

GLOBAL BIOIMAGING IMAGE DATA 2025 COURSE



Introduction to bioimage analysis

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One-stop shop for the materials in this course

- <https://hms-iac.github.io/GBI-india/>

I.A.C. (Image Analysis Collaboratory)

@ HMS since Sep '22

- **Support and collaboration:** for image and data analysis projects
- **Teaching:** at HMS and internationally
- **IT:** access to relevant solutions, open- and closed-source
- **R&D:** centered on methods and tools
- **Locations:** LHRRB 105 & Armenise 531D
- **Consults:** Contact Simon
 - simon@hms.harvard.edu



<https://iac.hms.harvard.edu/>



Image
Analysis
Collaboratory

Who we are, currently



Antoine A. Ruzette

Associate



Ranit Karmakar

Specialist Postdoc



Maria Theiss

Specialist Postdoc



Federico Gasparoli

Research Associate



Simon F. Nørrelykke

Director, Lecturer

Backgrounds in

Physics
Biology
Microscopy
Bioengineering
Computer Engineering

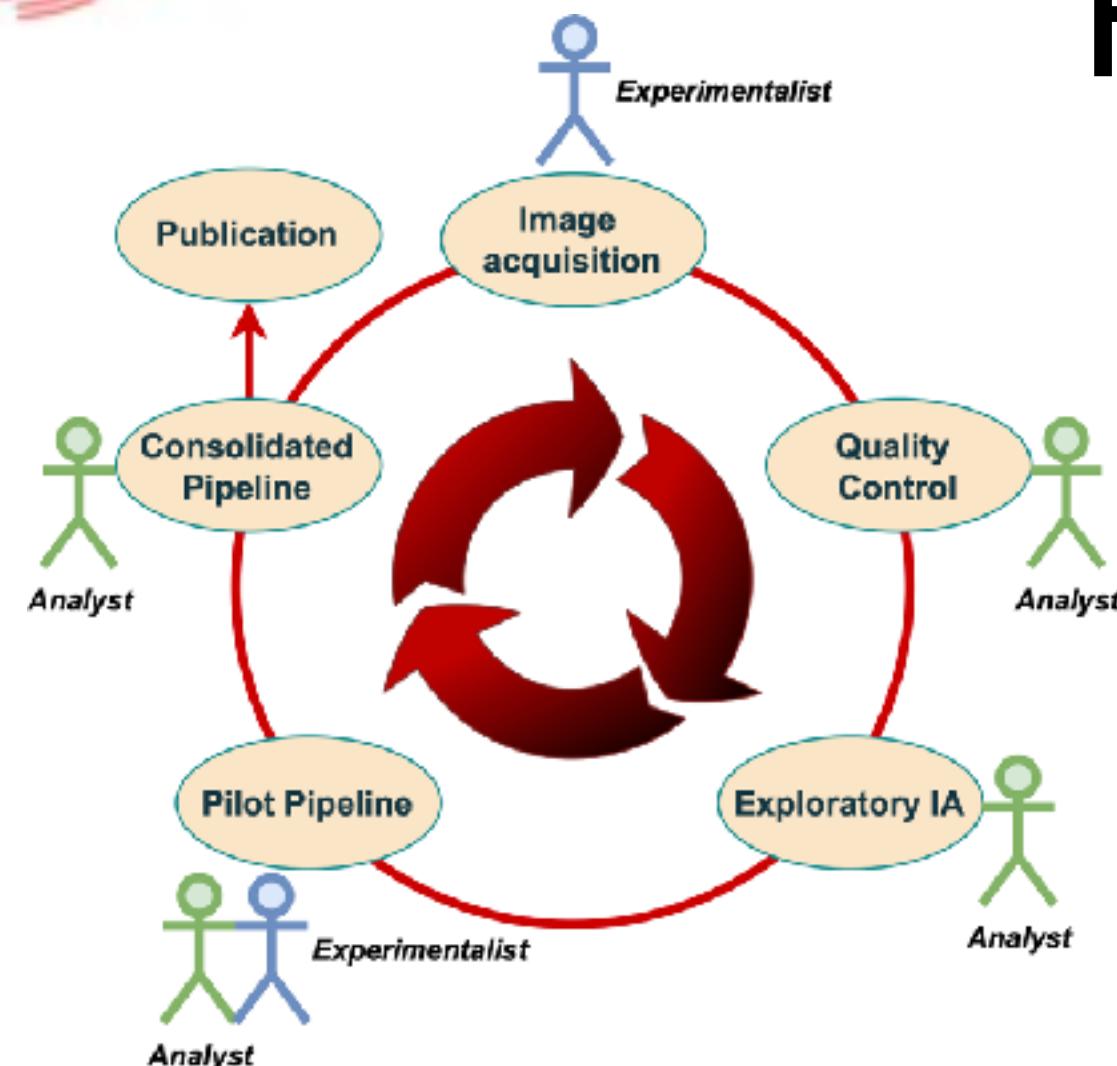
How we operate



Consultations
“Image Clinics”

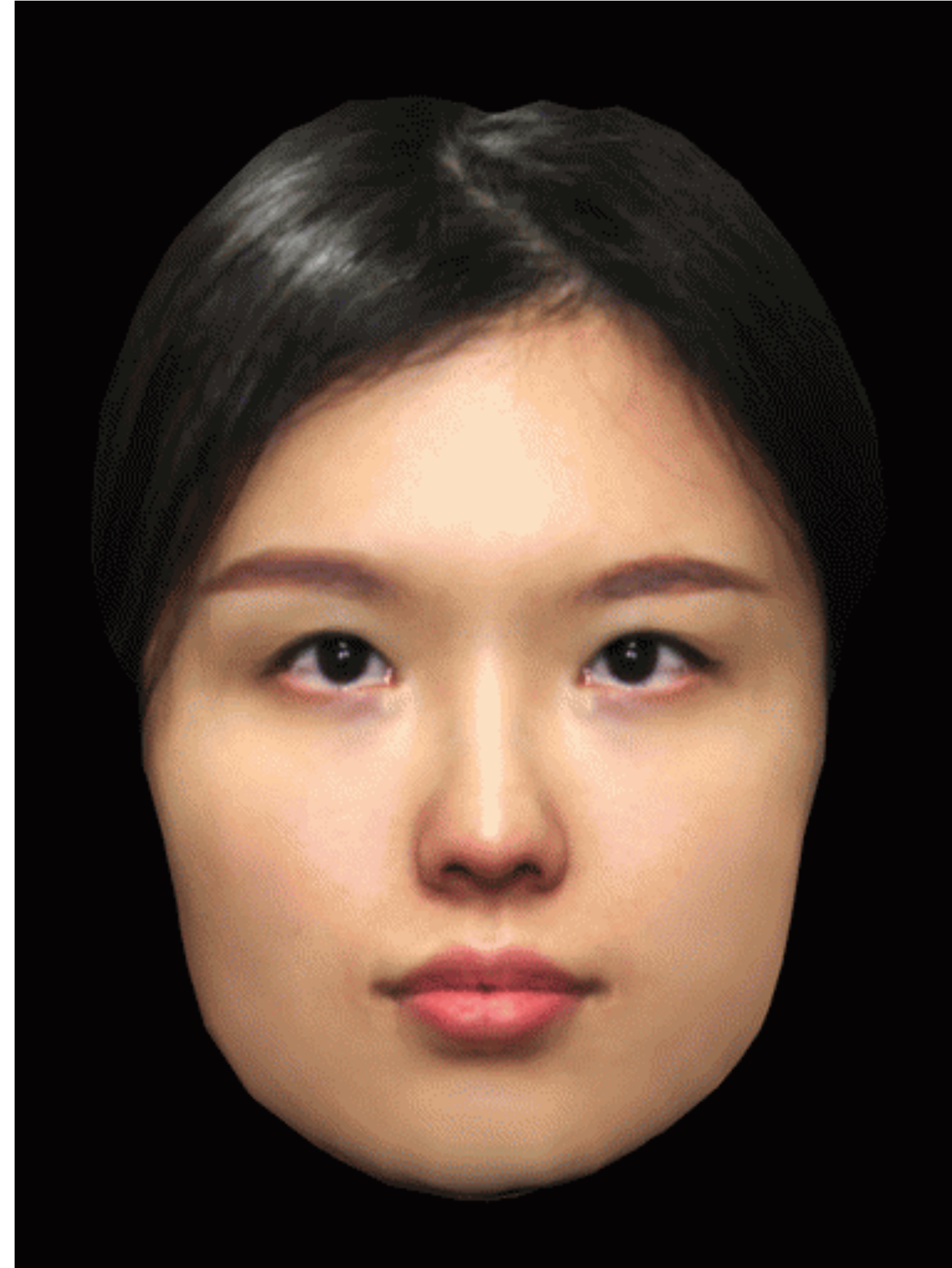


Teaching and Training
Courses in image analysis
Postdoc training program



Collaborations

Problem: Seeing is Believing



Mask rotates continuously in the same direction
(nose passing right to left on screen)

Can you see the inside of the mask?

Acknowledgements

- Szymon Stoma, ETH Zurich, Switzerland
 - slides and workflows
- Peter Bankhead, Edinburgh University, Scotland
 - slides and graphics
- CITE, Harvard Medical School
 - slides and graphics

Reasons to learn about image processing

- Make pretty pictures (processing)
 - publications, talks, websites, ...
- Get numbers out of pictures (analysis)
 - cell sizes, vessel lengths, GPF expression level, ...
- Make experiment possible (automation)
 - whole-genome screen: millions of images
- Objectivity and Reproducibility
 - in science, just do it!

Reasons not to learn about image processing

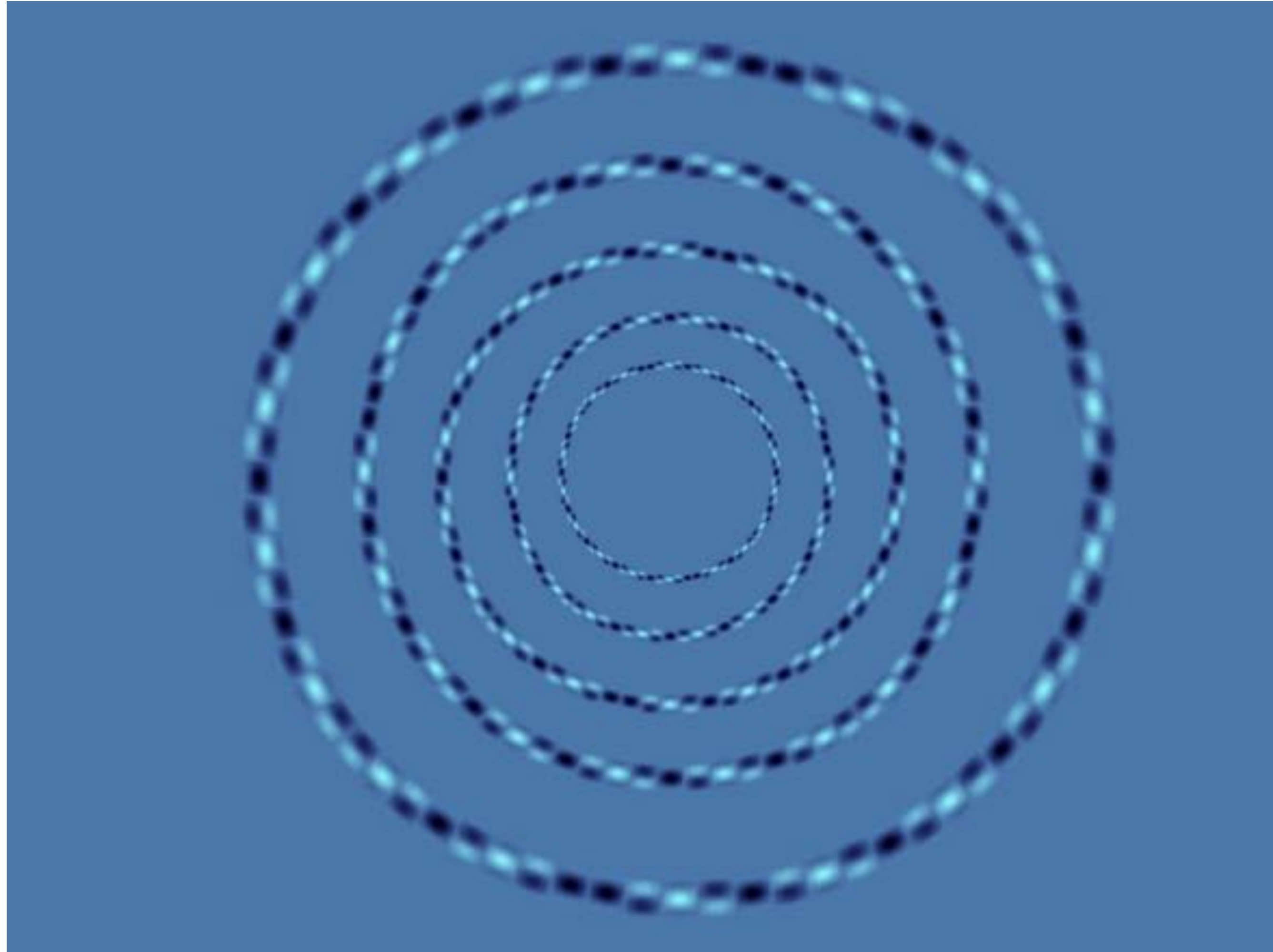
- none

Optical Illusions

—

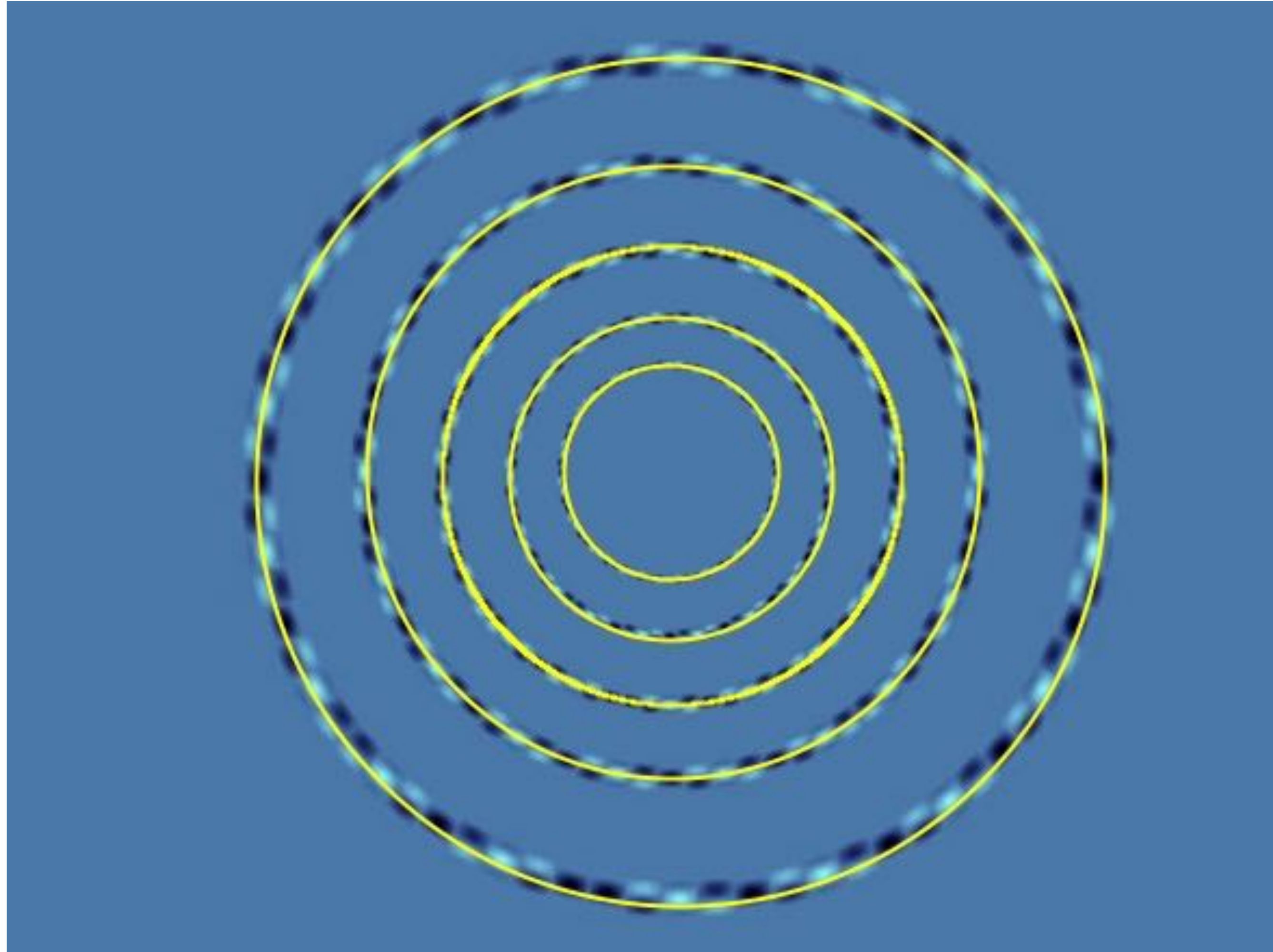
Why you should use a computer to
analyze images

Concentric circles !?



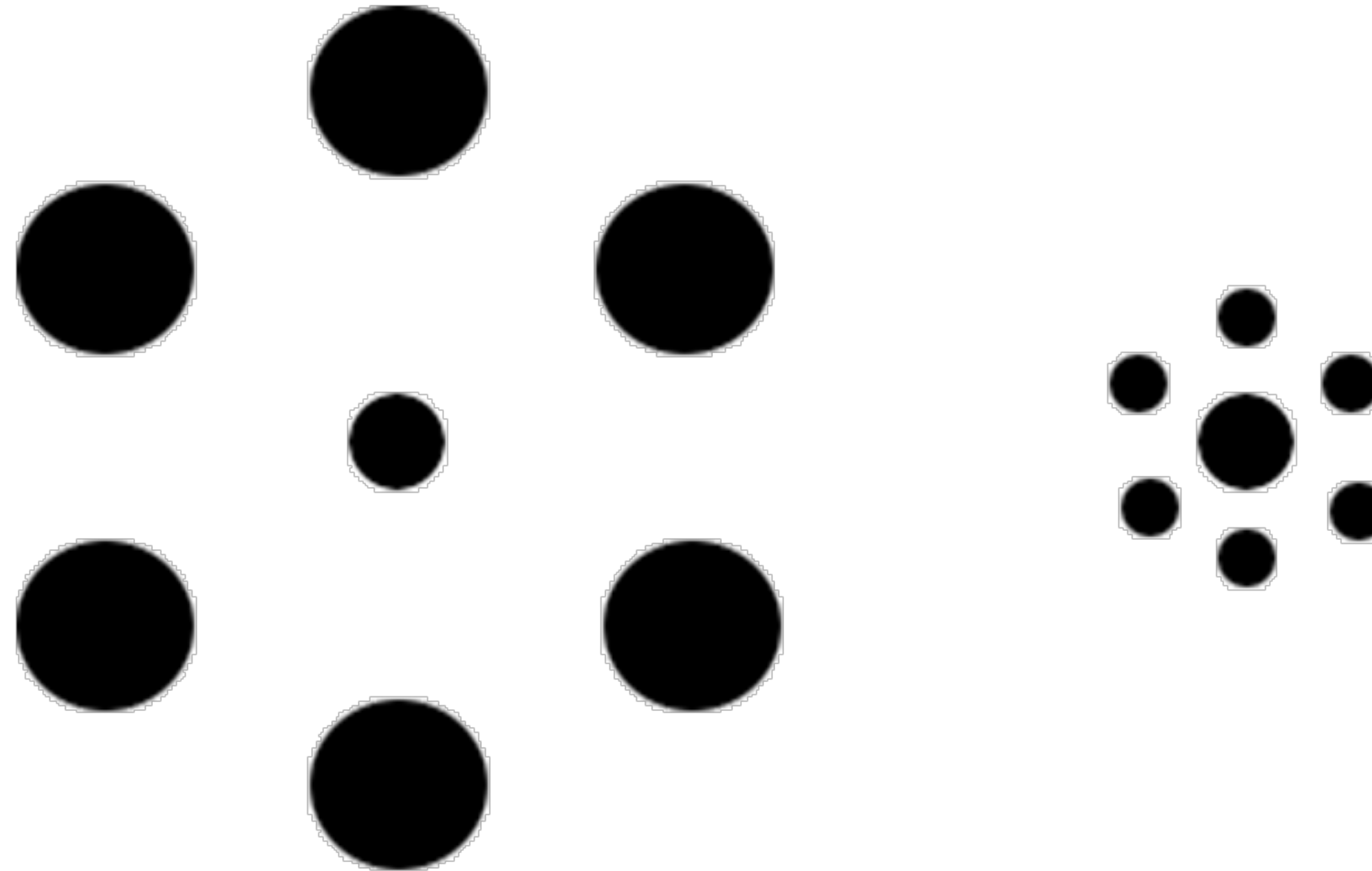
<https://www.moillusions.com/perfect-circles-optical-illusion/>

Concentric circles !?



<https://www.moillusions.com/perfect-circles-optical-illusion/>

Identical central discs?



Our size estimate is strongly influenced by the local neighbourhood

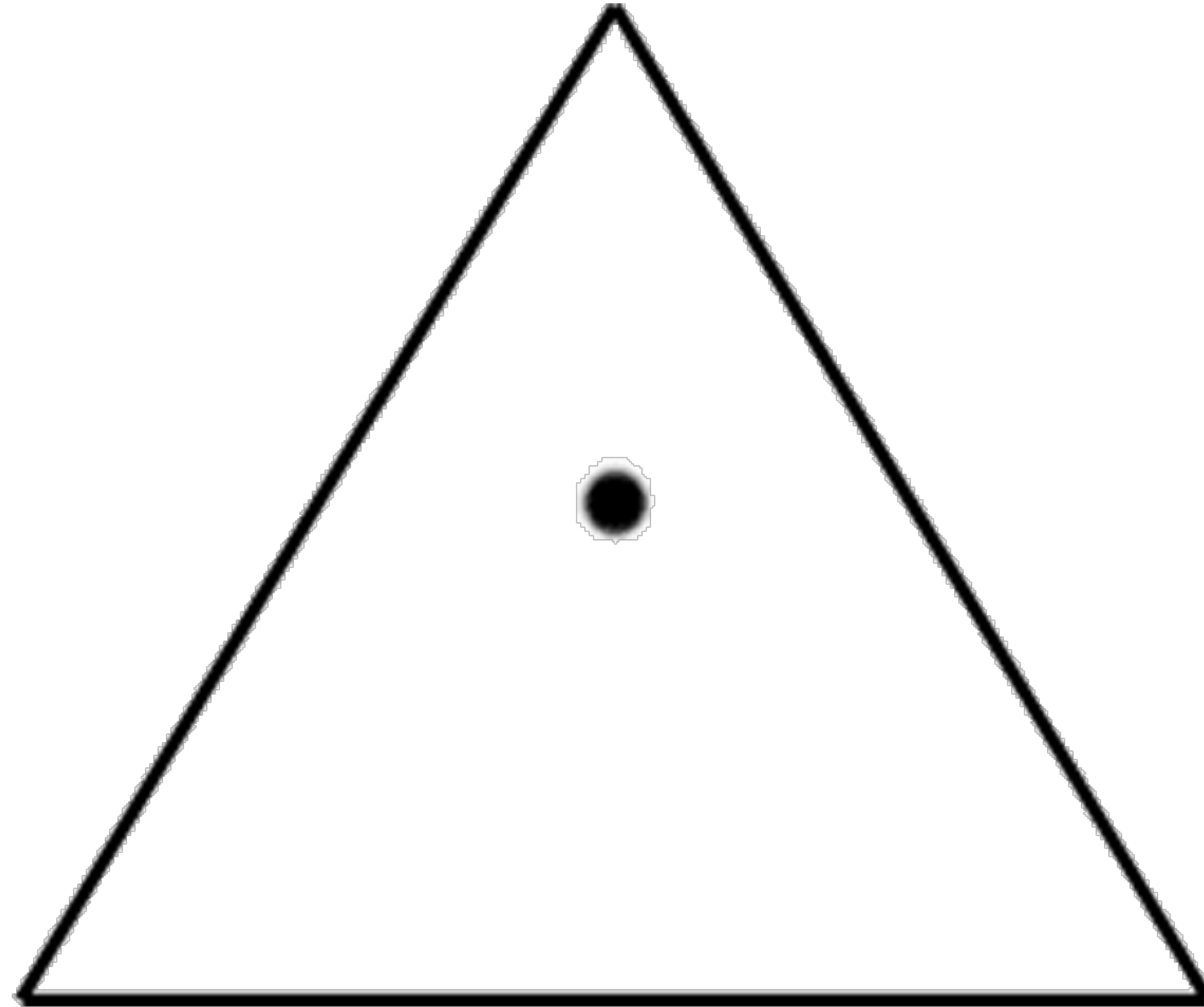
Identical central discs?



Yes, the discs are identical

<http://www.brainbashers.com>

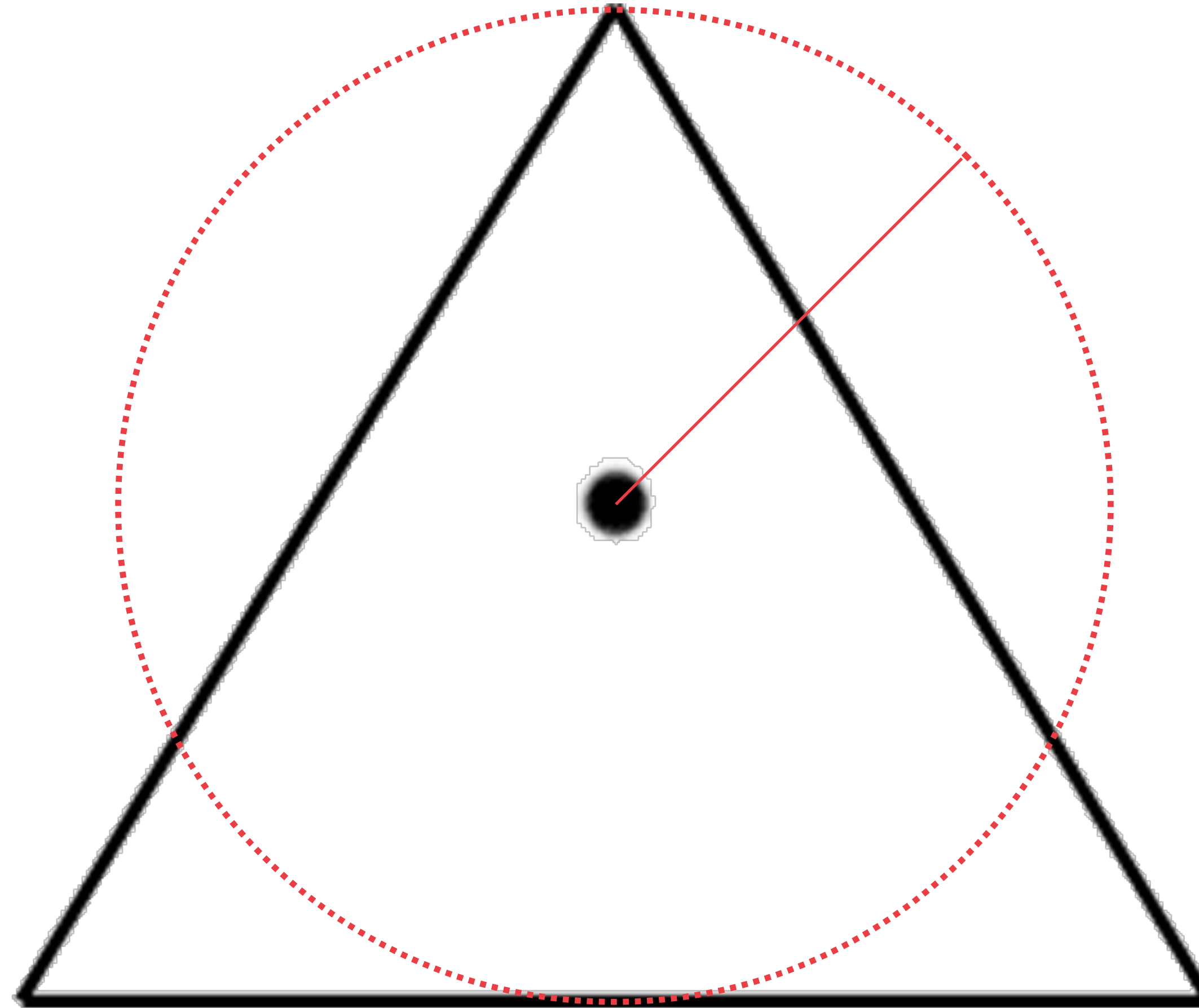
Is the dot half-way up?



Our sense of distance depends on neighbourhood

<http://www.brainbashers.com>

Is the dot half-way up?



Yes it is!

<http://www.brainbashers.com>

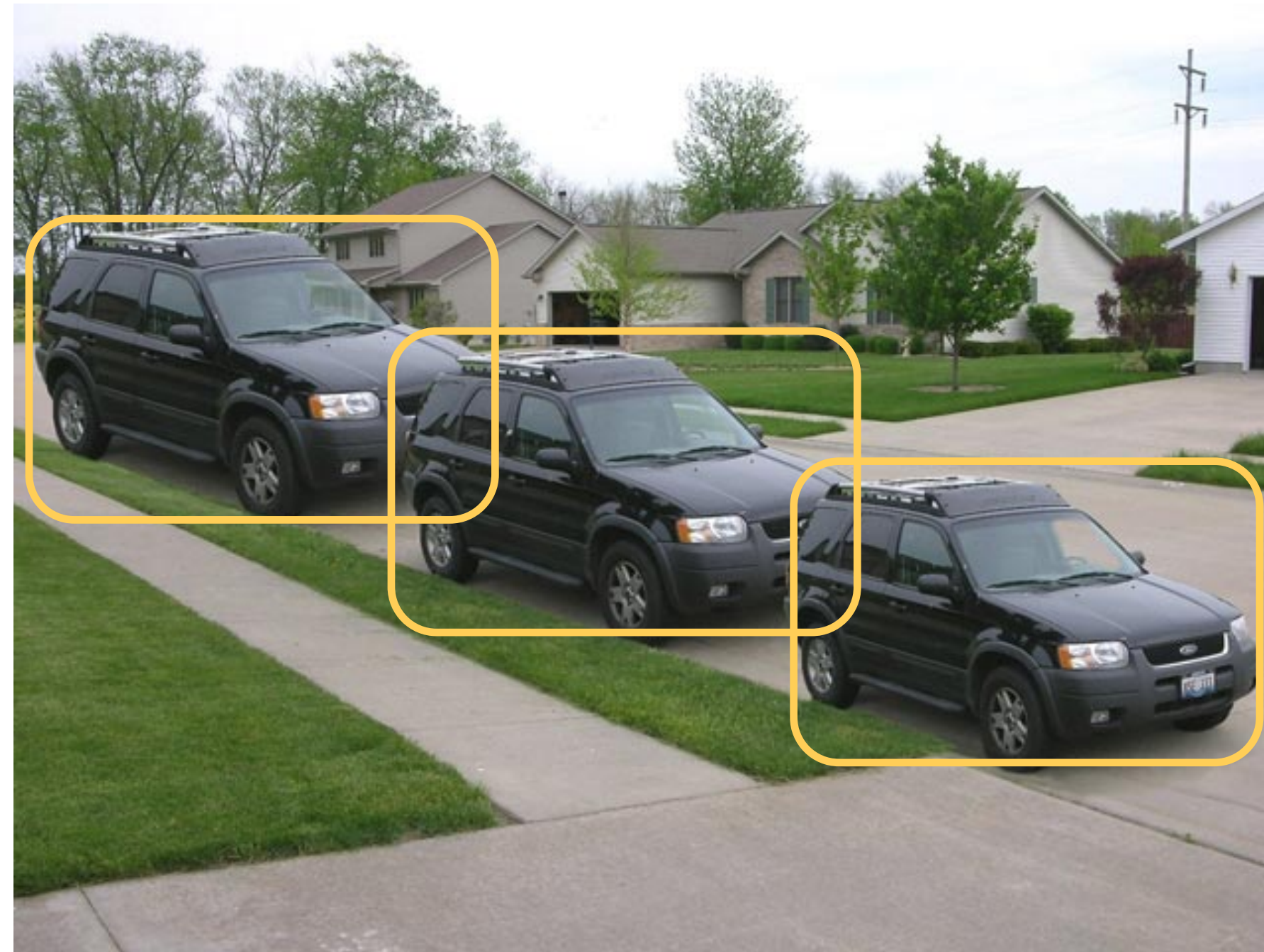
Which car is bigger?



Which car is bigger?



Which car is bigger?



All cars are same size

(Erroneous, application of subjective/perceptual constancy algorithm by your brain)

<http://www.brainbashers.com>

Are discs equally grey?



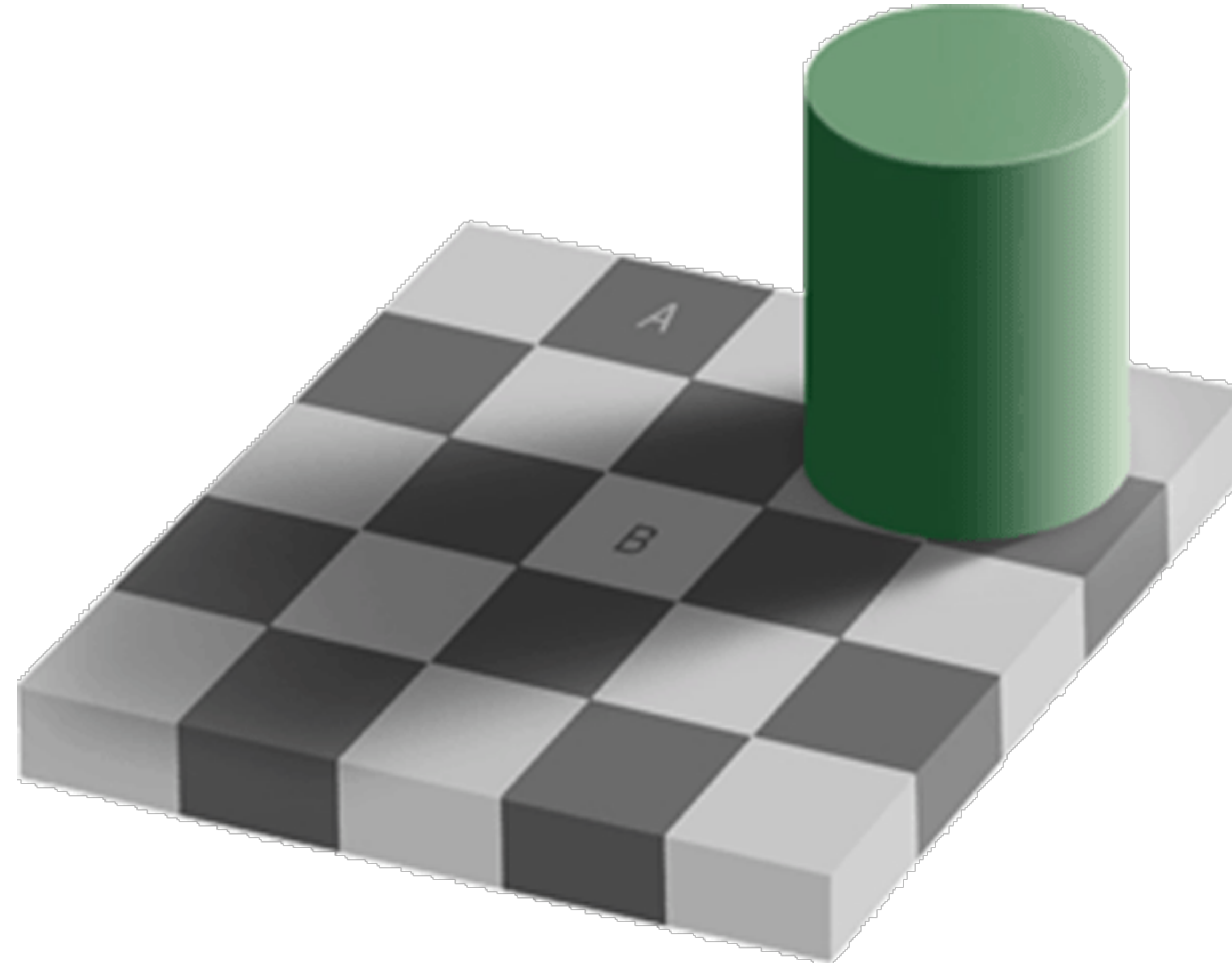
Intensity perception depends strongly on neighbourhood

Are discs equally grey?



Yes they are!

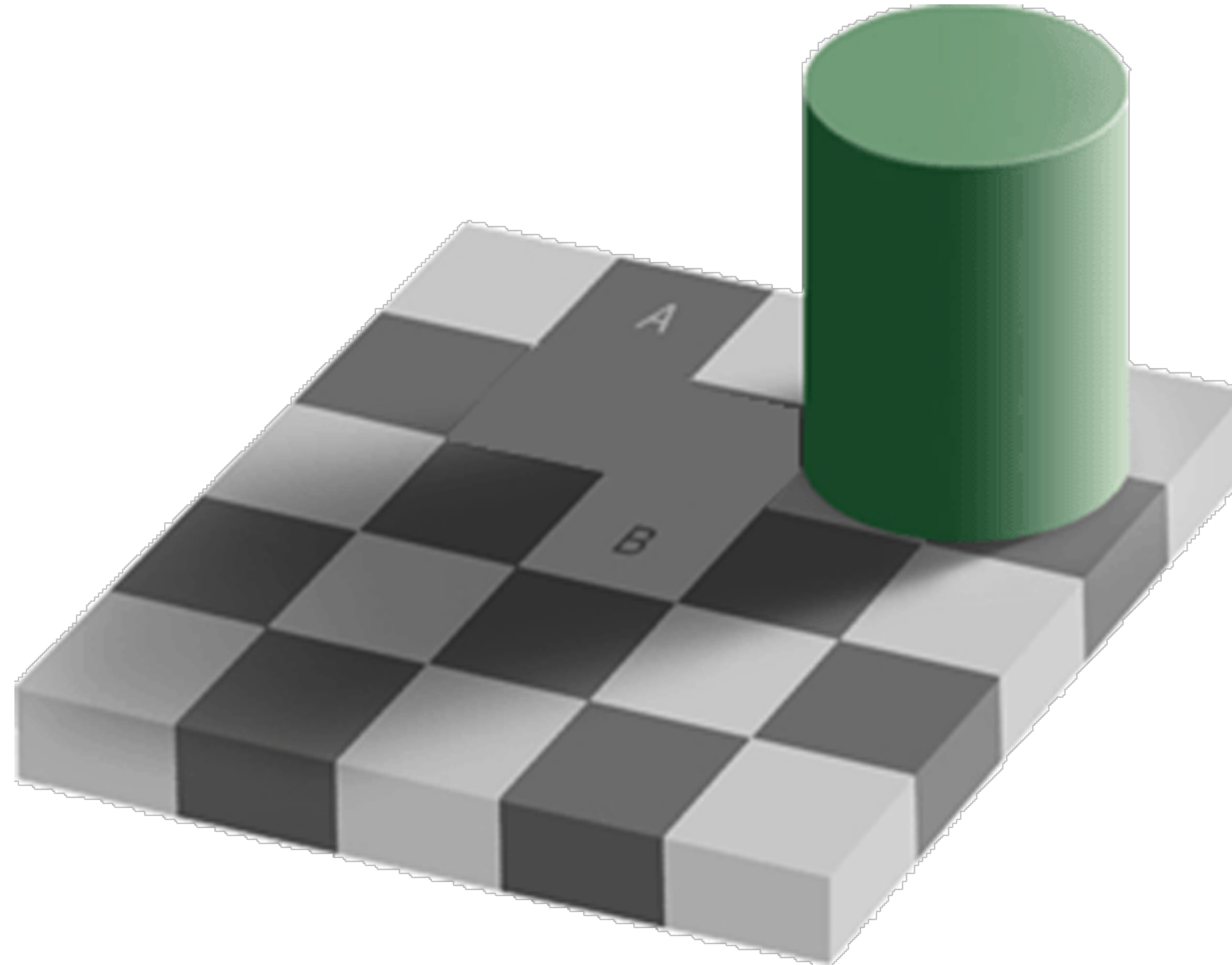
Are A and B equally grey?



Intensity perception depends strongly on neighbourhood

<http://www.brainbashers.com>

Are A and B equally grey?

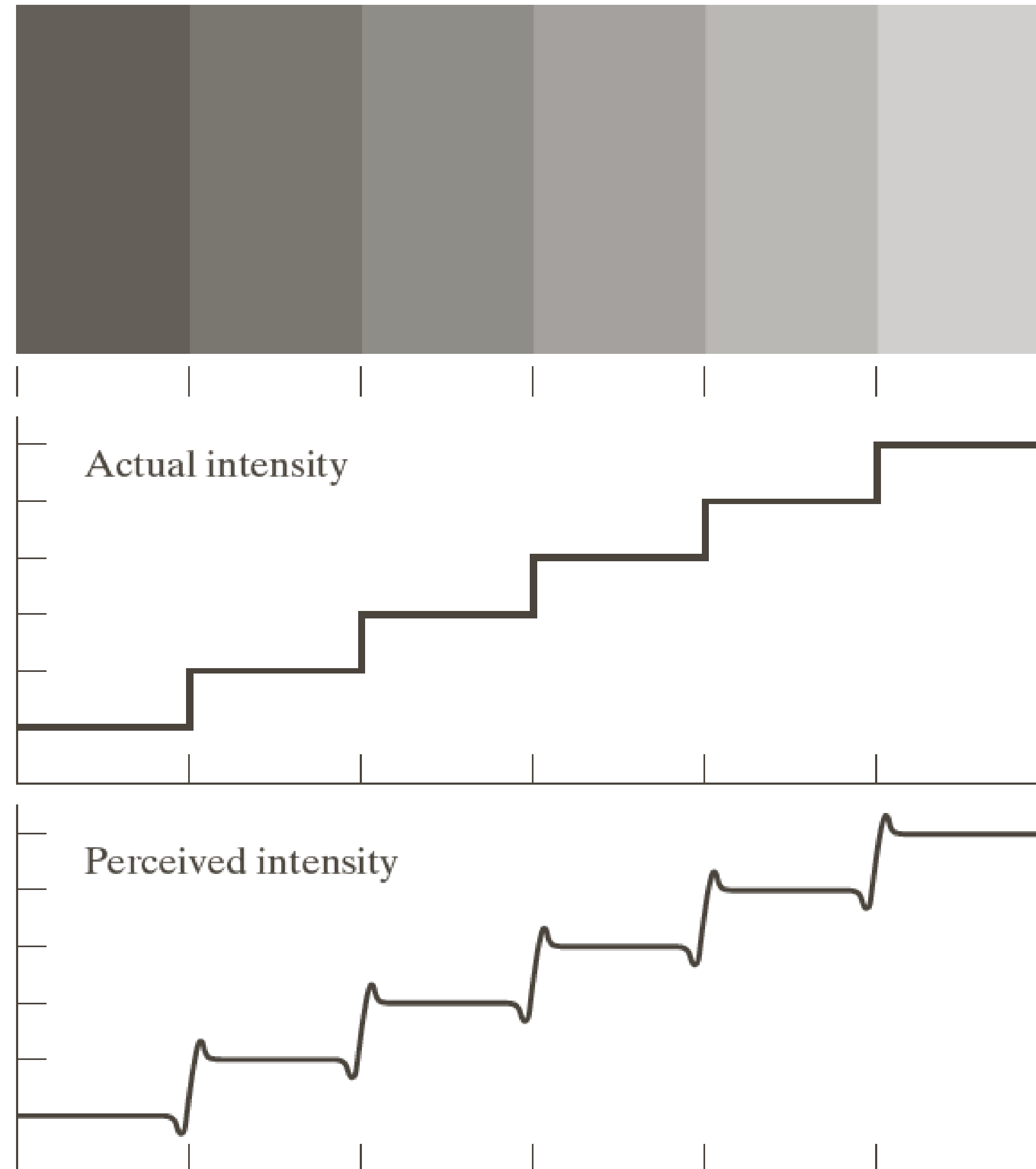


Yes they are!

<http://www.brainbashers.com>

Intensity Perception

The Mach band effect:
the *perceived* intensity
is not a simple
function of *actual*
intensity



Spot the Dog

—

Why you should use your Brain
(a neural network)

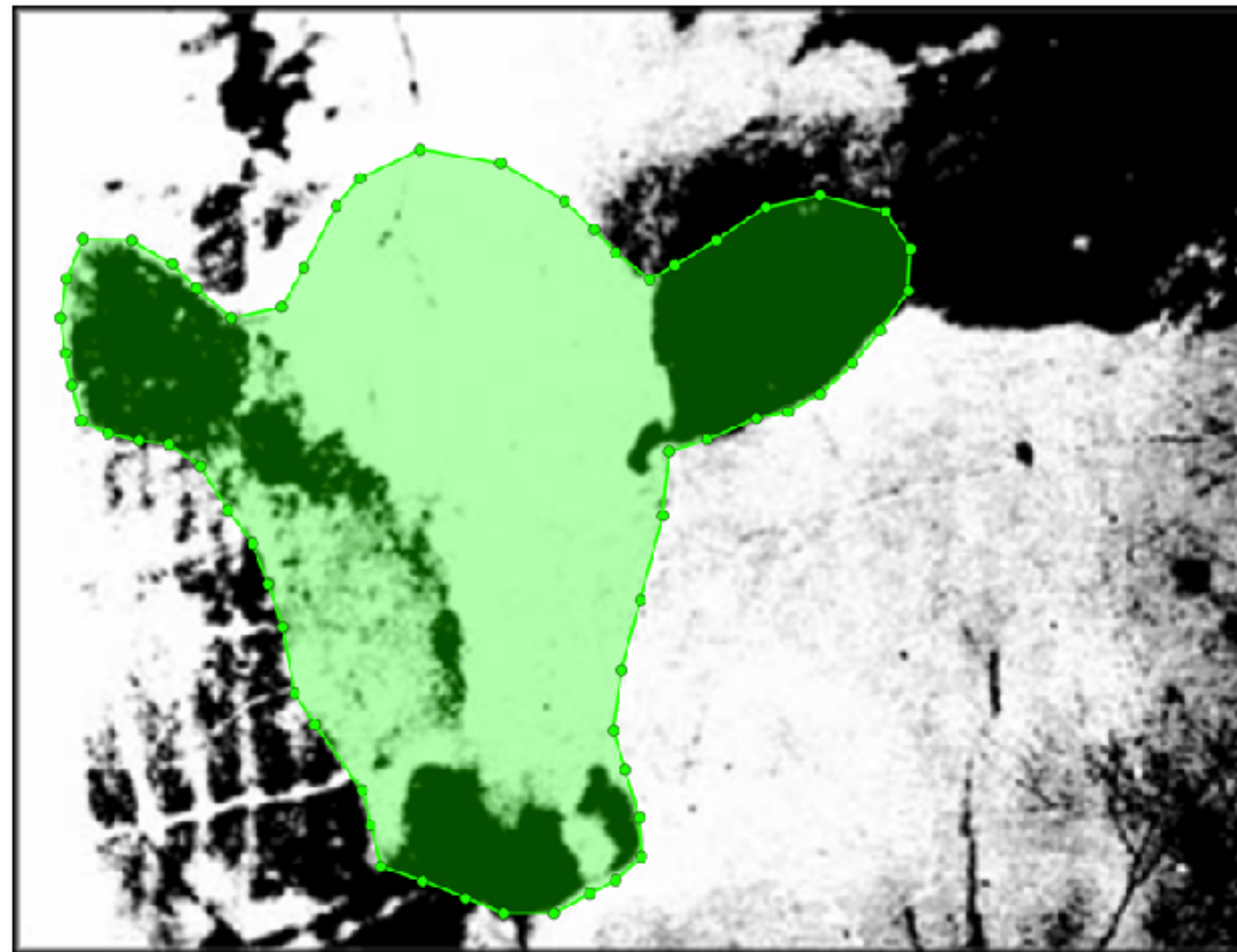
Bayesian Pattern Recognition



Yes, that is a cow!

<http://www.brainbashers.com>

Bayesian Pattern Recognition



Yes, that is a cow!

<http://www.brainbashers.com>

Using Prior Knowledge



Keyword: Dalmatian dog!

<http://www.brainbashers.com>

Using Prior Knowledge



Keyword: Dalmatian dog!

<http://www.brainbashers.com>

A brief history of image analysis

Bioimage analysis 37 years ago (1987)

- [NIH Image](#) is the only game in town (below \$150,000)
 - Written in Pascal for the Macintosh (Mac II) by Wayne S. Rasband
 - First released in 1987, shared on floppy disks
 - Supporting 8-bit 256 gray “colors”
 - If you wanted it, buy a Macintosh, or
- Windows supported through buggy closed-source C port *Scion Image*
 - Scion built frame-grabbers and wanted to expand to Windows
- Self-driving off-road in day and night time conditions demonstrated
- 1988: Yann LeCun publishes convolutional neural network [paper](#)
 - This is a technology, not a tool, few people notice, paper is fun to read today
 - Middle of second [AI winter](#) (1987-1993)



Bioimage analysis 27 years ago (1997)



- **ImageJ** (J for Java)---still the main game in town
 - Released in 1997 as rewrite of *NIH Image*
 - Apple in decline, the PC on the rise
 - 1995: Sun Microsystems created the Java programming language as an operating system–agnostic programming platform
 - Rasband wanted to support Windows, but not two sets of code
 - “*Instead of ‘write once, run everywhere’, Rasband found himself writing once and debugging everywhere.*”
- First autonomous coast-to-coast drive of the United States two years earlier

Bioimage analysis 12 years ago (2012)

- [AlexNet](#) is published (2012)
 - Wins the *ImageNet Large Scale Visual Recognition Challenge*
 - Error of 15.3%, more than 10.8 percentage points better than #2
 - Possible due to GPUs
- [CellProfiler](#) has been around for 6 years (2006)
- [Fiji](#) (Fiji is just ImageJ) has been around for 5 years (2007)
- [scikit-image](#) released 3 years ago (2009)
- Bitcoin grows from \$5 to \$13 (remember Silk Road?) (2012)
- U-Net, GANs, and [Jupyter](#) will appear in 2-3 years (2014/15)
- [AlphaGo](#) will beat Lee Sodol in 4 years (2016)
- [QuPath](#) is still 4 years in the future (2016)



CellProfiler™
cell image analysis software

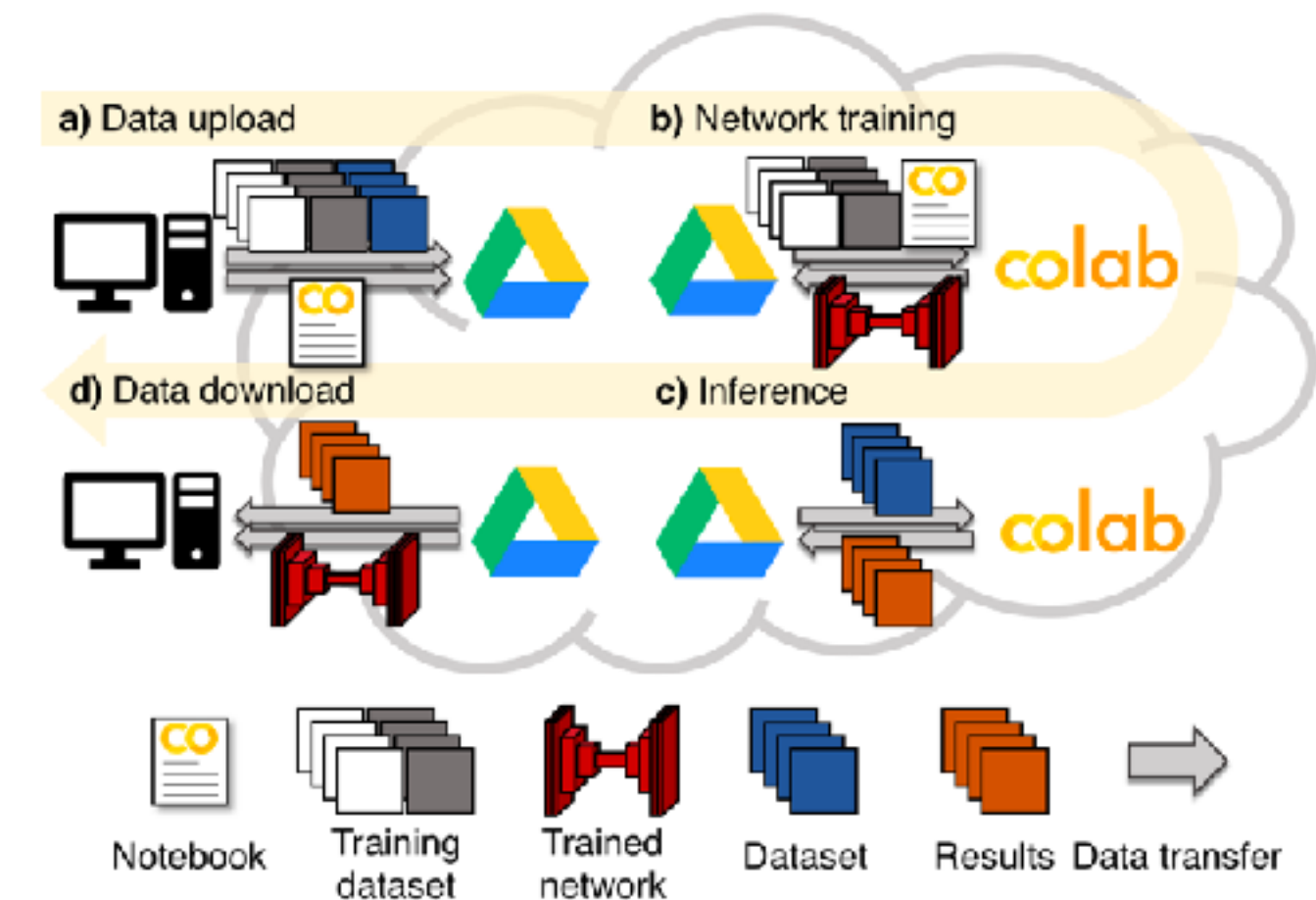


scikit-image
image processing in python



Bioimage analysis 4 years ago (2020)

- CellPose is out (2020)
 - “Cellpose: a generalist algorithm for cellular segmentation”
 - Trained on highly varied images of cells, over 70,000 segmented objects
 - Cells don't have to be star-shaped
 - Web-platform and Jupyter notebooks
 - <https://www.nature.com/articles/s41592-020-01018-x>
- ZeroCostDL4Mic available
 - [Paper](#) still a year in the future
 - Implementation of common DL technologies to microscopy imaging
 - Relies on GPUs and other infrastructure provided by Google Colab.
 - <https://github.com/HenriquesLab/ZeroCostDL4Mic/wiki>



Bioimage analysis now (2024)

- Increased integration of DL technologies into existing bioimage tools
 - CARE, Stardist, CellPose, [DeepCell](#) (MESMER) as plugins, modules, notebooks for
 - Fiji, CellProfiler, [Napari](#), ZeroCostDL4Mic, etc
- Most major Microscopy producers offer AI
 - Implemented in-house or through acquisition
 - Zeiss: Arivis (bought)
 - Leica: Aivia (bought)
 - Nikon: NIS.ai suite (in-house implementation of open-source technologies)
- “Smart microscopy” includes DL for event detection
 - <https://www.nature.com/articles/s41592-022-01589-x>
- Fully autonomous cars still in the future
 - Image analysis is hard if you don't understand what you are looking at



 GPT-3.5

 GPT-4 

ChatGPT

Create a workout plan
for resistance training

Design a database schema
for an online merch store

Explain why popcorn pops
to a kid who loves watching it in the microwave

Write an email
to request a quote from local plumbers

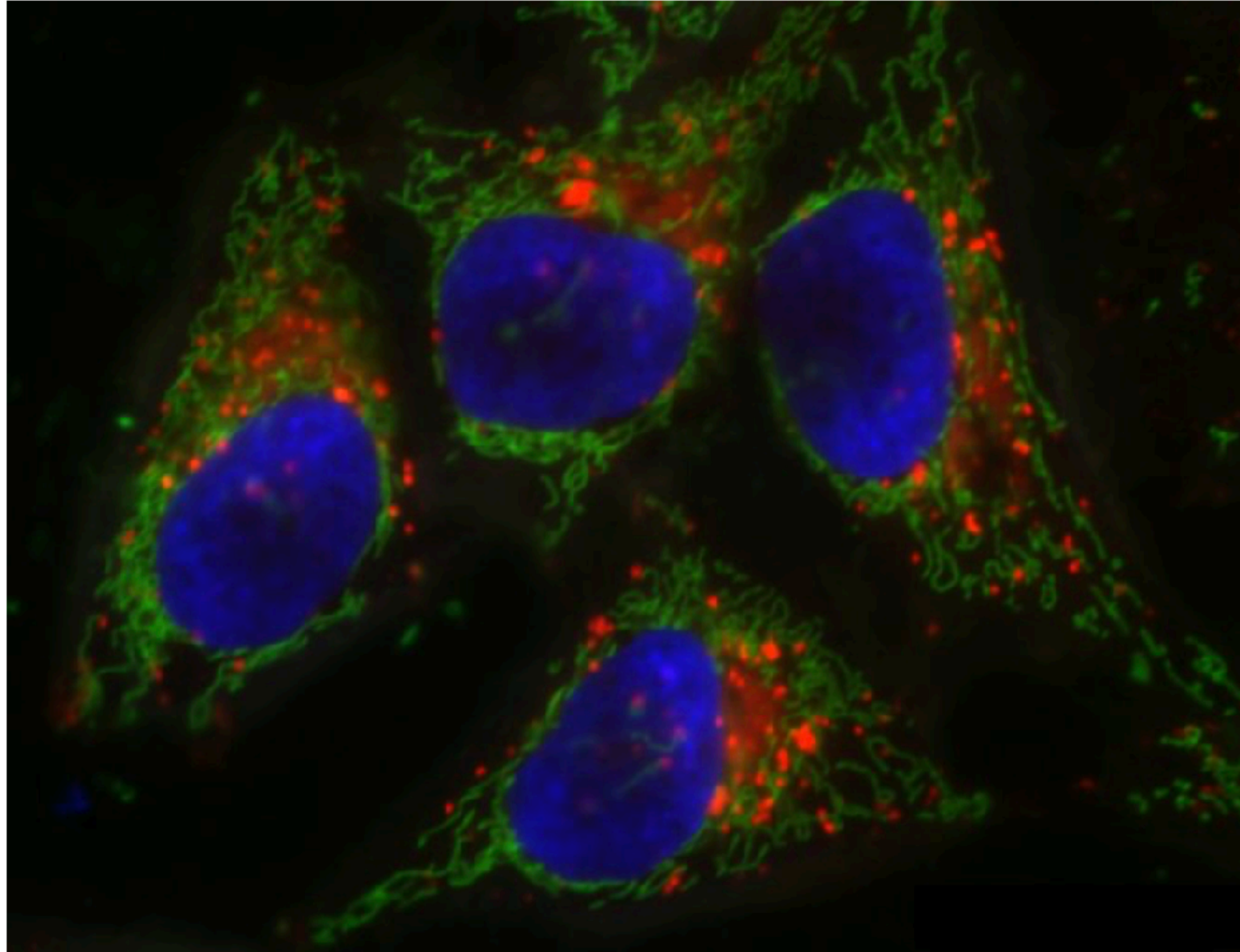
Please write an ImageJ macro that uses StarDist to segment an image from fluorescence microscopy



Free Research Preview. ChatGPT may produce inaccurate information about people, places, or facts. [ChatGPT September 25 Version](#)

Using ChatGPT-4 for Image Analysis

SI You

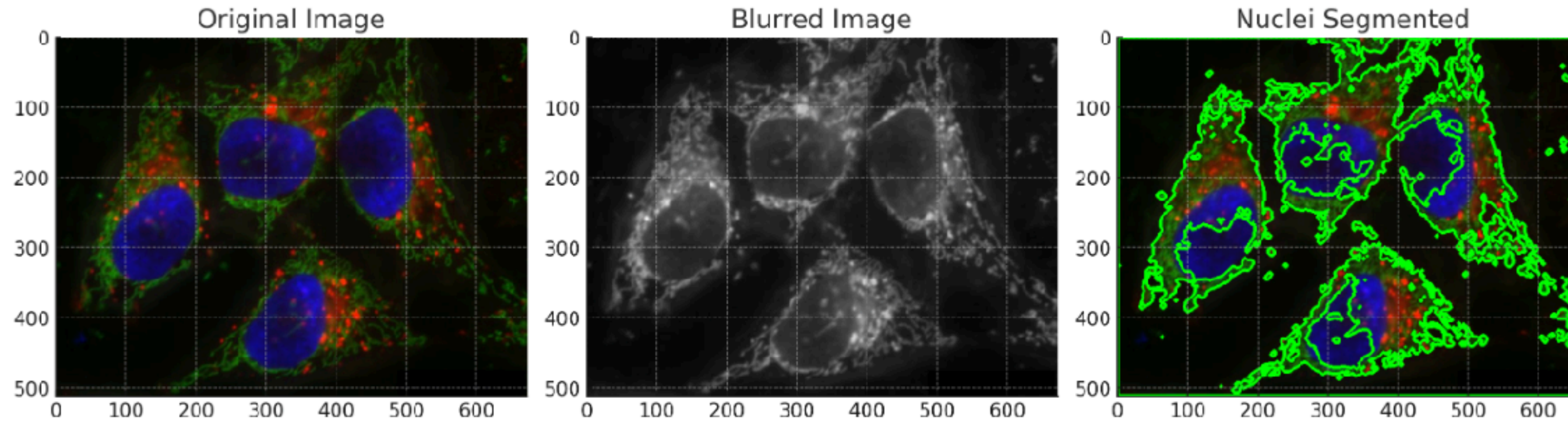


please segment the nuclei and show me the relevant code you used

Using ChatGPT-4 for Image Analysis



ChatGPT



The nuclei in the image have been segmented using the following steps:

- The image was converted to grayscale to simplify the analysis.
- Gaussian Blur was applied to reduce noise and detail in the image, which aids in thresholding and contour detection.
- Otsu's thresholding was performed to convert the grayscale image into a binary image, which helps in distinguishing the nuclei from the background.
- Contours were found in the binary image, which correspond to the boundaries of the nuclei.
- These contours were then drawn onto the original image to visually segment the nuclei.

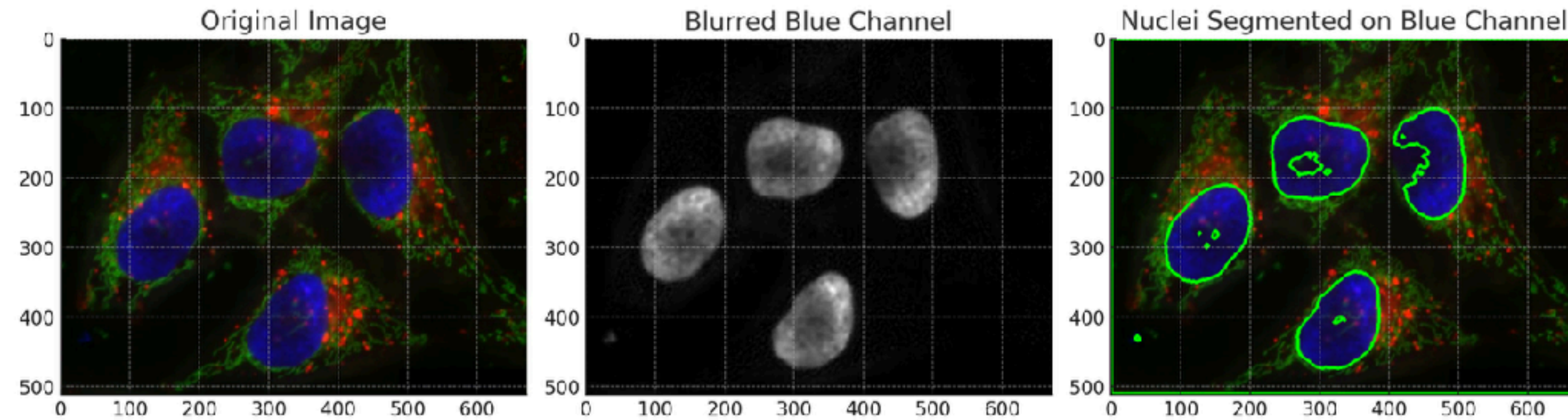
Using ChatGPT-4 for Image Analysis

```
python Copy code  
  
import cv2  
import numpy as np  
from matplotlib import pyplot as plt  
  
# Load the image  
image_path = '/mnt/data/hela-cells.jpg'  
image = cv2.imread(image_path)  
  
# Convert the image to gray scale  
gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY)  
  
# Apply GaussianBlur, which is good for edge detection  
blurred = cv2.GaussianBlur(gray, (5, 5), 0)  
  
# Perform Otsu's thresholding  
_, thresh = cv2.threshold(blurred, 0, 255, cv2.THRESH_BINARY_INV + cv2.  
  
# Find contours which will give us the nuclei boundaries  
contours, _ = cv2.findContours(thresh, cv2.RETR_TREE, cv2.CHAIN_APPROX_  
  
# Draw the contours on the original image  
contoured_image = image.copy()  
cv2.drawContours(contoured_image, contours, -1, (0, 255, 0), 3)  
  
# Save the output image with segmented nuclei  
output_path = '/mnt/data/hela-cells-segmented.jpg'  
cv2.imwrite(output_path, contoured_image)
```


Using ChatGPT-4 for Image Analysis

You
please use the blue channel for segmenting the nuclei

ChatGPT




The nuclei segmentation has been performed using the blue channel of the image with the following steps:

- The blue channel was extracted from the original image since the nuclei appear to be stained blue.
- Gaussian Blur was applied to the blue channel to smooth the image, which helps in reducing noise for thresholding.
- Otsu's thresholding was then used on the blurred blue channel to create a binary image where the nuclei are distinguished from the background.

Using ChatGPT-4 for Image Analysis

Here is the code that was used for this process:

python

 Copy code

```
# Extract the blue channel, as the nuclei are stained with a blue dye
blue_channel = image[:, :, 0]

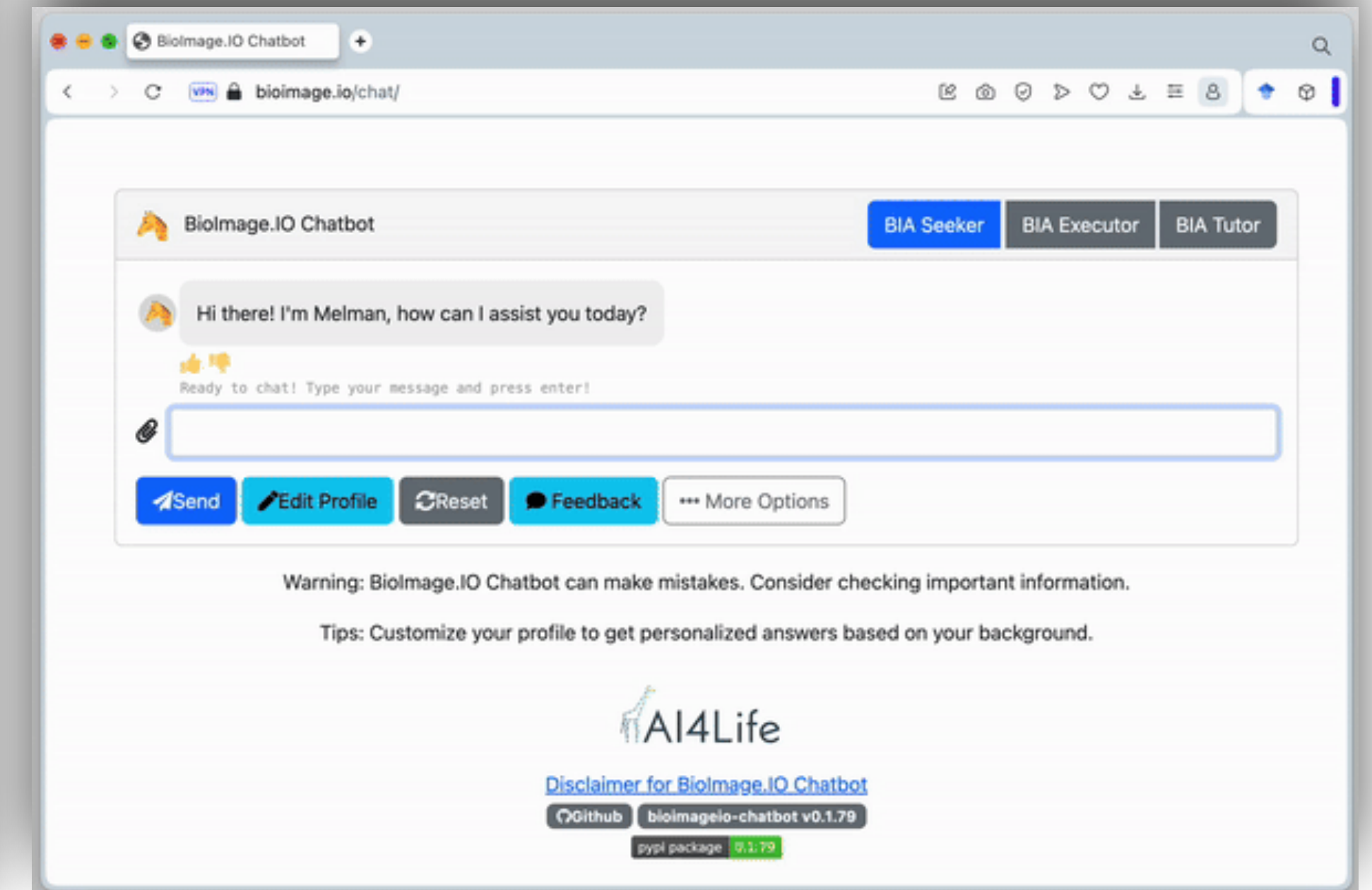
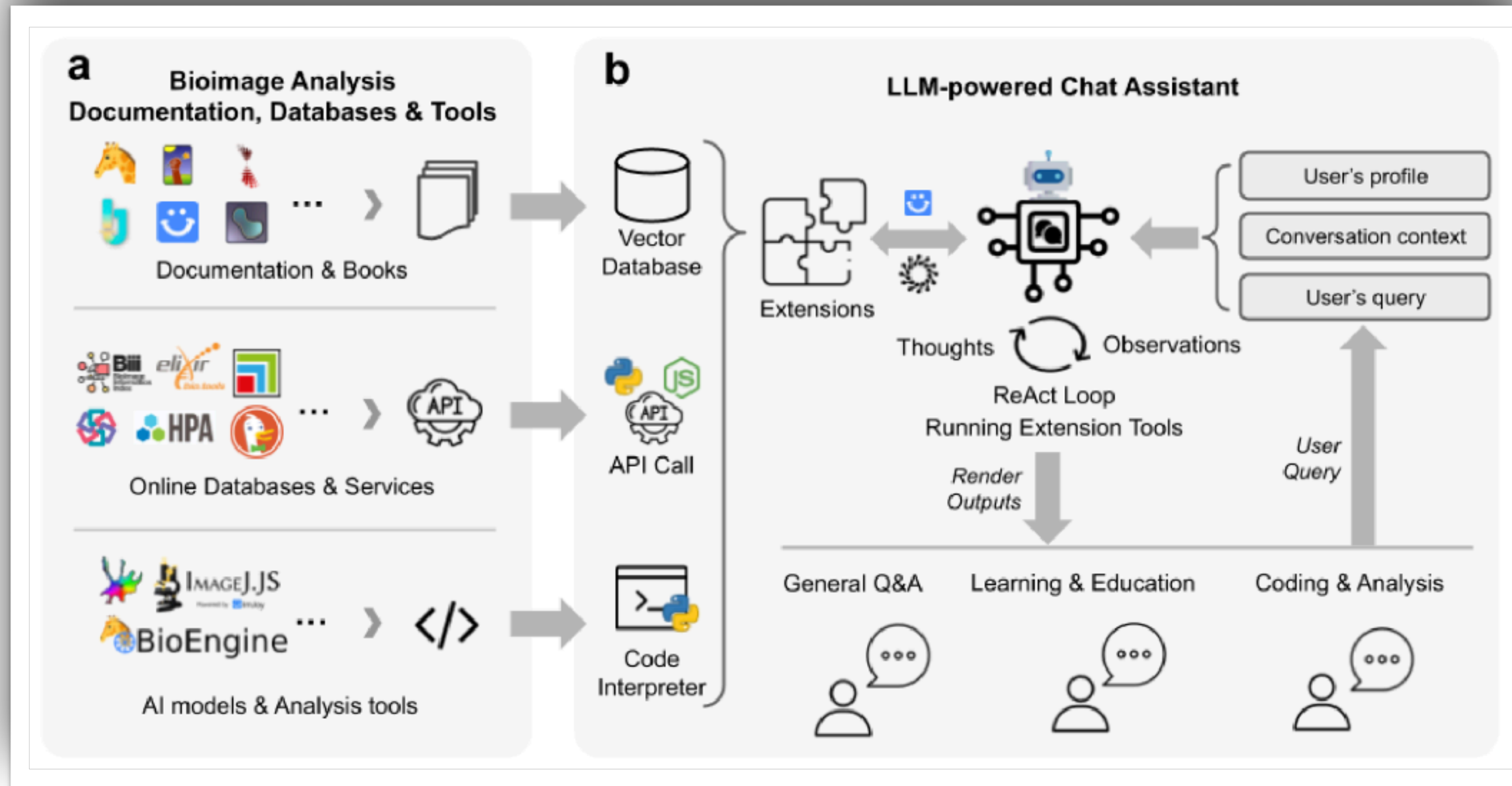
# Apply GaussianBlur to the blue channel
blurred_blue = cv2.GaussianBlur(blue_channel, (5, 5), 0)

# Perform Otsu's thresholding on the blue channel
_, blue_thresh = cv2.threshold(blurred_blue, 0, 255, cv2.THRESH_BINARY_)

# Find contours on the thresholded blue channel
contours_blue, _ = cv2.findContours(blue_thresh, cv2.RETR_TREE, cv2.CHAIN_APPROX_SIMPLE)

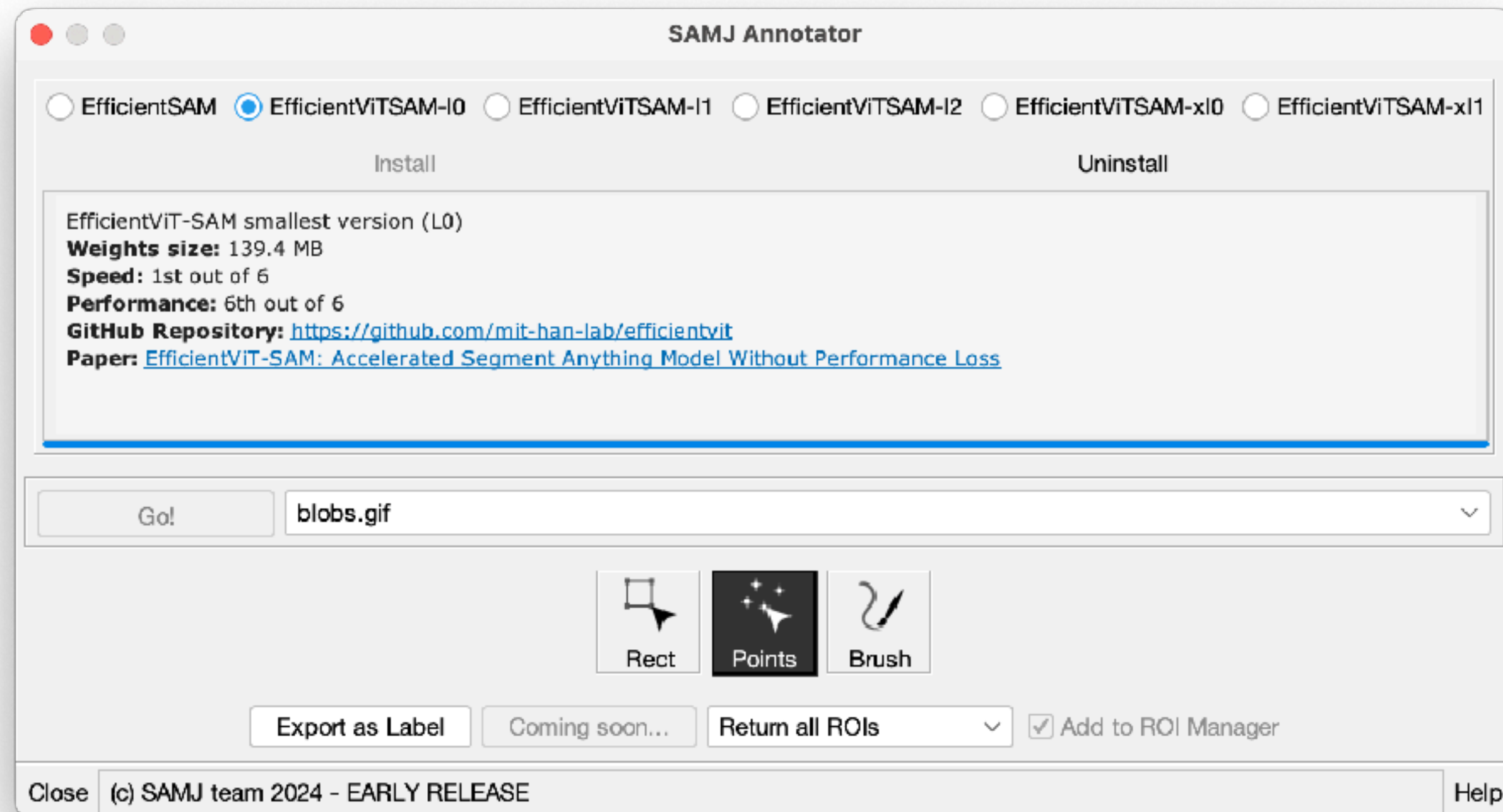
# Draw the contours on the original image to show the segmentation of nuclei
contoured_blue_image = image.copy()
cv2.drawContours(contoured_blue_image, contours_blue, -1, (0, 255, 0), 2)
```


BioImage Chatbot



Segment Anything in ImageJ/Fiji

Released around March 14th, 2024
Graphical User Interphase
No coding required



Example of point annotations

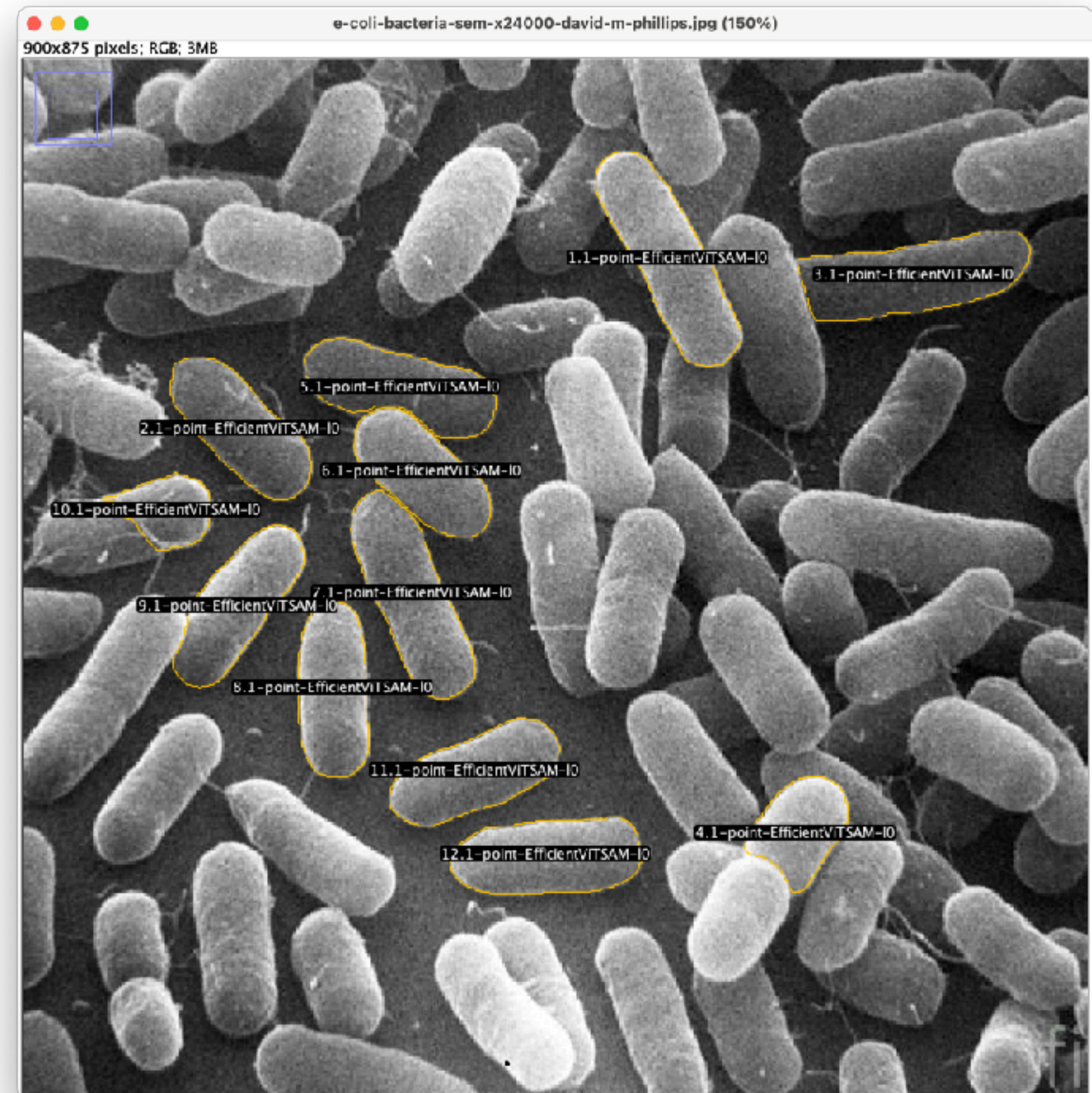
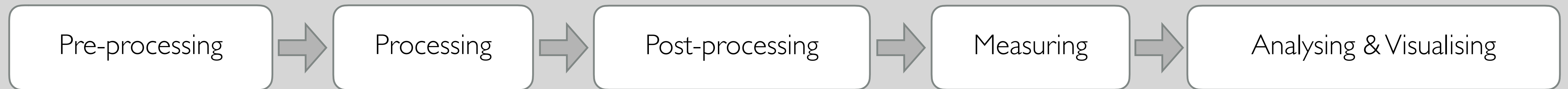


Image Analysis Workflow

- There are typically *five* steps in an image analysis
- Often a good idea to structure work along these lines before starting



Think of this even ***before*** you acquire the images!

otherwise image analysis may become only a ***post-mortem*** on your experiment



- **Data-wrangling in general**
 - Image format conversion, incl. lossless compression
 - Renaming
- **Restoration and Reconstruction**
 - Tomographic reconstruction
 - Registration (3D stacks)
 - Stitching (tiled images)
 - Illumination correction
 - Intensity normalisation
 - Deconvolution
 - Filtering (smoothing, sharpening, etc)
 - Grayscale morphological operations
- **Color deconvolution**
- **Channel alignment (chromatic aberrations)**
- ...



- **Image Segmentation**
 - Thresholding
 - Pixel Clustering
 - Edge detection
 - Region growing
 - Level set methods
 - Watershed
 - Model based
 - Multi-scale
 - Trainable pixel-classification
- **Spot detection**
- **Tracking**
- ...



- **Filtering** detected objects based on
 - Size, shape, intensity
 - Texture, location, neighbours
- **Binary morphological operations**
 - Open/close
 - Skeletonise
 - Hit-or-miss transform
- ...



- **Object measures**
 - Size, shape, intensity
 - Texture, positions
 - Local neighbourhood
- **Image measures**
 - Object count
 - Area covered
 - Total intensity
- **Co-localisation**
- ...

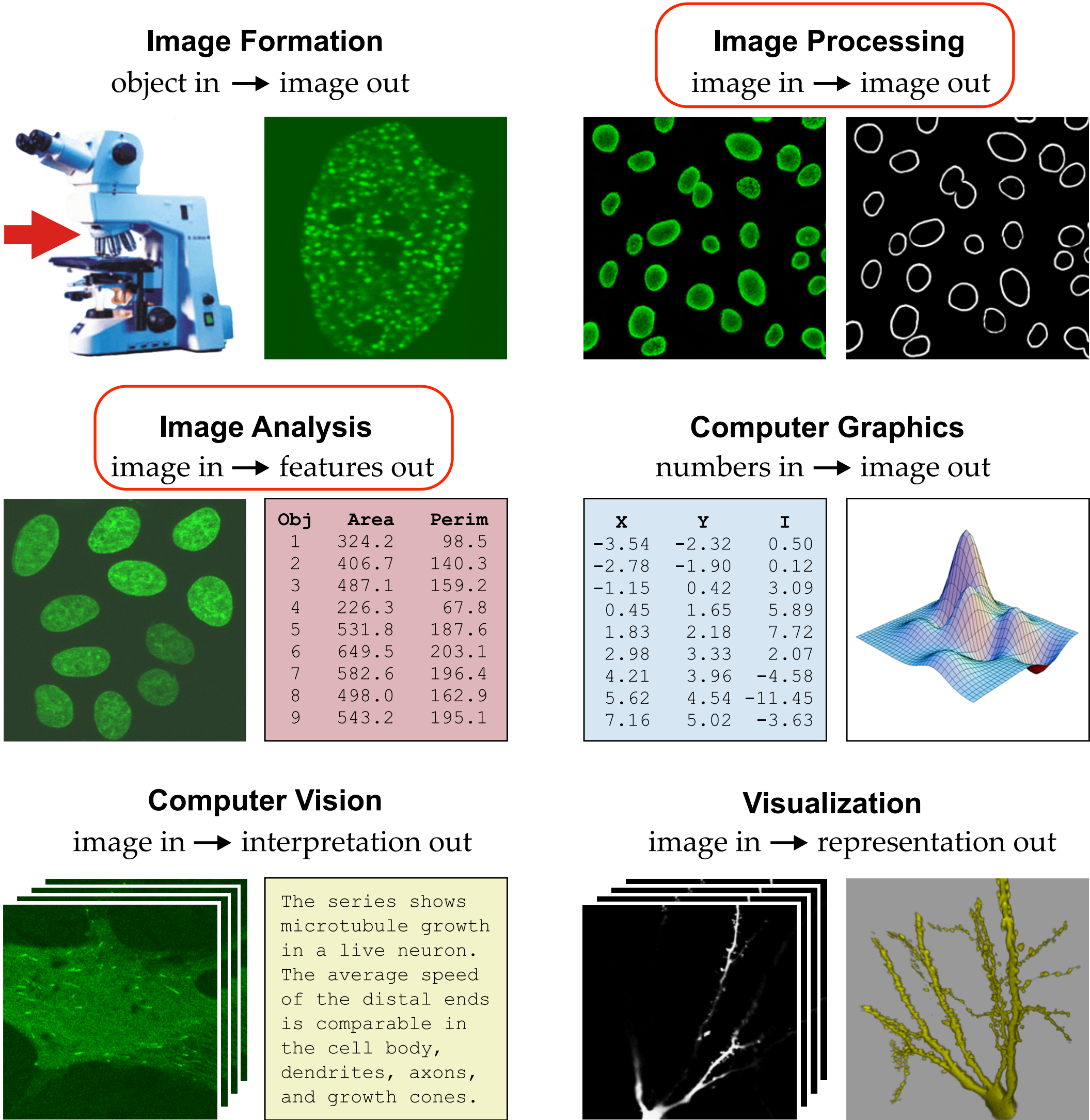


- Exploratory data analysis
- Filtering of numerical data based on measured values
- Summary statistics
- Plotting numerical values
- Making movies and montages of images
- Record entire work-flow for later reference
 - Write down what you did and why (each step)
 - Use a version control system
 - Document your code
- ...

What about Deep Learning?

- Do I still need to know about image analysis?
 - *Yes! But what you need to know may differ*
- What parts of image analysis can Deep Learning replace?
 - *Classification, segmentation, restoration, ...*
- Can I trust the results from Artificial Intelligence?
 - *Can you trust the results from other algorithms?*
 - *Have good controls, verify results!*

Processing vs. Analysis



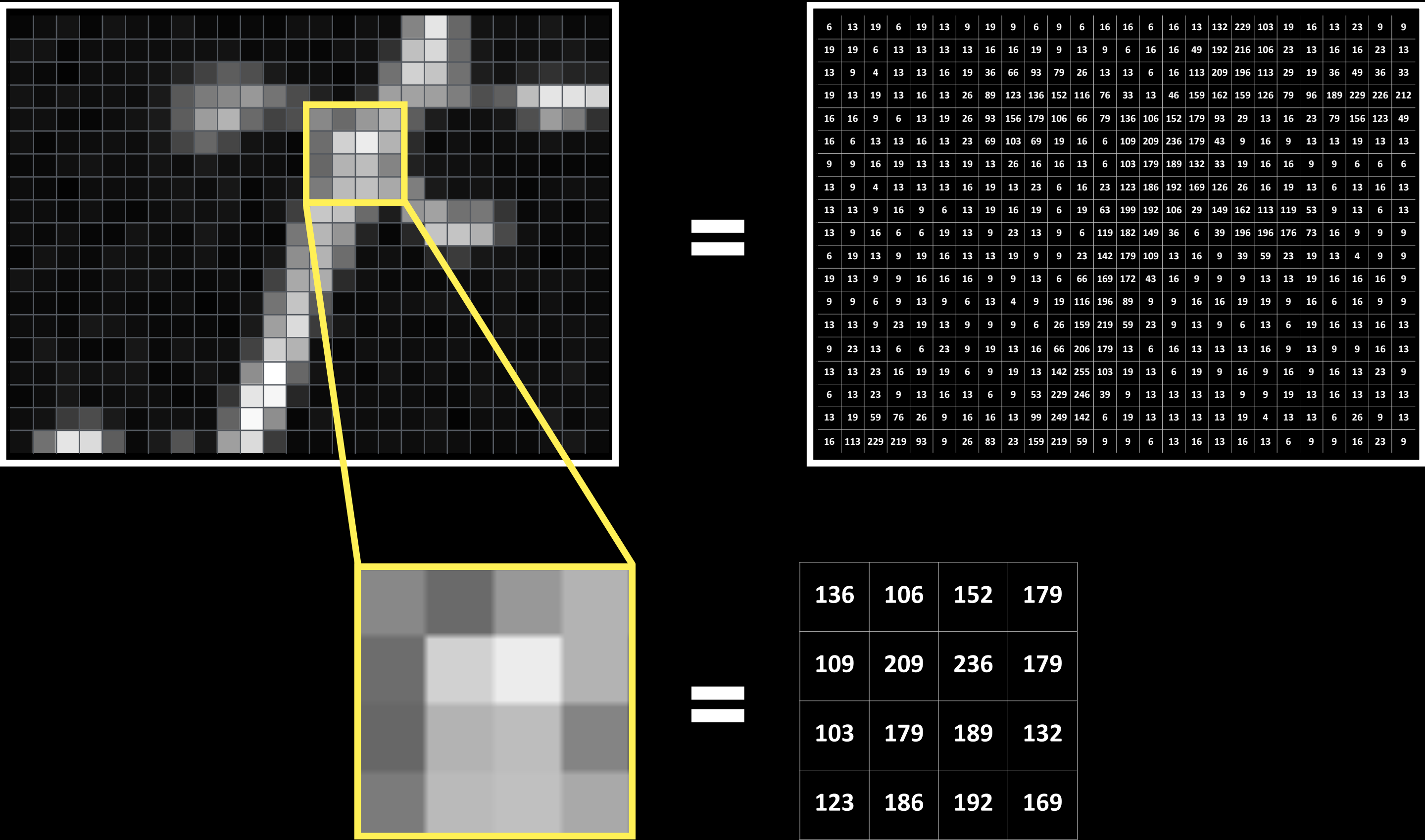
What we see...

...is *not* what we've got!

- Computer screens have 256 grey-levels (8-bit)
 - RGB: $256^3 = 16'777'216$ colours (24-bit, true-color)
 - This is about what the human eye can perceive
- Scientific images are often 16-bit = 65'536 grey-levels
 - The human eye can perceive less than that
- Your monitor shows ***less than 0.4%*** of what is in a good tiff-image

what is an image?

A digital image is a matrix of numbers!



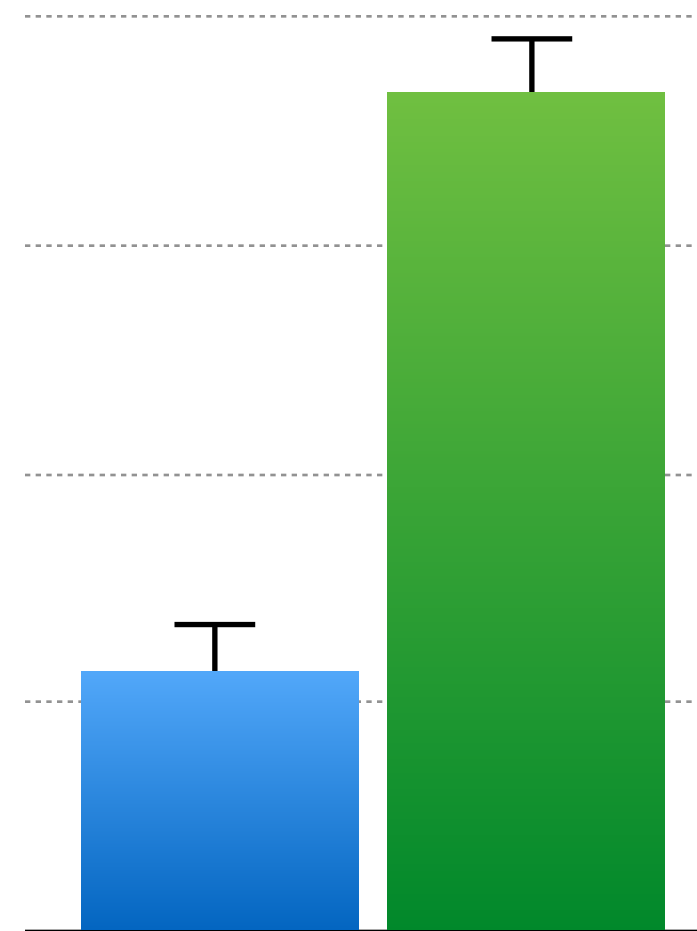
Pixel = Picture Element

Images in publications and presentations
should be used to **communicate** a finding...
not **be** the finding

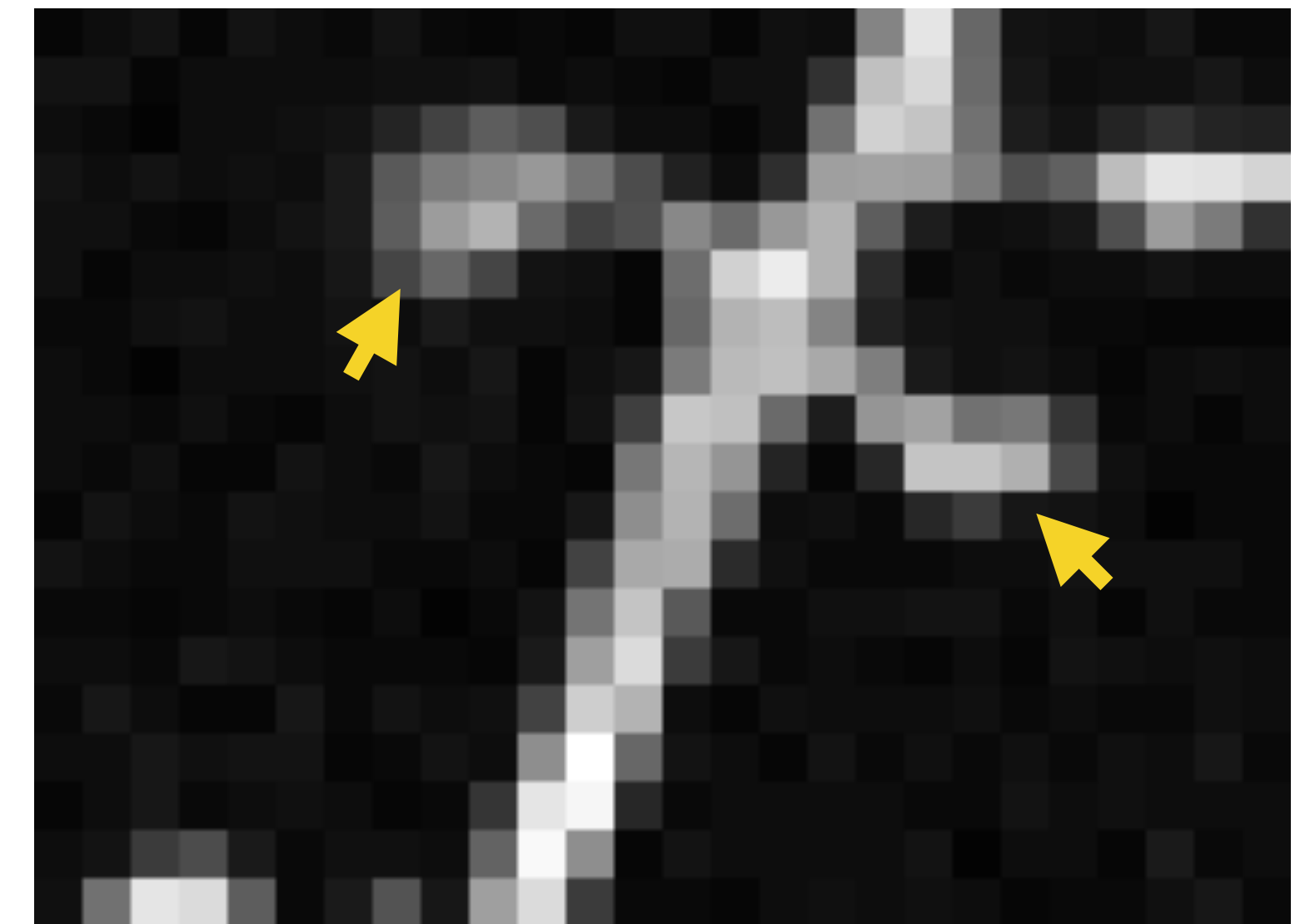
this is your **data**

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

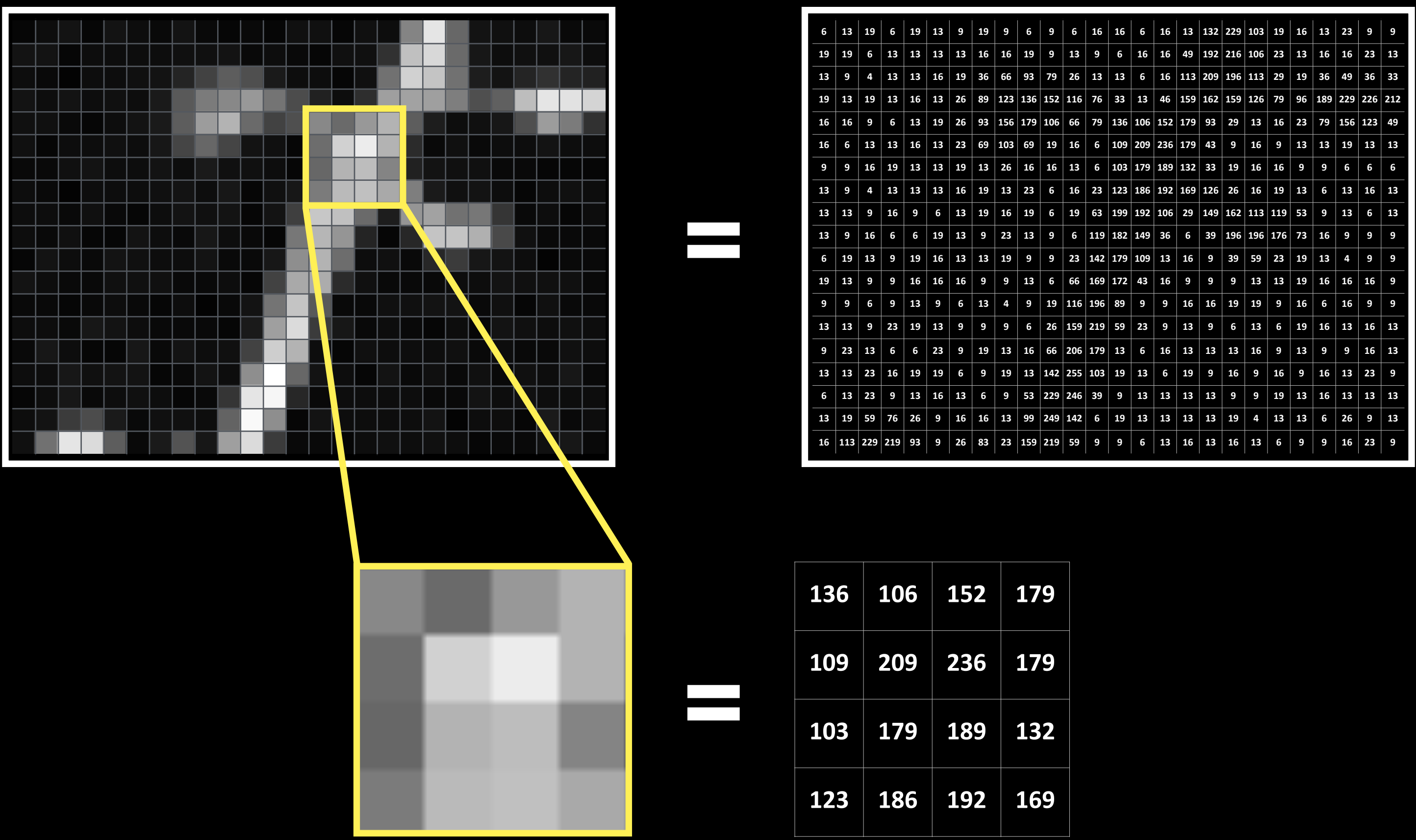
this is your **result**



this just helps to
communicate the result



A digital image is a matrix of numbers!



Where do these numbers come from?

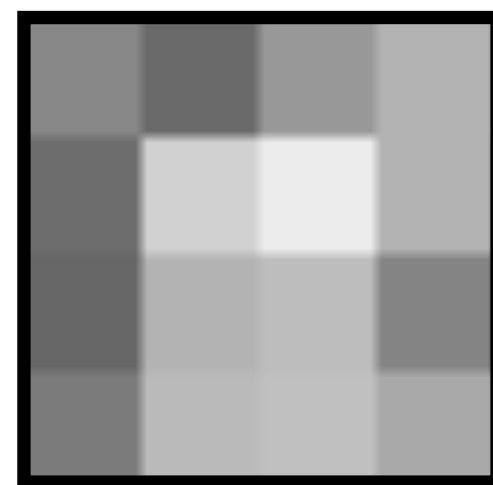
Bit depth

Detectors in Fluorescence Microscopy

The detectors used in fluorescence microscopy are **monochromatic**.

Microscope cameras are **not able to distinguish between different wavelengths** (they just collect photons), you need **fluorescence filters** to separate your fluorophores.

The detector converts photons in digital numbers (linear relation).



=

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Each pixel in the digital image has **one digital value** that **depends on** the **intensity** of the signal emitted by the **sample**.

Digital Values = Pixel Intensity Value

The **range** of possible **digital values** is defined by the **bit depth**.

Bit Depth

The **bit depth** defines the range of possible **digital values** that each pixel can have, usually **8**, **12** or **16 bit**.

The **bit depth** is expressed in **grey values**.

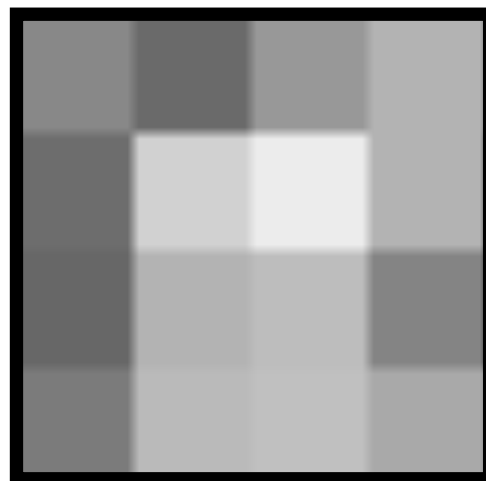
bit depth of the image = bit depth of the detector
(Unless you change that during acquisition)

x bit = a range of 2^x grey values

8 bit image = each pixel can have 2^8 grey values = 256 grey values = range 0-255

12 bit image = each pixel can have 2^{12} grey values = 4096 grey values = range 0-4095

16 bit image = each pixel can have 2^{16} grey values = 65536 grey values = range 0-65535



=

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

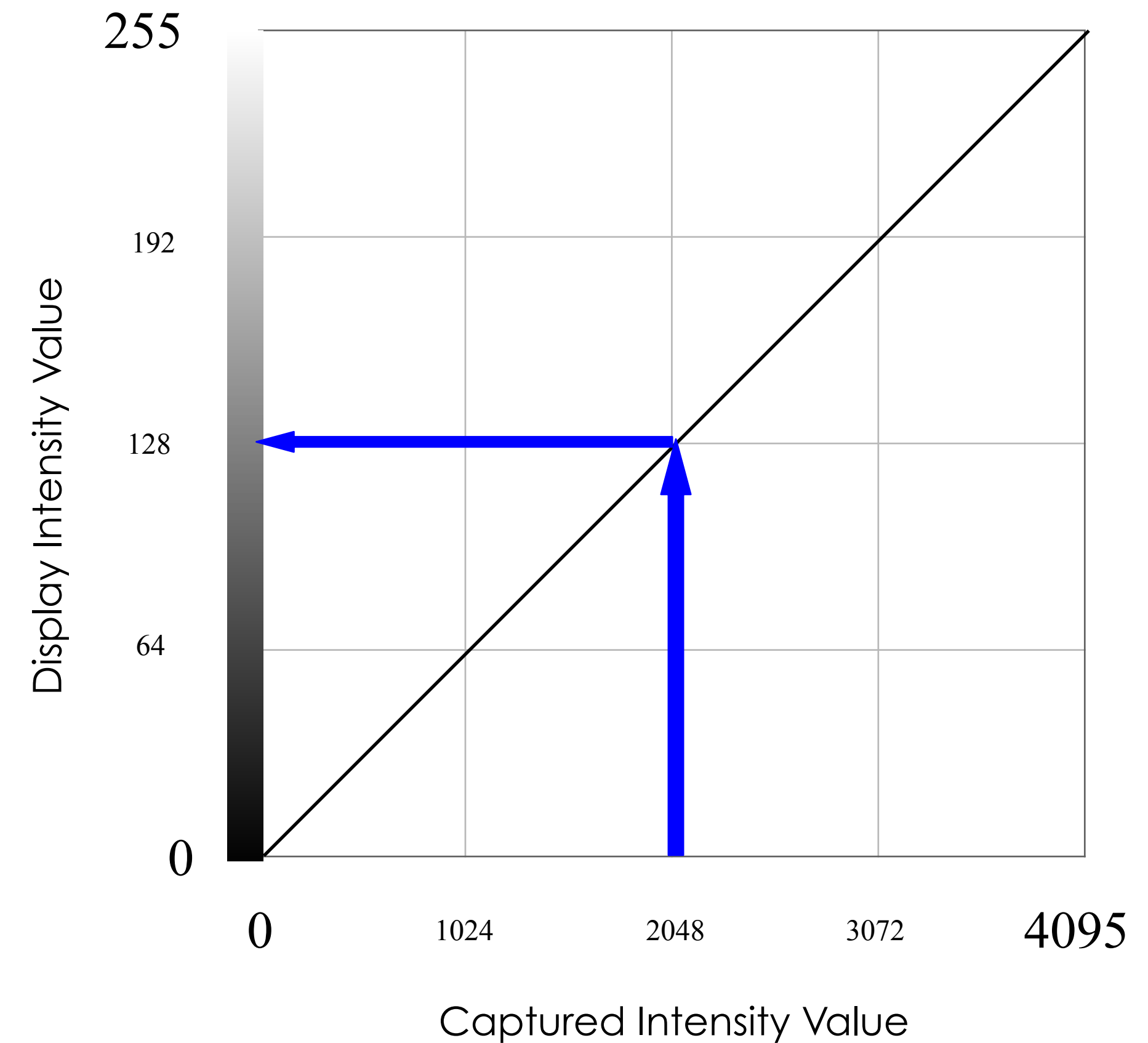
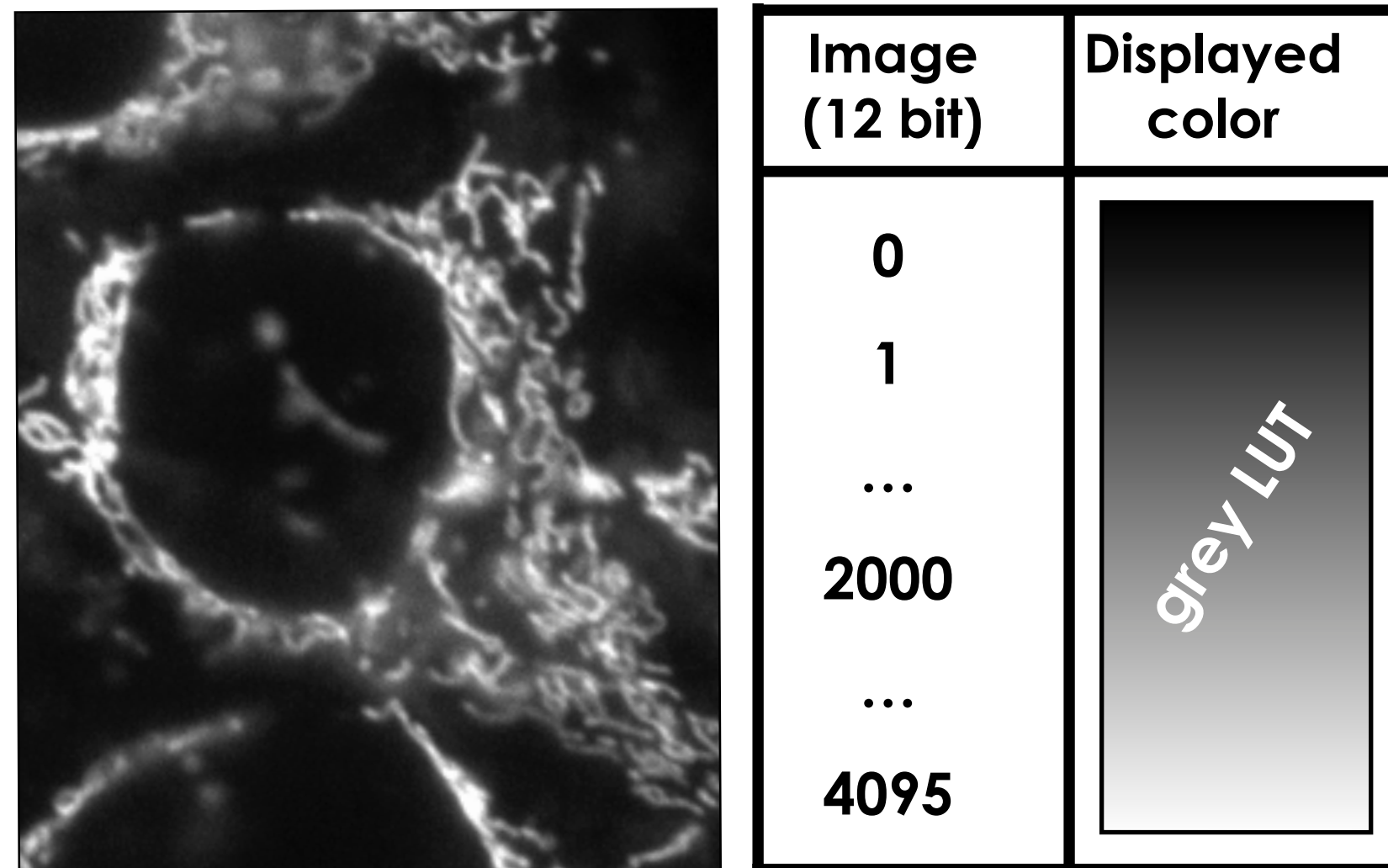
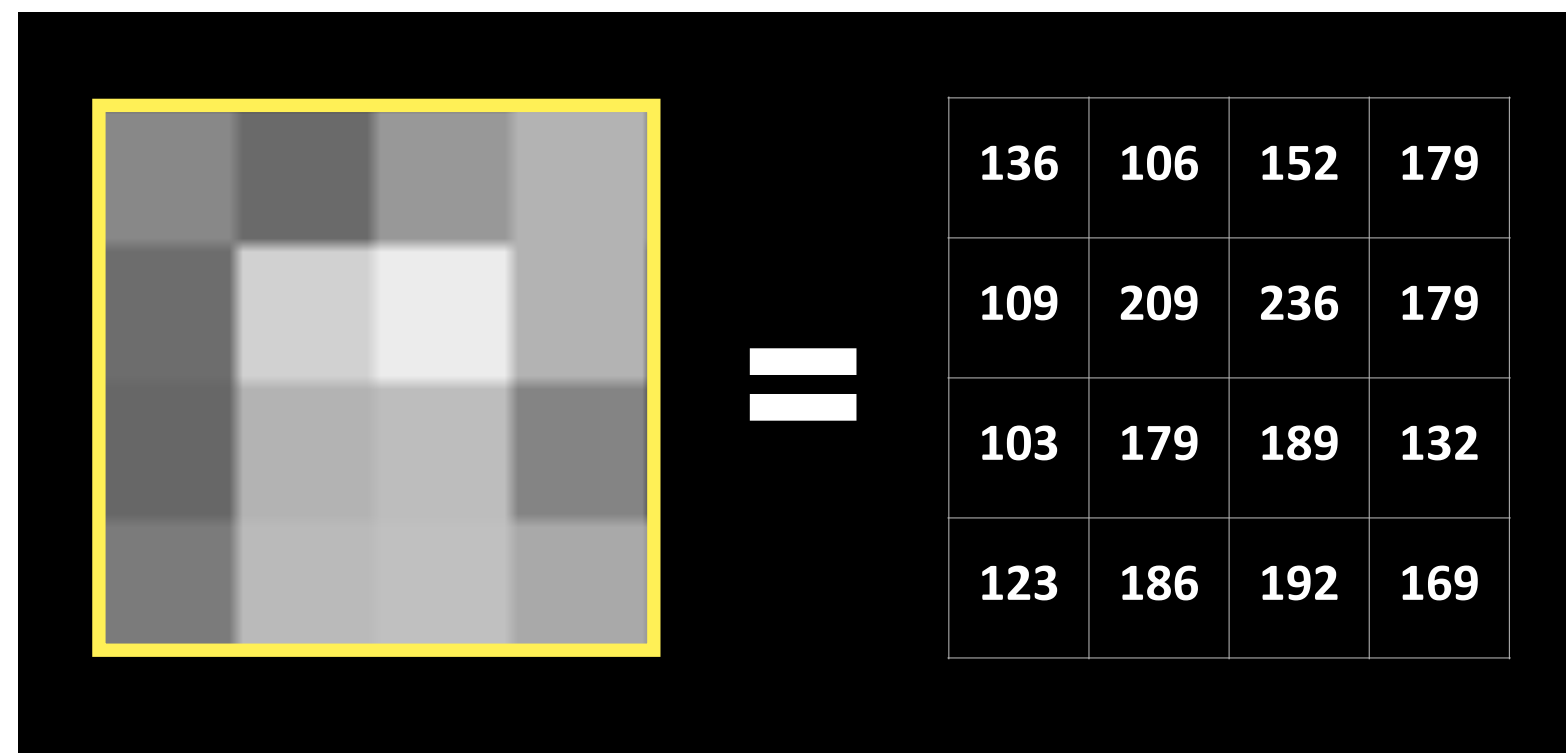
Digital Value = Pixel Intensity Value = Grey Value

display your images

Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are displayed

LUTs do not change the pixel values

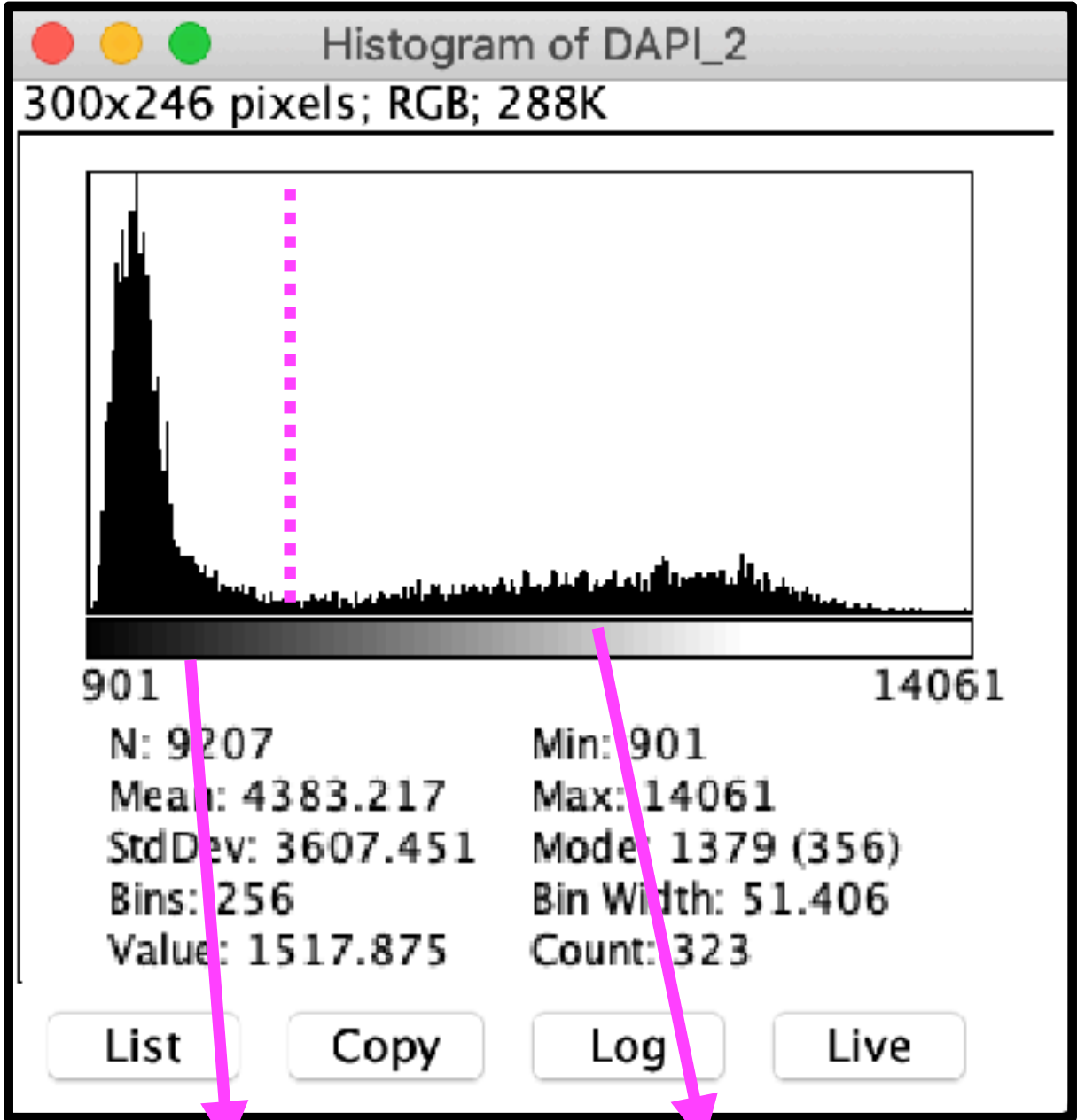




Histogram

Analyze > Histogram

h

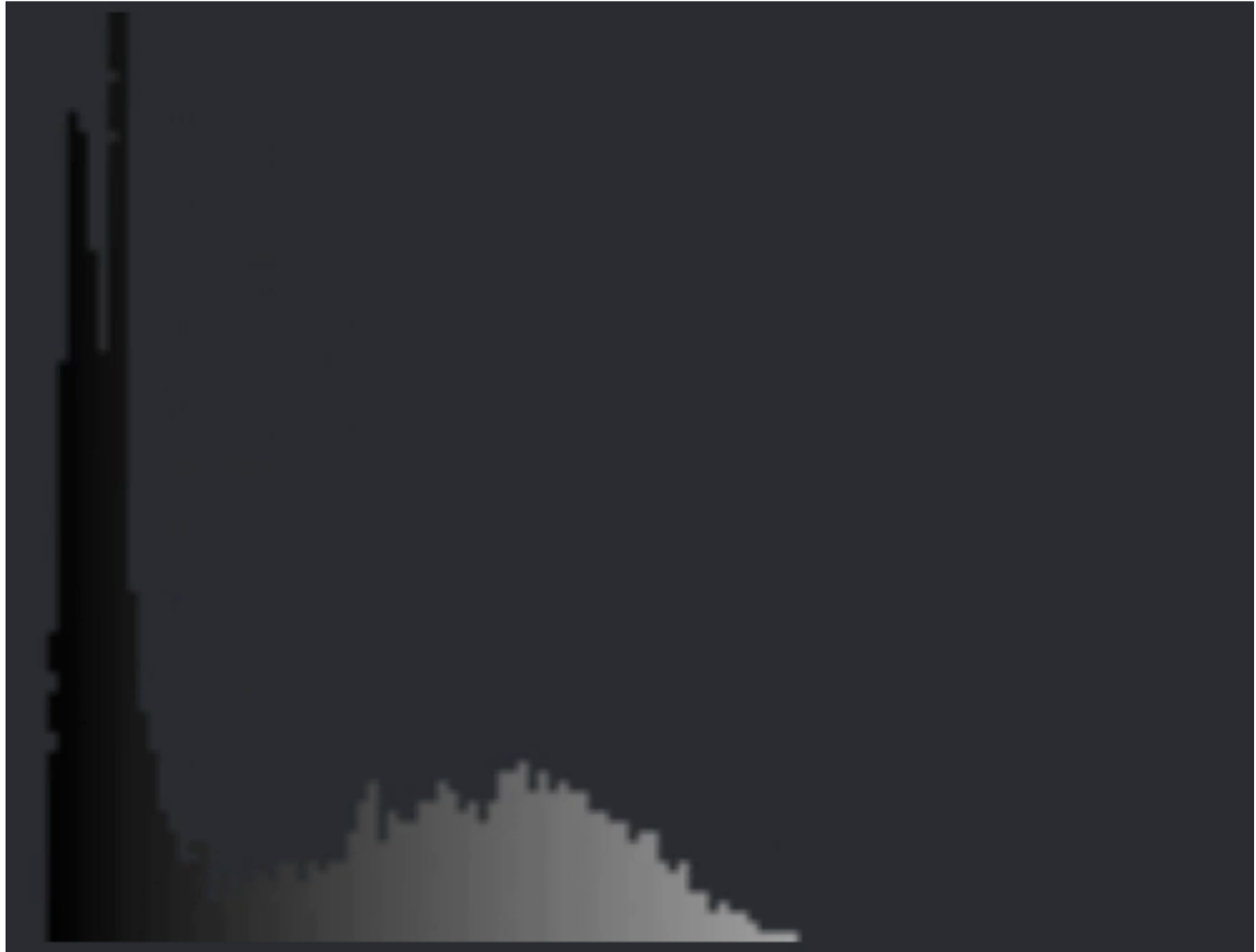


background

foreground

jaehyuk-lee: <https://jaehyuk-lee.com/animated-image-histogram/>

Pixel Count



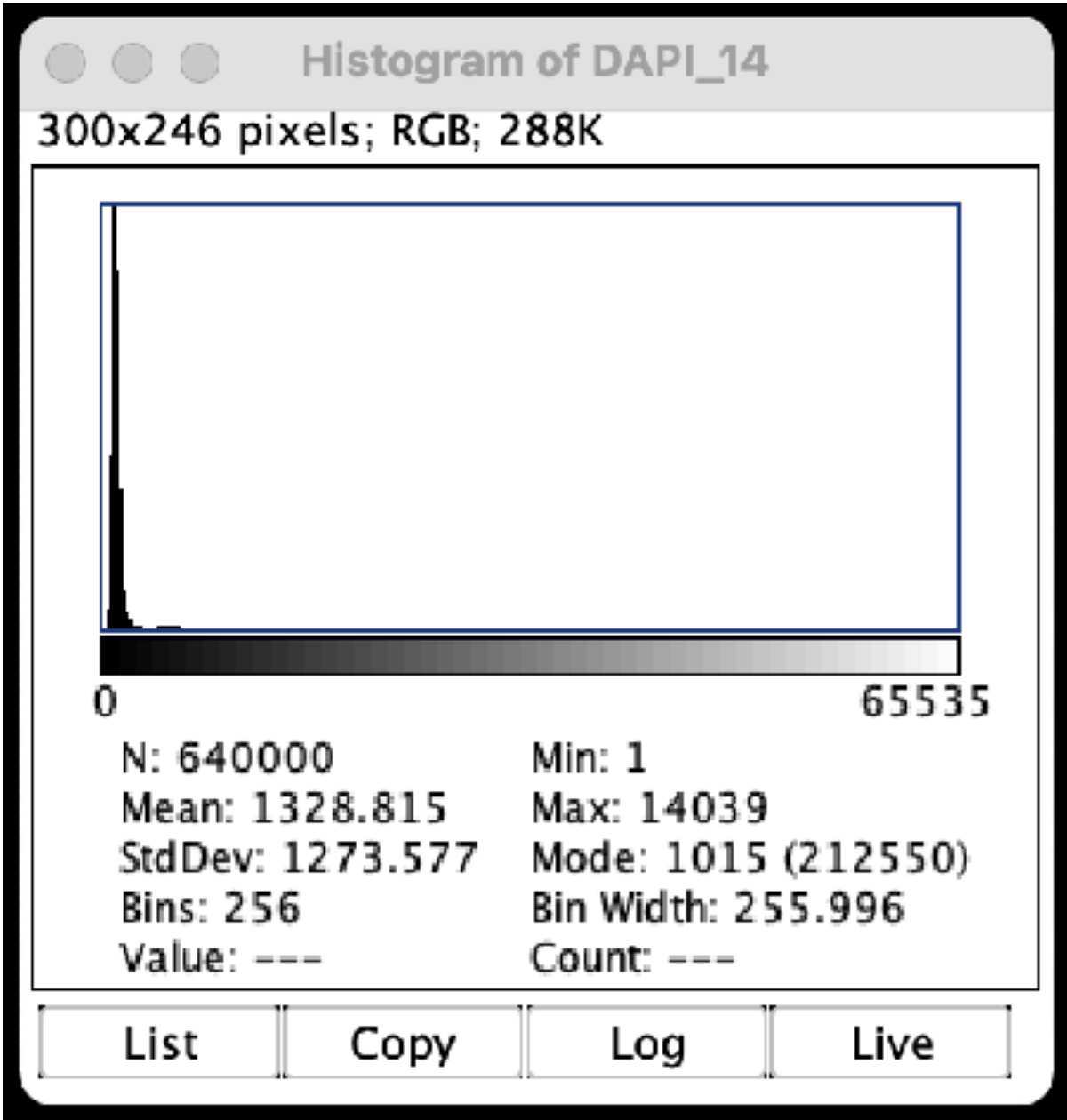
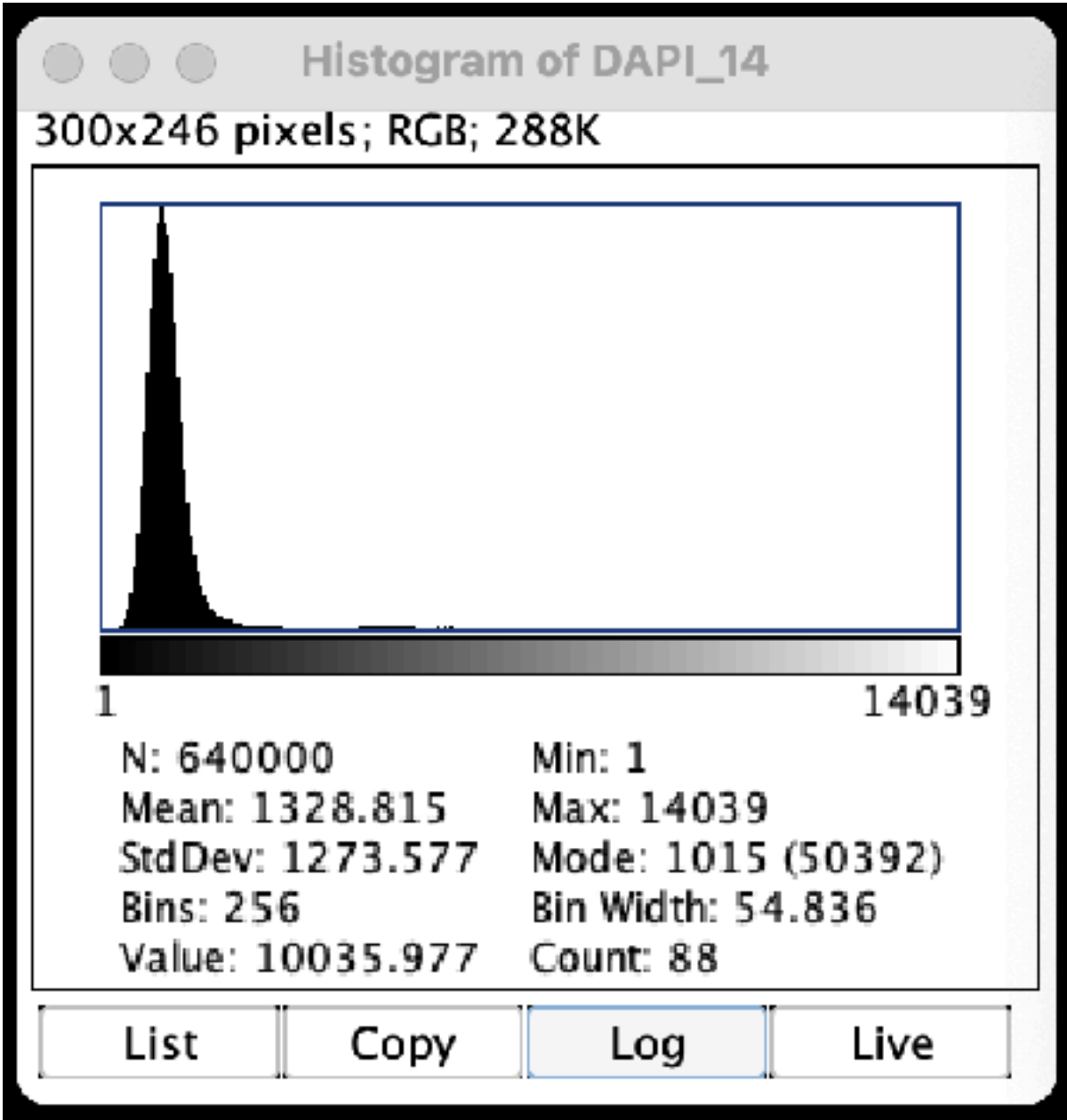
Pixel Values



Histogram

Analyze > Histogram

h

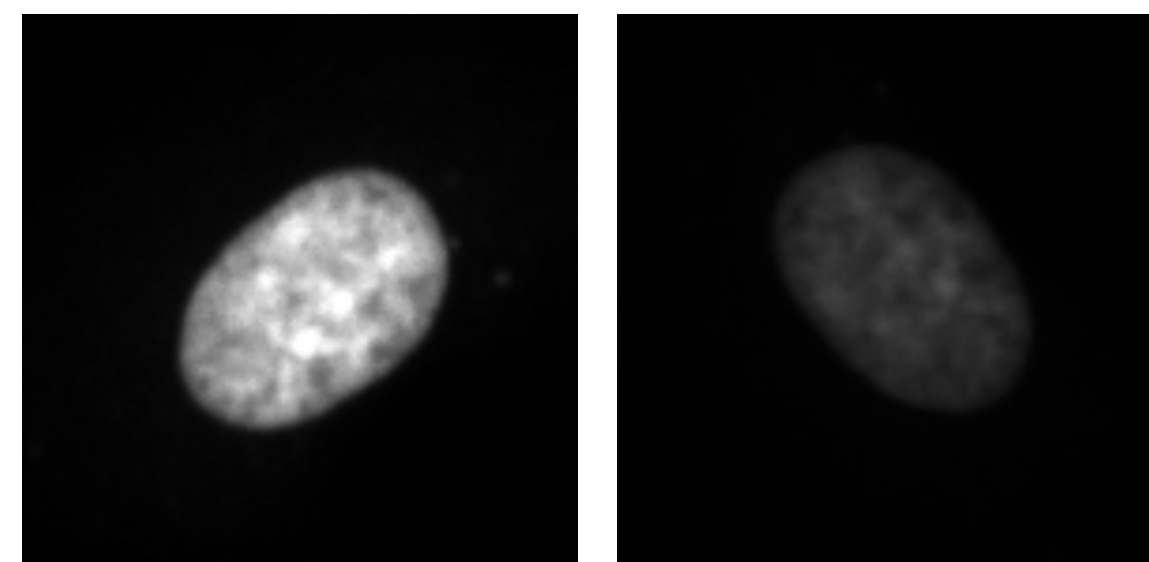


Fiji auto-adjust the range (default option)

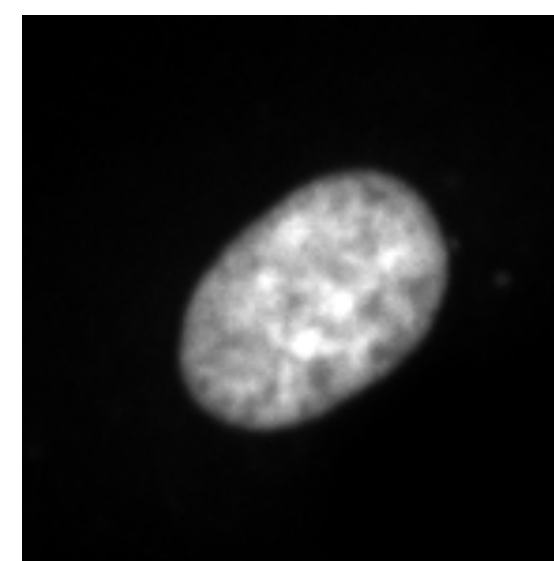
Bit depth

Display a file: Brightness & Contrast

Which image has more fluorescence?



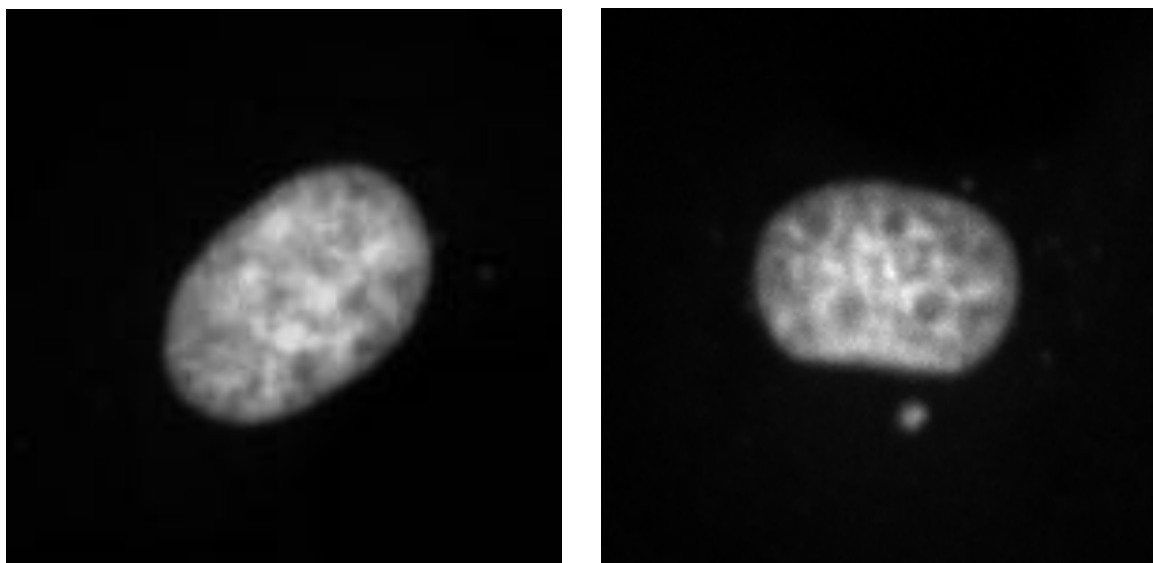
Mean:	4803	4803
Display range:	188-16828	188-45514



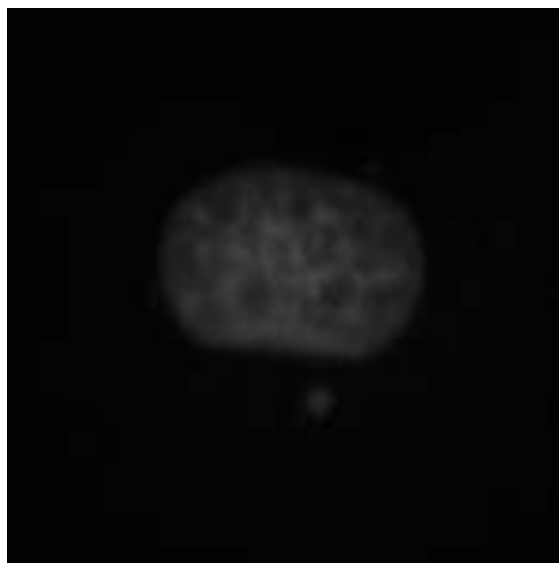
Mean:	4803	4803
Display range:	188-16828	188-16828

Display a file: Brightness & Contrast

Which image has more fluorescence?



Mean:	4803	2074
Display range:	188-19540	112-7768



Mean:	4803	2074
Display range:	188-19540	188-19540

Do NOT trust your eyes,
rely on numbers!

Images and Colors

Lookup Tables (LUTs)

LUT = how the grey values are displayed

LUTs do not change the pixel values

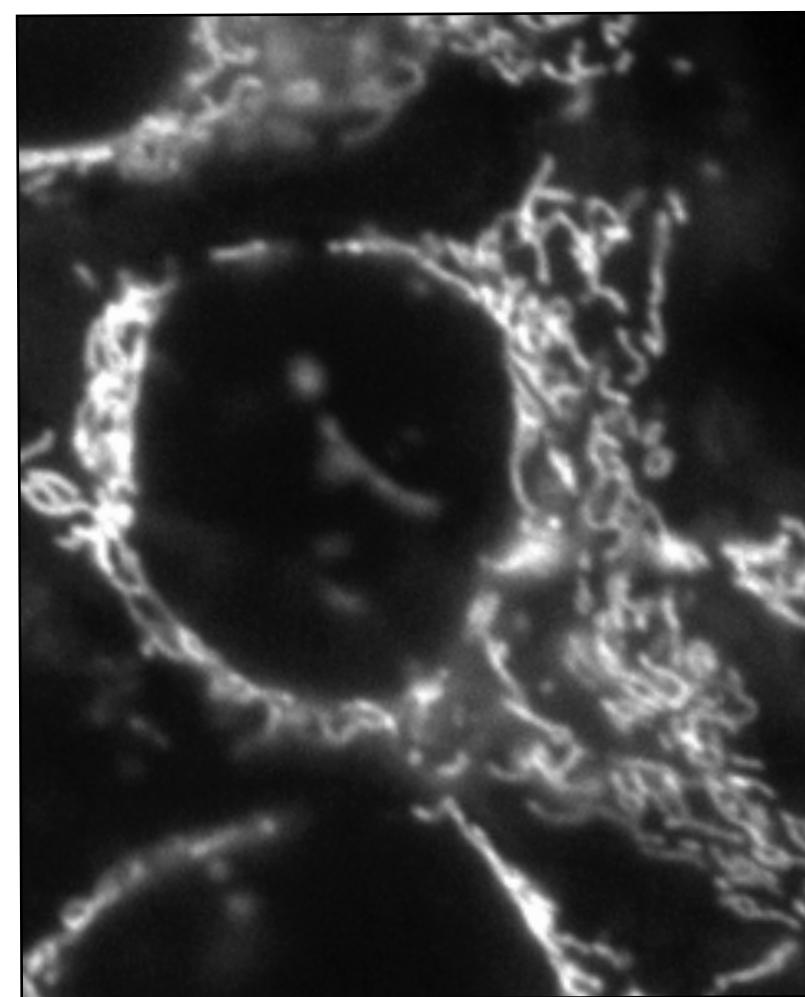
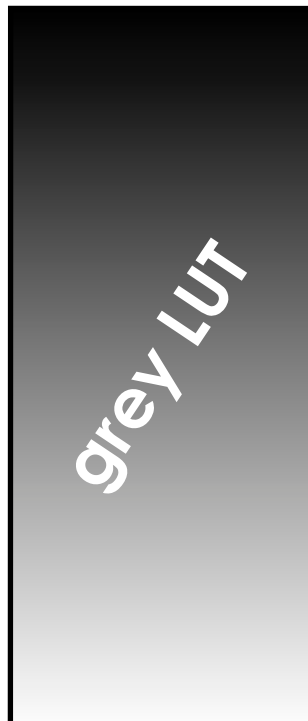


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	

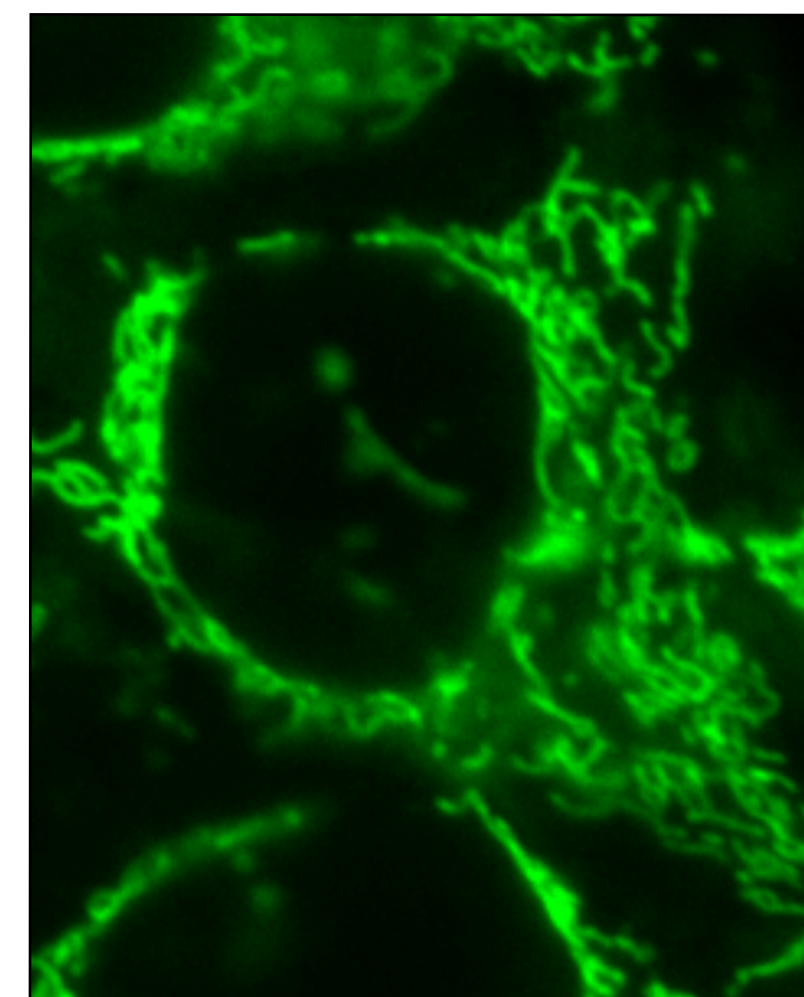

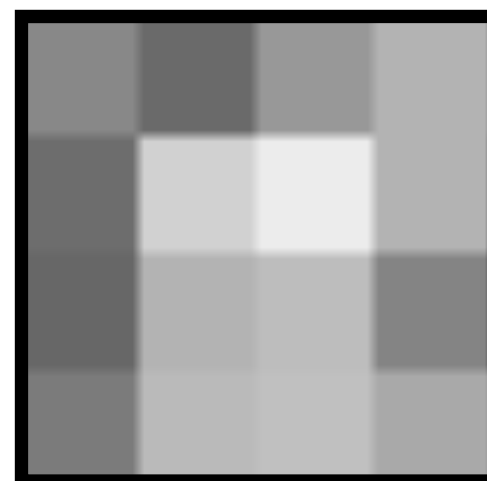
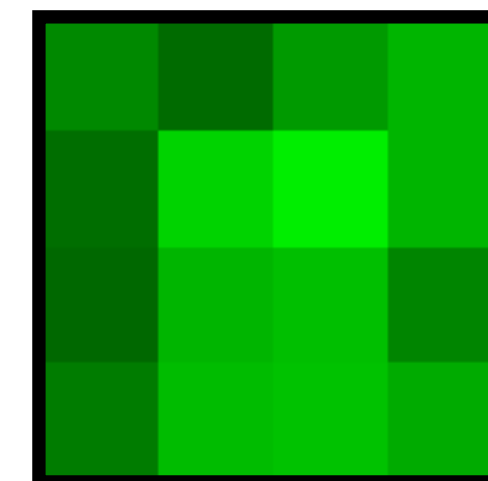


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	



=

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169



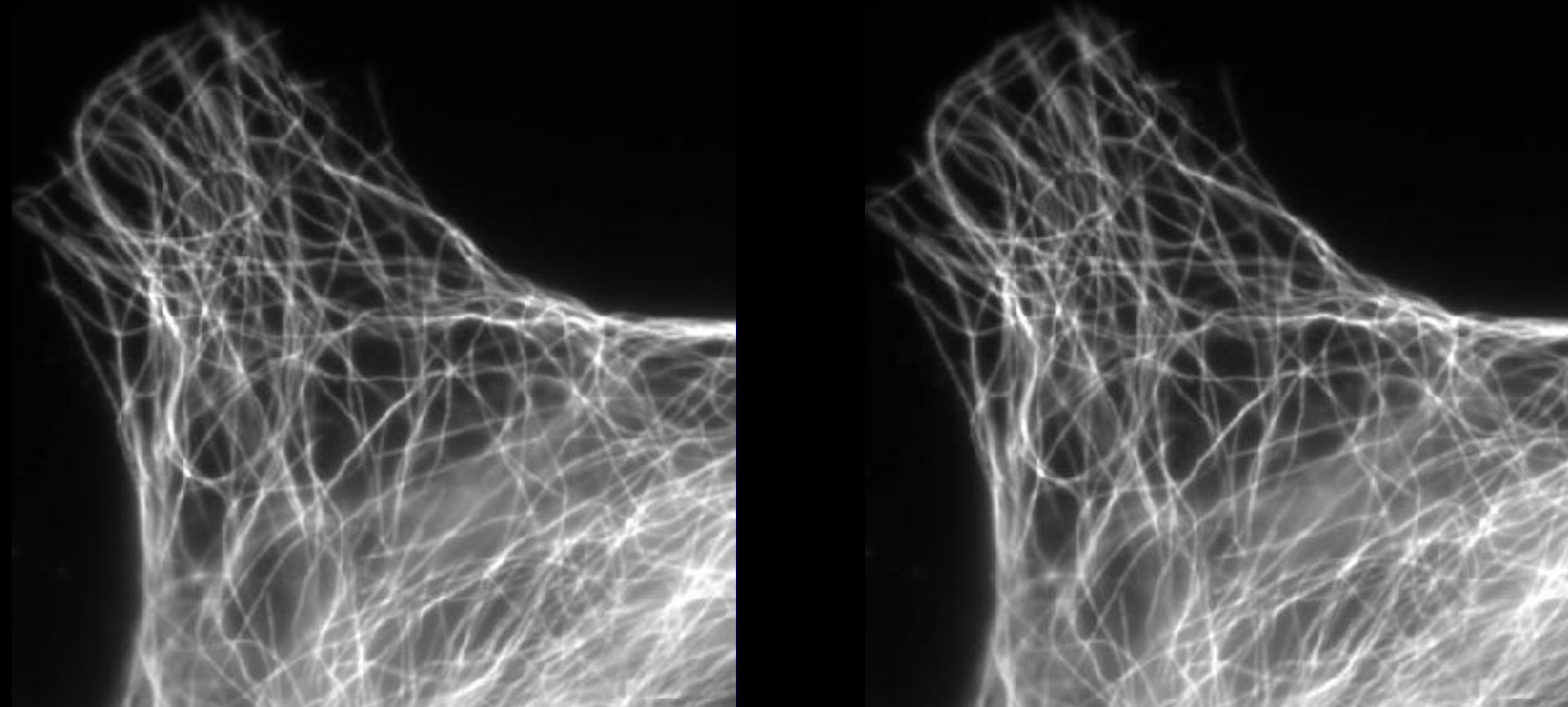
=

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Images and Colors

Choose the right LUT

Which is brighter?



The human eye evaluates intensity best in grayscale

If you are imaging for example a blue fluorophore, you
are NOT FORCED to display it in blue!

Images and Colors

Choose the right LUT

Color blind people don't distinguish some colors

POINTS OF VIEW

Color blindness

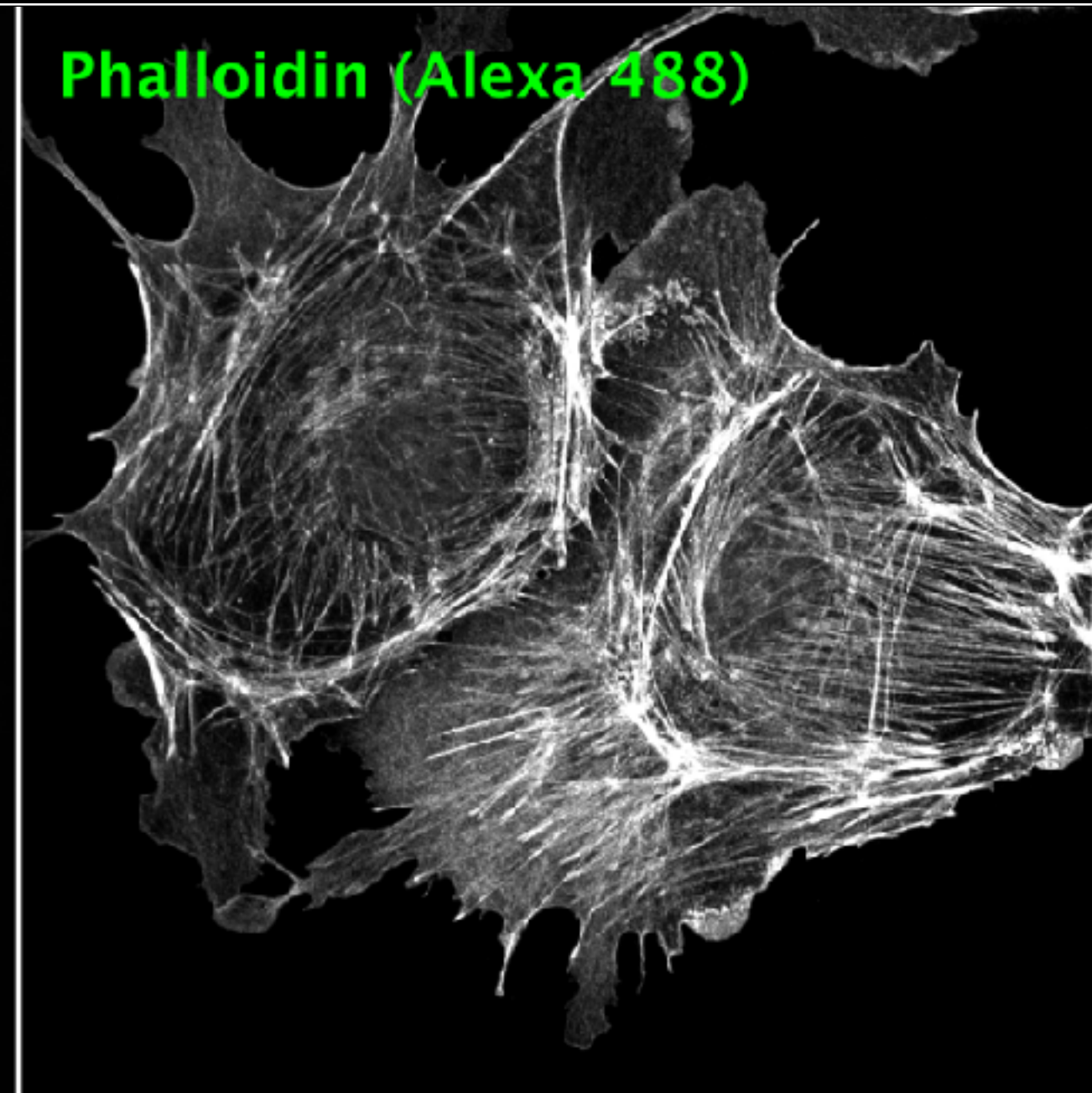
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"If a submitted manuscript happens to go to three male reviewers of Northern European descent, the chance that at least one will be color blind is 22 percent"

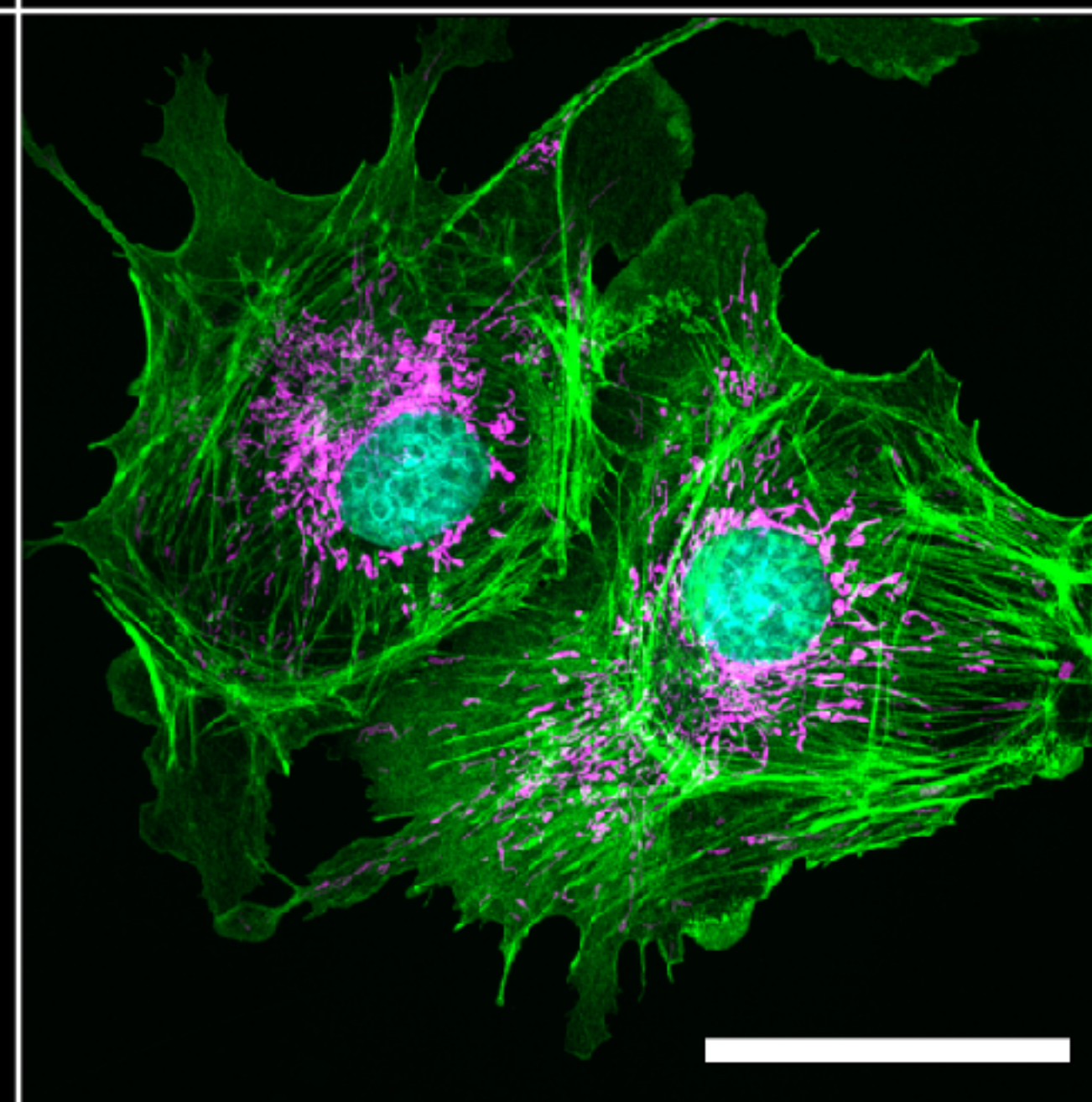
DAPI

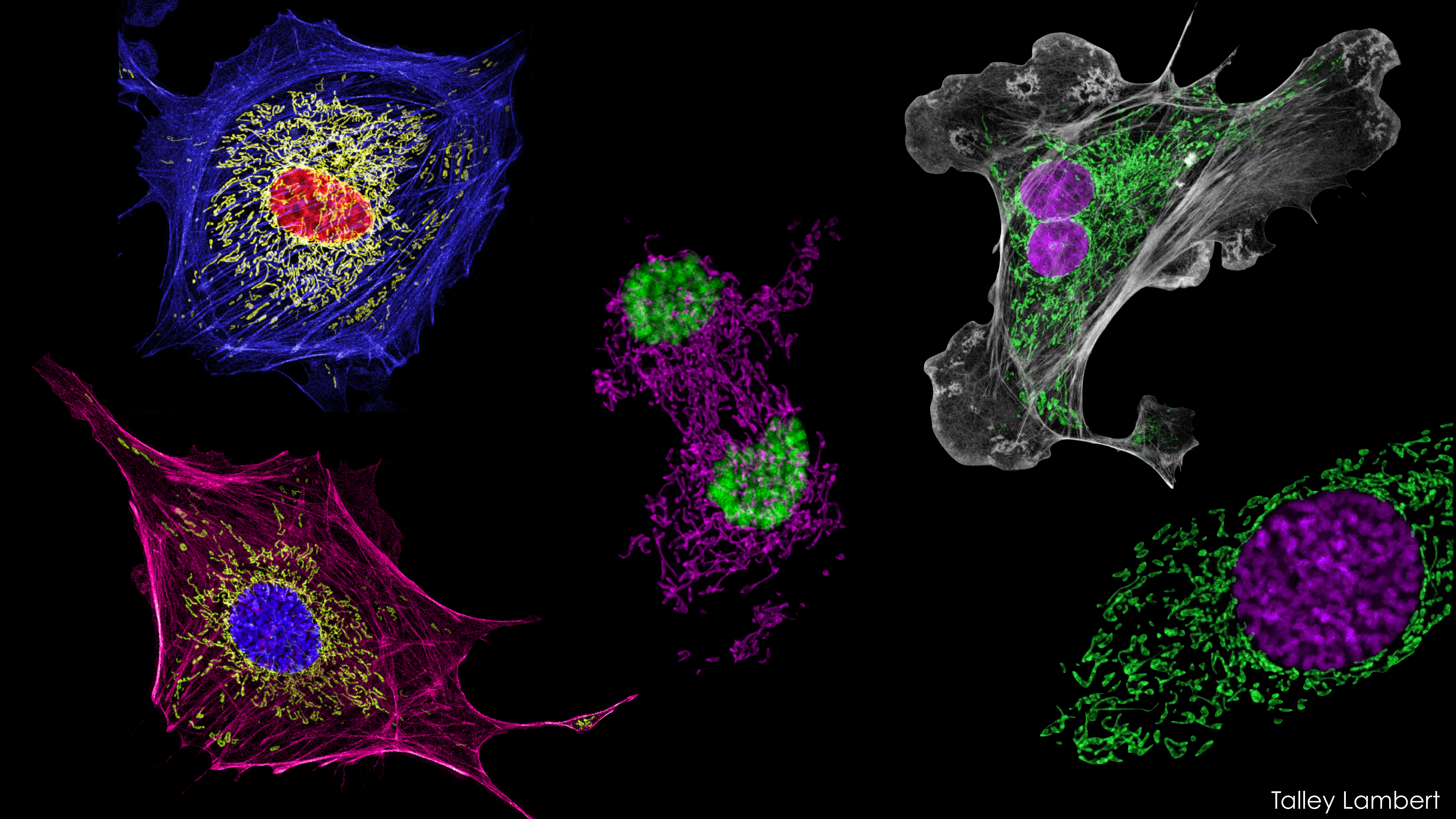


Phalloidin (Alexa 488)



MitoTracker Red





Further Learning (<https://iac.hms.harvard.edu/resources/>)



image.sc

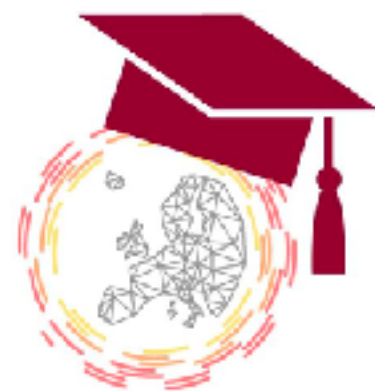
Forum: Knowledge exchange and support

- <https://forum.image.sc/>



Online book with code: Introduction to Bioimage Analysis

- <https://bioimagebook.github.io/>



Online training: NEUBIAS Academy

- <https://eubias.org/NEUBIAS/training-schools/neubias-academy-home/>
- <https://www.youtube.com/c/NEUBIAS>



Fiji manual from Monash University

- https://bridges.monash.edu/articles/educational_resource/Fiji_Training_Manual_v6_4_/20033513