Literature Review 101 - Advanced Microscopy and Image Analysis

1. Why use advanced microscopy and image analysis at all?
2. What is a microscope exactly?
3. What is an image? And what can we analyze?
4. Parting thoughts about the assigned paper

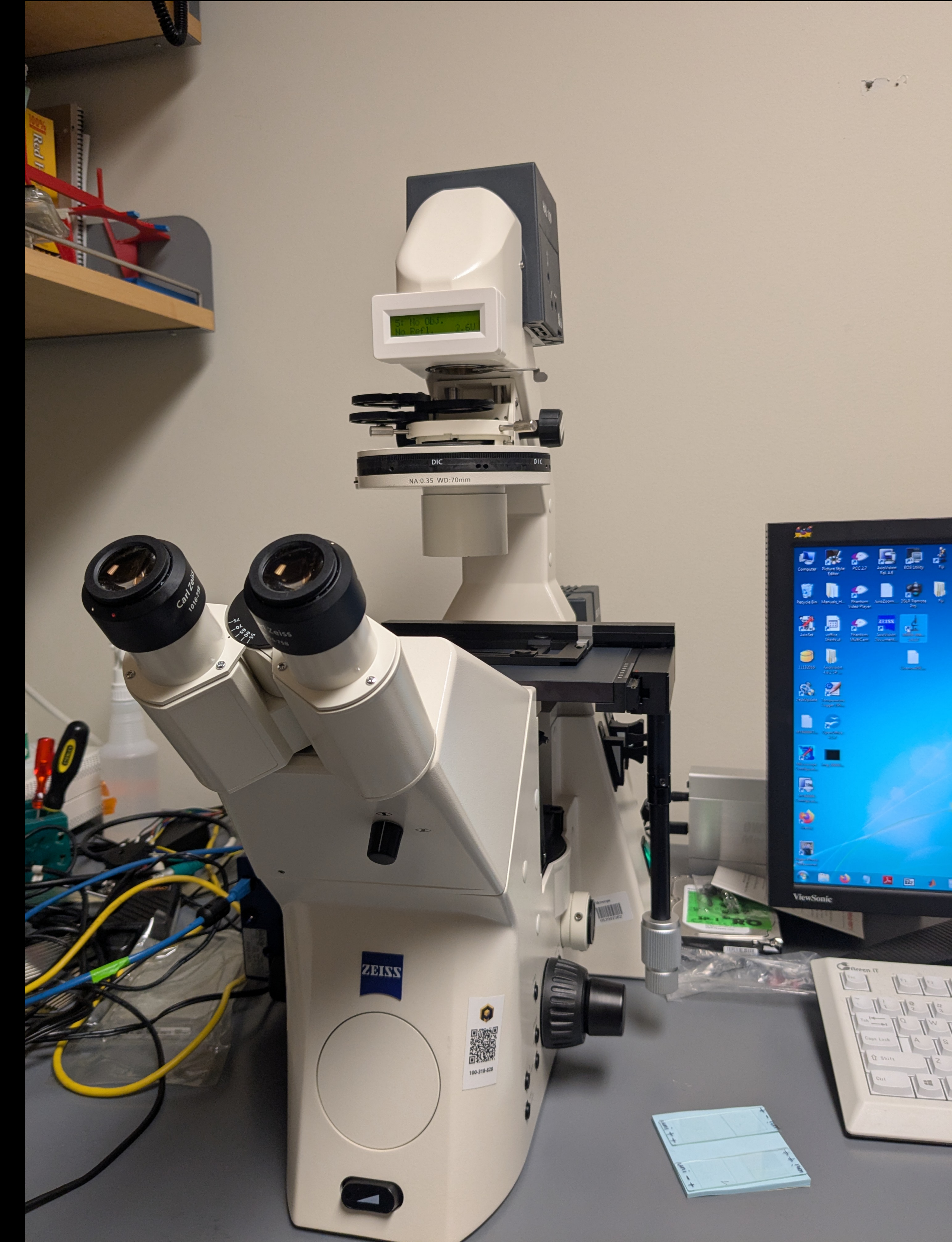
Ximena Garcia Arceo
ximena.GarciaArceo@ucsf.edu
Postdoc, Wallace Marshall lab

Thursday April 23, 2026

Who was used a microscope for research?

Why use a (light) microscope?

What kinds of questions/phenomena are best suited for microscopy?



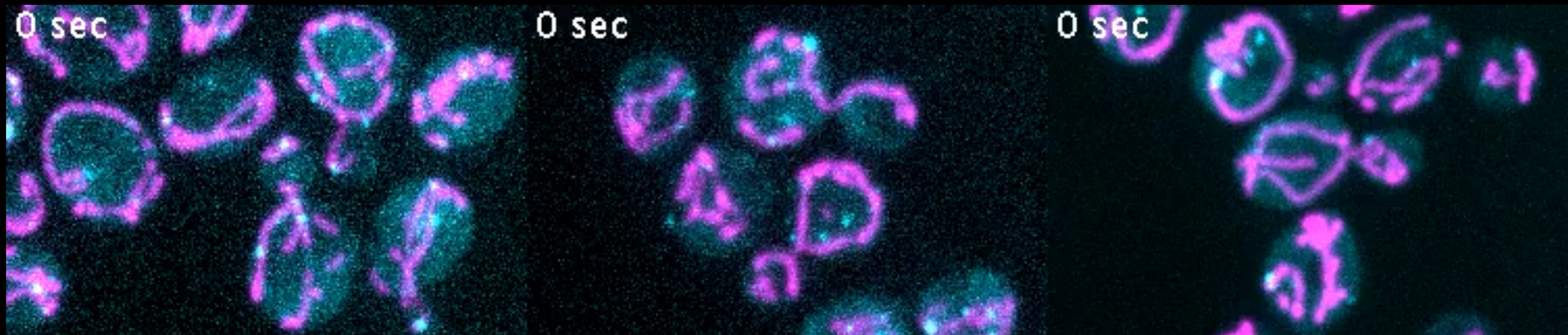
One of 3 “big” microscopes in Marshall lab

Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

- Ex. During my PhD, I studied mRNA localization to mitochondria
    - mRNA diffuse on and around mitochondria in *living* cells
    - Microscopy gave me absolute numbers whereas sequencing reports relative localization, & allows me to quantify mitochondrial volume

Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

- Ex. During my PhD, I studied mRNA localization to mitochondria
  - mRNA diffuse on and around mitochondria in *living* cells
  - Microscopy gave me absolute numbers whereas sequencing reports relative localization, & allows me to quantify mitochondrial volume
  -



Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

- Ex. During my PhD, I studied mRNA localization to mitochondria
    - mRNA diffuse on and around mitochondria in *living* cells
    - Microscopy gave me absolute numbers whereas sequencing reports relative localization, & allows me to quantify mitochondrial volume

- Phenomena of interest is dynamic, takes place every few seconds or minutes
- <u>Where</u> or <u>how big</u> something is
- <u>How often</u> something happens
- Motion/motility
- Co-localization
- Absolute numbers

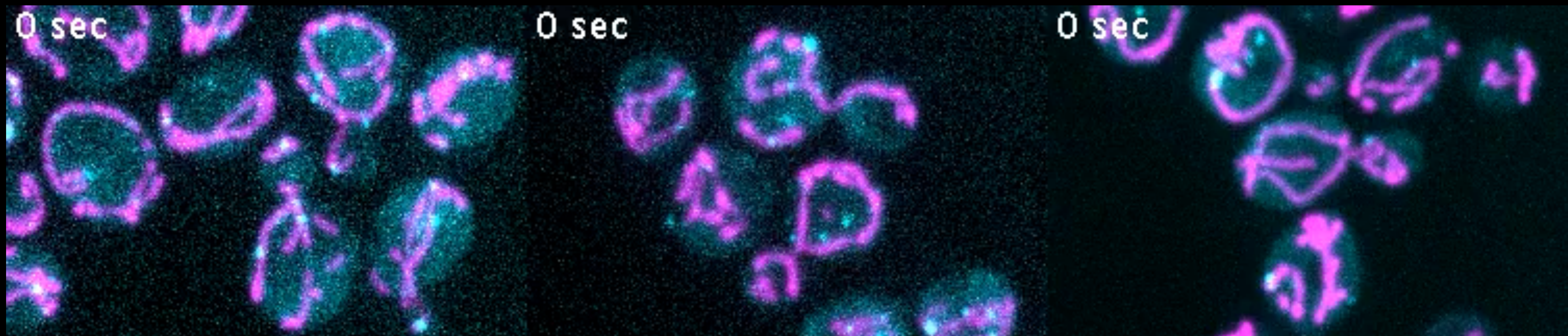
What are some tradeoffs for using microscopy instead of other techniques?

- Ex. During my PhD, I studied mRNA localization to mitochondria
    - Image analysis is usually low throughput compared to sequencing
    - Troubleshoot fluorescent proteins or dyes

-

Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

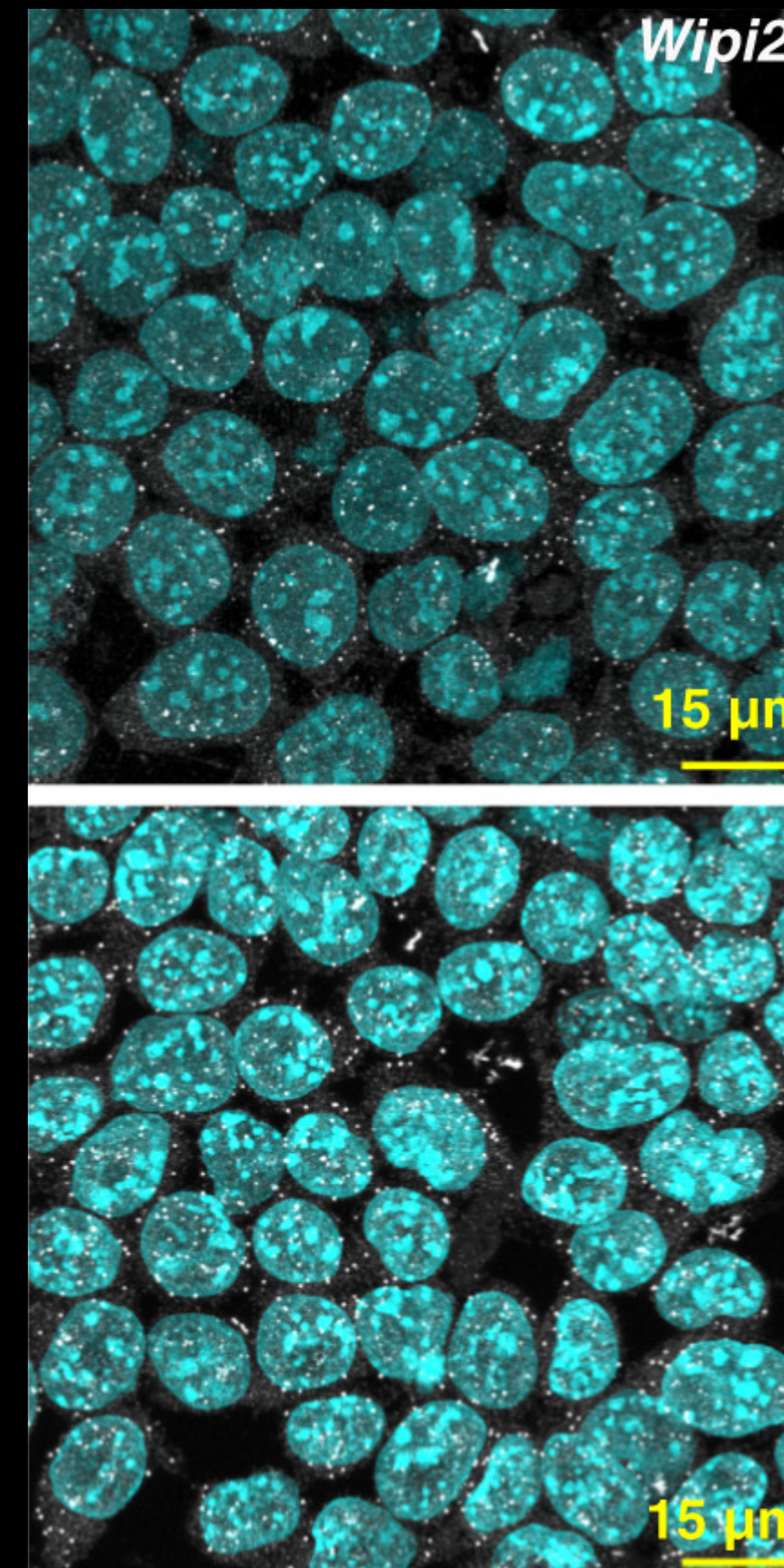
- Phenomena of interest is dynamic, takes place every few seconds or minutes
- Where or how big something is
- How often something happens
- Motion/motility
- Co-localization
- Absolute numbers



Why use a (light) microscope for an experiment? What kinds of questions are best suited for (light) microscopy?

- Phenomena of interest is dynamic, takes place every few seconds or minutes
- Where or how big something is
- How often something happens
- Motion/motility
- Co-localization
- Absolute numbers

Noise in # mRNAs/cell
for 9 genes in +/- drug

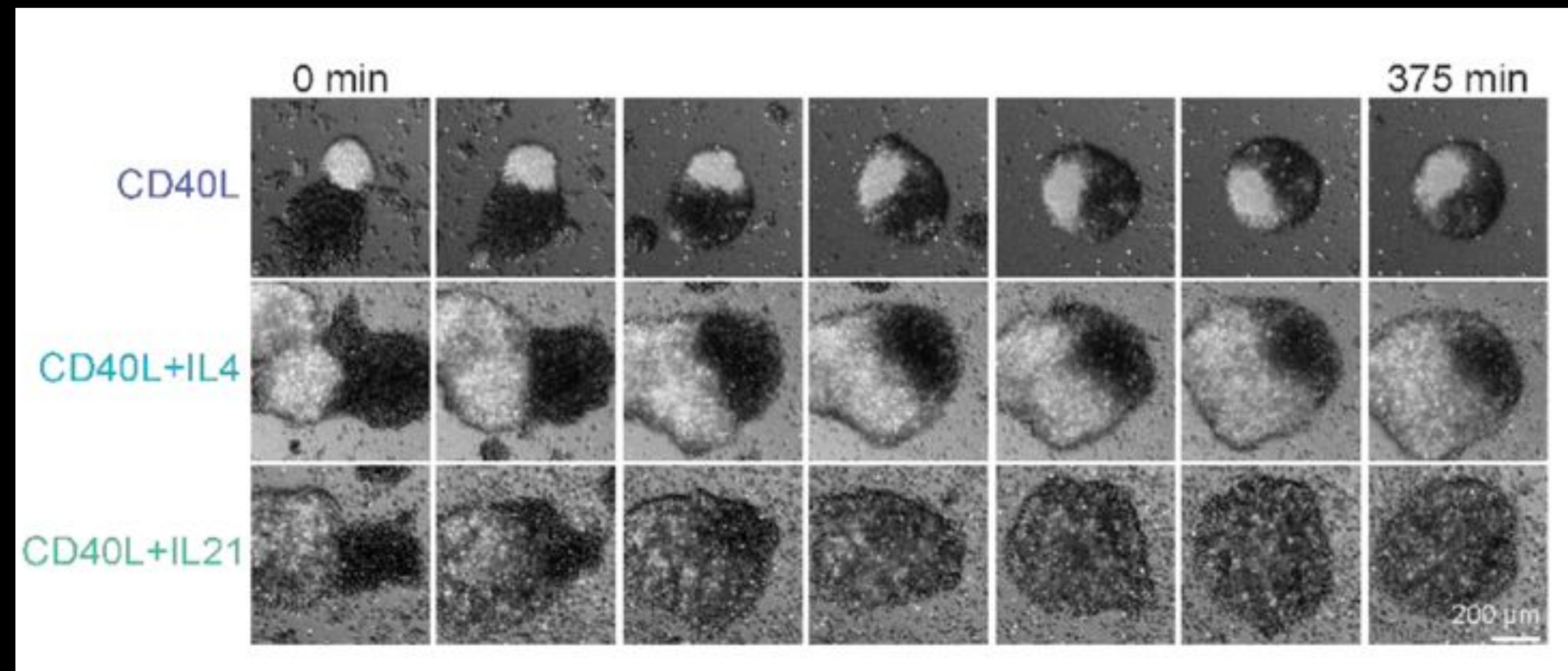


Khetan, Calia, Zuckerman et al (2024)

Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

- Phenomena of interest is dynamic, takes place every few seconds or minutes
- Where or how big something is
- How often something happens
- Motion/motility
- Co-localization
- Absolute numbers

Individual cell motility and populations “mixing” over time in +/- cytokines

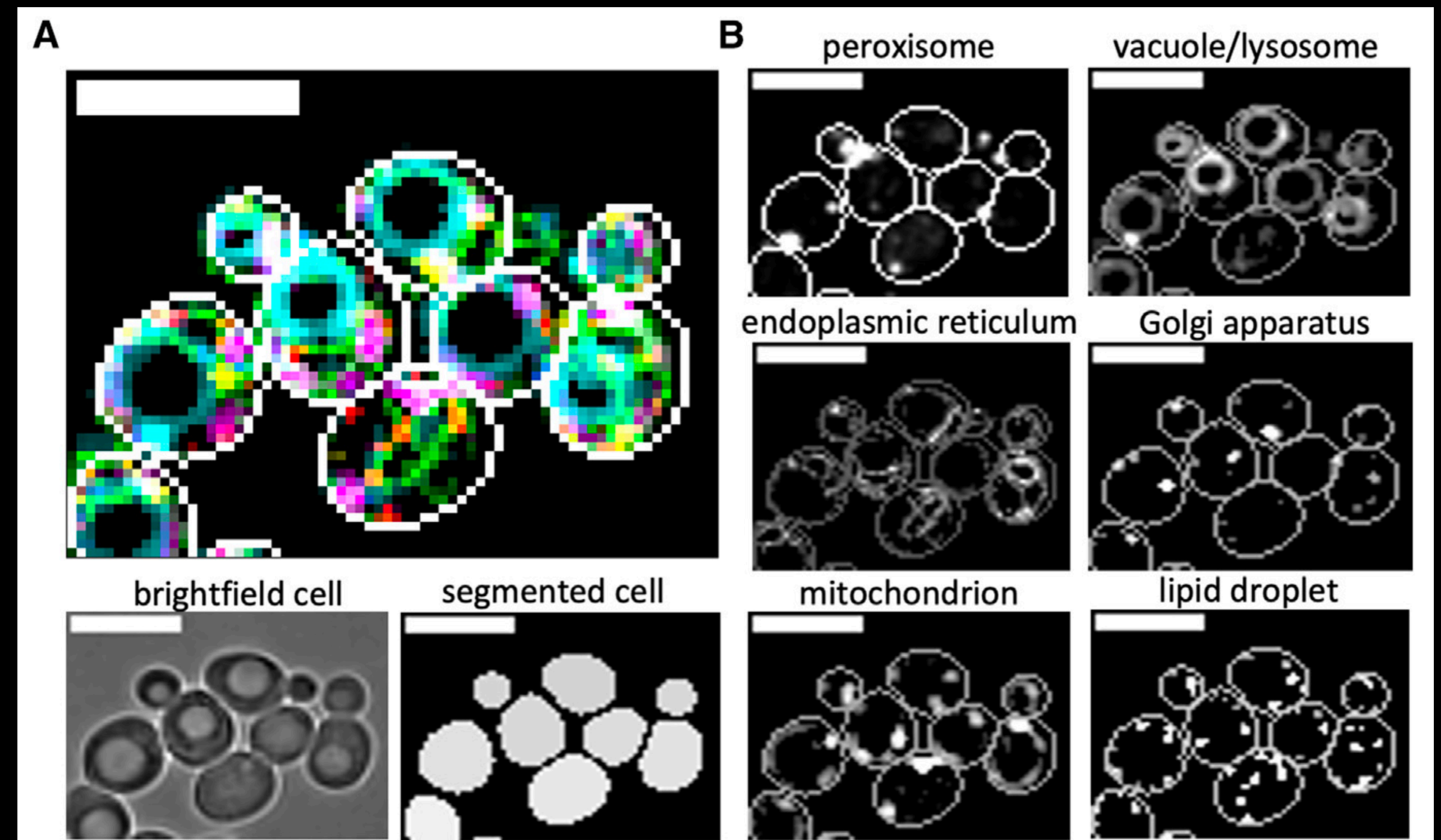


Ghose et al (2025)

Why use a (light) microscope for an experiment?
What kinds of questions are best suited for (light) microscopy?

- Phenomena of interest is dynamic, takes place every few seconds or minutes
- Where or how big something is
- How often something happens
- Motion/motility
- Co-localization
- Absolute numbers

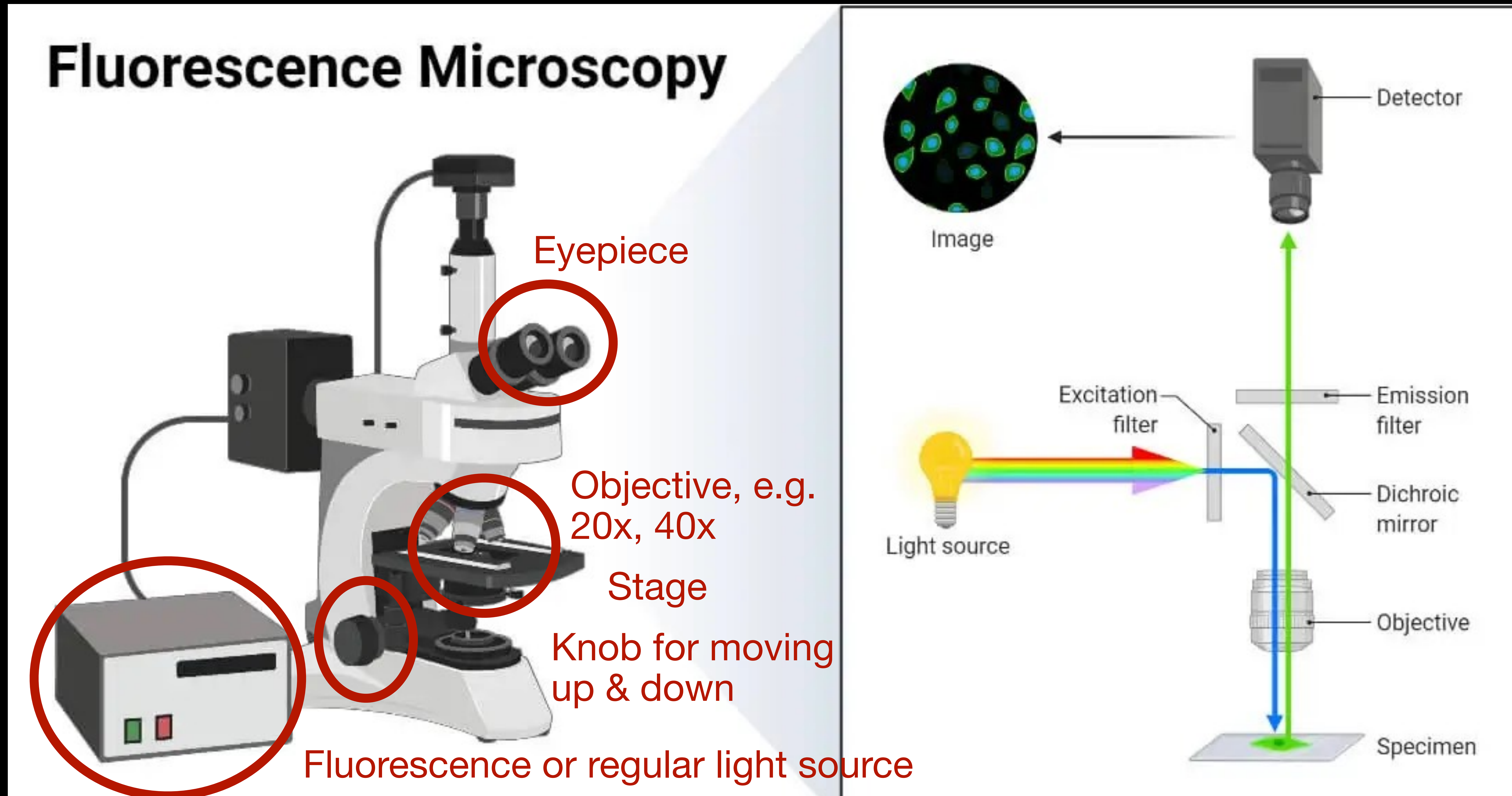
Visualized all major organelles in diverse nutrient, growth and drug conditions



Live cells - peroxisomes (blue), vacuoles (teal), endoplasmic reticulum (green), Golgi apparatus (yellow), mitochondria (magenta), lipid droplets (red)

Wang et al (2025)

What is a microscope exactly?

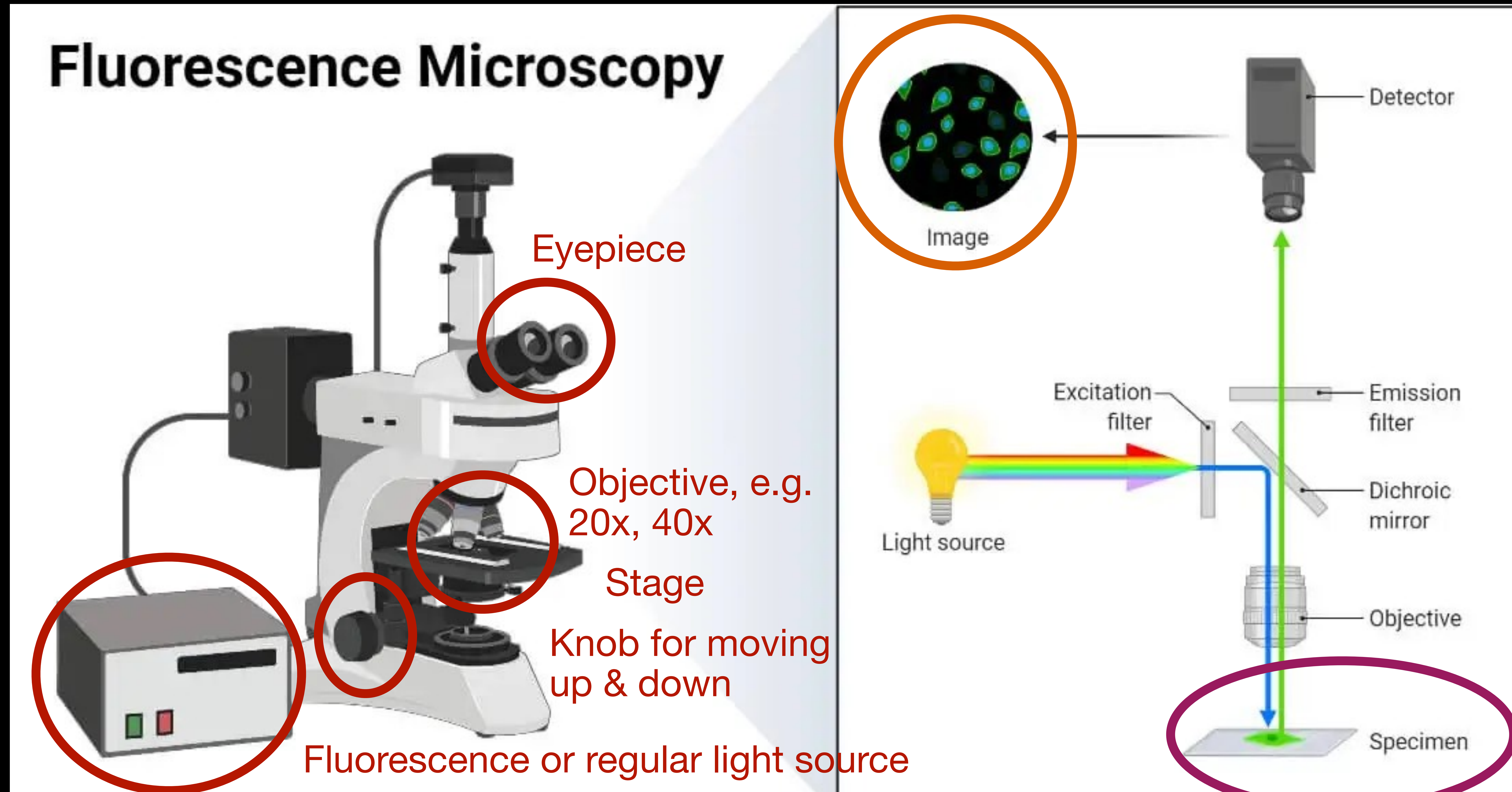


Minimal or beginner's microscopy

https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/

(Light) microscopy “modules”

Processing, quantification, making figures

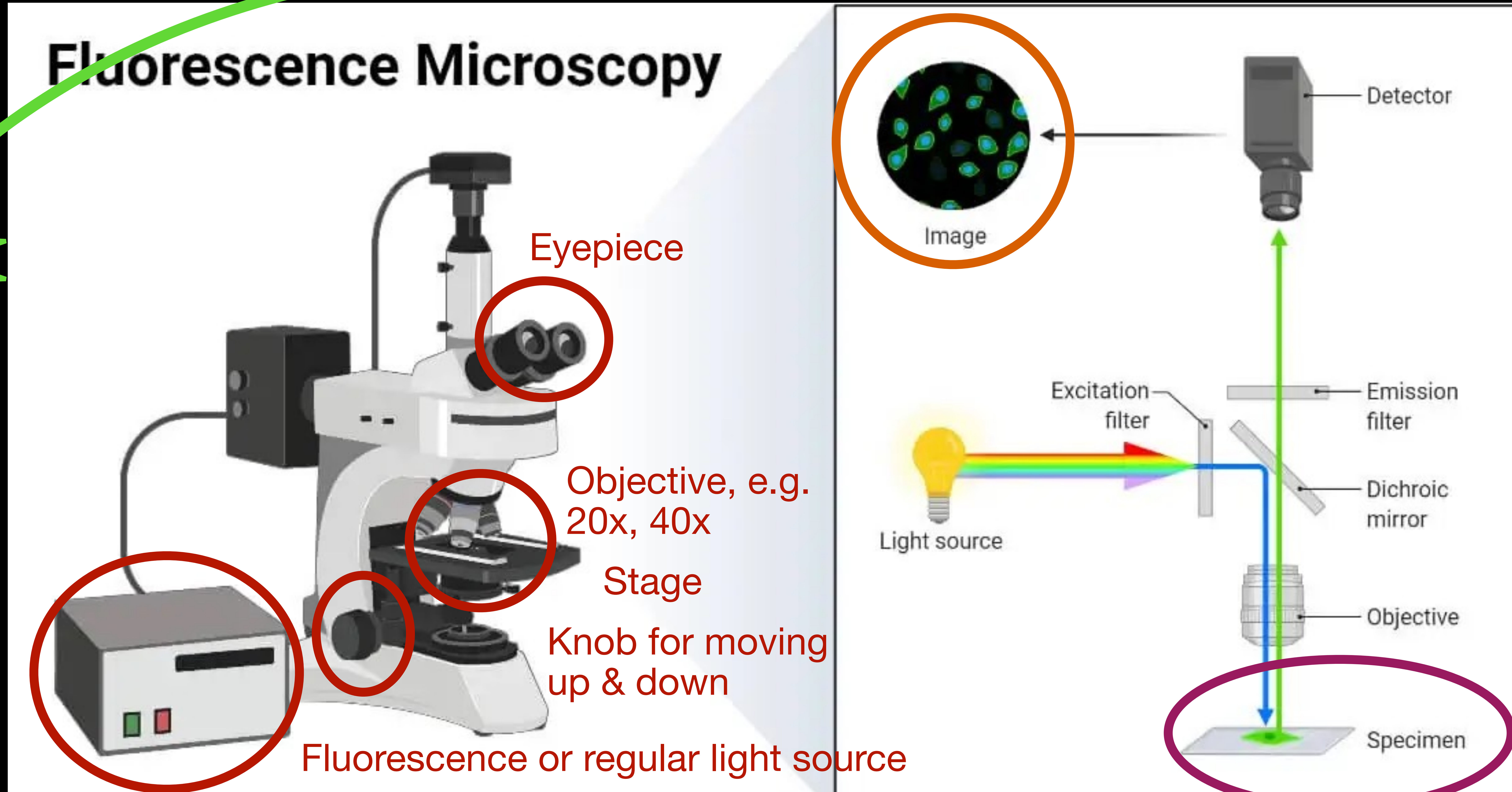


Microscope experiments

Mol bio, cell bio, biochemistry, engineering

https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/

(Light) microscopy “modules”



https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/

Some challenges can be redressed, some you have to live with

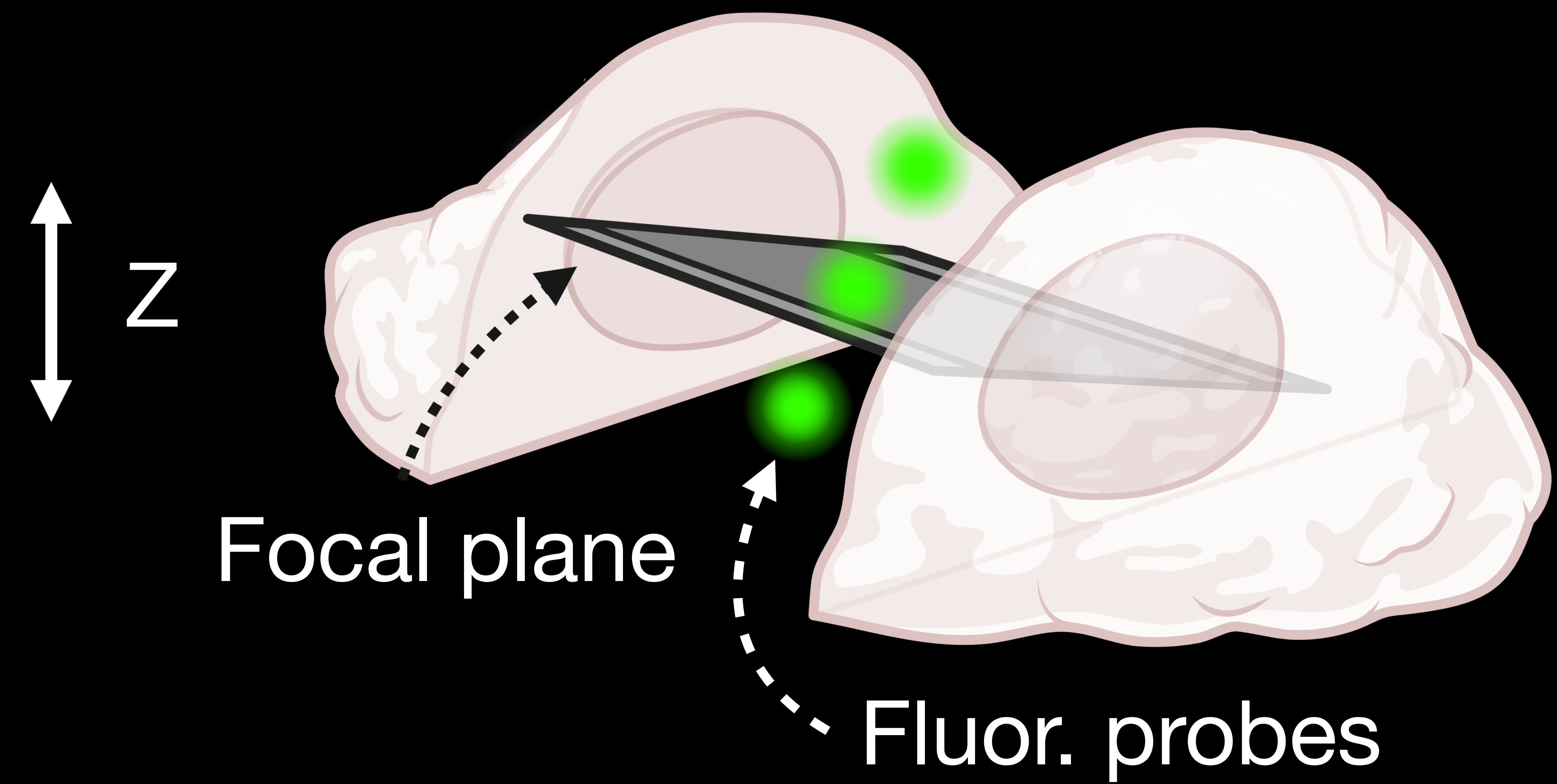
- “Better” image analysis is often not the answer
- I’m assuming that dyes, probes, and media are already as optimal as is feasible

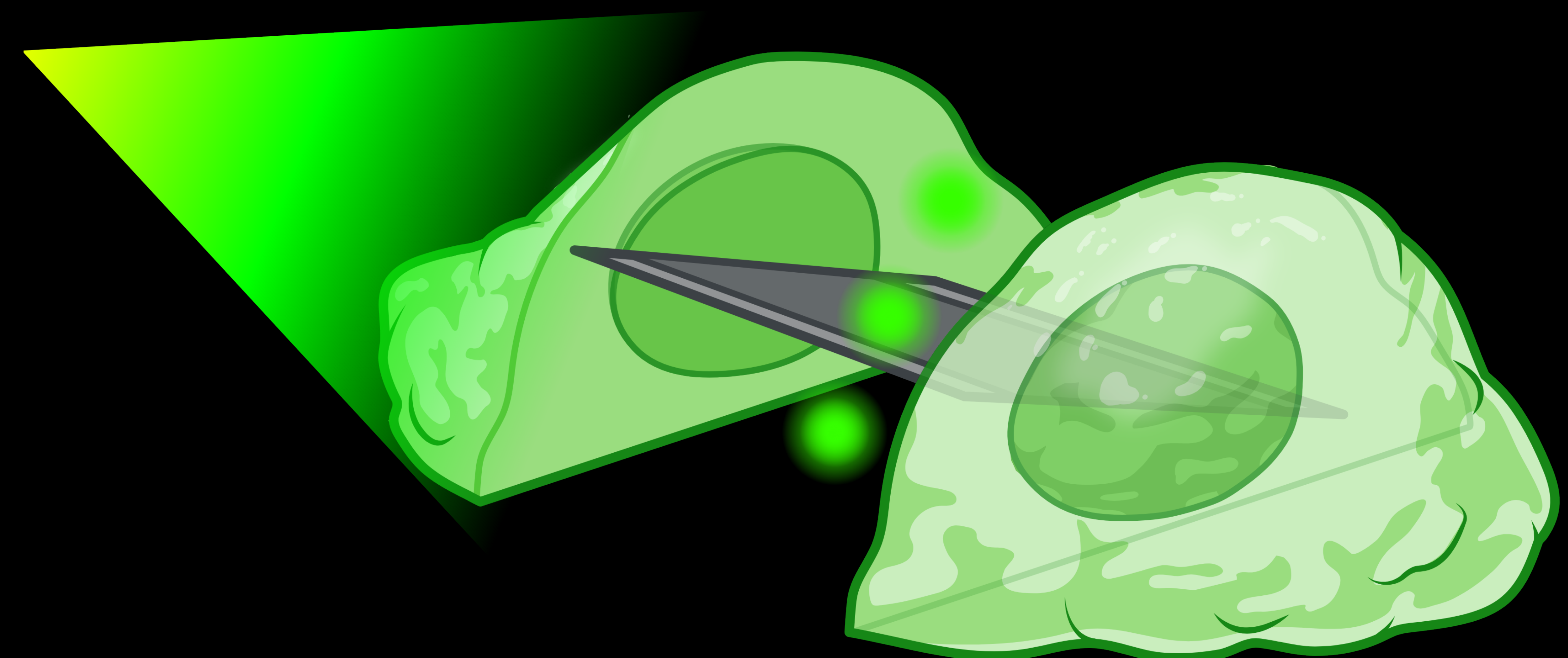
- Photobleaching of fluorescent dye or protein
  - Possible solution: decrease laser intensity, don’t take images of the same cells over and over again, ★ only use the most expensive and specialized microscope ever invented ★
- Photodamage of specimen (fluorescence)
  - Same as above
- Out of focus light (a type of signal to noise ratio issue)
  - Hardware solutions: high numerical aperture, confocal or structured illumination (i.e. use a different microscope)
- Backscattering (only an issue if your lab builds their own microscope)
- Samples move
- Microscope environment =\= Specimen’s preferred environment
- *What about you all?*

} Shorter experiments, Engineer the slides, or Improve microscope chamber

A note on out of focus light

- The laser hits the entire sample, not just the z-plane in focus





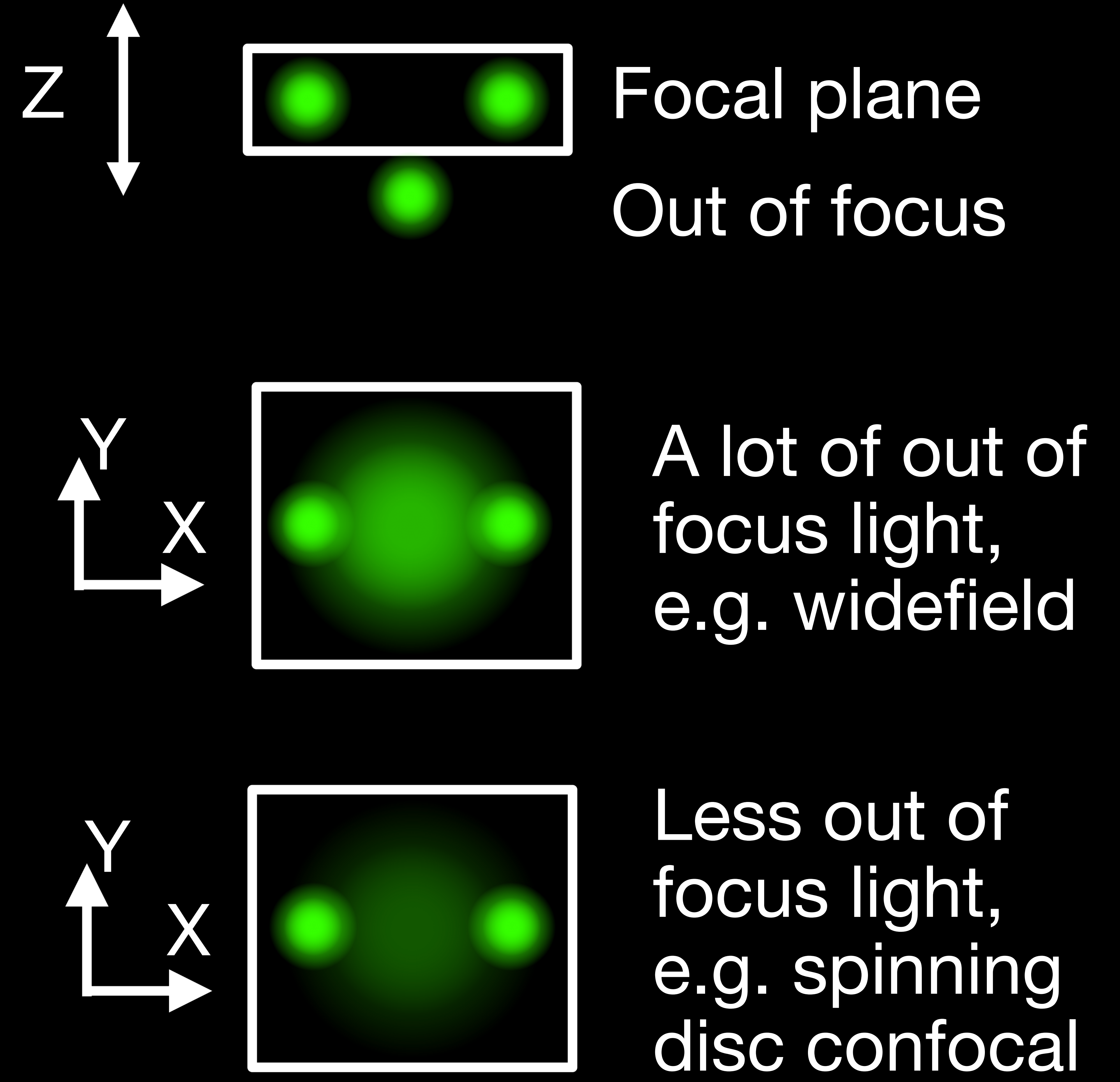
A note on out of focus light

- The laser hits the entire sample, not just the z-plane in focus
- Out of focus objects look faint, big and blurry in the detector

Z

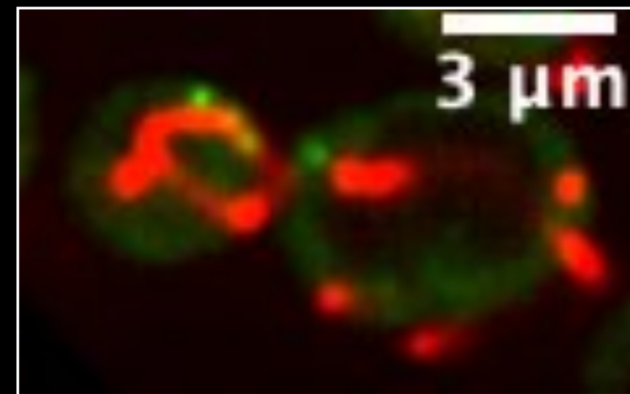
Focal plane

Fluor. probes

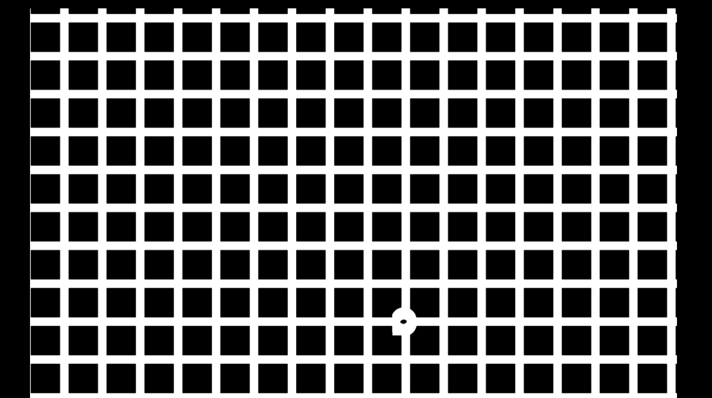


Common image processing techniques are the result of foundational mathematical operations

Math is to image processing as molecular biology/biochemistry is to PCR, cloning, running a gel, etc



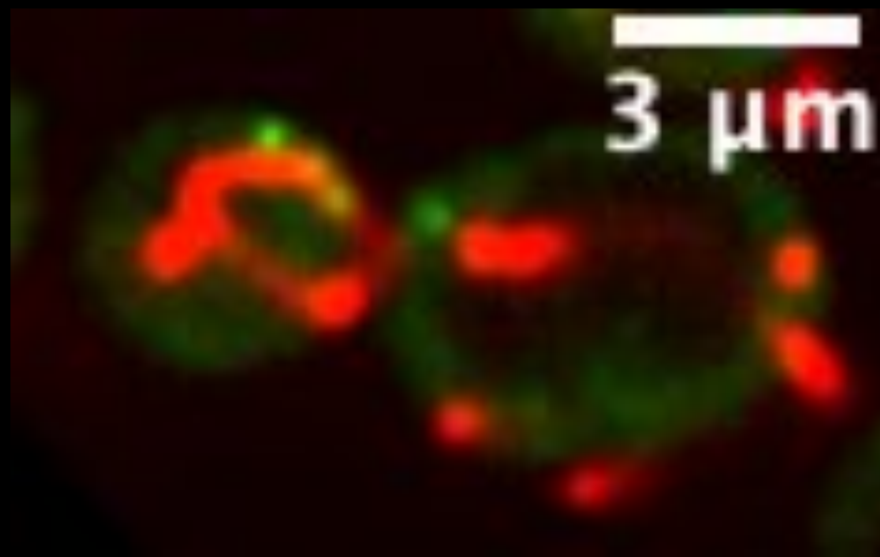
- Noise reduction
- Edge detection
- Face or object detection
- Enhanced contrast/sharpened
- Segmentation
- Histogram equalization (composite of multiple color channels)



- Convolution
- Deconvolution
- Linear filters (order of operations do not matter)
- Nonlinear algorithms (order of operations matters)

What is an image?

- Photons hit the detector and generate an image
- More photons at a single spot = brighter pixel
- Image = matrix or array of pixels
- 16-bit image, each pixel can have value between 0 and 2^(16)
- Voxel = 3-D pixel



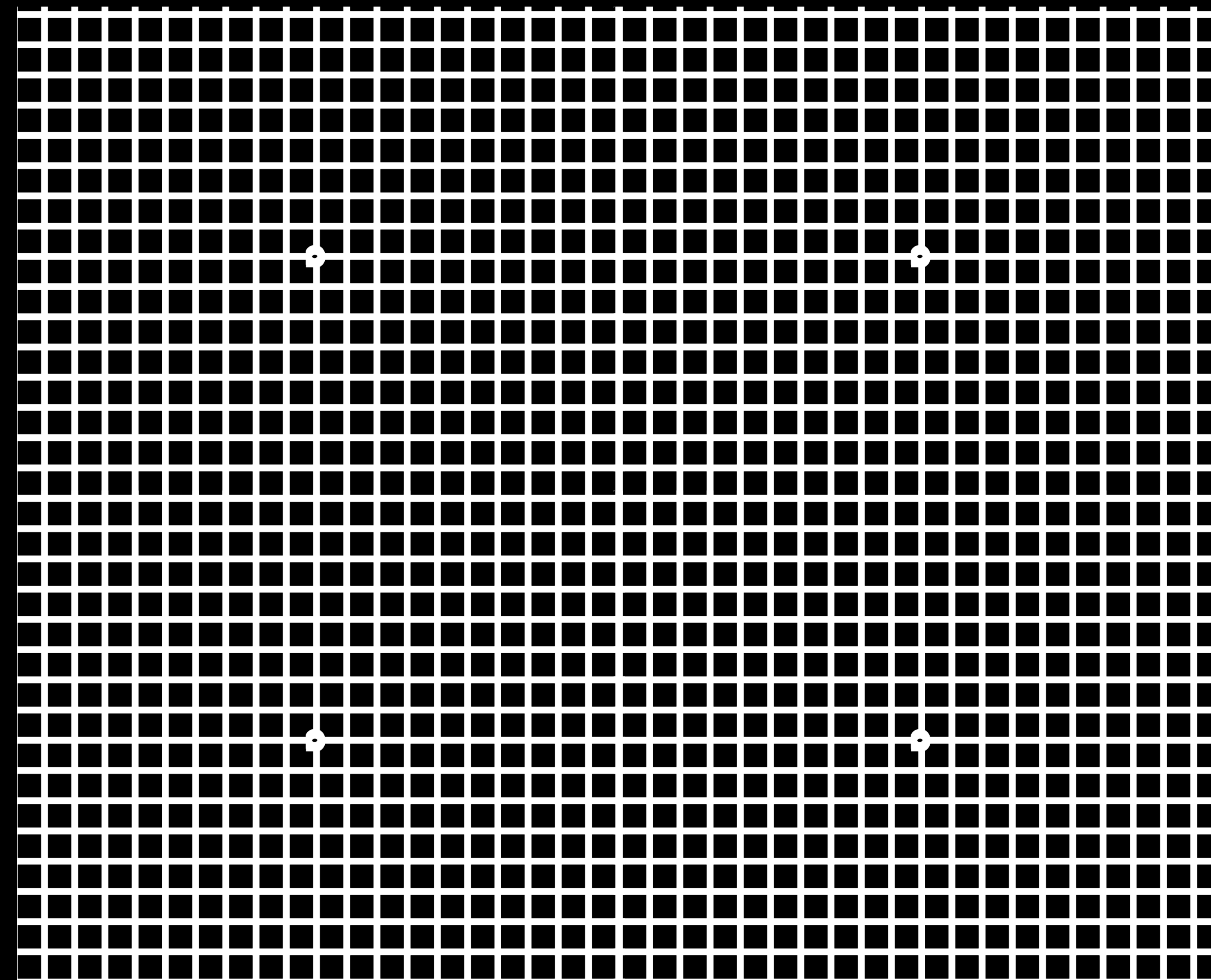
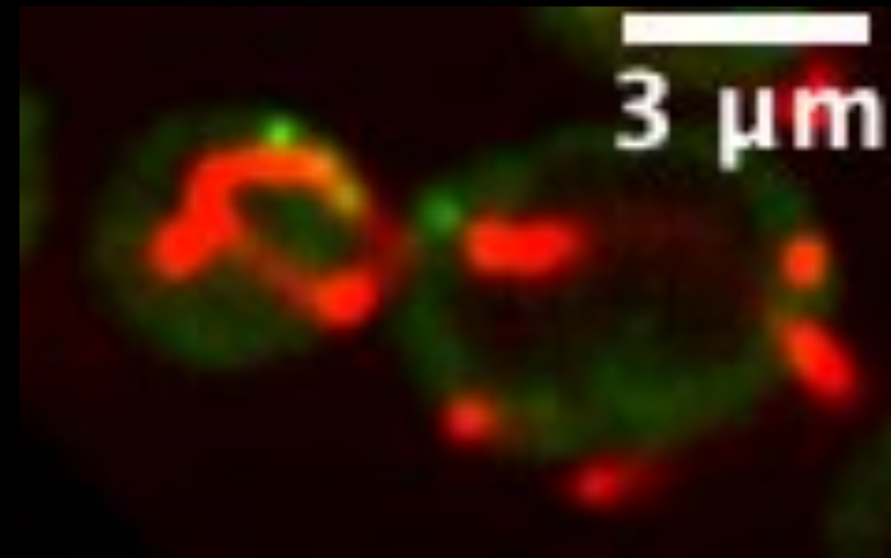
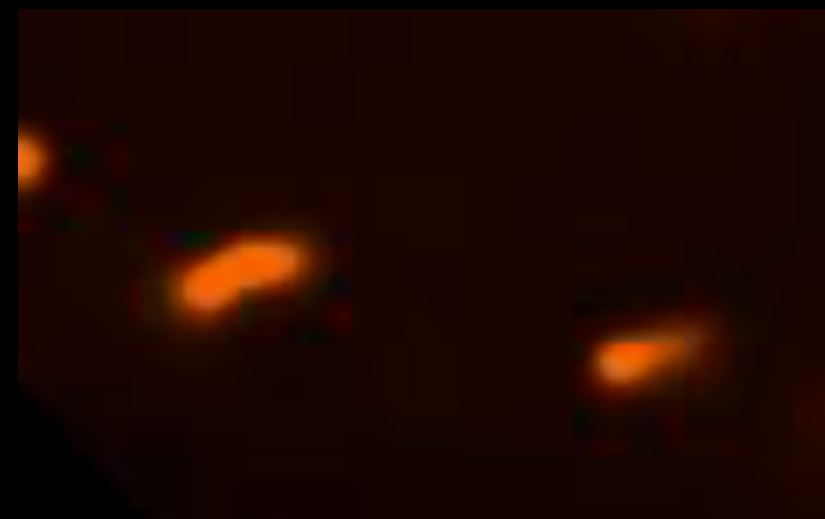
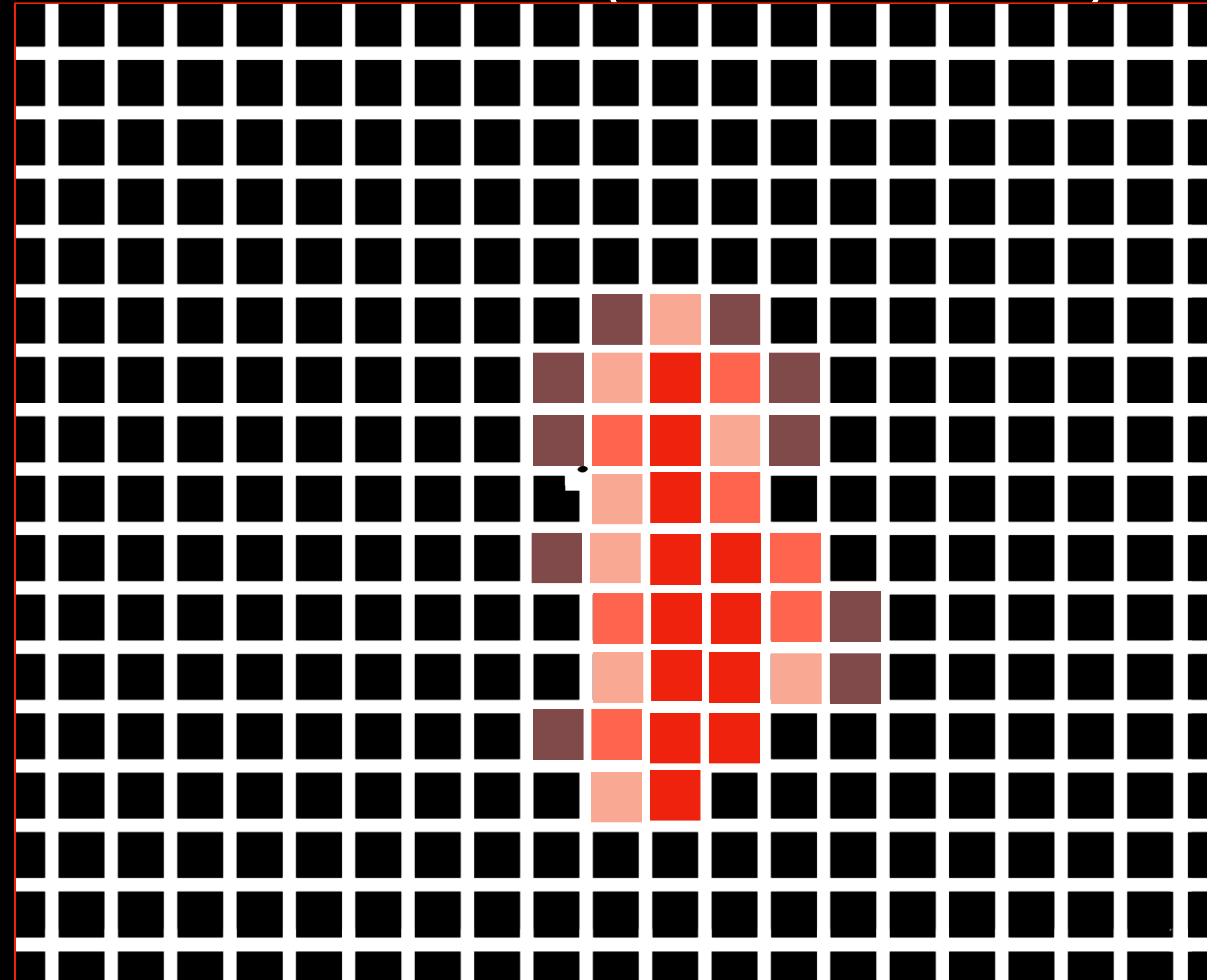


Image processing and analysis = performing math operations on a matrix

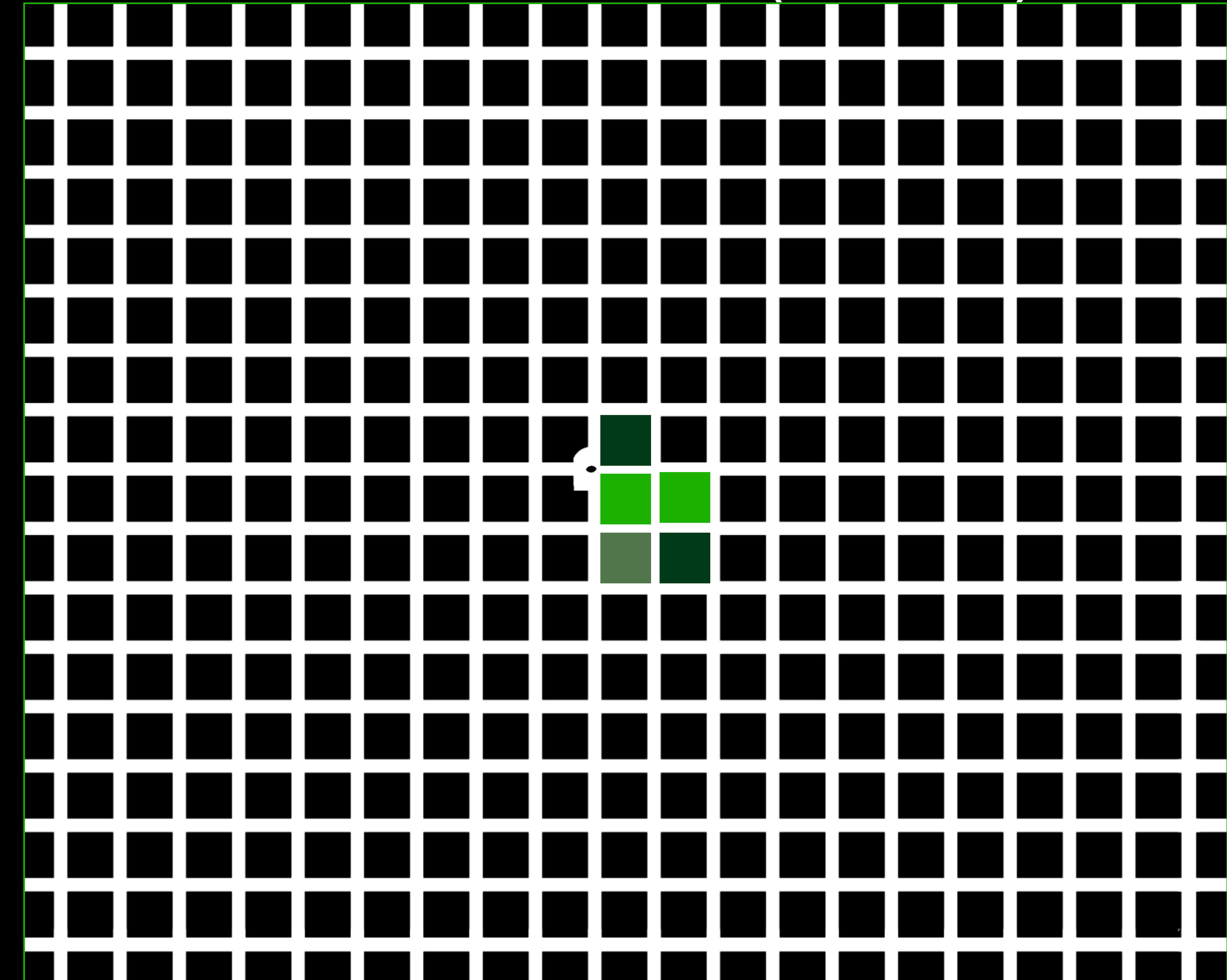




Red channel (mitochondria)

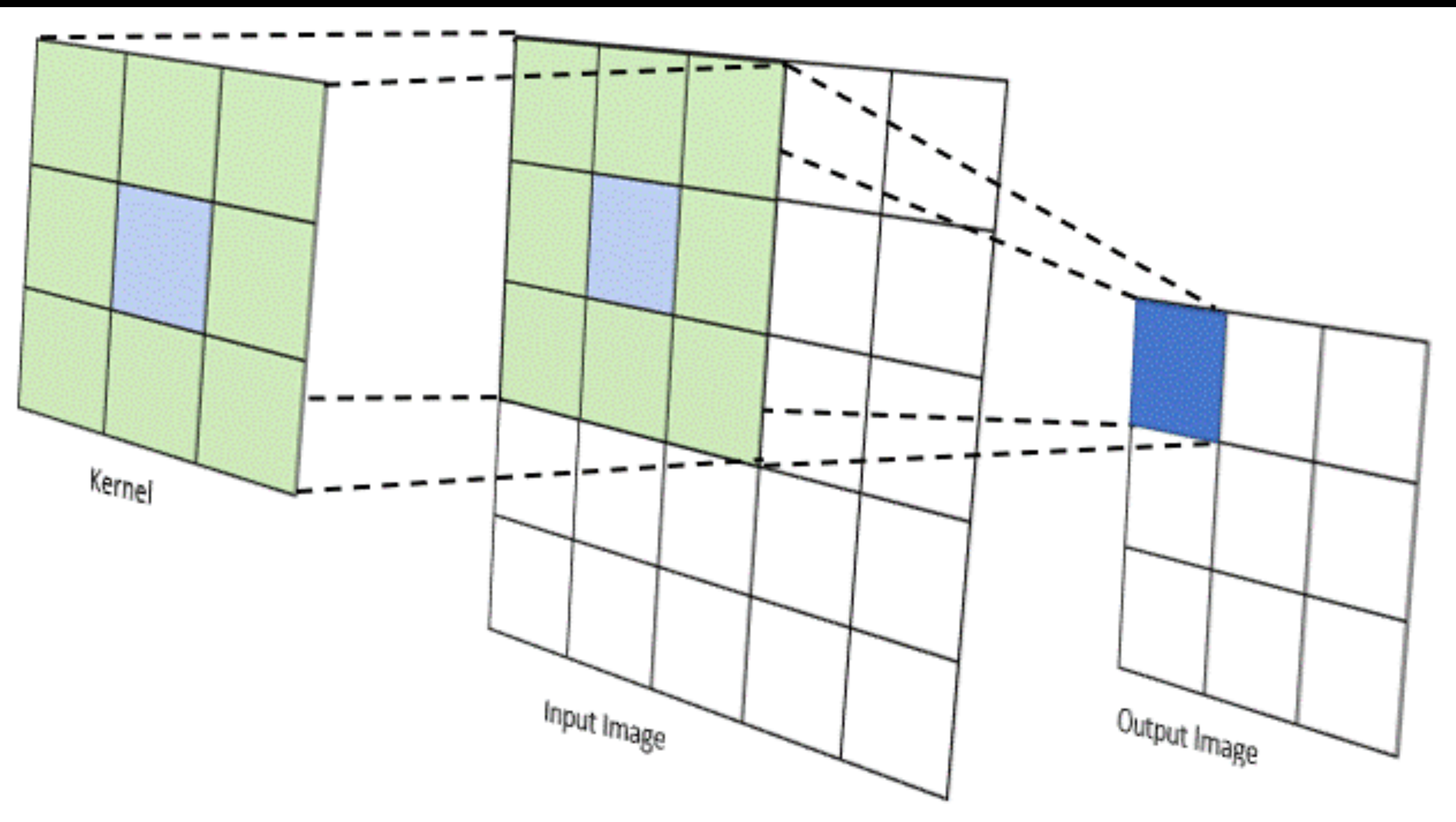


Green channel (mRNA)



Kernels!

- Filters are defined by their kernel
-

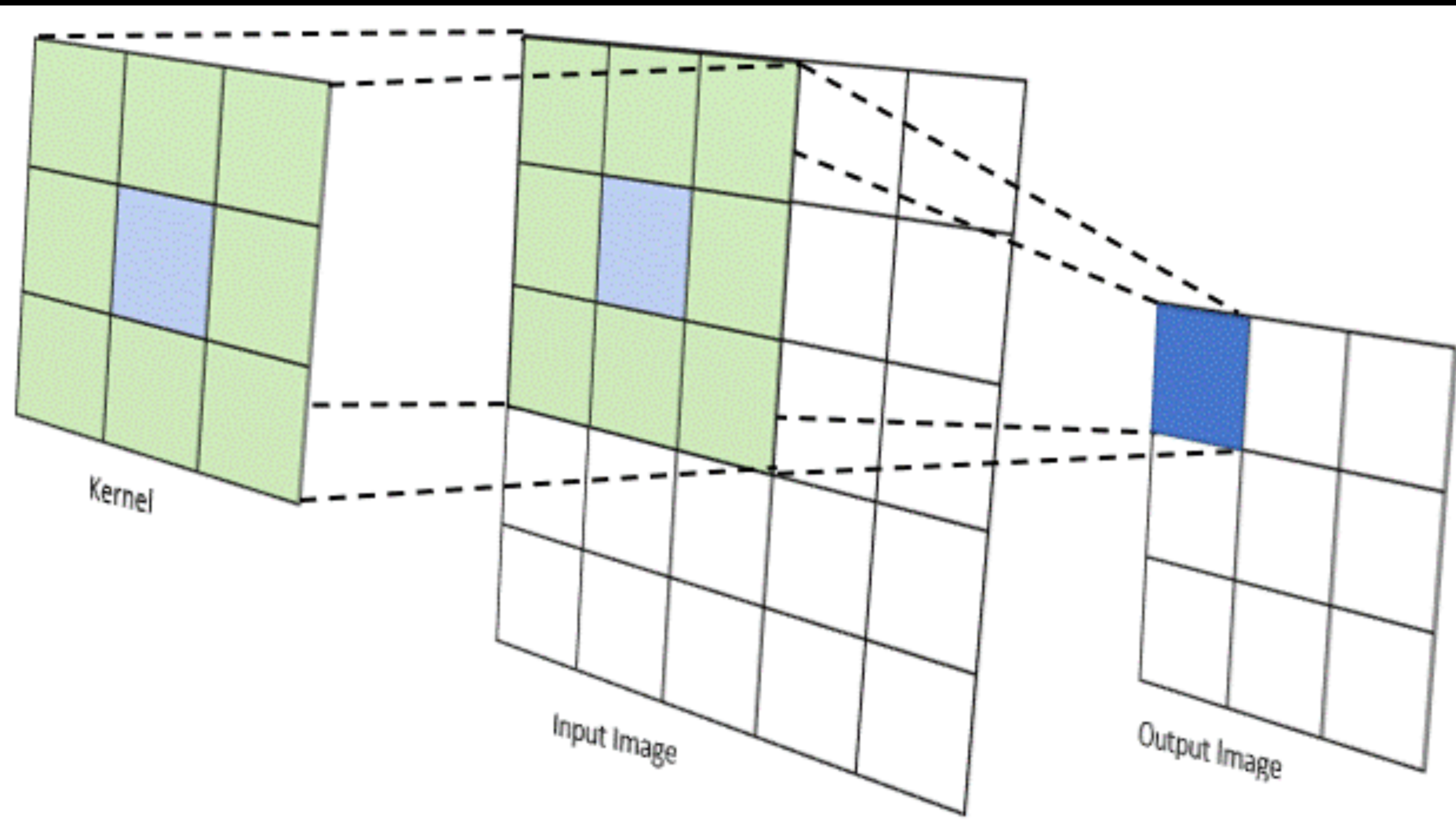


https://medium.com/@timothy_terati/image-convolution-filtering-a54dce7c786b

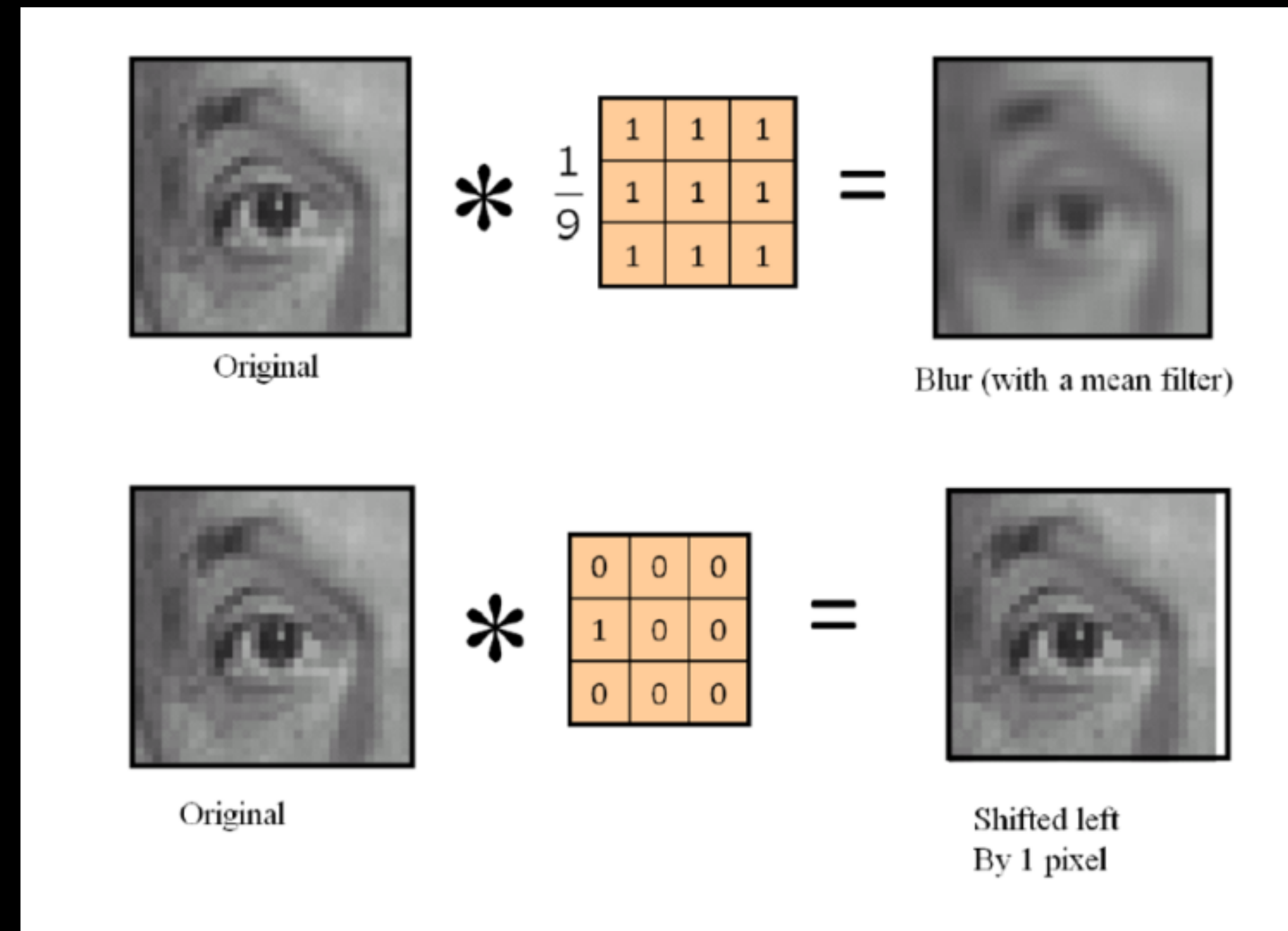
https://m.blog.naver.com/framkang/220561249726?view=img_1

Kernels!

- Filters are defined by their kernel
- Visit https://setosa.io/ev/image-kernels/



Input image | Kernel | Output

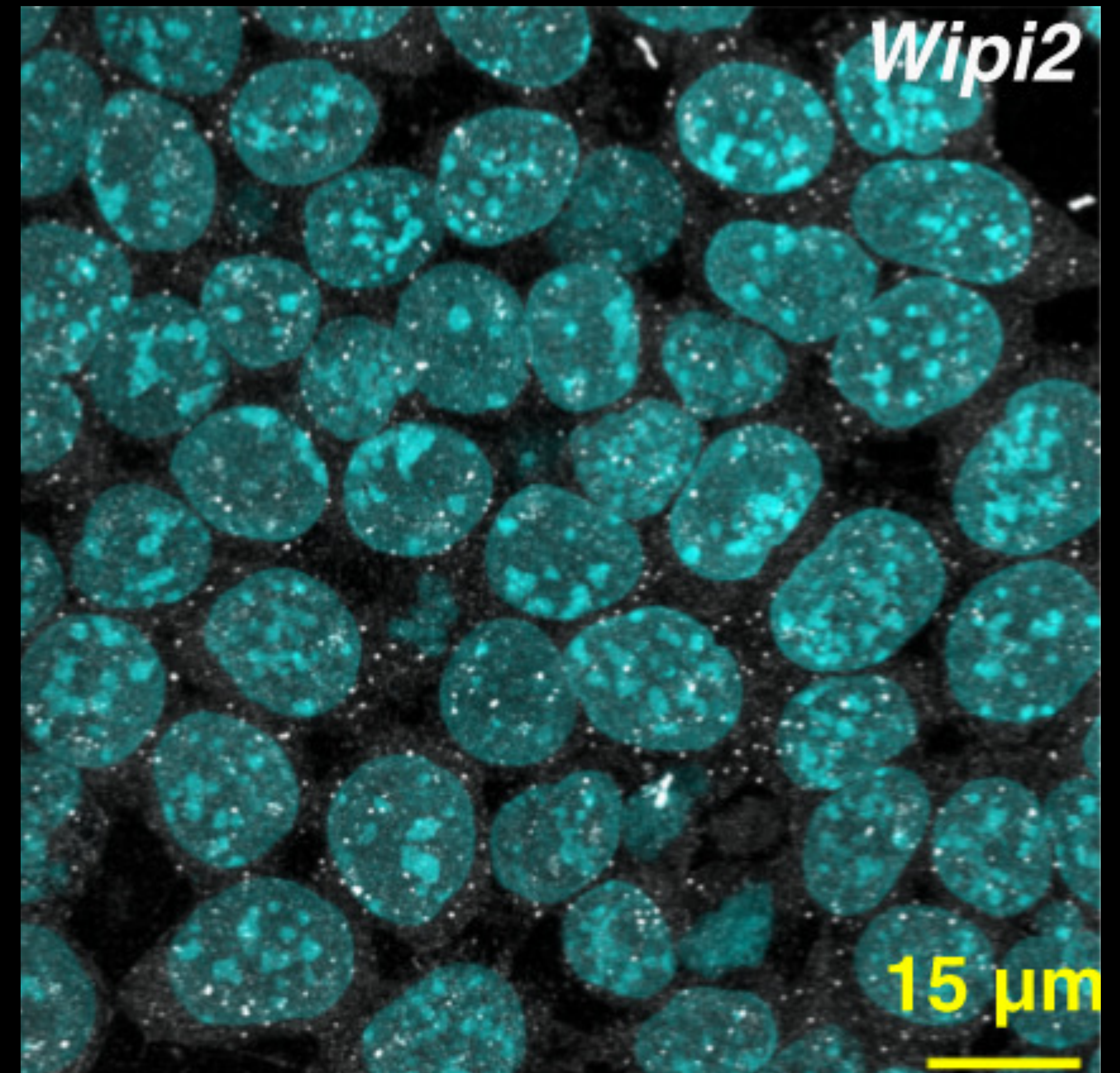


https://medium.com/@timothy_terati/image-convolution-filtering-a54dce7c786b

https://m.blog.naver.com/framkang/220561249726?view=img_1

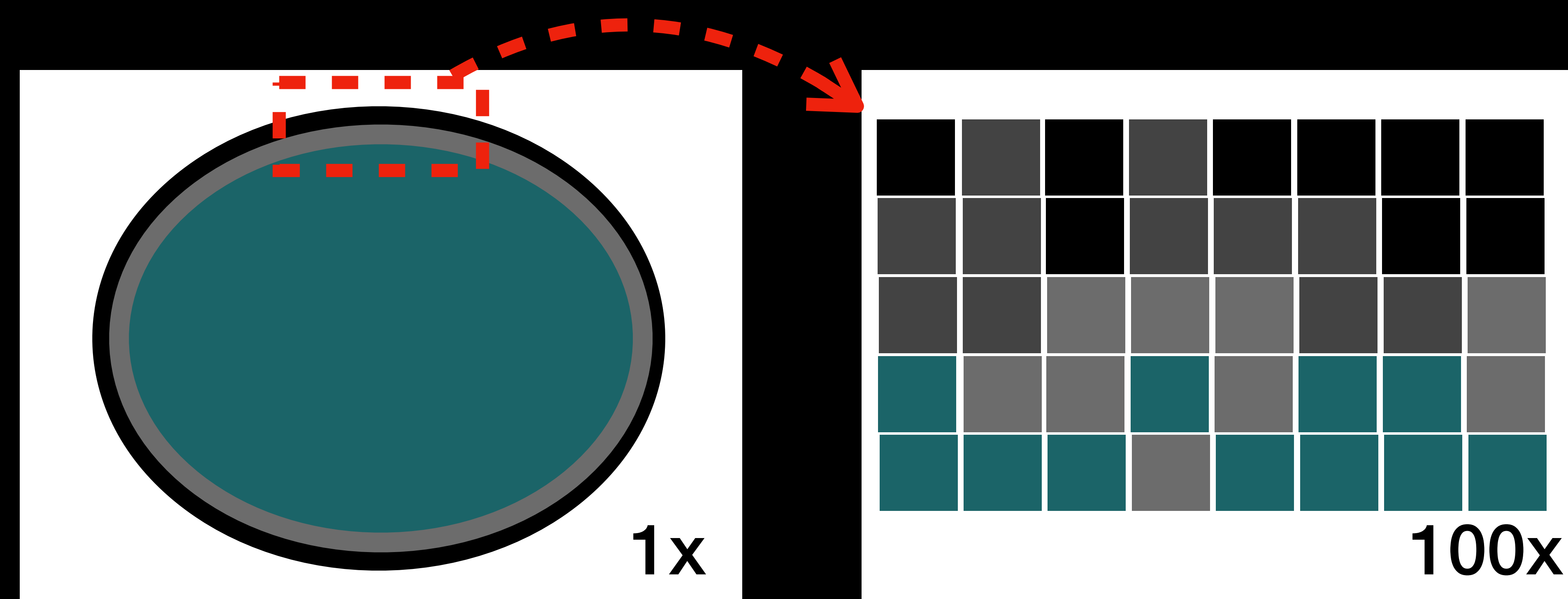
What kind of image processing techniques or mathematical operations would help me build a cell segmentation pipeline?

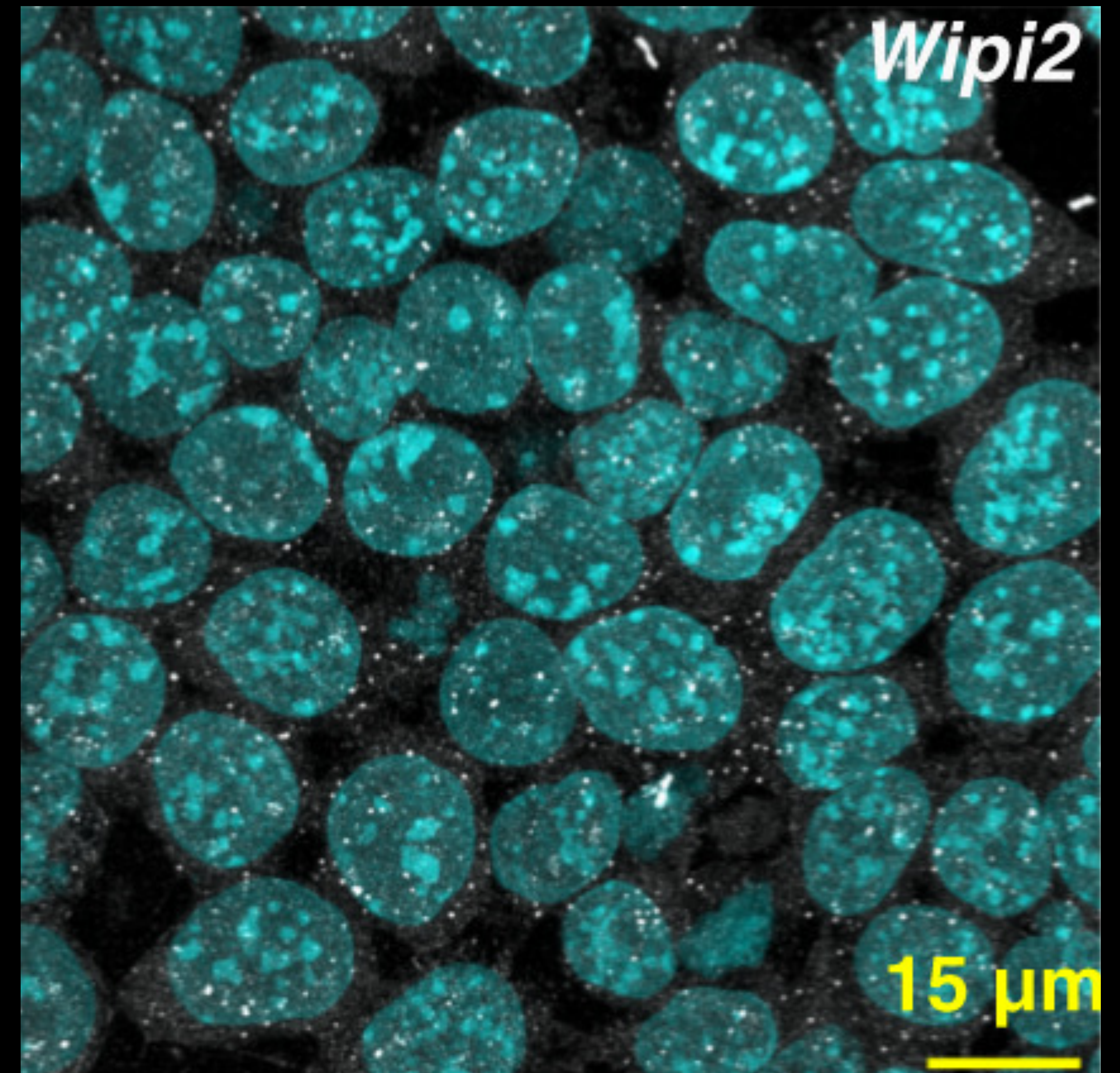
-



What kind of image processing techniques or mathematical operations would help me build a cell segmentation pipeline?

- Enhance contrast (within > 2 pixels)
- Local smoothing (within 2 pixels)
- Edge detection
- We are looking for a continuous curve where pixel values got from light blue to black
- Curves can touch but not overlap





References

- Microscopy
  - https://zeiss-campus.magnet.fsu.edu/articles/basics/index.html
  - https://microbenotes.com/fluorescence-microscope-principle-instrumentation-applications-advantages-limitations/
- Image Analysis
  - https://noirlab.edu/public/media/archives/techdocs/pdf/techdoc027.pdf
  - https://www.cl.cam.ac.uk/teaching/1920/AdvGraphIP/01_Intro_to_image_processing.pdf
  - Math and Matlab: https://eeweb.engineering.nyu.edu/~yao/EL5123/lecture1_intro.pdf
  - Visualizing kernels: https://setosa.io/ev/image-kernels/