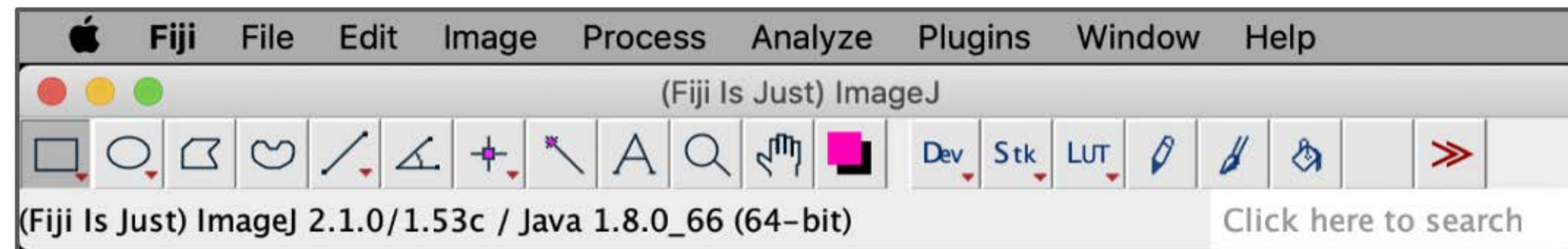


Introduction to image analysis using ImageJ/Fiji



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



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

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

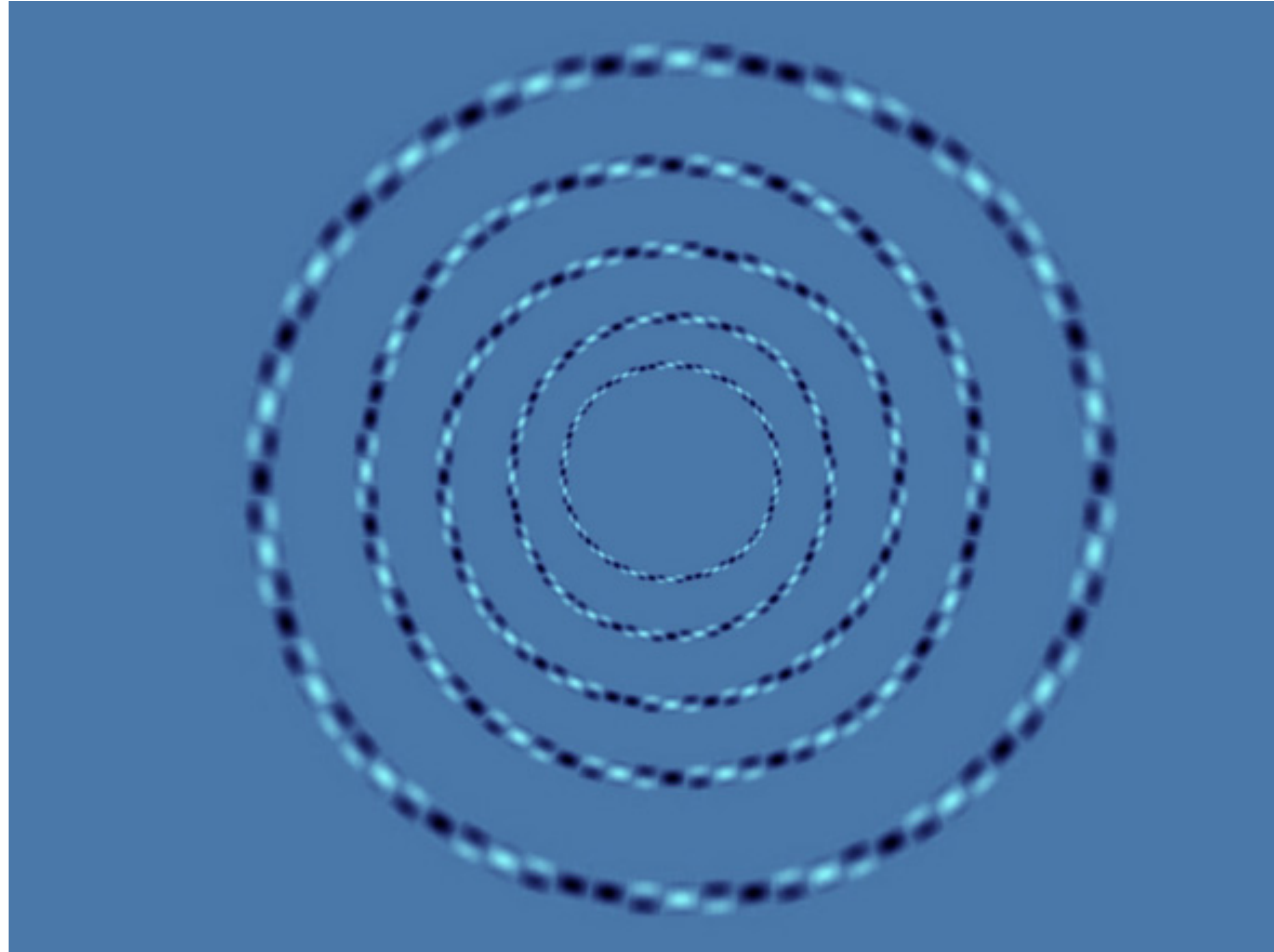
1. My **name** is *Donald Duck*
2. My **position** is as a *10th year postdoc*
3. My **lab** is *Mad City Labs*
4. My model **system** is *blue whales*
5. I have **data** from *confocal microscopy*

Optical Illusions

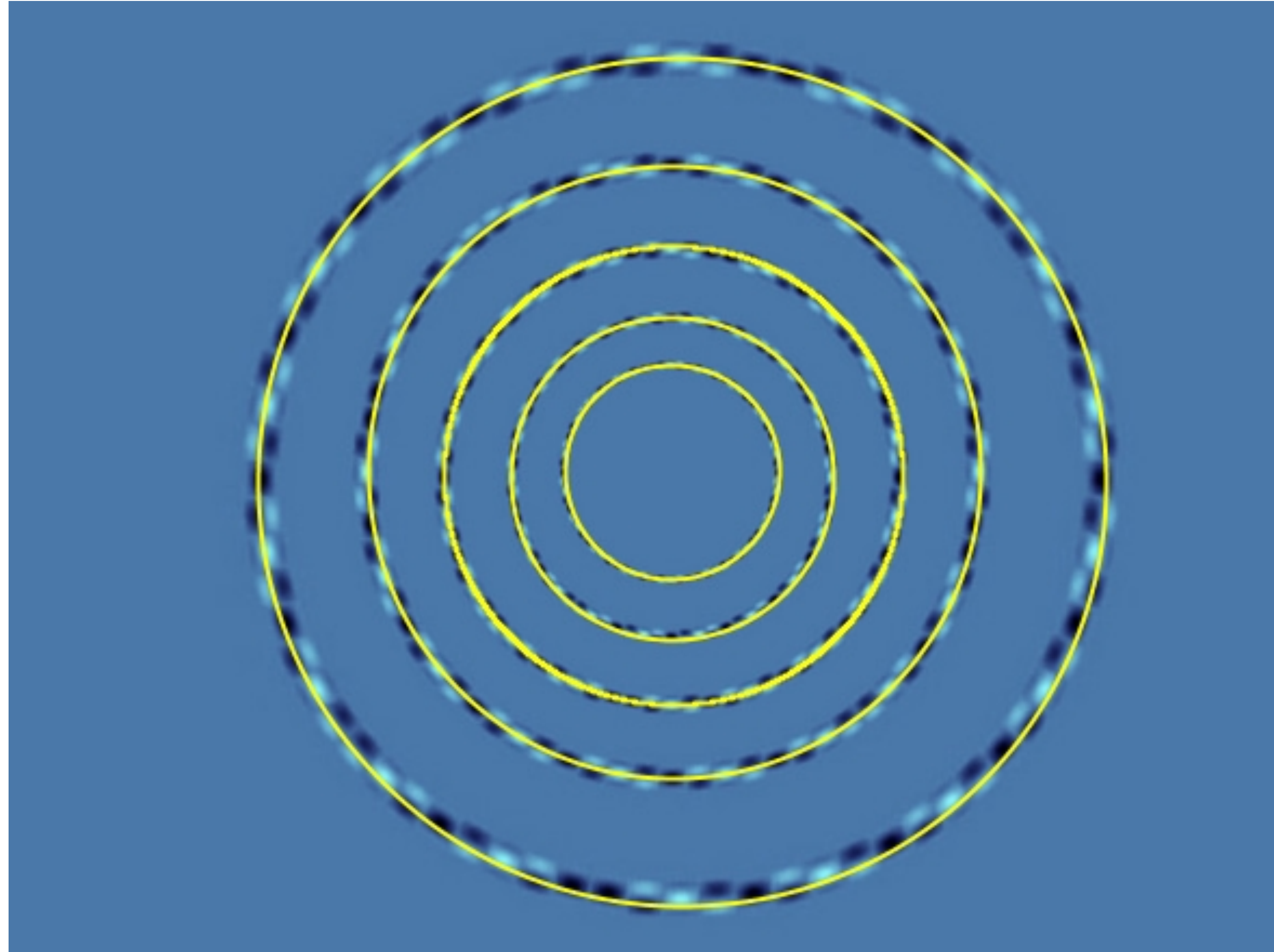
—

Why should you analyze images
with computers

Color perception and pattern recognition is individual – science less so

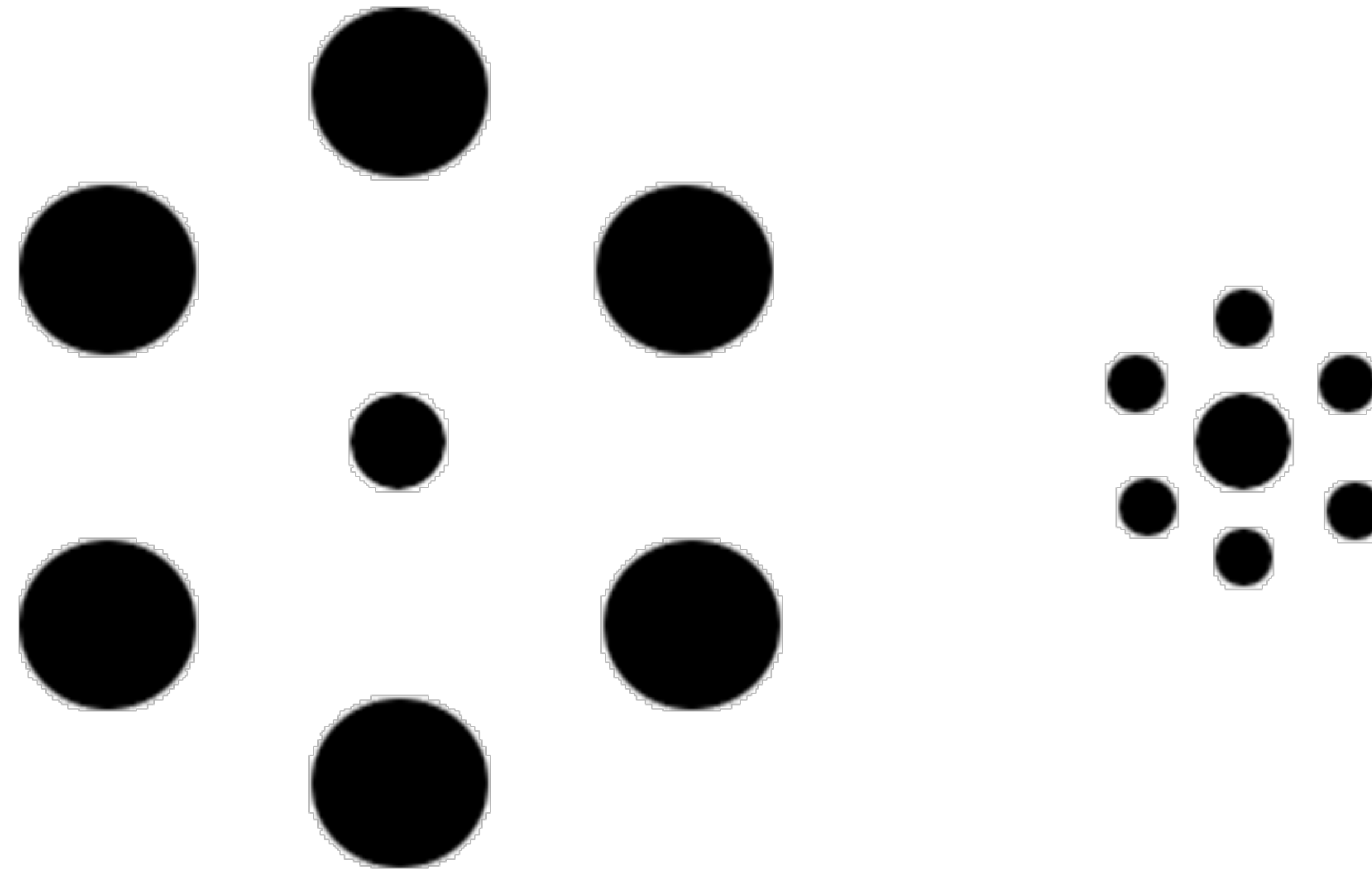


Concentric circles !?



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

Identical central discs?



Our size estimate is strongly influenced by the local neighbourhood

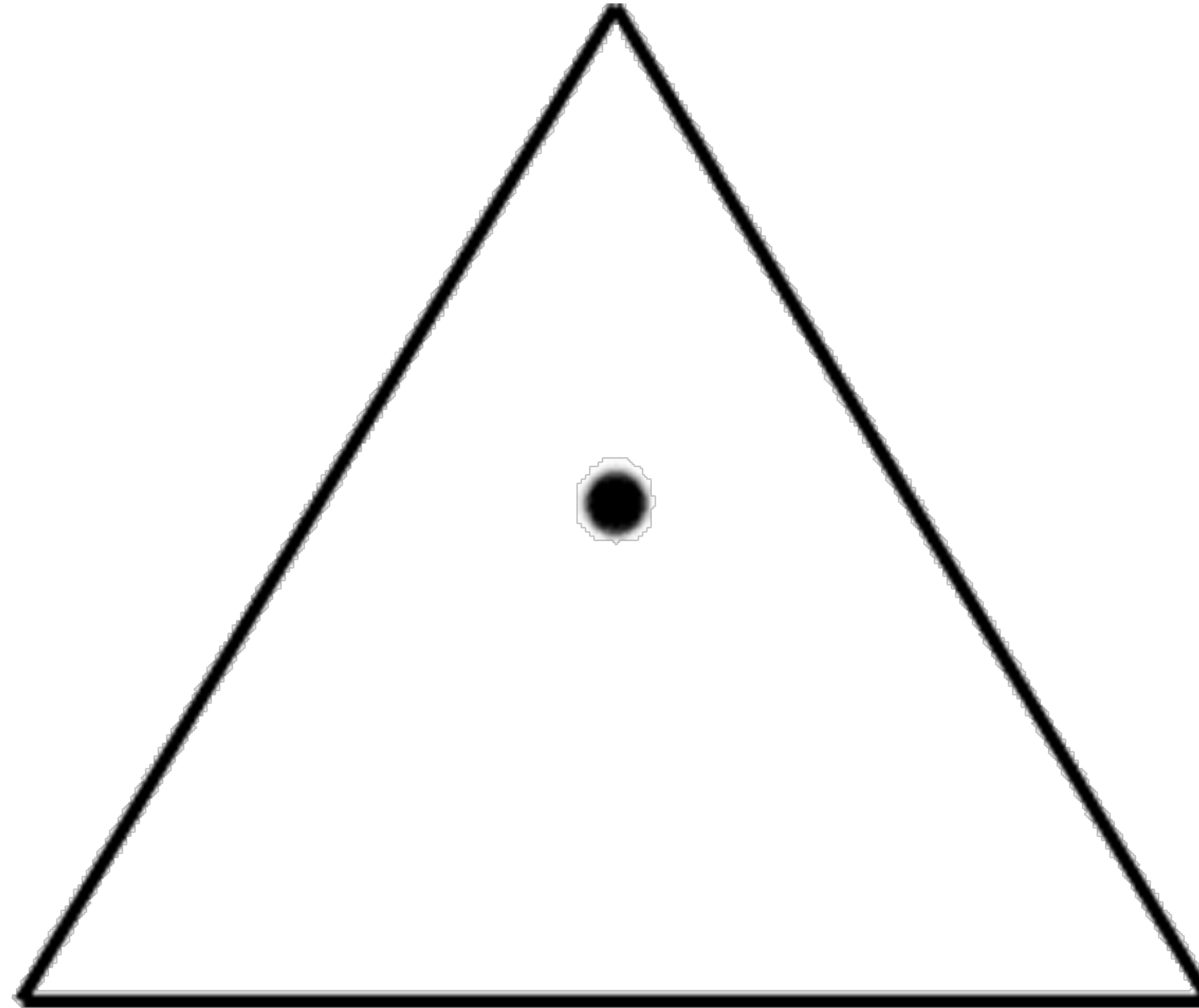
Identical central discs?



Yes, the discs are identical

<http://www.brainbashers.com>

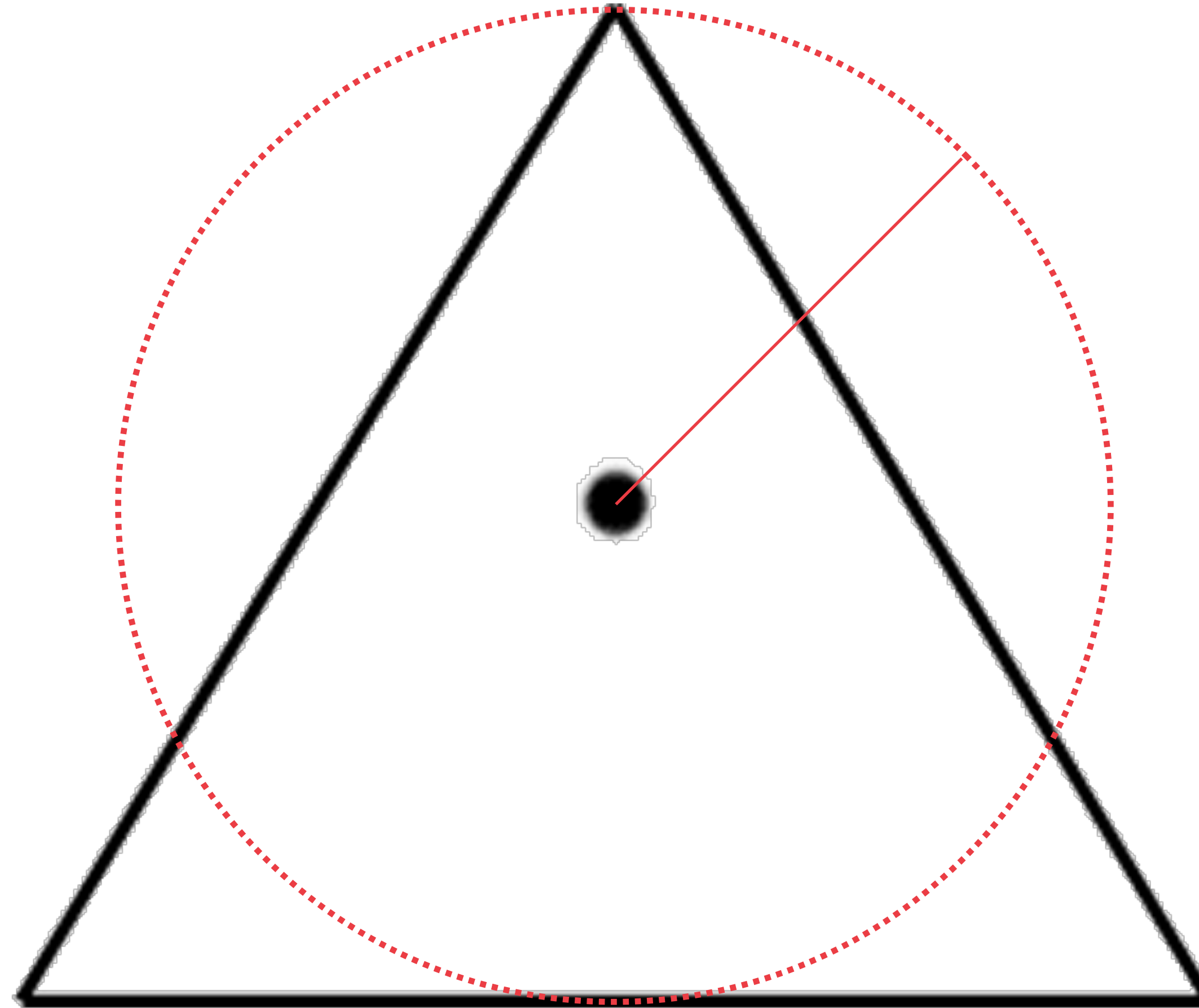
Is the dot half-way up?



Our sense of distance depends on neighbourhood

<http://www.brainbashers.com>

Is the dot half-way up?



Yes it is!

<http://www.brainbashers.com>

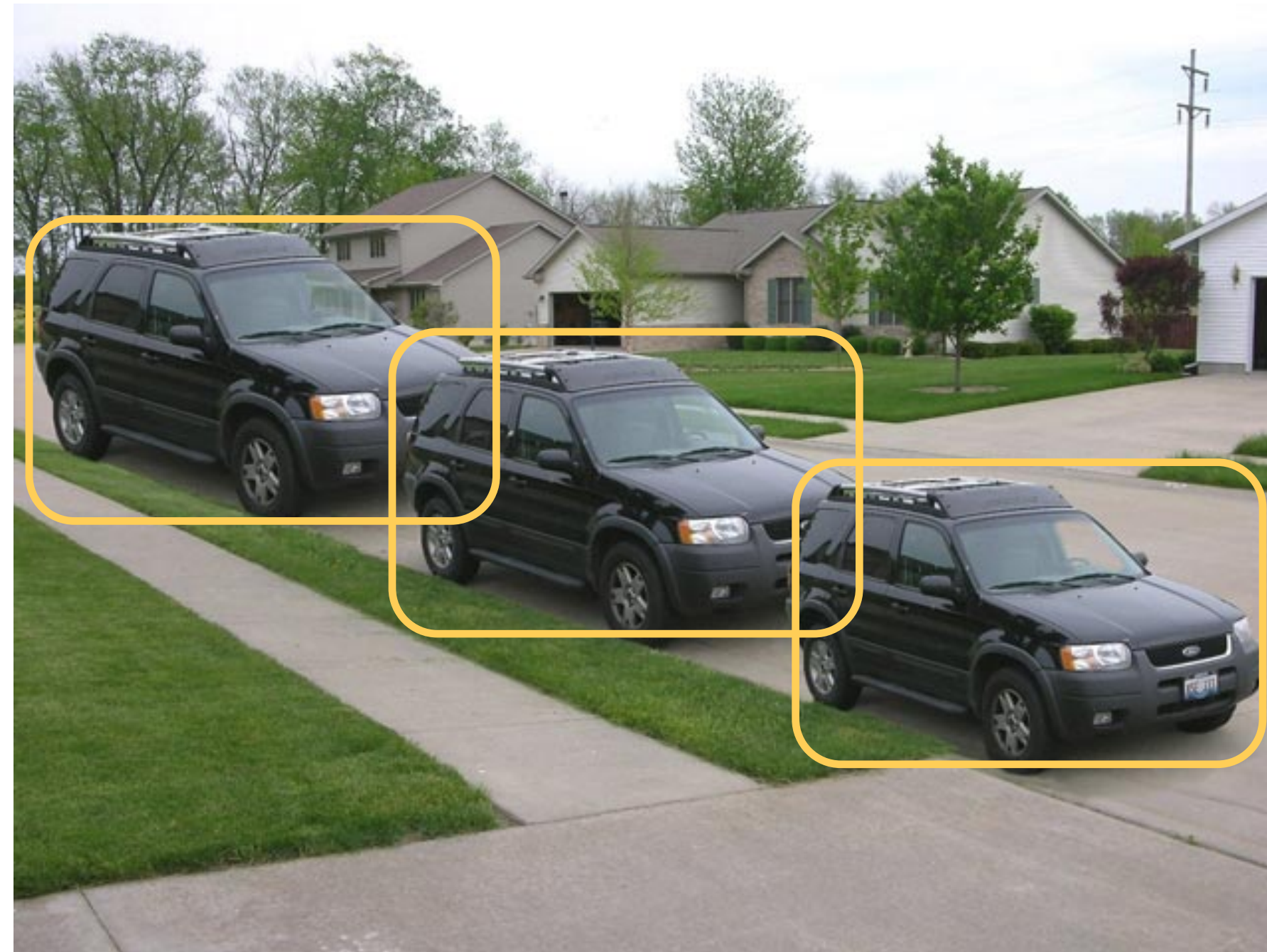
Which car is bigger?



Which car is bigger?



Which car is bigger?

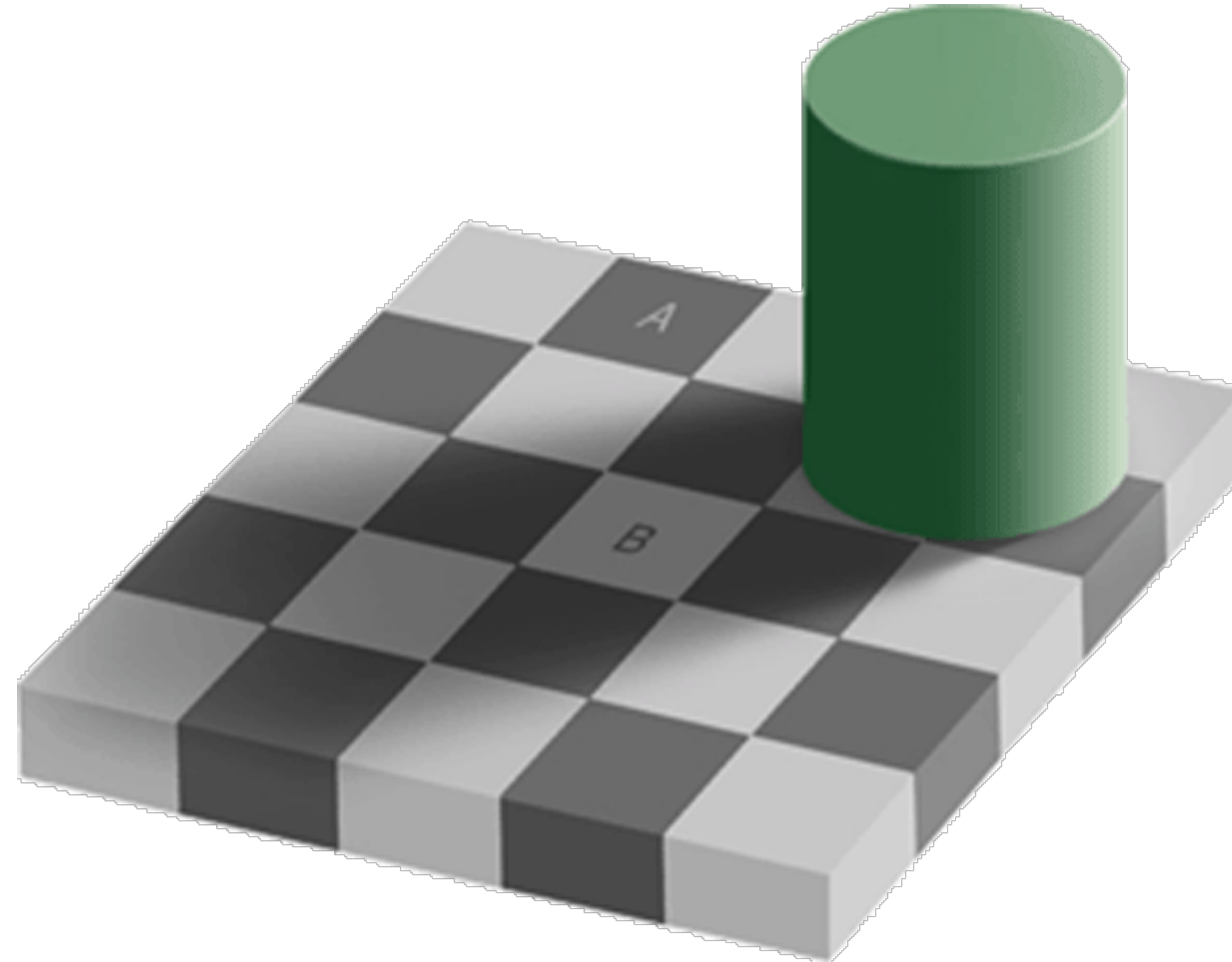


All cars are same size

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

<http://www.brainbashers.com>

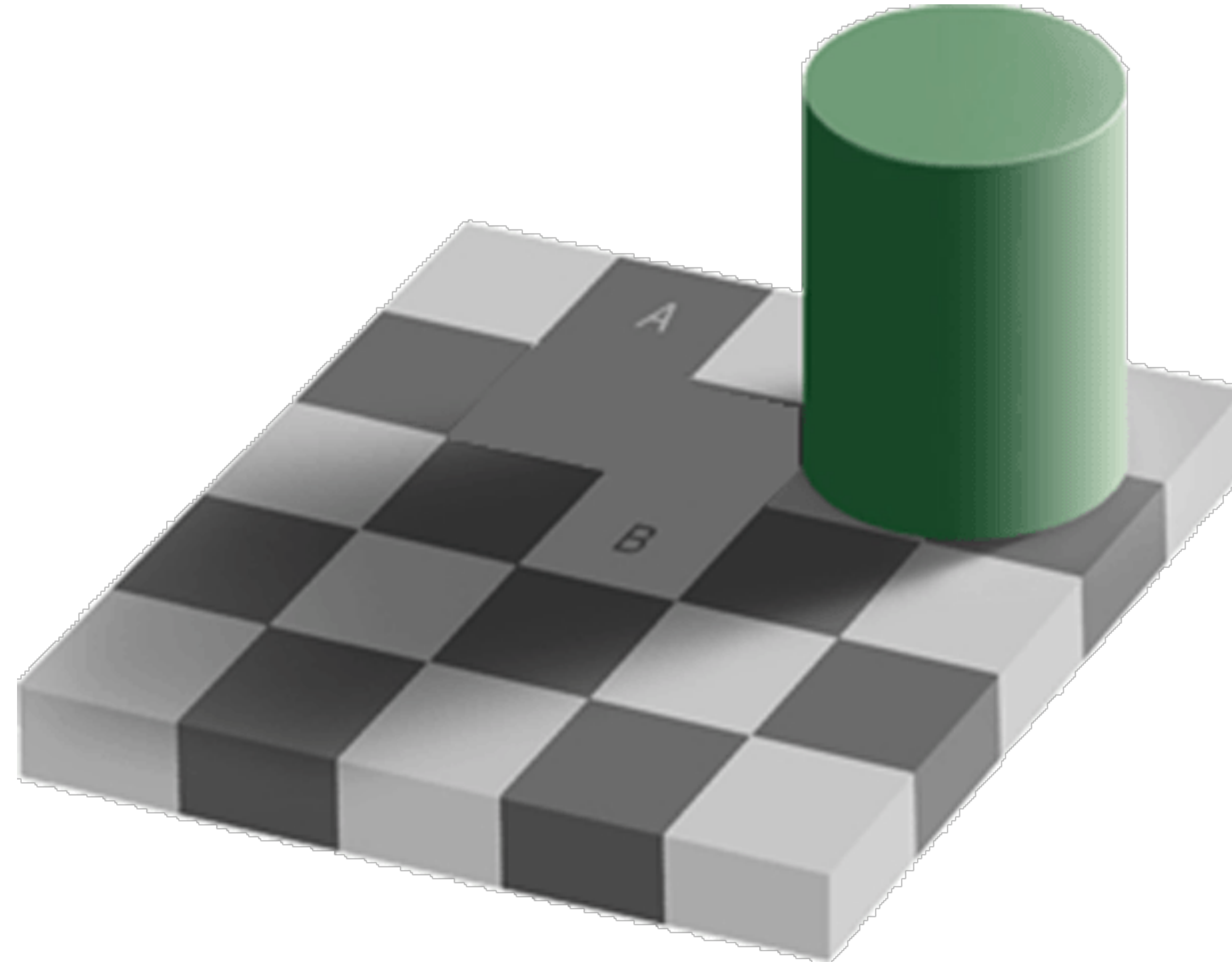
Are A and B equally grey?



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)

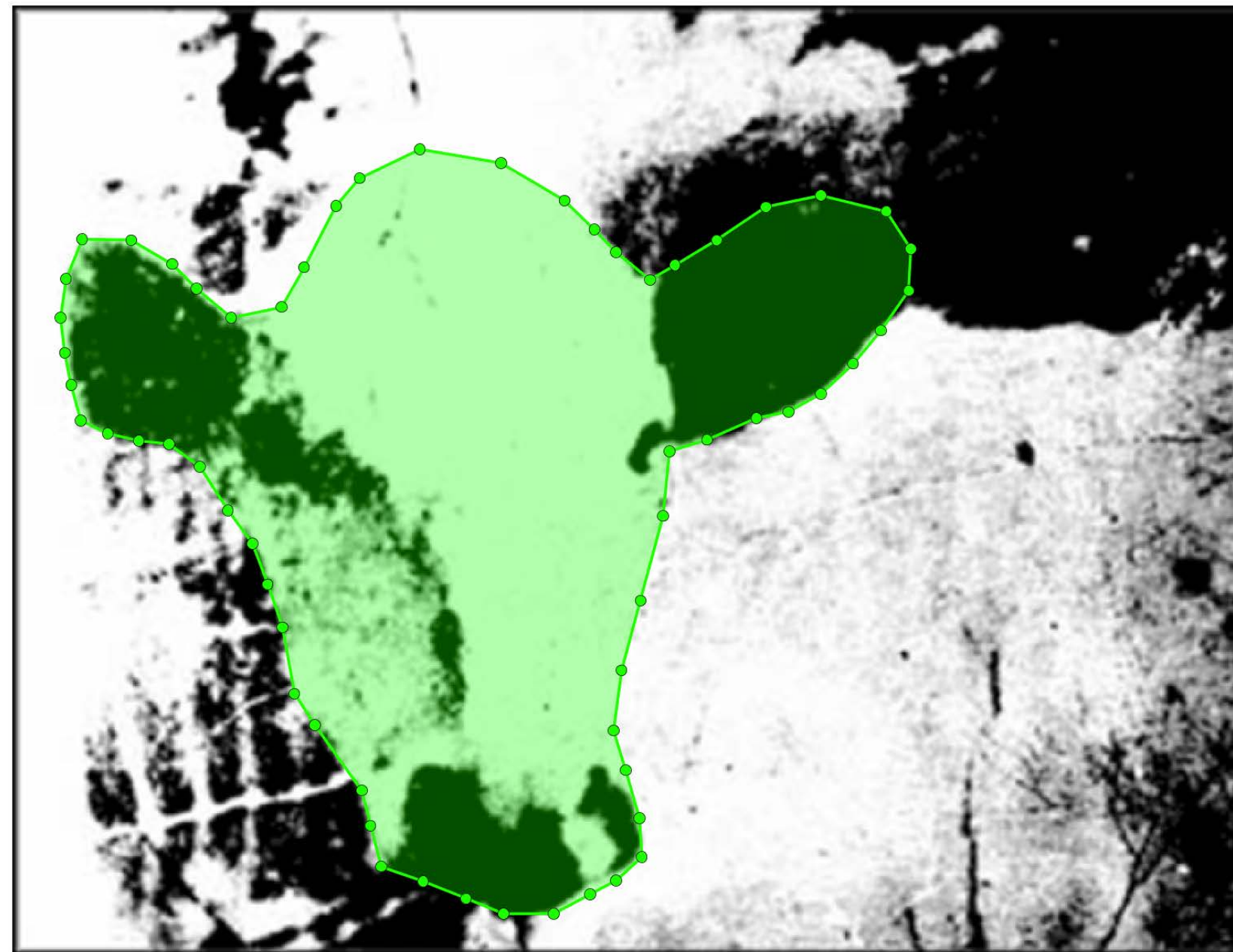
Pattern Recognition



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**

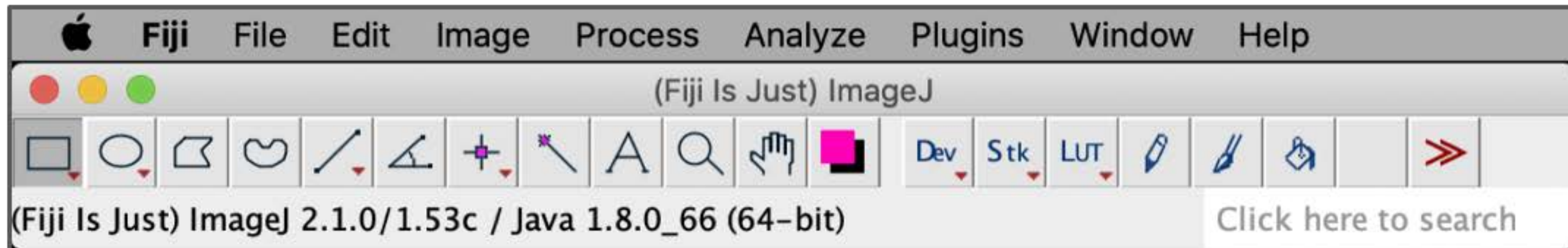
It is a “batteries included” distribution of ImageJ2

Easy to install (“portable” application)

Include a collection of Plugins

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

Documentation

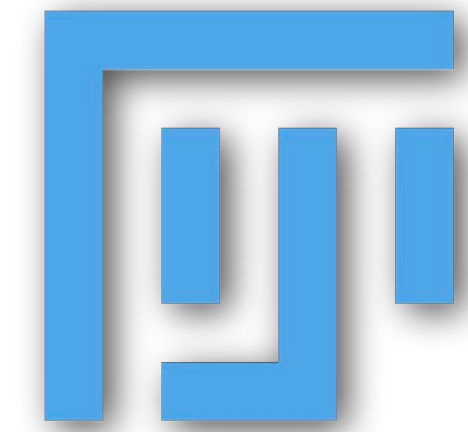


12 years ago...

- [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)



CellProfiler[™]
cell image analysis software



scikit-image
image processing in python



4 years ago...

- CellPose is out (2020)

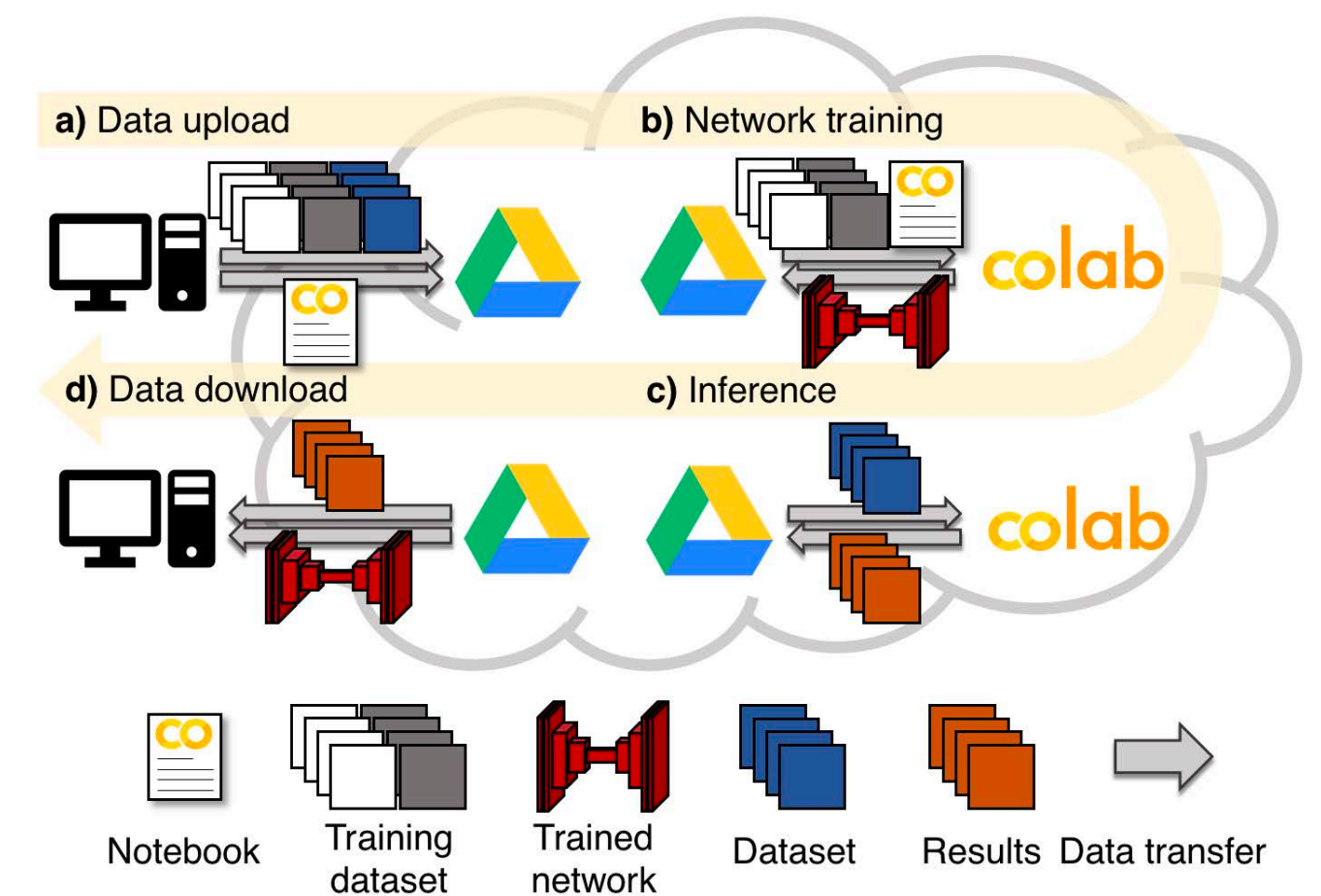
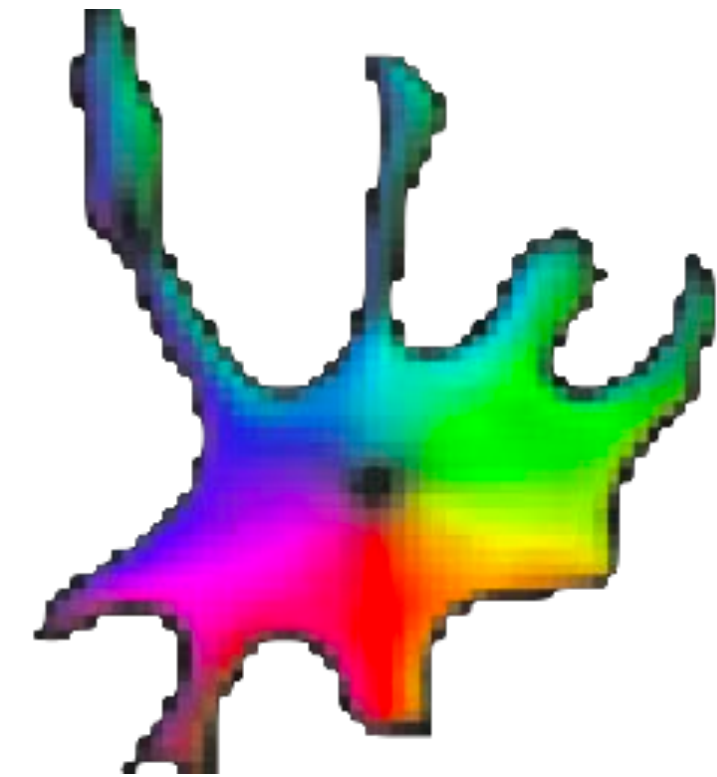
- “Cellpose: a generalist algorithm for cellular segmentation”
- Trained on highly varied images of cells, over 70,000 segmented objects
- Cells don't have to be star-shaped
- Web-platform and Jupyter notebooks
- <https://www.nature.com/articles/s41592-020-01018-x>

- 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

All Topics	AGAVE	AICSImageIO	Arktekt	Aydin	BAND
BIAFLOWS	BiaPy	Bili	BiofilmQ	Bio-Formats	BioImageIO
BoneJ	BrainGlobe	Cellpose	CellProfiler	CLIJ	CytoMAP
Cytomine	DAIM	DeepLabCut	Fiji	FLIMLib	GerBI
Icy	IDR	ilastik	ImageJ	ImageJ2	ImgLib2
ImJoy	ImSwitch	iRODS	JIPipe	JuliaImages	Mars
MCMICRO	MIA	MIB	µManager	MoBIE	ModularImageAnalysis
MorphoGraphX	MorphoNet	napari	NEUBIAS	NFDI4BIOIMAGE	OME
OMERO	OmeSliCC	OpenIRIS	OpenSPIM	Orbit	Piximi
PolusAI	PYME	Python-Microscope	QUAREP-LiMi	QuPath	scenery
SCIFIO	scikit-image	sciview	SmartMicroscopy	SR-Tesseler	StarDist
TeamTomo	TissUUmaps	vedo			
... Your Icon Here					

RELATED COMMUNITIES

- µforum
- STK
- KNIME

Citation Details

Article

Fiji: an open-source platform for biological-image analysis

Nature Methods, 2012, Volume 9, Number 7, Page 678
Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, Curtis Rueden, Stephan Saalfeld, Benjamin Schmid, Jean-Yves Tinevez, Daniel J. Storer, Daniel J. White, Volker Hartenstein, Kevin Eliceiri, Pavel Tomancak, Albert Cardona

[Read Online](#)

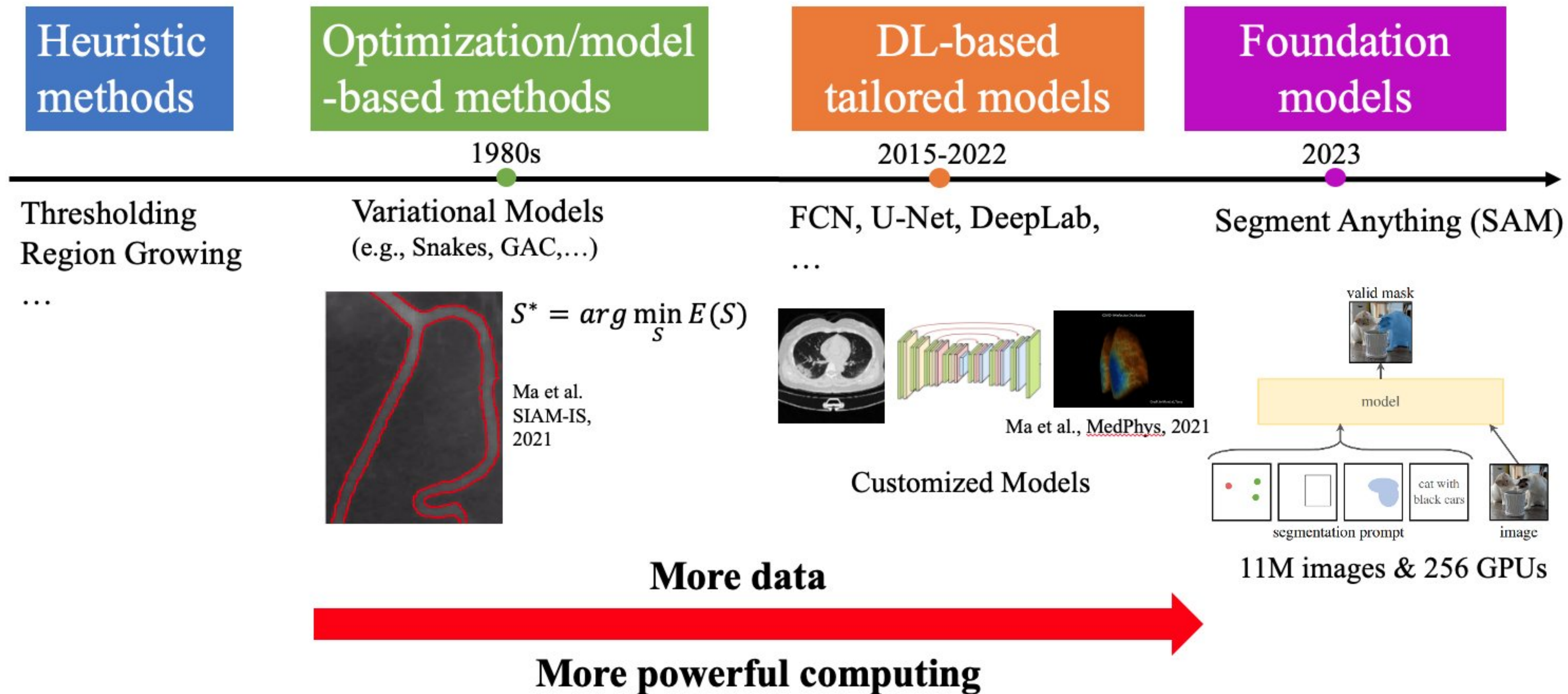
4680 ITEMS CITE THIS ARTICLE WITH YEAR: 2022

30162 ITEMS CITE THIS ARTICLE
33431 ITEMS CITE THIS ARTICLE
39921 ITEMS CITE THIS ARTICLE



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

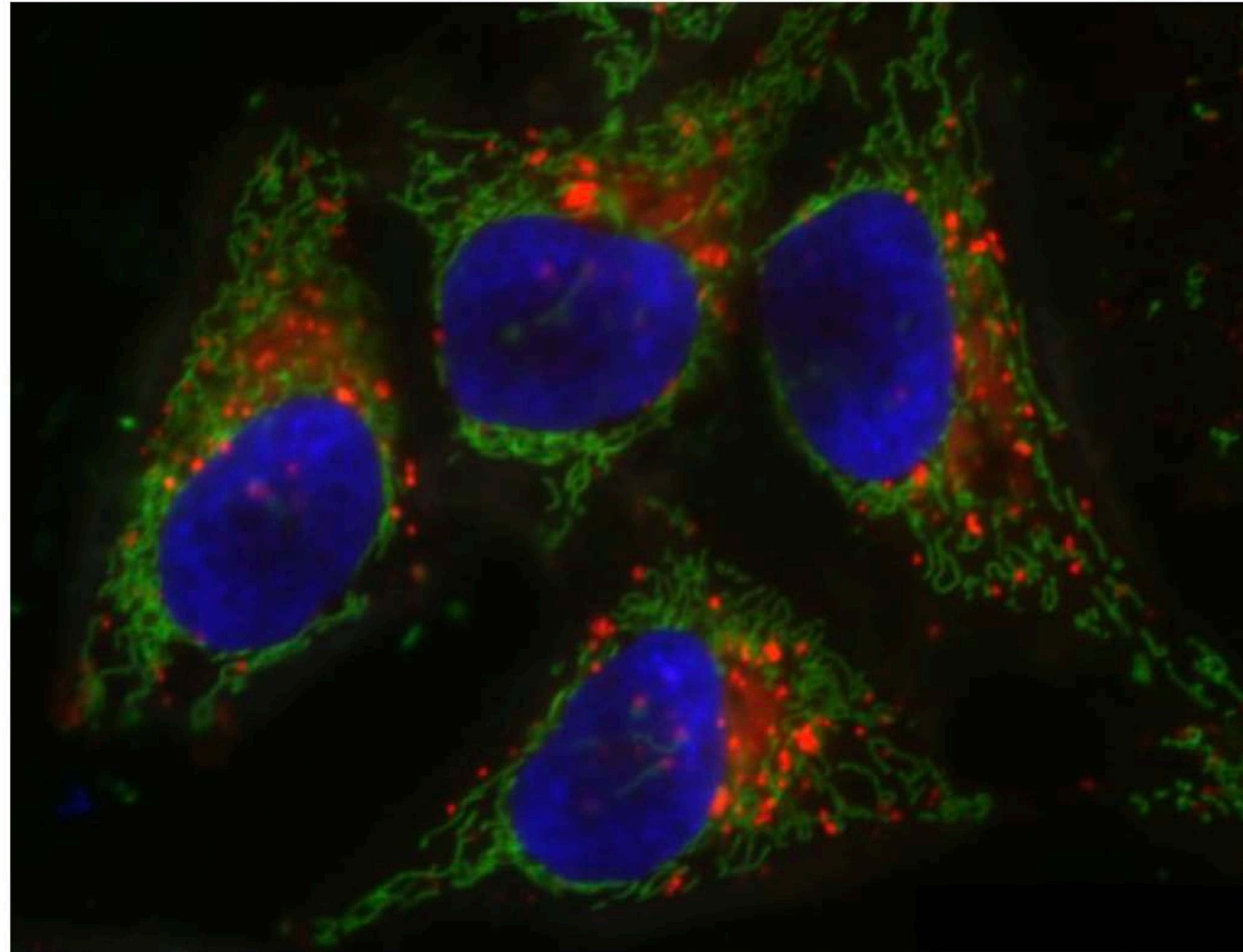
Write an email
to request a quote from local plumbers

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



Using ChatGPT for Image Analysis

SI You

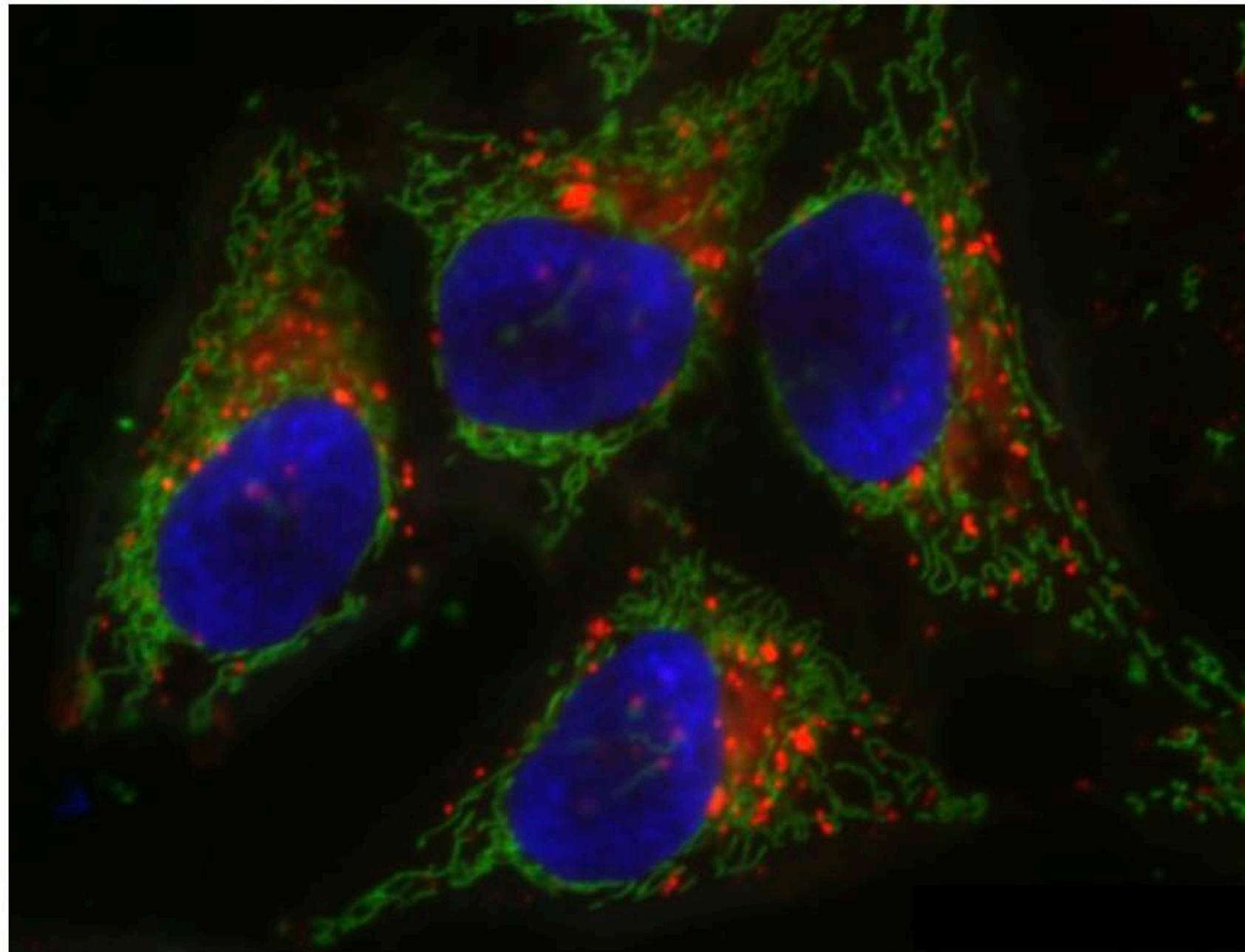


please segment the nuclei and show me the relevant code you used

Using ChatGPT for Image Analysis

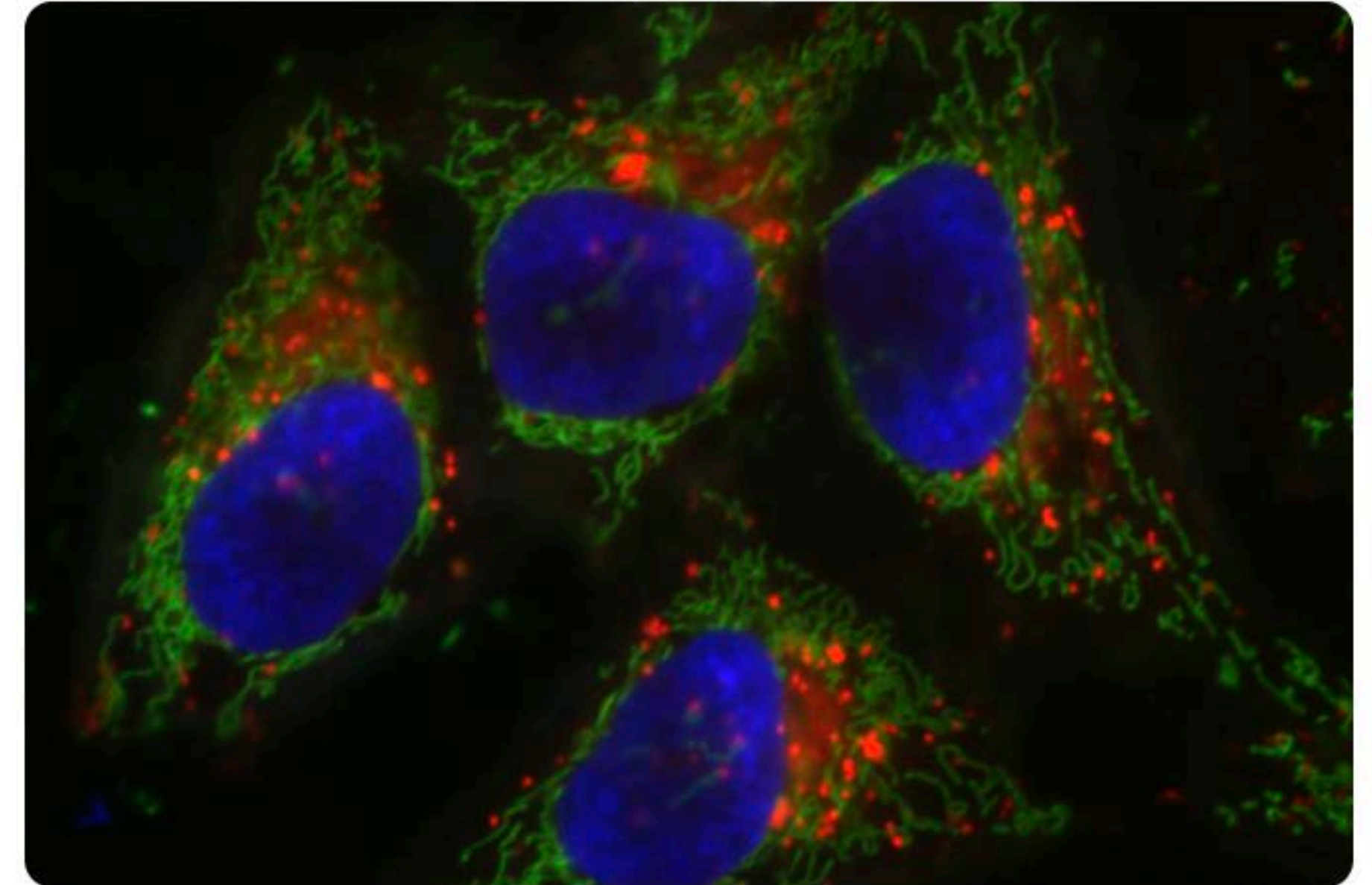
GPT-4, May 2024

SI You



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

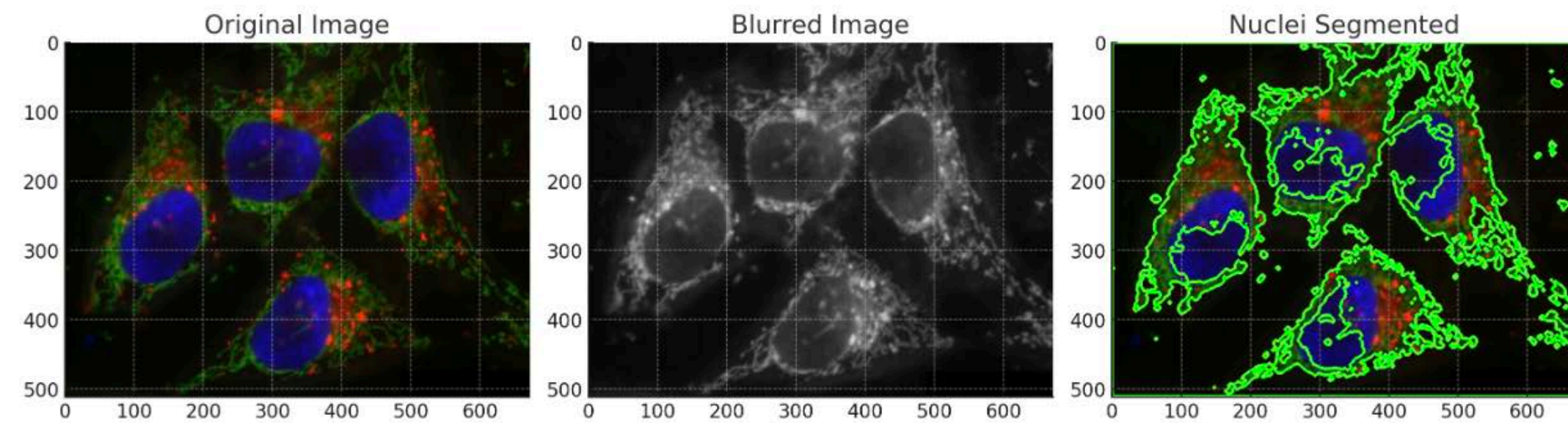
GPT-4o, Oct. 2024



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

Using ChatGPT for Image Analysis

GPT-4, May 2024

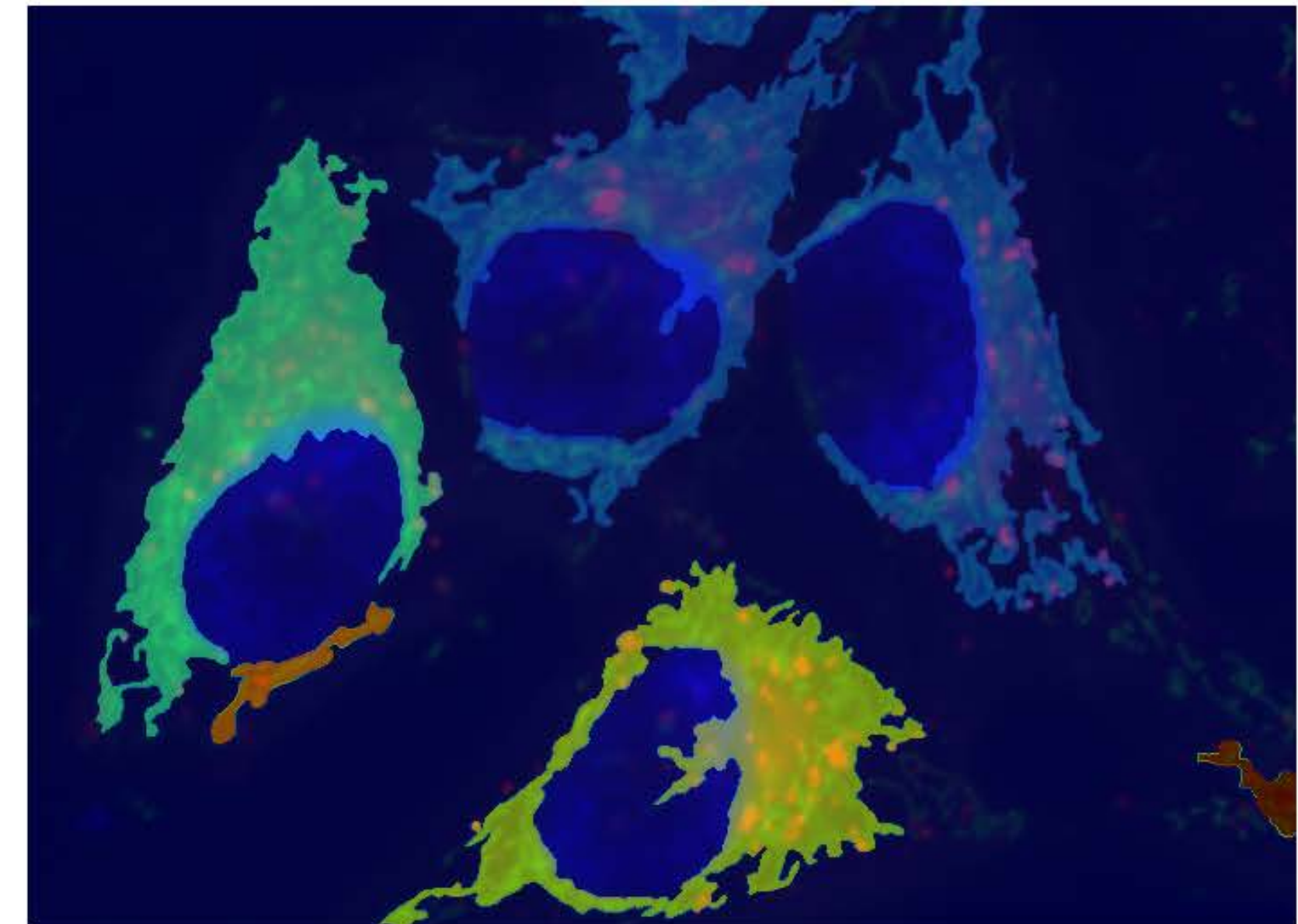


The nuclei in the image have been segmented using the following steps:

- 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.

GPT-4o, Oct. 2024

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)
```

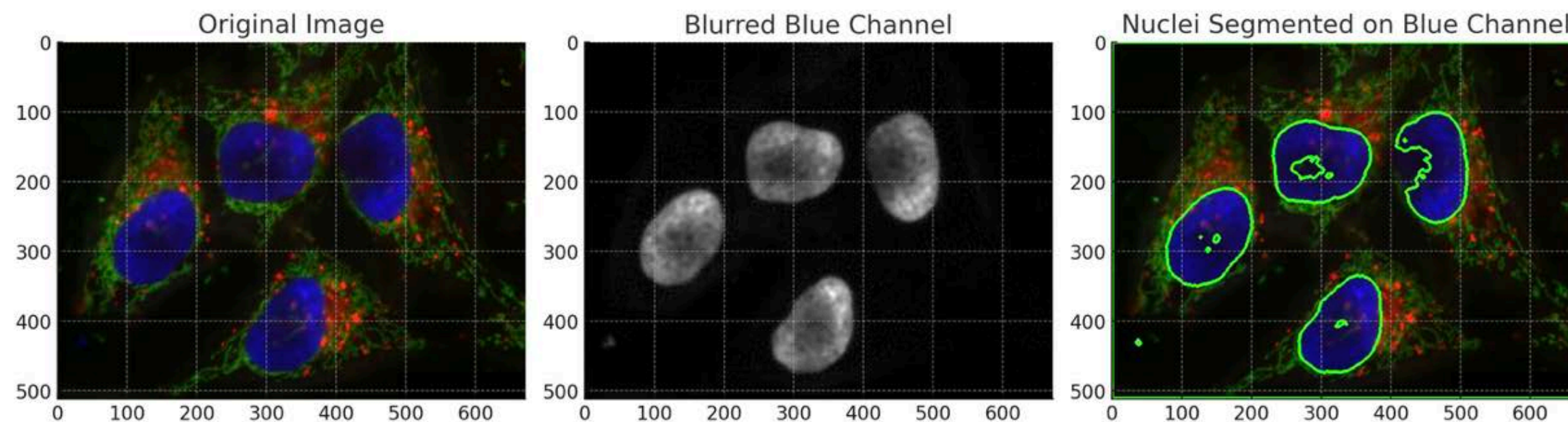

Using ChatGPT for Image Analysis

GPT-4, May 2024

GPT-4o, Oct. 2024

You
please use the blue channel for segmenting the nuclei

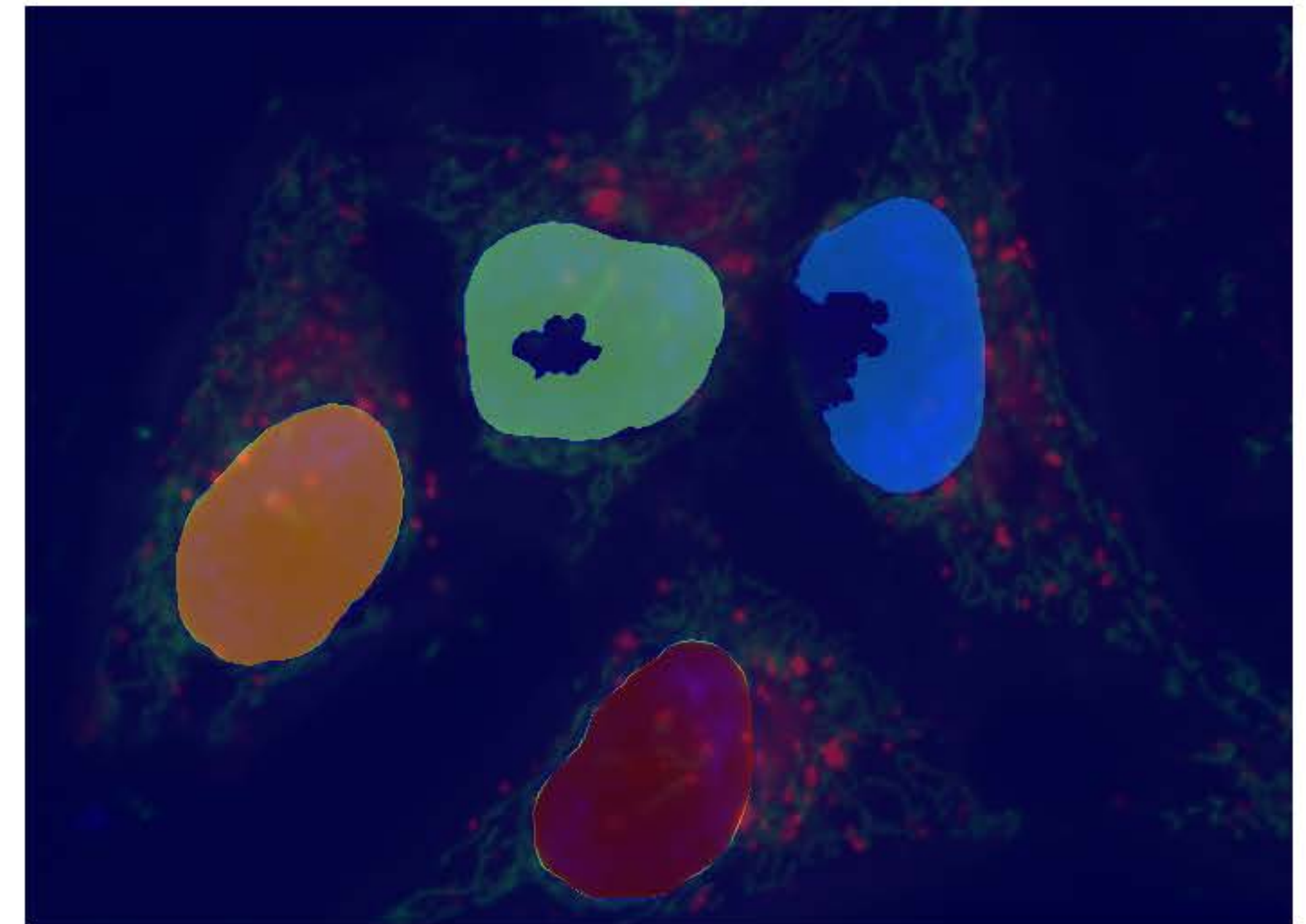
ChatGPT



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.

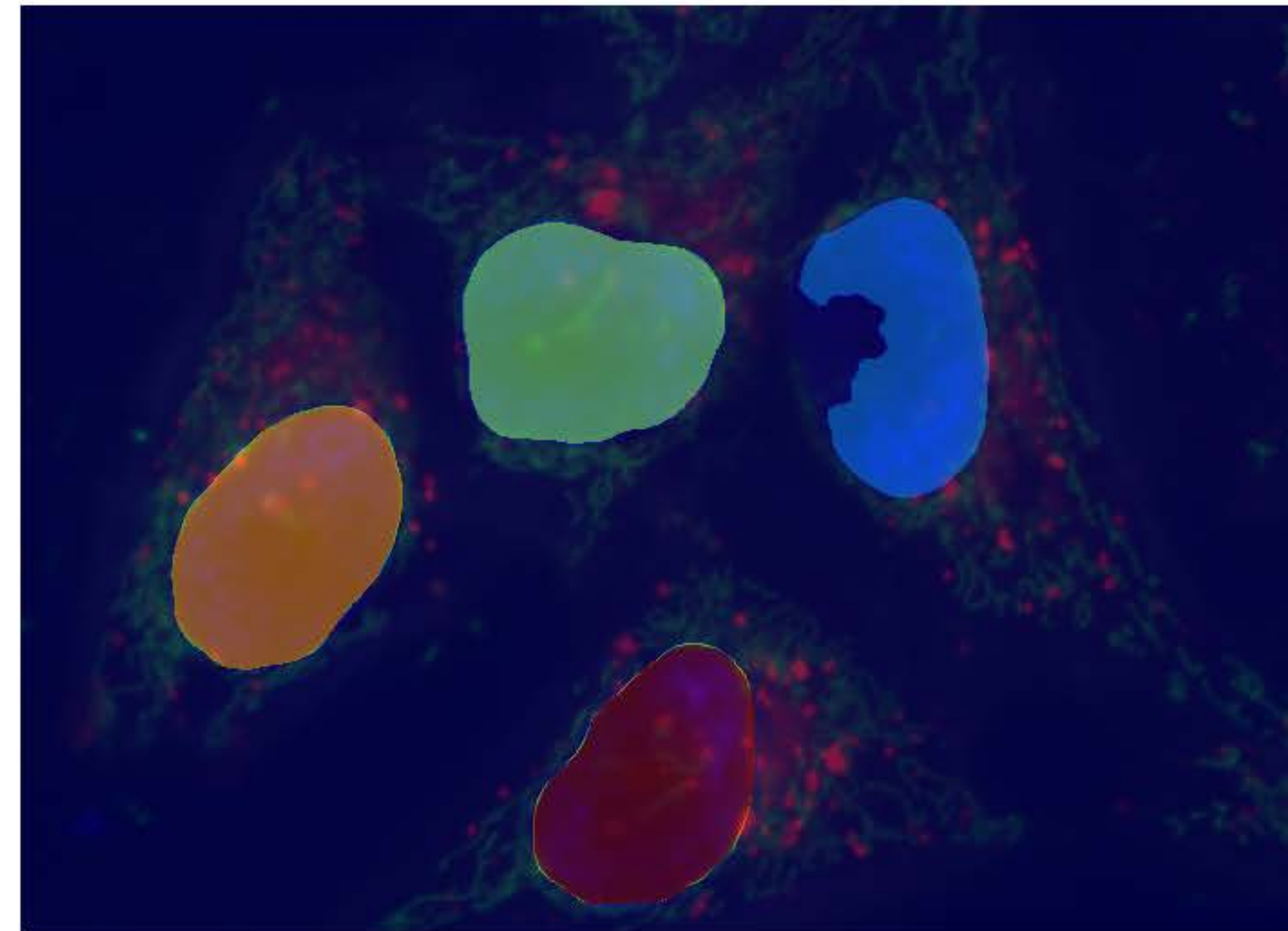
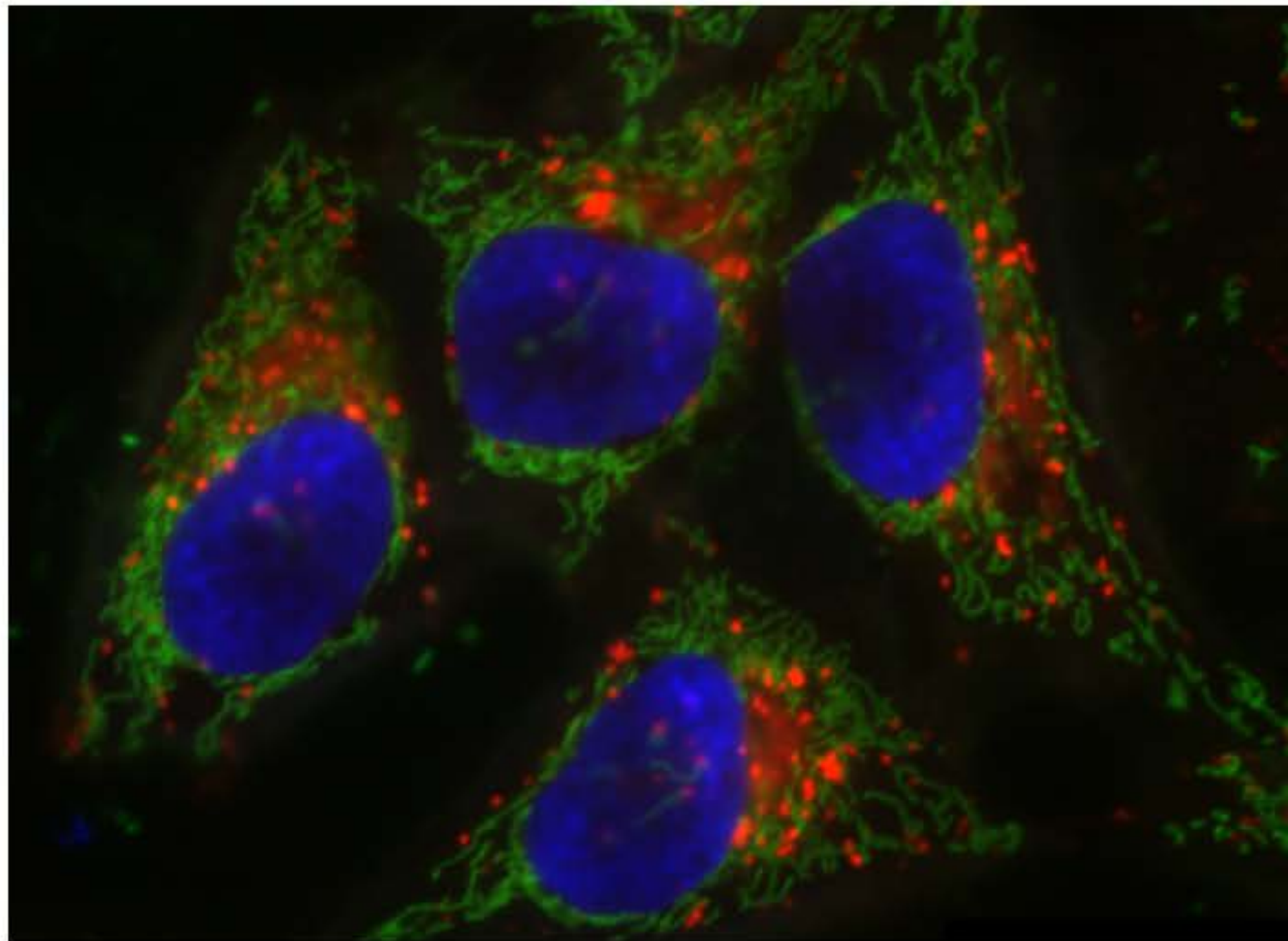
Segmented Nuclei (Blue Channel)



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

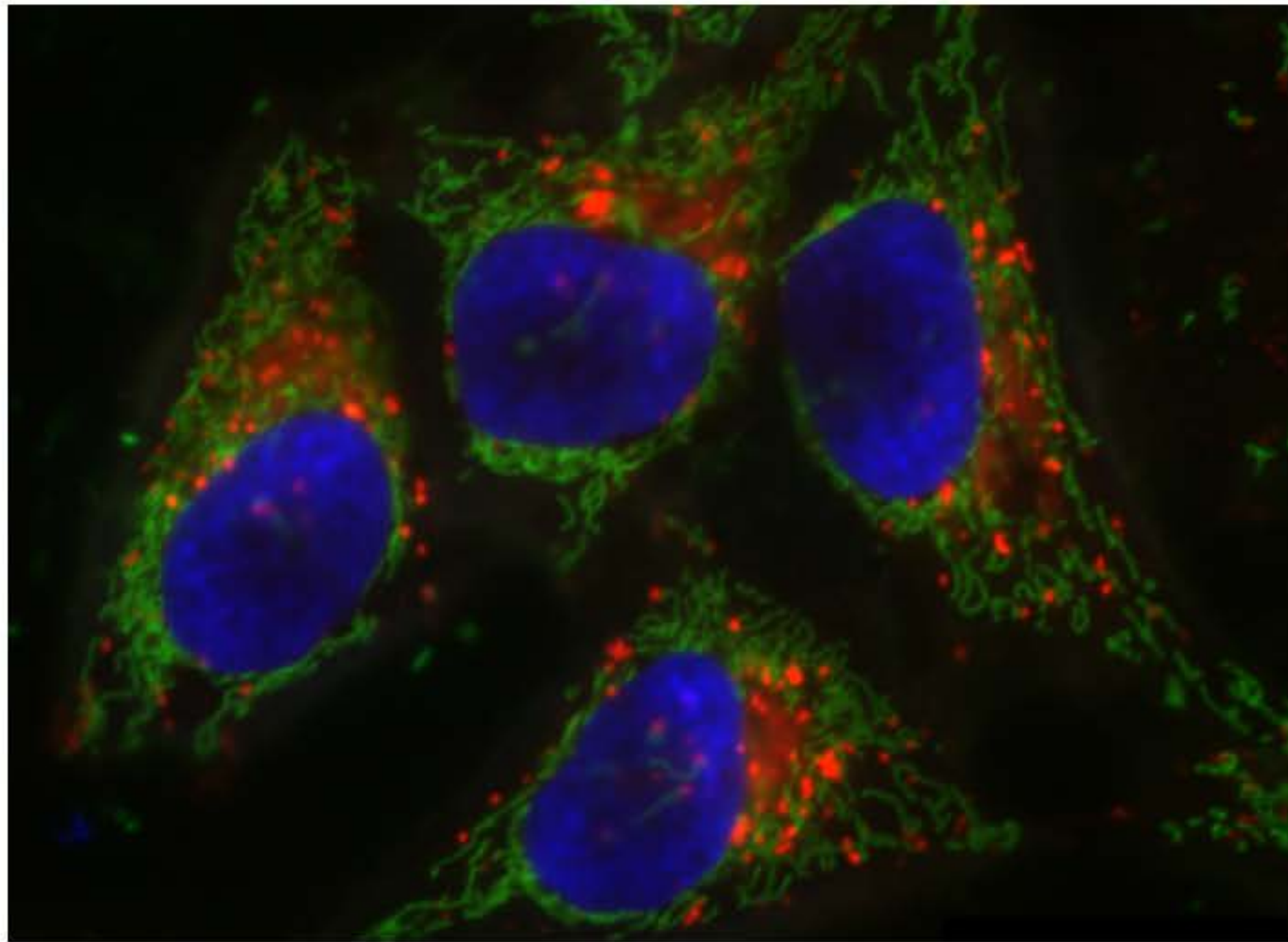


Using ChatGPT-4o for Image Analysis

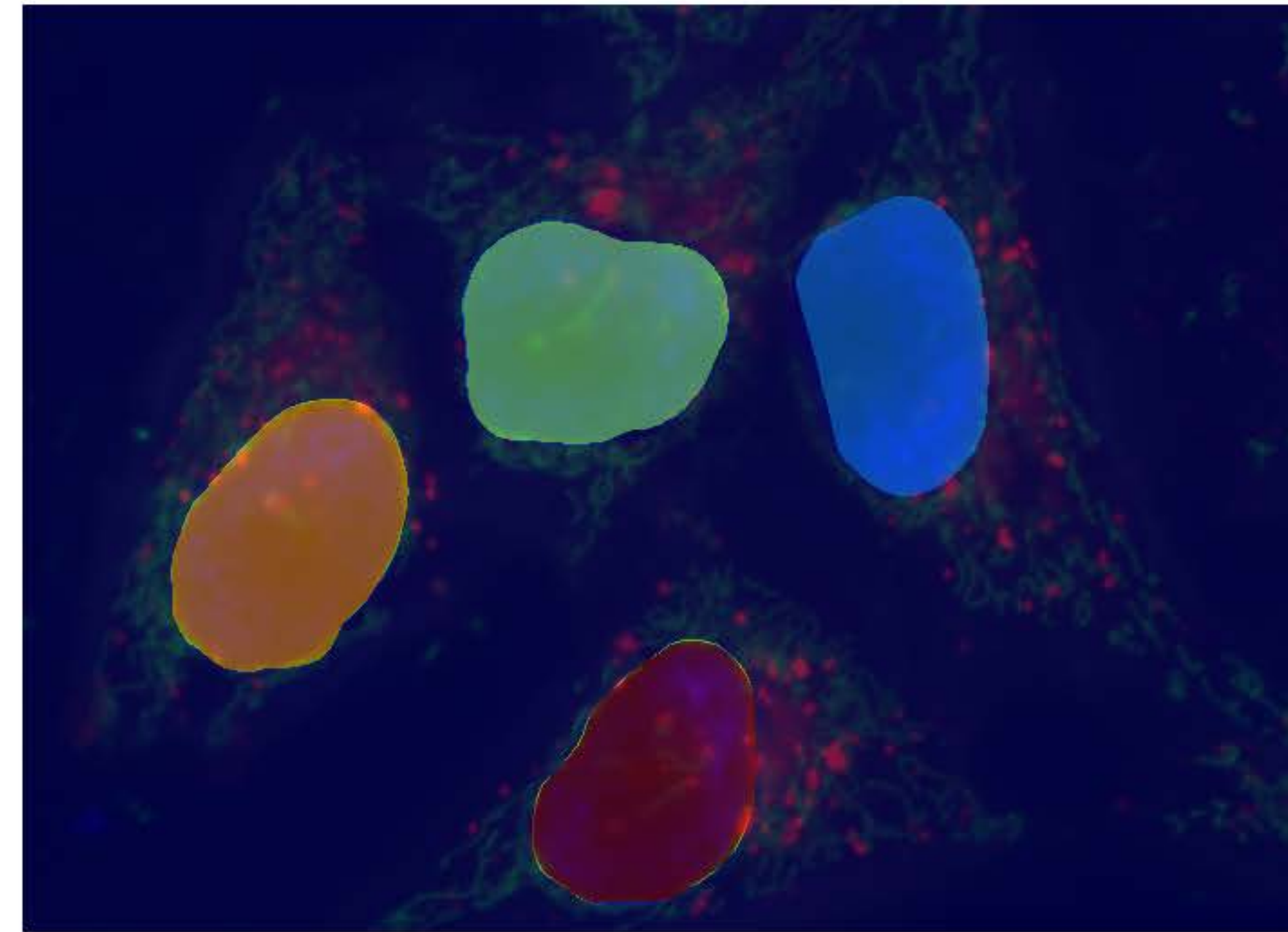
GPT-4o, Oct. 2024 - it helps to know what to ask

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

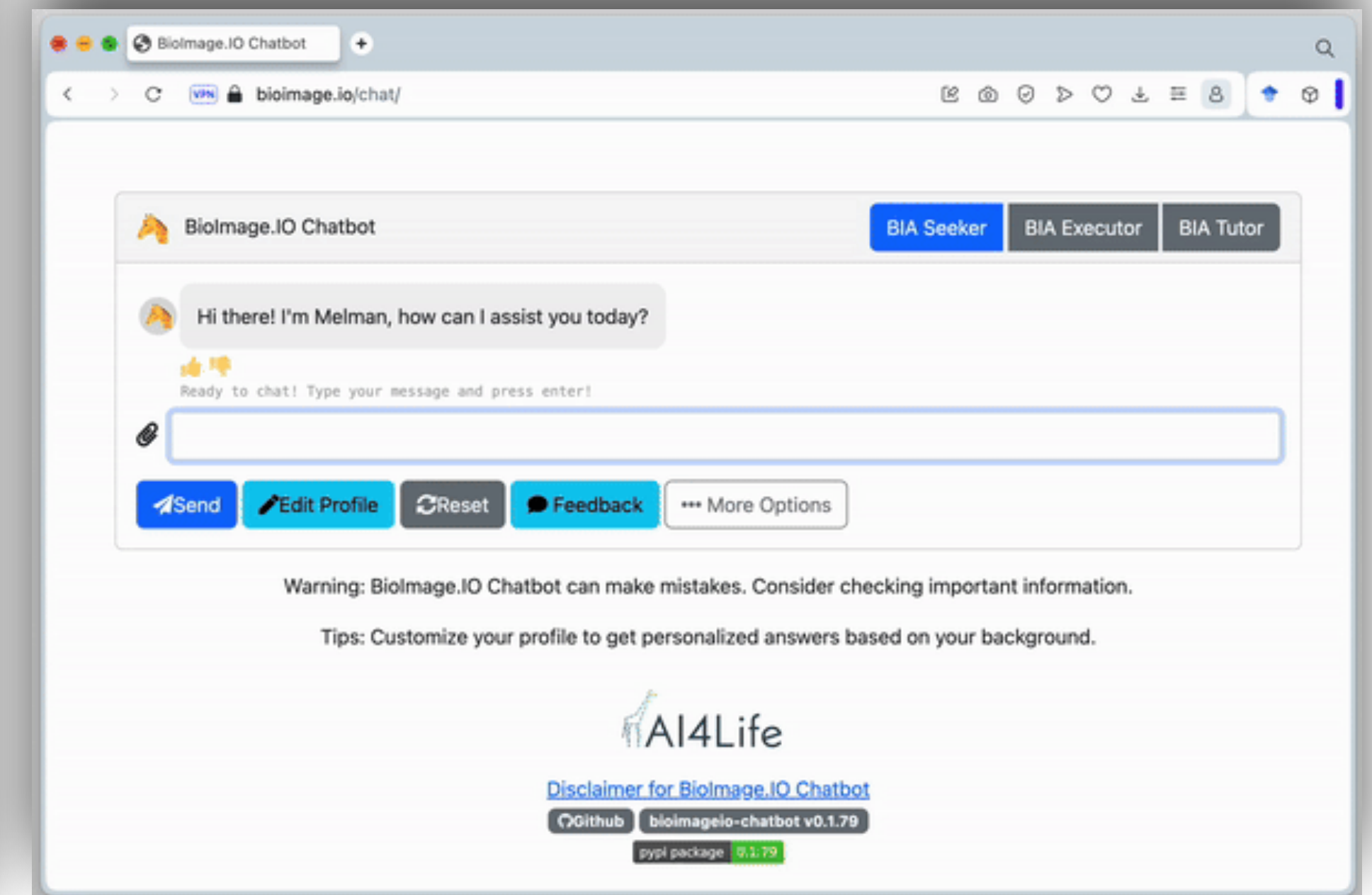
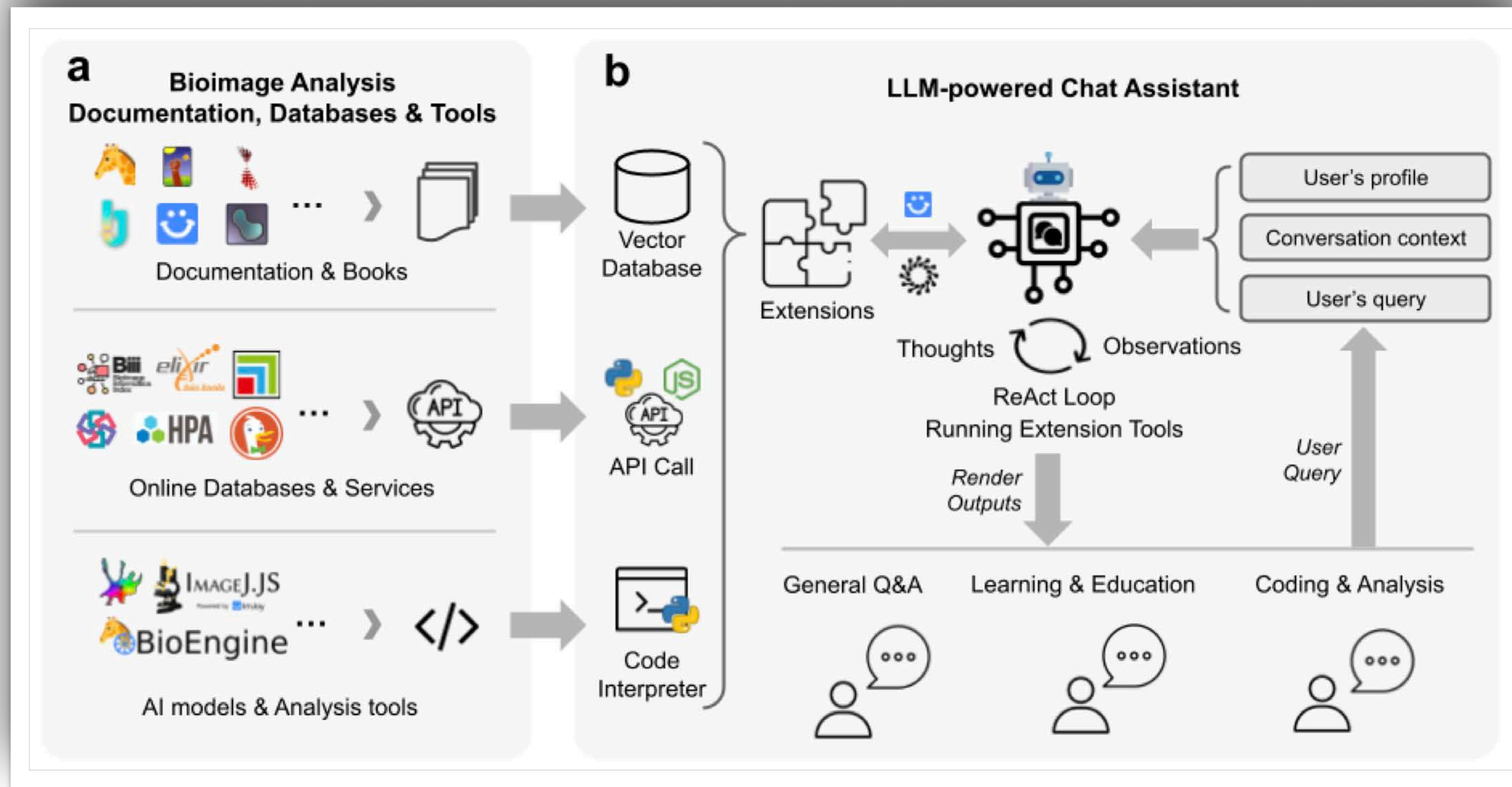
Original Image



Convex Hull for Low Circularity Nuclei



BioImage Chatbot



napari-omega



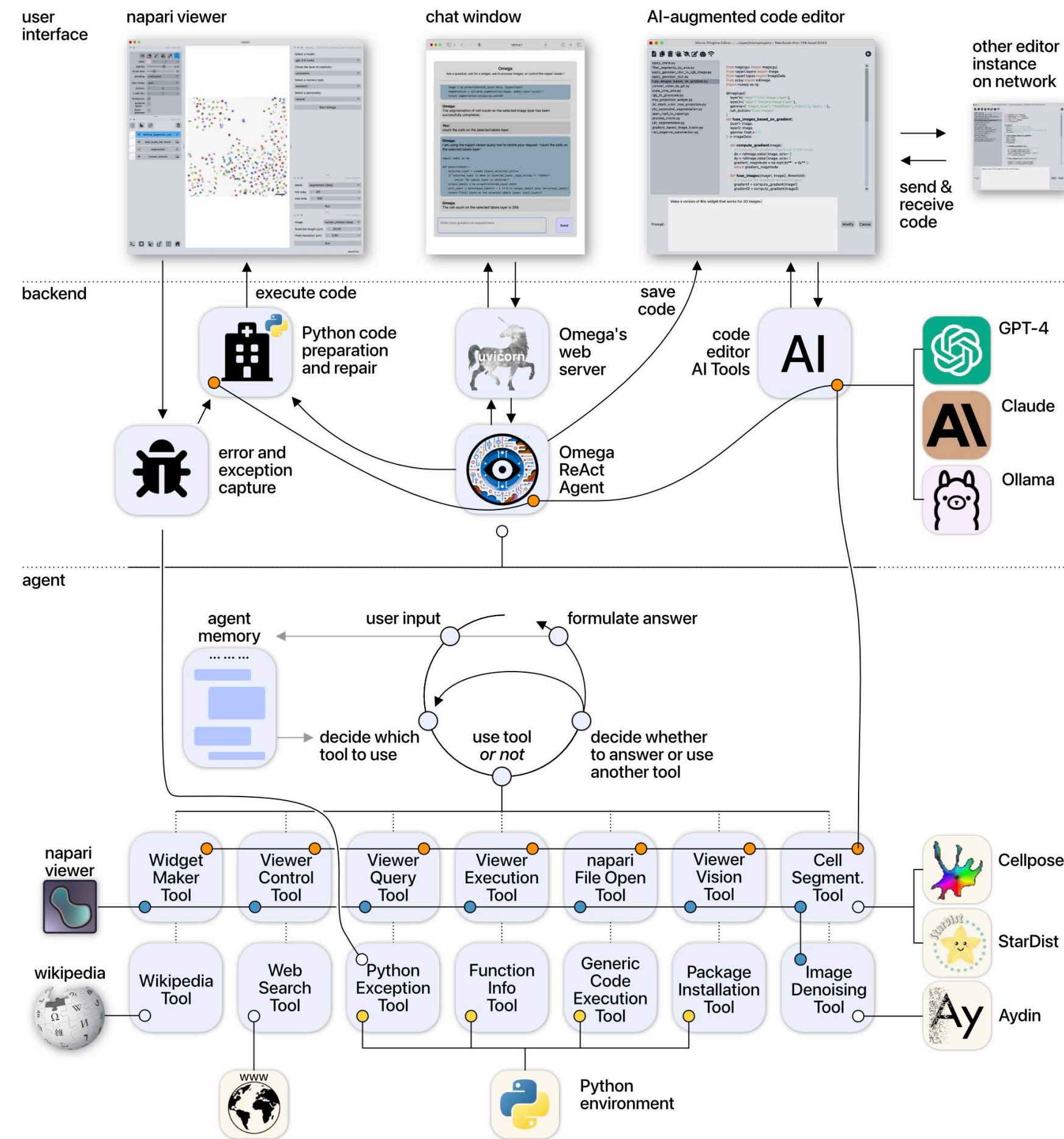
Omega – Harnessing for Bioimage Analysis

Loïc A. Royer^{1, *}

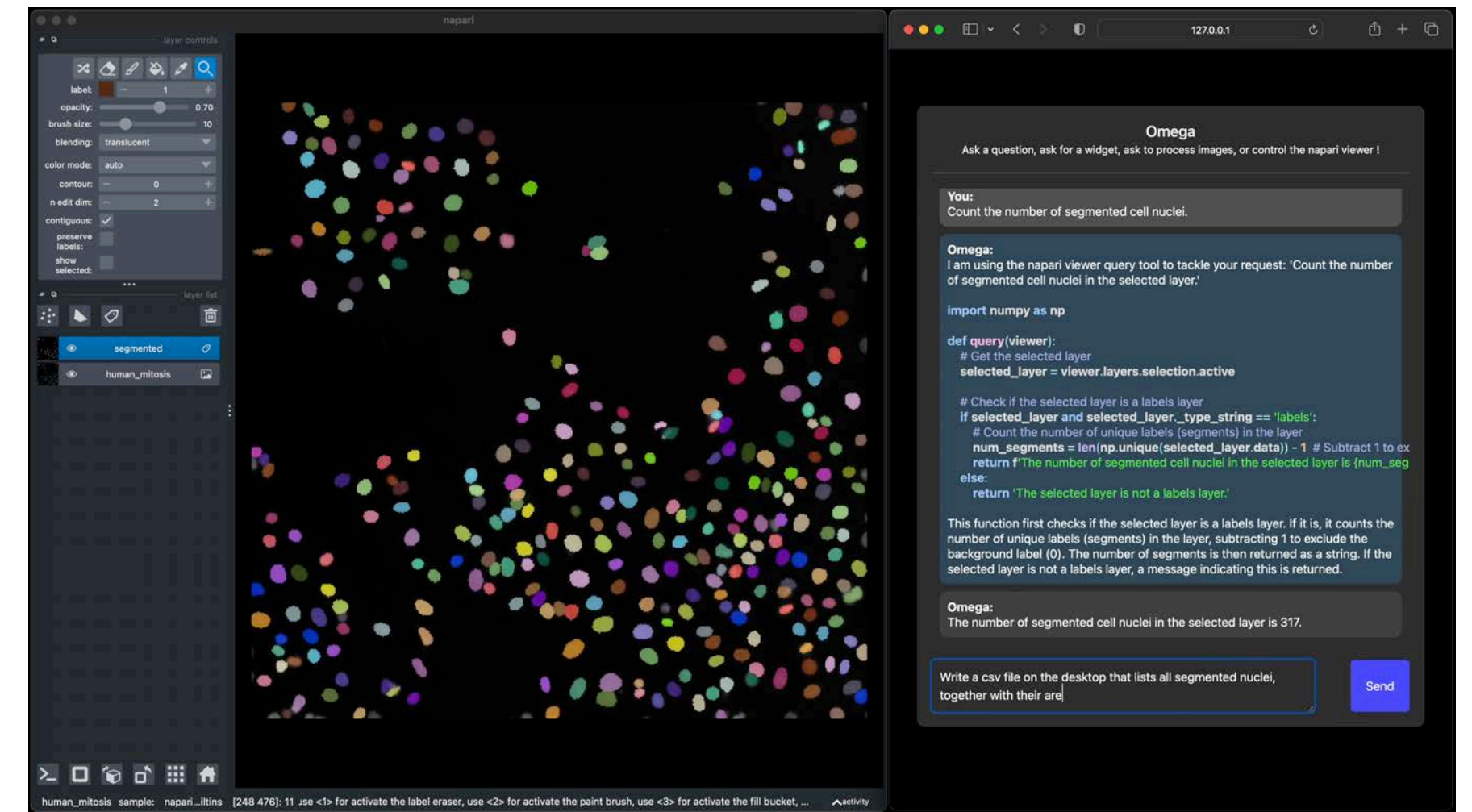
¹Chan Zuckerberg Biohub, San Francisco, USA.

*Correspondence: loic.royer@czbiohub.org

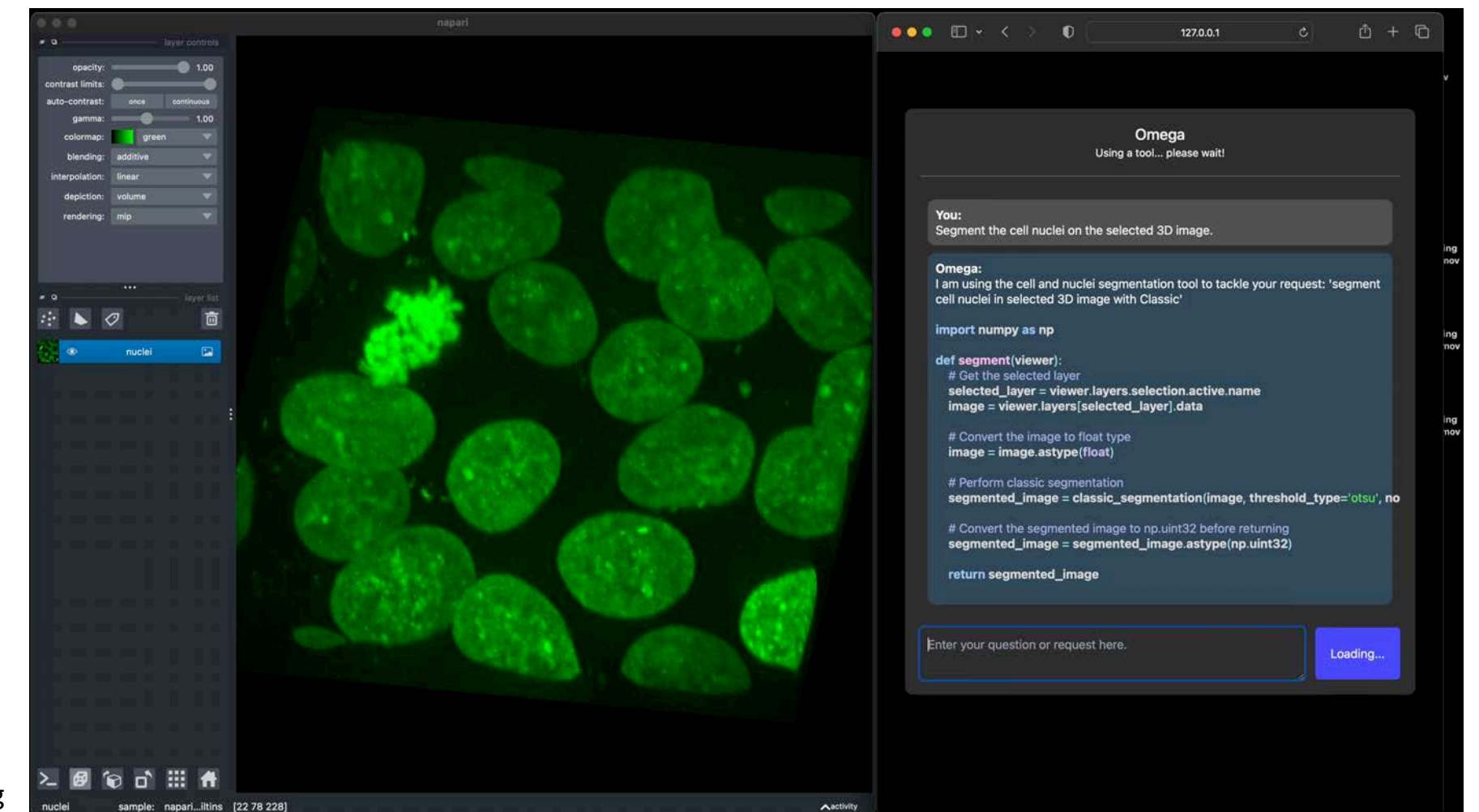
[10.5281/zenodo.10828225](https://doi.org/10.5281/zenodo.10828225)



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  UndoRedo

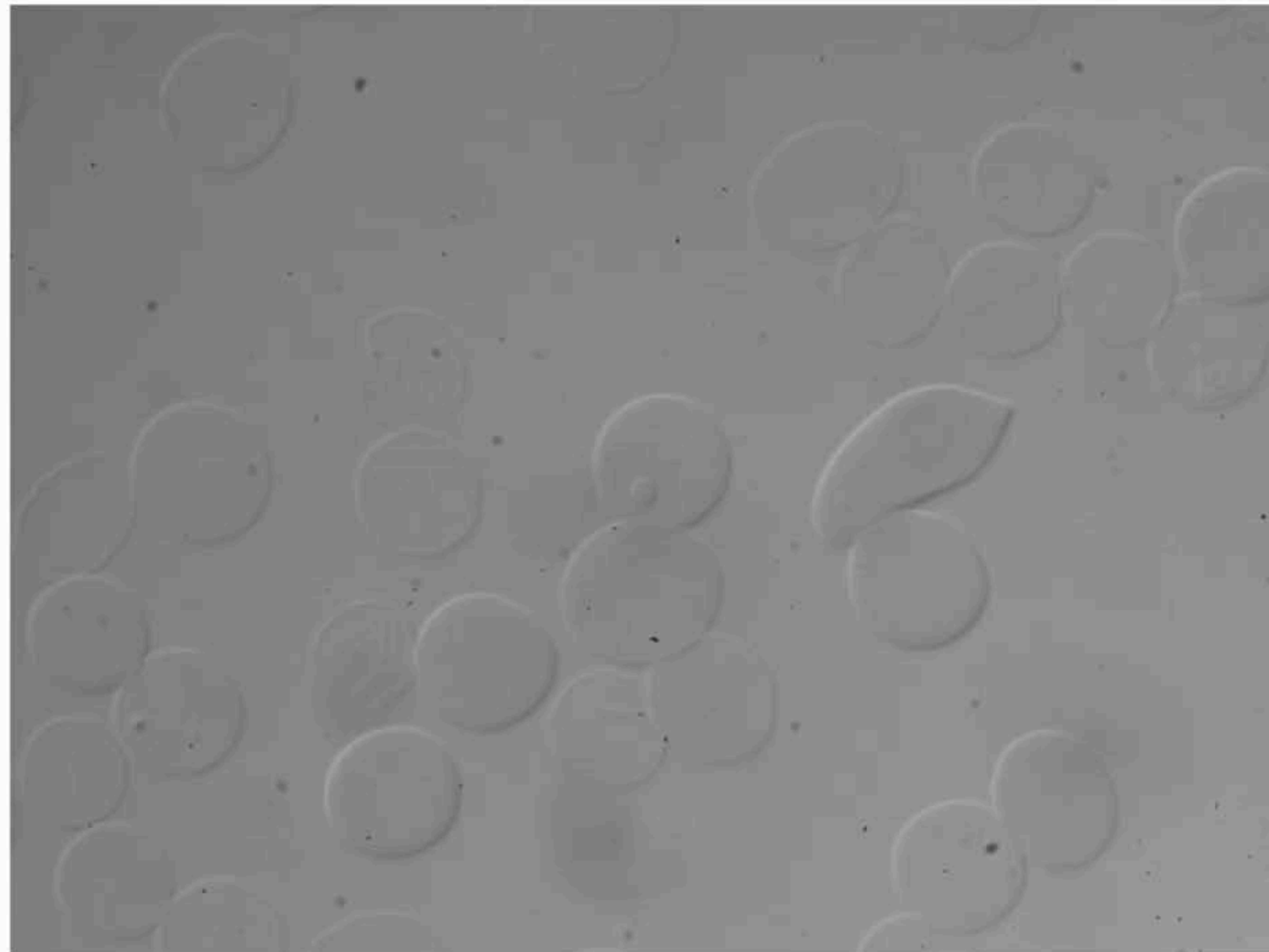
 Multi-mask

 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



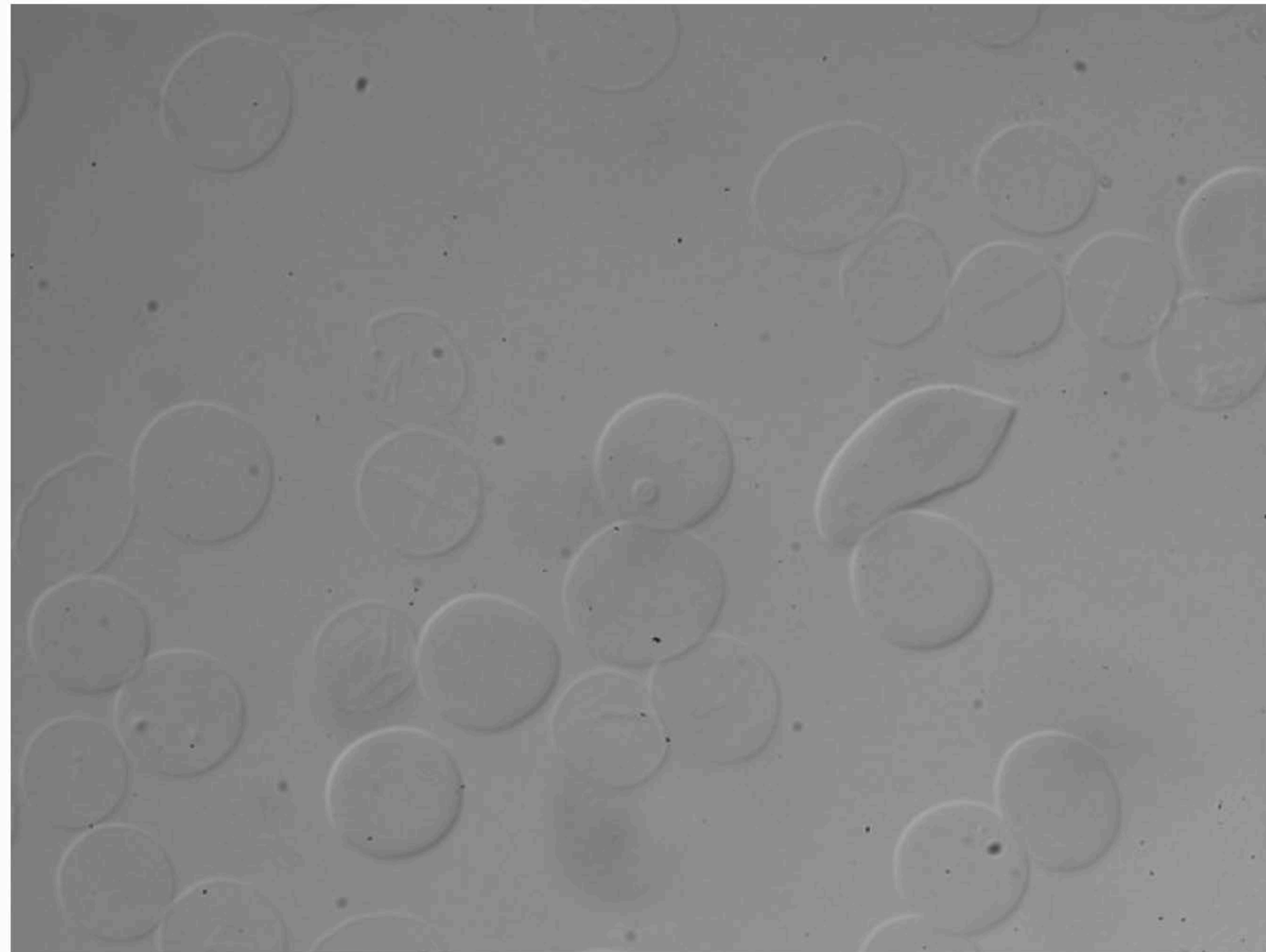
Box



Everything

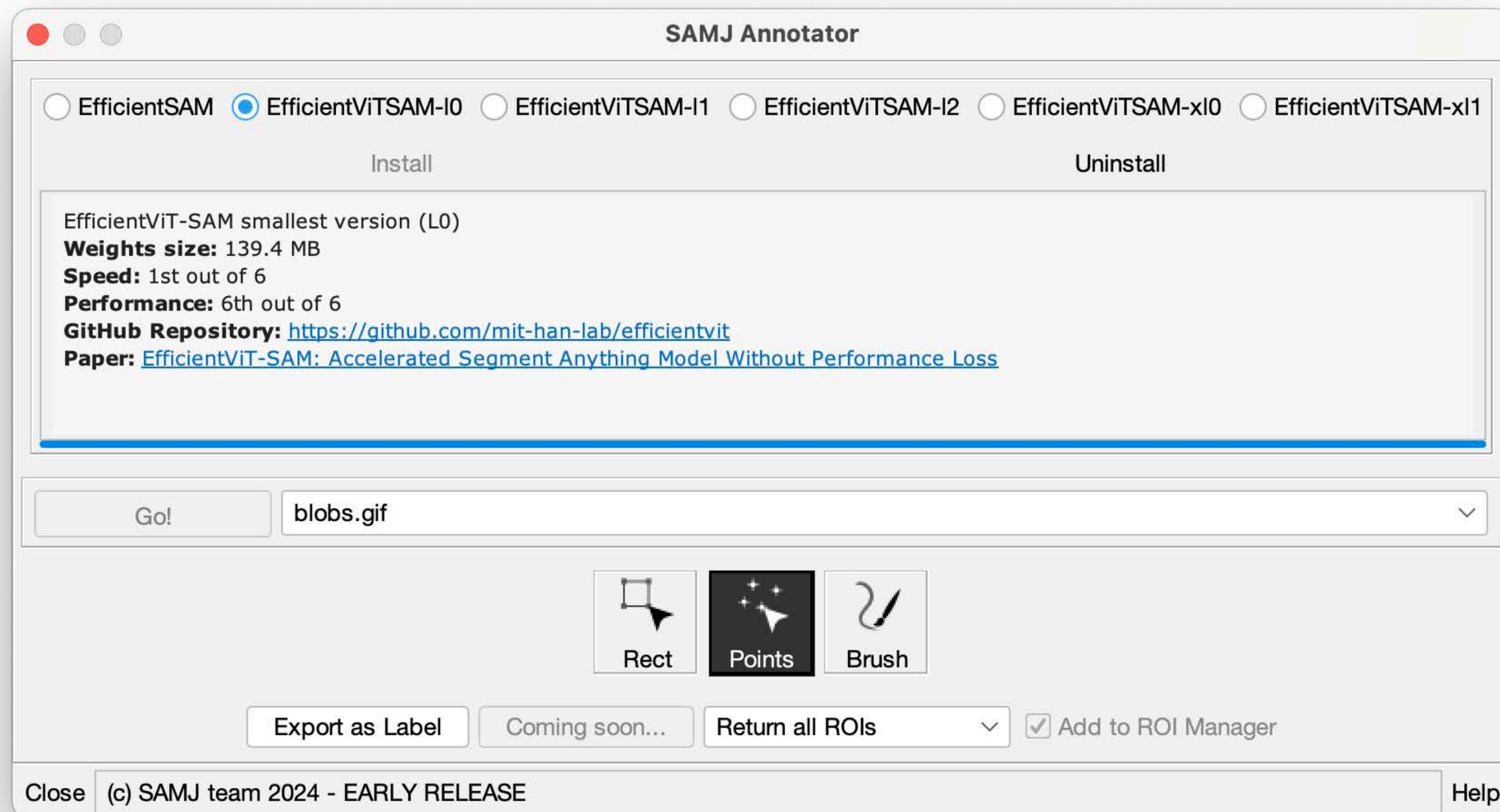


Cut-Outs



Segment Anything in ImageJ/Fiji

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



Example of point annotations

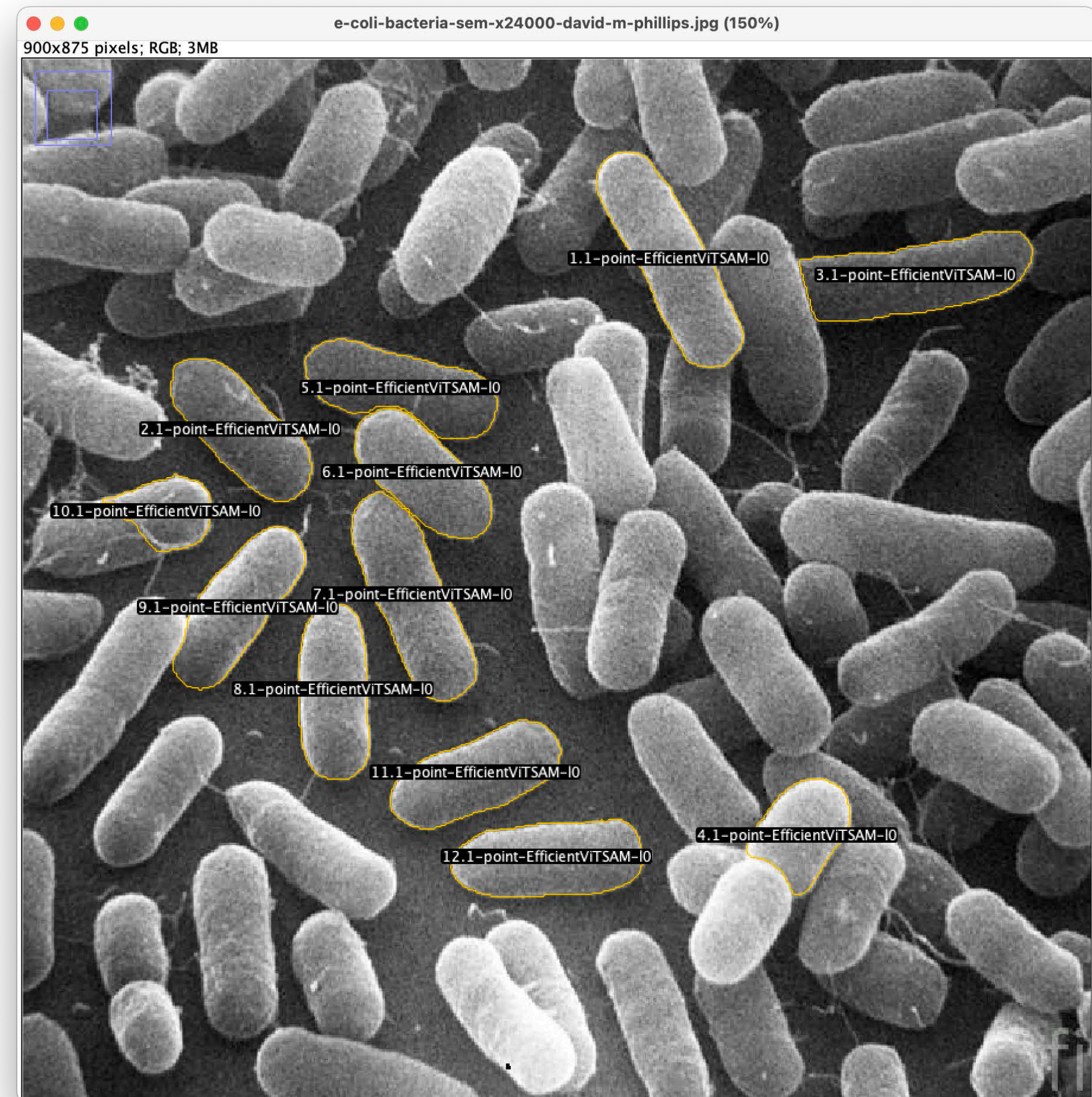


Image Analysis Workflow

- 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

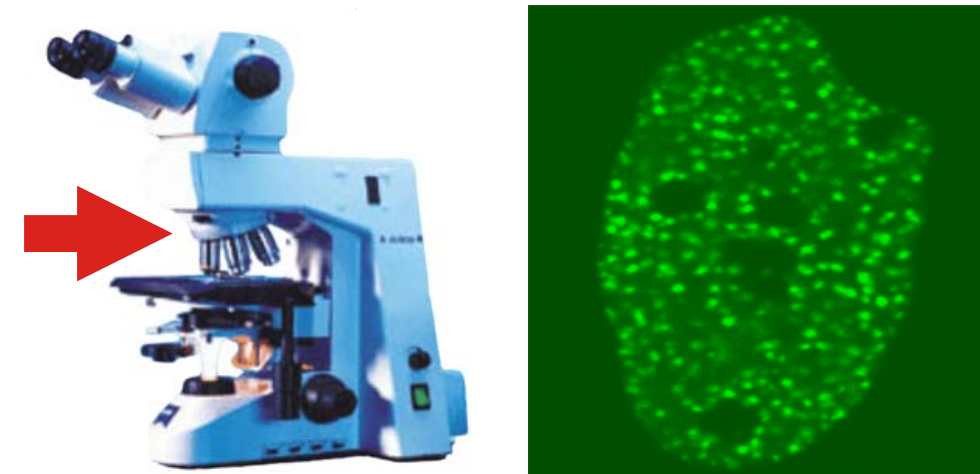


Image Processing

image in → image out

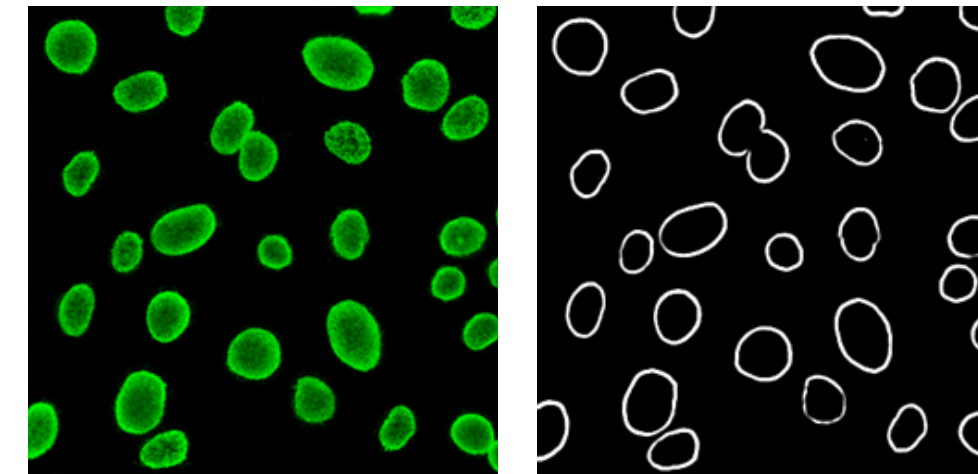
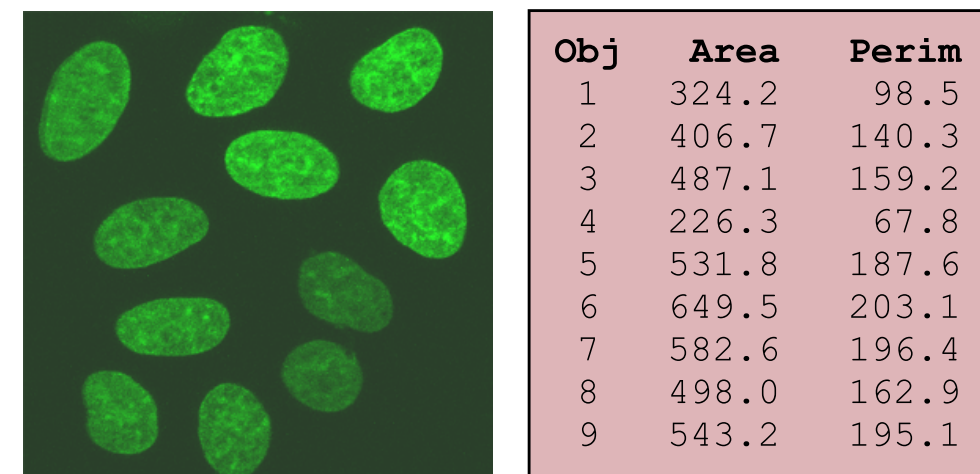


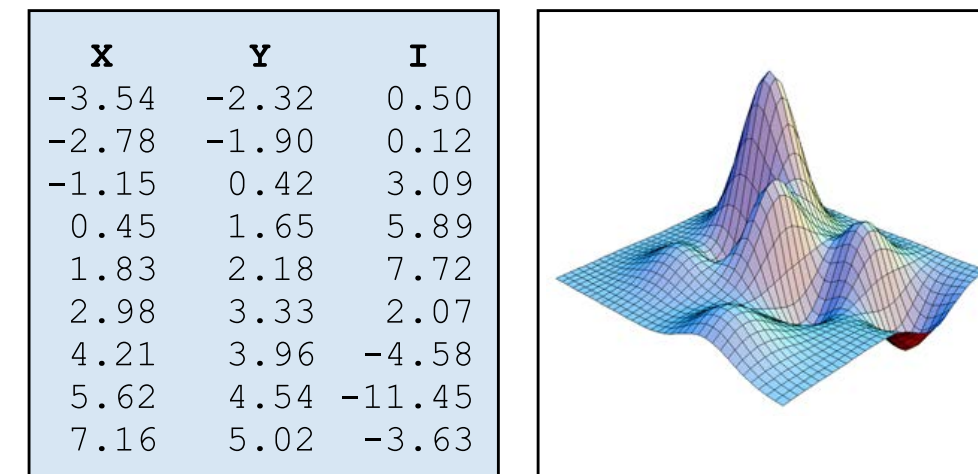
Image Analysis

image in → features out



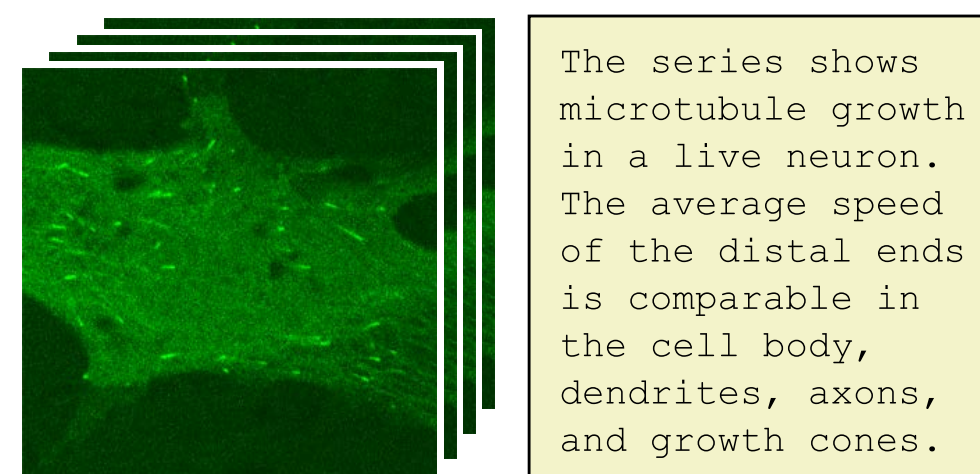
Computer Graphics

numbers in → image out



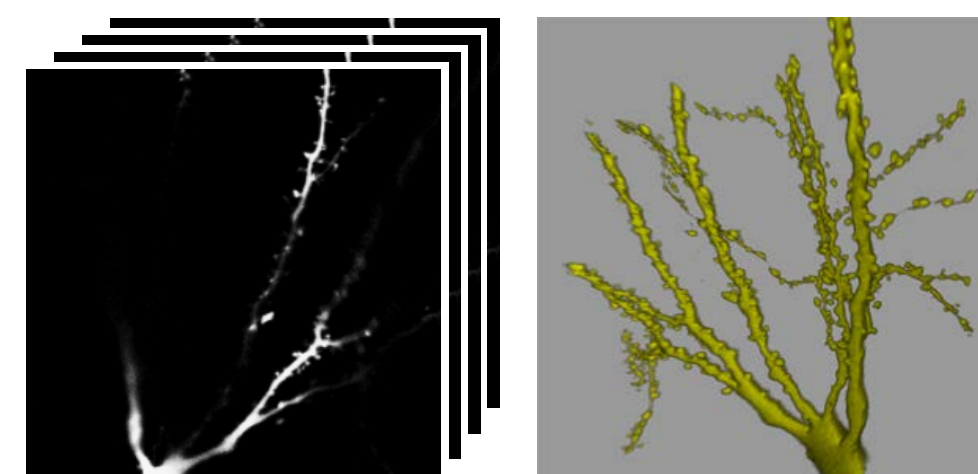
Computer Vision

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/>