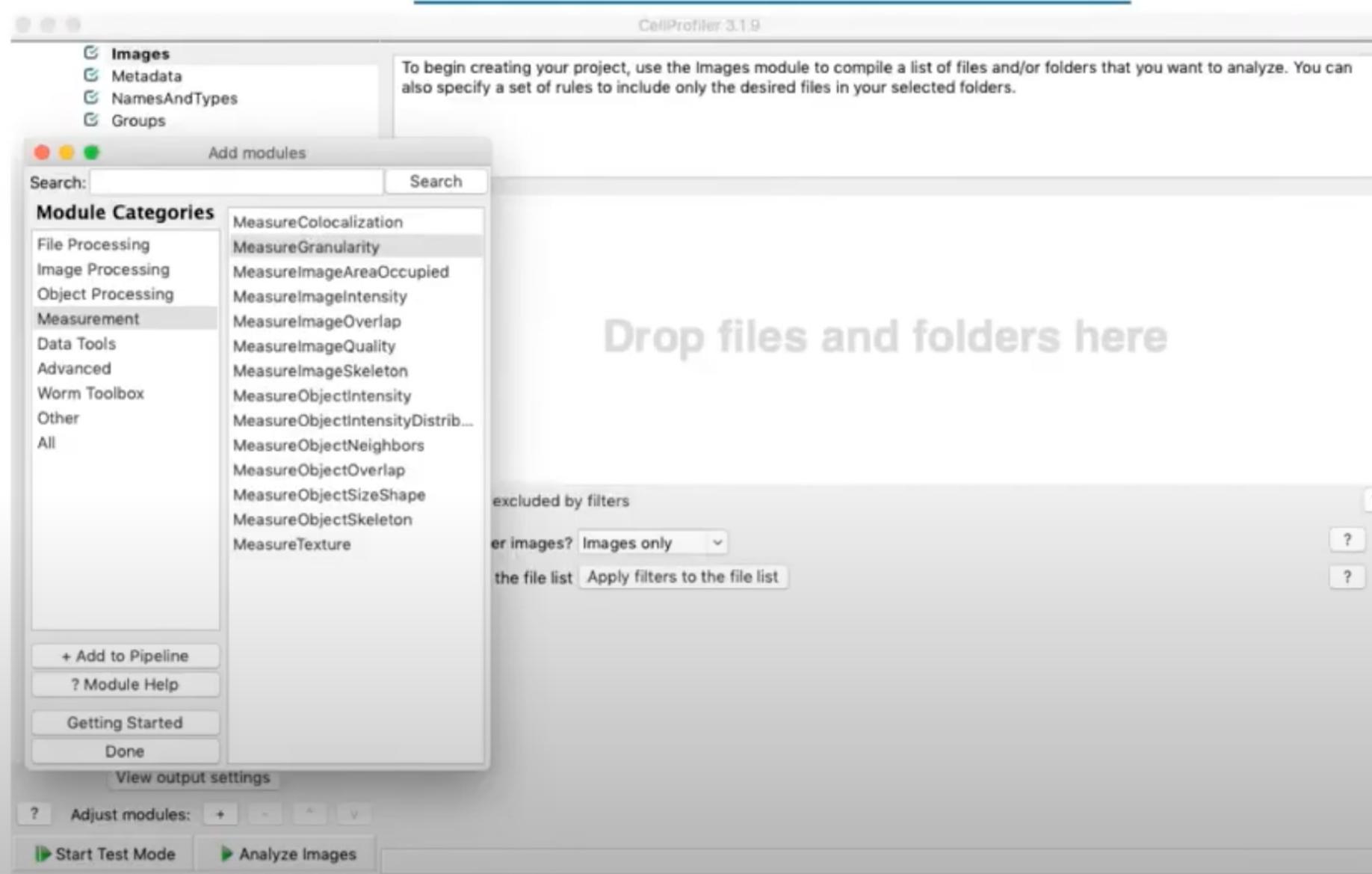
# Separating touching objects

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- Over-adjusting can produce more improper segmentation than it solves
- The proper settings are usually a matter of trial and error, and trying to globally maximize good segmentations rather than tuning to one image

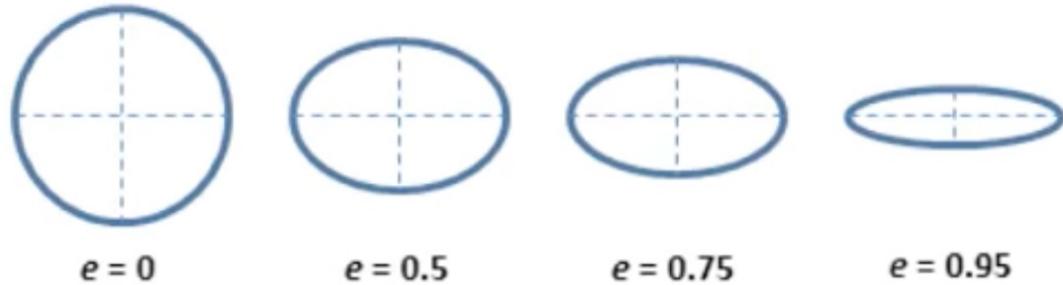
# Software can help us build up big measurement suites for our images



# Objects can be measured for their... size and shape

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- Area
- Perimeter
- Eccentricity (circle = 1, line = 0)
- MajorAxisLength
- MinorAxisLength
- Orientation
- Solidity

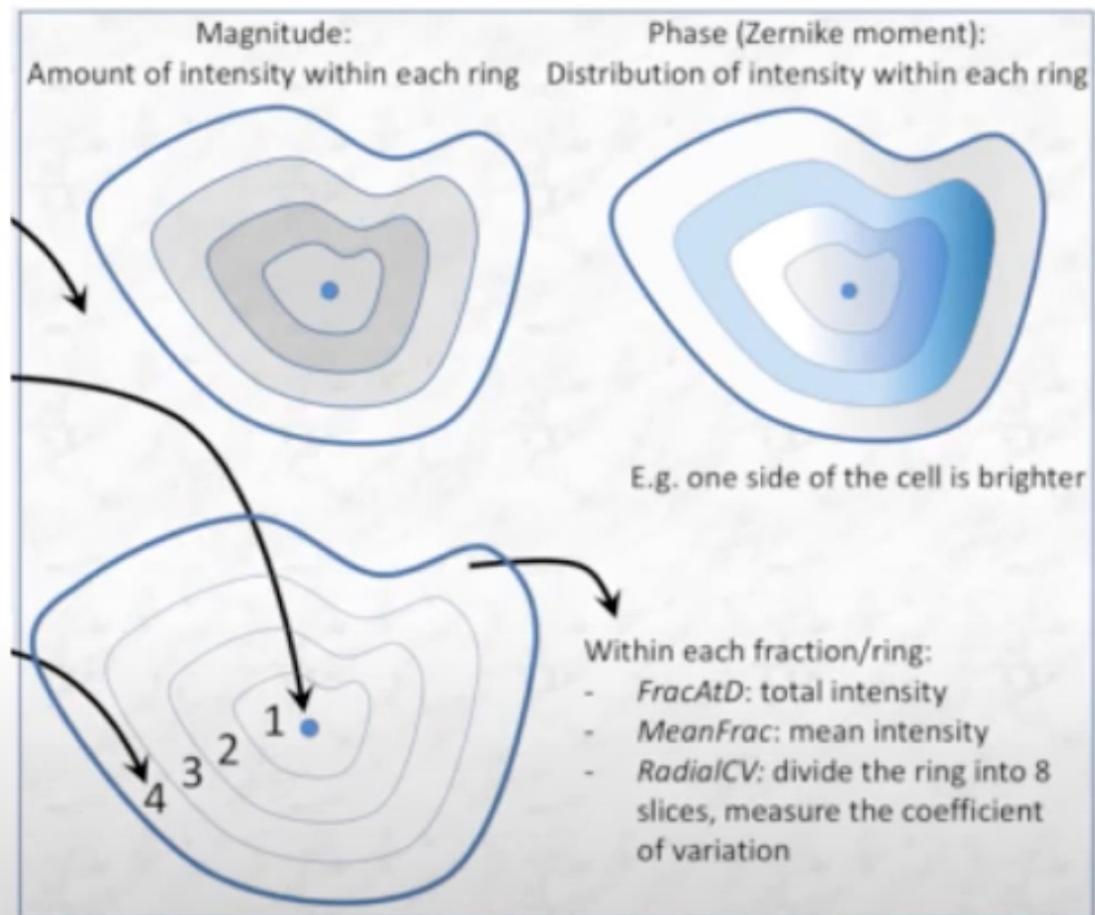


# Objects can be measured for their... intensity

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- Integrated intensity: Sum of the pixel intensities within an object
- Mean, median, standard deviation intensities
- Maximal and minimal pixel intensities
- Lower/Upper quartile of the intensity
  
- Object intensities may be measured from any channel, not just the channel used to identify the object
  - Example: GFP intensity may be measured using nuclei objects identified with DAPI

# Objects can be measured for their... intensity distribution



# Objects can be measured for their... intensity distribution

Calculate intensity Magnitudes and phase

Maximum zernike moment 9

Select an image to measure image\_input (from NamesAndTypes)

Add another image

Select objects to measure Cell (from IdentifySecondaryObjects)

Object to use as center? These objects

Add another object

Scale the bins?  Yes  No

Number of bins 4

Scale the bins?  Yes  No

Number of bins 4

Maximum radius 35

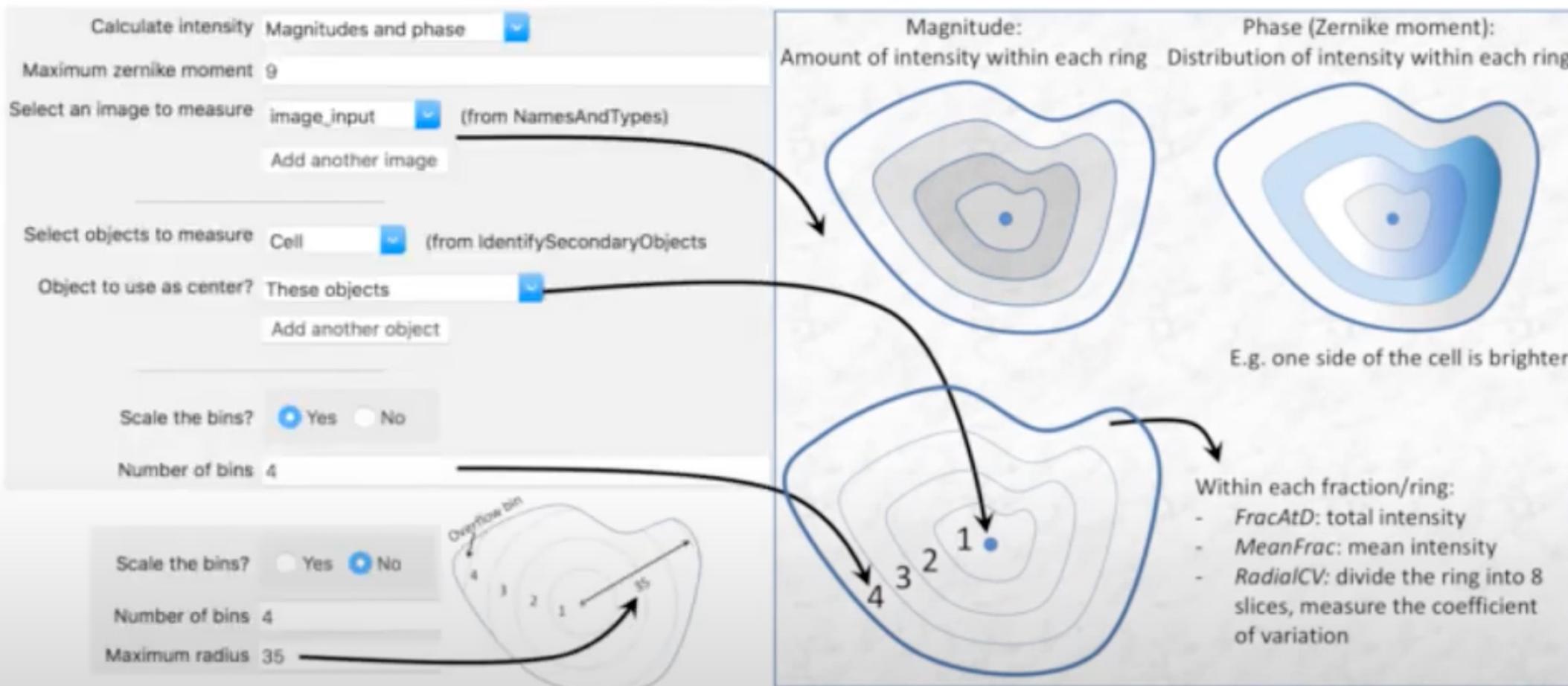
Magnitude: Amount of intensity within each ring

Phase (Zernike moment): Distribution of intensity within each ring

E.g. one side of the cell is brighter

Within each fraction/ring:

- *FracAtD*: total intensity
- *MeanFrac*: mean intensity
- *RadialCV*: divide the ring into 8 slices, measure the coefficient of variation



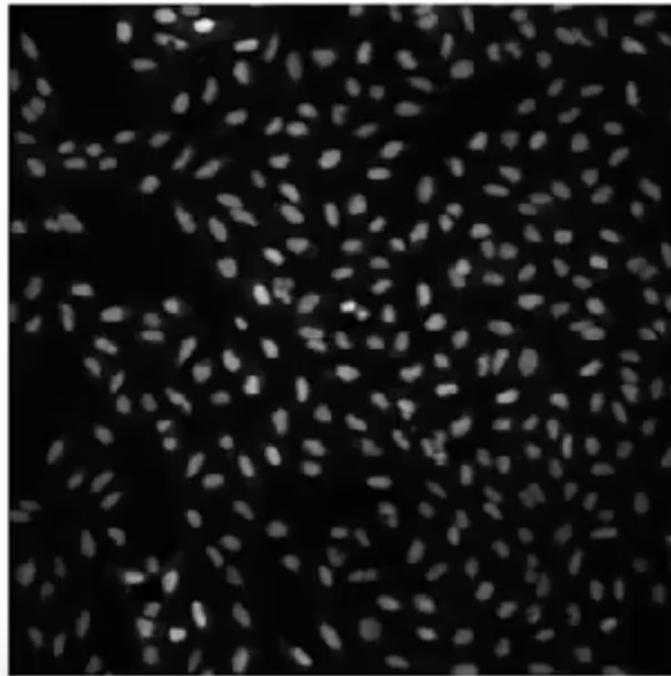
## Objects can be measured for their... texture and granularity

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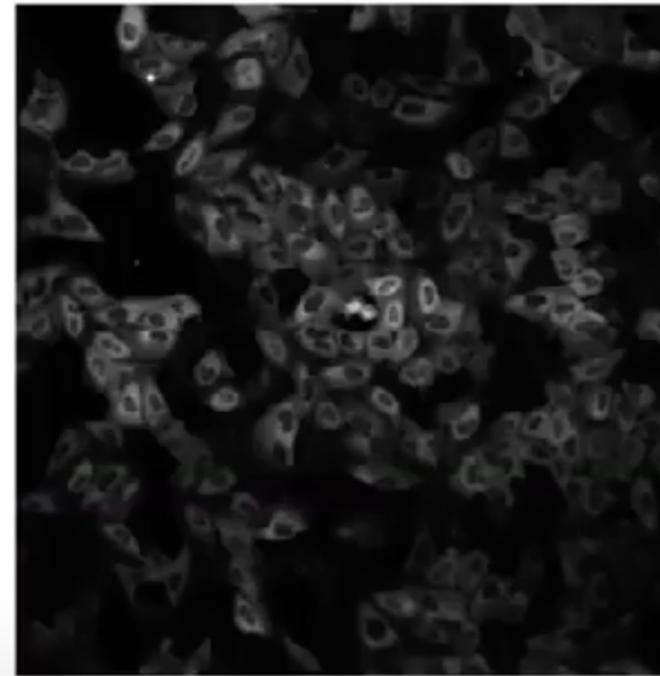
- Goal: Determine whether the staining pattern is smooth on a particular scale
  - Selection of the appropriate texture scale is essentially empirical
  - A higher number measures larger patterns of texture
  - Smaller numbers measure more localized (finer) patterns of texture
- To collect the most information, look at multiple scales

# Objects can be measured for their... colocalization

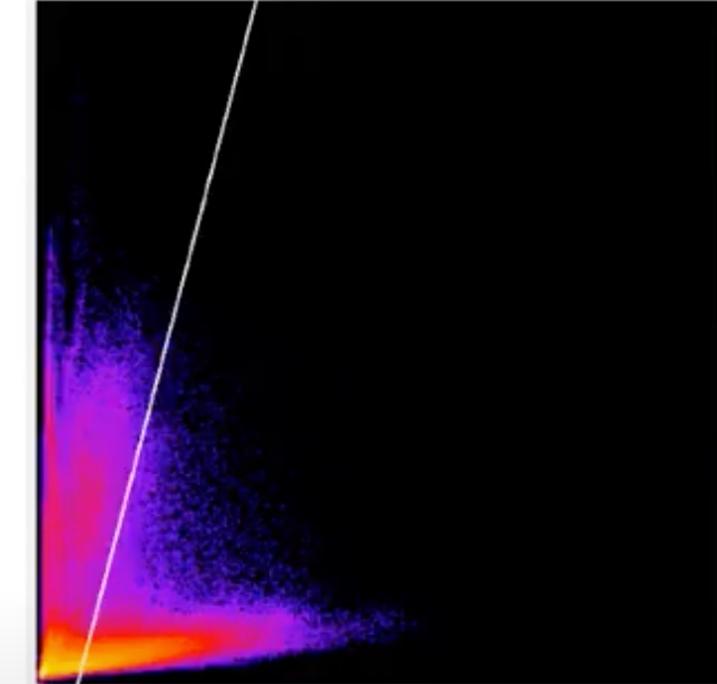
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DAPI

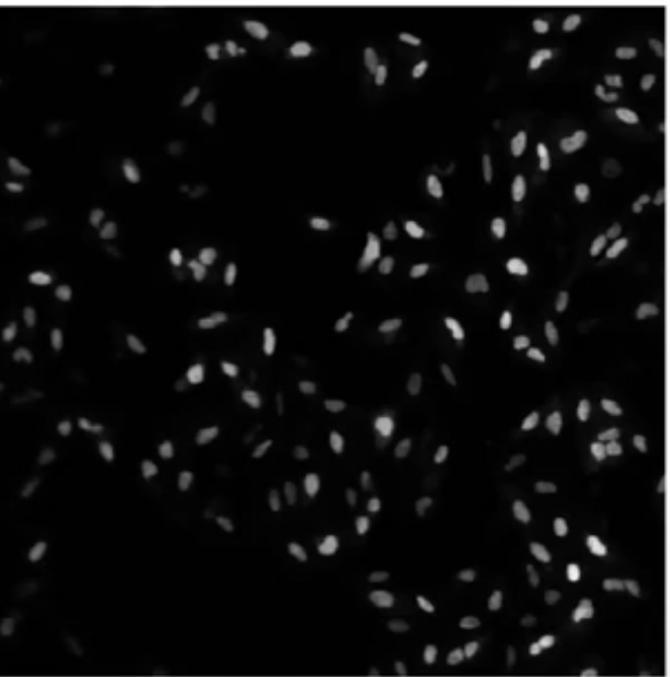


GFP

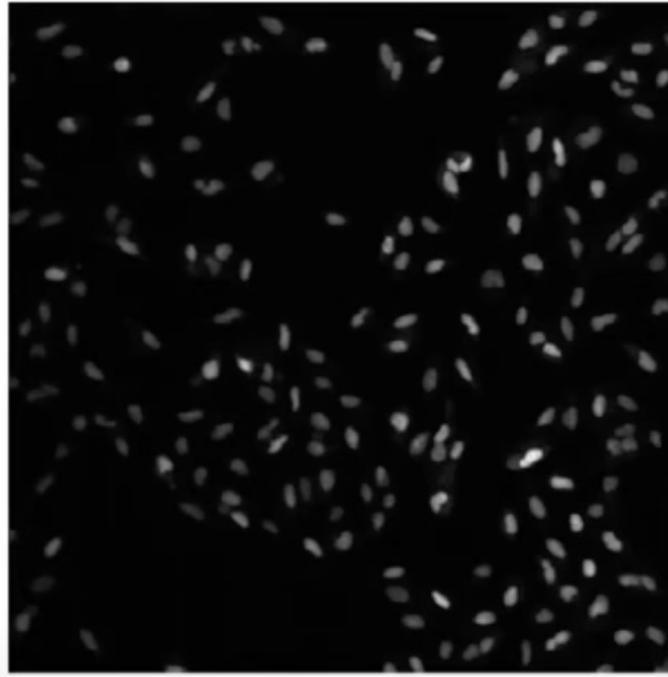


# Objects can be measured for their... colocalization

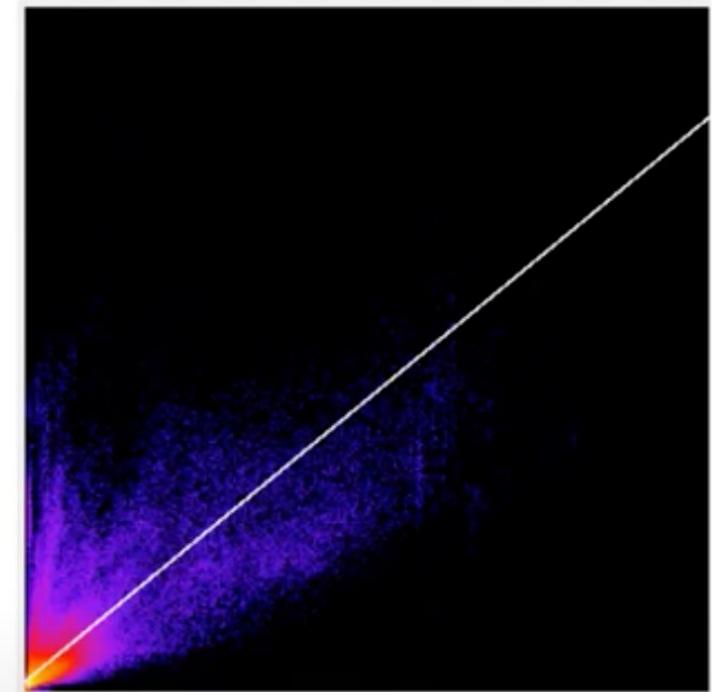
---



DAPI



GFP



# Information is hidden in images

## Label-free cell cycle



Minh  
Doan



Claire  
McQuin



Holger  
Hennig,  
Paul  
Rees,



Huw  
Summers,  
Swansea U



Andrew  
Filby,  
Swansea U



Fabian  
Theis,  
Newcastle U



Helmholtz  
Zentrum,

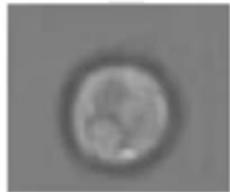
# Information is hidden in images

## Label-free cell cycle

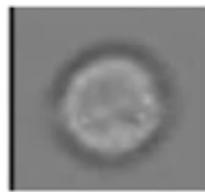
G1



S



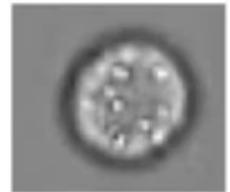
G2



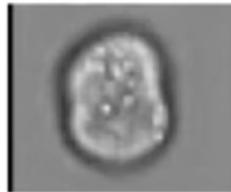
Prophase



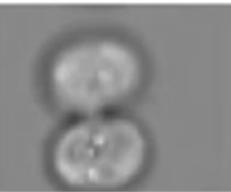
Metaphase



Anaphase



Telophase



actual phase:

G1

S

G2

Prophase

Metaphase

Anaphase

Telophase

	G1	S	G2	Prophase	Metaphase	Anaphase	Telophase
G1	0.92	0.07	0.03	0.00	0.00	0.00	0.00
S	0.23	0.64	0.11	0.00	0.00	0.00	0.00
G2	0.01	0.08	0.90	0.01	0.00	0.00	0.00
Prophase	0.00	0.04	0.18	0.58	0.01	0.00	0.00
Metaphase	0.00	0.01	0.15	0.02	0.82	0.02	0.00
Anaphase	0.00	0.00	0.13	0.01	0.03	0.87	0.02
Telophase	0.02	0.05	0.02	0.09	0.00	0.01	0.91

predicted phase



Minh  
Doan



Claire  
McQuin



Holger  
Hennig,  
Paul  
Rees,



Huw  
Summers,  
Swansea U

Andrew  
Filby,  
Newcastle U

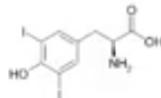


Fabian  
Theis,  
Helmholtz  
Zentrum,



# Morphological profiling can predict small molecule activity

Treat cells  
with compounds



Imaging



Image  
analysis



Match  
morphological  
profiles



Vebjorn  
Ljosa →  
Rob ter  
Horst

Actual class	Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS
Actin disruptors	5											
Aurora kinase inhibitors		12										
Cholesterol-lowering			6									
DNA damage	1			7	2							
DNA replication				1	6							
Eg5 inhibitors						12						
Epithelial						1	7					
Kinase inhibitors							5					
Microtubule destabilizers					1			13				
Microtubule stabilizers								9				
Protein degradation								7				
Protein synthesis									8			

Class predicted by morphology

	Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	5												100 %
Aurora kinase inhibitors		12											100 %
Cholesterol-lowering			6										100 %
DNA damage	1			7	2								74 %
DNA replication				1	6								81 %
Eg5 inhibitors						12							100 %
Epithelial						1	7						91 %
Kinase inhibitors							5						100 %
Microtubule destabilizers				1				13					93 %
Microtubule stabilizers								9					100 %
Protein degradation								7					96 %
Protein synthesis									8				100 %

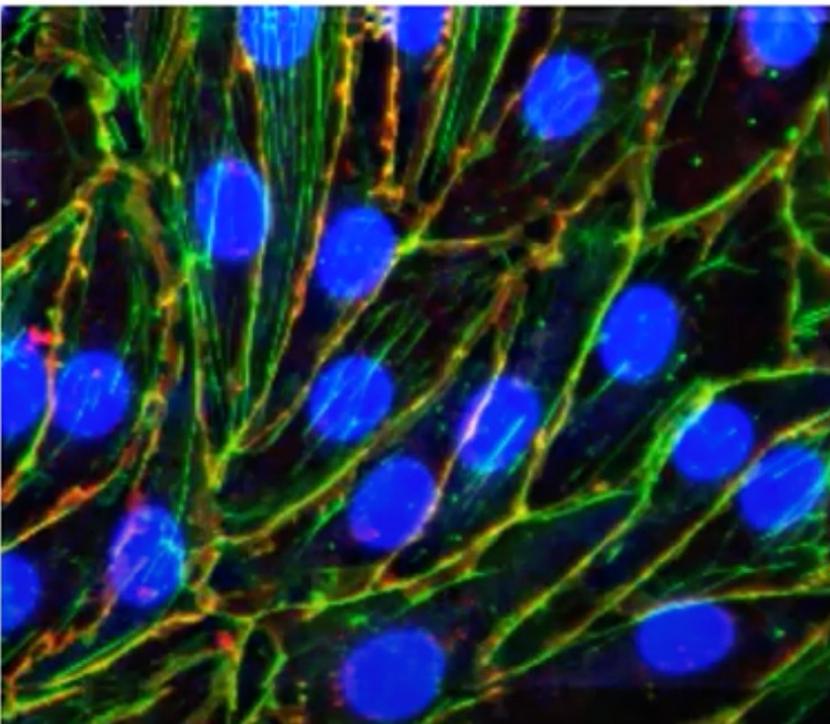
Overall accuracy: 94 %



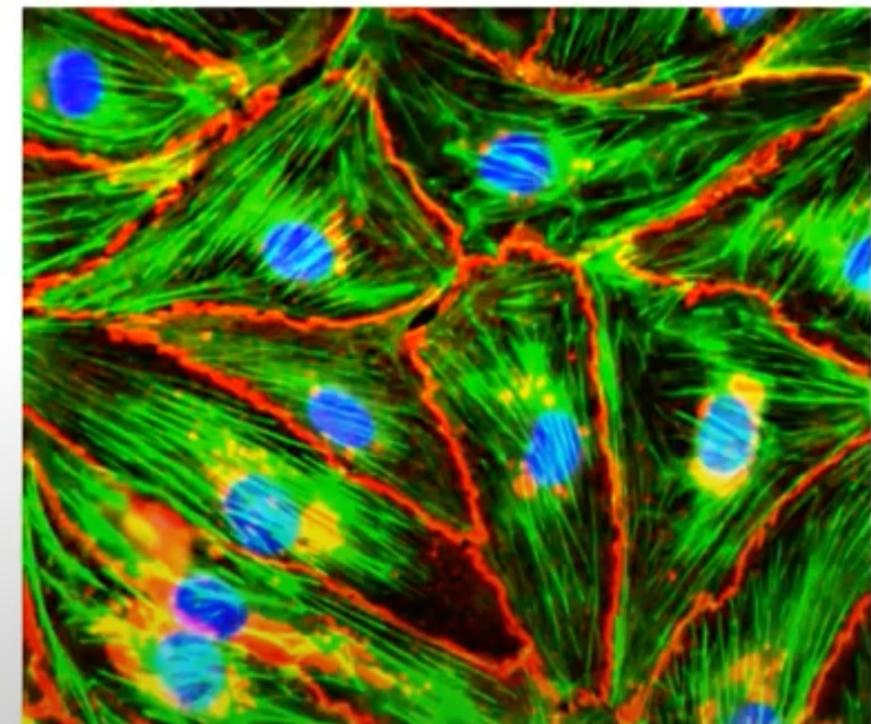
# Morphological profiling can identify drugs for disease

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Healthy



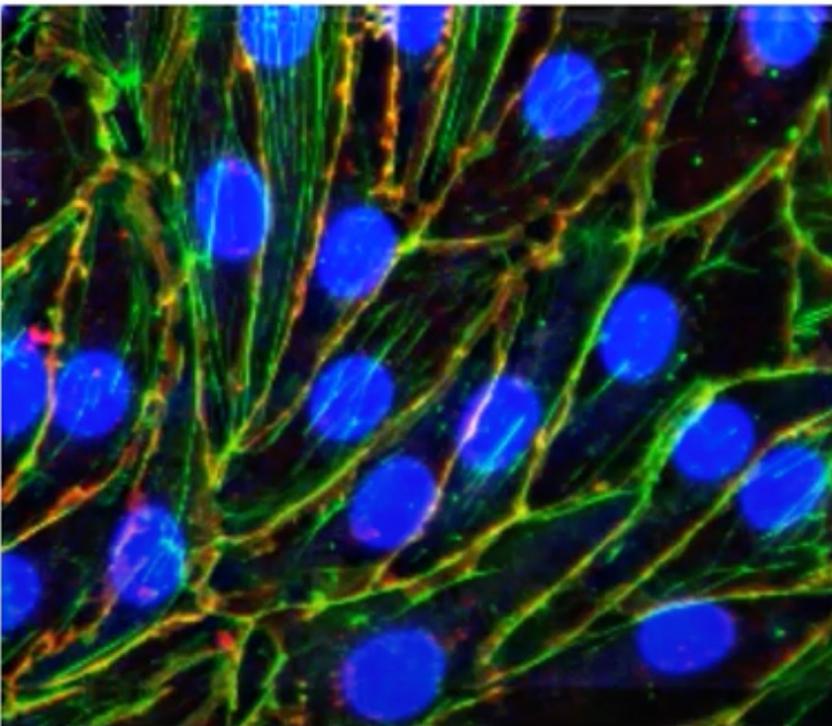
Disease (CCM knockdown)



# Morphological profiling can identify drugs for disease

+ drug?

Healthy



Disease (CCM knockdown)

