

Using Cellpose and StarDist in CellProfiler

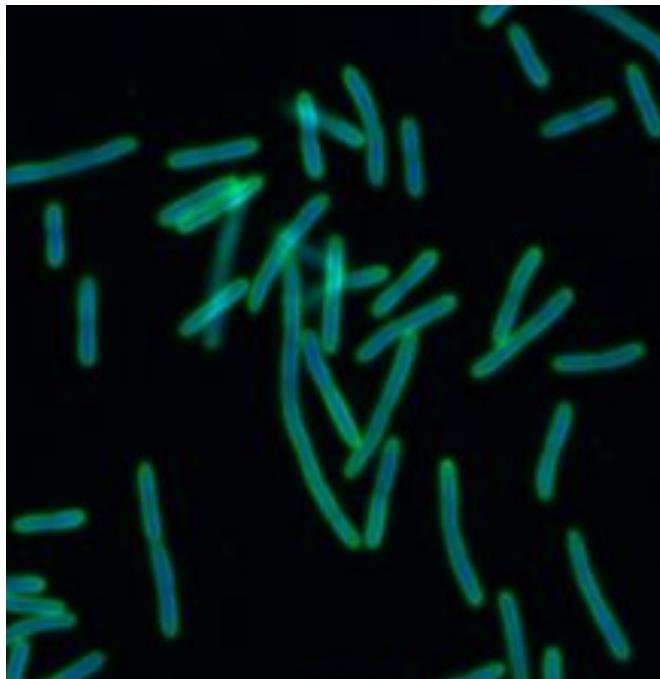
Rebecca Senft and Barbara Diaz-Rohrer

Broad Institute of MIT

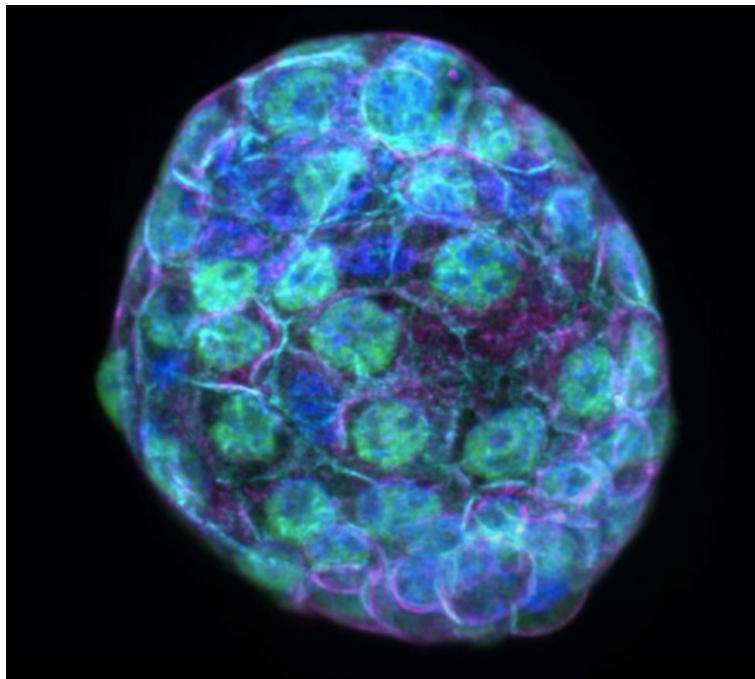
Outline

- When is classical segmentation not enough?
 - Overview of stardist and cellpose
 - Demo datasets:
 - 3D blastoids
 - 2D bacteria
 - 2D neurons in tissue
 - Considerations for using the RunStarDist and RunCellpose CellProfiler plugins
 - How to train your own model
 - Resources for further learning
- Download materials at:
<http://broad.io/CPSDtutorial>

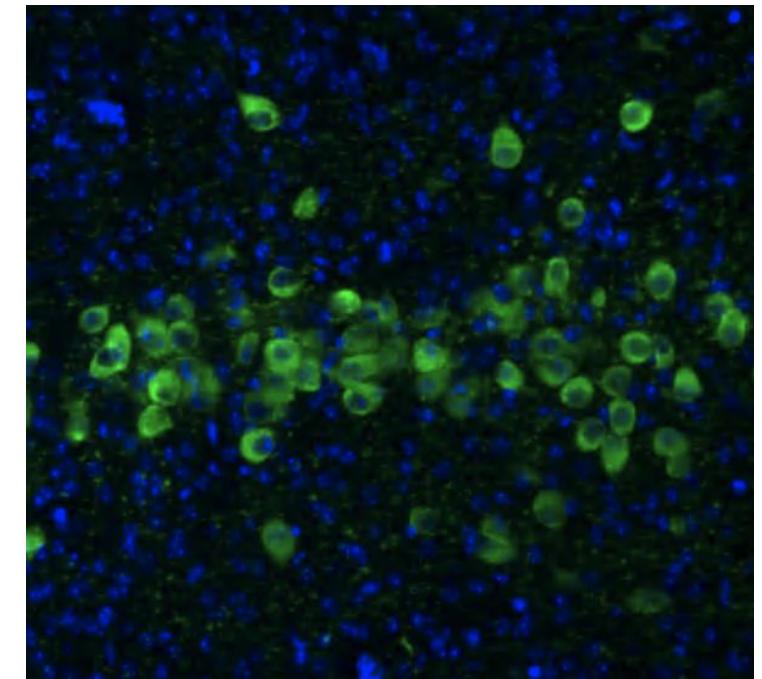
Some images are difficult to segment



- Irregular-shaped objects
- Non-round objects

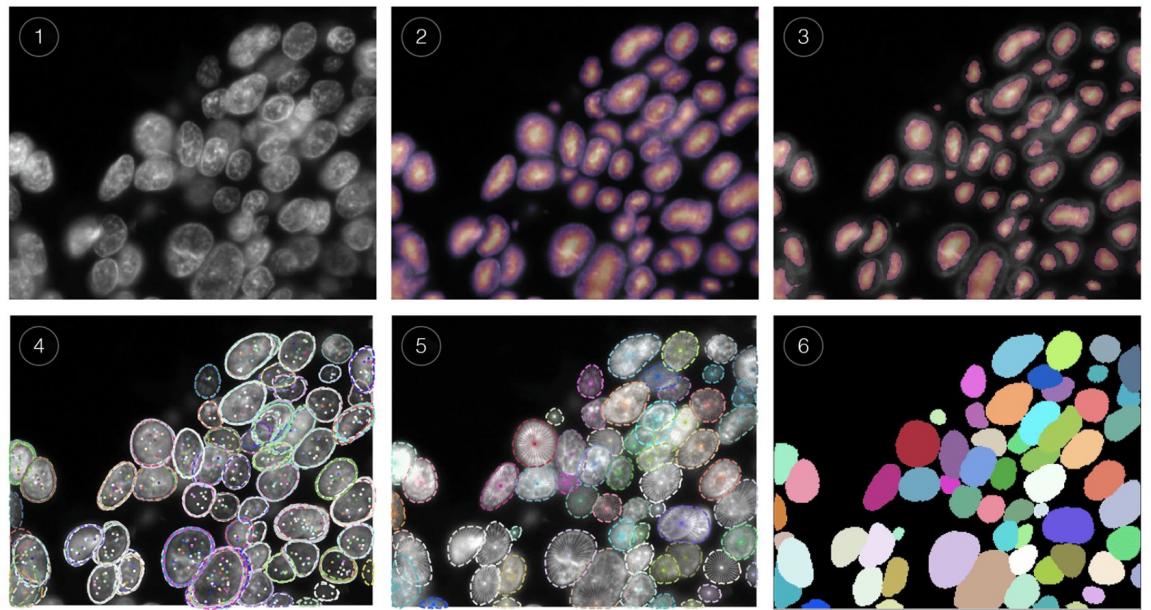
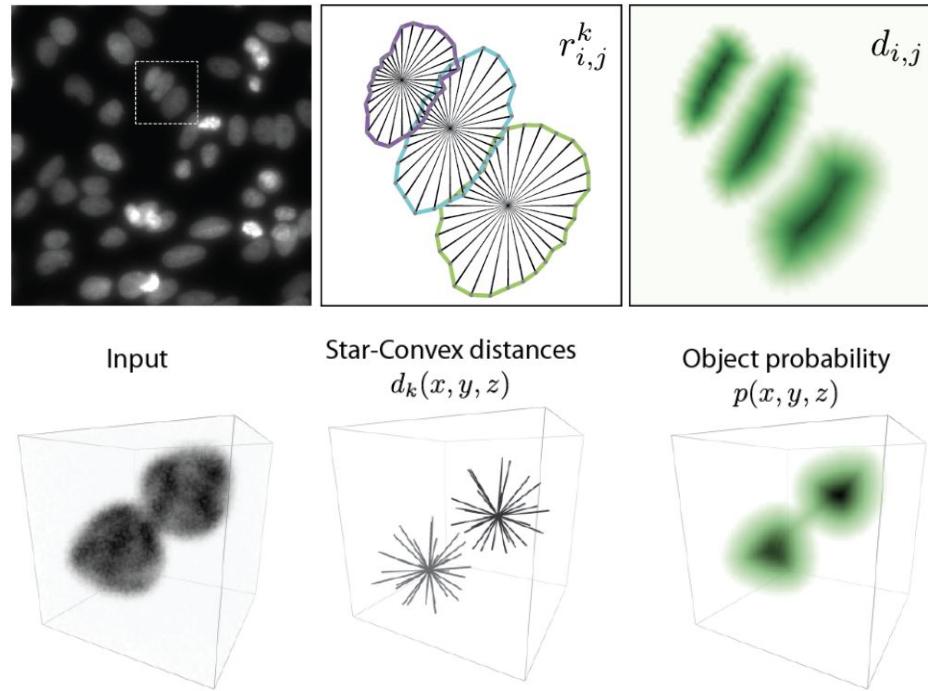


- 3D images
- Clustered cells



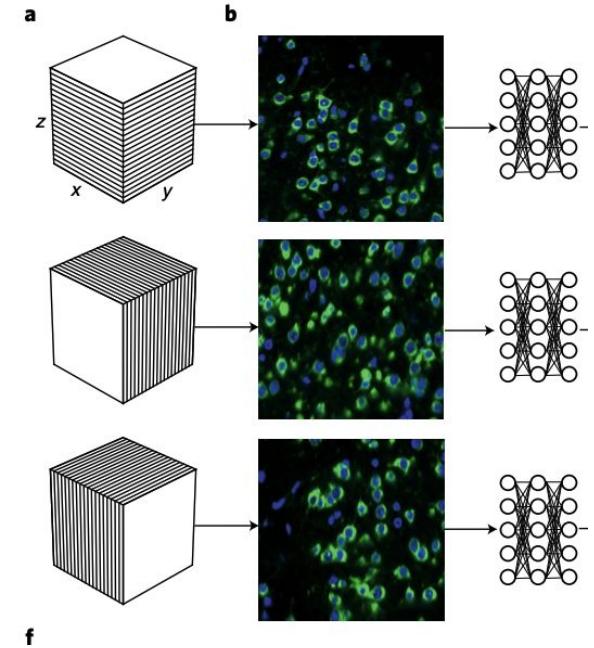
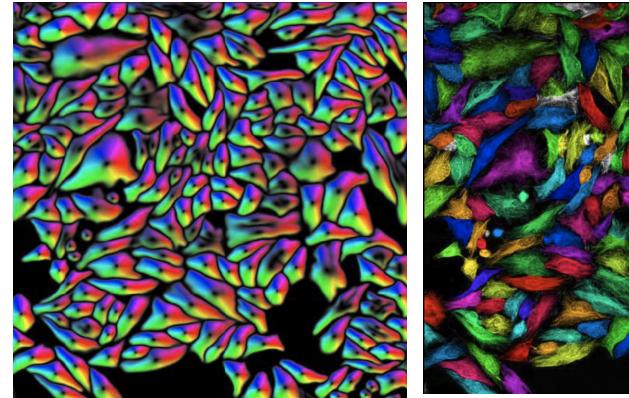
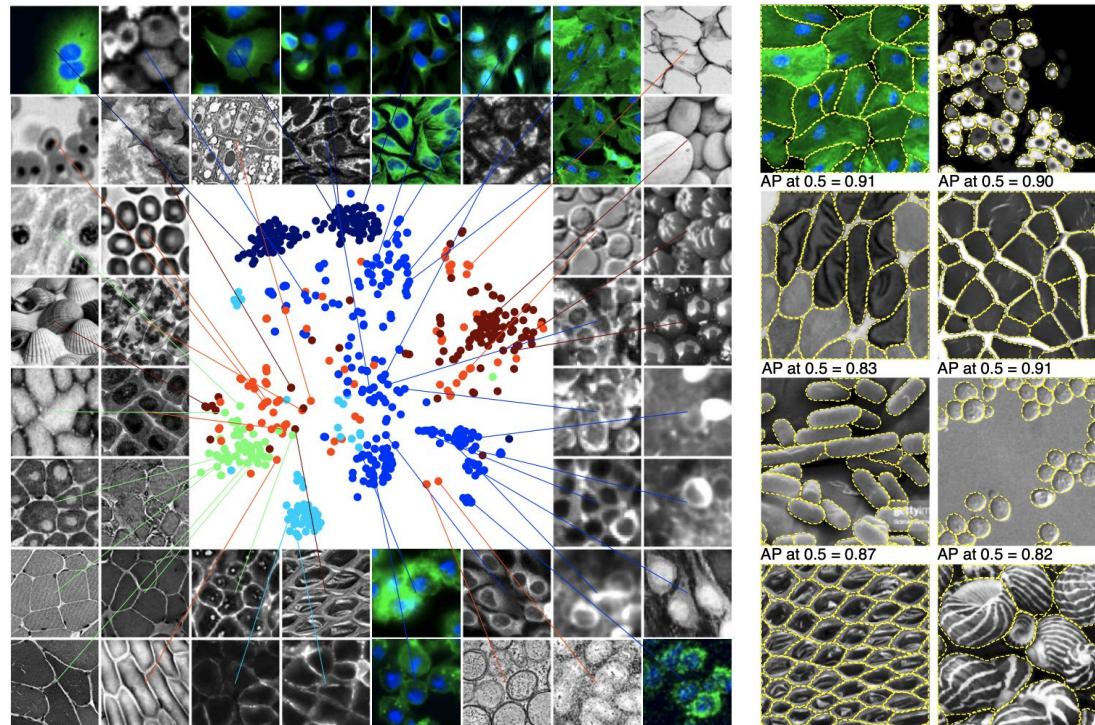
- Donut-shaped cells
- Heterogeneous brightness and background

StarDist - Object Detection with Star-convex Shapes



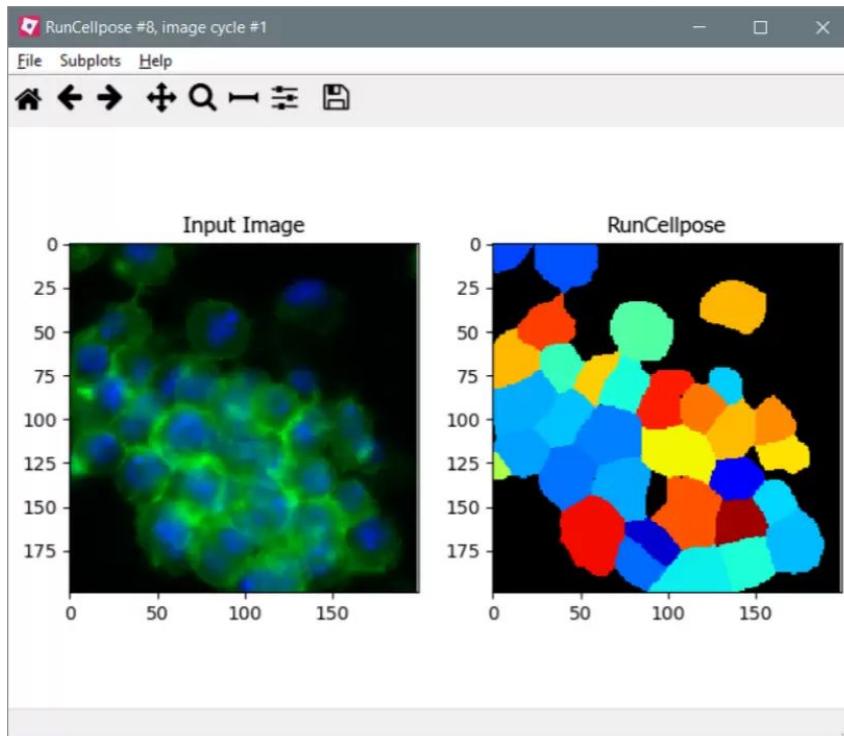
Cellpose - a generalist algorithm for cellular segmentation

Cellpose was built from training with >600 varied 2D images of cells and altogether >70,000 objects



RunCellpose and RunStarDist plugins bring these tools to CellProfiler

RunCellpose

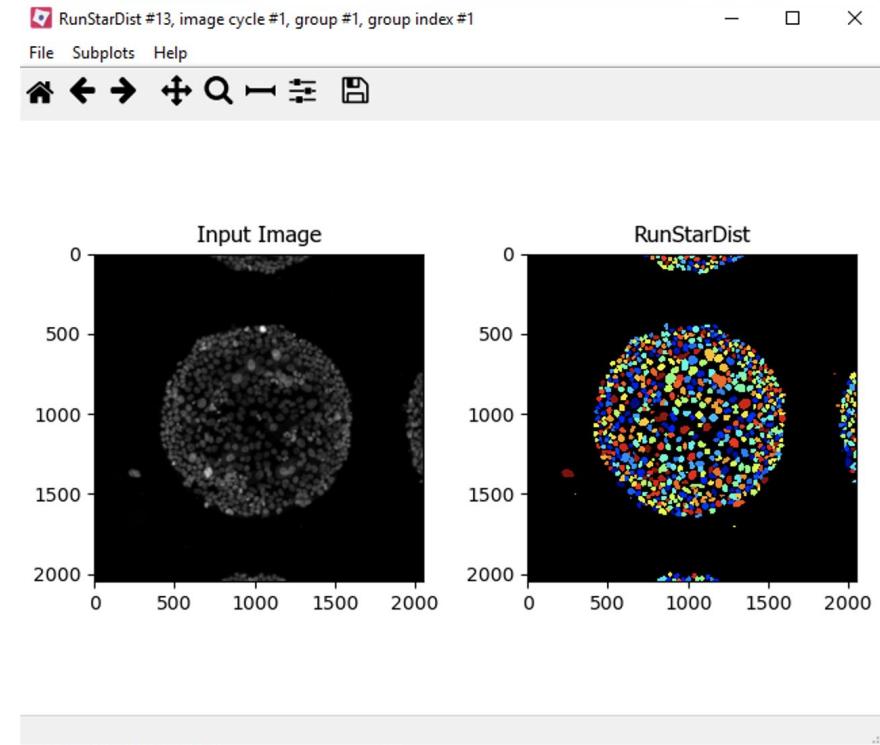


Beth Cimini



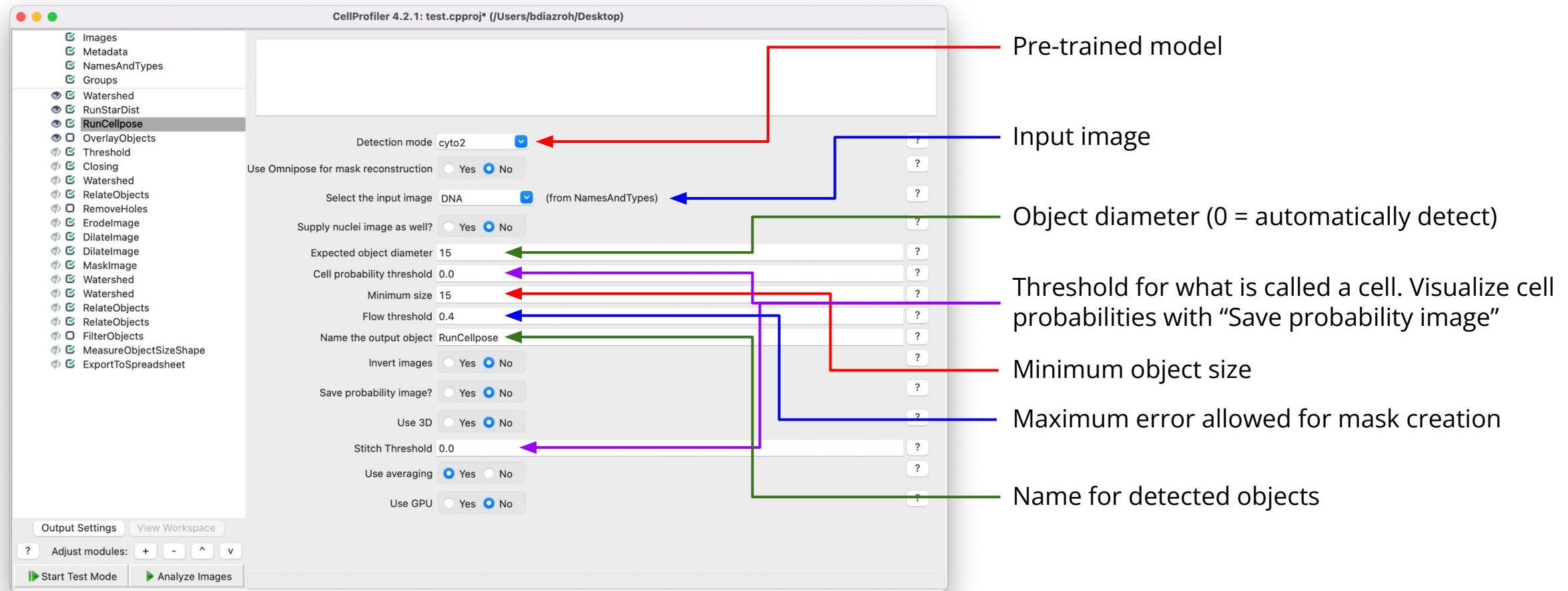
David Stirling

RunStarDist

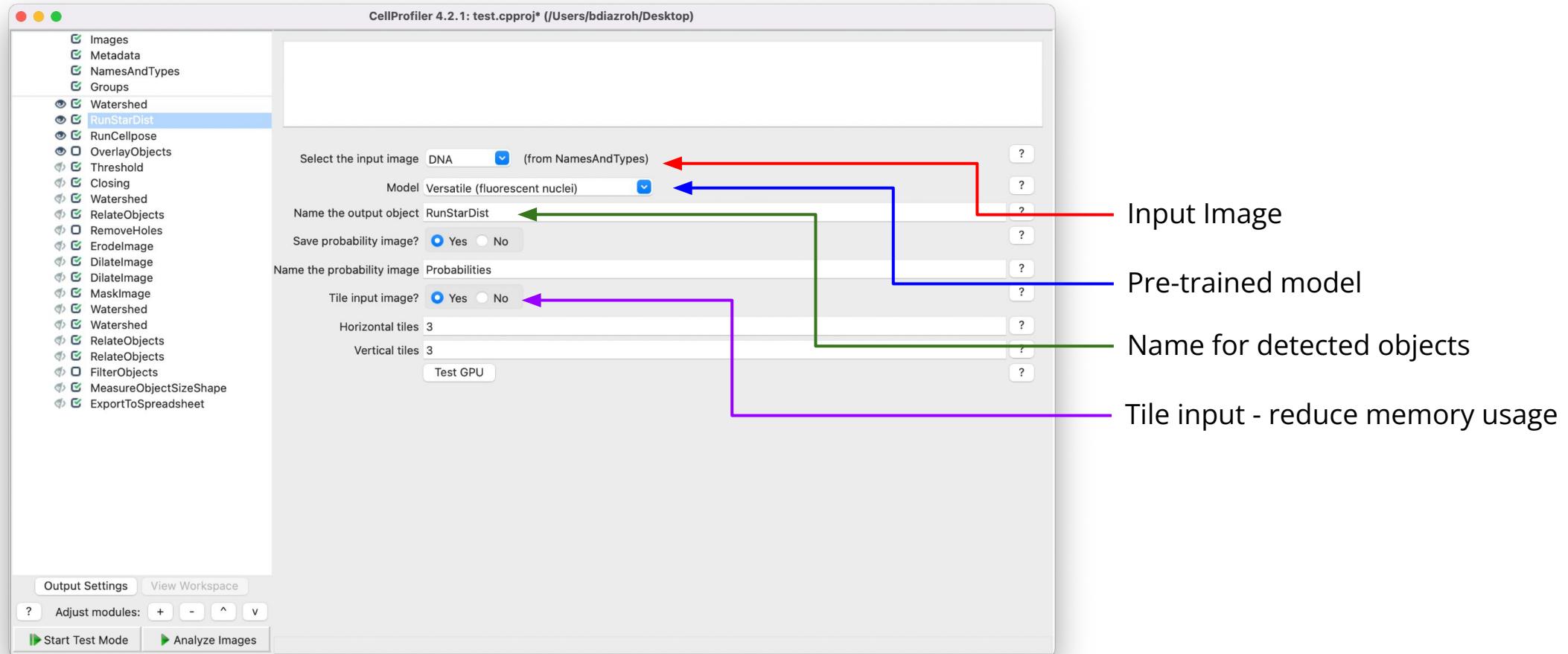


Plugins and installation instructions available at github.com/CellProfiler/CellProfiler-plugins

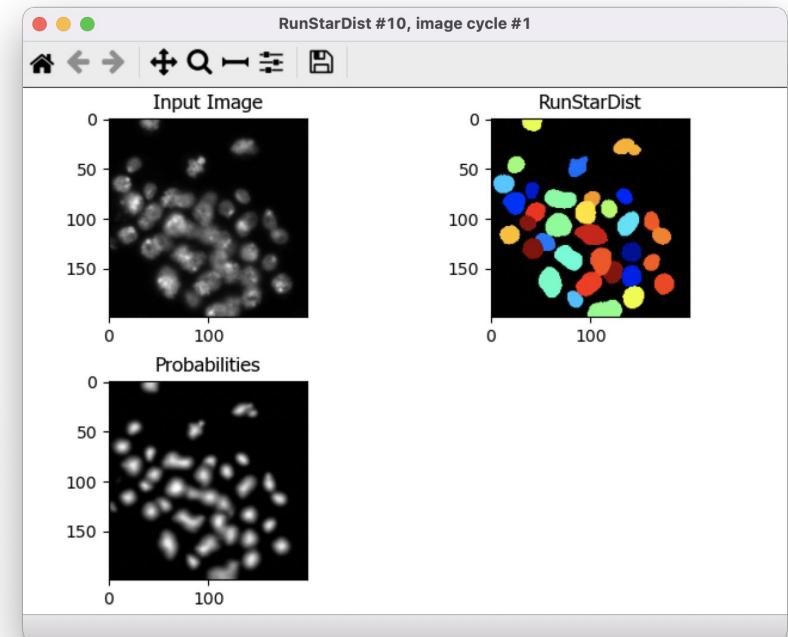
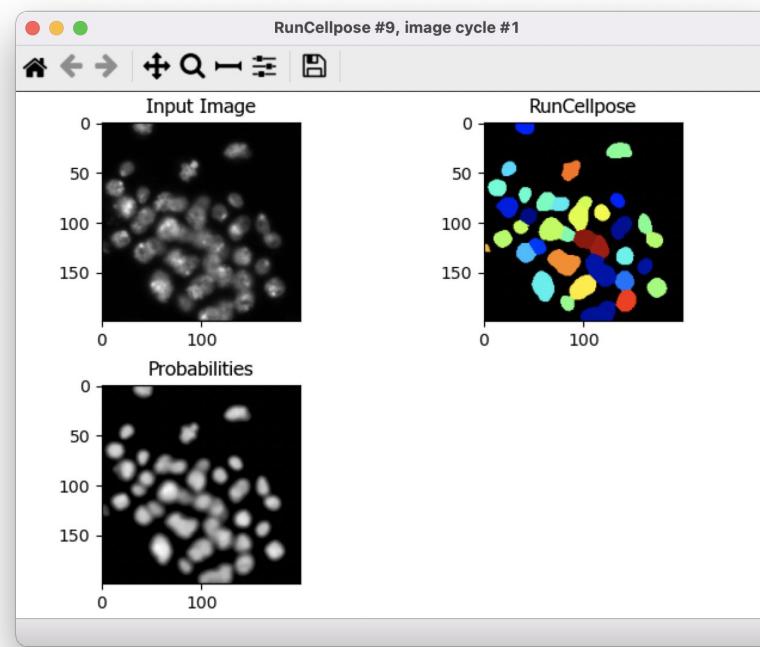
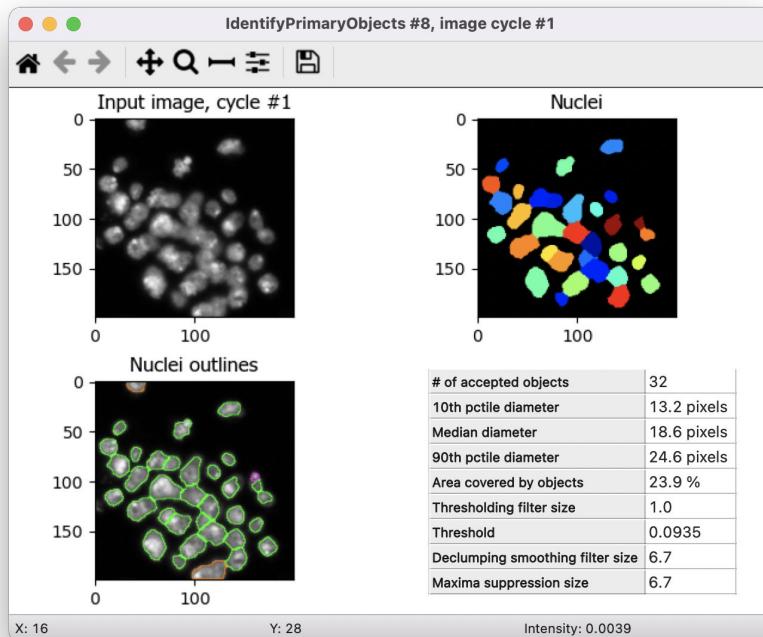
How to use RunCellpose



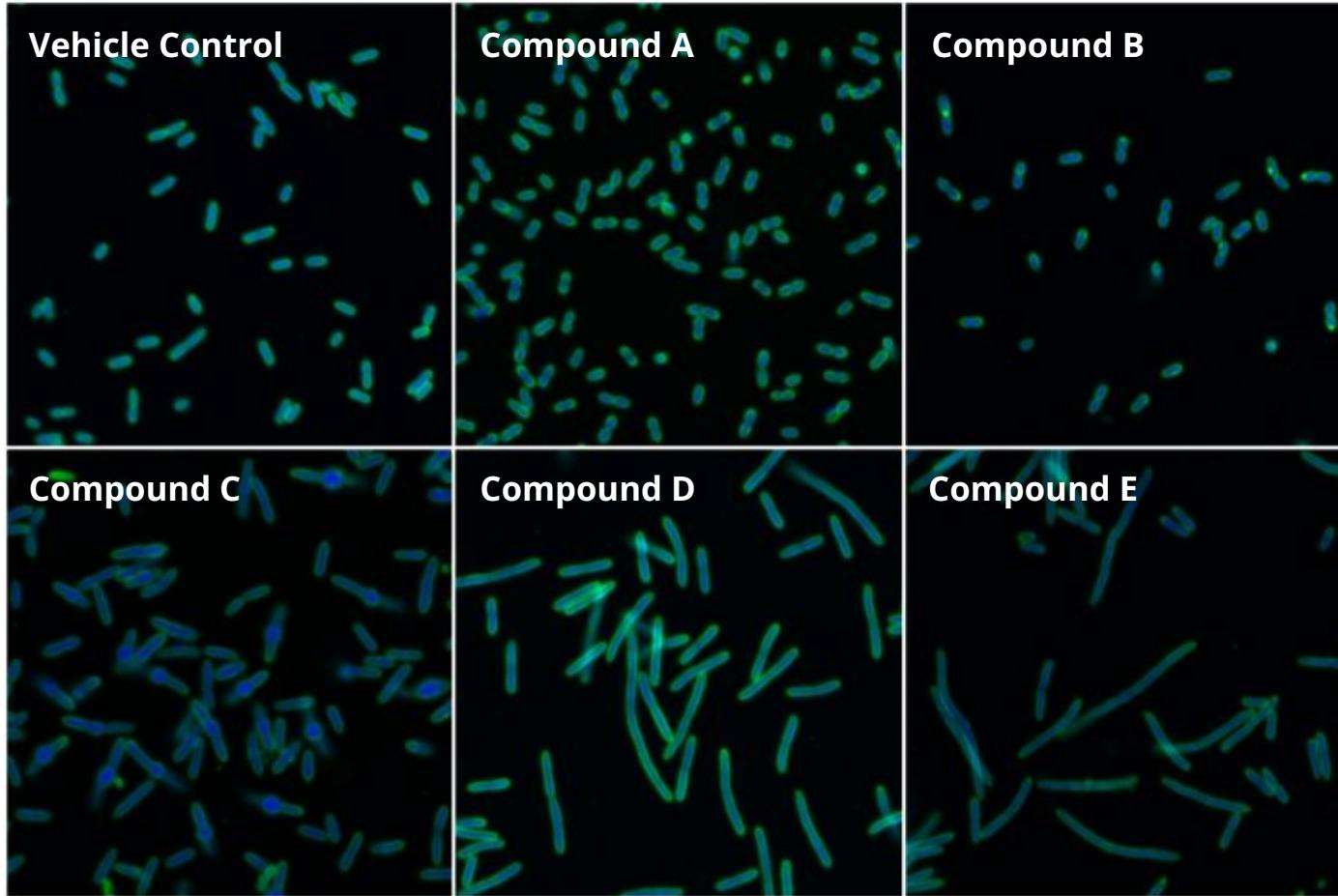
How to use RunStarDist



DEMO Fly data



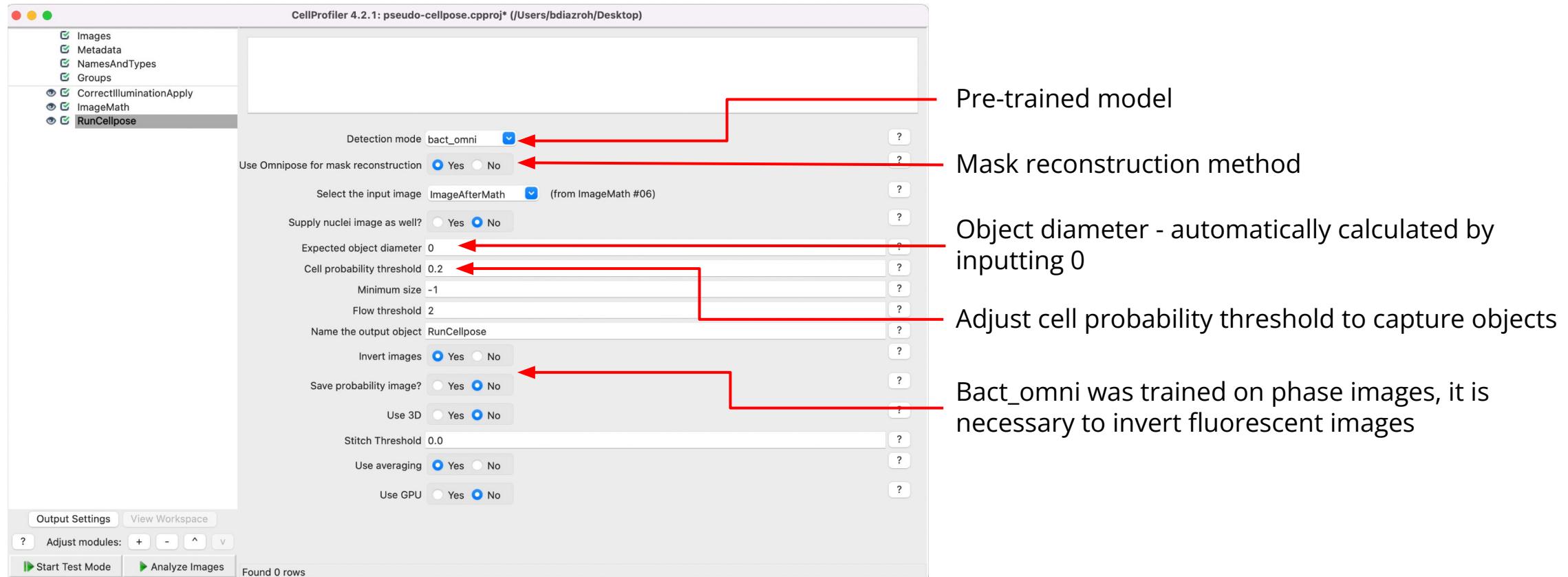
Example 1: Bacteria (2D)



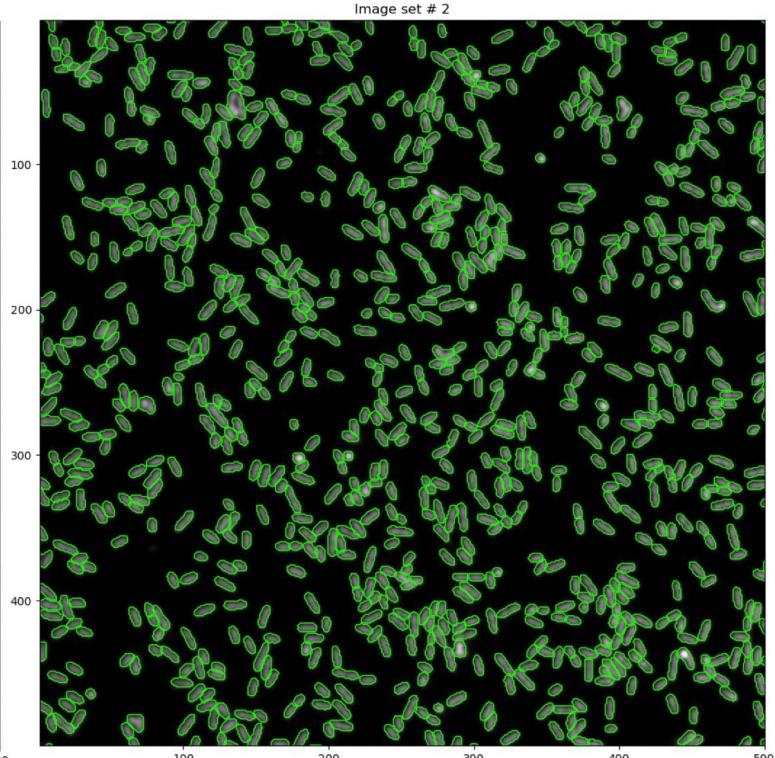
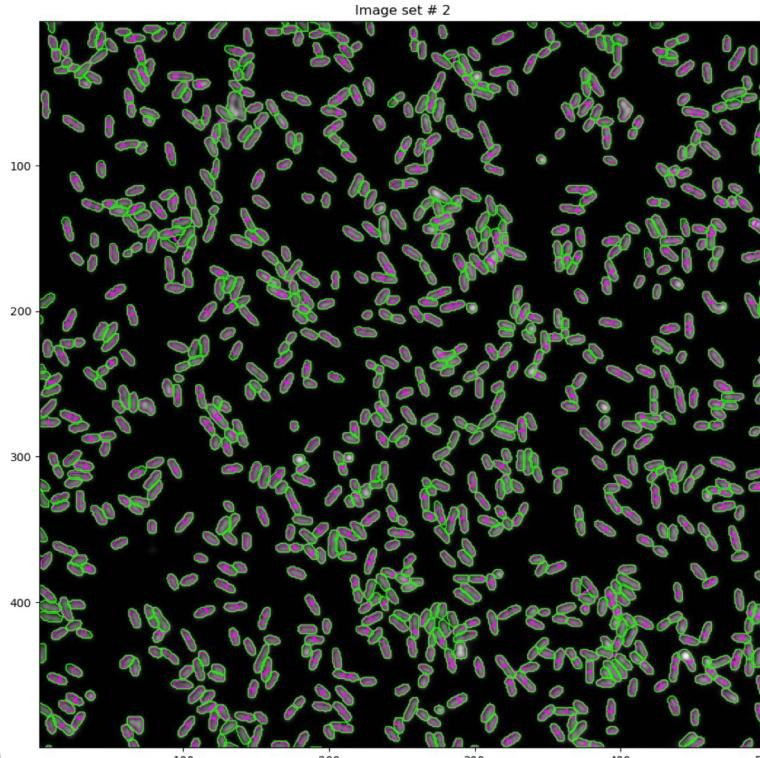
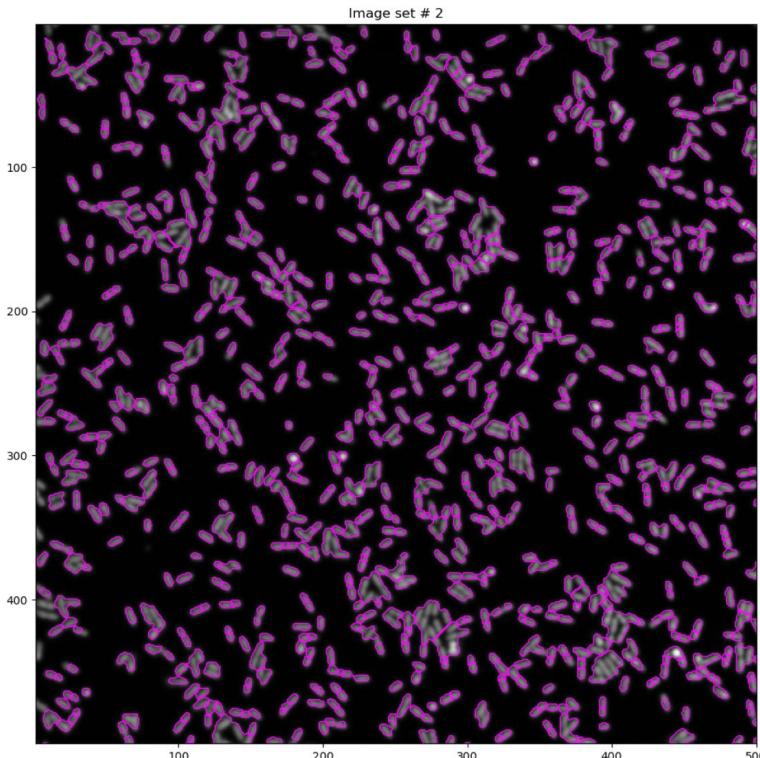
Why are these cells challenging to segment?

- Heterogeneous size and shape
- Cells overlapping
- Crowded

Example 1: Bacteria (2D)



Example 1: Bacteria (2D)

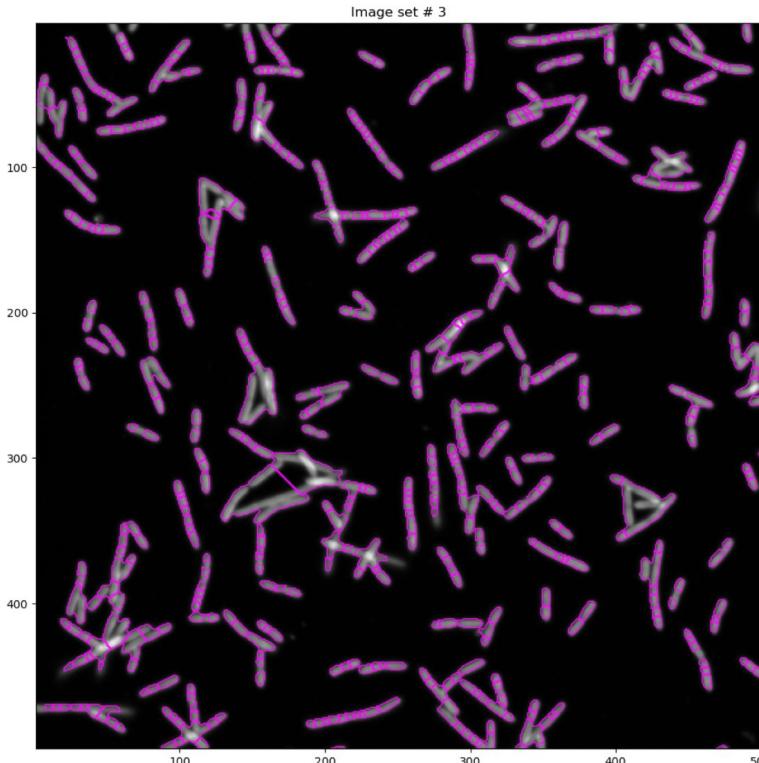


IdentifyPrimaryObjects

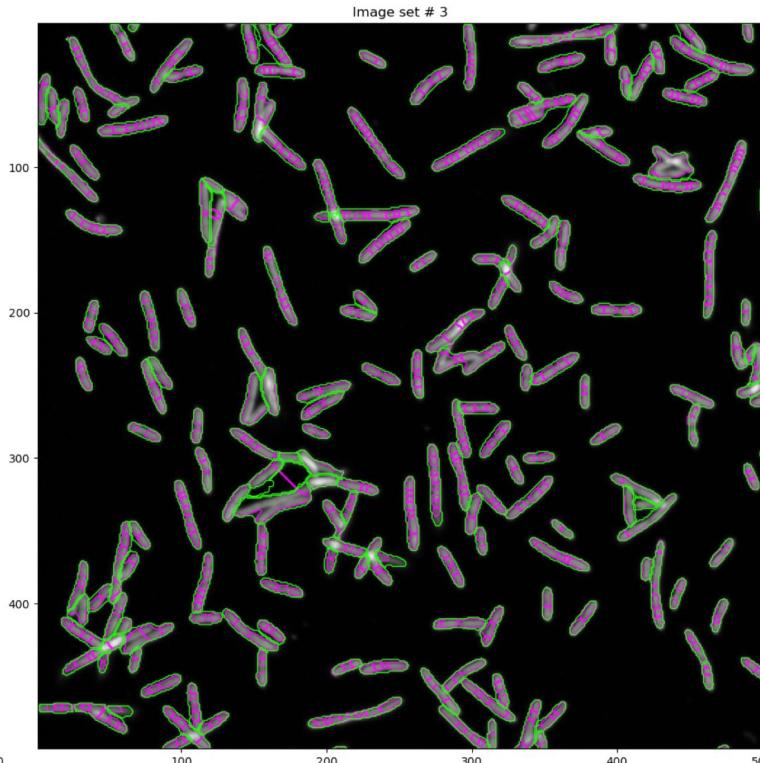
RunCellpose

Images courtesy of the Hung Lab, Broad Institute

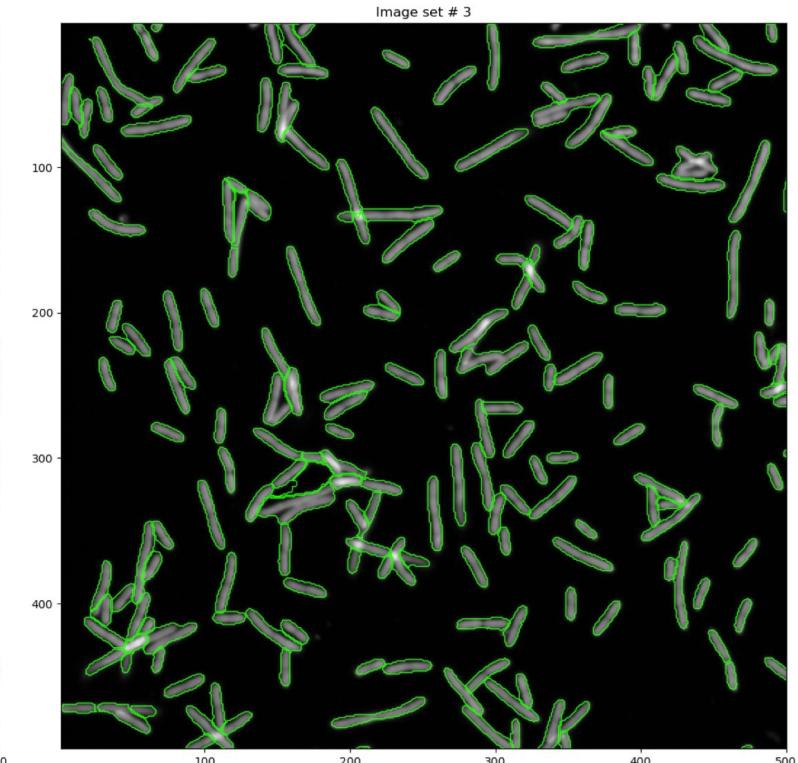
Example 1: Bacteria (2D)



IdentifyPrimaryObjects

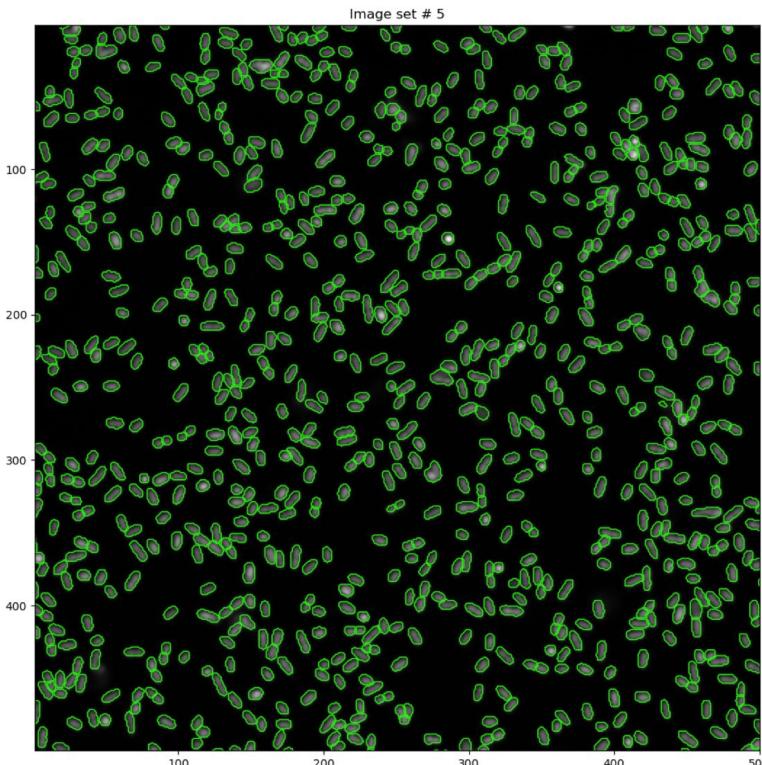


RunCellpose

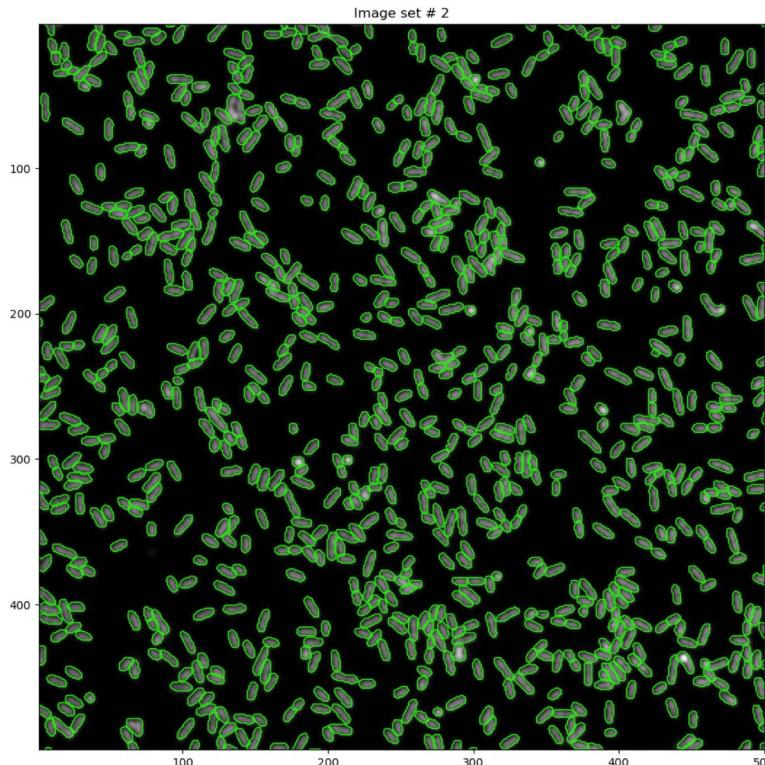


Images courtesy of the Hung Lab, Broad Institute

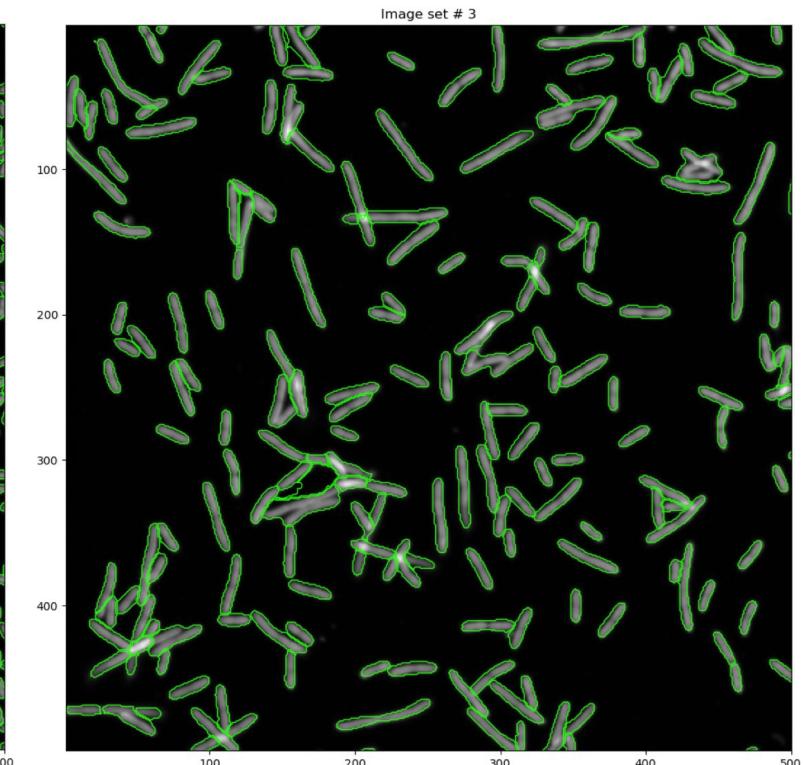
Example 1: Bacteria (2D)



Compound B



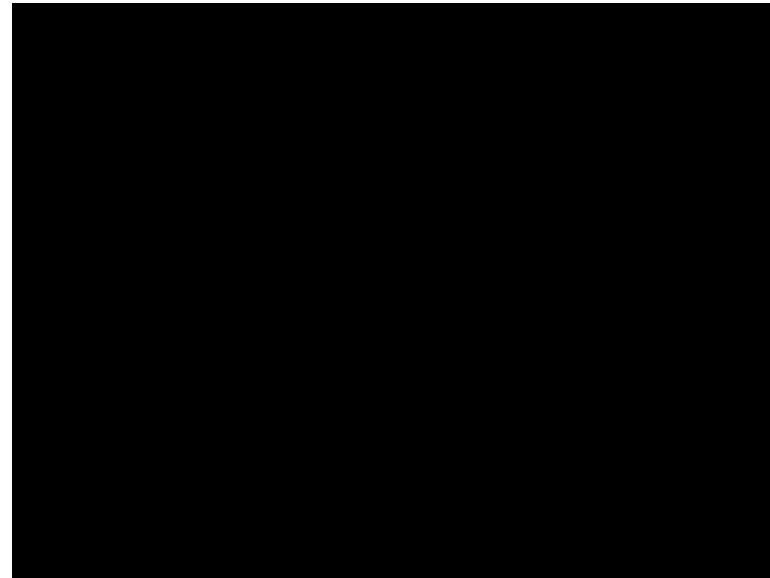
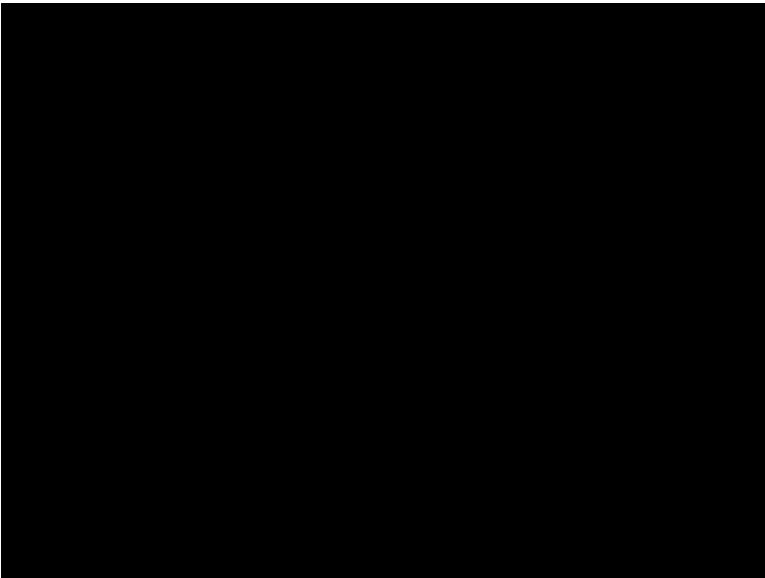
Vehicle Control



Compound D

Images courtesy of the Hung Lab, Broad Institute

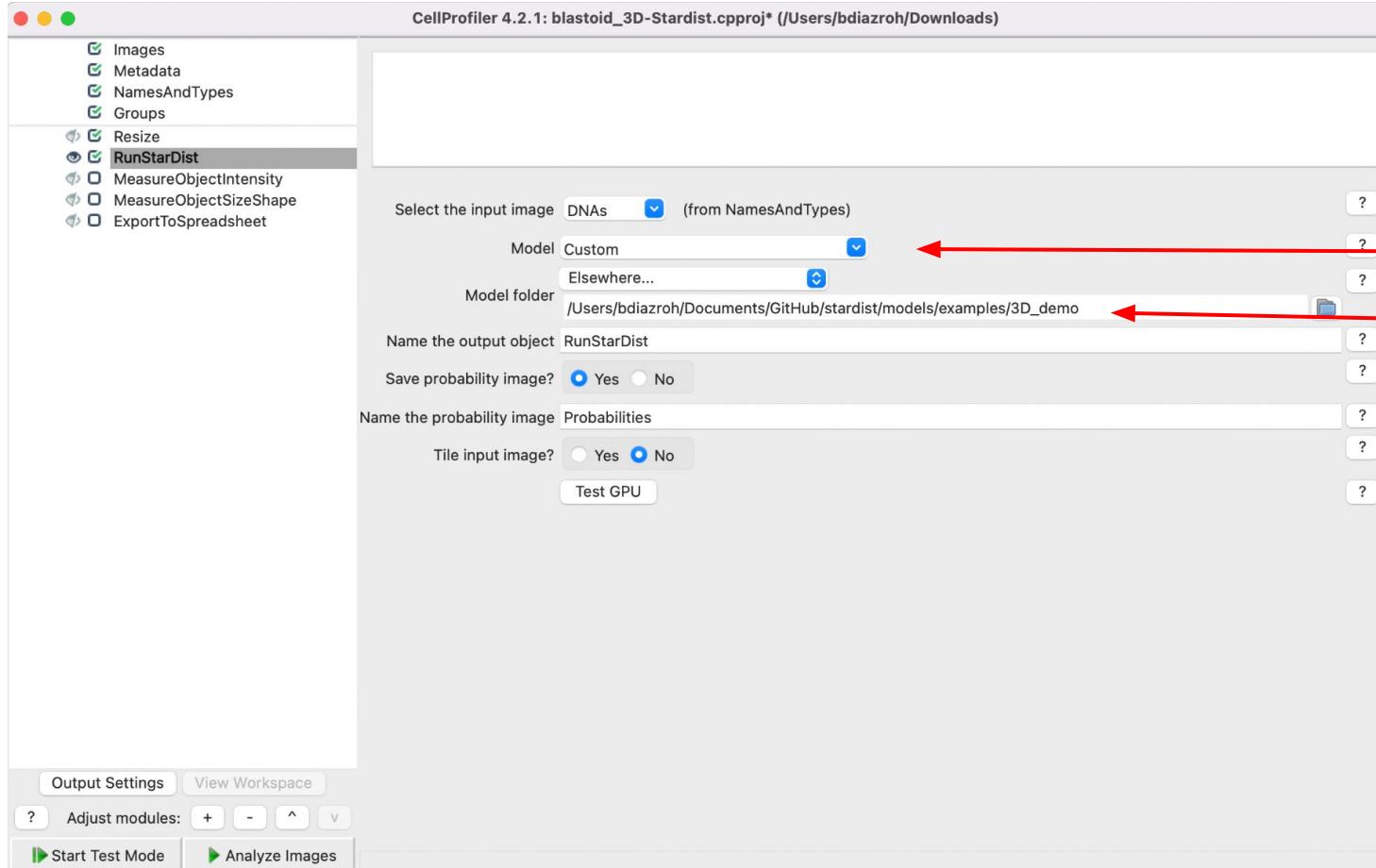
Example 2: Blastoids (3D)



Why are these cells challenging to segment?

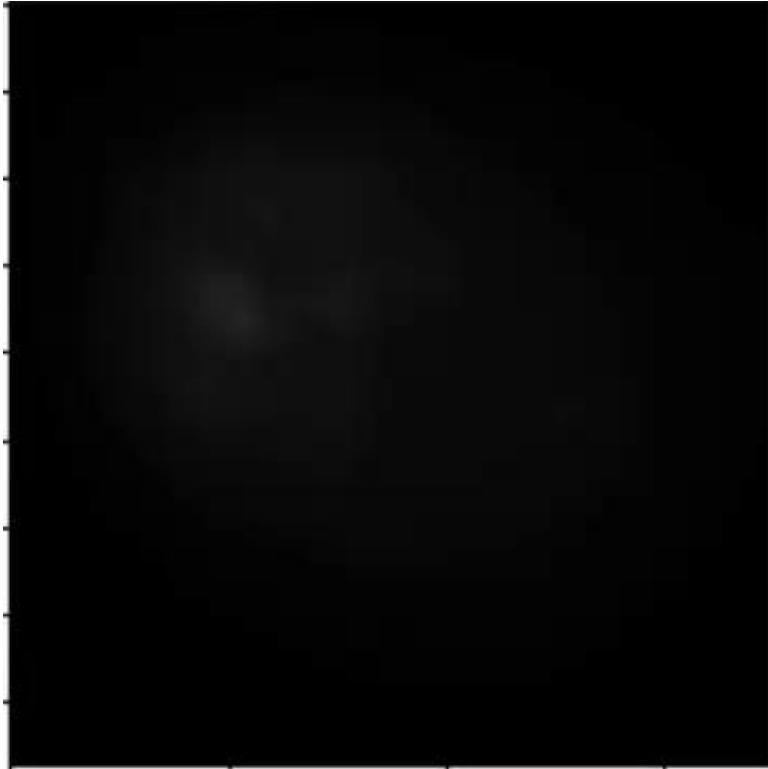
- Heterogeneous size and shape
- Cells overlapping
- Bleaching

Example 2: Blastoids (3D)



Custom model
Folder where your
model is stored

Example 2: Blastoids (3D)



Watershed

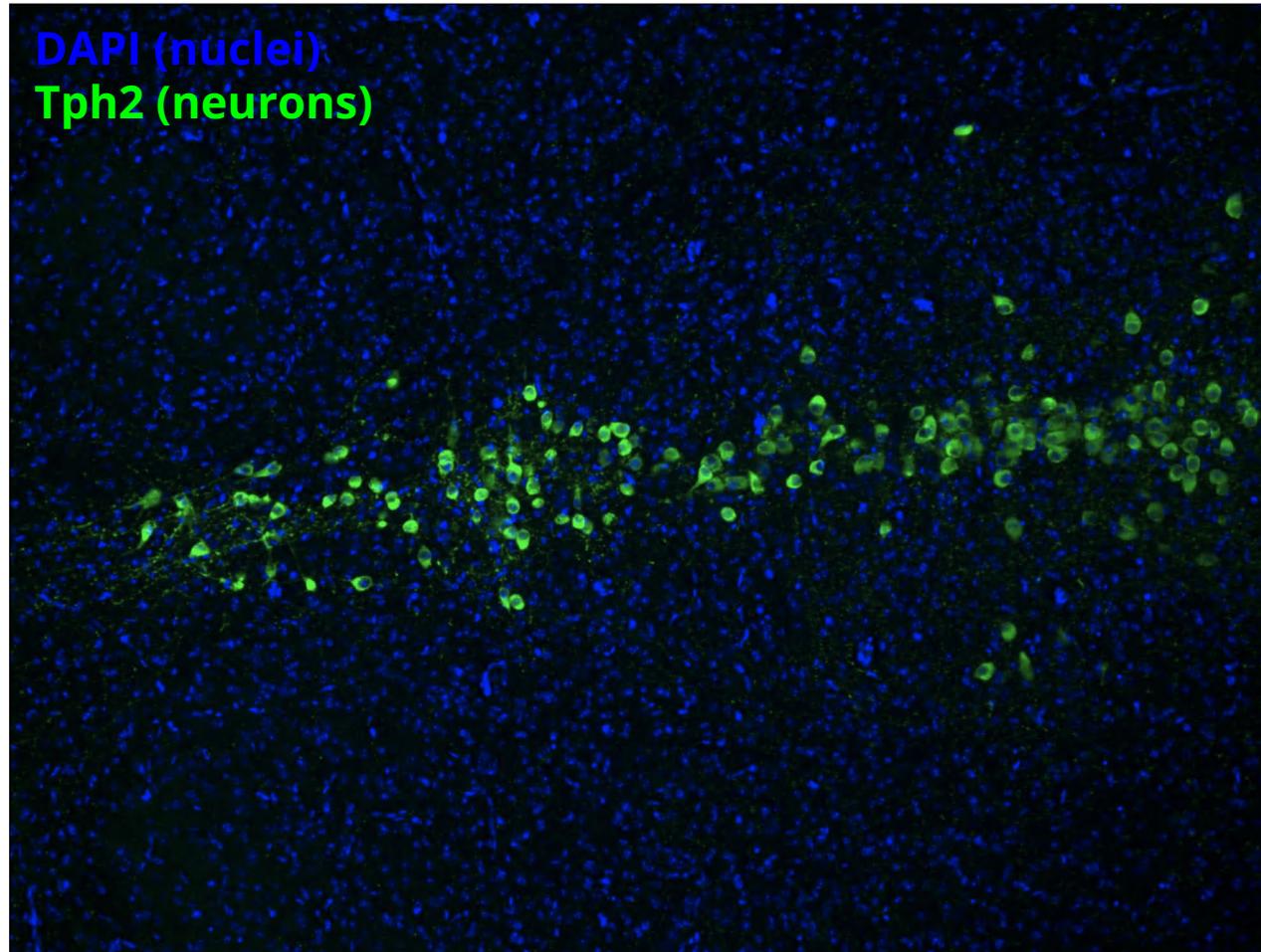


RunStarDist

Images courtesy of the Rivron Lab - IMBA Vienna BioCenter

Example 3: Neurons in brain sections (2D)

Fluorescent neurons in mouse brain sections

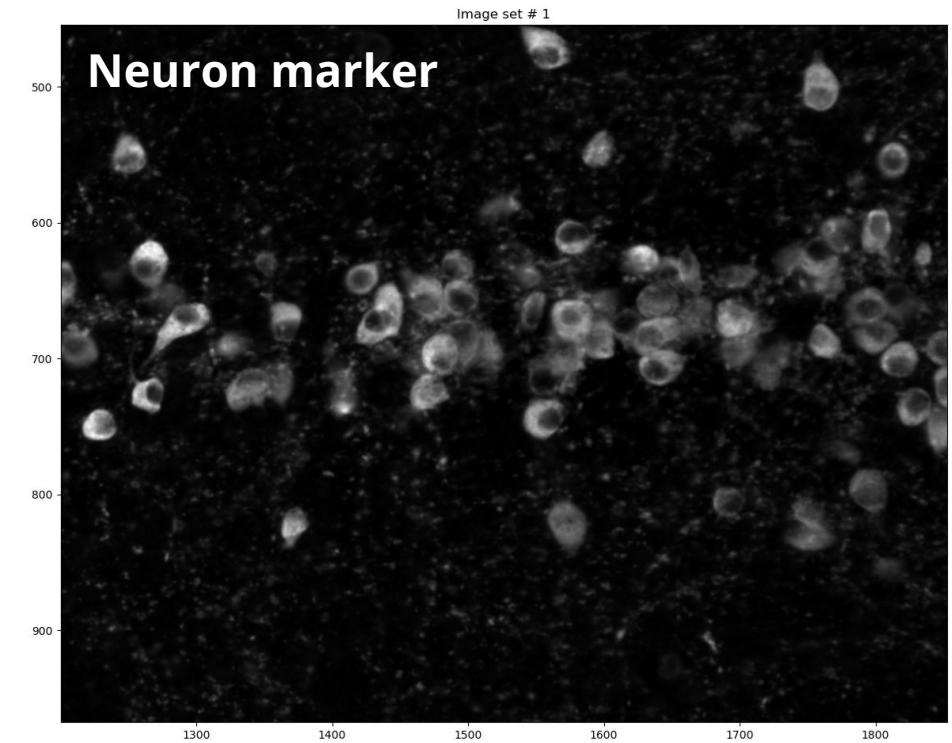


Images courtesy of the Dymecki lab, Harvard Medical School

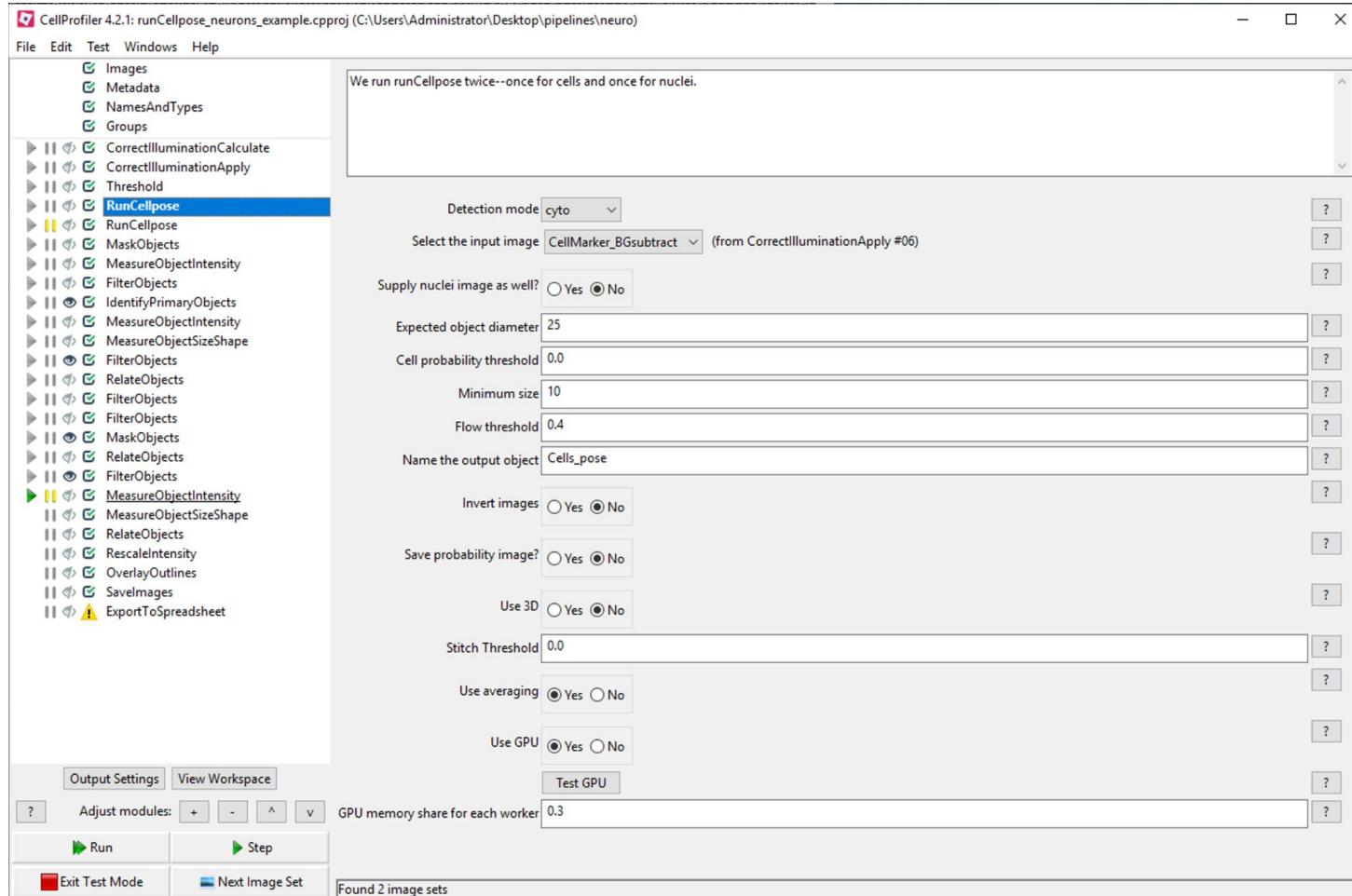
Example 3: Neurons in brain sections (2D)

Why are these cells challenging to segment?

- Heterogeneous brightness
- Lots of fluorescent fibers in the same region
- Donut-shaped intensity (hole in middle where nucleus is)
- Cells of interest are rare within the section but clustered together where they are found

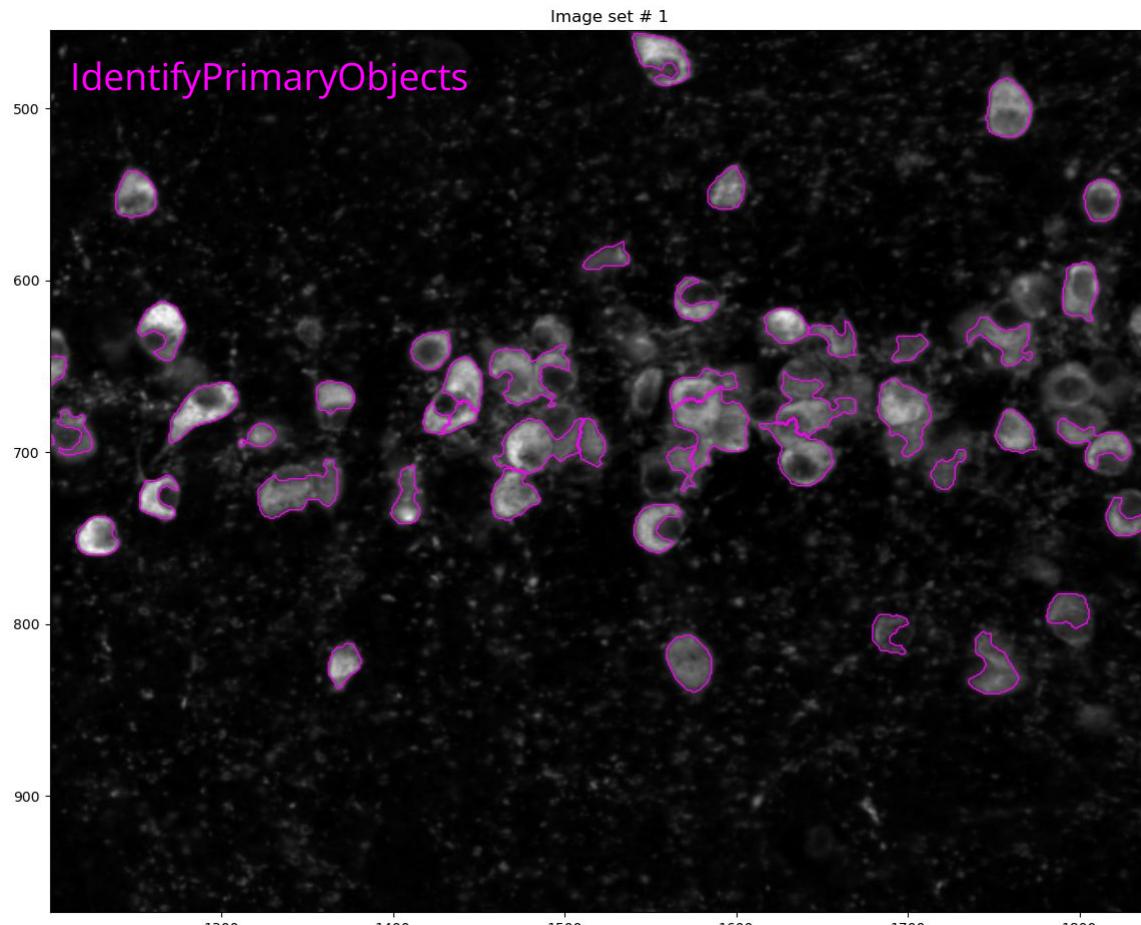


Example 3: Neurons in brain sections (2D)



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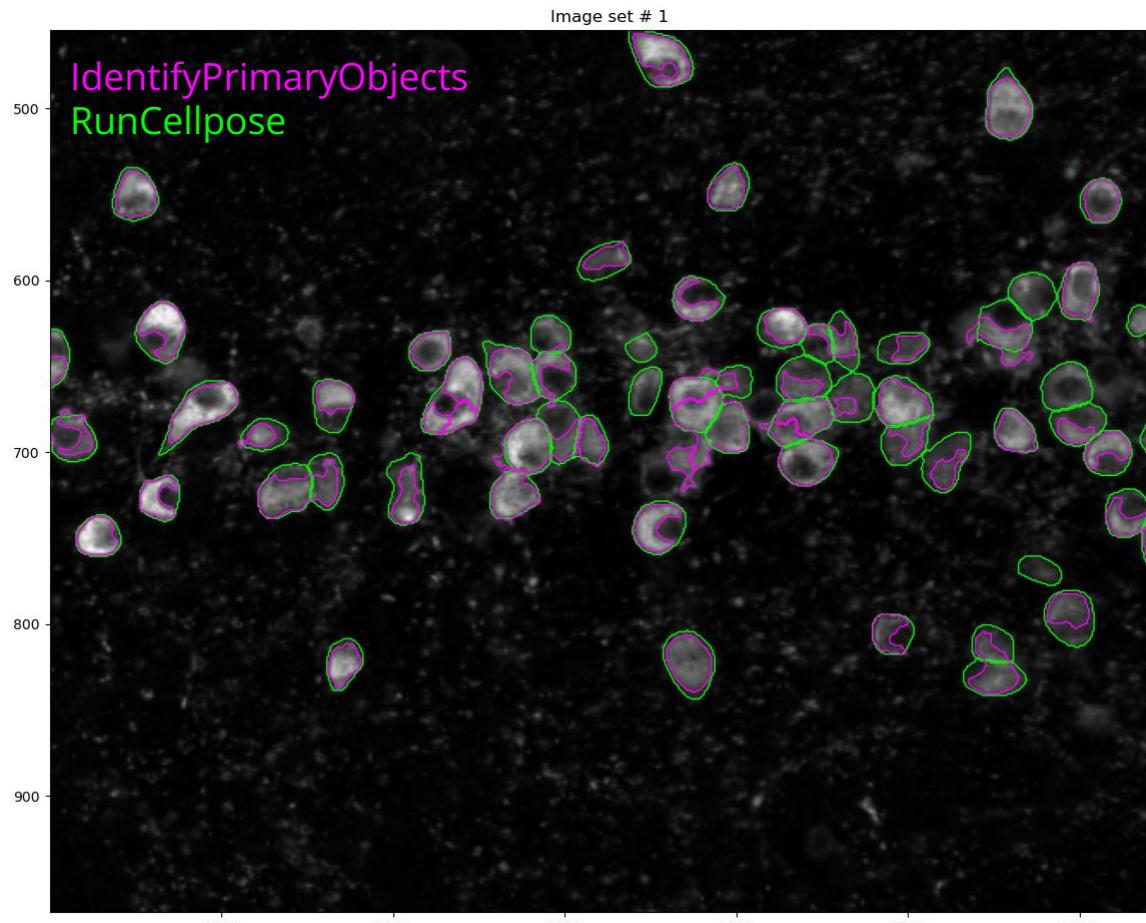
Comparison to IdentifyPrimaryObjects:



Images courtesy of the Dymecki lab, Harvard Medical School

Example 3: Neurons in brain sections (2D)

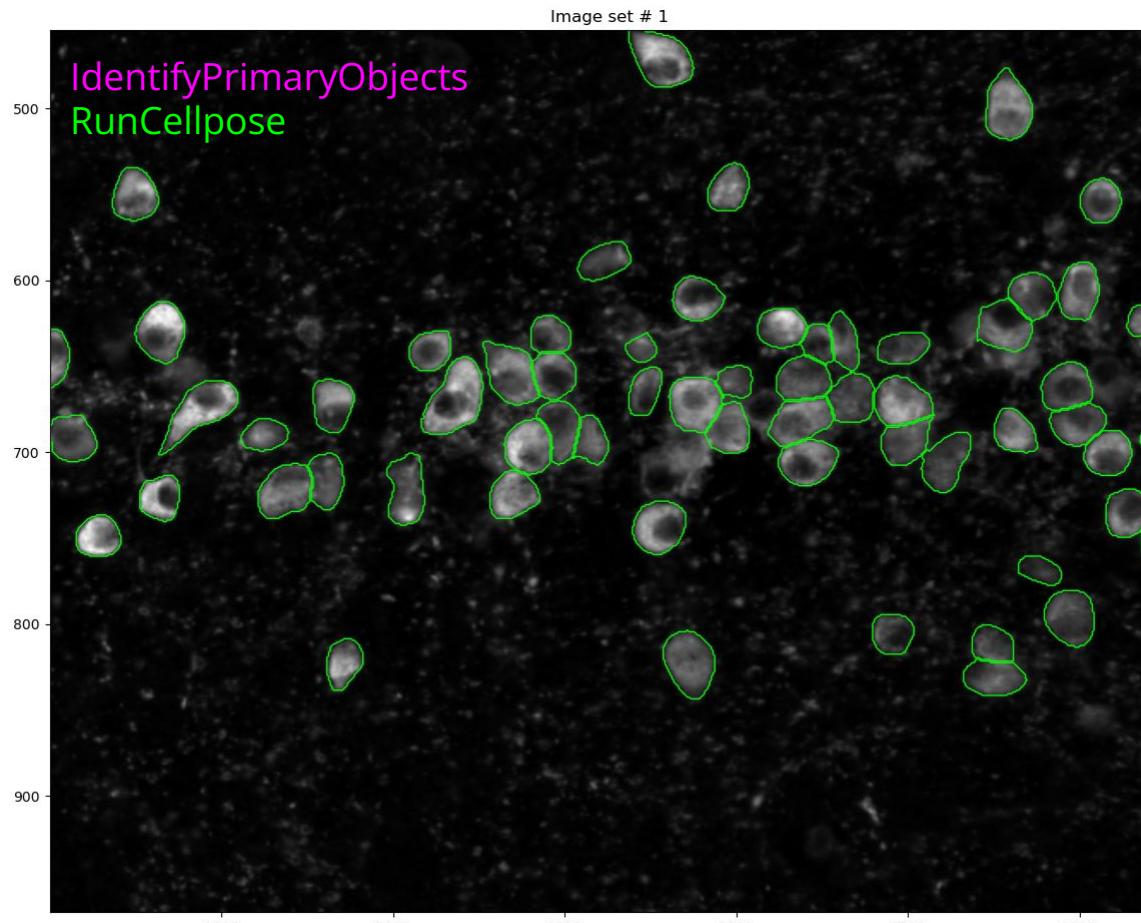
Comparison to IdentifyPrimaryObjects:



Images courtesy of the Dymecki lab, Harvard Medical School

Example 3: Neurons in brain sections (2D)

Comparison to IdentifyPrimaryObjects:



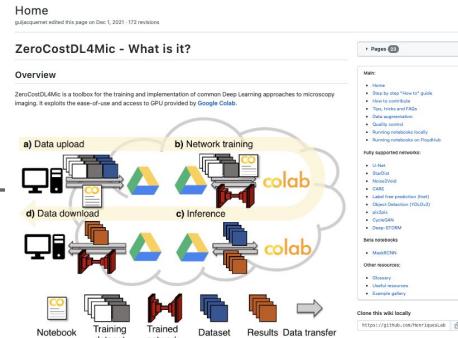
Images courtesy of the Dymecki lab, Harvard Medical School

Considerations for using these plugins in your pipelines

- RunStarDist is generally faster and since it assumes star-convex shapes, is best suited for nuclei or roundish cells
- RunCellpose and the omnipose models within are a good starting point for segmenting non-convex cells
- For either tool, a GPU will help increase speed/efficiency dramatically

How to train your own model

- Training a is computationally costly
You need a GPU!
- ZerocostDL4Mic - Google colab notebook (free*)
 - CellPose and StarDist
 - Images and ground truth
 - Runs in browser
 - Use your custom model in CellProfiler
- <https://github.com/HenriquesLab/ZeroCostDL4Mic/wiki>



Implemented networks

ZeroCostDL4Mic provides fully annotated Google Colab notebooks for popular pre-existing networks. These cover a range of important image analysis tasks (e.g. segmentation, denoising, restoration, label-free prediction).

- Fully supported - considered mature and thoroughly tested by our team.
- Under beta-testing - an early prototype of networks that may not be stable yet.

We welcome network contributions from the research community. If you wish to contribute, please read our guidelines first.

How to get the notebooks and test datasets?

Both fully supported and beta-testing versions of the individual notebooks can be directly opened from GitHub into Colab by clicking one of the respective links in the table below. You will need to create a local copy of your Google Drive in order to save the notebook locally. This will allow you to run the notebook without an internet connection. Once you have done this, you selected to install the relevant packages, load the training dataset, train, check on test datasets and perform inference and predictions on unseen data.

With the exception of the U-net training data, we provide training and test datasets that were generated by our lab. These can also be downloaded from Zenodo using the various links below. The U-net data was obtained from the RGB segmentation contest.

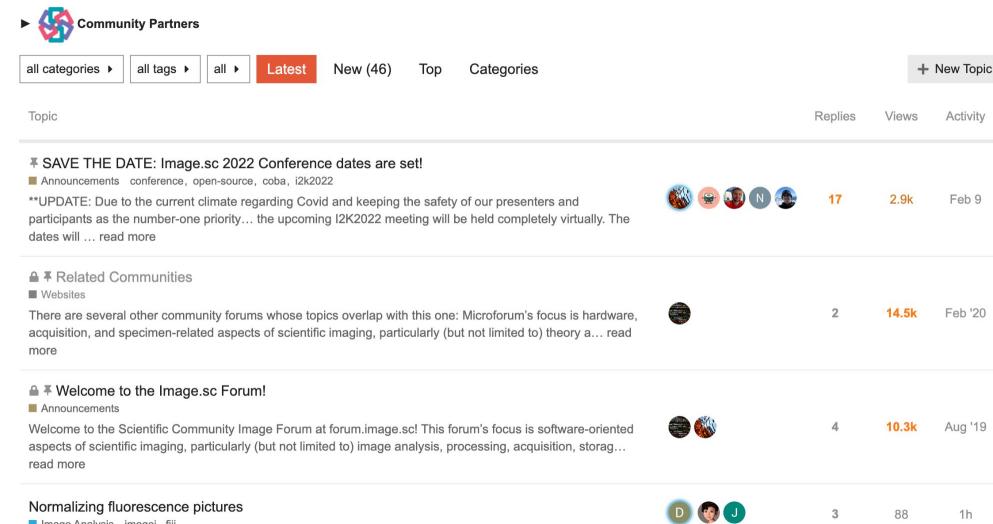
Segmentation networks

Network	Paper(s)	Tasks	Status	Link to example training and test dataset	Direct link to the notebook in Colab
U-Net (2D)	here and here	Binary segmentation	Fully supported	here	Open in Colab
U-Net (3D)	here	Binary segmentation	Fully supported	EPFL dataset	Open in Colab
U-Net (2D) multilabel	here and here	Semantic segmentation	Under beta-testing	here	Open in Colab
DeepNetSeg	here	Joint denoising and binary segmentation	Fully supported	Available soon	Open in Colab
StarDist (2D)	here and here	Binary segmentation	Fully supported	here	Open in Colab
StarDist (3D)	here and here	Instance segmentation	Fully supported	from StarDist github	Open in Colab
Cellpose (2D and 3D)	here	Instance segmentation (Cells or Nuclei)	Fully supported	Coming soon	Open in Colab
SpineNet (2D)	here	Instance segmentation	Fully supported	here	Open in Colab
EmbedSeg (2D)	here	Instance segmentation	Under beta-testing	here	Open in Colab

Lucas von Chamier*, Romain F. Laine*, Johanna Jukkala, Christoph Spahn, Daniel Krentzel, Elias Nehme, Martina Lerche, Sara Hernández-pérez, Pieta Mattila, Eleni Karinou, Séamus Holden, Ahmet Can Solak, Alexander Krull, Tim-Oliver Buchholz, Martin L Jones, Loic Alain Royer, Christophe Leterrier, Yoav Shechtman, Florian Jug, Mike Heilemann, Guillaume Jacquemet, Ricardo Henriques. Democratizing deep learning for microscopy with ZeroCostDL4Mic. *Nature Communications*, 2021. DOI: <https://doi.org/10.1038/s41467-021-22518-0>

I need more help!

- Plugins found at: github.com/CellProfiler/CellProfiler-plugins
- Help installing plugins:
github.com/CellProfiler/CellProfiler-plugins/tree/master/Instructions
- Help installing CellProfiler from source: github.com/CellProfiler/CellProfiler/wiki
- For further help, make a forum post at image.sc



The screenshot shows the homepage of the image.sc forum. At the top, there's a navigation bar with links for 'Community Partners', 'all categories', 'all tags', 'all', 'Latest' (which is highlighted in red), 'New (46)', 'Top', 'Categories', and '+ New Topic'. Below the navigation, there's a section titled 'Topic' with three recent posts:

- SAVE THE DATE: Image.sc 2022 Conference dates are set!**
Announcements conference, open-source, coba, i2k2022
**UPDATE: Due to the current climate regarding Covid and keeping the safety of our presenters and participants as the number-one priority... the upcoming I2K2022 meeting will be held completely virtually. The dates will ... [read more](#)
- Welcome to the Image.sc Forum!**
Announcements
Welcome to the Scientific Community Image Forum at forum.image.sc! This forum's focus is software-oriented aspects of scientific imaging, particularly (but not limited to) image analysis, processing, acquisition, storag... [read more](#)
- Normalizing fluorescence pictures**
Image Analysis ImageJ, Fiji
Normalizing fluorescence pictures

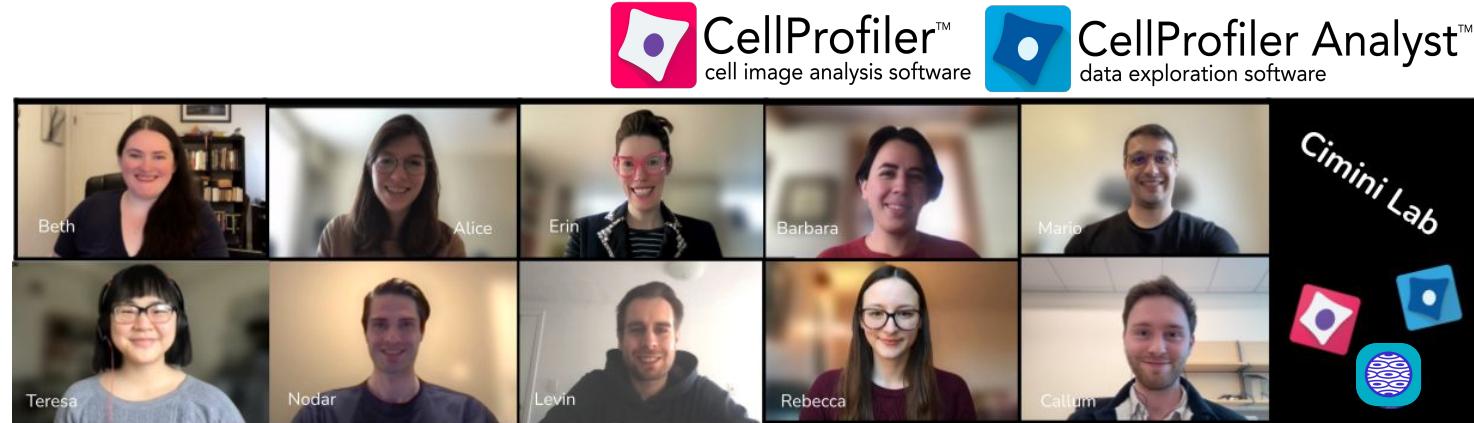
Each post includes a small user icon, the number of replies, views, and the date it was posted.

Acknowledgements

Broad Institute Imaging Platform

Cimini lab members

Beth Cimini
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Alice Lucas
Becki Ledford
Levin Moser



David Stirling

Carpenter-Singh lab members

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Shantanu Singh
Srinivas Niranj Chandrasekaran
John Arévalo
Marzieh Haghghi
Yu Han
Serena Larew
Robert van Dijk



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