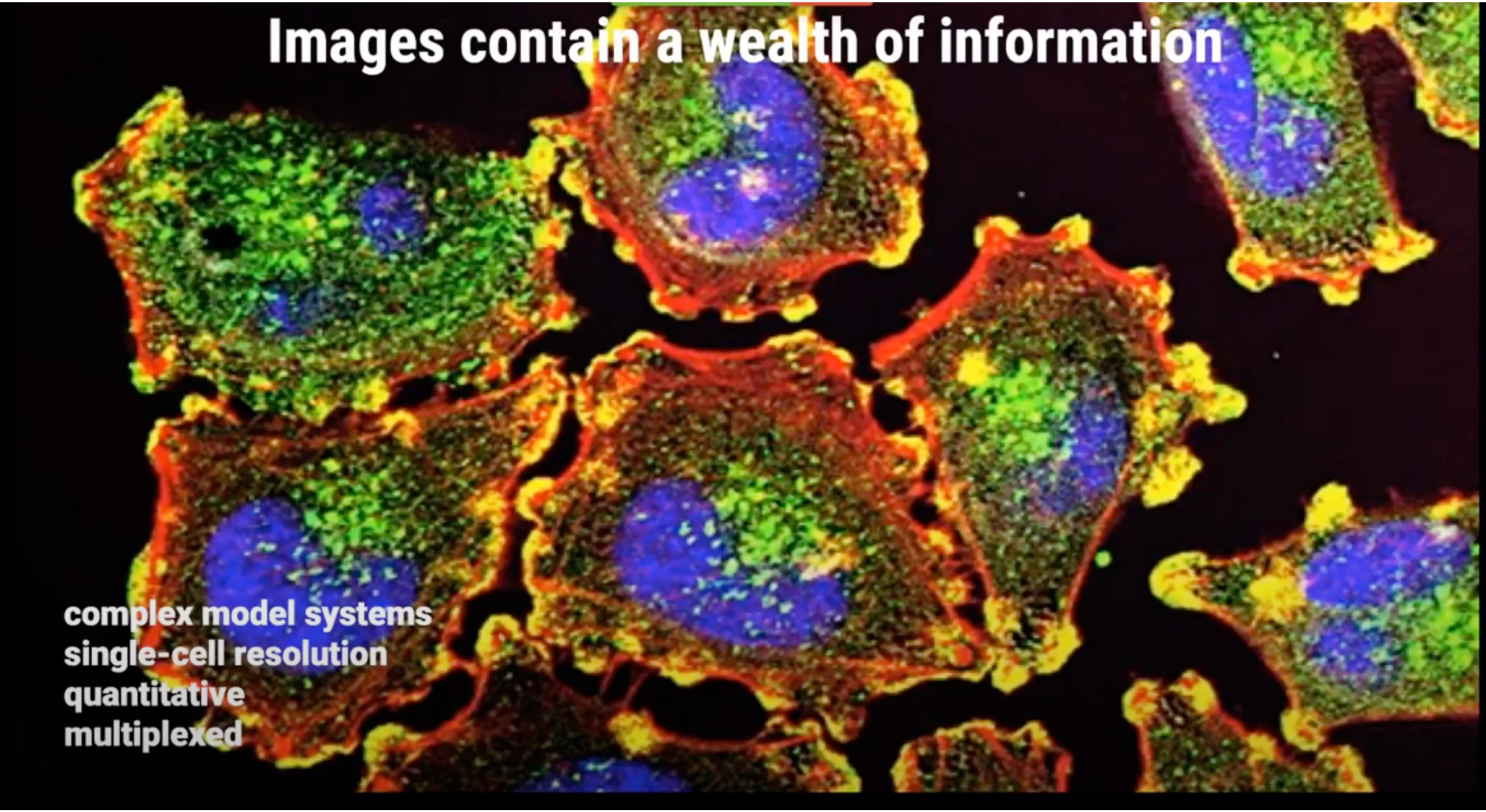
Intelligent Analysis of Biomedical Images

Presenter: Mohammad H. Rohban, Ph.D.

Fall 2023

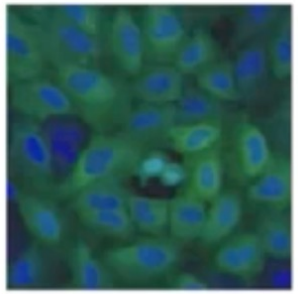
Courtesy: Slides are adopted from the Beth Cimini's presentation (March 27, 2020)

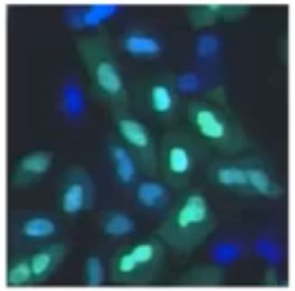
Images contain a wealth of information



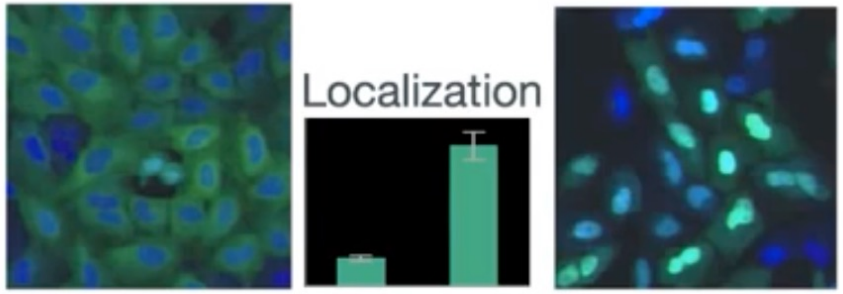
complex model systems
single-cell resolution
quantitative
multiplexed

Visual appearance can be quantified





Visual appearance can be quantified



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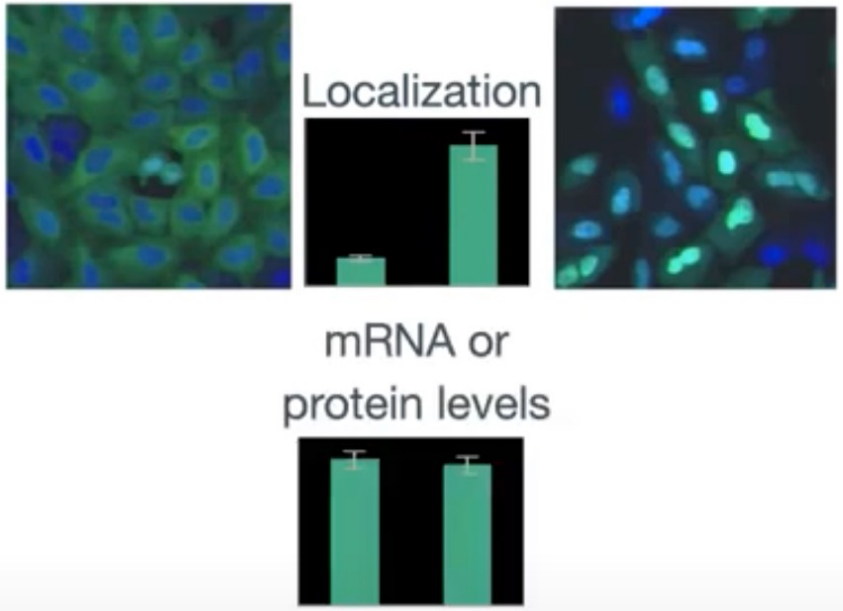

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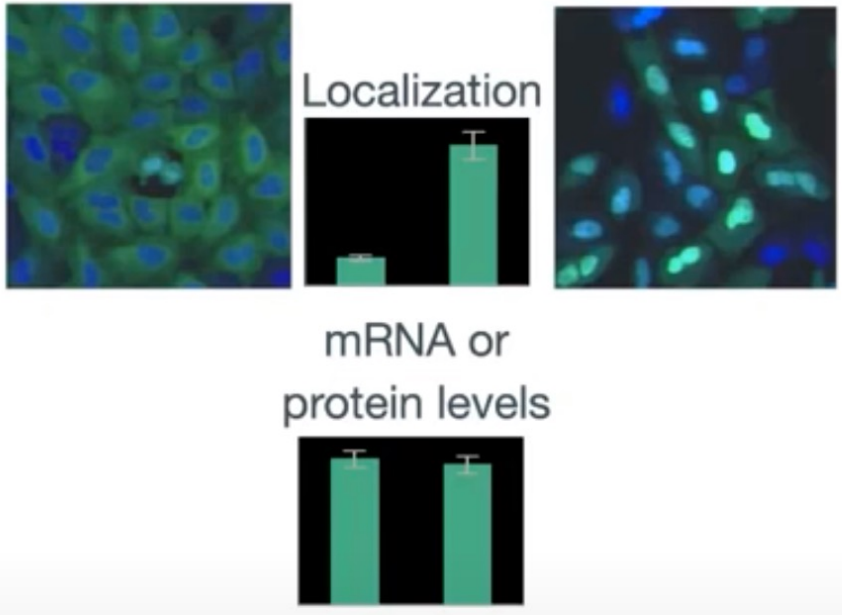


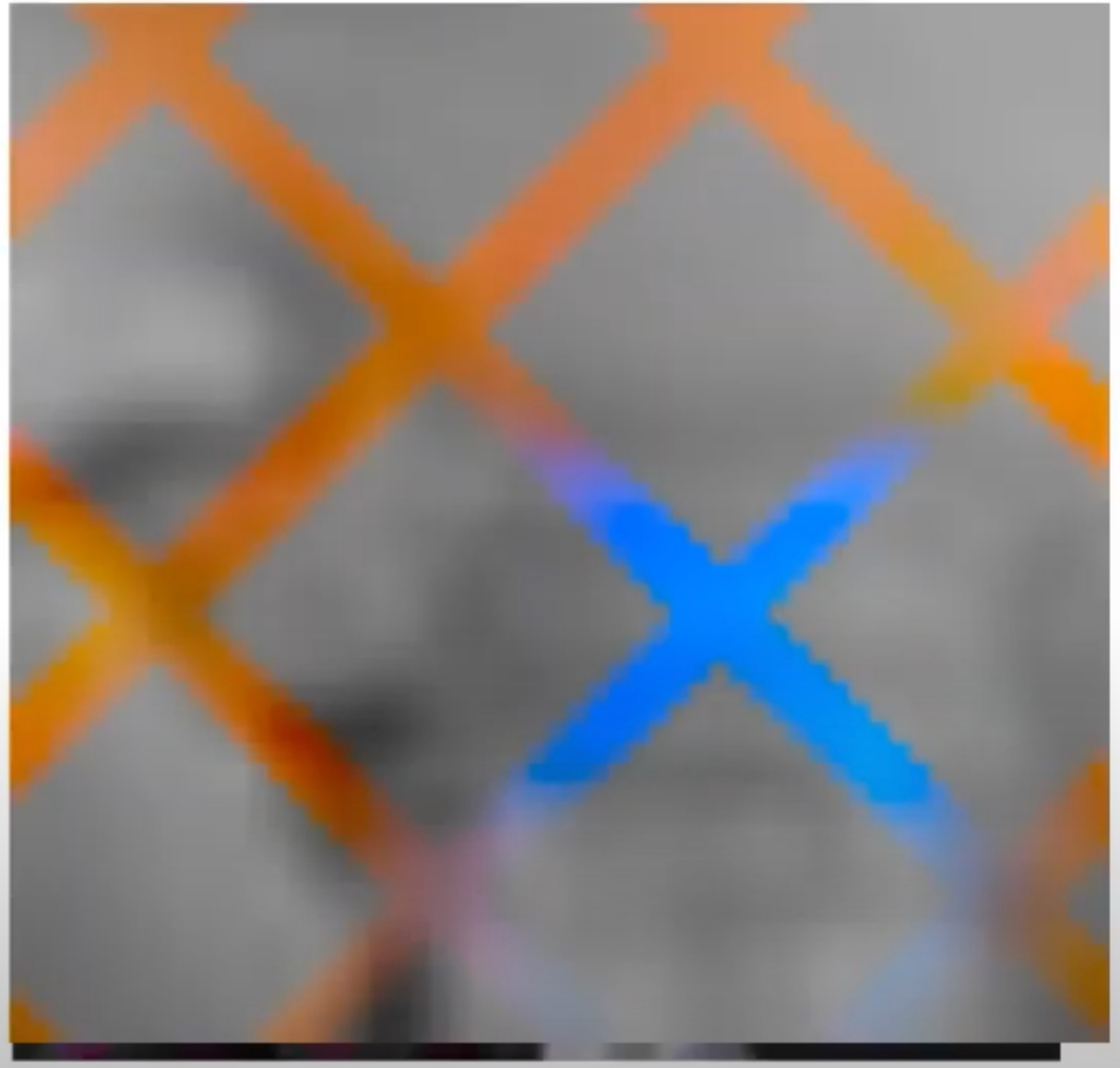
- Automatic image analysis is
  - Objective
  - Quantitative, with statistics
  - Can measure multiple properties at once
  - Can quantify heterogeneity (single-cell measurements)
  - Distinguishes subtle changes, even those undetectable by eye
  - Faster, less tedious

Human brains are not built for quantification

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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ImageJ

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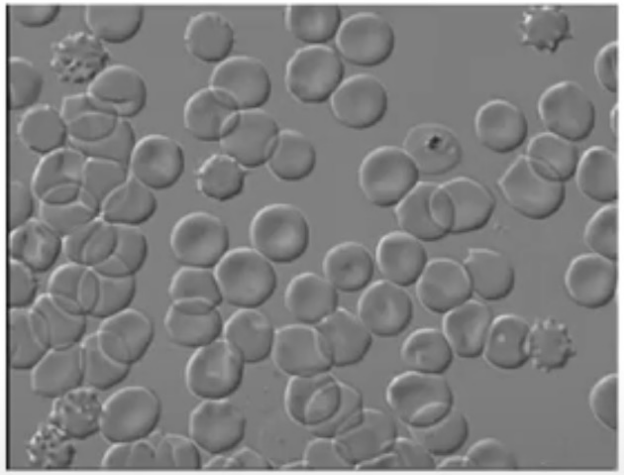
Advantages- very fast image processing, lots of plugins for measurement, image processing, even gel analysis

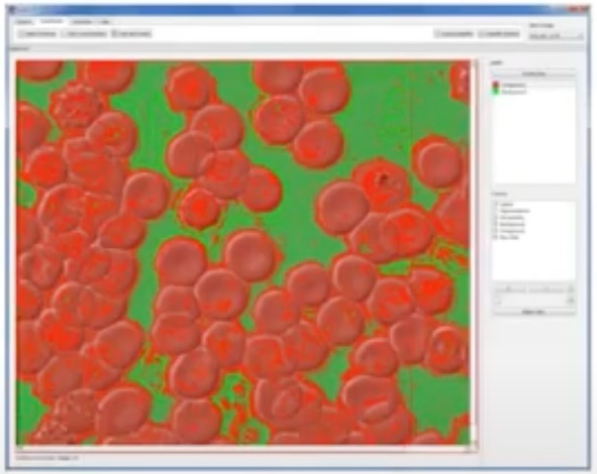
Disadvantages- need to be able to write scripts and/or macros in order to apply the same analysis to many images

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

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

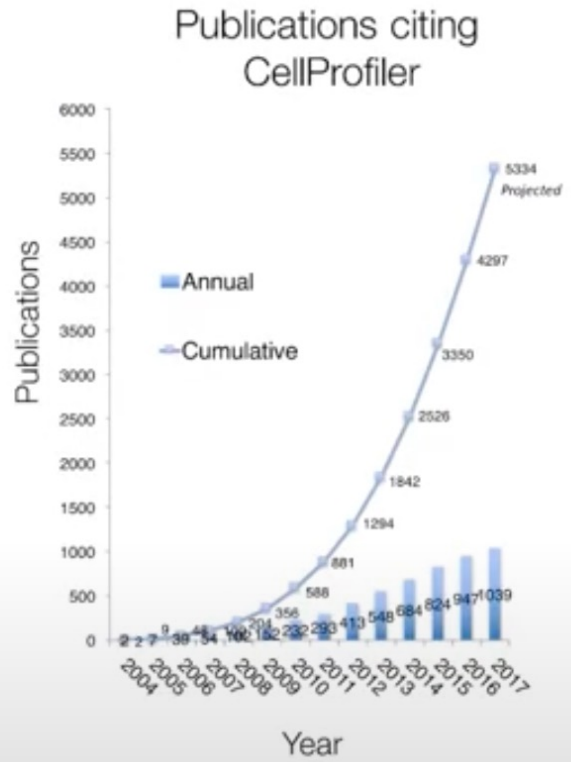
- Available from www.cellprofiler.org for Windows, Mac and Linux

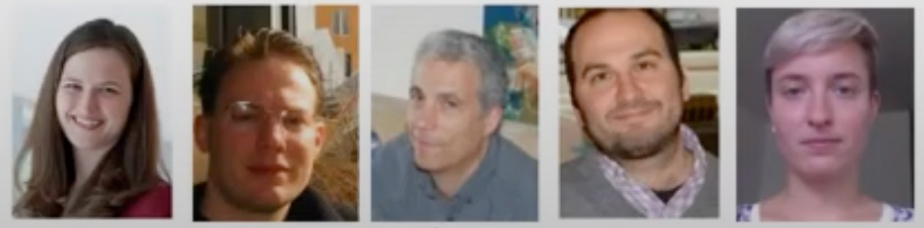
CellProfiler

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.)*





Anne Carpenter | Ray Jones | Lee Kamentsky | Allen Goodman | Claire McQuin

Object identification

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

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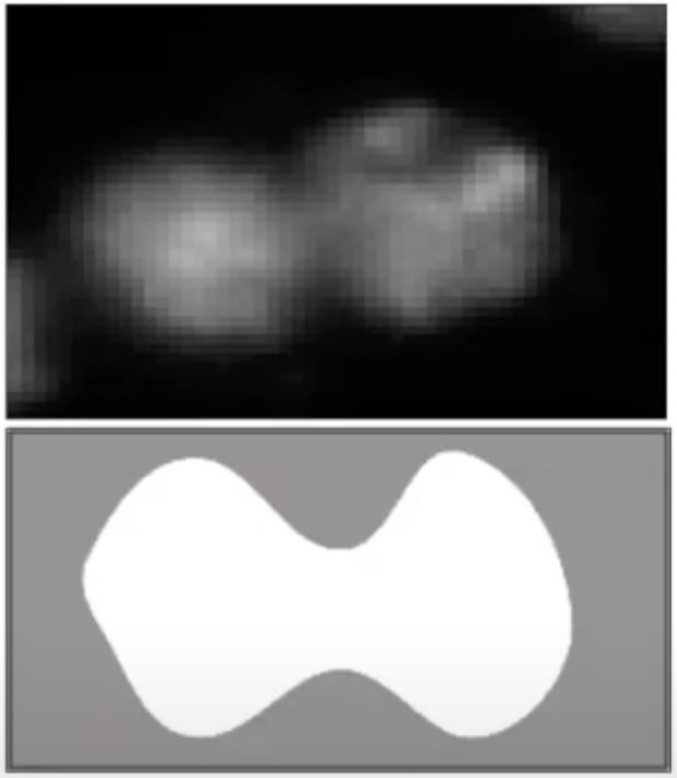


Step 1: Distinguish the foreground from the background (thresholding)

Object identification

- 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

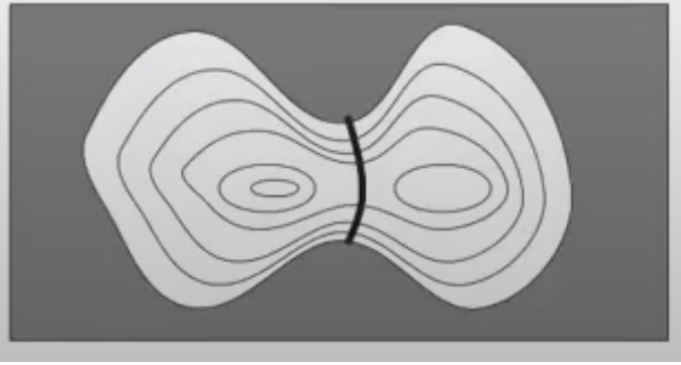
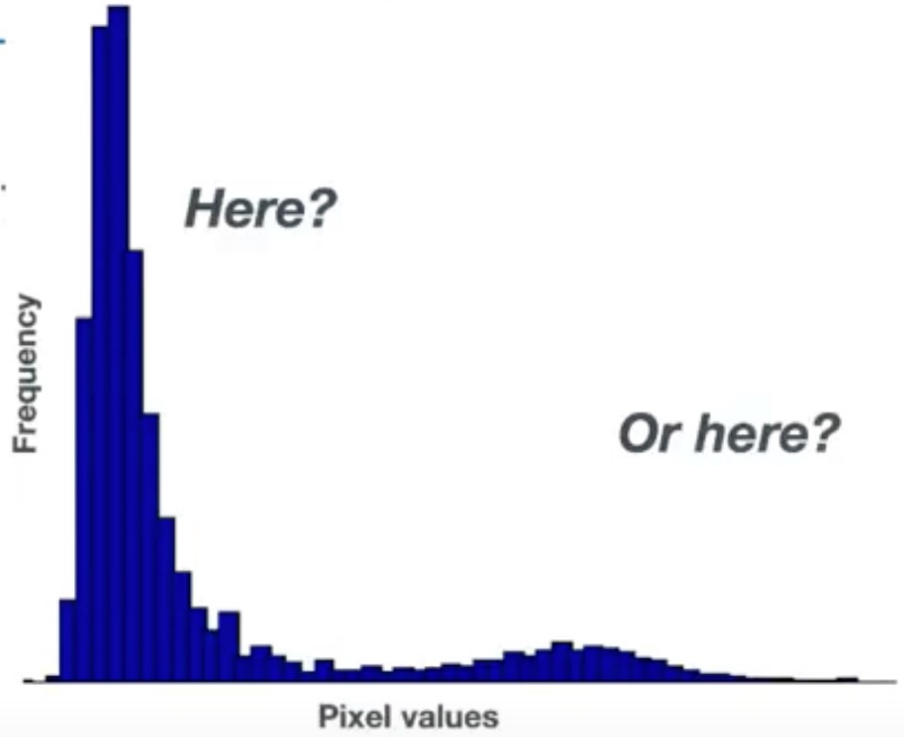


Image thresholding

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

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



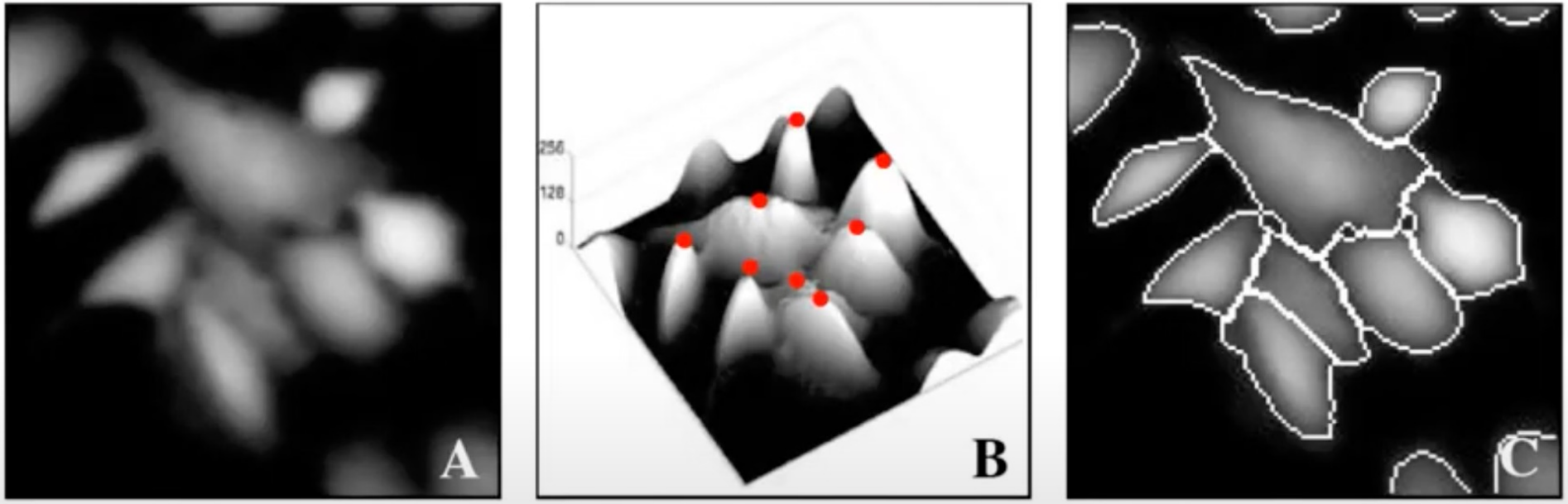
Separating touching objects

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?

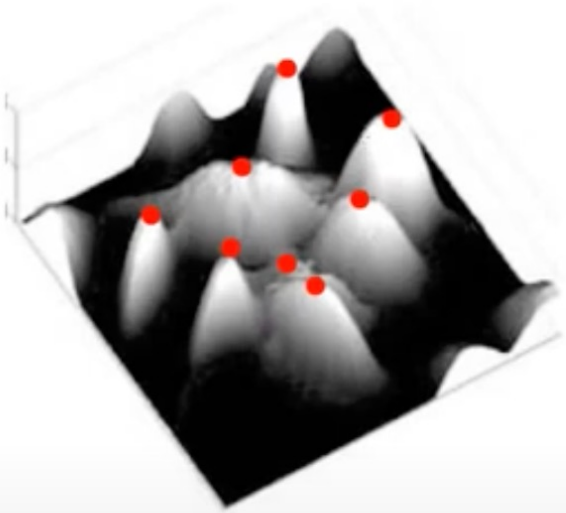


- We need to distinguish multiple objects contained in the same “clump”

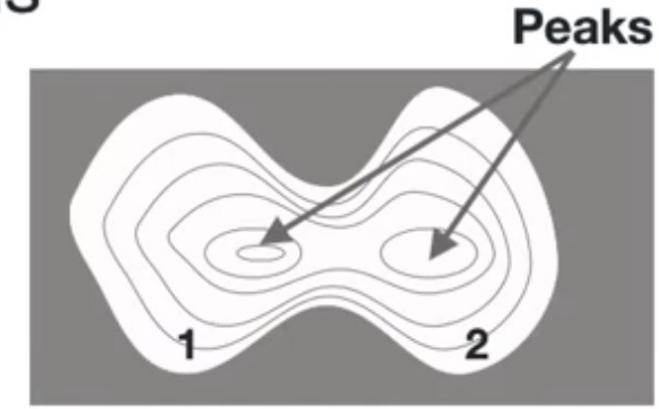
Images from Carolina Wahlby

Separating touching objects

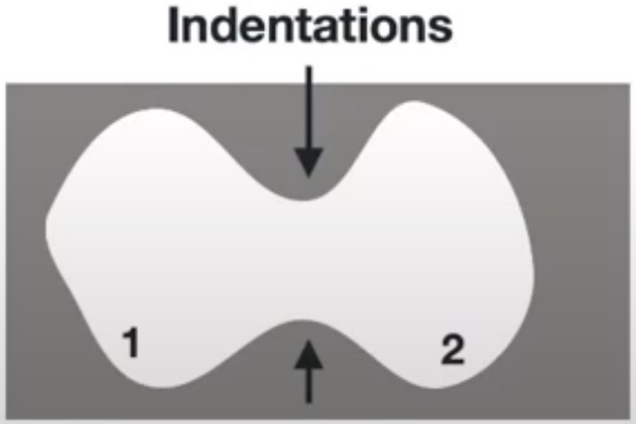
Clump identification: Two options



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



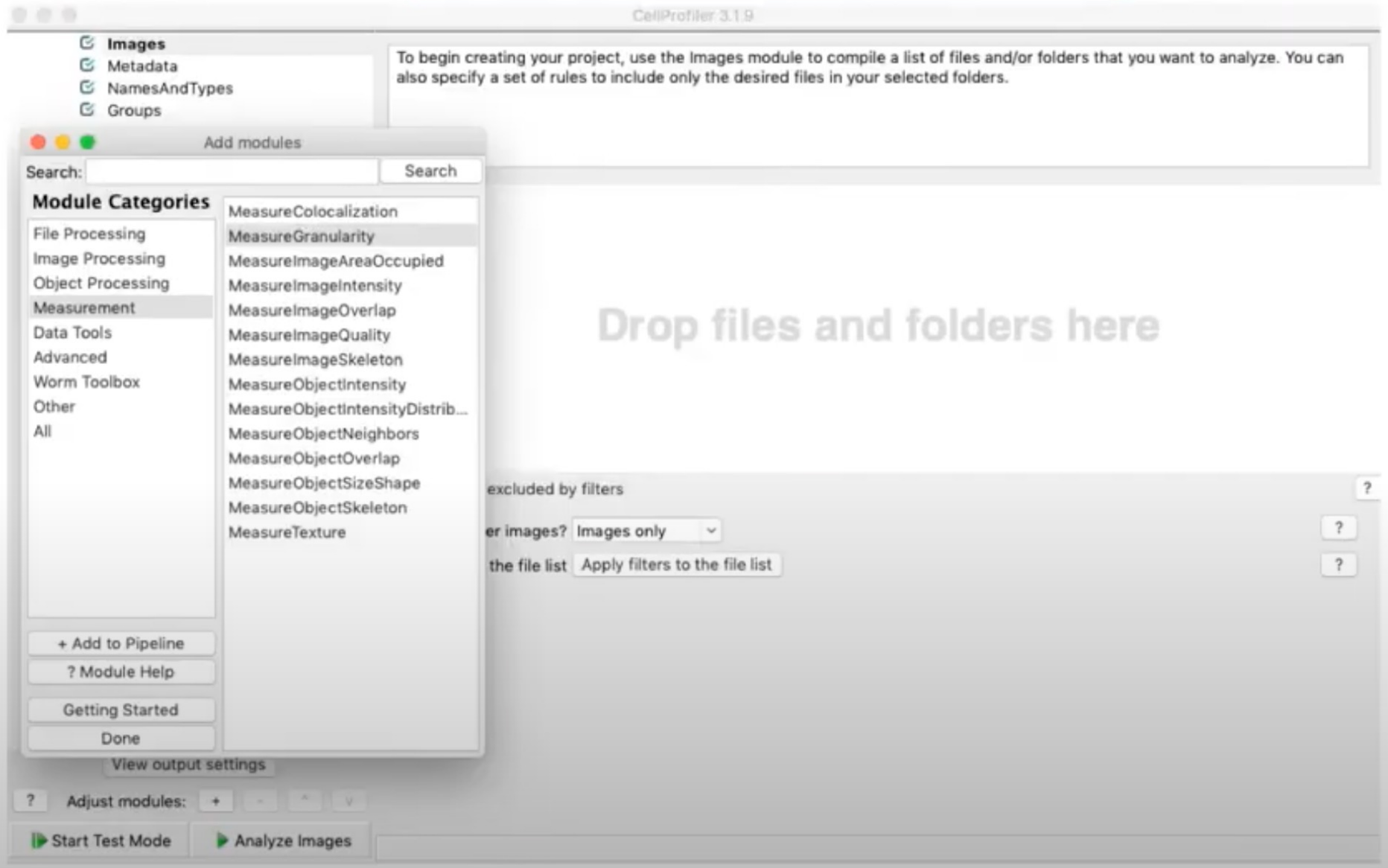
- **Shape:** Works best if objects have indentations where clumps touch (esp. if objects are round)



Separating touching objects

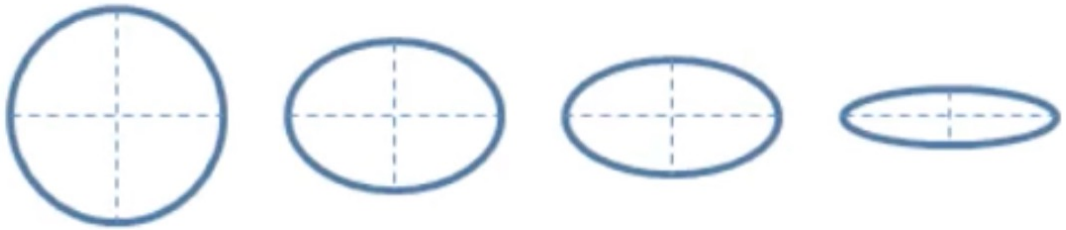
- Over-adjusting can produce more improper segmentation than it solves
- The proper settings are usually a matter of trial and error, and trying to globally maximize good segmentations rather than tuning to one image

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



Objects can be measured for their… size and shape

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



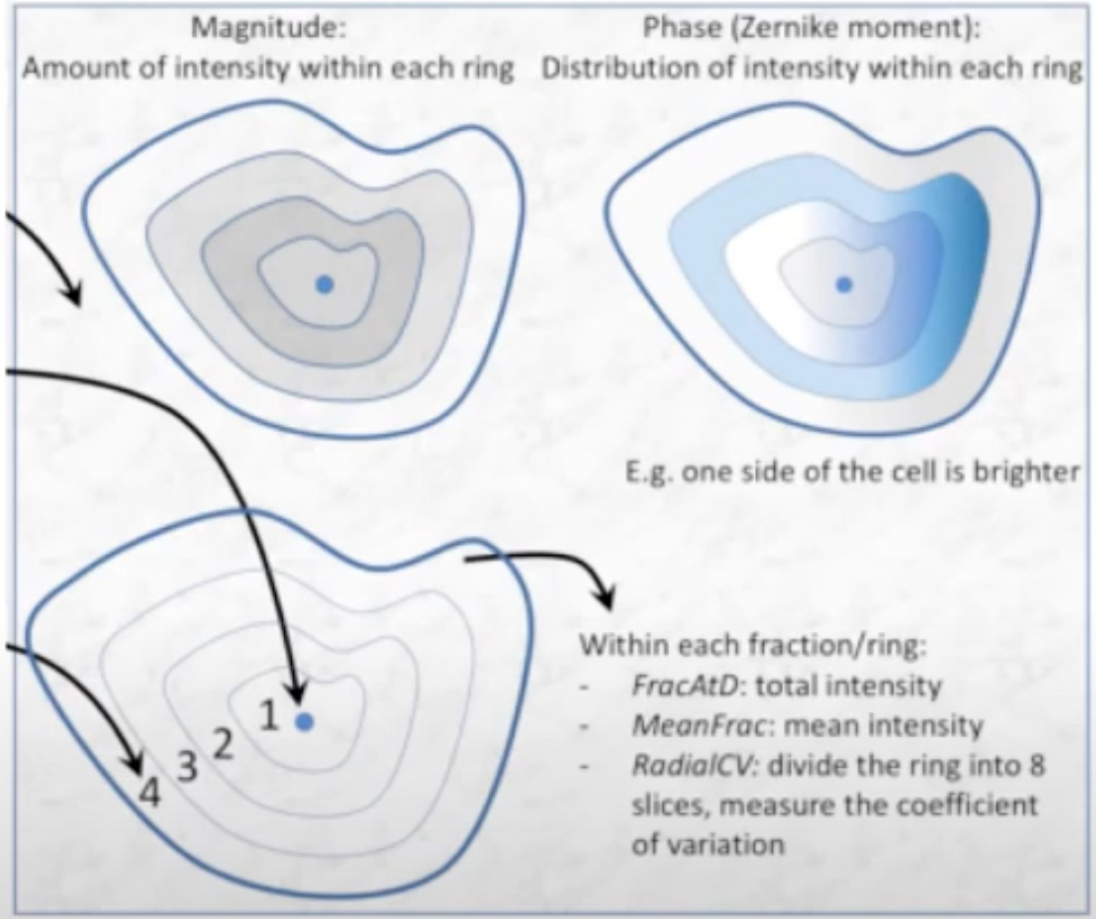
e = 0 *e* = 0.5 *e* = 0.75 *e* = 0.95

Objects can be measured for their… intensity

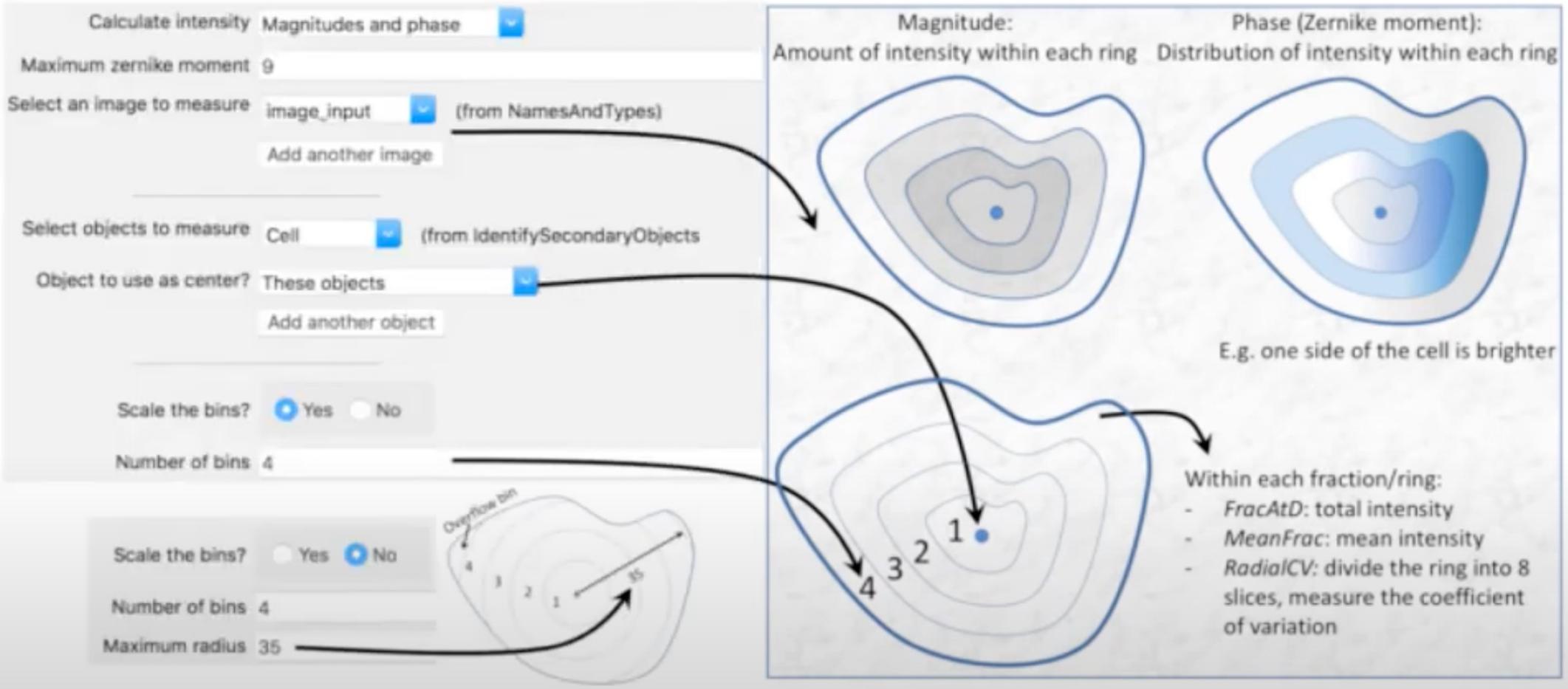
- Integrated intensity: Sum of the pixel intensities within an object
- Mean, median, standard deviation intensities
- Maximal and minimal pixel intensities
- Lower/Upper quartile of the intensity

- 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



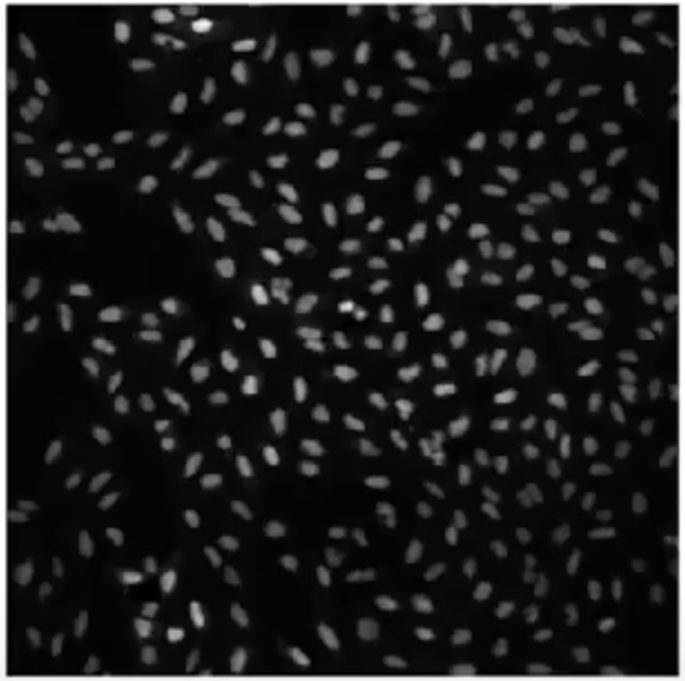
Objects can be measured for their… intensity distribution



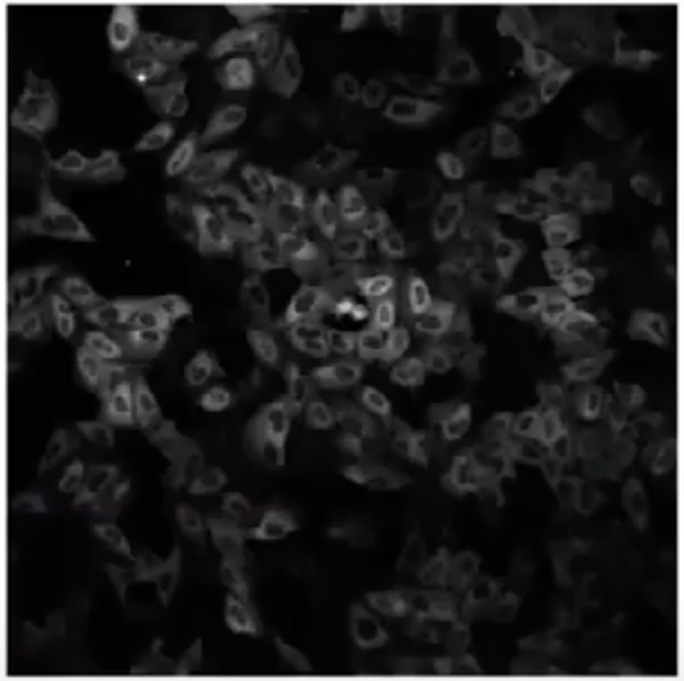
Objects can be measured for their… texture and granularity

- 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

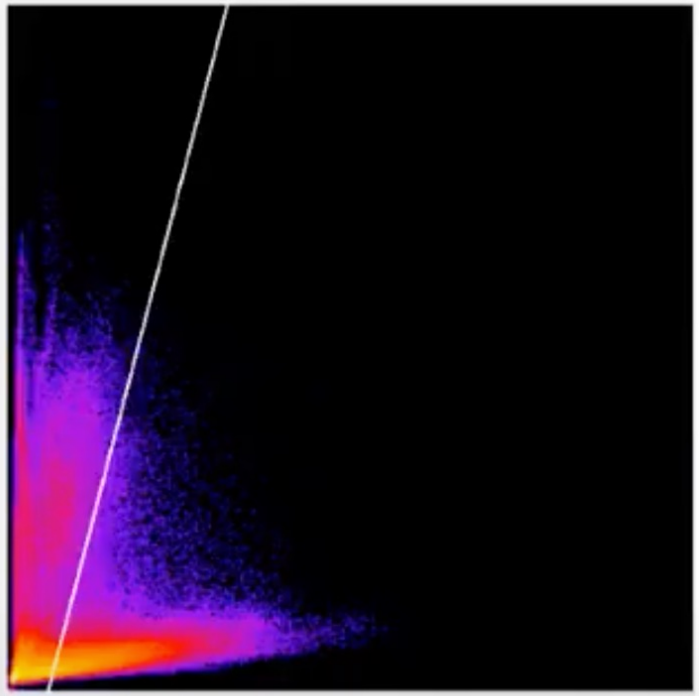
Objects can be measured for their… colocalization



DAPI

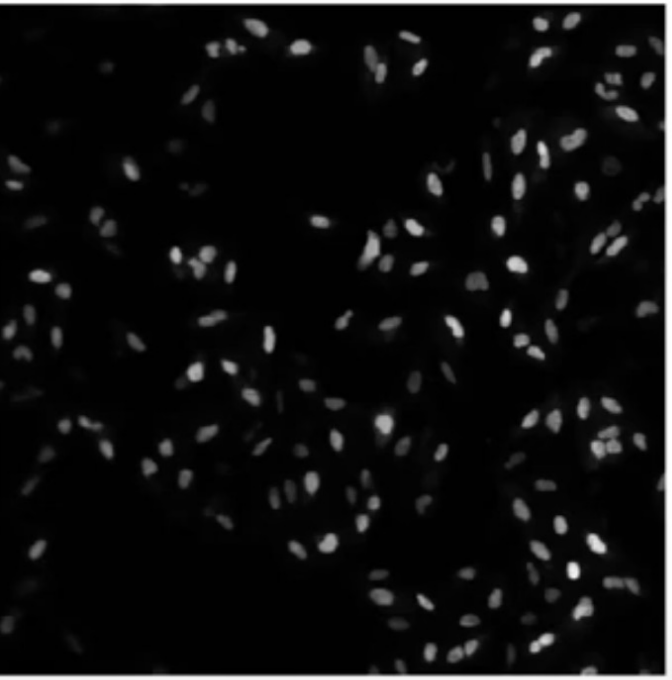


GFP

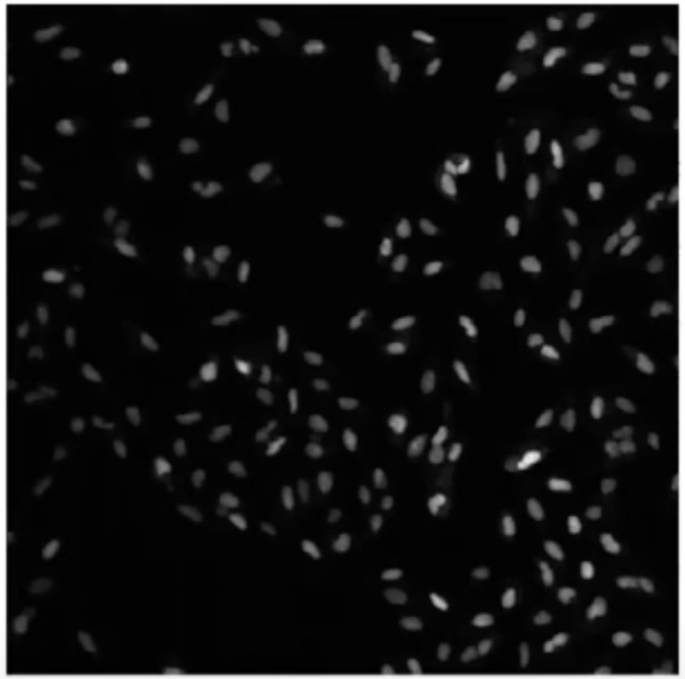


FIJI, Coloc2- https://imagej.net/Coloc_2

Objects can be measured for their… colocalization



DAPI



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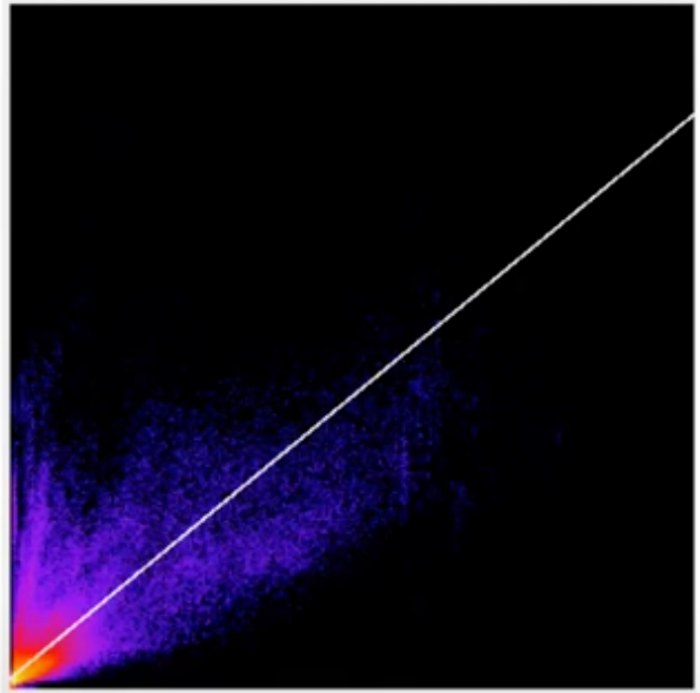
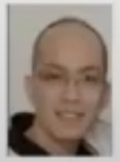
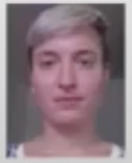


FIJI, Coloc 2- https://imagej.net/Coloc_2

Information is hidden in images

Label-free cell cycle


Minh Doan


Claire McQuin


Holger Hennig, Rostock U


Paul Rees, Swansea U


Huw Summers, Swansea U

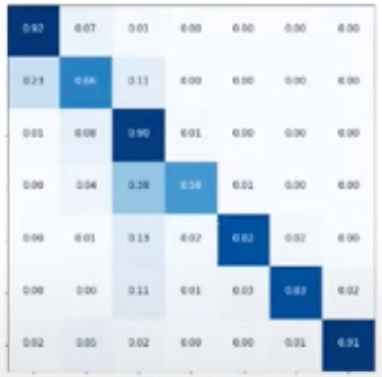

Andrew Filby, Newcastle U


Fabian Theis, Helmholtz Zentrum,

Information is hidden in images

Label-free cell cycle

actual phase:
G1
S
G2
Prophase
Metaphase
Anaphase
Telophase



predicted phase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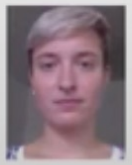
Claire McQuin


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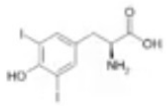
Andrew Filby, Newcastle U


Fabian Theis, Helmholtz Zentrum,

Morphological profiling can predict small molecule activity

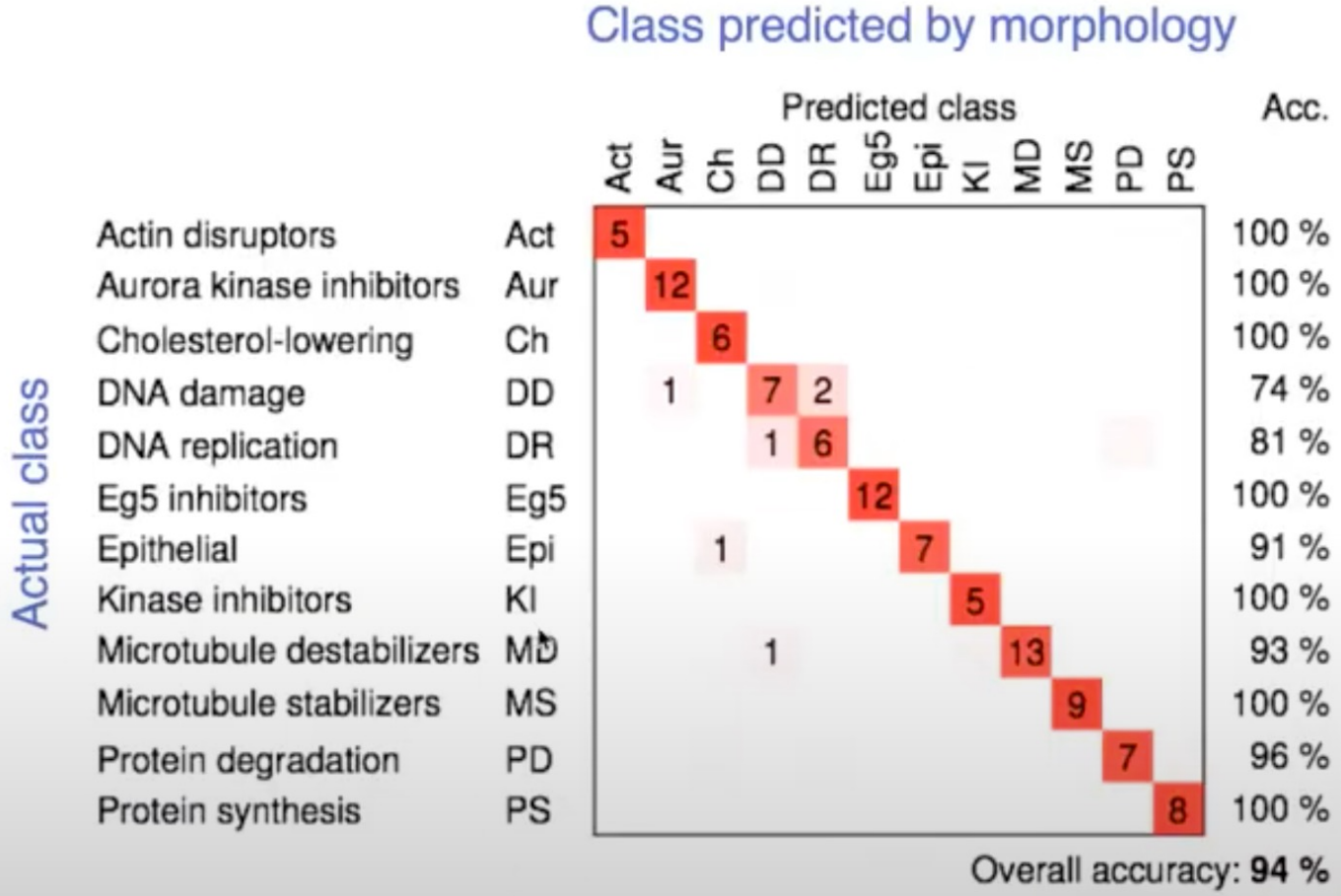
Treat cells with compounds



Imaging

Image analysis

Match morphological profiles



Vebjorn Ljosa

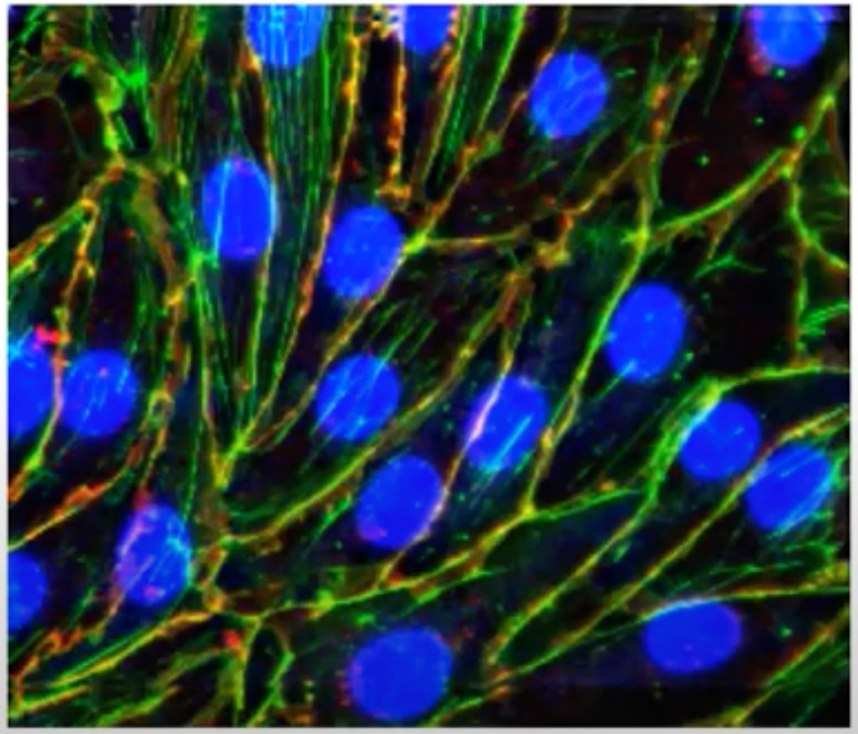
Rob ter Horst

Ljosa, et al. J Biomol Screening 2013

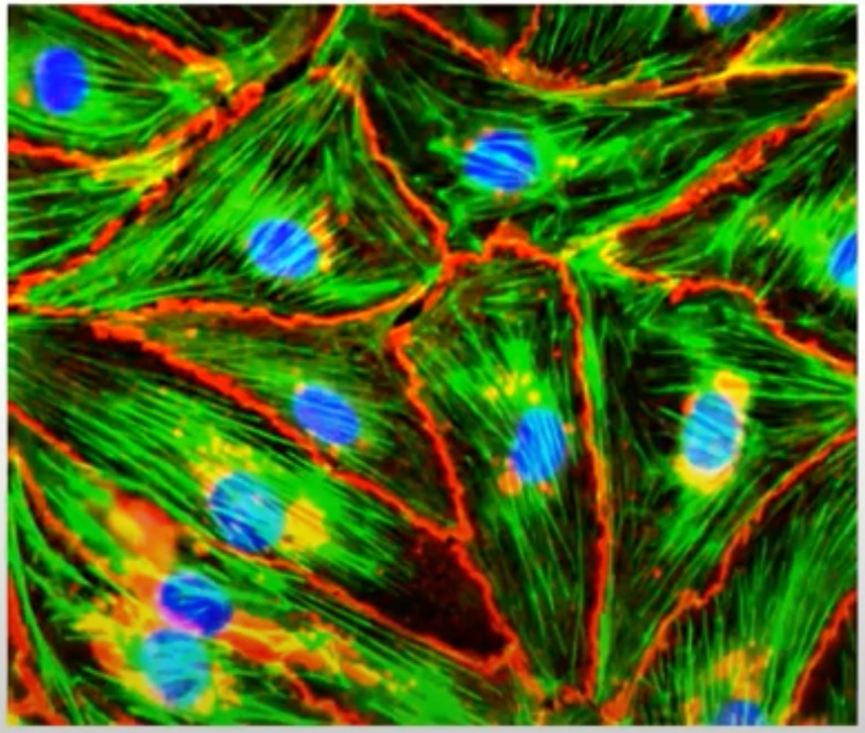
AstraZeneca

Morphological profiling can identify drugs for disease

Healthy

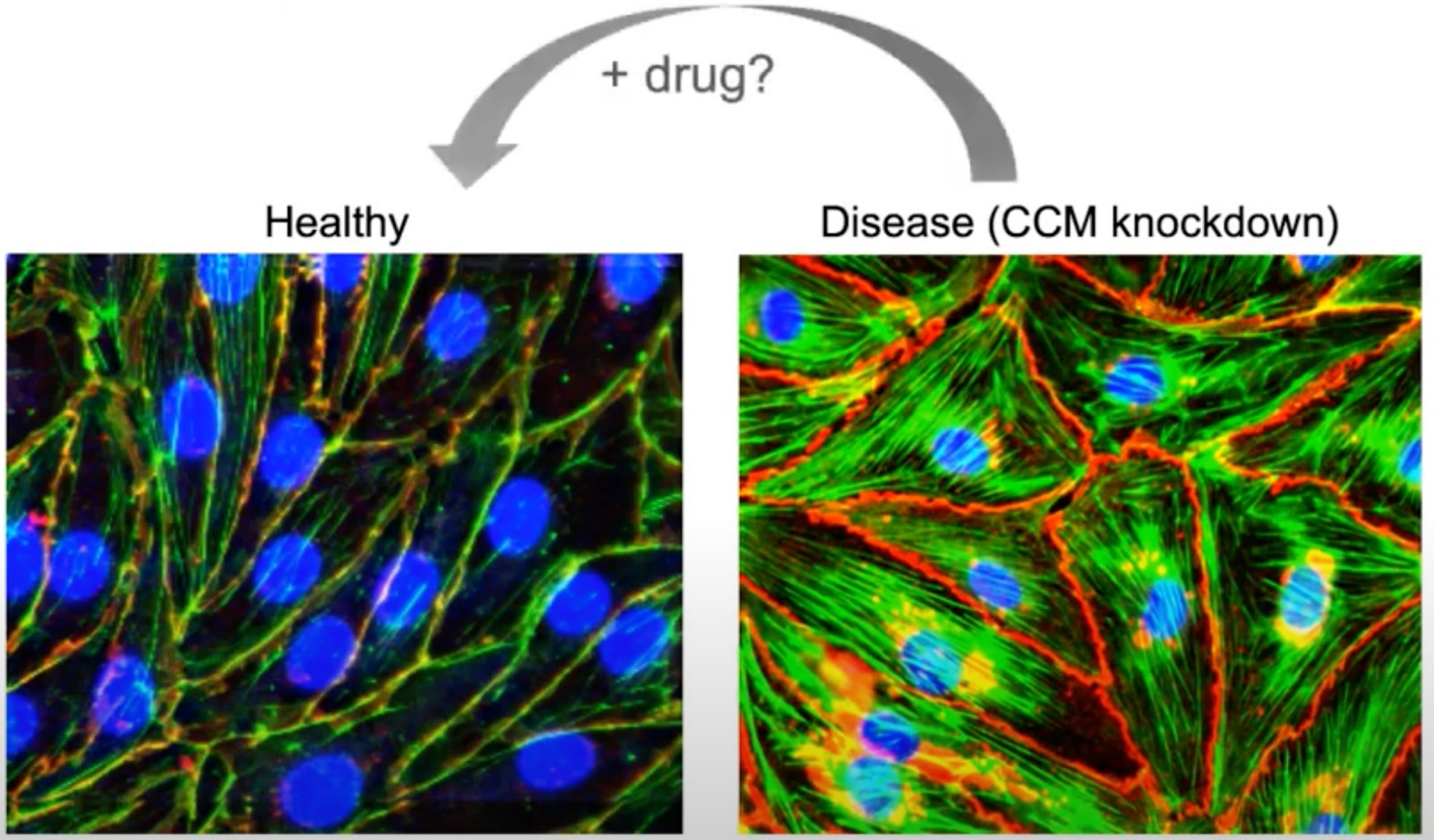


Disease (CCM knockdown)



Gibson, et al. Circulation 2015

Morphological profiling can identify drugs for disease



Gibson, et al. Circulation 2015