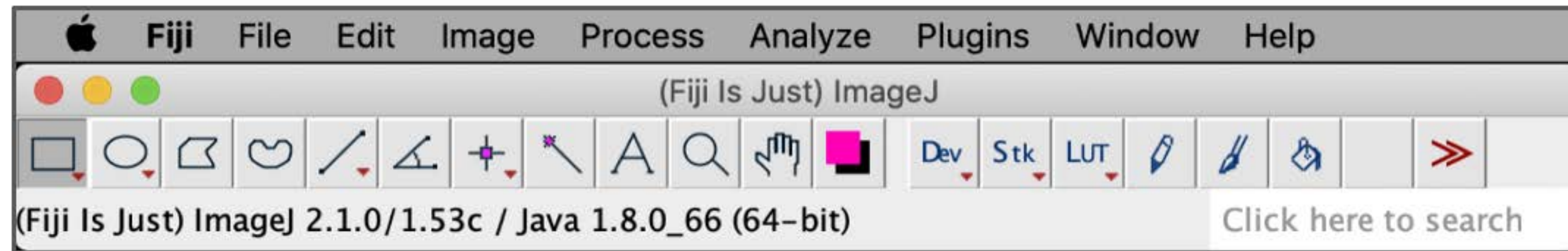


# Introduction to image analysis using ImageJ/Fiji



Federico Gasparoli, Ranit Karmakar, Antoine Ruzette, Maria Theiss & Simon F. Nørrelykke  
*Image Analysis Collaboratory, HMS*

# Get the course materials

**<https://hms-iac.github.io/fiji-workshop>**

One-stop resource for everything we'll cover today

- **Download all slides (PDF)**
- **Download all exercises (PDF)**
- **Download all images (ZIP)**

# 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](mailto: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

# Learning objectives

1. **Motivate** the use of algorithms in image analysis
2. **Introduce** some image-analysis nomenclature
3. **Learn** to use Fiji effectively and reproducibly

# Reasons for Learning About Image Processing

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

# Reasons for Not Learning About Image Processing

- ...

**none**

# Acknowledgements

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



# Self-introductions

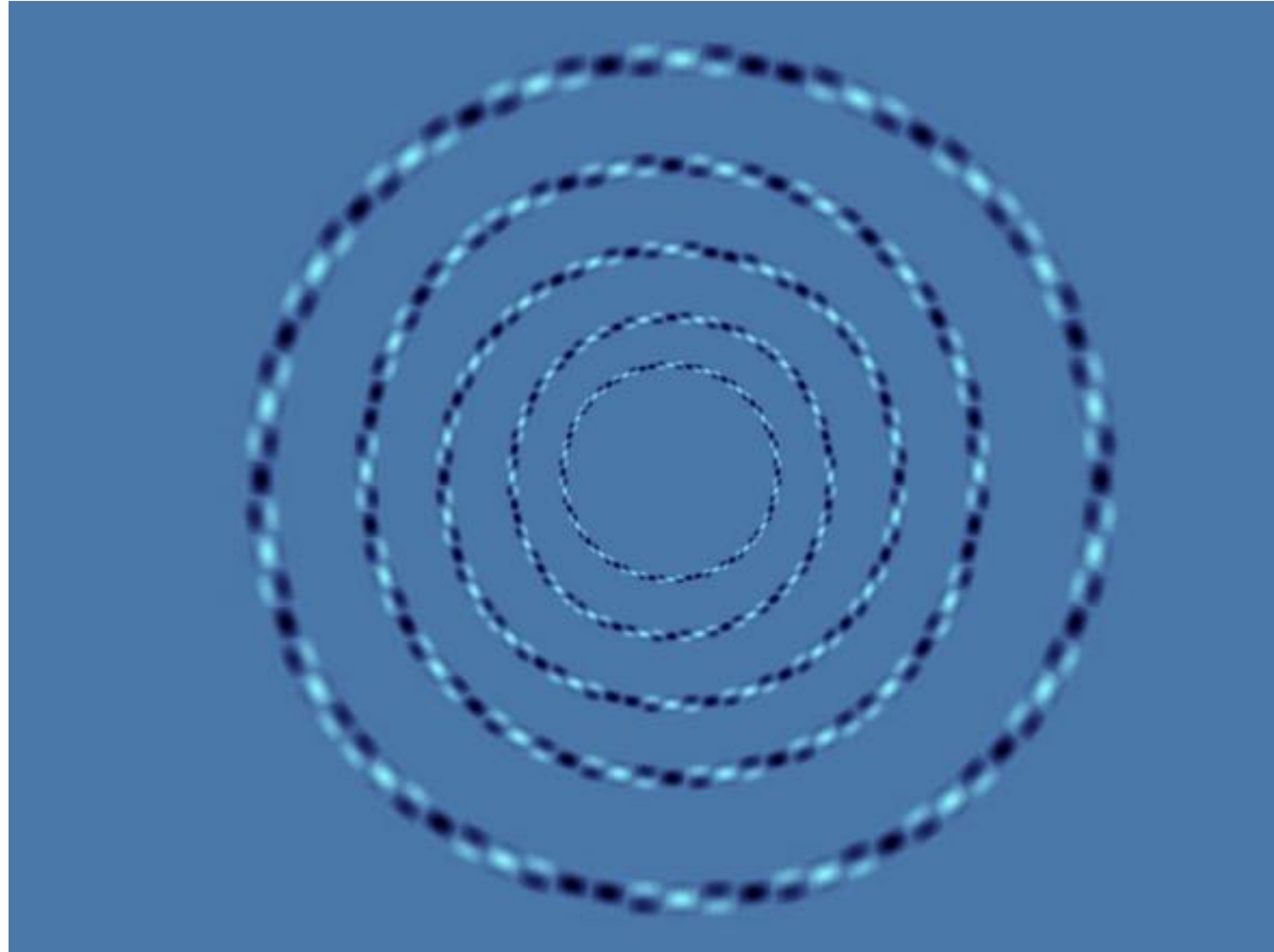
1. My **name** is *Donald Duck*
2. My **position** is as a *10th year postdoc*
3. My **lab** is *Mad City Labs*
4. My model **system** is *blue whales*
5. I have **data** from *confocal microscopy*

# Optical Illusions

—

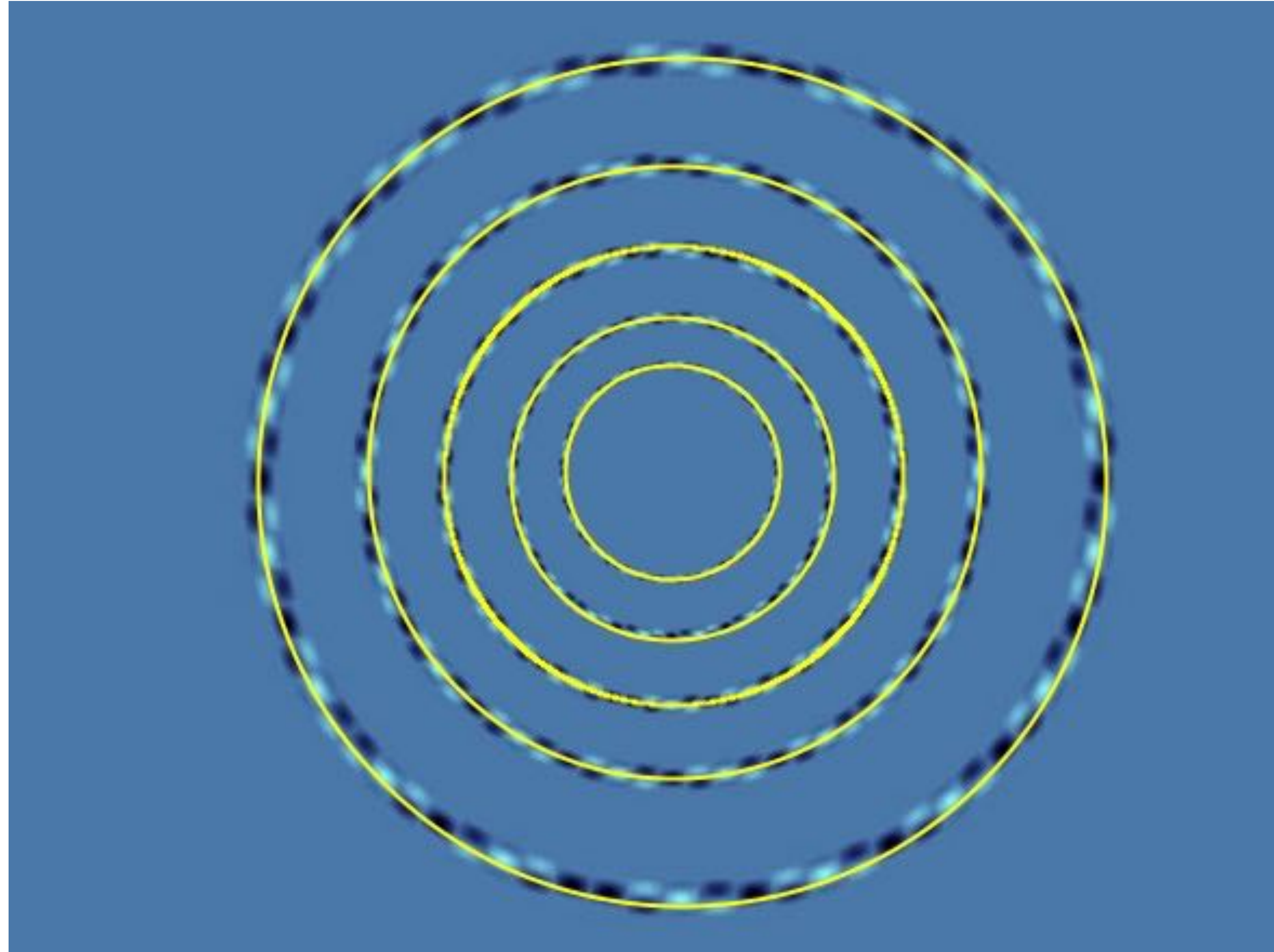
Why should you analyze images  
with computers

# Color perception and pattern recognition is individual – science less so



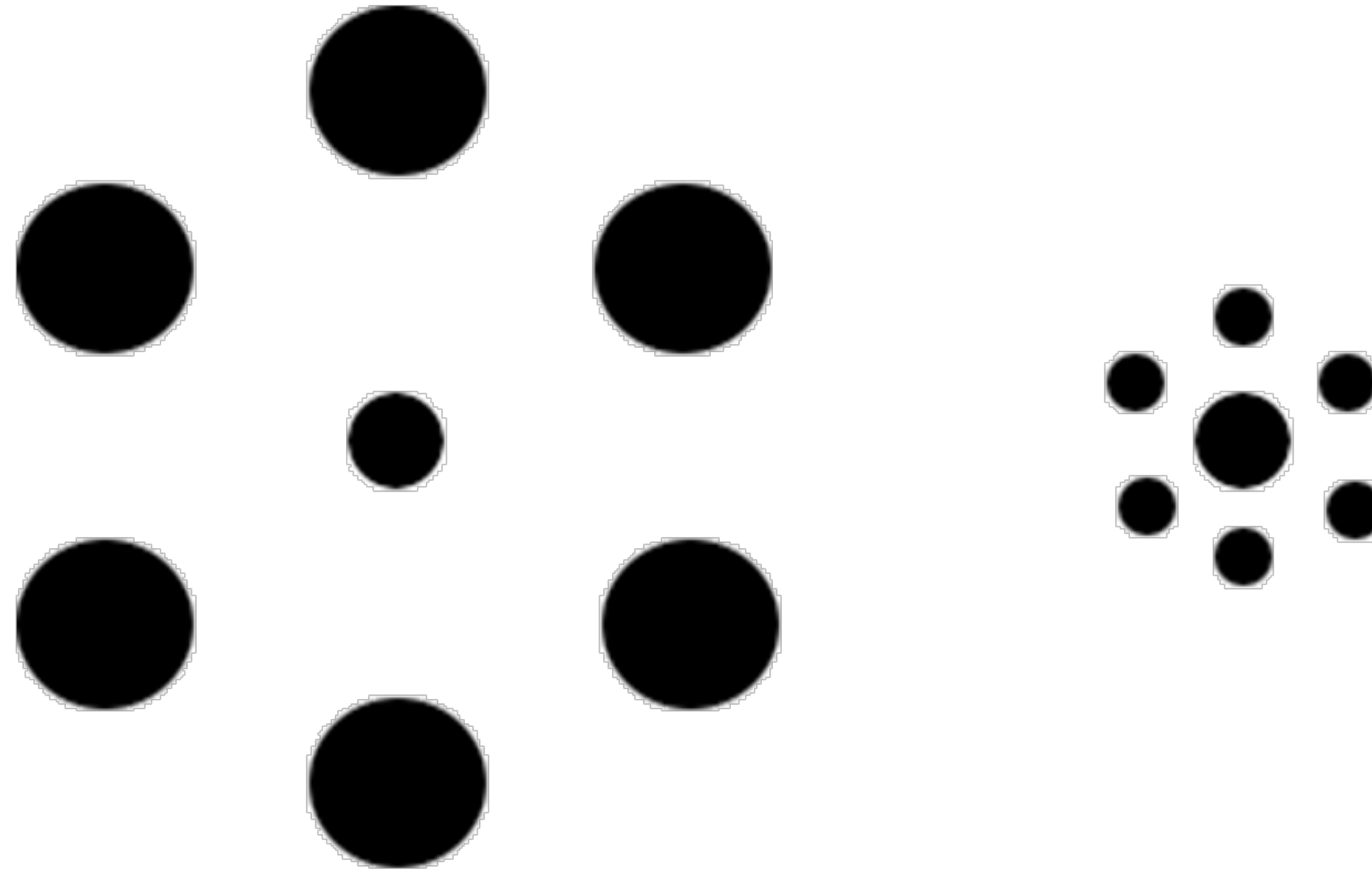
<https://www.moillusions.com/perfect-circles-optical-illusion/>

# Concentric circles !?



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

# Identical central discs?



Our size estimate is strongly influenced by the local neighbourhood

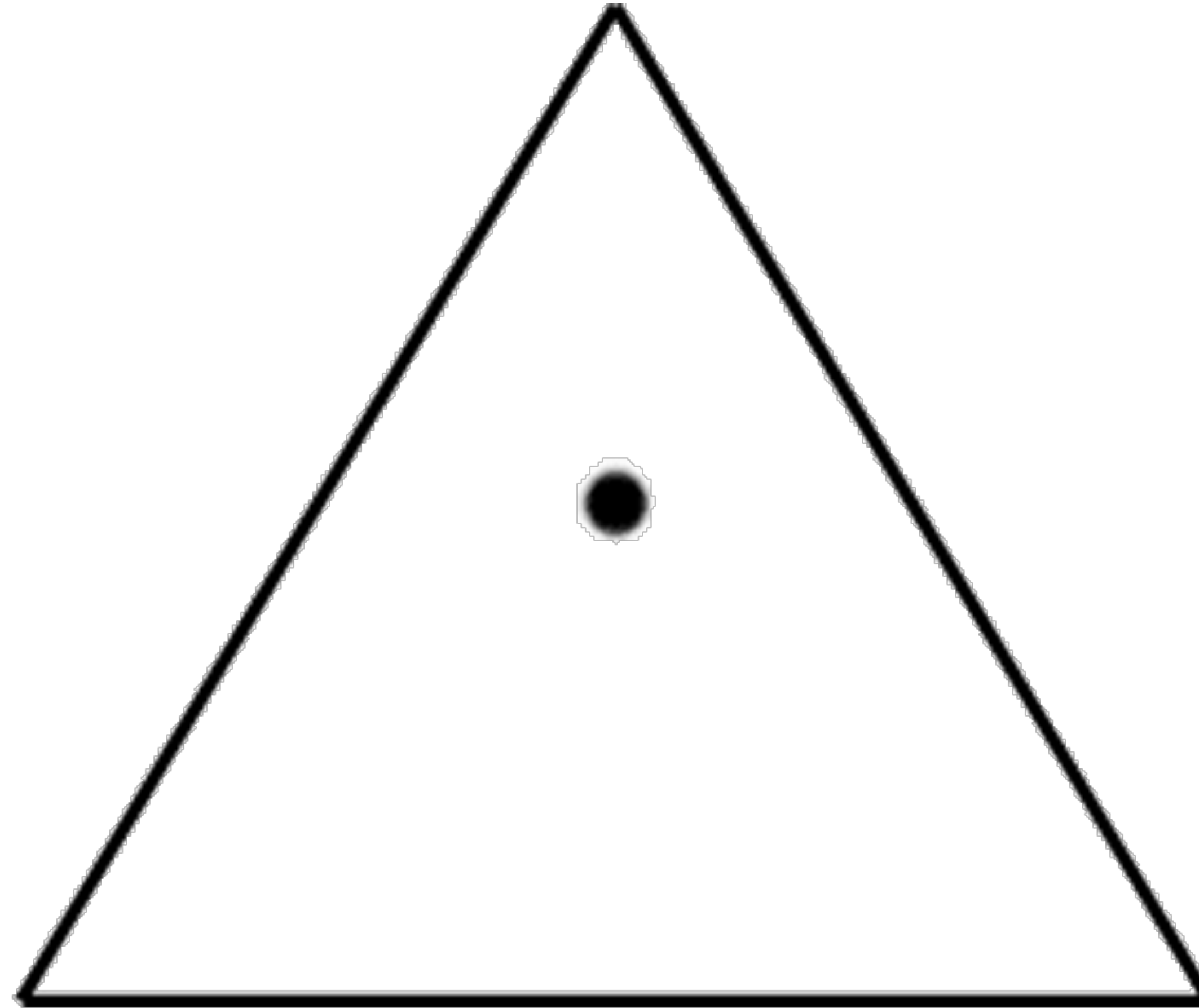
# Identical central discs?



Yes, the discs are identical

<http://www.brainbashers.com>

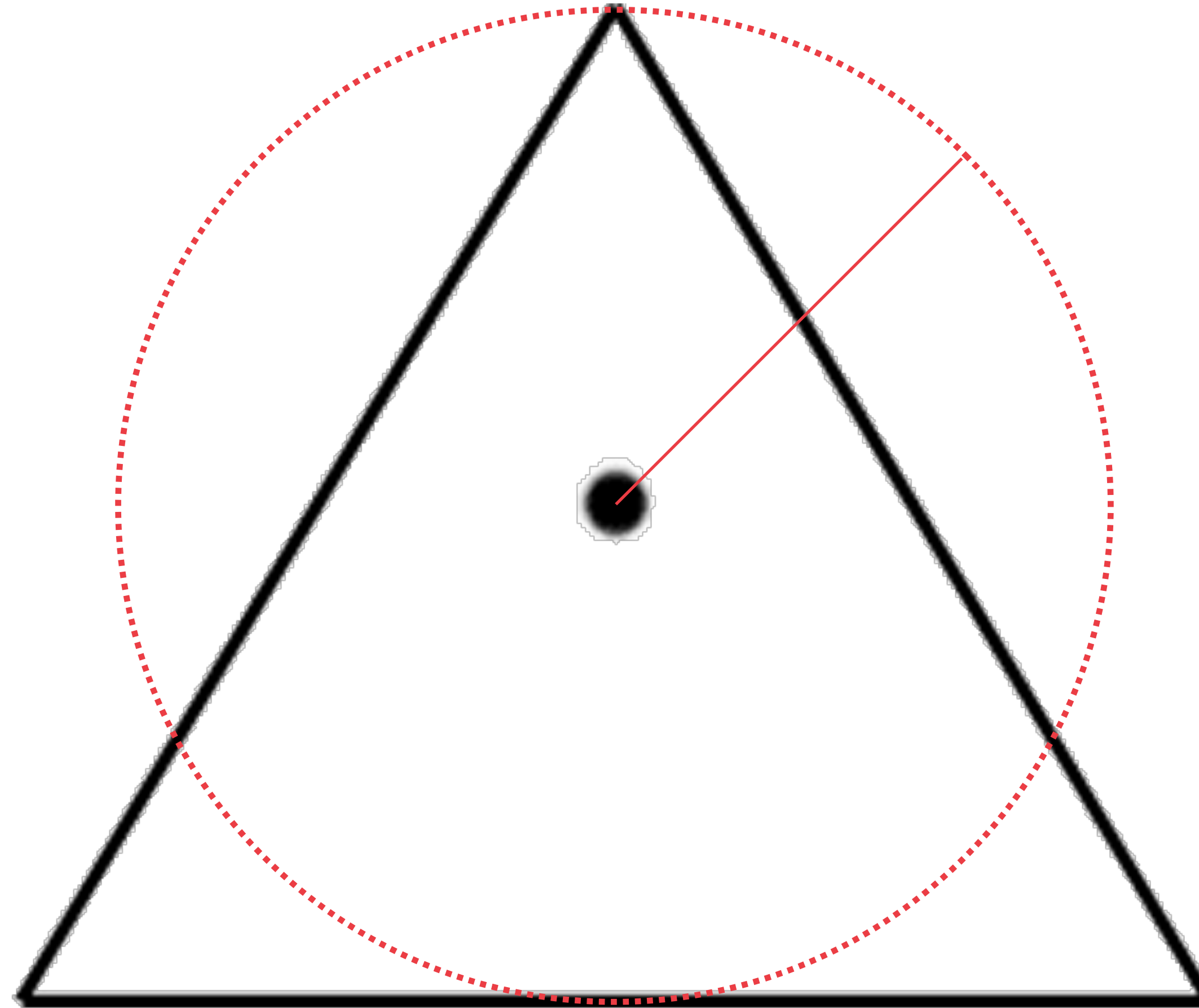
**Is the dot half-way up?**



Our sense of distance depends on neighbourhood

<http://www.brainbashers.com>

**Is the dot half-way up?**



Yes it is!

<http://www.brainbashers.com>



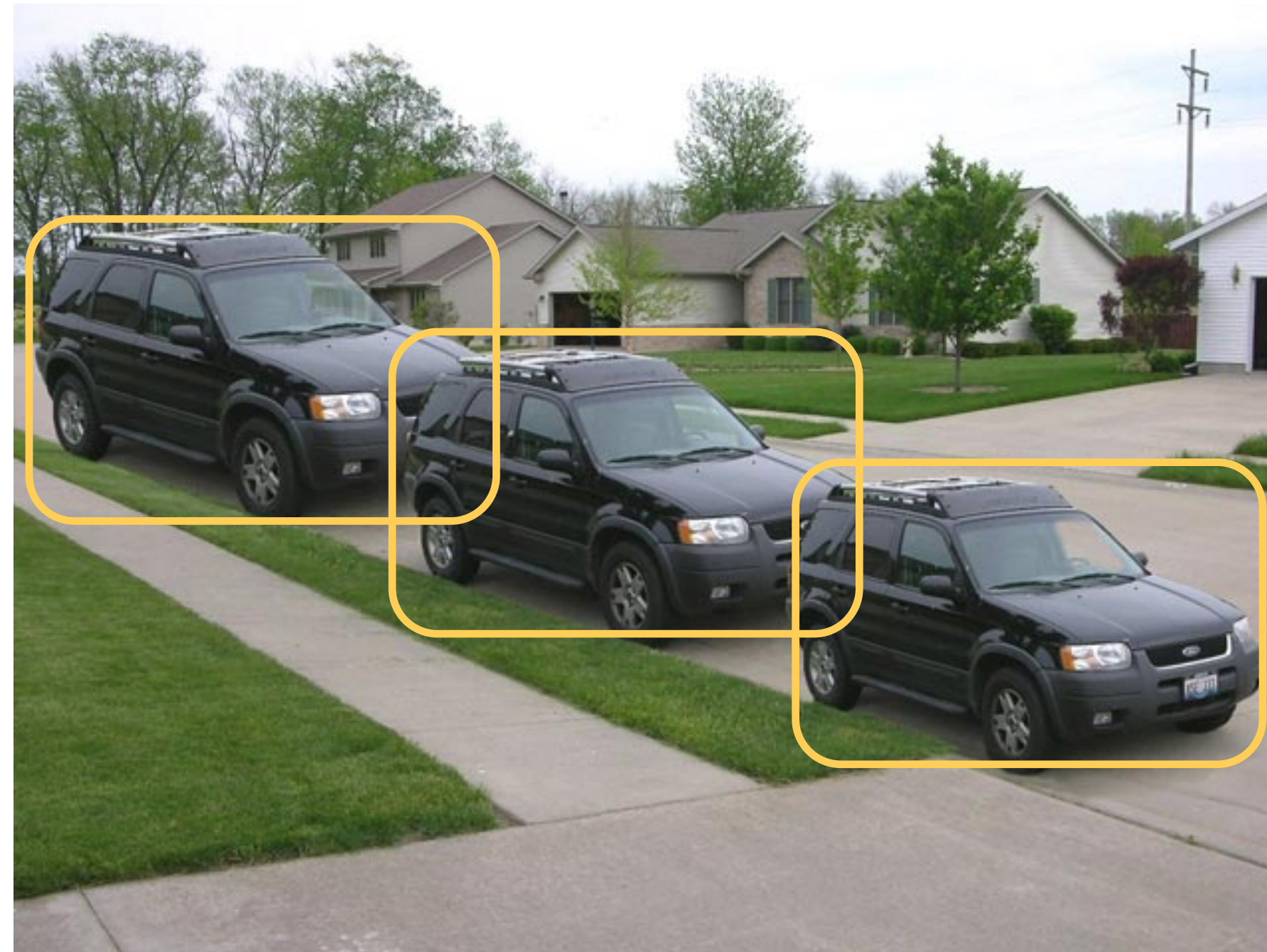
# Which car is bigger?



# Which car is bigger?



# Which car is bigger?

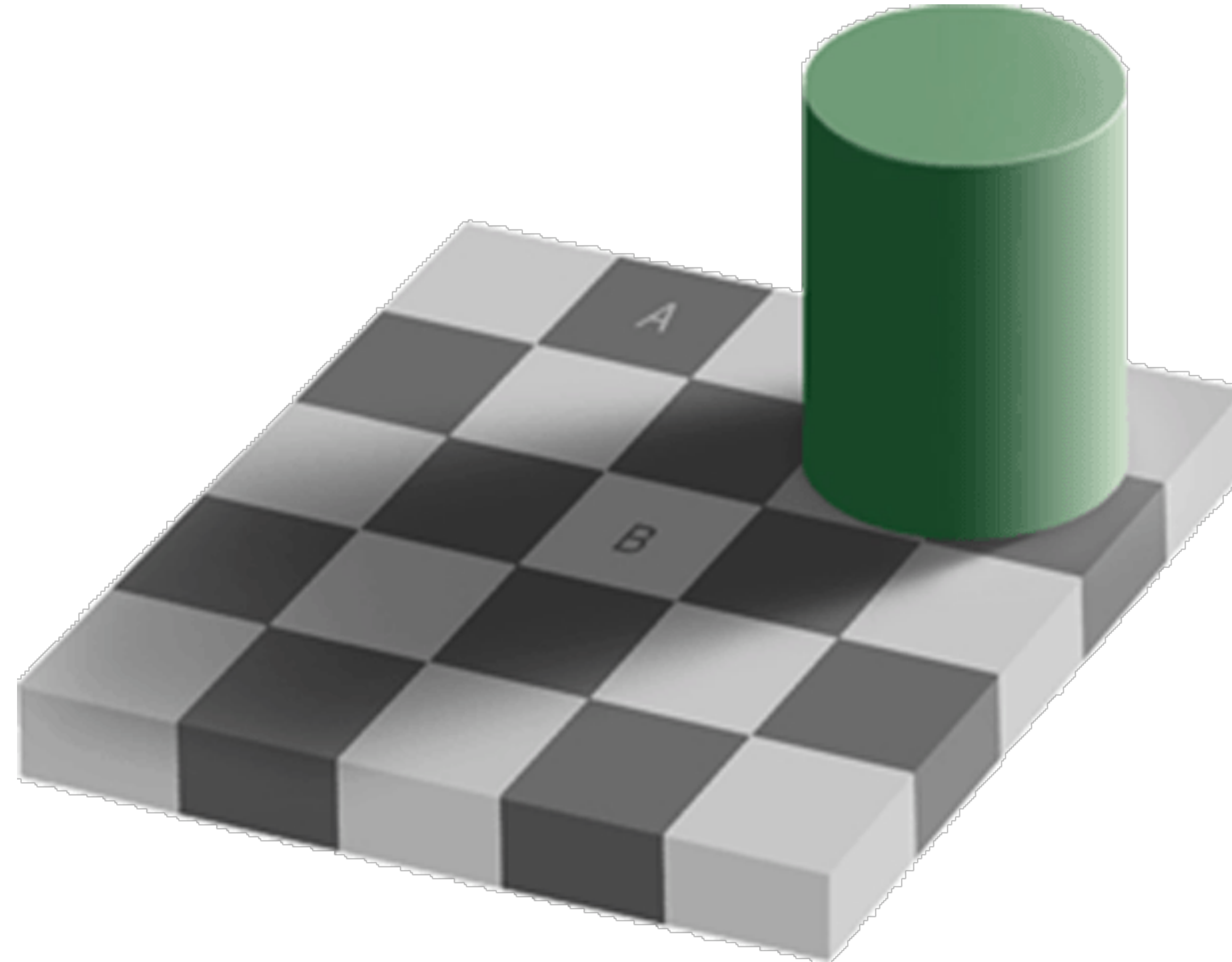


All cars are same size

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

<http://www.brainbashers.com>

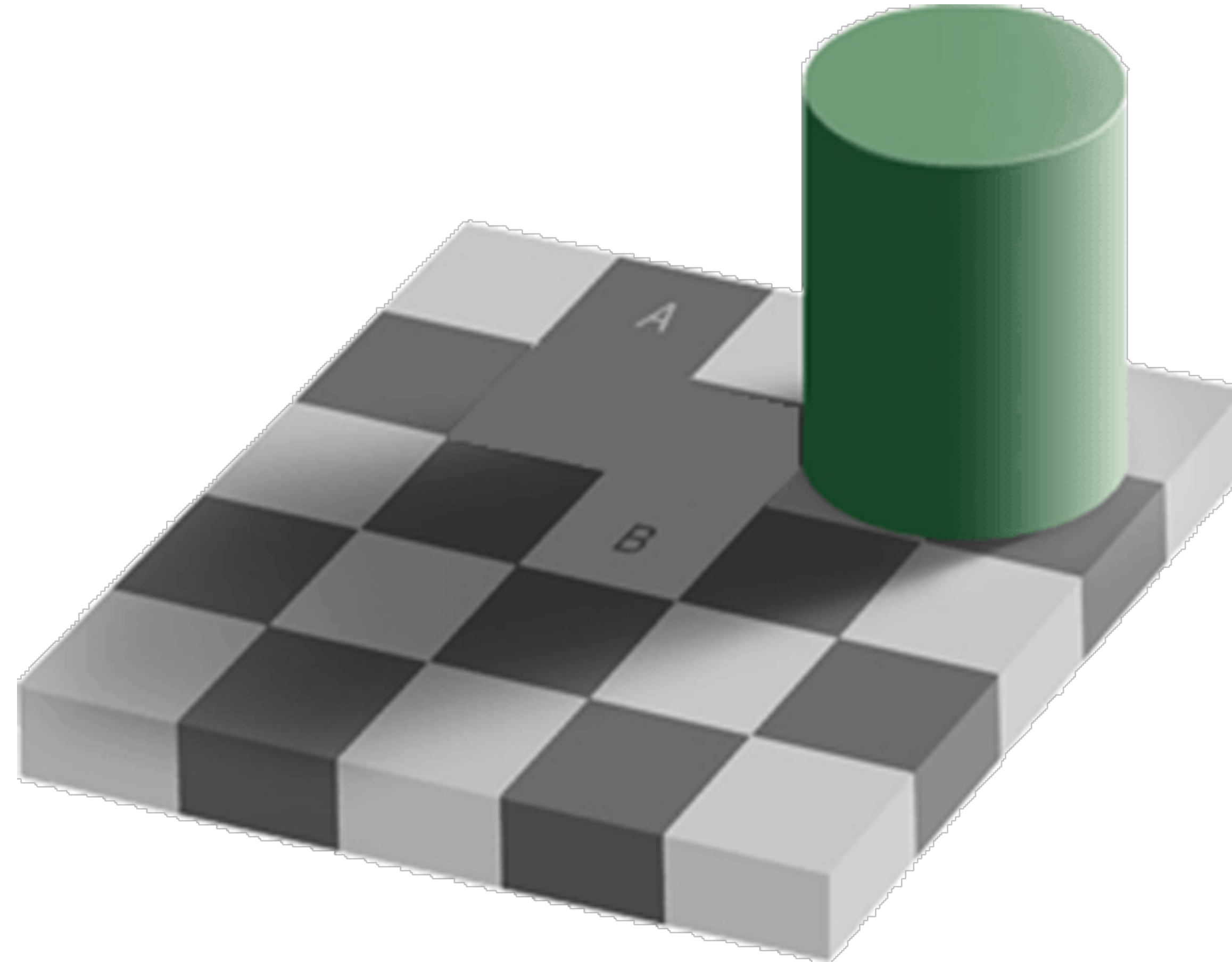
**Are A and B equally grey?**



Intensity perception depends strongly on neighbourhood

<http://www.brainbashers.com>

**Are A and B equally grey?**



Yes they are!

<http://www.brainbashers.com>

# Spot the animal

—

Why you should still use your brain  
(a pre-trained neural network)

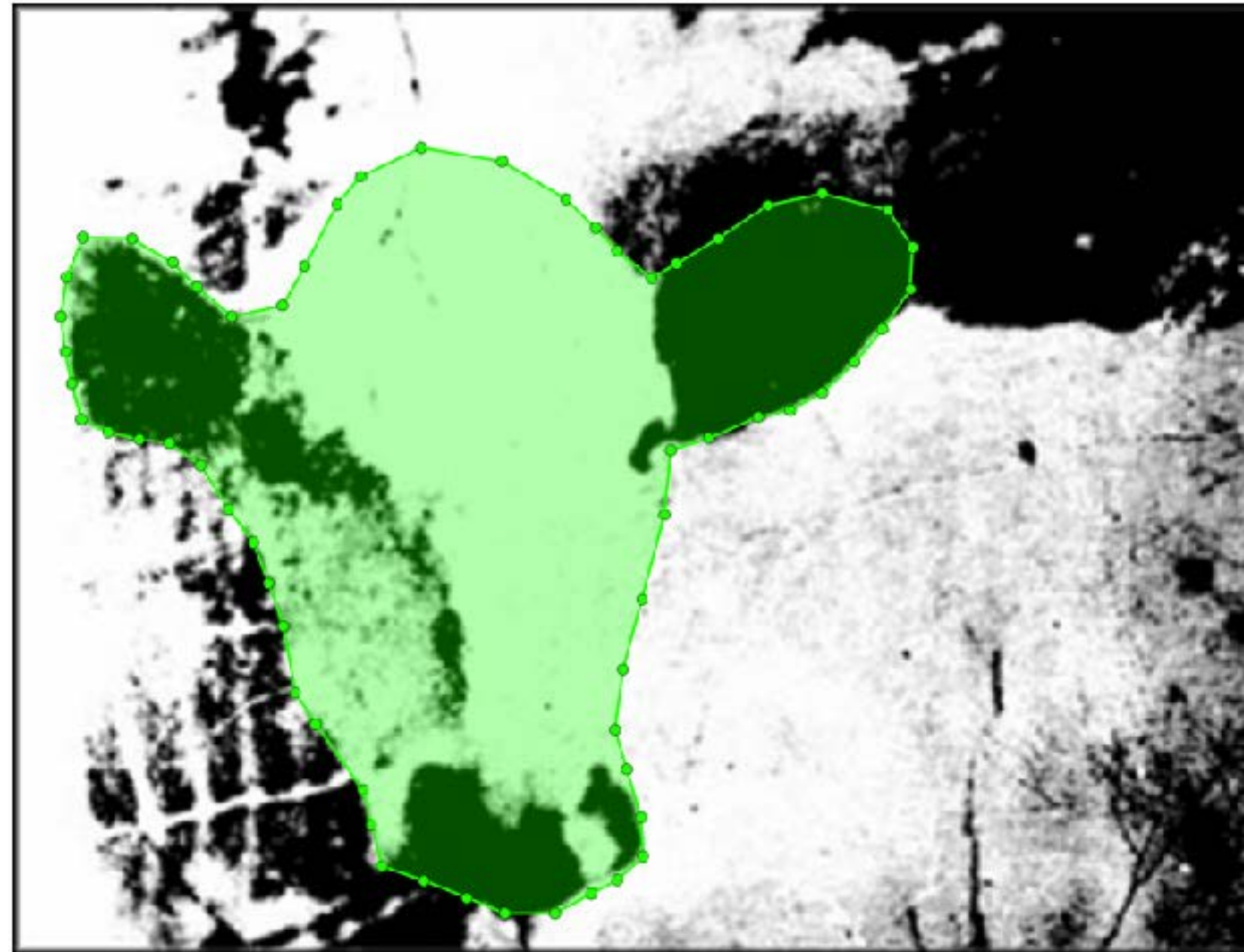
# Pattern Recognition



Yes, that is a cow!

<http://www.brainbashers.com>

# Pattern Recognition



Yes, that is a cow!

<http://www.brainbashers.com>



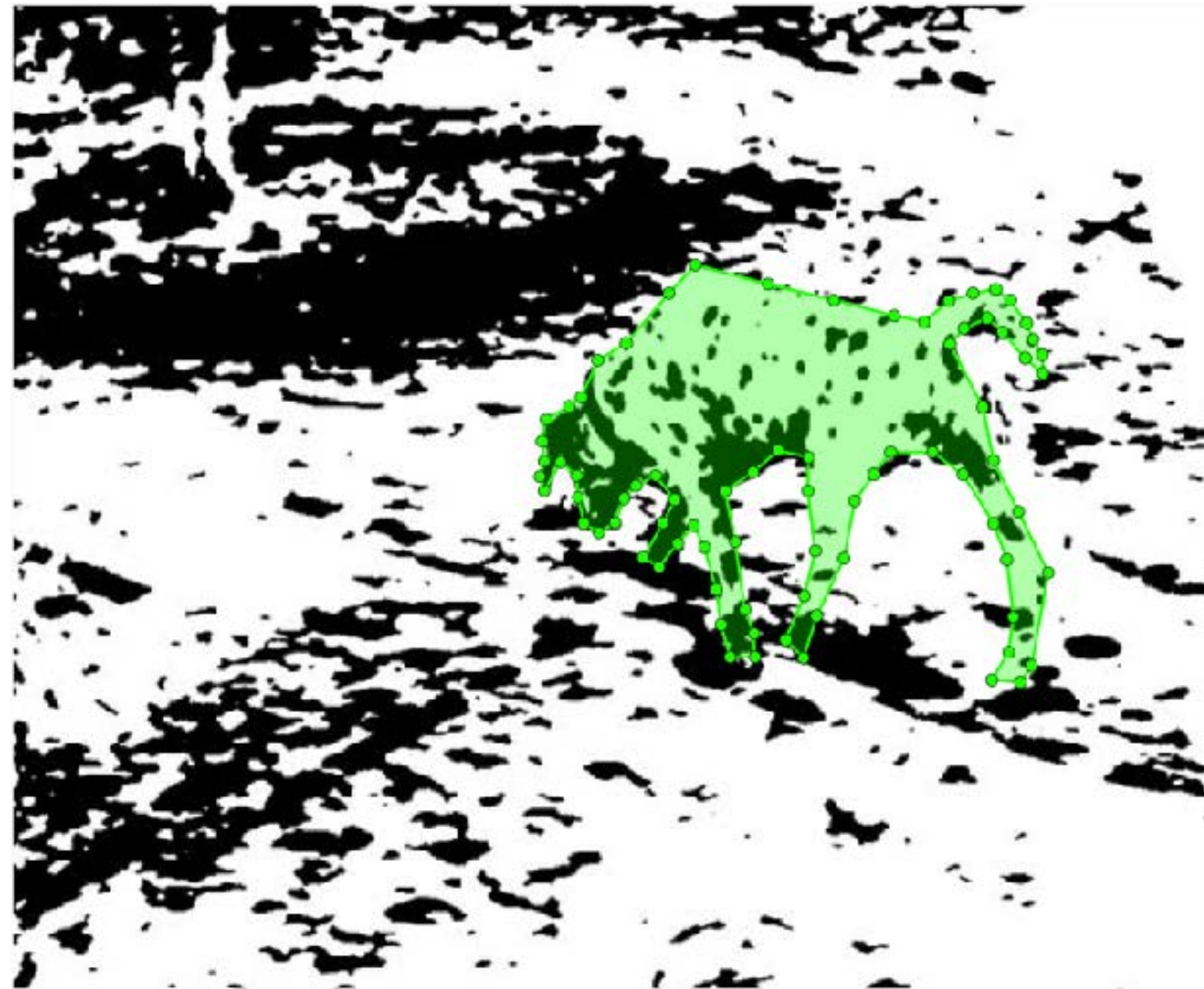
# Using Prior Knowledge



Keyword: Dalmatian dog!

<http://www.brainbashers.com>

# Using Prior Knowledge



Keyword: Dalmatian dog!

<http://www.brainbashers.com>

# A brief history of image analysis

—

ImageJ centric

# 37 years ago...

**NIH IMAGE**

## What is ImageJ?



[ImageJ](#) is an open source image processing and image analysis software for multi-dimensional image data with a focus on scientific imaging.

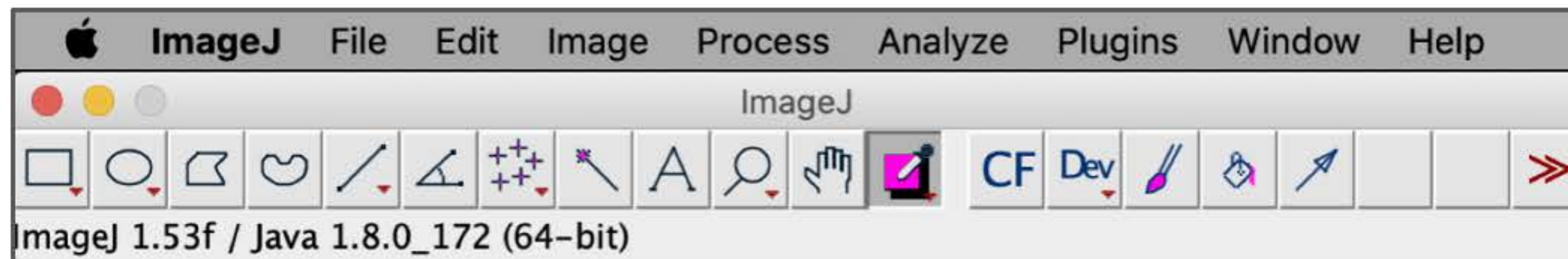
1987 - [NIH Image](#): written in Pascal for the Macintosh by [Wayne Rasband](#)

1997 - [ImageJ](#) 1.x: written in Java and also compatible with Windows PC

Since then, [ImageJ](#) has been maintained and upgraded (e.g. [ImageJ2](#), [Fiji](#), ...)

[ImageJ GitHub](#)

[Fiji GitHub](#)





# What is Fiji?

Fiji stands for **Fiji Is Just ImageJ**

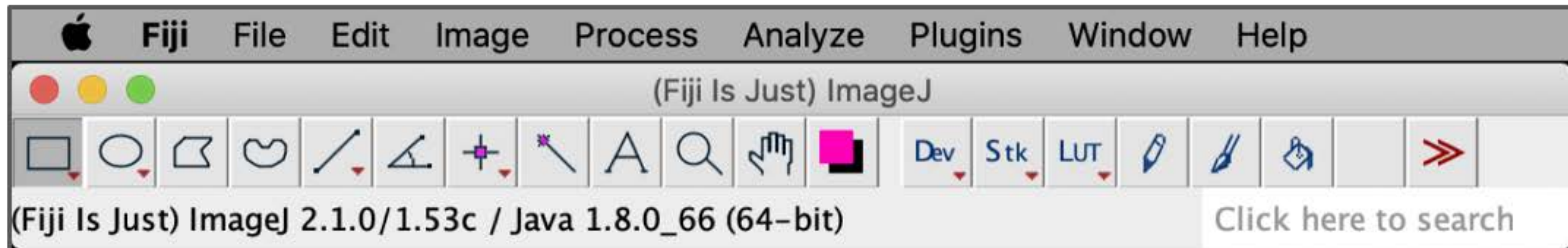
It is a “batteries included” distribution of ImageJ2

Easy to install (“portable” application)

Include a collection of Plugins

Easy to update (<https://imagej.net/list-of-update-sites/>)

Documentation



# 12 years ago...

- [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)



# 4 years ago...

- CellPose is out (2020)

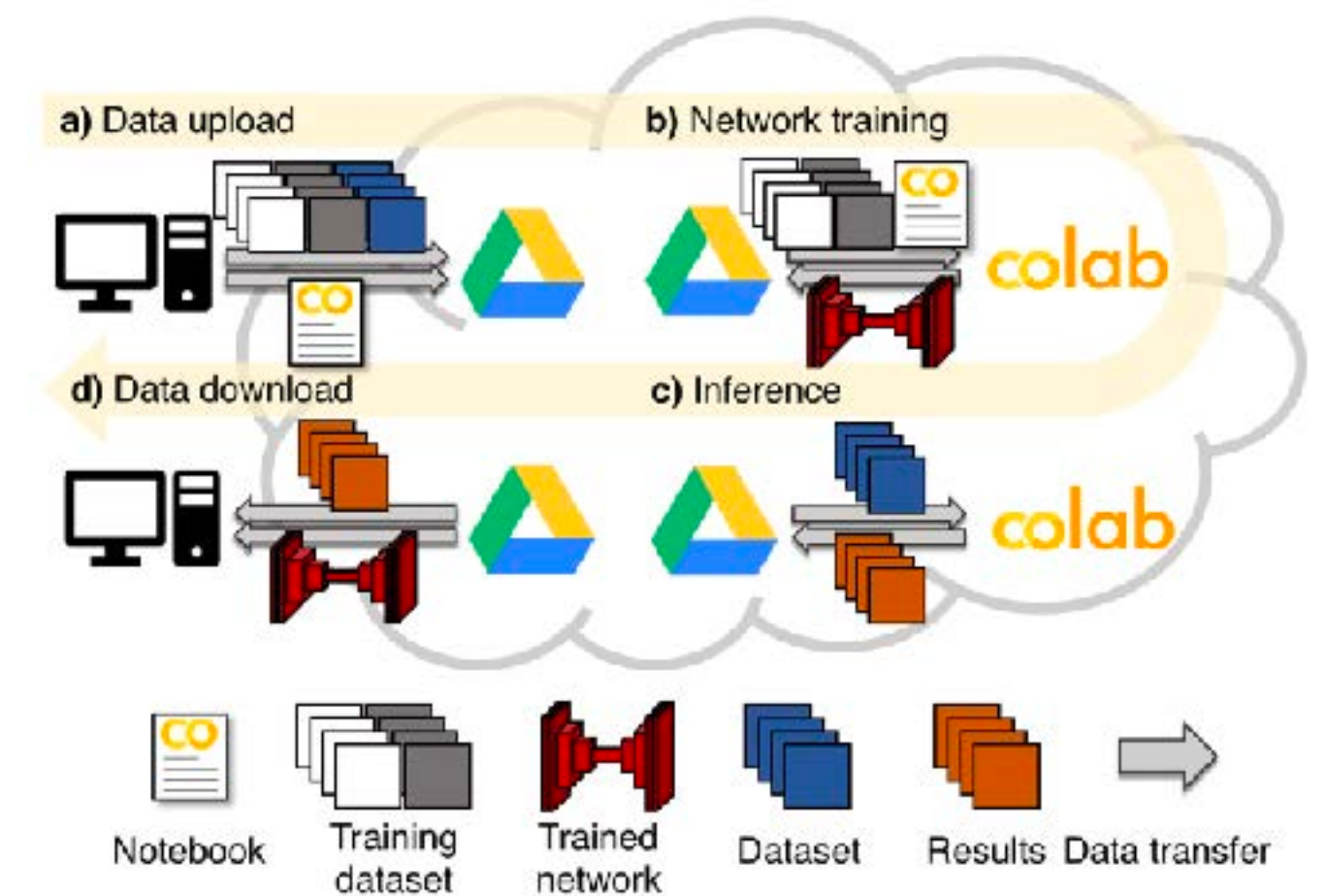
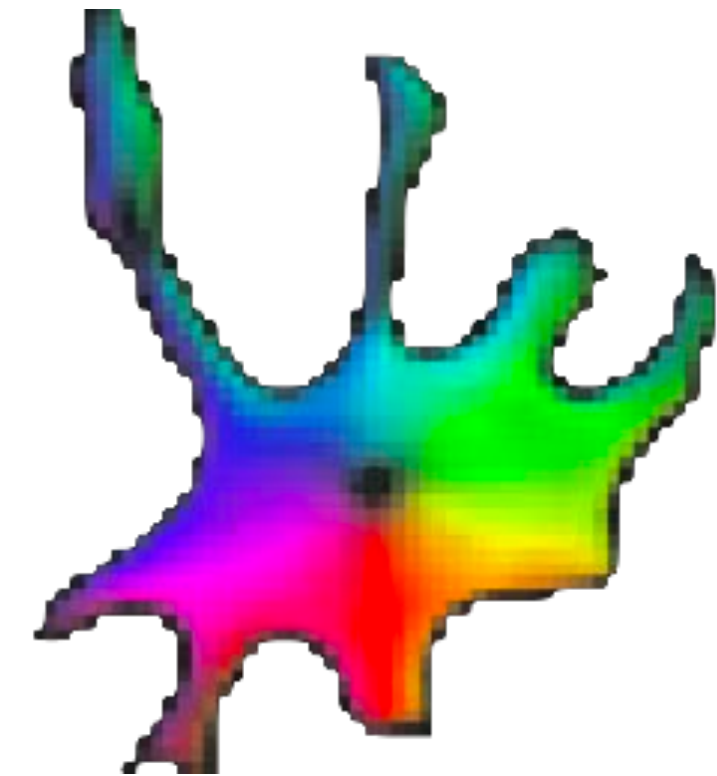
- “Cellpose: a generalist algorithm for cellular segmentation”
- Trained on highly varied images of cells, over 70,000 segmented objects
- Cells don't have to be star-shaped
- Web-platform and Jupyter notebooks
- <https://www.nature.com/articles/s41592-020-01018-x>

- 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>

- *Vision Transformers* (2020)

- “An Image is Worth 16x16 Words: Transformers for Image Recognition at Scale”, Dosovitskiy et al.
- <https://arxiv.org/abs/2010.11929>



# Today...

## Image Analysis Software

The image displays a comprehensive list of image analysis software tools, organized in a grid. Each tool is represented by its logo and name. The tools are arranged in six columns and approximately 15 rows. The first column includes 'All Topics', 'BIAFLOWS', 'BoneJ', 'Cytomine', 'Icy', 'ImJoy', 'MCMICRO', 'MorphoGraphX', 'OMERO', 'PolusAI', 'SCIFIO', and 'TeamTomo'. The second column includes 'AGAVE', 'BiaPy', 'BrainGlobe', 'DAIM', 'IDR', 'ImSwitch', 'MIA', 'MorphoNet', 'OmeSliCC', 'PYME', 'scikit-image', and 'TissUUmaps'. The third column includes 'AICSImageIO', 'Bili', 'Cellpose', 'DeepLabCut', 'ilastik', 'iRODS', 'MIB', 'napari', 'OpenIRIS', 'Python-Microscope', 'sciview', and 'vedo'. The fourth column includes 'Arktekt', 'BiofilmQ', 'CellProfiler', 'Fiji', 'ImageJ', 'JIPipe', 'JIPipe', 'µManager', 'NEUBIAS', 'OpenSPIM', 'QUAREP-LiMi', and 'SmartMicroscopy'. The fifth column includes 'Aydin', 'Bio-Formats', 'CLIJ', 'FLIMLib', 'ImageJ2', 'JuliaImages', 'MoBIE', 'NFDI4BIOIMAGE', 'Orbit', 'QuPath', and 'SR-Tesseler'. The sixth column includes 'BAND', 'BioImageIO', 'CytoMAP', 'GerBI', 'ImgLib2', 'Mars', 'ModularImageAnalysis', 'OME', 'Piximi', 'scenery', and 'StarDist'. At the bottom left, there is a section for 'RELATED COMMUNITIES' with logos for 'µforum', 'STK', and 'KNIME'. At the bottom right, a 'Citation Details' box for Fiji is shown, highlighting its citation information: 'Fiji: an open-source platform for biological-image analysis', Nature Methods, 2012, Volume 9, Number 7, Page 678. The citation details box also shows '30162 ITEMS CITE THIS ARTICLE' from Crossref, '33431 ITEMS CITE THIS ARTICLE' from Web of Science, and '39921 ITEMS CITE THIS ARTICLE' from another source. A footer at the very bottom reads 'forum.image.sc'.

... Your Icon Here

RELATED COMMUNITIES

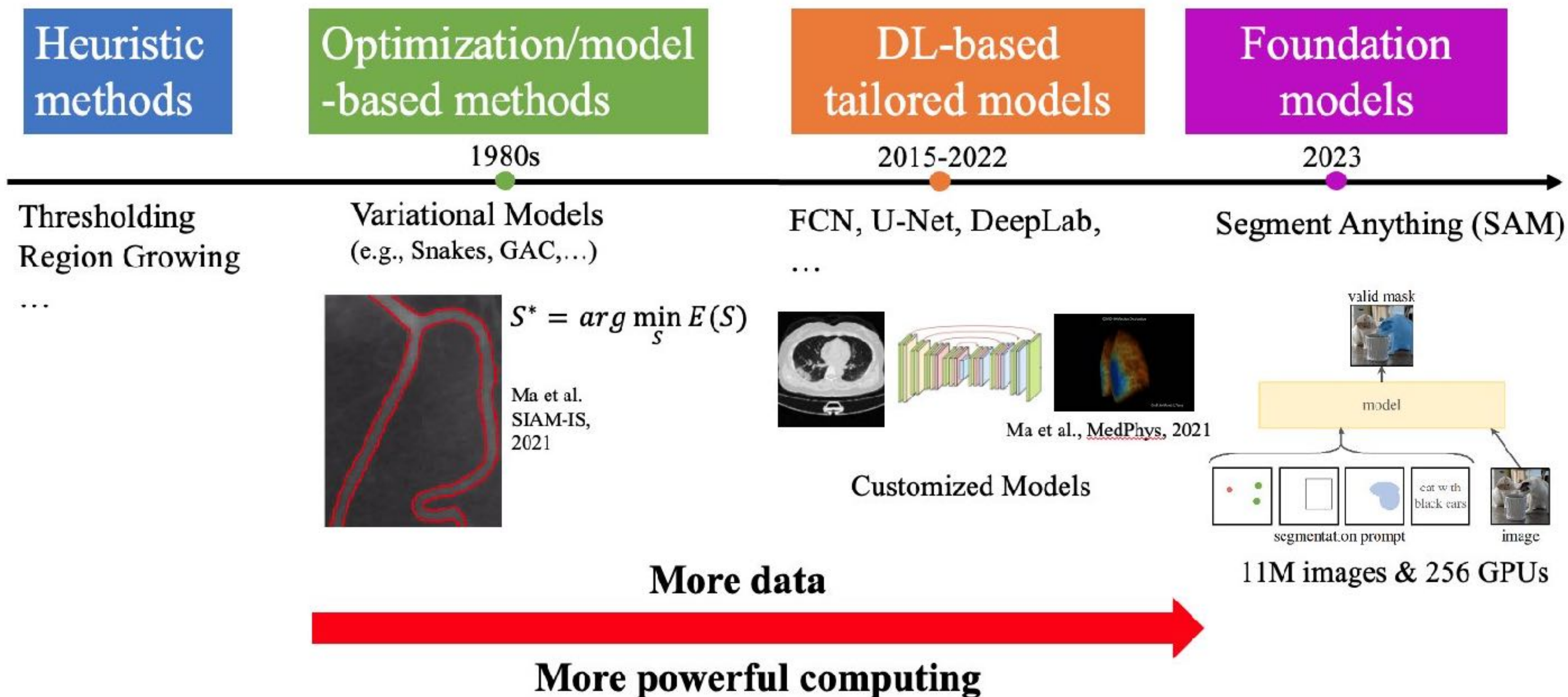
µforum STK KNIME

**forum.image.sc**



# History of Segmentation

## Segmentation Paradigm Over the Past Half Century



English as the next  
programming language

—

LLMs, chatbots and foundation  
models for bio image analysis



⚡ 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

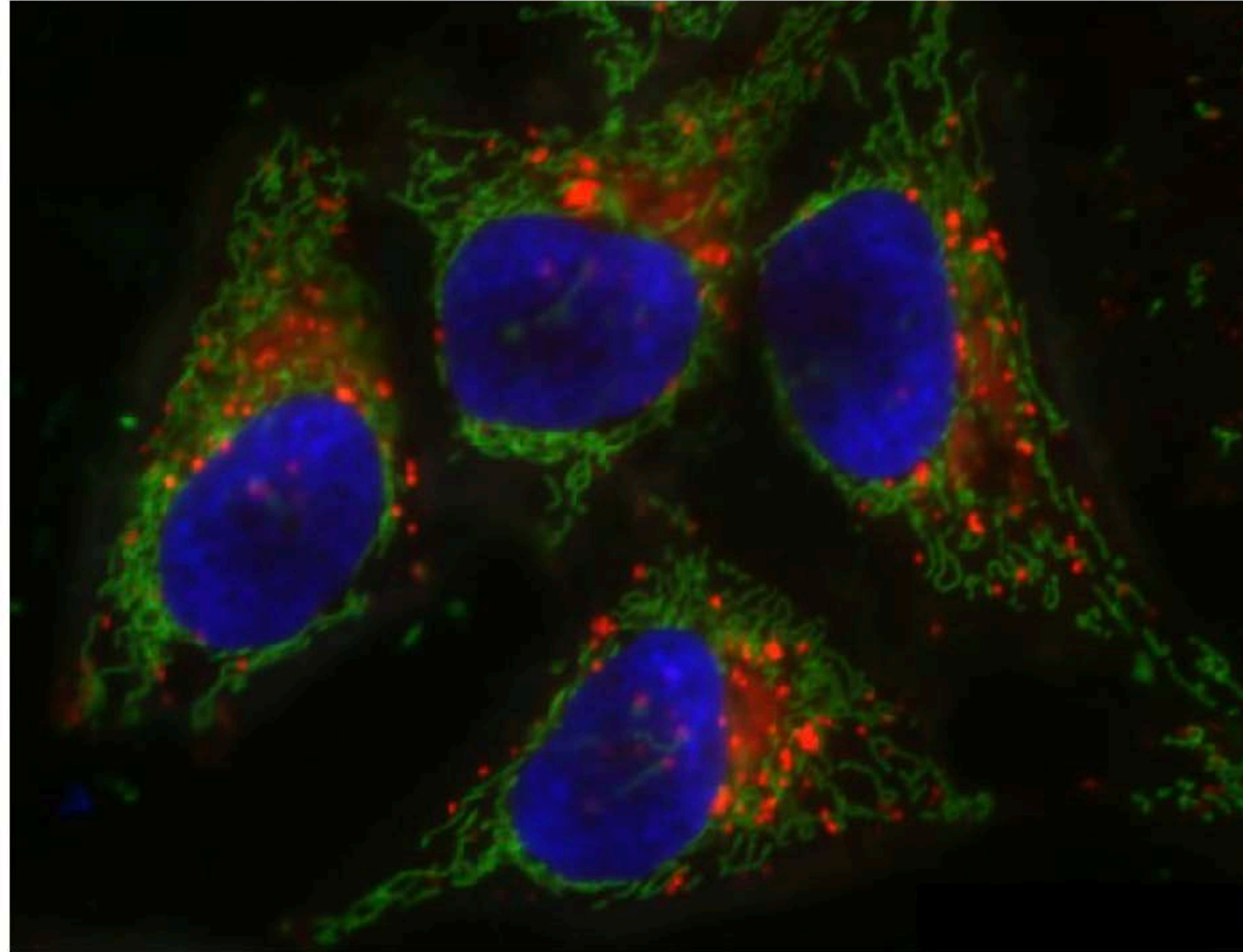
**Write an email**  
to request a quote from local plumbers

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



# Using ChatGPT for Image Analysis

SI You

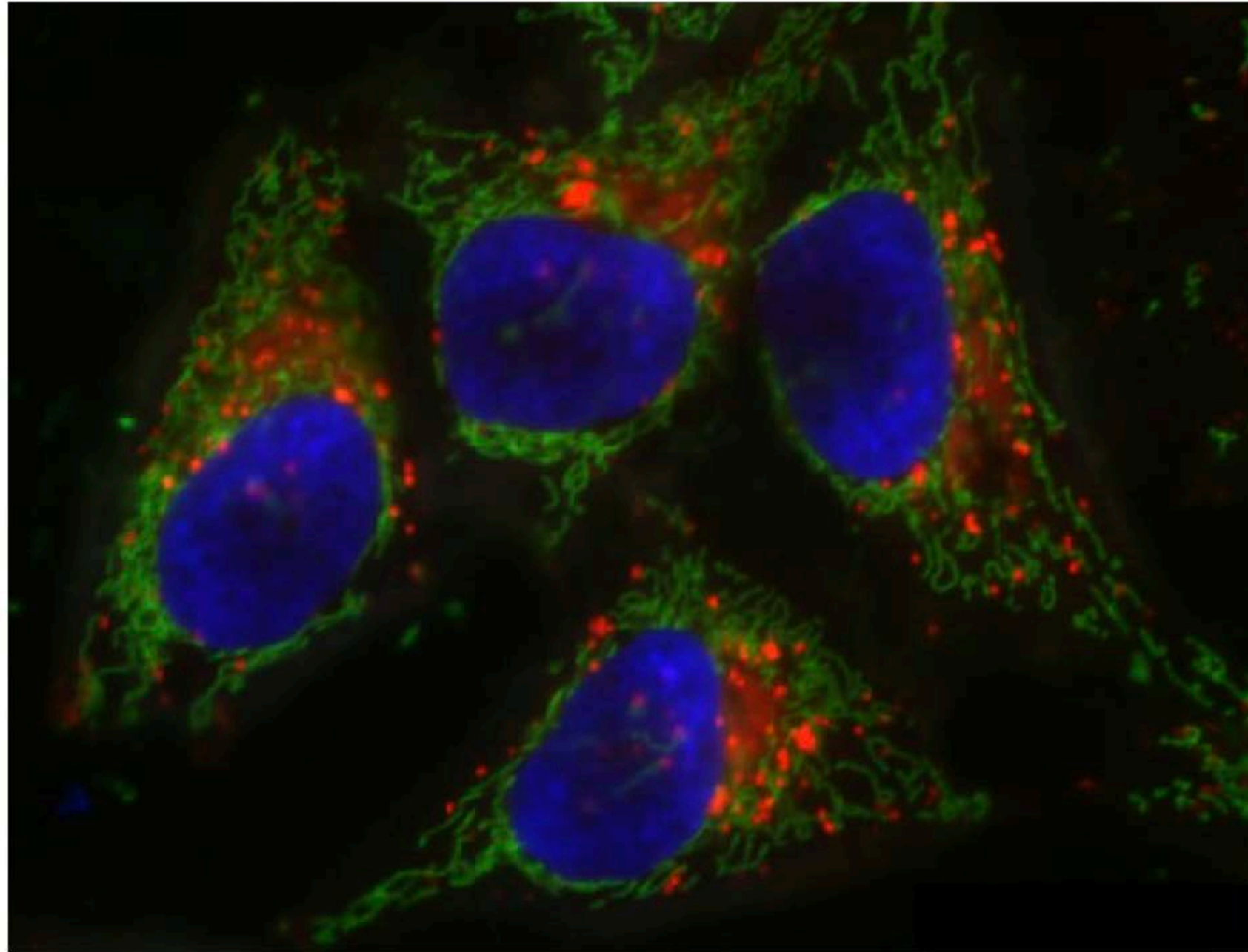


please segment the nuclei and show me the relevant code you used

# Using ChatGPT for Image Analysis

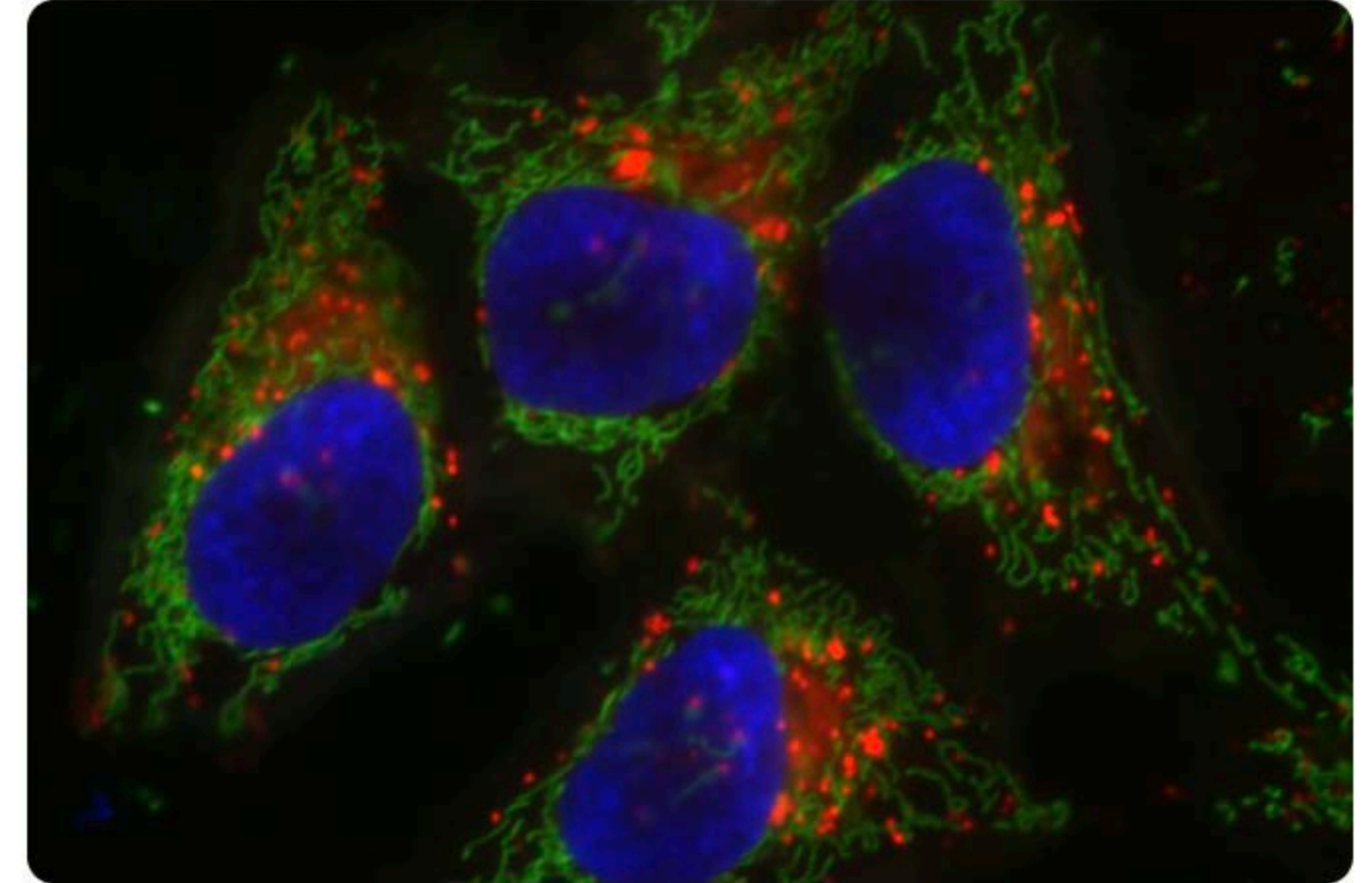
GPT-4, May 2024

SI You



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

GPT-4o, Oct. 2024



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

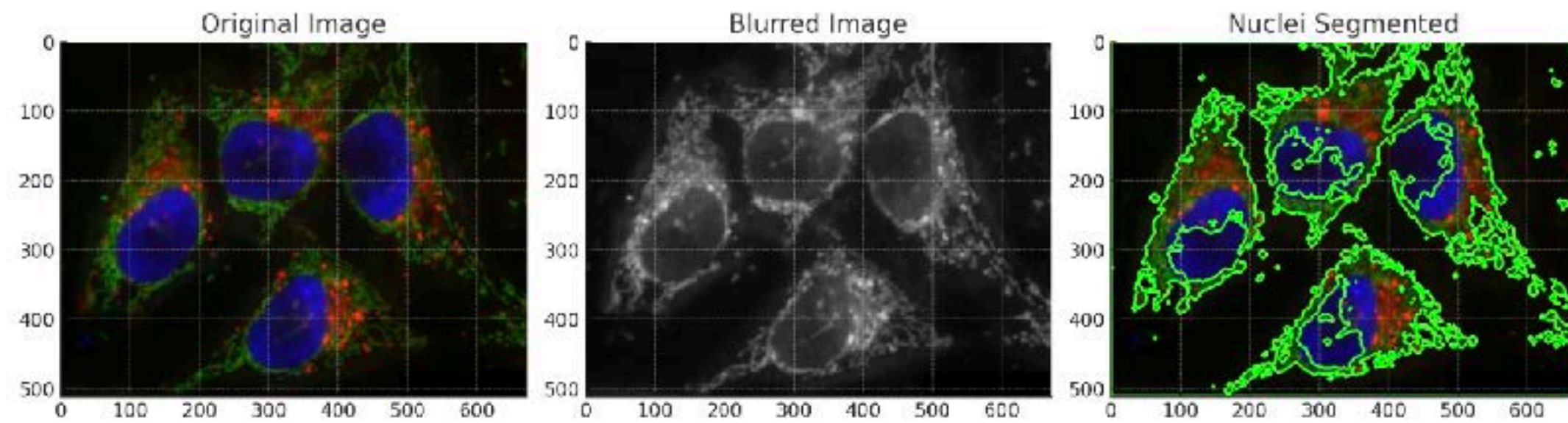
# Using ChatGPT for Image Analysis

GPT-4, May 2024

GPT-4o, Oct. 2024



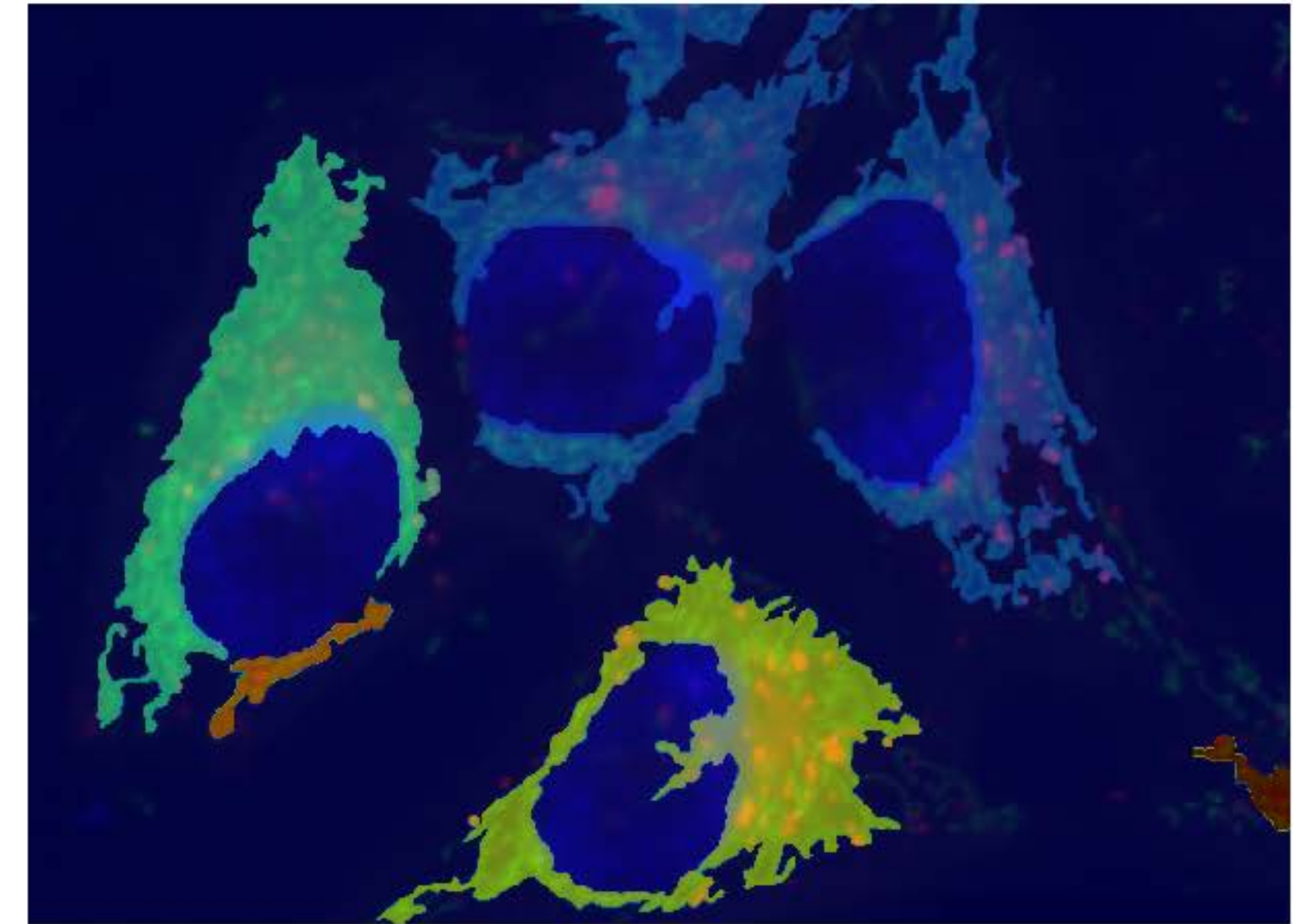
ChatGPT



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.

Segmented Nuclei



# Using ChatGPT for Image Analysis

GPT-4, May 2024

```
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.THRESH_OTSU)

# Find contours which will give us the nuclei boundaries
contours, _ = cv2.findContours(thresh, cv2.RETR_TREE, cv2.CHAIN_APPROX_SIMPLE)

# 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)
```

GPT-4o, Oct. 2024

```
python Copy code

from skimage import io, filters, measure, morphology, color
import matplotlib.pyplot as plt
import numpy as np

# Load the image
image_path = '/mnt/data/hela-cells.png'
image = io.imread(image_path)

# Convert to grayscale
gray_image = color.rgb2gray(image)

# Apply Gaussian filter to smooth the image
smoothed = filters.gaussian(gray_image, sigma=1)

# Use Otsu's threshold to create a binary mask of the nuclei
threshold_value = filters.threshold_otsu(smoothed)
binary_mask = smoothed > threshold_value

# Remove small objects and fill holes to improve segmentation
cleaned_mask = morphology.remove_small_objects(binary_mask, min_size=500)
cleaned_mask = morphology.remove_small_holes(cleaned_mask, area_threshold=500)

# Label the segmented regions
labeled_nuclei = measure.label(cleaned_mask)
```

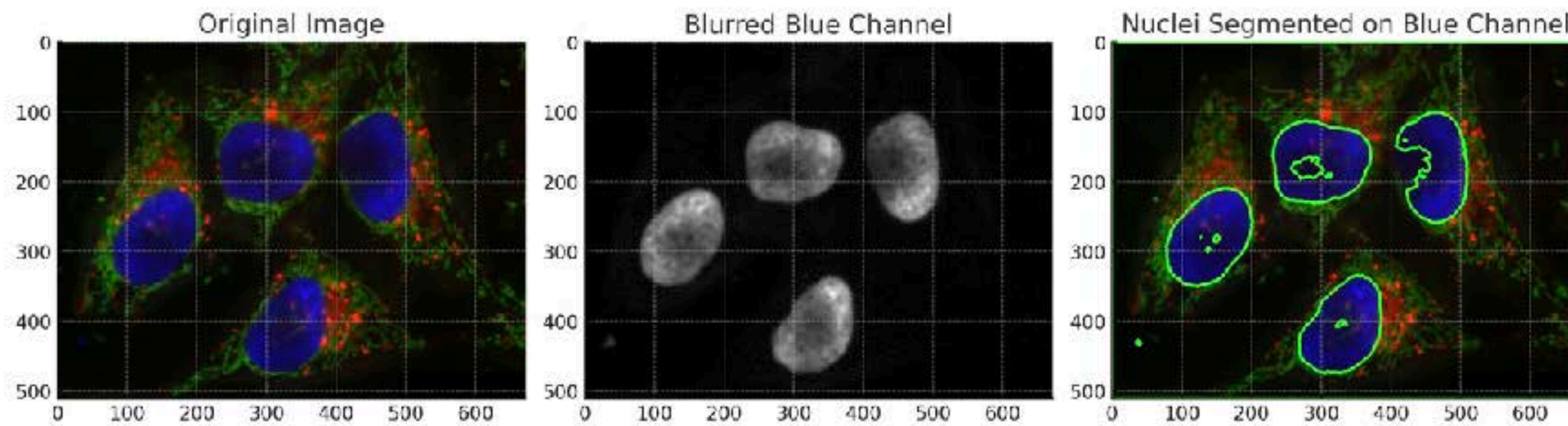
# Using ChatGPT for Image Analysis

GPT-4, May 2024

GPT-4o, Oct. 2024

**You**  
please use the blue channel for segmenting the nuclei

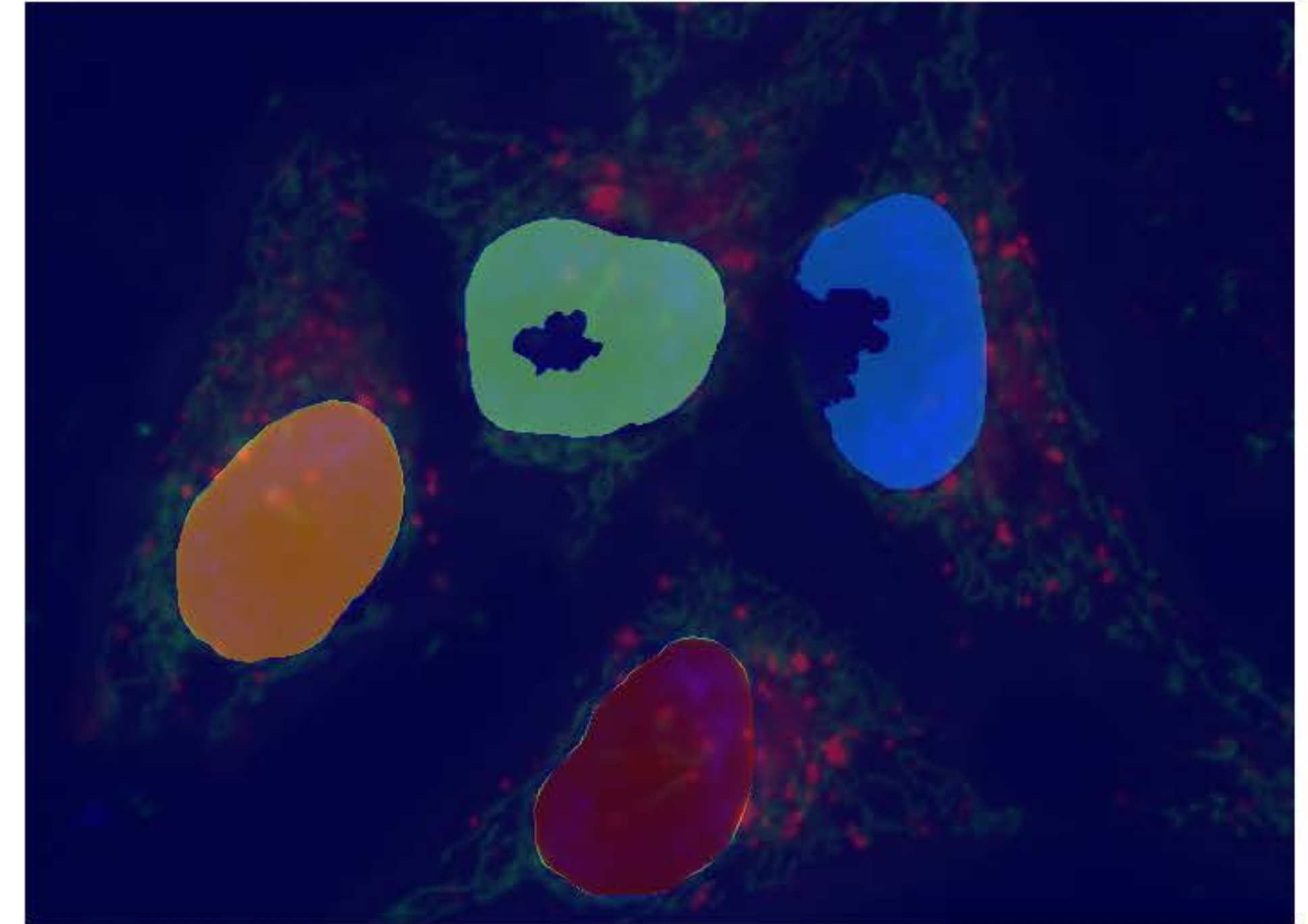
**ChatGPT**



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.

Segmented Nuclei (Blue Channel)

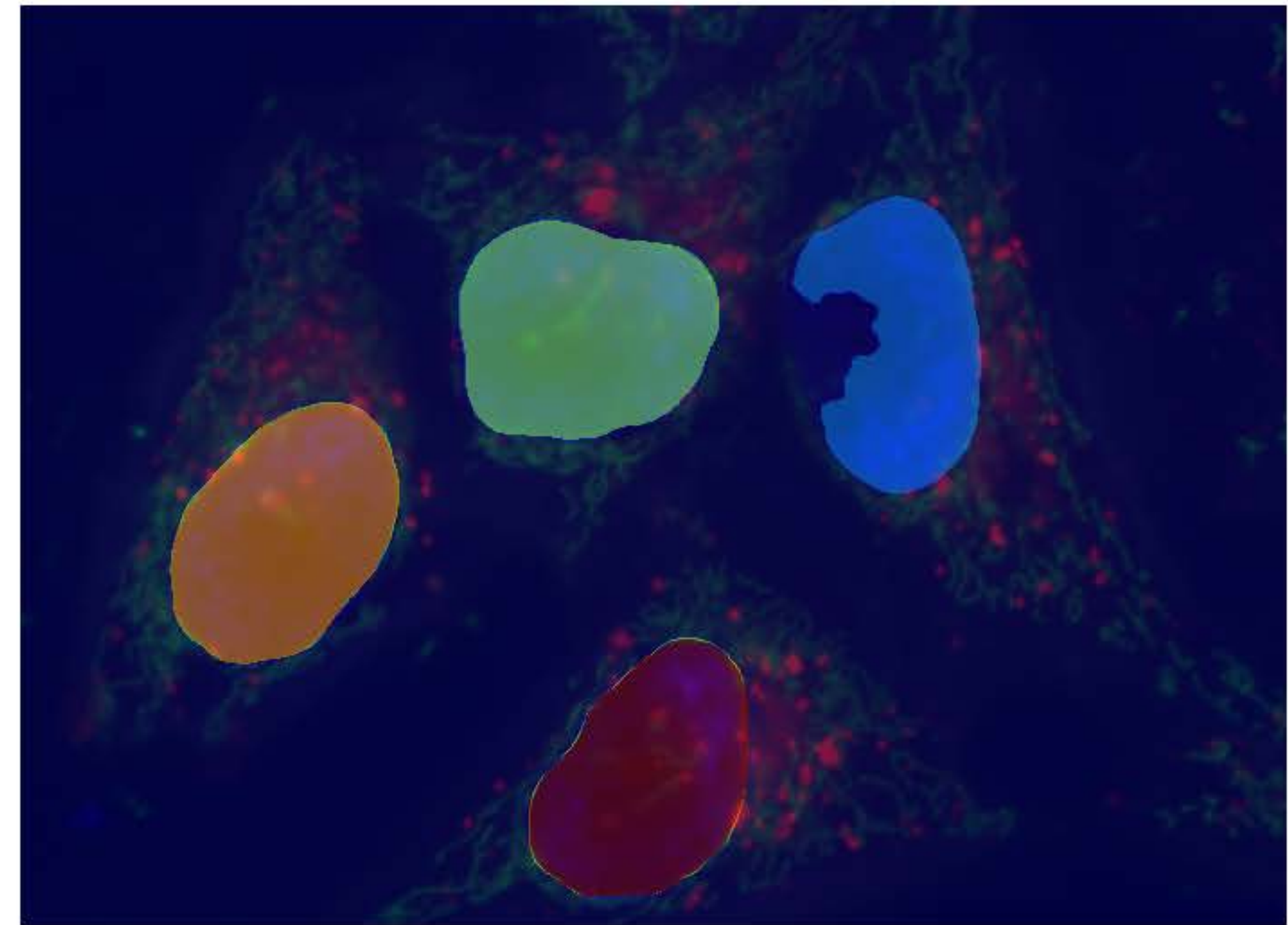
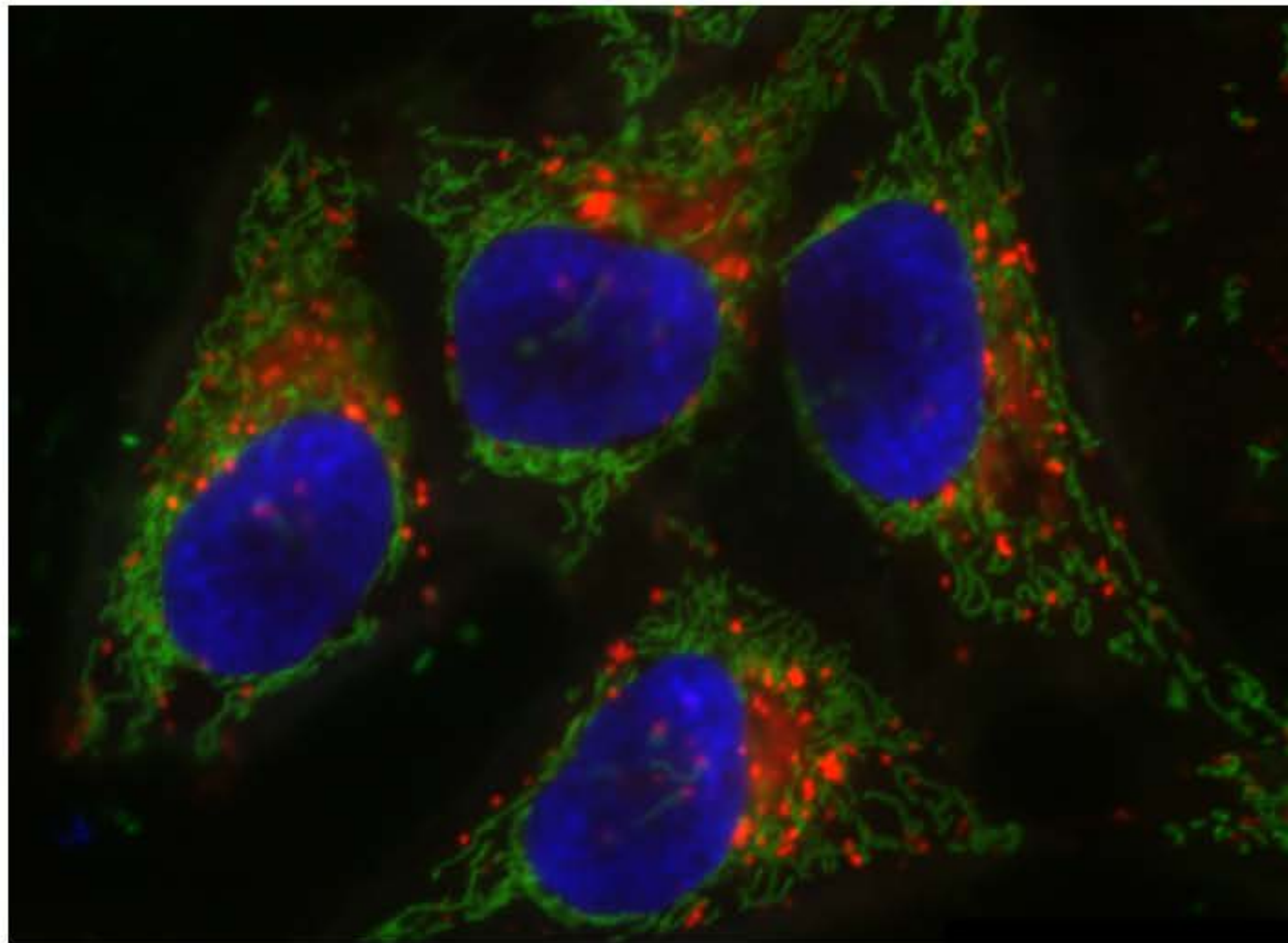




# Using ChatGPT-4o for Image Analysis

GPT-4o, Oct. 2024 - prompted to close larger holes

make sure to close larger holes, and increase smoothing sigma to 3

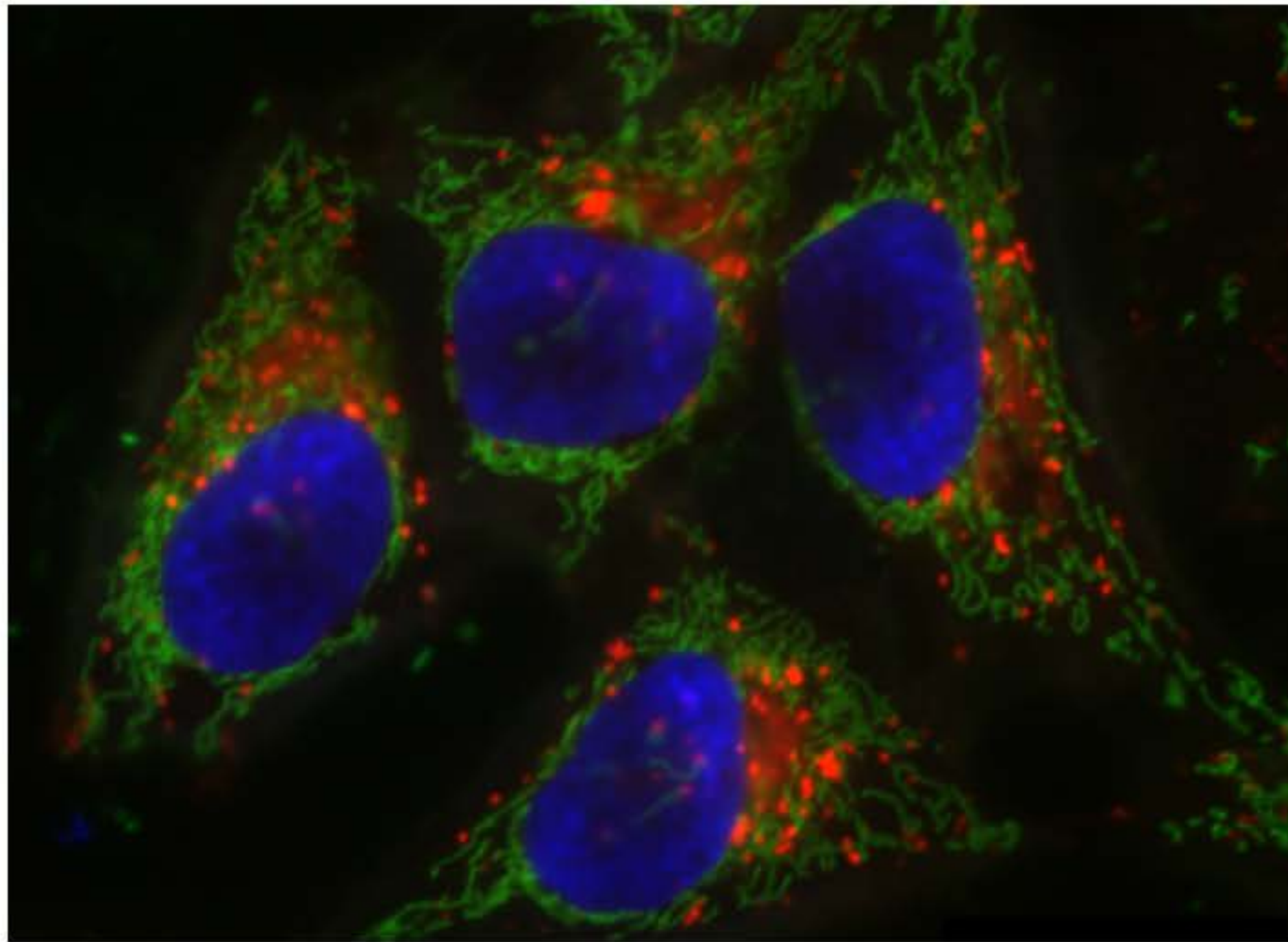


# Using ChatGPT-4o for Image Analysis

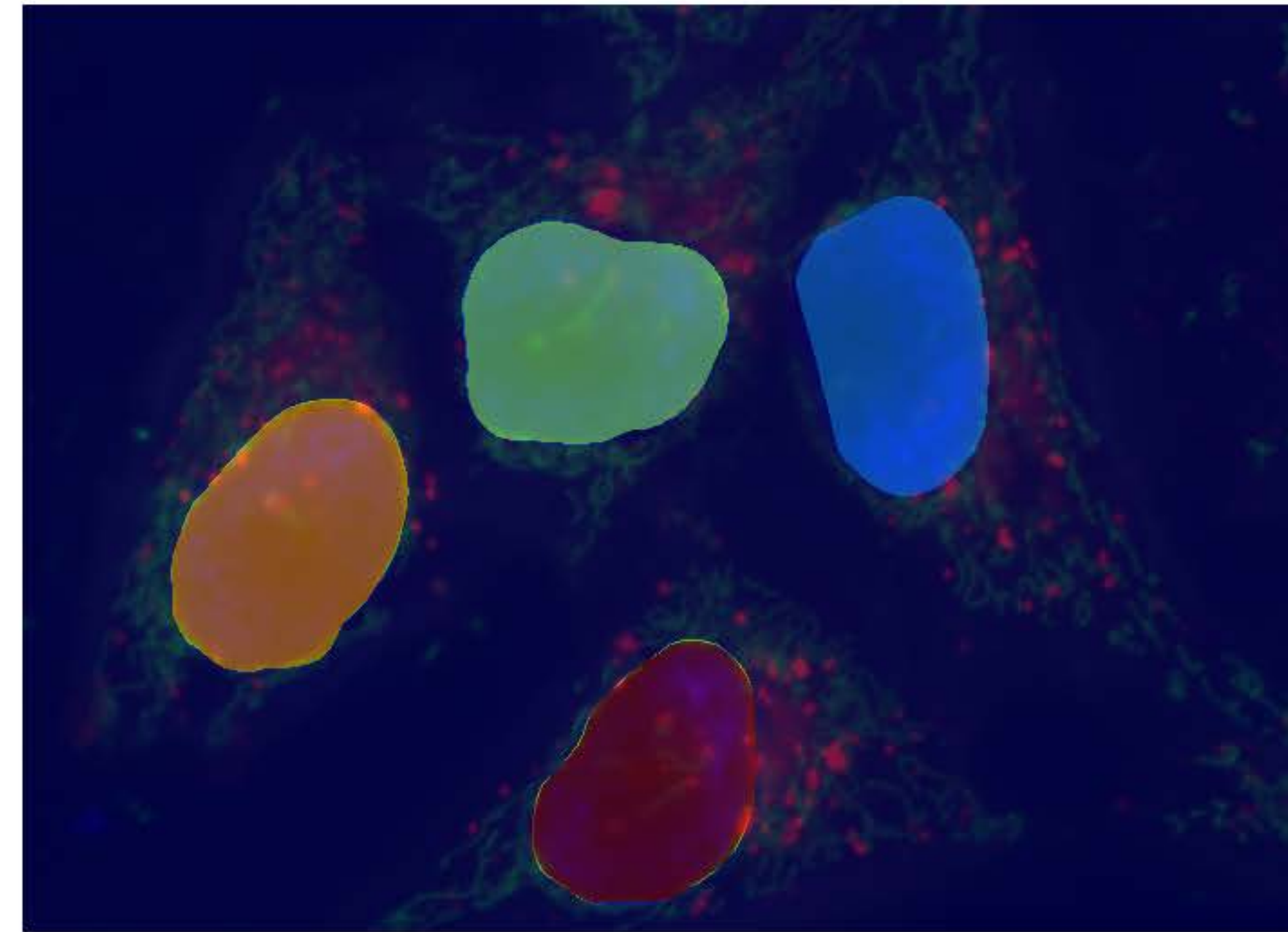
GPT-4o, Oct. 2024 - it helps to know what to ask

compute the convex hull for shapes with a low circularity, to approximate the characteristic shape of a nuclei

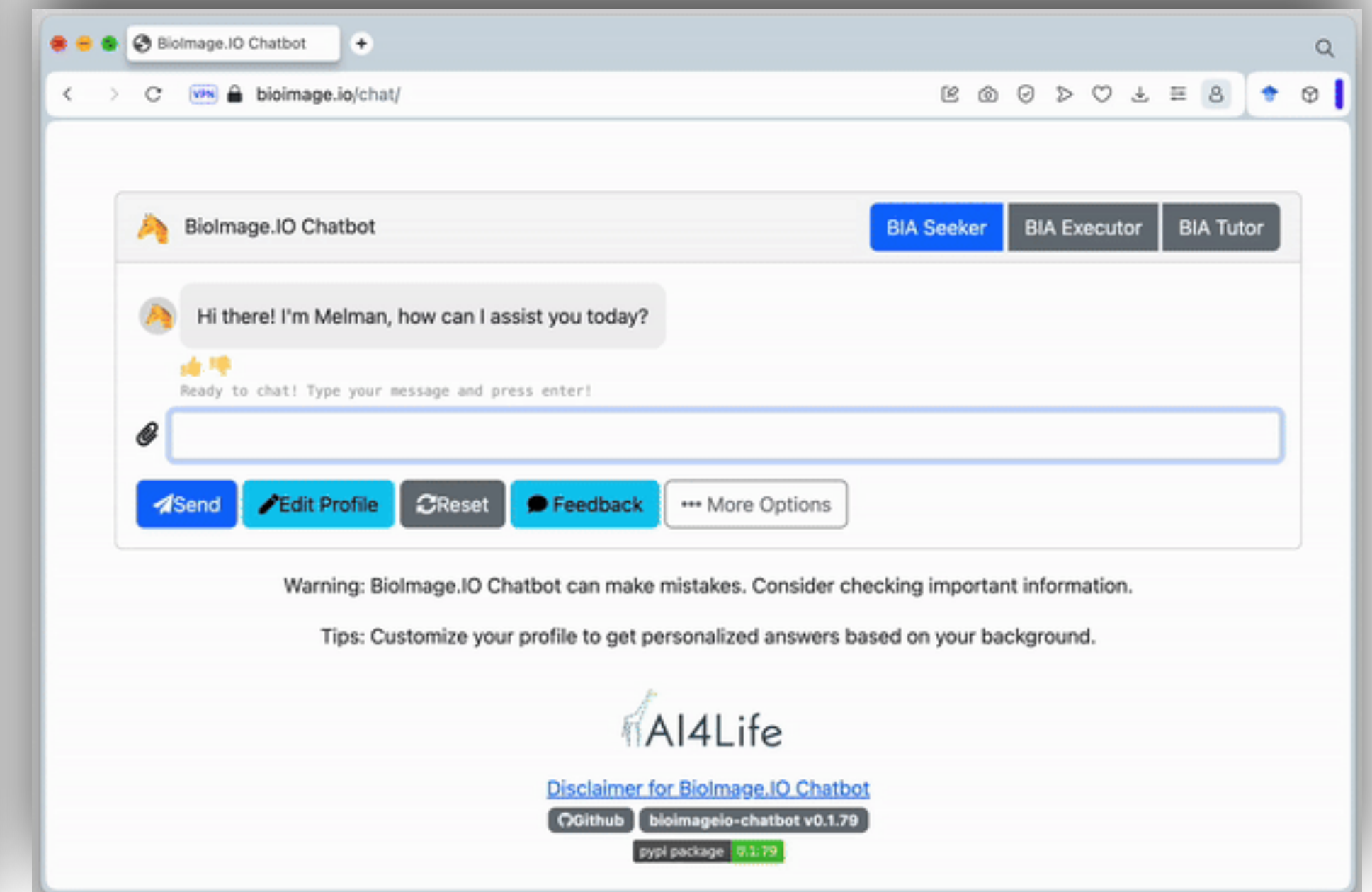
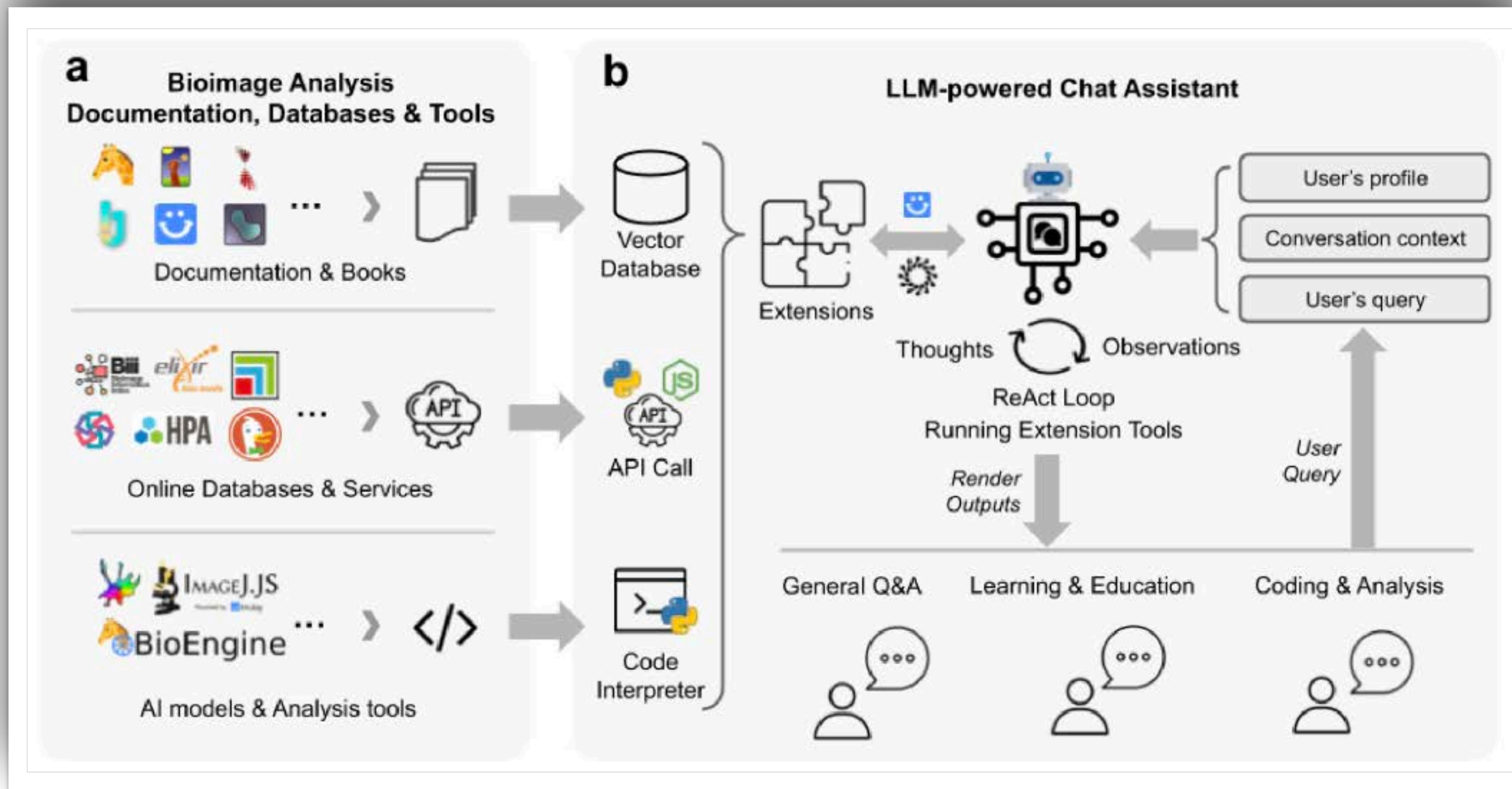
Original Image



Convex Hull for Low Circularity Nuclei



# BioImage Chatbot



# napari-omega



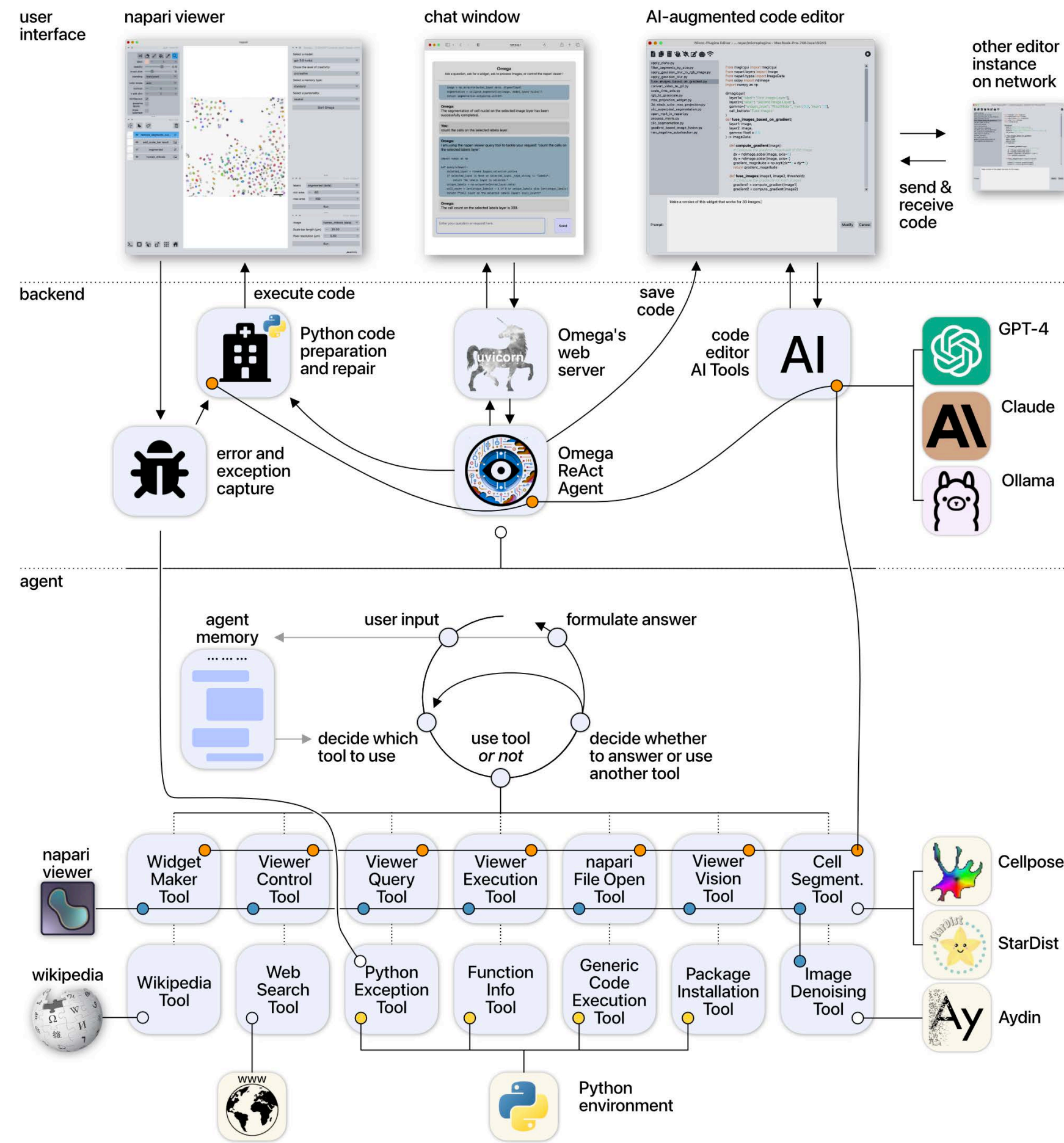
## Omega – Harnessing for Bioimage Analysis

Loïc A. Royer<sup>1, \*</sup>

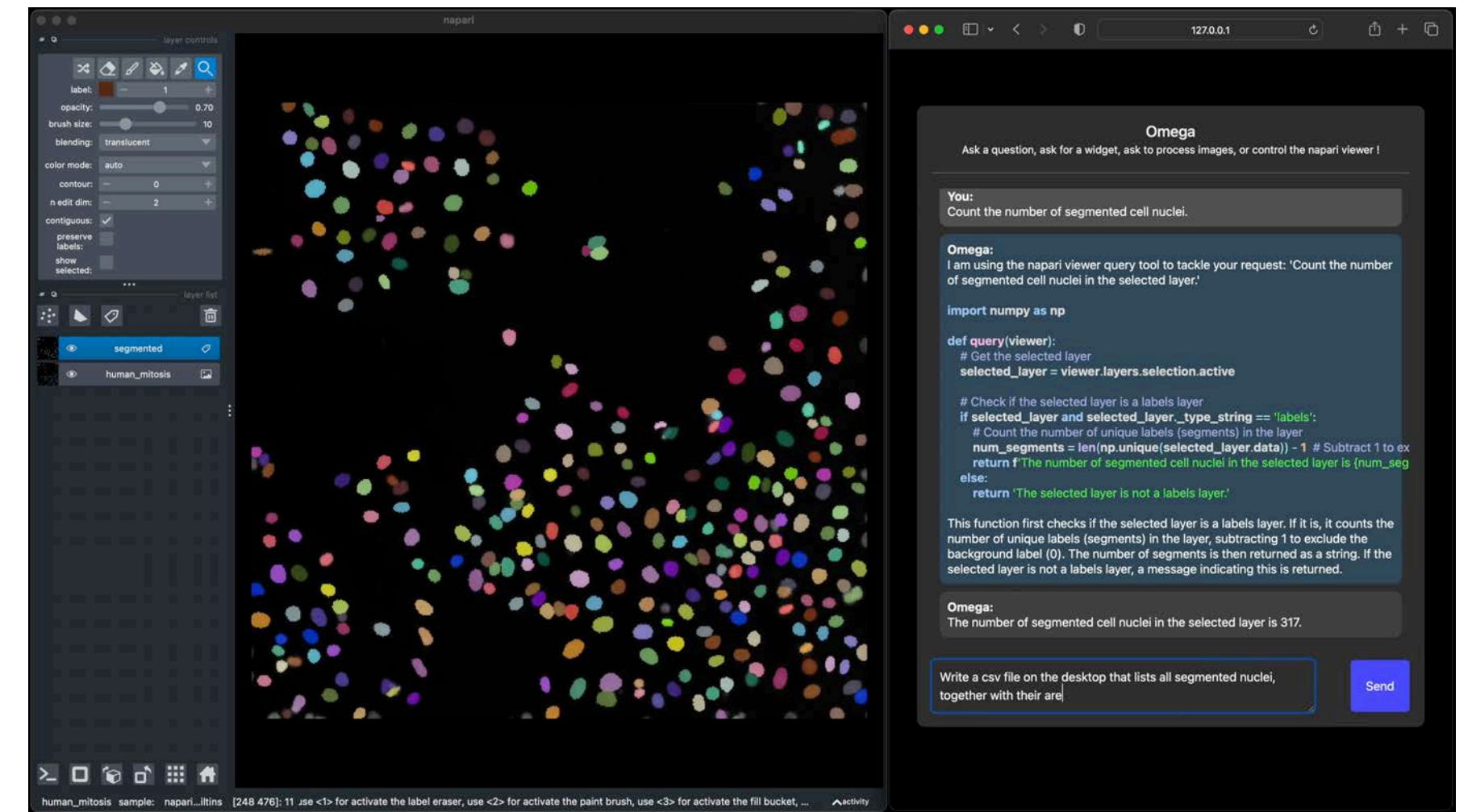
<sup>1</sup>Chan Zuckerberg Biohub, San Francisco, USA.

\*Correspondence: [loic.royer@czbiohub.org](mailto:loic.royer@czbiohub.org)

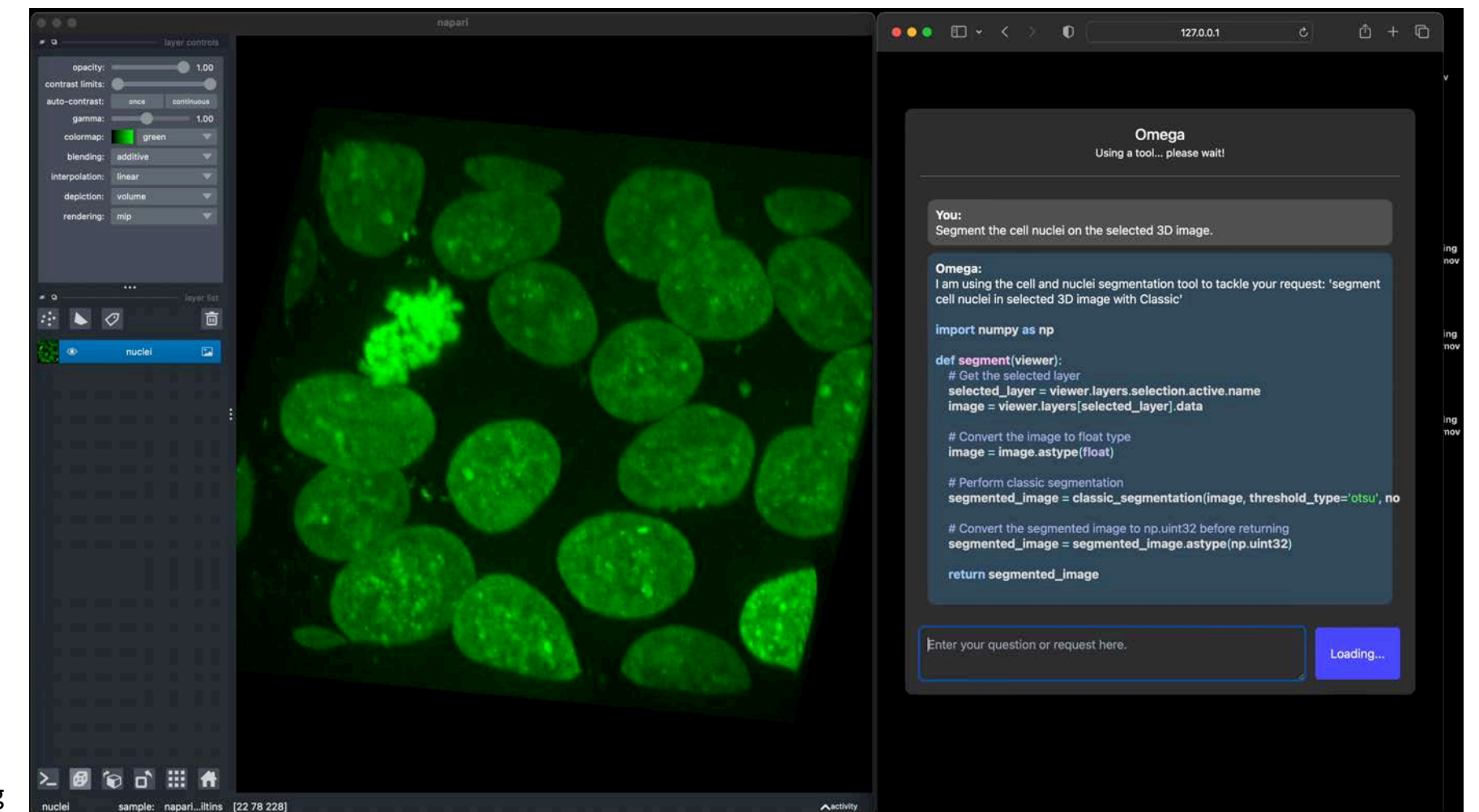
[10.5281/zenodo.10828225](https://doi.org/10.5281/zenodo.10828225)



**Supplementary Figure 3.** Omega’s System Architecture. Diagram illustrating the Omega system architecture, encompassing both the user interface and backend components. The user interface is displayed at the top, consisting of the napari viewer, chat window, and AI-enhanced code editor. Key backend elements include the Omega ReAct agent, web server, AI tools within the editor, and the Python code repair module. The architecture employs a cascaded design, with a central dialog loop orchestrated by a primary language model. This model coordinates a suite of specialized tools, each powered by a secondary tool-specific language model that can access online resources, interact with the napari viewer, and integrate functionalities from third-party libraries such as Cellpose and StarDist.



**Supplementary Video 1.** Omega can segment nuclei with StarDist and perform follow-up analysis. The video showcases Omega’s ability to segment cell nuclei in a 2D image using StarDist. Omega successfully segments the nuclei and adds a label layer to the napari viewer. With further instructions, Omega can count the segmented nuclei and create a CSV file on the desktop folder of the machine. This file contains coordinates and areas of all segments, sorted by decreasing area, with one segment per row. Omega also opens the file using the system’s default CSV viewer. The video has been sped up by a factor of 2.



**Supplementary Video 2.** Omega can segment nuclei in a 3D image. This video shows how Omega segments the nuclei in a 3D image displayed in the napari viewer. Omega uses a specialized tool for cell and nuclei segmentation and employs a 'classic' approach that combines single thresholding, specifically Otsu, with watershed splitting to prevent under-segmentation. After segmentation, Omega adds a labels layer to the viewer, and we inquire about the number of segments detected. The response is 27. The video has been sped up by a factor of 2.

Human red blood cells  
DIC microscopy

## Tools

Upload Gallery

### Hover & Click

Click an object one or more times. Shift-click to remove regions.




Add Mask




Remove Area

Res  Undo/Redo

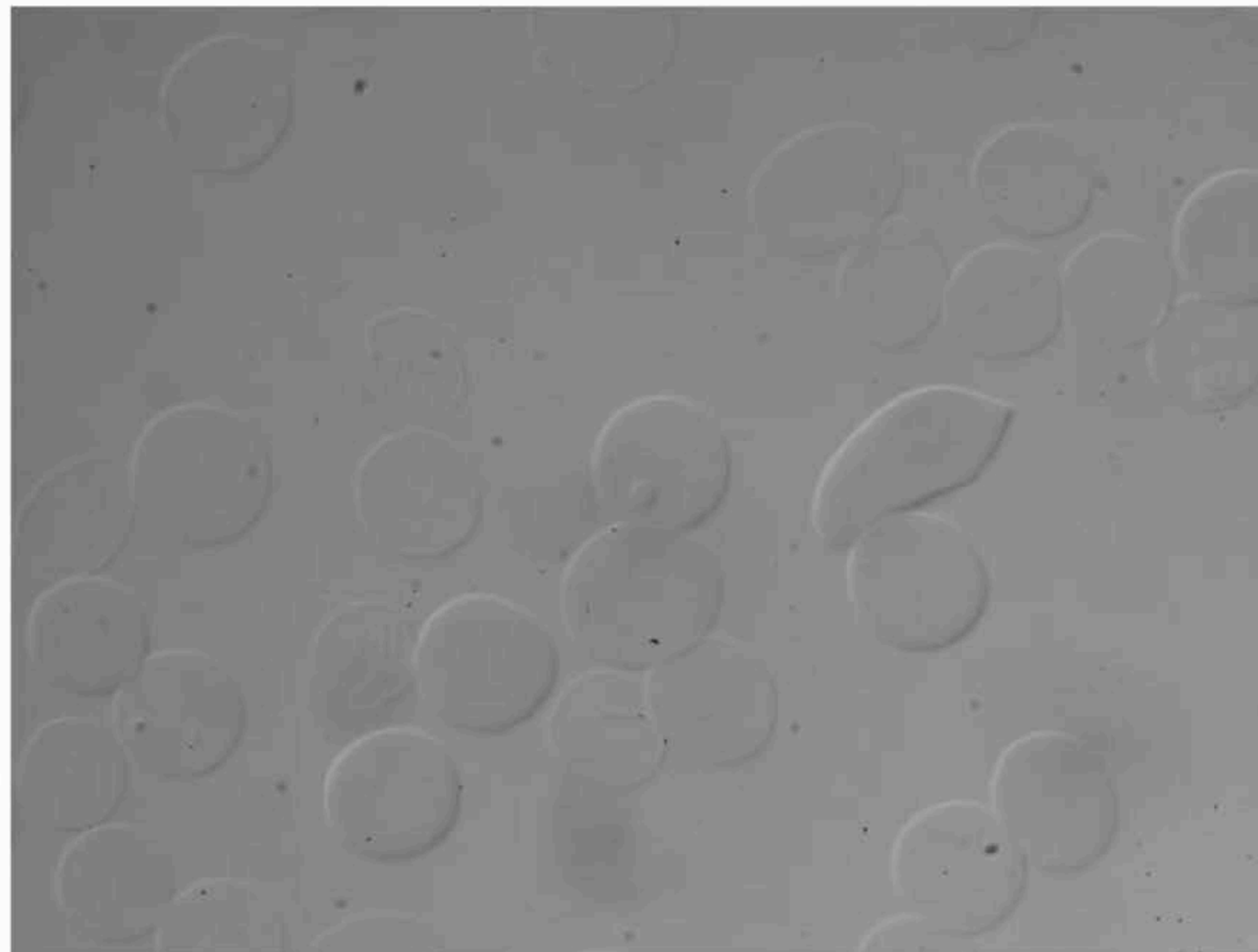
 Multi-mask

 Cut out object

 Box

 Everything

 Cut-Outs



"[BBBC009v1](#) from the Broad Bioimage Benchmark Collection [[Ljosa et al., Nature Methods, 2012](#)]."



## Tools

Upload Gallery

### Hover & Click

Click an object one or more times. Shift-click to remove regions.



Add  
Mask



Remove  
Area

ResetUndoRedo



Multi-mask



Cut out object



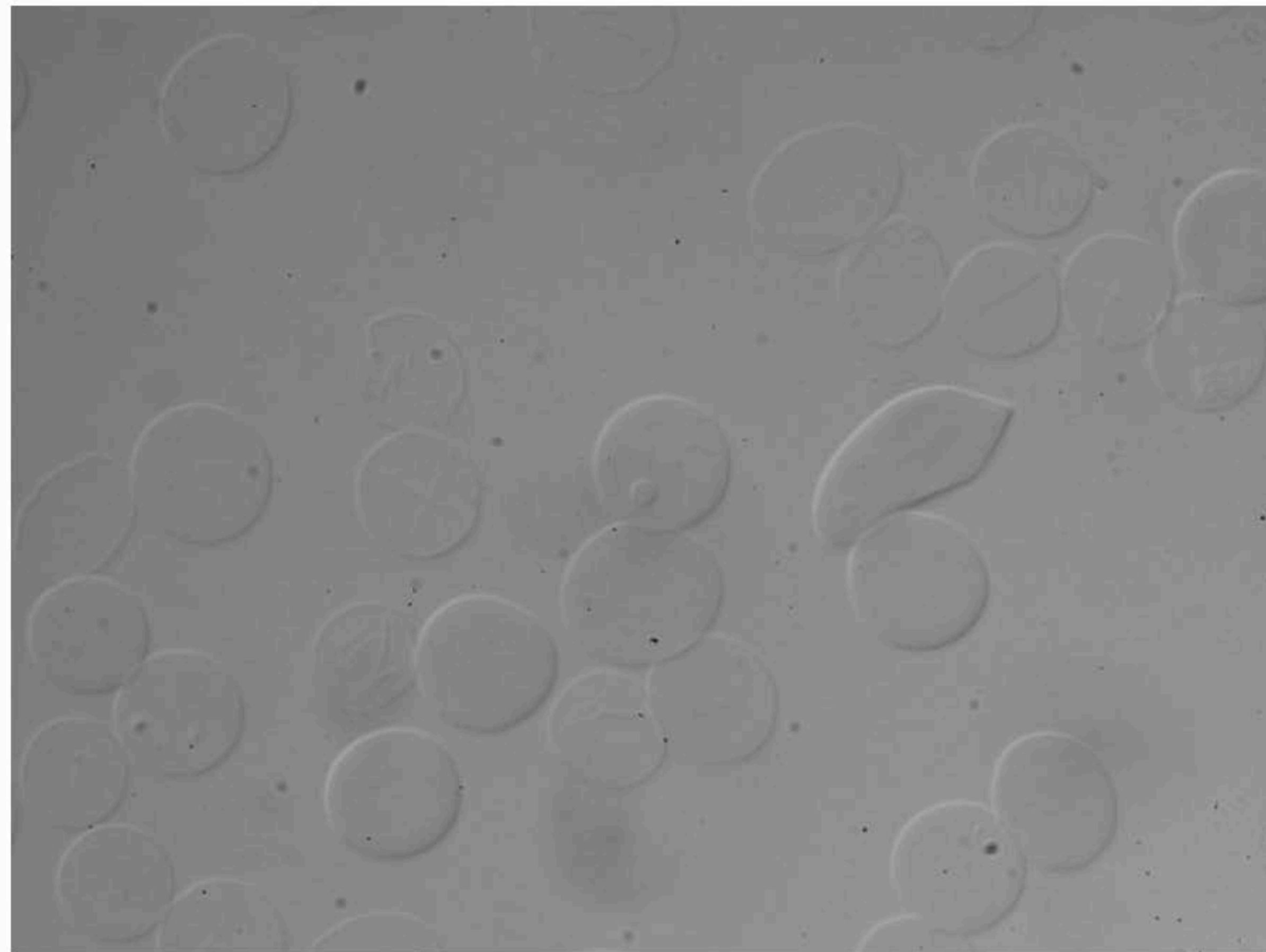
Box



Everything



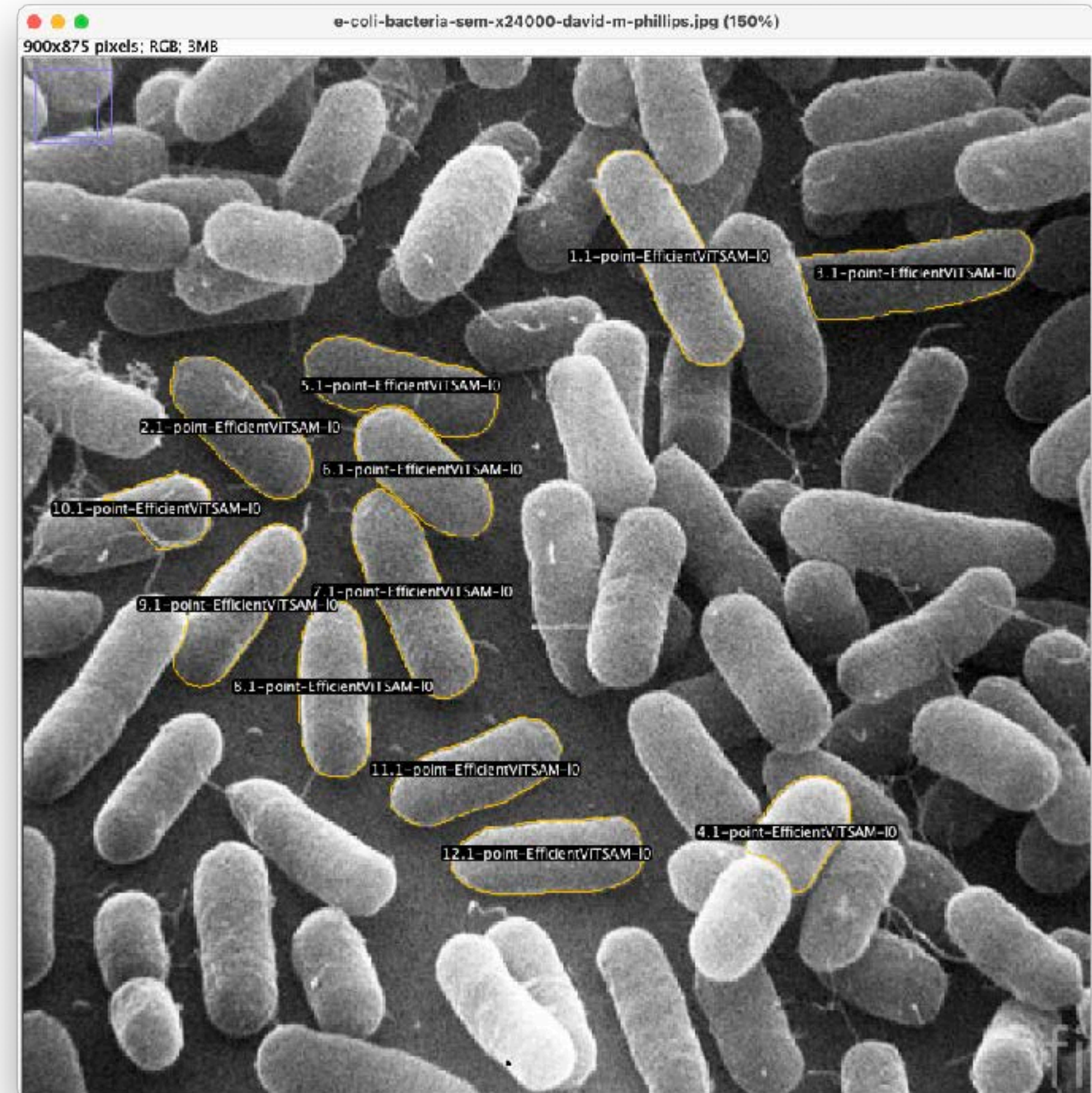
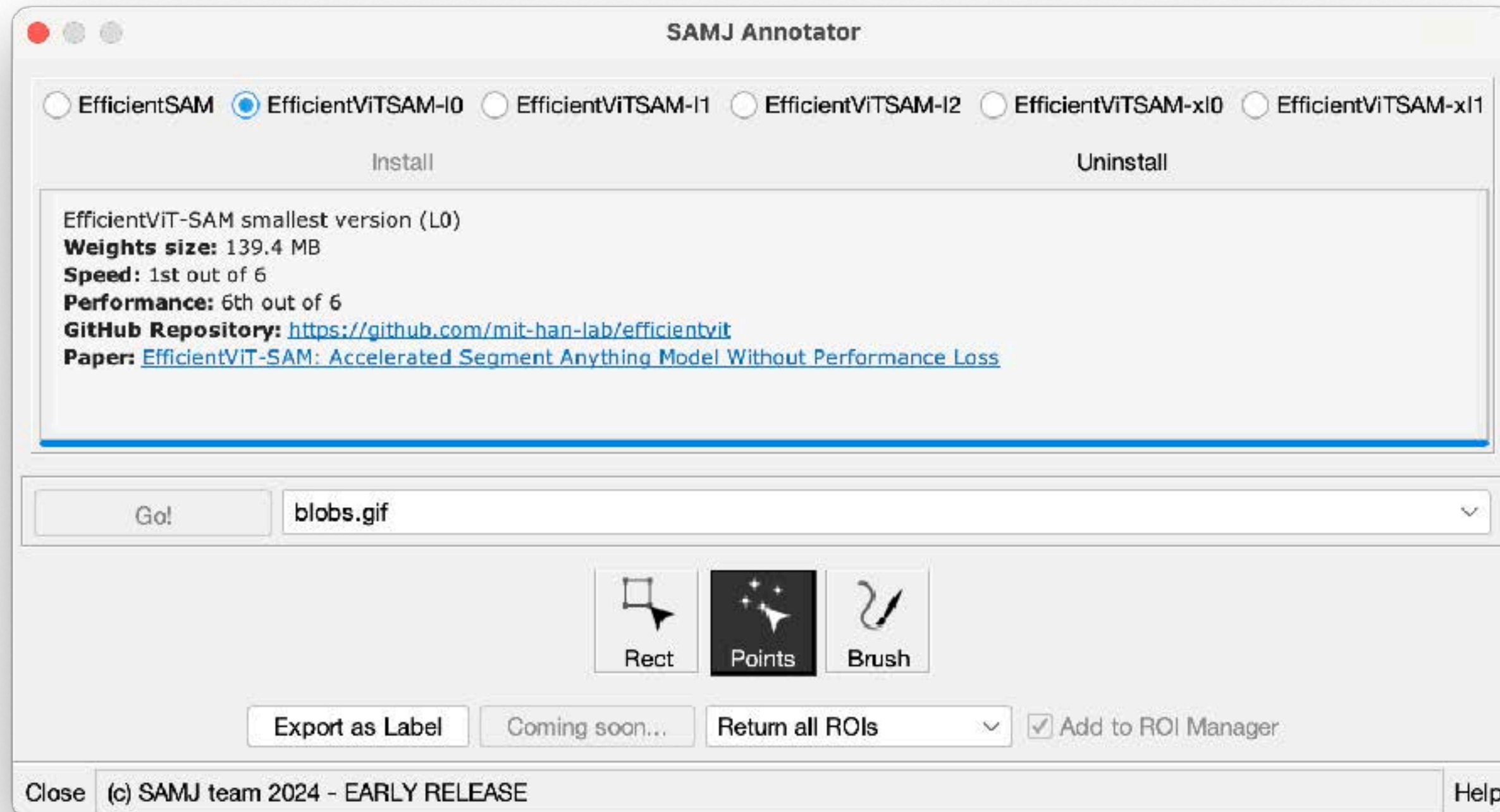
Cut-Outs



# 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**
- ...

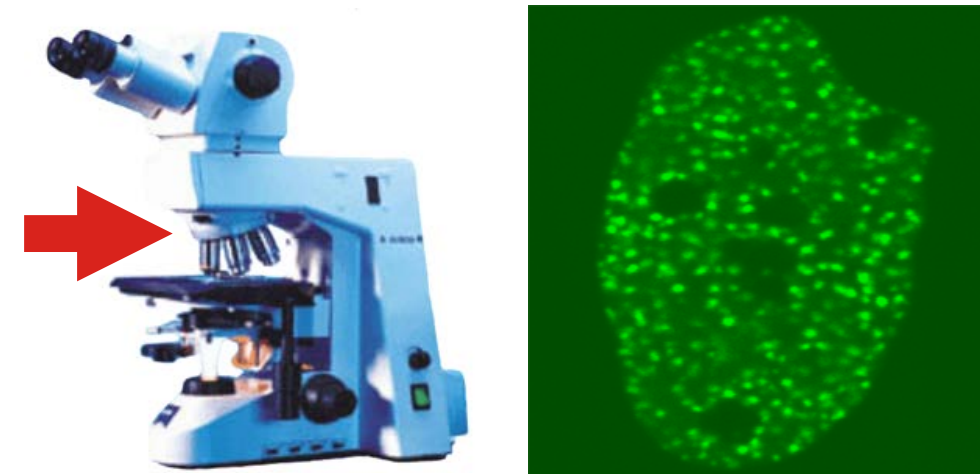


- Exploratory data analysis
- Filtering of numerical data based on measured values
- Summary statistics
- Plotting numerical values
- Making movies and montages of images
- Record entire work-flow for later reference
  - Write down what you did and why (each step)
  - Use a version control system
  -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

# Processing vs. Analysis

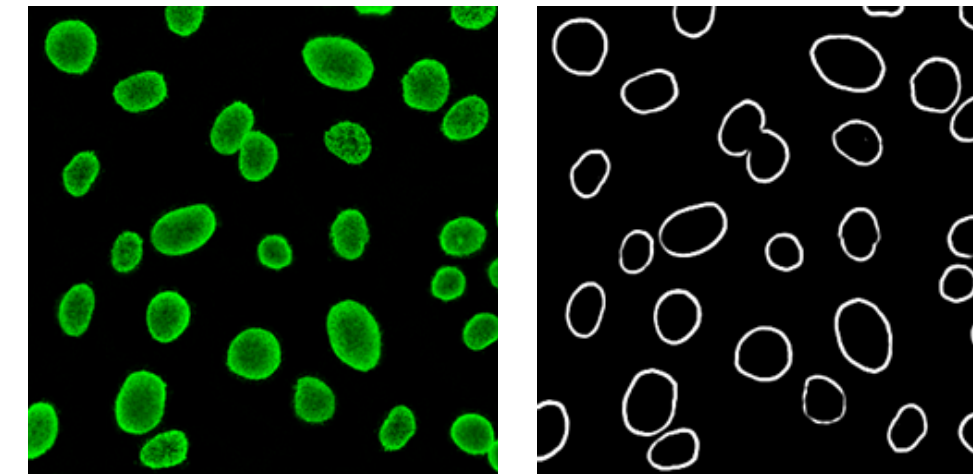
## Image Formation

object in → image out



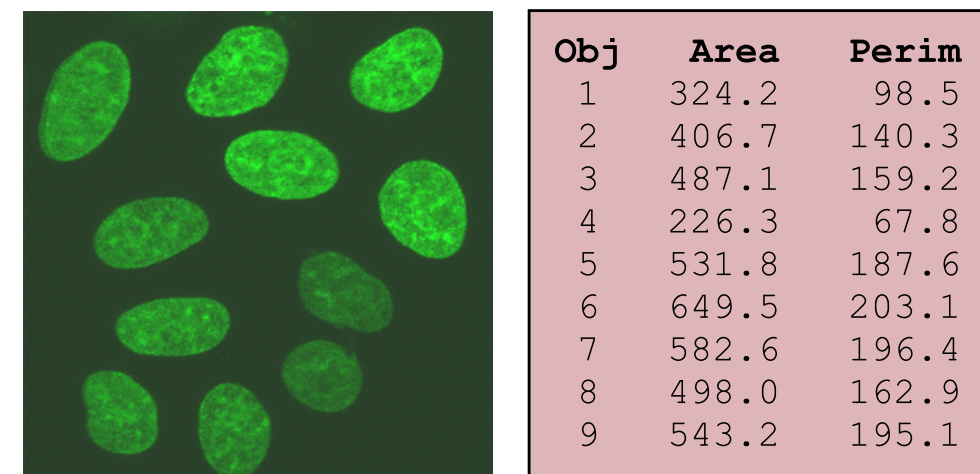
## Image Processing

image in → image out



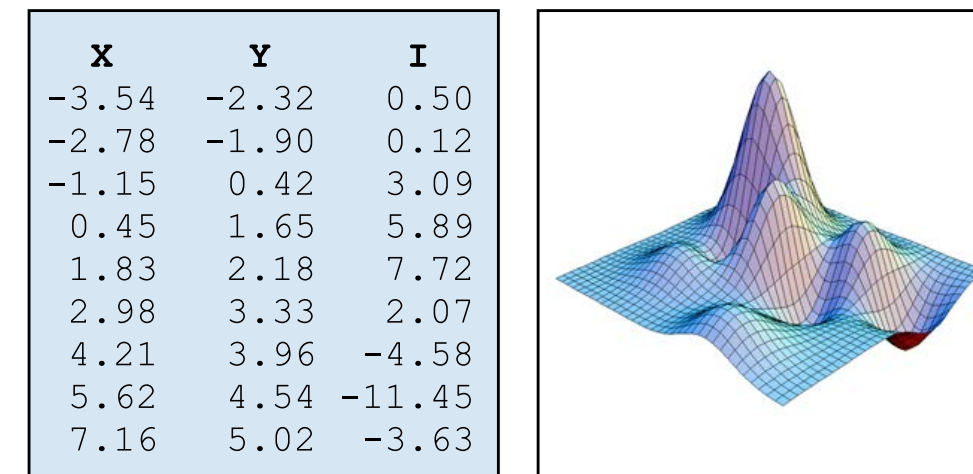
## Image Analysis

image in → features out



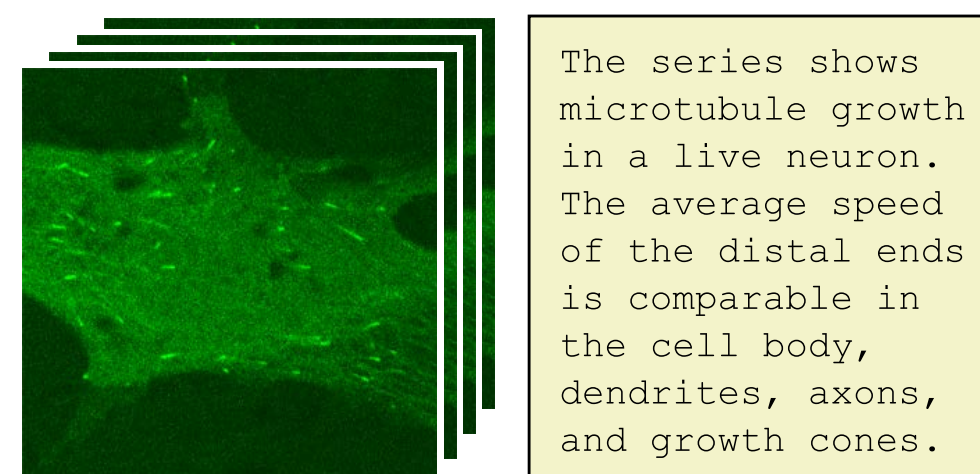
## Computer Graphics

numbers in → image out



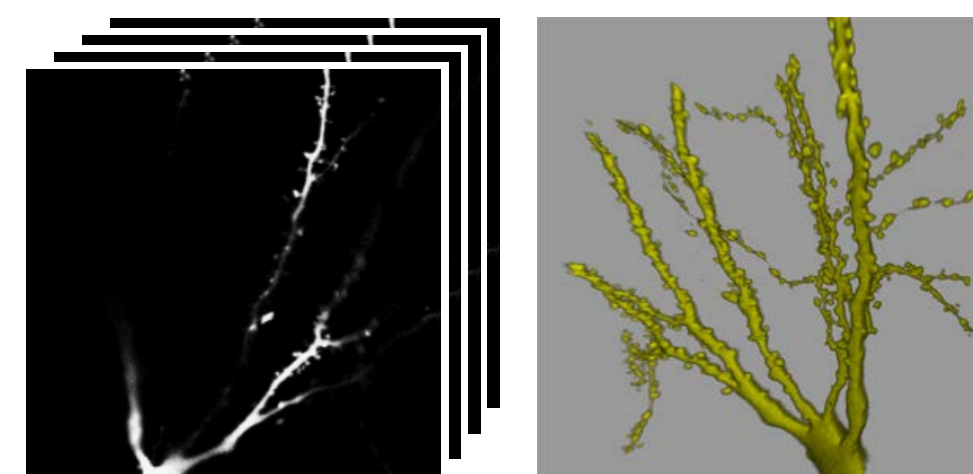
## Computer Vision

image in → interpretation out



## Visualization

image in → representation out



# Debugging Fiji and getting help — a workflow

1. **Restart Fiji**
2. **Ask** you right neighbor
3. **Ask** you left neighbor
4. **Ask** an instructor
5. **Search** <https://forum.image.sc/>