Using Cellpose and StarDist in CellProfiler

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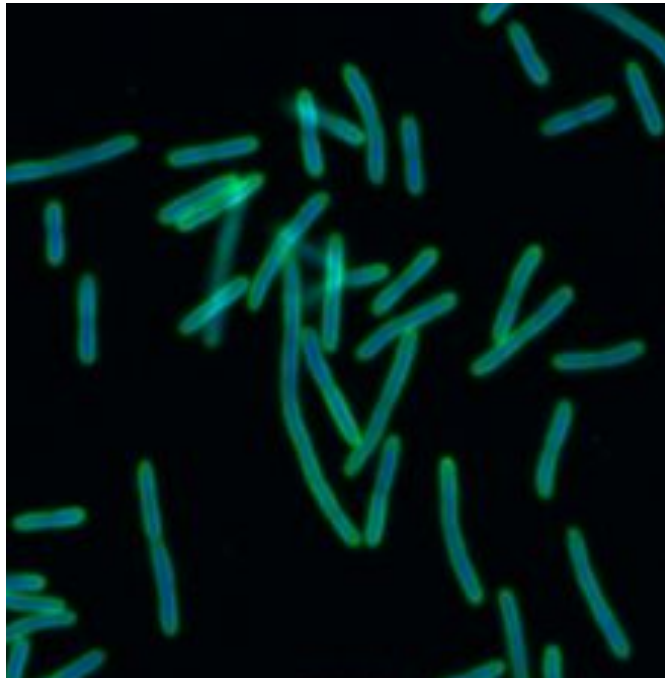
Outline

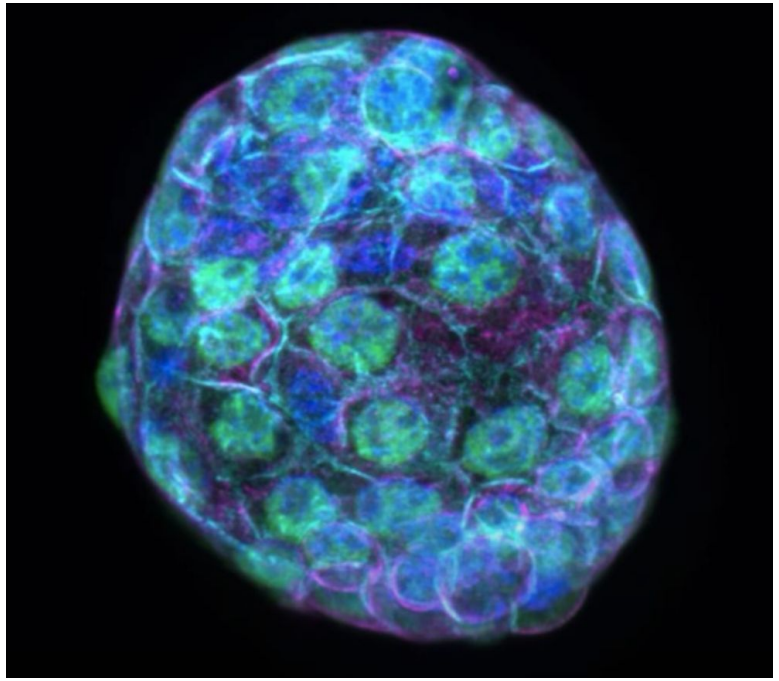
- When is classical segmentation not enough?
- Overview of stardist and cellpose
- Demo datasets:
  - 3D blastoids
  - 2D bacteria
  - 2D neurons in tissue
- Considerations for using the RunStarDist and RunCellpose CellProfiler plugins
- How to train your own model
- Resources for further learning

Download materials at:
http://broad.io/CPSDtutorial

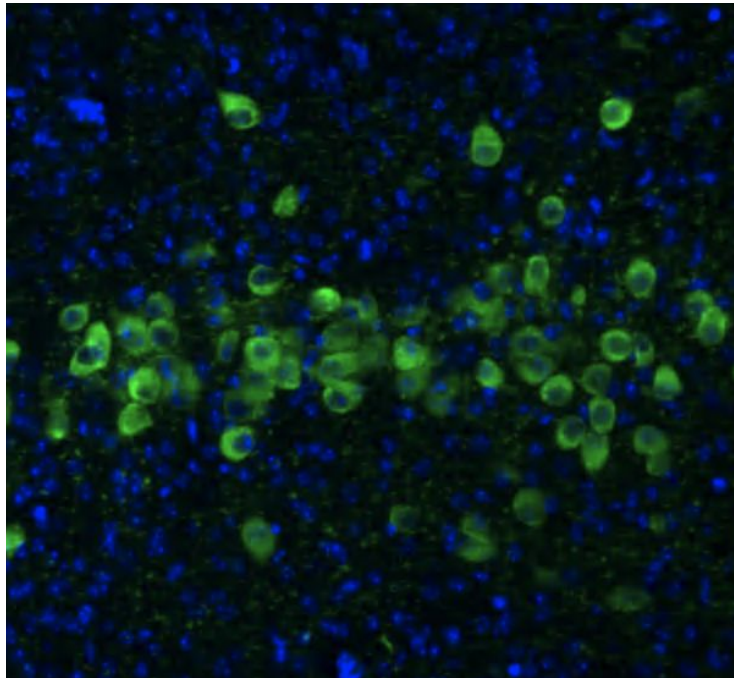
Some images are difficult to segment



- Irregular-shaped objects
- Non-round objects

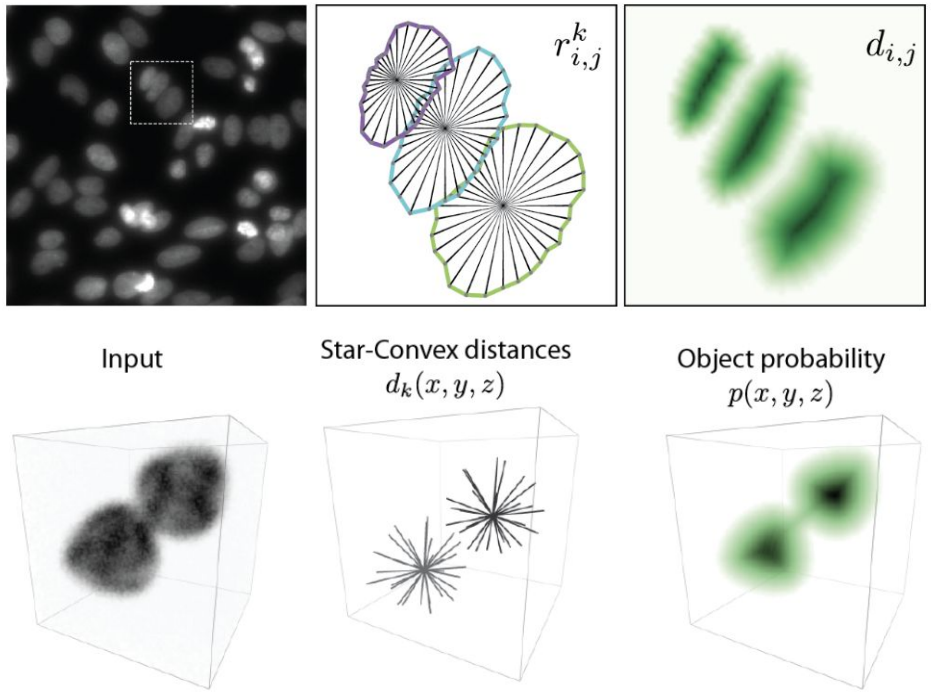


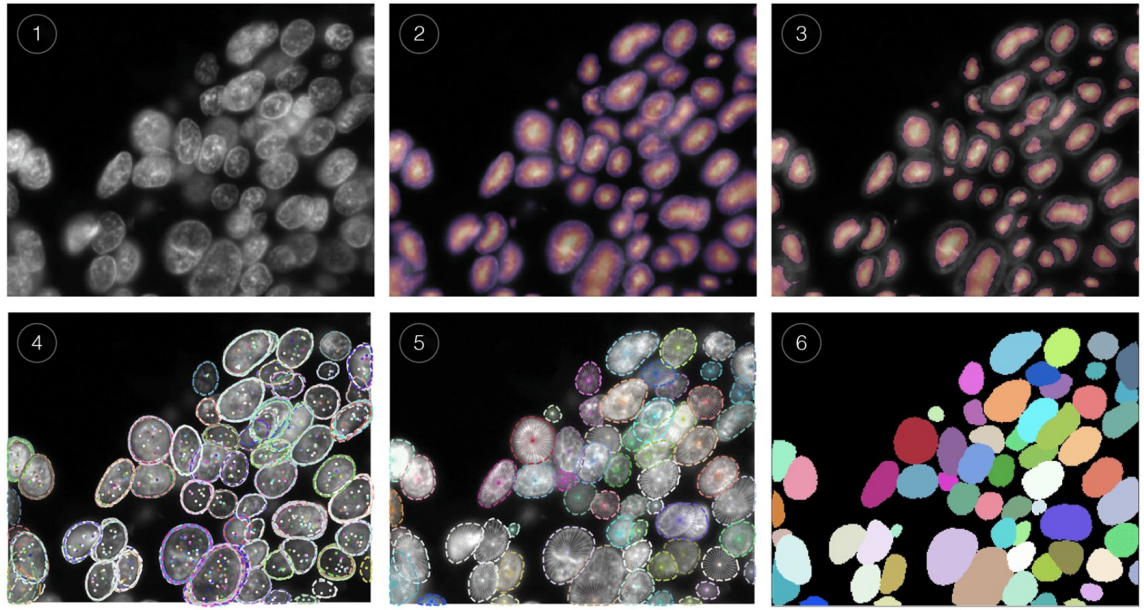
- 3D images
- Clustered cells



- Donut-shaped cells
- Heterogeneous brightness and background

StarDist - Object Detection with Star-convex Shapes

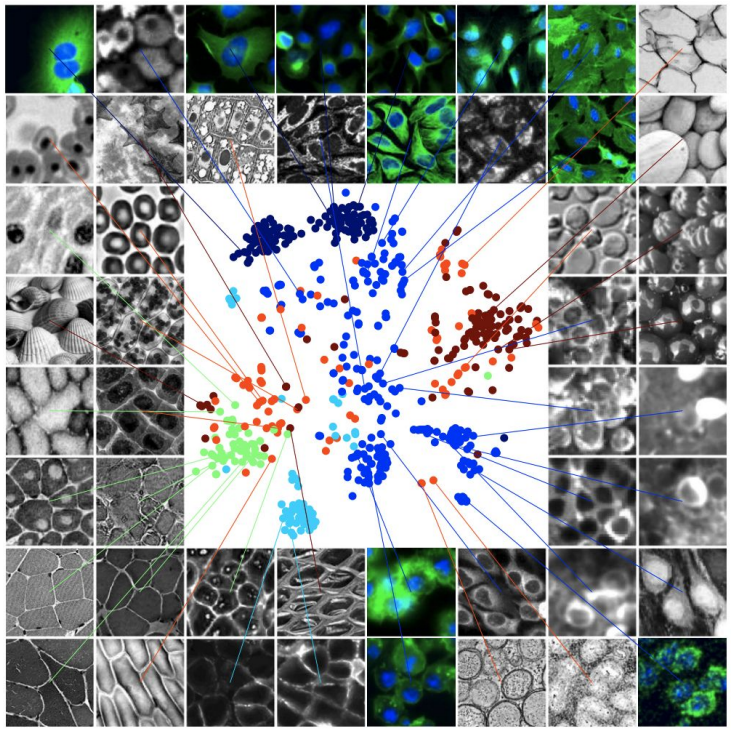


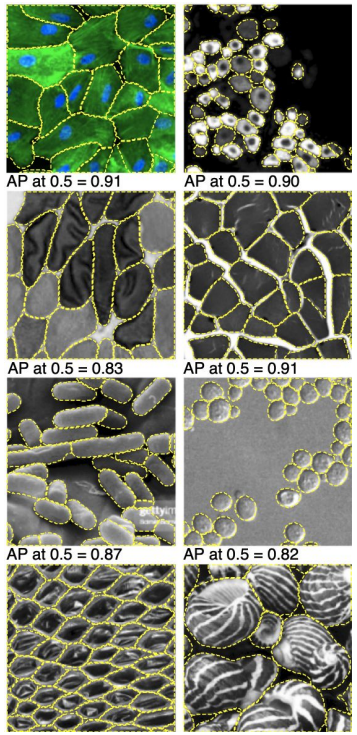


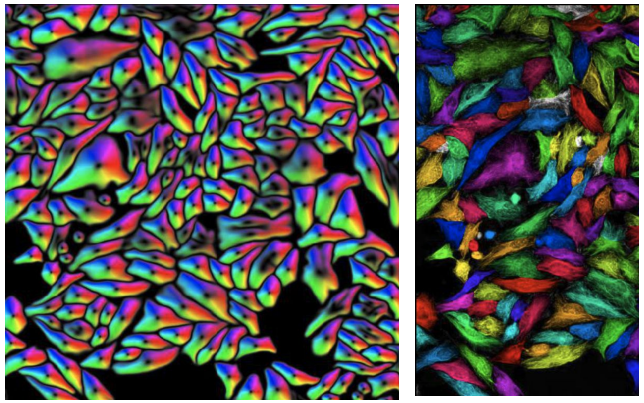
Schmidt, Weigert, Broaddus and Myers, 2018 *International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI)* doi: 10.1007/978-3-030-00934-2_30

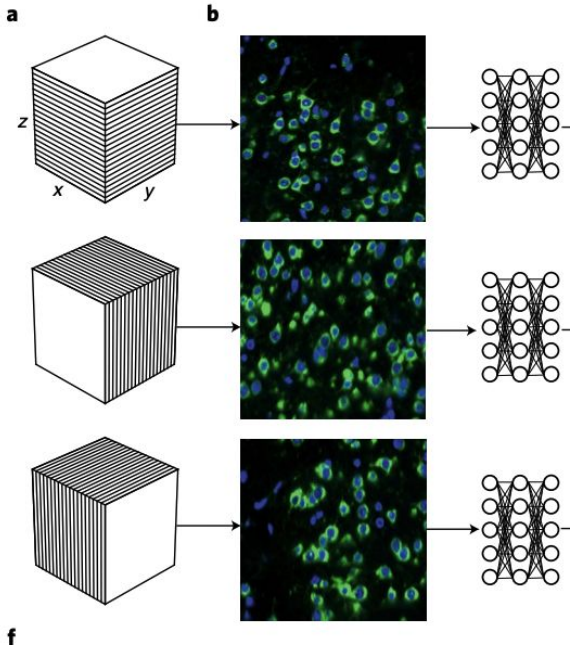
Cellpose - a generalist algorithm for cellular segmentation

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









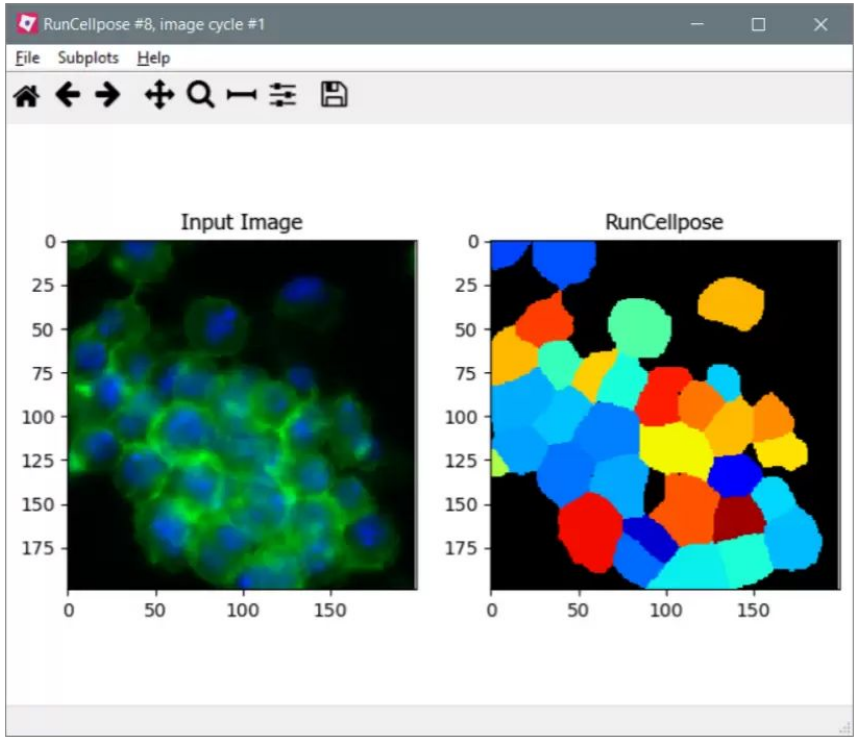
Stringer, Wang, Michaelos, and Pachitariu, 2020 *Nature Methods* doi: 10.1038/s41592-020-01018-x

RunCellpose and RunStarDist plugins bring these tools to CellProfiler

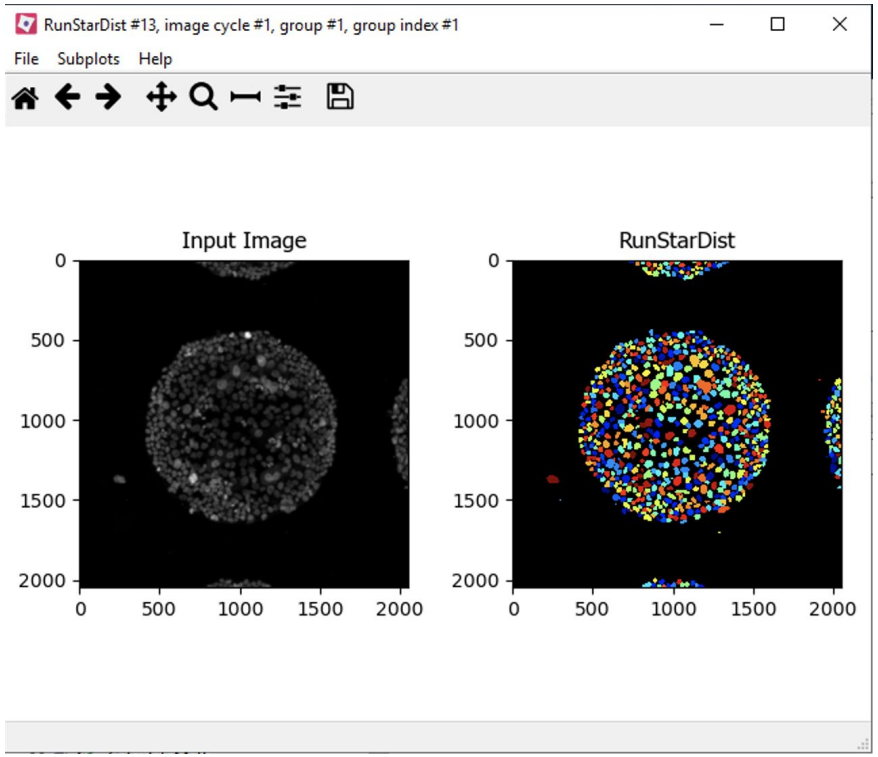
Beth Cimini

David Stirling

RunCellpose



RunStarDist



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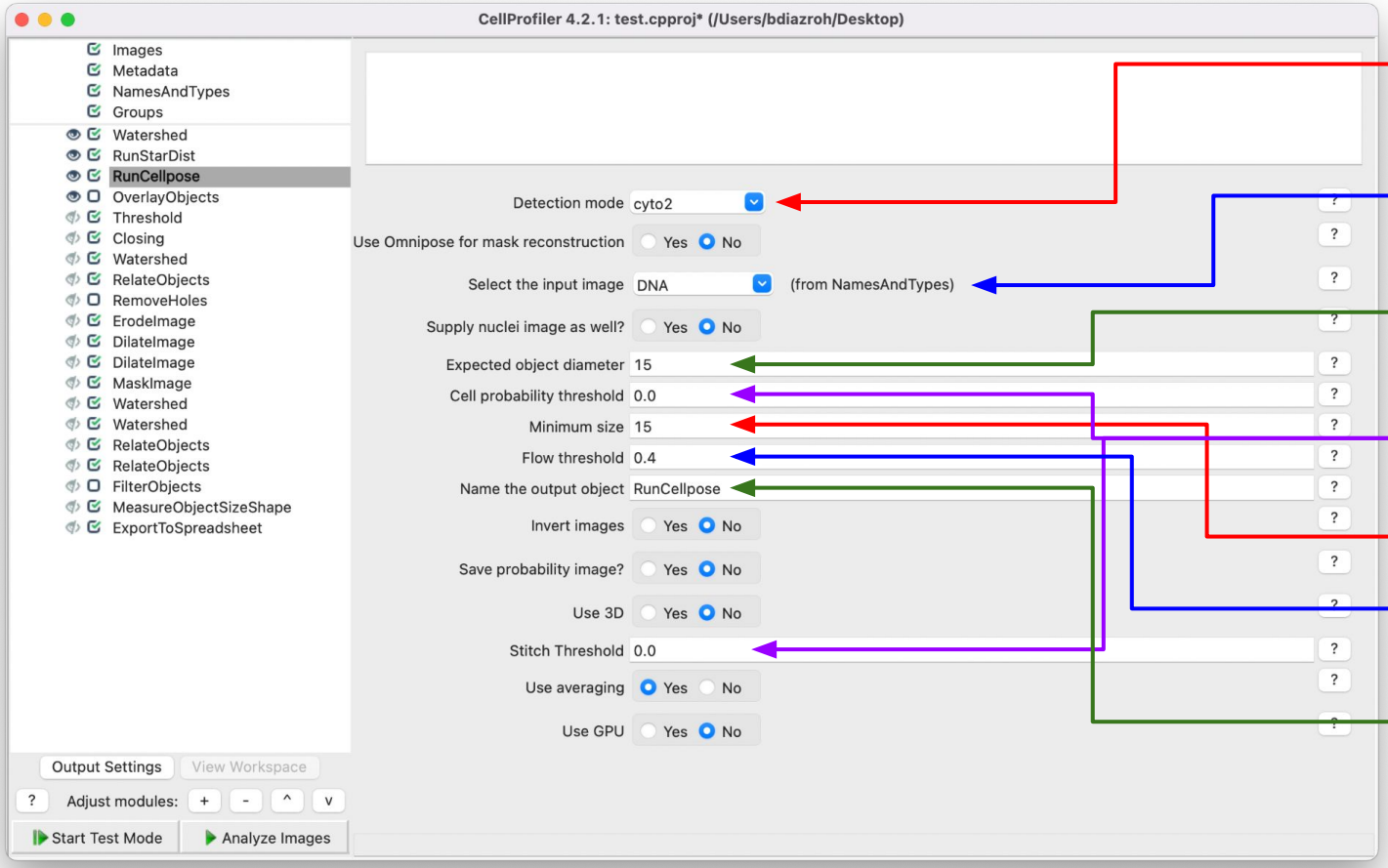
Plugins and installation instructions available at github.com/CellProfiler/CellProfiler-plugins

How to use RunCellpose



Pre-trained model

Input image

Object diameter (0 = automatically detect)

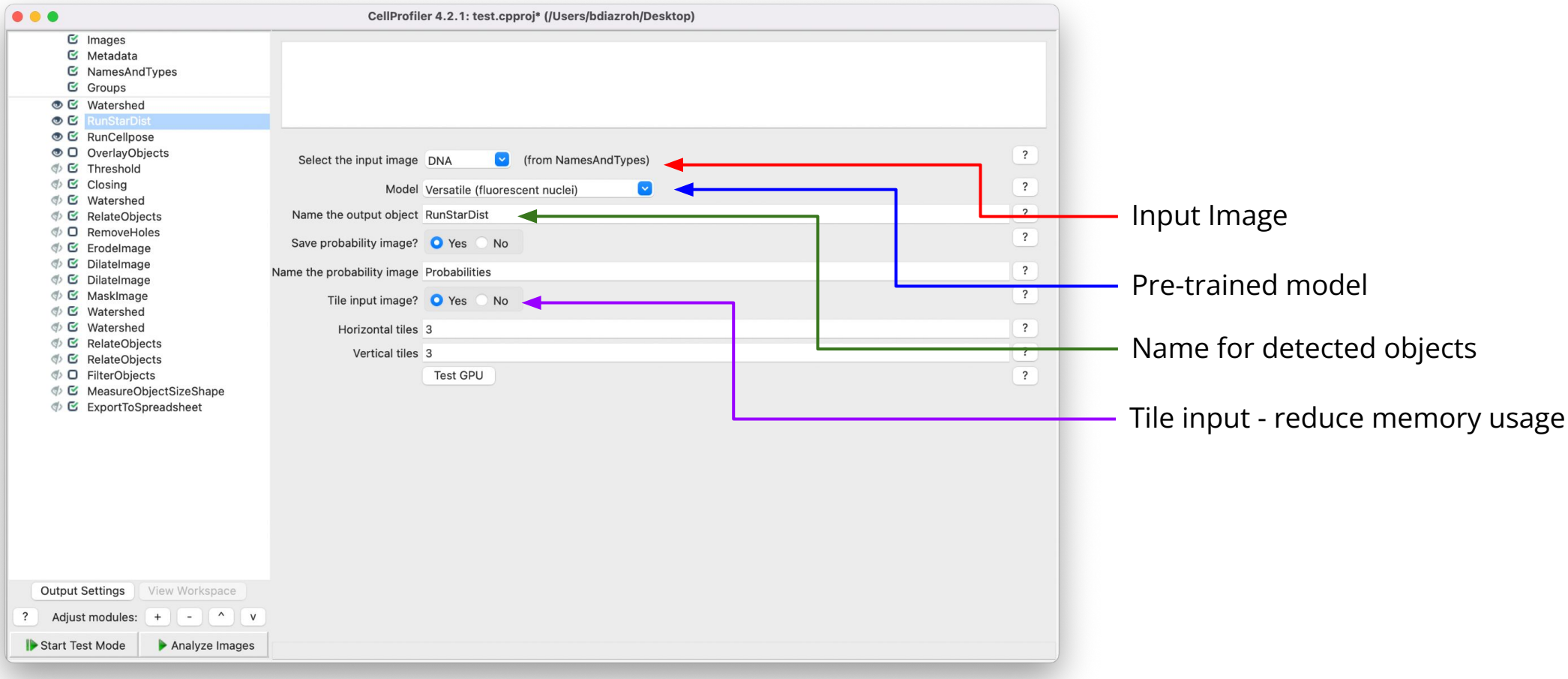
Threshold for what is called a cell. Visualize cell probabilities with “Save probability image”

Minimum object size

Maximum error allowed for mask creation

Name for detected objects

How to use RunStarDist



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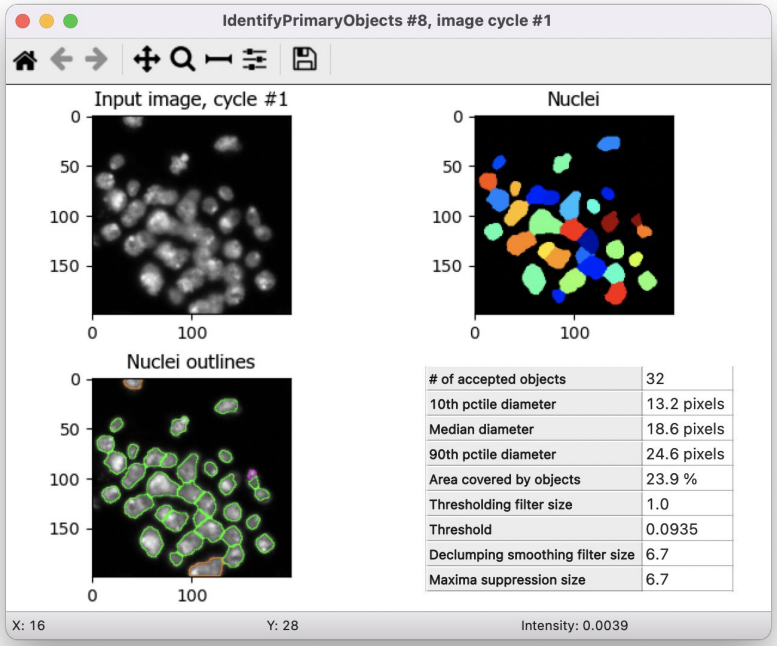
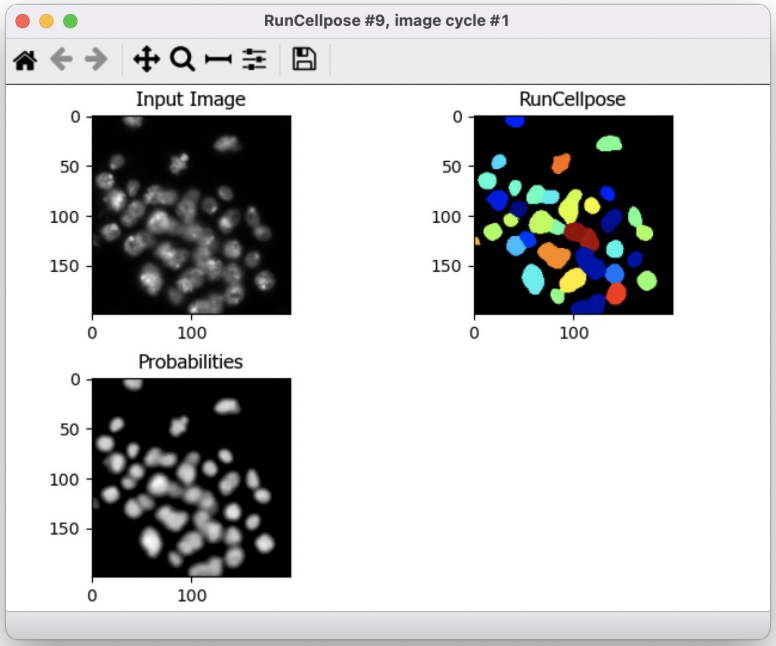
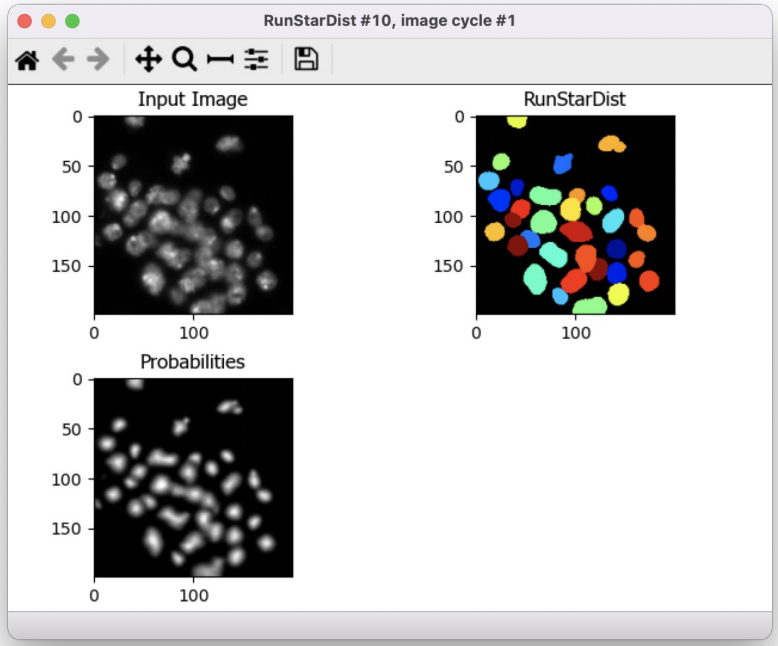
DEMO Fly data







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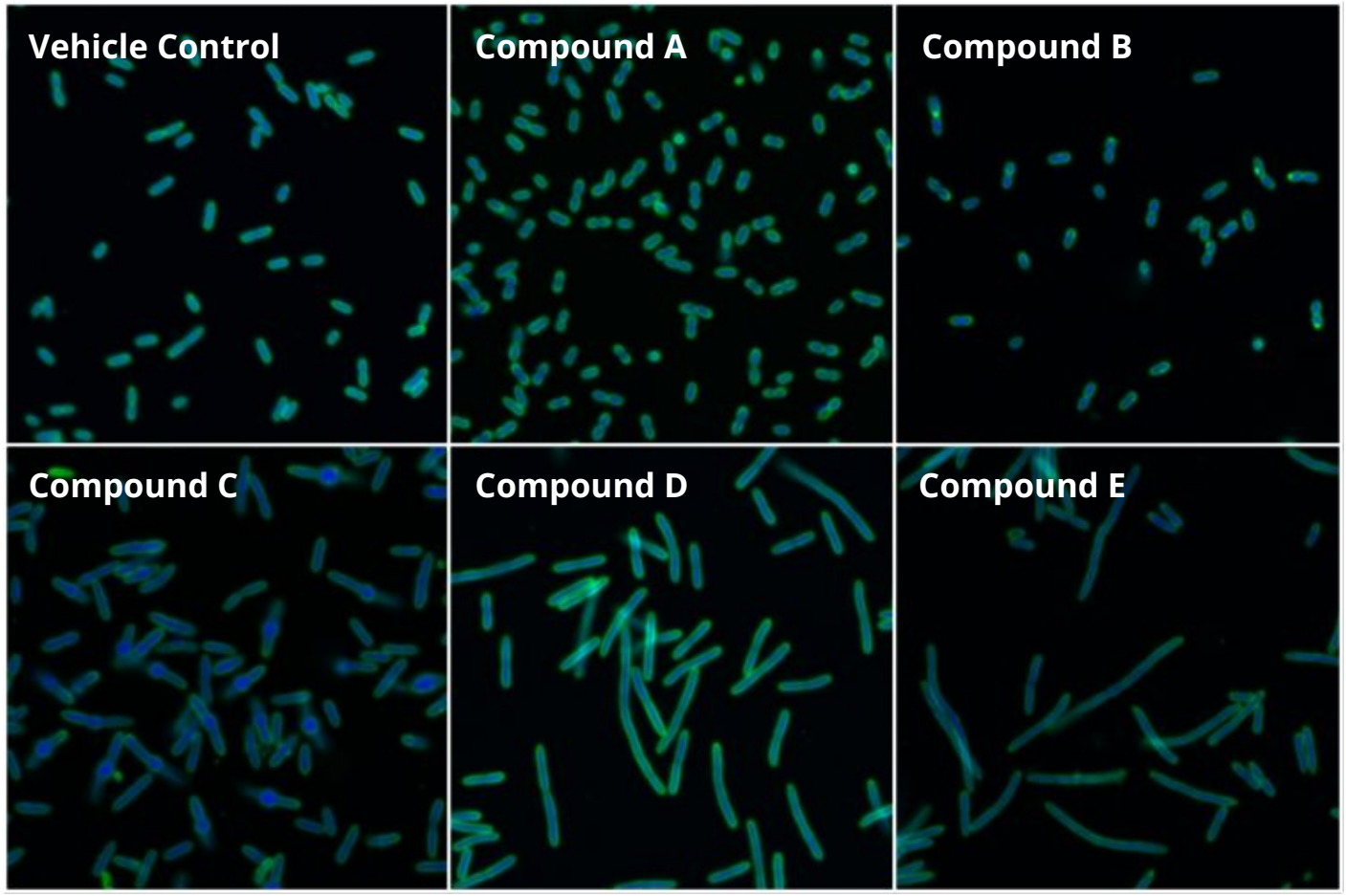
Example 1: Bacteria (2D)



Why are these cells challenging to segment?

- Heterogeneous size and shape
- Cells overlapping
- Crowded

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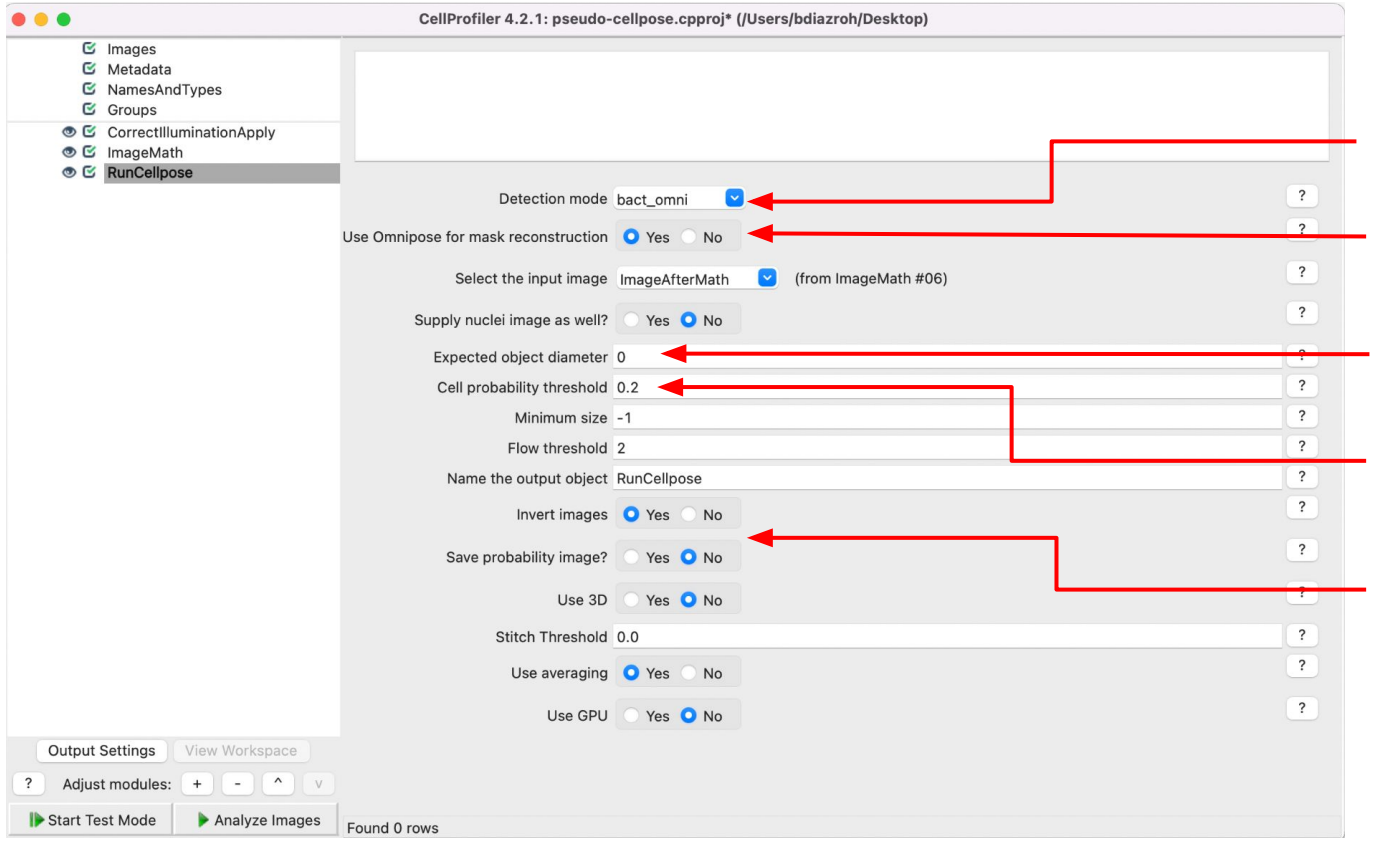
Example 1: Bacteria (2D)



Pre-trained model

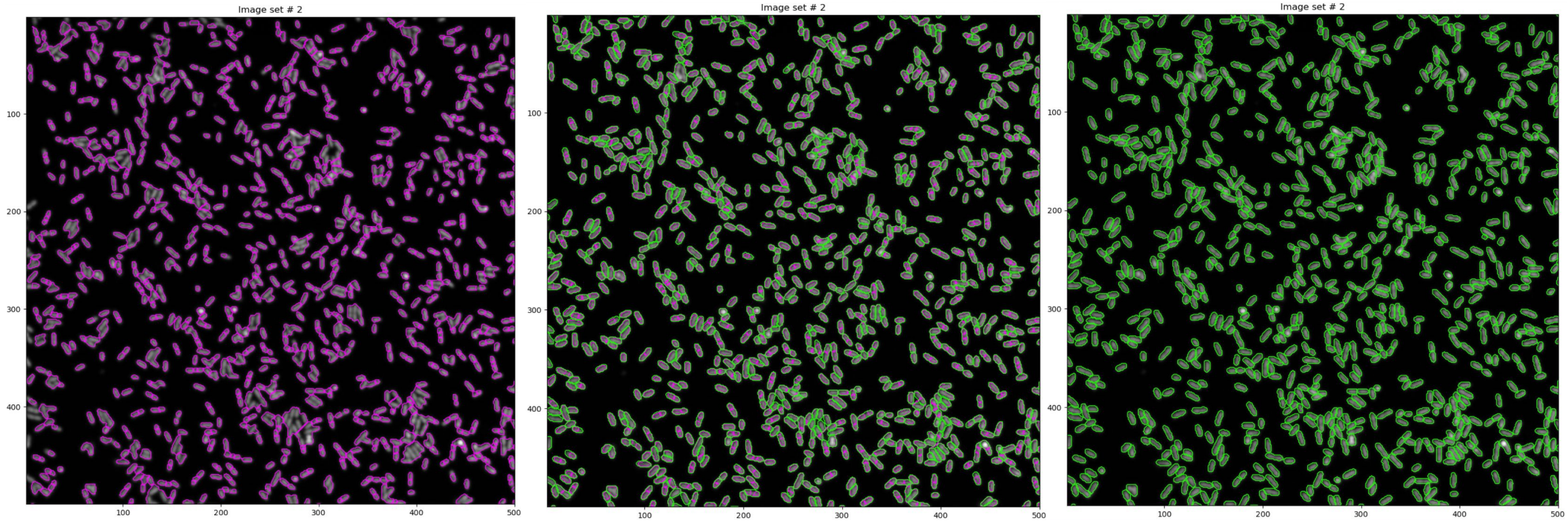
Mask reconstruction method

Object diameter - automatically calculated by inputting 0

Adjust cell probability threshold to capture objects

Bact_omni was trained on phase images, it is necessary to invert fluorescent images

Example 1: Bacteria (2D)



IdentifyPrimaryObjects

RunCellpose

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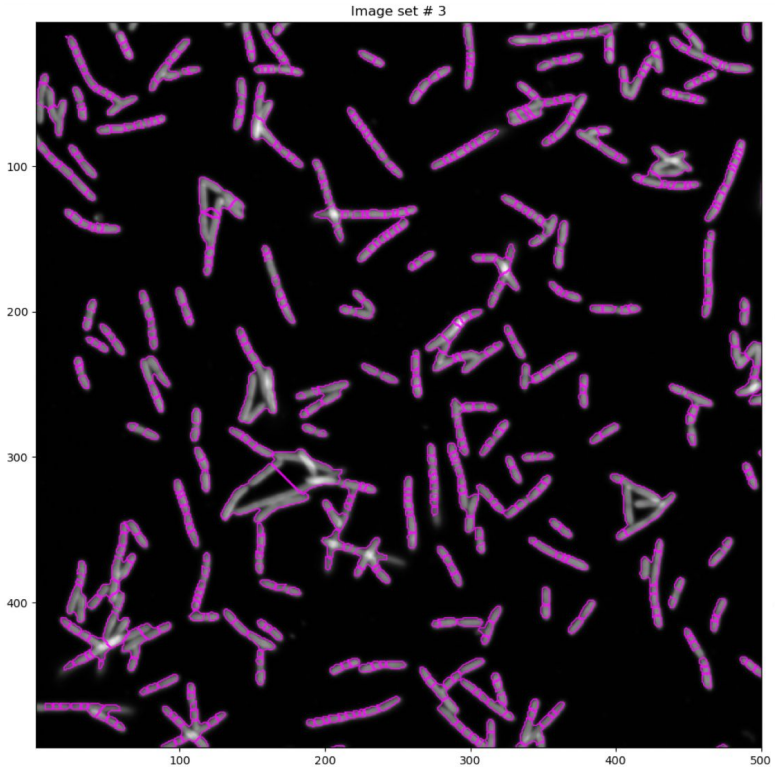
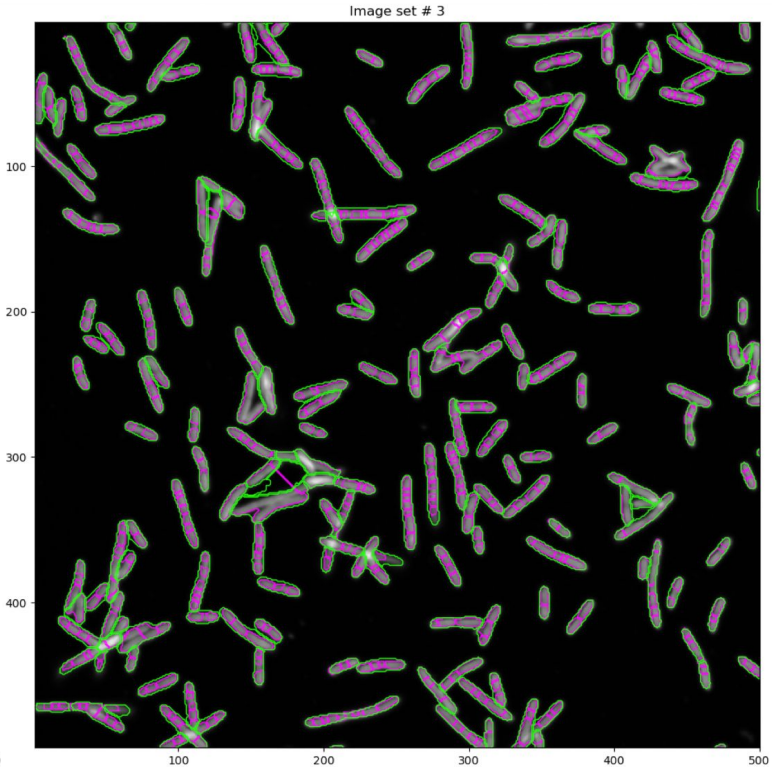
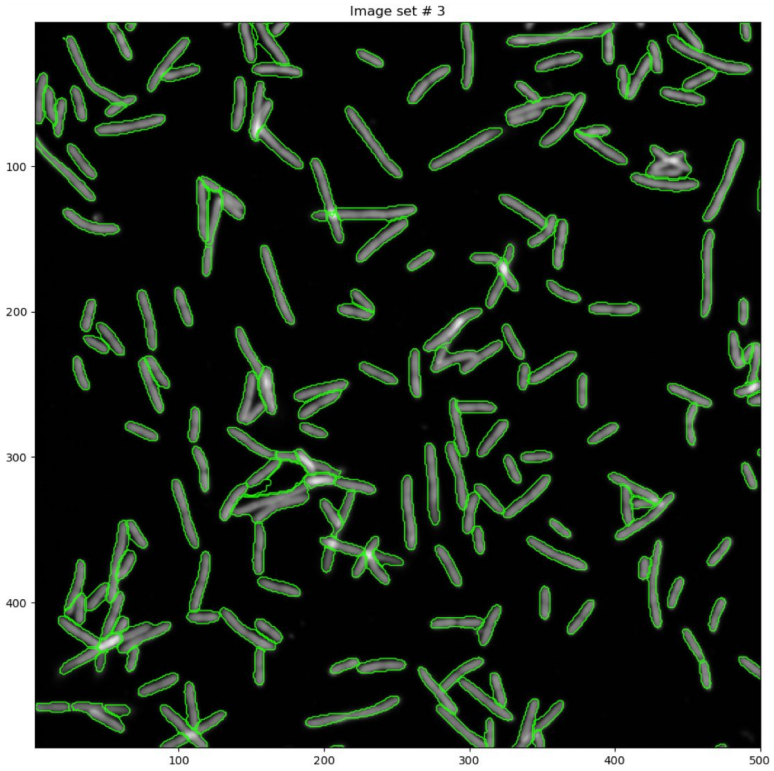


IdentifyPrimaryObjects





RunCellpose

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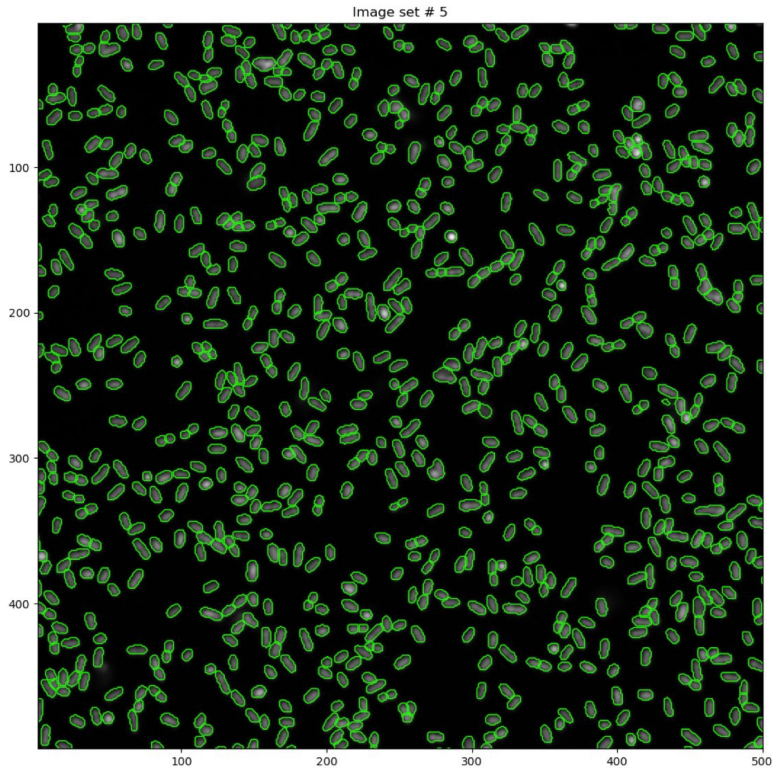
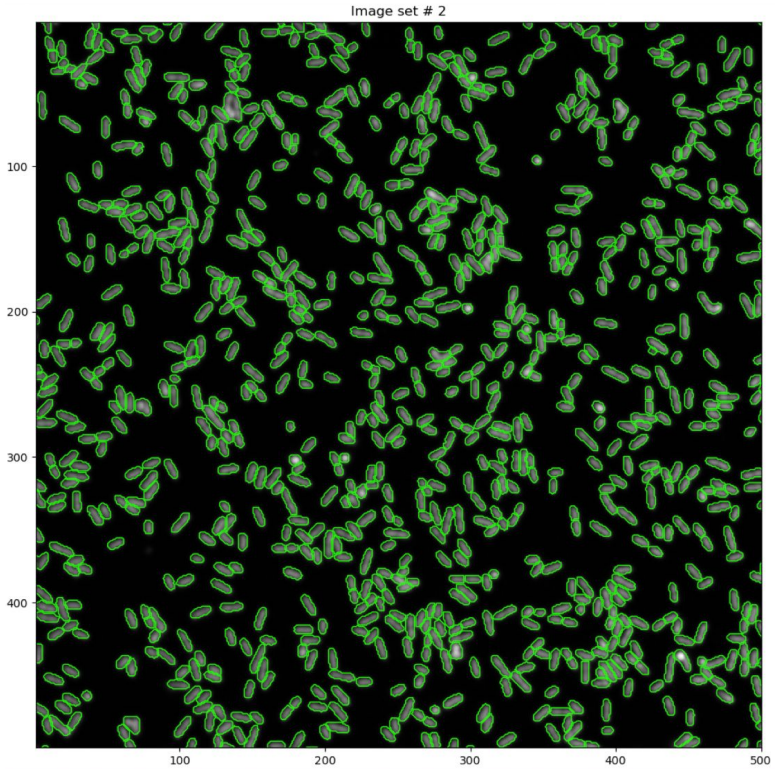
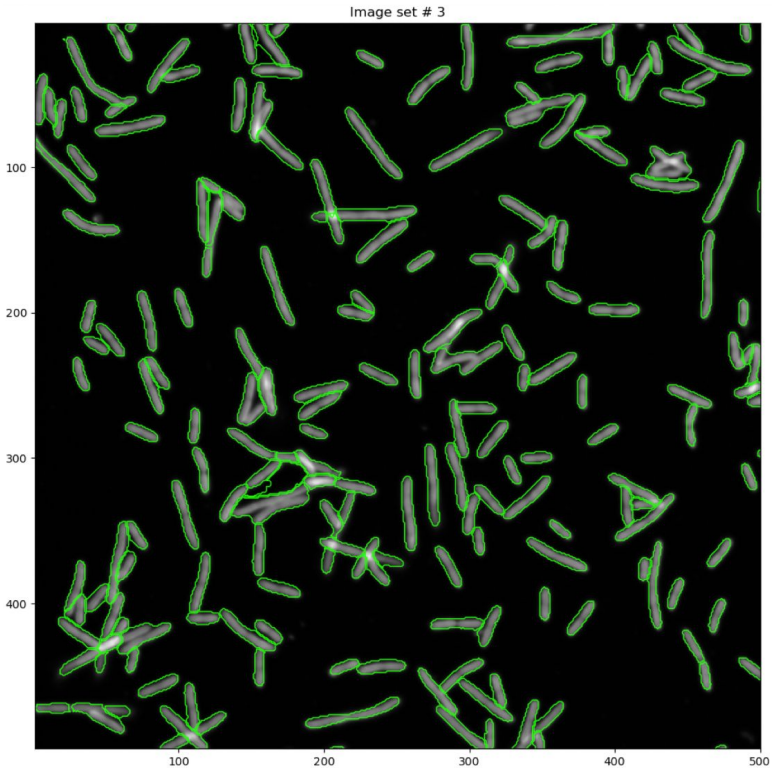
Example 1: Bacteria (2D)



Compound B



Vehicle Control



Compound D

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Example 2: Blastoids (3D)

Why are these cells challenging to segment?

- Heterogeneous size and shape
- Cells overlapping
- Bleaching

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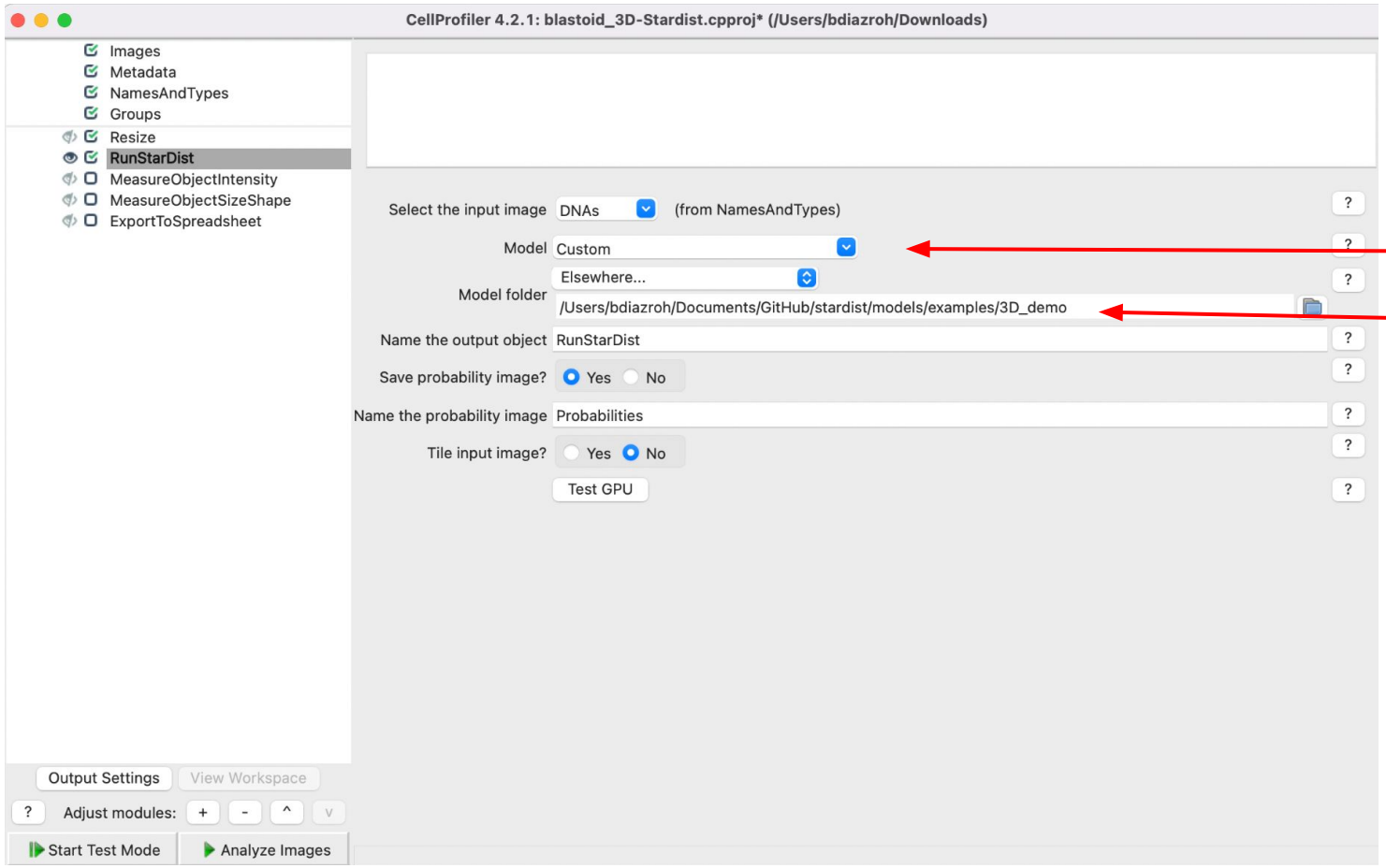
Images courtesy of the Rivron Lab - IMBA Vienna BioCenter

Example 2: Blastoids (3D)

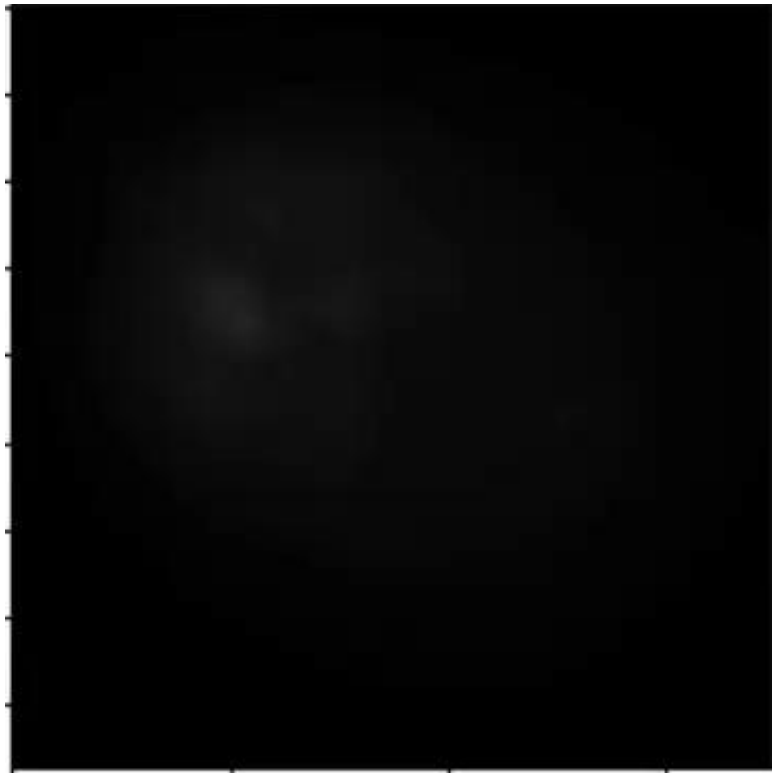


Custom model

Folder where your model is stored

Images courtesy of the Rivron Lab - IMBA Vienna BioCenter

Example 2: Blastoids (3D)



Watershed

RunStarDist

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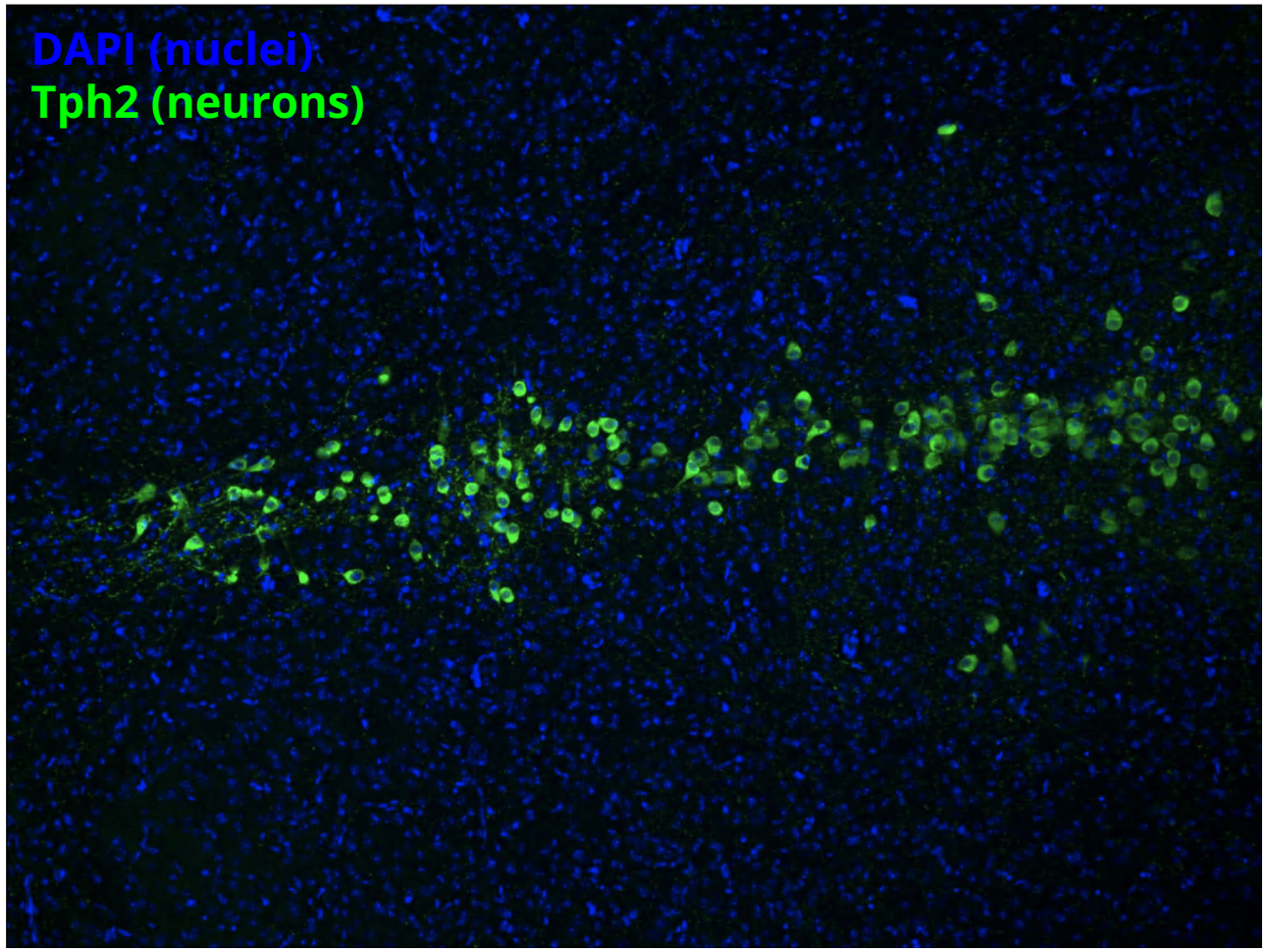
Images courtesy of the Rivron Lab - IMBA Vienna BioCenter

Example 3: Neurons in brain sections (2D)

Fluorescent neurons in mouse brain sections

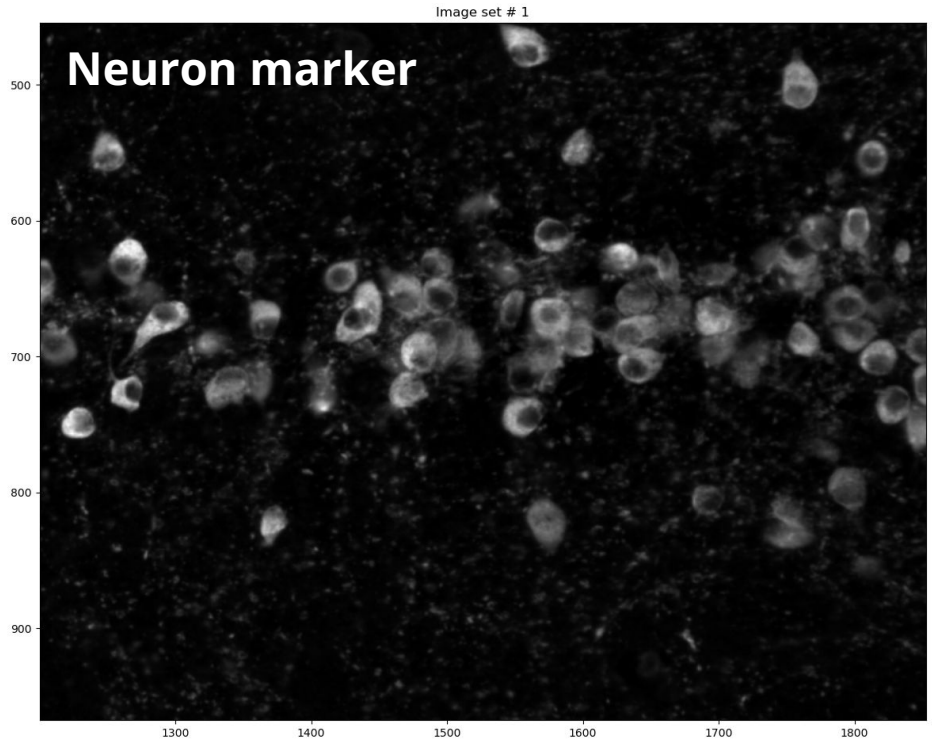


Images courtesy of the Dymecki lab, Harvard Medical School

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



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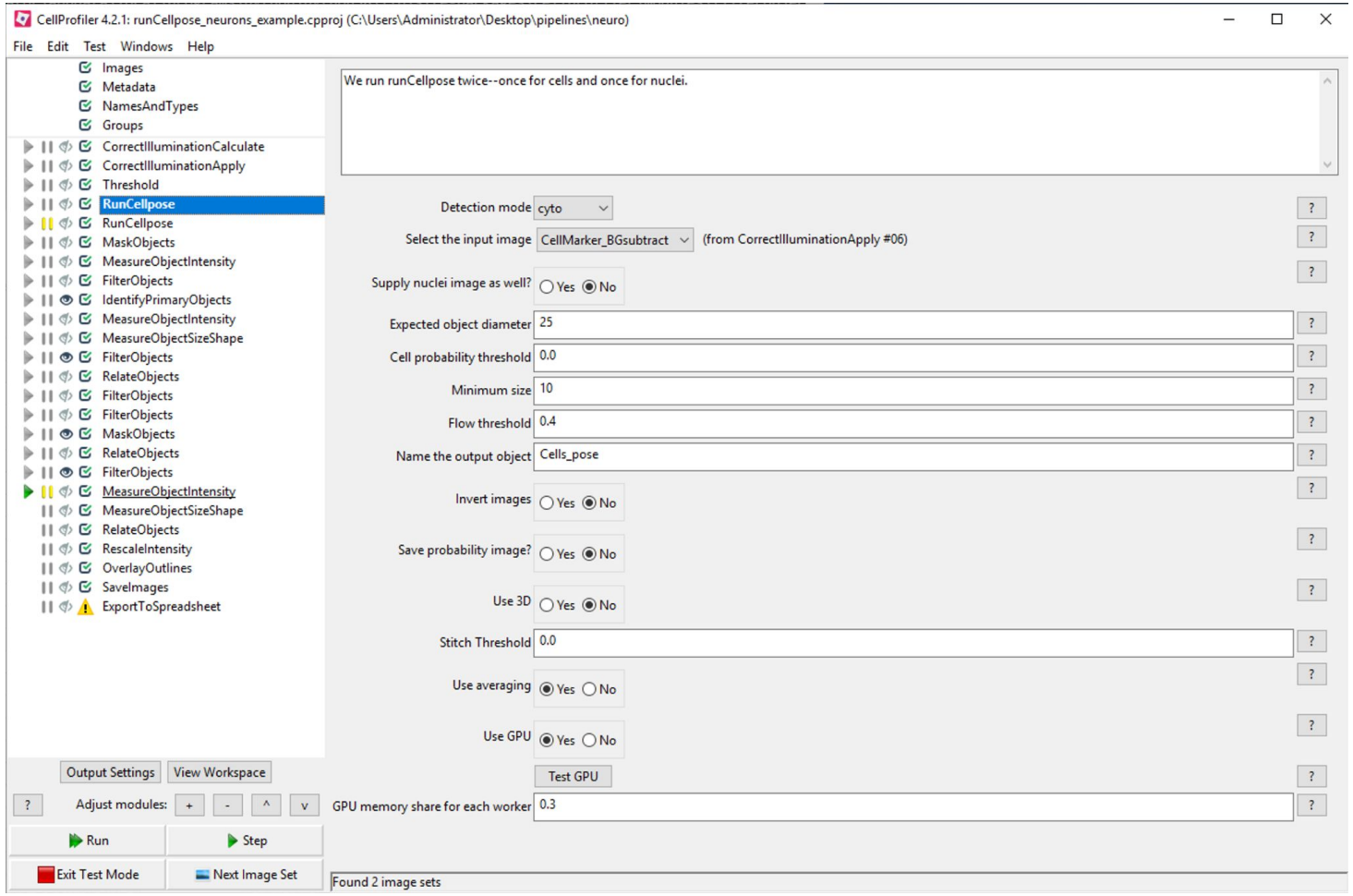
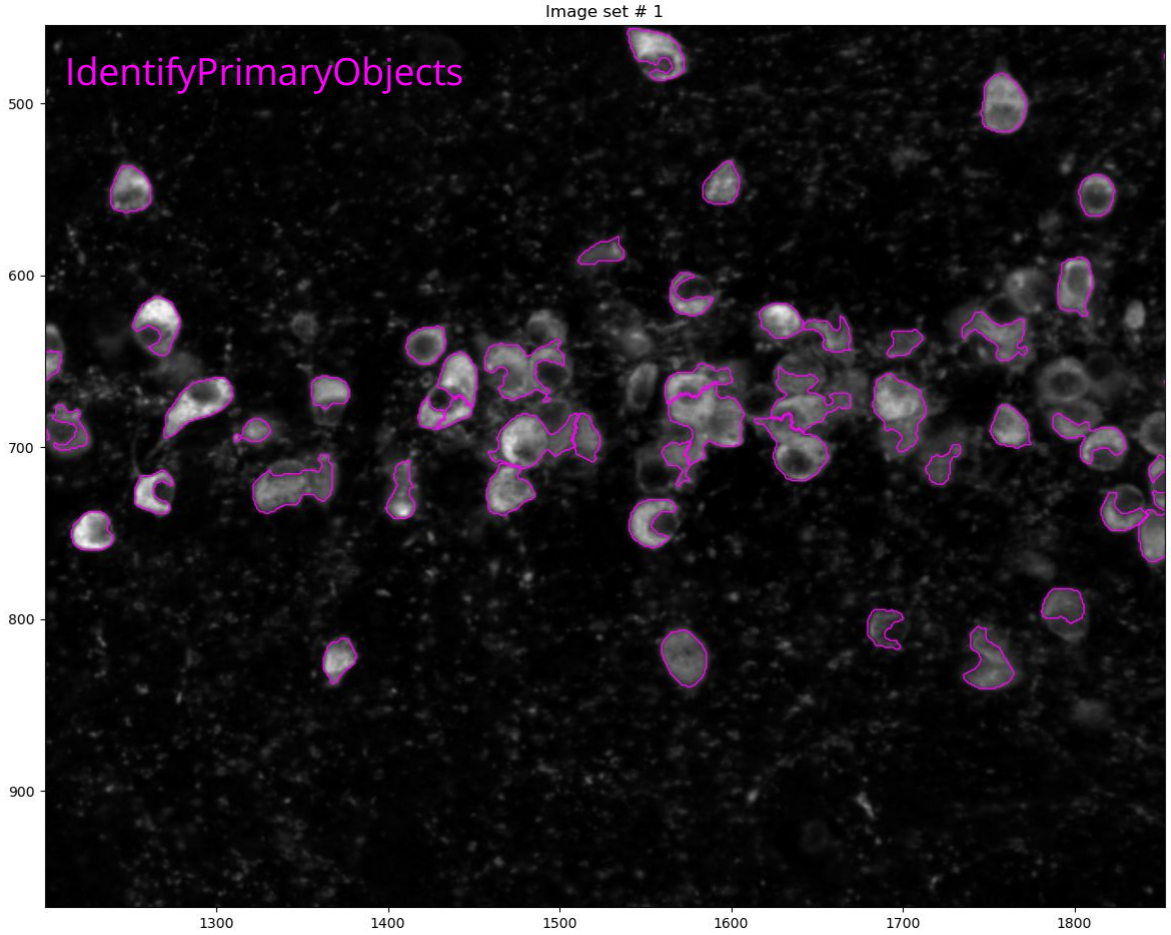
Images courtesy of the Dymecki lab, Harvard Medical School

Example 3: Neurons in brain sections (2D)



Example 3: Neurons in brain sections (2D)

Comparison to IdentifyPrimaryObjects:



Images courtesy of the Dymecki lab, Harvard Medical School

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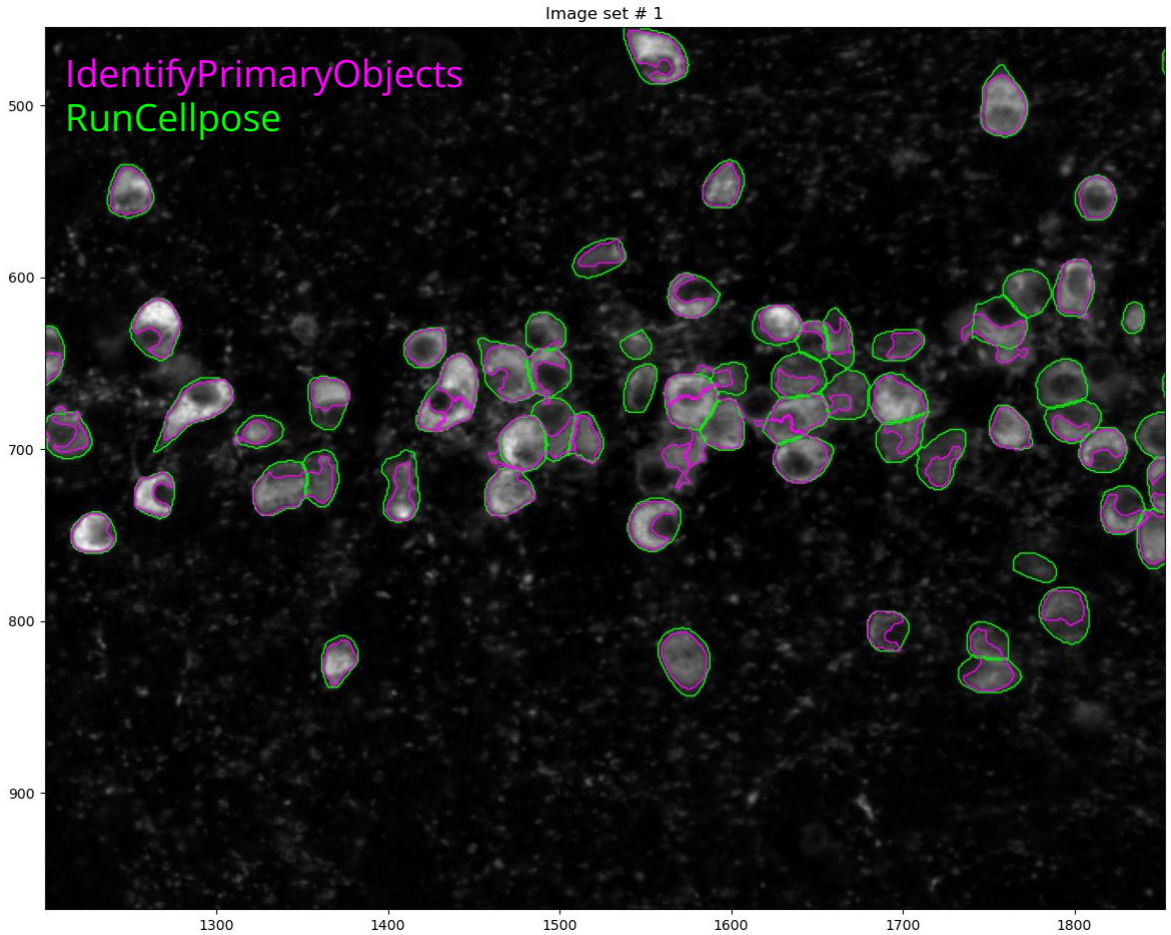

Images courtesy of the Dymecki lab, Harvard Medical School

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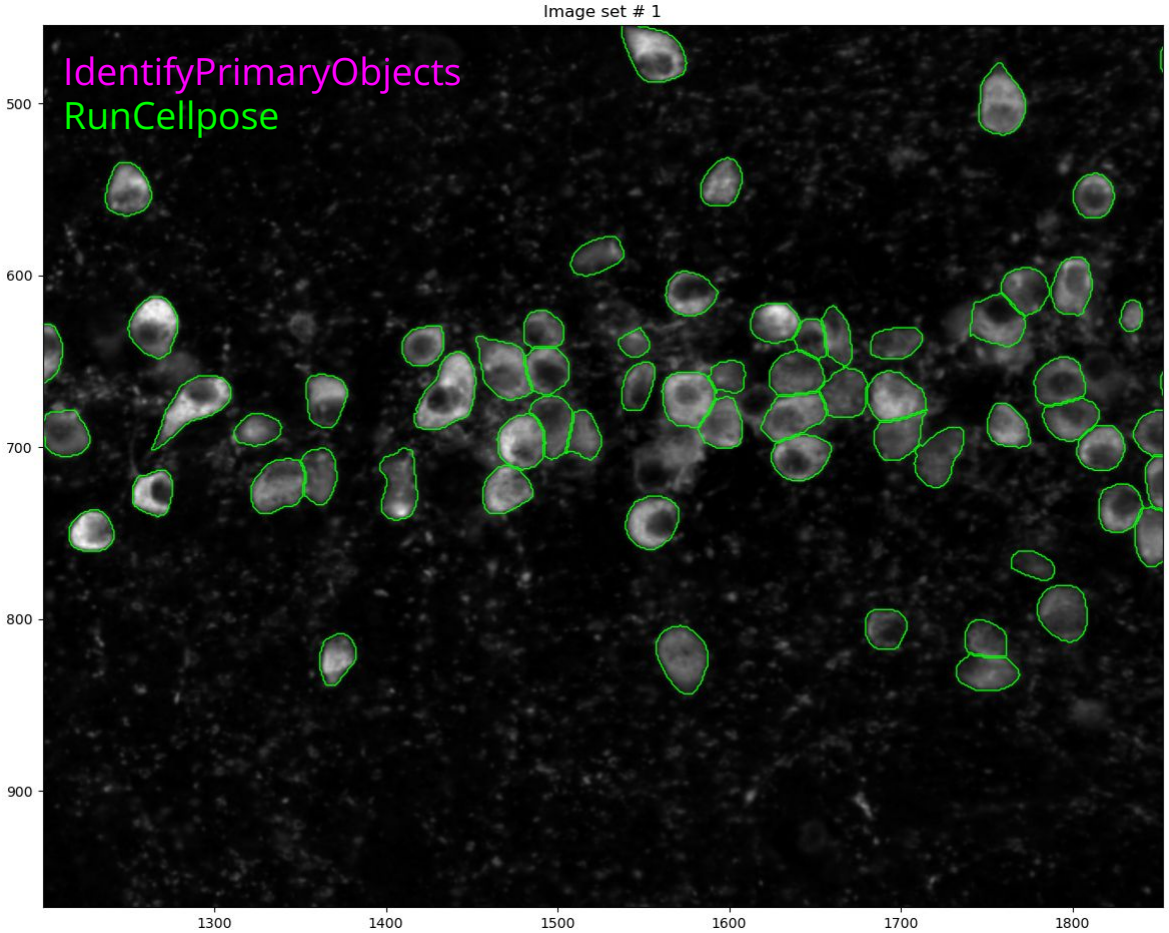


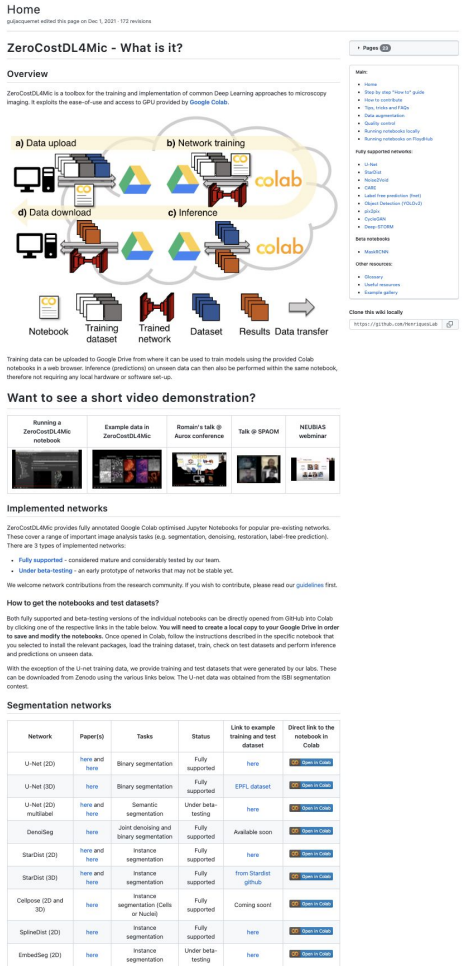
Images courtesy of the Dymecki lab, Harvard Medical School

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



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. Democratising deep learning for microscopy with ZeroCostDL4Mic. Nature Communications, 2021. DOI: https://doi.org/10.1038/s41467-021-22518-0*

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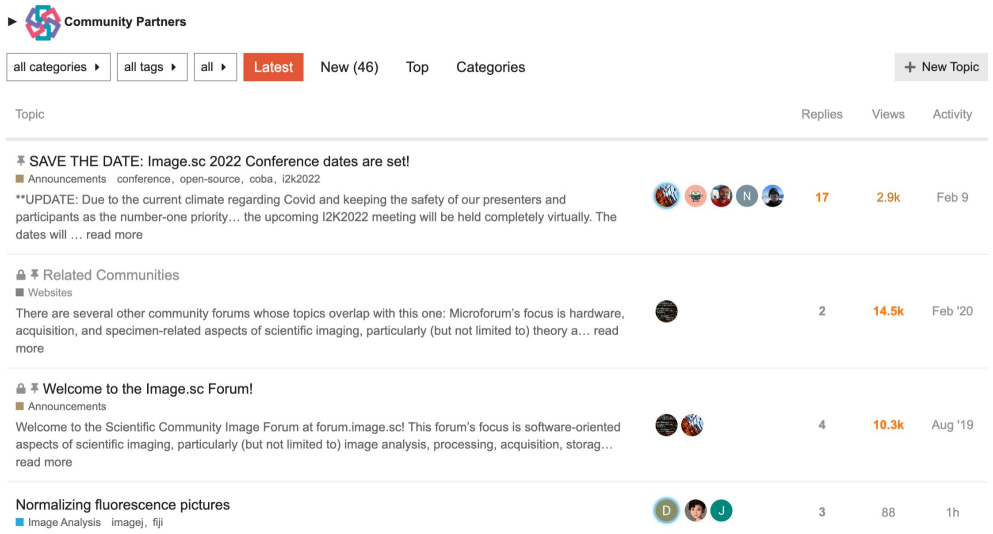
I need more help!

- 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*



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Acknowledgements

Broad Institute Imaging Platform

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Becki Ledford
Levin Moser

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CellProfiler Analyst™ data exploration software


David Stirling

Carpenter-Singh lab members
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COBA

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