Sep 27, 2024

A CellProfiler computational pipeline to quantify localization of PPM1H on mitochondria

DOI
dx.doi.org/10.17504/protocols.io.j8nlk8qk6l5r/v1

Ebsy Jaimon[1], Suzanne R Pfeffer[1]

[1]Stanford University School of Medicine and Aligning Science Across Parkinson's

ASAP Collaborative Rese… Suzanne Pfeffer

Suzanne R Pfeffer
Stanford University School of Medicine

DOI: dx.doi.org/10.17504/protocols.io.j8nlk8qk6l5r/v1

Protocol Citation: Ebsy Jaimon, Suzanne R Pfeffer 2024. A CellProfiler computational pipeline to quantify localization of PPM1H on mitochondria. **protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlk8qk6l5r/v1**

Protocol status: Working
We use this protocol and it's working

Created: September 25, 2024

Last Modified: September 27, 2024

Protocol Integer ID: 108397

Keywords: ASAPCRN, PPM1H phosphatase, mitochondrial localization

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-000463

Abstract

Here we present a CellProfiler (1) software pipeline to quantify the localization of PPM1H on mitochondria. In this work, we have fragmented the mitochondria by hypotonic swelling to facilitate localization and highlight membrane contacts (2); PPM1H-mApple labeled pixels that coincide with GFP-Mito labeled pixels are scored. Wild-type MEFs expressing PPM1H-mApple and GFP-Mito are treated with oligomycin/antimycin (4 hours) and then incubated for 2 minutes in a hypotonic medium. Images of live cells are acquired using a spinning disk confocal microscope. The multi-channel Z-stack images are maximum intensity projected and used for CellProfiler analysis.

Materials

1) .TIFF files from Spinning disk confocal microscope
2) FIJI/imageJ
3) CellProfiler software 4.04 or later*

*Stirling DR, Swain-Bowden MJ, Lucas AM, Carpenter AE, Cimini BA, Goodman A (2021). CellProfiler 4: improvements in speed, utility and usability. BMC Bioinformatics, 22 (1), 433. PMID: 34507520 PMCID: PMC8431850.

Batch process images and import files into CellProfiler

1 Use the FIJI macro as described in **dx.doi.org/10.17504/protocols.io.3byl4bpo8vo5/v1** to maximum intensity Z project the images. Open the folder with the images that need to be processed, choose the channel and the output folder, run code for maximum intensity Z projection, and save the files as .TIFF.

2 Open CellProfiler software. Go to the Images module, drag and drop the maximum intensity projected .TIFF files as indicated. Select “no filtering” in the filter images option.

3 Go to the Metadata module to extract information describing the images.

3.1 In the Metadata module, choose Yes for Extract metadata.

3.2 Metadata extraction method: Extract from image file headers

3.3 Extract metadata from: All images

3.4 Click “Extract metadata”

3.5 Click on Add another extraction method

3.6 Metadata extraction method: Extract from file/folder names

3.7 Metadata source: File name

3.8 Enter regular expression to extract from file name:
“Regex” will be ^_(?P<image_number>[0-9]{2}) for an example file name “Image_22_w1561 Confocal.TIF_max.tif”. This step helps to extract the image number (22) from the file name.
In Regex, ^ indicates the beginning of the file name and (?P<image_number>[0-9]{2}) tells the program to name the captured field “image_number” and recognize two digits that follow. Click the magnifying glass icon on the right to check the accuracy of regular expression.

3.9 Metadata data type: Text

3.10 Click on “update” to populate the metadata field

4 Go to the NamesAndTypes module to give a name to each channel.

4.1 Assign a name to: Images matching rules

4.2 Process as 3D: No

4.3 Match “All” of the following rules

4.4 Select the rule criteria: “File/Does/Contain/w1561”

4.5 Name to assign these images: PPM1H_mApple

4.6 Select the image type: Grayscale image

4.7 Set intensity range from: Image metadata

4.8 Click on Add another image

4.9 Match “All” of the following rules

4.10 Select the rule criteria: “File/Does/Contain/w2488”

4.11 Name to assign these images: GFP_Mito

4.12 Select the image type: Grayscale image

4.13 Set intensity range from: Image metadata

4.14 Click on “update” to populate the names and types field

5 Go to the Groups module and choose No for Do you want to group your images?

Segment GFP-Mito objects

6 Steps 6 and 7 help to reduce noise.

6.1 Click on the “+” sign at the bottom left next to Adjust Modules. Under the module categories, click Advanced, and then choose Gaussian filter.

6.2 Select the input image: GFP_Mito

6.3 Name the output image: Gaussian filter_mito

6.4 Sigma: 2 [Note that larger sigmas induce more blurring]

7 Add ImageMath module to the pipeline

7.1 Operation: Subtract

7.2 Name the output image: ImageAfterMath_mito

7.3 Image or measurement? Image

7.4 Select the first image: GFP-Mito

7.5 Multiply the first image by 1.0

7.6 Image or measurement? Image

7.7 Select the second image: GaussianFilter_mito

7.8 Multiply the second image by 1.0

7.9 Add another image

7.10 Raise the power of the result by 1.0

7.11 Multiply the result by 1.0

7.12 Add to result 0.0

7.13 Set values less than 0 equal to 0? Yes

7.14 Set values greater than 1 equal to 1? Yes

7.15 Replace invalid values with 0? Yes

7.16 Ignore the imaging masks? No

8 Add IdentifyPrimaryObjects module to the pipeline.

8.1 Use advanced settings? Yes

8.2 Select the input image: ImageAfterMath_mito

8.3 Name the primary objects to be identified: Mitochondriaobjects

8.4 Typical diameter of objects, in pixel units (Min, Max): 1,20

8.5 Discard objects outside the diameter range: Yes

8.6 Discard objects touching the border of the image: No

8.7 Threshold strategy: Global

8.8 Thresholding method: Otsu

8.9 Two-class or three-class thresholding? Two classes

8.10 Threshold smoothing scale: 1

8.11 Threshold correction factor: 1

8.12 Lower and upper bounds on threshold 0.0001 and 1.0

8.13 Log transform before thresholding? No

8.14 Method to distinguish clumped objects? Intensity

8.15 Method to draw dividing lines between clumped objects: Intensity

8.16 Automatically calculate size of smoothing filter for declumping? Yes

8.17 Automatically calculate minimum allowed distance between local maxima? Yes

8.18 Speed up by using lower-resolution image to find local maxima? Yes

8.19 Display accepted local maxima? No

8.20 Fill holes in identified objects? After both thresholding and declumping

8.21 Handling of objects if excessive number of objects identified? Continue

9 Check by selecting “Start Test Mode” and select the green triangle next to the IdentifyPrimaryObjects module each time a parameter is changed to find the best parameters for each image set. Test using multiple images to ensure that the settings work for other images in the project.

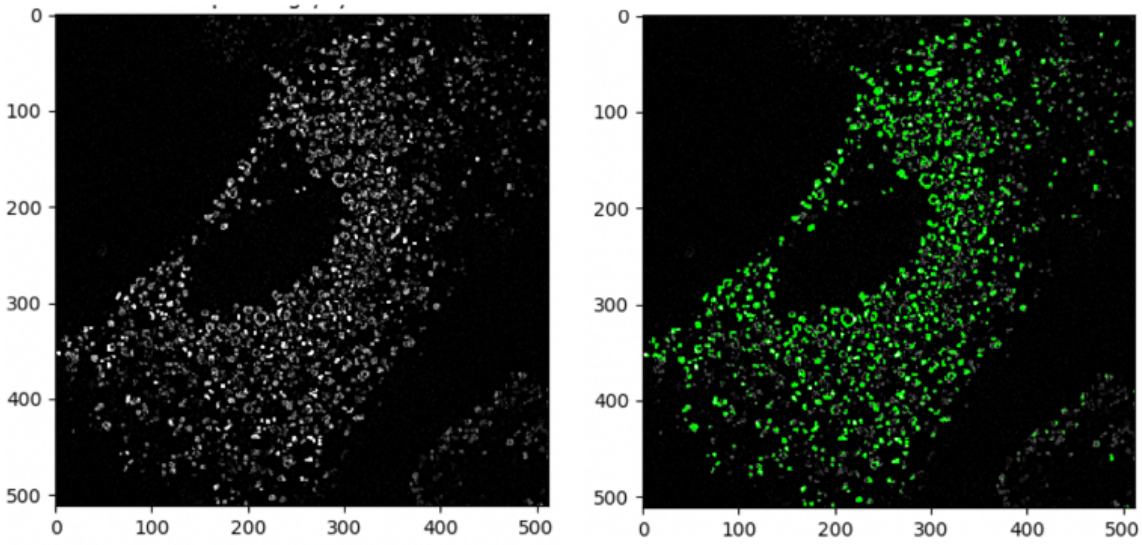

Figure 1: Example of input image (at left) and segmented GFP-Mito objects (at right). Green outlines represent valid objects.

Segment PPM1H-mApple objects

10 Add Gaussian filter module to the pipeline

10.1 Select the input image: PPM1H_mApple

10.2 Name the output image: Gaussian filter_ppm1h

10.3 Sigma: 10 [Note that larger sigmas induce more blurring]

11 Add ImageMath module to the pipeline

11.1 Operation: Subtract

11.2 Name the output image: ImageAfterMath_ppm1h

11.3 Image or measurement? Image

11.4 Select the first image: PPM1H_mApple

11.5 Multiply the first image by 1.0

11.6 Image or measurement? Image

11.7 Select the second image: GaussianFilter_ppm1h

11.8 Multiply the second image by 1.0

11.9 Add another image

11.10 Raise the power of the result by 1.0

11.11 Multiply the result by 1.0

11.12 Add to result 0.0

11.13 Set values less than 0 equal to 0? Yes

11.14 Set values greater than 1 equal to 1? Yes

11.15 Replace invalid values with 0? Yes

11.16 Ignore the imaging masks? No

12 Add IdentifyPrimaryObjects module to the pipeline.

12.1 Use advanced settings? Yes

12.2 Select the input image: ImageAfterMath_ppm1h

12.3 Name the primary objects to be identified: PPM1Hobjects

12.4 Typical diameter of objects, in pixel units (Min, Max): 1,20

12.5 Discard objects outside the diameter range: Yes

12.6 Discard objects touching the border of the image: No

12.7 Threshold strategy: Global

12.8 Thresholding method: Otsu

12.9 Two-class or three-class thresholding? Two classes

12.10 Threshold smoothing scale: 1.2

12.11 Threshold correction factor: 1

12.12 Lower and upper bounds on threshold 0.001 and 1.0

12.13 Log transform before thresholding? No

12.14 Method to distinguish clumped objects? Intensity

12.15 Method to draw dividing lines between clumped objects: Intensity

12.16 Automatically calculate size of smoothing filter for declumping? Yes

12.17 Automatically calculate minimum allowed distance between local maxima? Yes

12.18 Speed up by using lower-resolution image to find local maxima? Yes

12.19 Display accepted local maxima? No

12.20 Fill holes in identified objects? After both thresholding and declumping

12.21 Handling of objects if excessive number of objects identified? Continue

13 Check by selecting “Start Test Mode” and select the green triangle next to the IdentifyPrimaryObjects module each time a parameter is changed to find the best parameters for each image set. Test using multiple images to ensure that the settings work for other images in the project.

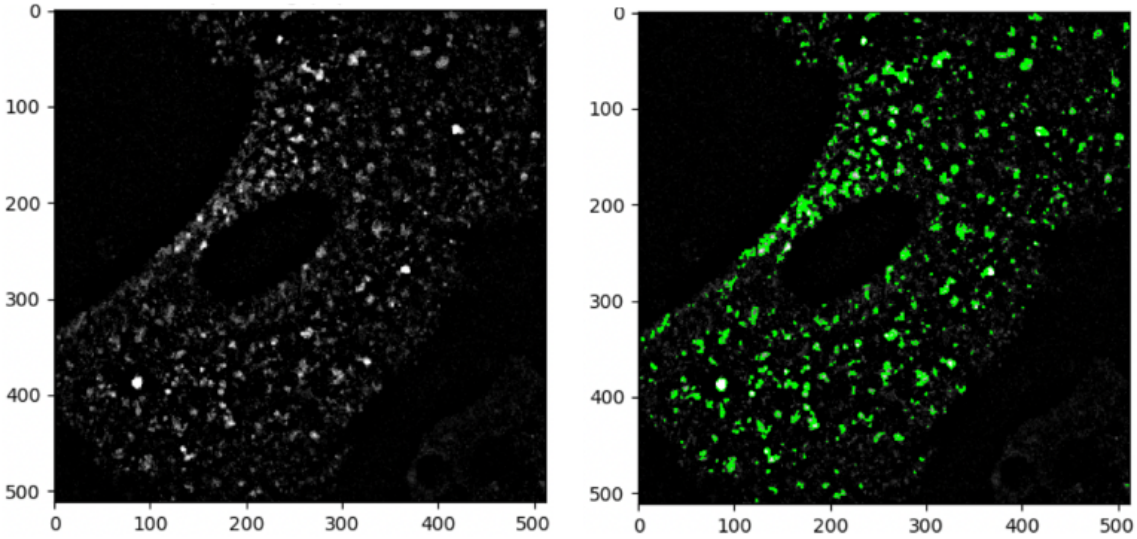

Figure 2: Example of input image (at left) and segmented PPM1H-mApple objects (at right). Green outlines represent valid objects.

Segment regions where PPM1H-mApple overlaps with GFP-Mito

14 Add MaskObjects module

14.1 Select objects to be masked: PPM1H objects

14.2 Name the masked objects: Maskedppm1h

14.3 Mask using a region defined by other objects or by binary image? Objects

14.4 Select the masking object: Mitochondriaobjects

14.5 Invert the mask? No

14.6 Handling of objects that are partially masked: Keep overlapping region

14.7 Numbering of resulting objects: Renumber

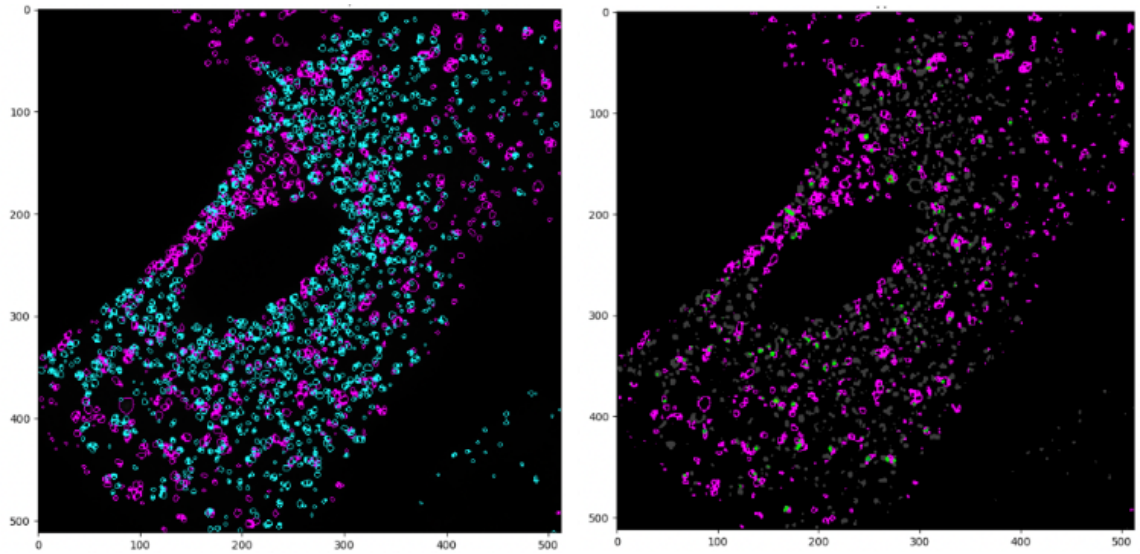

Figure 3: Example of overlay of PPM1H-mApple objects (magenta) and GFP-Mito objects (cyan) (at left). Shown at right is the segmentation used to identify overlapping objects. Green outlines are PPM1H-mApple objects overlapping with GFP-Mito objects and magenta outlines are PPM1H-mApple objects that don't overlap.

Measure the intensity

15 Add MeasureObjectIntensity module.

15.1 Select images to measure: PPM1H_mApple

15.2 Select objects to measure: Choose Maskedppm1h and PPM1Hobjects

16 Add ExportToSpreadsheet module from the + at the bottom to export the measurements into files that can be opened in Excel.

16.1 Select the column delimiter: Tab

16.2 Output file location: choose a folder where images will be saved

16.3 Add a prefix to file names? Yes

16.4 File name prefix: Add experiment identifier

16.5 Overwrite existing files without warning? No

16.6 Add image metadata columns to your object data file? Yes

16.7 Add image file and folder names to your object data file? No

16.8 Representation of Nan/Inf: NaN

16.9 Select the measurements to export? Yes

16.10 Click Press button to select measurements: Under “Maskedppm1h” choose Intensity -> IntegratedIntensity -> PPM1H ->mApple. Under “PPM1Hobjects” choose Intensity -> IntegratedIntensity -> PPM1H -> mApple

16.11 Calculate the per-image mean values for object measurements? No

16.12 Calculate the per-image median values for object measurements? No

16.13 Calculate per-image standard deviation values for object measurements? No

16.14 Create GenePattern GCT file? No

16.15 Export all measurement types? No

16.16 Data to export: Maskedppm1h

16.17 Use the object name for the file name? Yes

16.18 Click Add another data set

16.19 Data to export: PPM1Hobjects

16.20 Combine these object measurements with those of the previous object? Yes

17 Save the pipeline from File-Save Project and click on Analyze Images on the bottom left. The pipeline will run and export the data to the folder previously specified. The output .csv file will have distinct columns indicating image number, intensity of PPM1H-mApple labeled pixels that coincide with GFP-Mito labeled pixels, and intensity of all PPM1H-mApple labeled pixels.

Protocol references

(1) Stirling DR, Swain-Bowden MJ, Lucas AM, Carpenter AE, Cimini BA, Goodman A (2021). CellProfiler 4: improvements in speed, utility and usability. BMC Bioinformatics, 22 (1), 433.

(2) King C, Sengupta P, Seo AY, Lippincott-Schwartz J. ER membranes exhibit phase behavior at sites of organelle contact. Proc Natl Acad Sci U S A. 2020 Mar 31;117(13):7225-7235. doi: 10.1073/pnas.1910854117.