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CellProfiler Pipeline to Obtain Pearson's correlation coefficients for TMEM55B or pRab10 and RILPL1

Chloe A Hecht[1,2], Suzanne Pfeffer[1,2]

[1]Department of Biochemistry, Stanford University School of Medicine; [2]Aligning Science Across Parkinson's Disease

Chloe A Hecht

ABSTRACT

We present here a CellProfiler software pipeline to quantify the intensity of endogenous TMEM55B or pRab10 in LRRK2 R1441C or VPS35 D620N MEF cells transfected with a Myc-RILPL1 plasmid detected using an anti-Myc antibody in conjunction with 4X expansion super-resolution microscopy or regular immunofluorescence microscopy. This protocol works in conjunction with Nikon Spinning disk confocal images acquired with Metamorph software (Molecular Devices, LLC) or Zeiss laser scanning confocal microscope acquired using Zen 3.4. Acquired files have a .nd or a .czi format respectively; we use the associated .TIF raw files in this pipeline. Images are maximum intensity projected from the Z-stack acquired during imaging and used for CellProfiler analysis.

MATERIALS

Latest edition CellProfiler 4.04 (or later)
Maximum intensity projection raw .TIF files that accompany .nd files by Metamorph
or
Maximum intensity projection raw .TIF files that accompany .czi files by Zen 3.4

Immunofluoresence Microscopy TMEM55B or pRab10 and M..

1 The method involves the following steps:
Step 1 - Import data and extract metadata from file names
Step 2 - Group individual channels from each image
Step 3 - Rescale image intensities
Step 4 - Identify primary objects as RILPL1 positive cells
Step 5 - Measure colocalization
Step 6 - Save identified masked cells
Step 7 - Export to Spreadsheet

2 Select **Images** module at the upper right and drop images as indicated

3 In the **Metadata** tab. Extract metadata? Yes.

3.1
- Metadata extraction method: Extract from image file headers
- Extract metadata from: All images
- Metadata data type: Text
- Hit “extract metadata”
- Hit “update” to populate the metadata field

4 For .nd files captured using Metamorph:
In the **NamesAndTypes** tab:

4.1
- Assign a name to: image matching rules
- Process as 3D? No
- Match *All* of the following rules: File/Does/Contain/w1405
- Name to assign to these images “DAPI”
- Select the image type: Grayscale image
- Set intensity range from: Image metadata
- Add two more channels and repeat the above commands, naming the channels
- File/Does/Contain: w2488 as “RILPL1”
- File/Does/Contain: w3561 as “TMEM55b”
- Hit “update” so Cellprofiler will process the commands and sort the images based on channel.

4.2 For .czi files acquired using Zen 3.4:
In the **NamesAndTypes** tab:

4.3
- Add two more images using “add another image tab” at the bottom
- In the first image block:
- Assign a name to: image matching rules
- Process as 3D? No
- Match All of the following rules
- Select the rule criteria: Metadata does Have C matching, 0
- Name to assign these images: TMEM55b
- Select the image type: Grayscale image
- Set intensity range from: Image metadata
- In the second image block:
- Assign a name to: image matching rules
- Process as 3D? No
- Match All of the following rules
- Select the rule criteria: Metadata does Have C matching, 0
- Name to assign these images: RILPL1
- Select the image type: Grayscale image
- Set intensity range from: Image metadata
- In the third image block:
- Assign a name to: image matching rules
- Process as 3D? No
- Match All of the following rules
- Select the rule criteria: Metadata does Have C matching, 0
- Name to assign these images: DAPI
- Select the image type: Grayscale image
- Set intensity range from: Image metadata
- Image set matching method: order
- Hit “update” at the bottom
- The chart should populate with values in three channels: TMEM55b, RILPL1, and DAPI

5 For both types of images:
In the **Groups** tab:

5.1
- Do you want to group your images? Yes
- Metadata category: treatment.
- Cellprofiler should sort based on the treatment as identified in the metadata file name expression tab.

6 Using the “+” sign at the bottom next to Adjust Modules, add **RescaleIntensity** module

6.1
- Select the input image: RILPL1
- Name the output image: rescale_RILPL1
- Rescaling method: Divide each image by the same value
- Divisor value: 0.08 for R1441C MEF or 0.3 for VPS35 D620N MEF

6.2

Note

- The RILPL1 channel should be significantly oversaturated. This is so cell profiler can easily identify mycRILPL1 positive cells, and not cells without mycRILPL1 transfection.
- This can be checked by clicking “Start Test Mode” and hitting the green triangle next to the RescaleIntensity module.

7 Add **IdentifyPrimaryObjects** module from the “+” sign at the bottom:

7.1
- Use advanced settings? Yes
- Select the input image: rescale_RILPL1
- Name the primary objects to be identified: RILPL1cell
- Diameter of objects, in pixel units: 50 - 600 for R1441C MEF or 150-700 for VPS35 D620N MEF

Note

- This will be different for each image set.
- This can be checked by clicking “Start Test Mode” and hitting the green triangle next to the IdentifyPrimaryObjects module.

- Discard objects outside the diameter range? Yes
- Discard objects touching the border of the image? No
- Threshold strategy? Global
- Thresholding method? Otsu
- Two-class or three-class thresholding? Two classes

- Threshold smoothing scale 1.7 for R1441C MEF or 3 for VPS35 D620N MEF
- Threshold correction factor .6 for R1441C MEF or 0.4 for VPS35 D620N MEF

> Note
>
> - These values will need to be optimized for each image set.
> - Again, this can be checked by clicking “Start Test Mode” and hitting the green triangle next to the IdentifyPrimaryObjects module each time a parameter is changed to find the best parameters for each image set.

- Lower and upper bounds on threshold? 0 to 1 for R1441C MEF or 0.002 to 1.0 for VPS35 D620N MEF
- Log transform before thresholding? No
- Method to distinguish clumped objects? Intensity
- Method to draw dividing lines between clumped objects? Shape
- Automatically calculate size of smoothing filter for declumping? Yes
- Automatically calculate size for smoothing filter for declumping? Yes
- Automatically calculate minimum allowed distance between local maxima? Yes
- Speed up by using lower-resolution image to find local maxima? Yes
- Display accepted local maxima? No
- Fill holes in identified objects? After both thresholding and declumping
- Handling of objects if excessive number of objects identified? Continue

8 Add **MeasureColocalization** module from the “+” sign at the bottom

8.1
- Select images to measure: pRab10 or TMEM55b and RILPL1
- Set threshold as percentage of maximum intensity for the images: 15
- Select where to measure correlation: within objects, RILPL1cell

> Note
>
> This allows for colocalization to be measured only in mycRILPL1 positive cells.

- Run all metrics: No
- Calculate correlation and slope metrics? Yes
- Select “No” for the rest of the colocalization measures

Note

For our purposes we were only interested in the Pearson's Correlation coefficient.

9 Add the **ConvertObjectstoImage** module and the **SaveImages** module from the + at the bottom

9.1 In the **ConvertObjectstoImage** module:

- Select the input object: RILPL1cell
- Name the output image: IdentifyRILPL1
- Select the color format: Color
- Select the colormap: default

In the **SaveImages** module:

- Select type of image to save: image
- Select the image to save: IdentifyRILPL1
- Constructing file names: From image filename
- Image name for file prefix: RILPL1
- Append a suffix to the image file name? No
- Saved file format: tiff
- Image bit depth: 8-bit integer
- Save with lossless compression? Yes
- Output file location: choose a folder where images should be saved
- Overwrite existing files without warning? Yes
- When to save? Every cycle
- Record the file and path information to the saved image? No
- Create subfolders in the output folder? No

Note

These two modules allow you to save CellProfiler's interpretations of identified cells. This is useful when checking each image to ensure a good interpretation.

10 Add the **ExportToSpreadsheet** module from the + at the bottom

10.1

- Select the column delimiter: Tab
- Output file location: choose a folder where you want the images to be saved.
- Add a prefix to file names? Yes. Add date and experiment identifier. Ie. RILPL1March_
- Overwrite existing files without warning? Yes
- Note: This allows you to run the pipeline multiple times without having to individually ask the pipeline to rewrite a file.
- Add image metadata columns to your object data file? Yes
- Add image file and folder names to your object data file? Yes
- Representation of Nan/Inf: NaN
- Select measurements to export? Yes
- Press button to select measurements: under “RILPL1cell” select: Correlation, FileName, and PathName
- Calculate the per-image mean values for object measurements? No
- Calculate the per-image median values for object measurements? No
- Calculate the per-image standard deviation values for object measurements? No
- Create GenePattern GCT file? No
- Export all measurement types? No
- Export all measurement types? Yes
- Click “Analyze Images”
- The pipeline will run and export the data to the folder previously specified

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Step 4 - Export the data

12 Select **Images** module at the upper right and drop images into box as indicated

13 In the **Metadata** tab. Extract metadata? No.

14 In the **NamesAndTypes** tab:

14.1
- assign a name to: image matching rules
- Process as 3D? No
- Match All of the following rules: File/Does/Contain/w1405
- Name to assign to these images “DAPI”
- Select the image type: Grayscale image
- Set intensity range from: Image metadata
- Add two more channels and repeat the above commands, naming the channels
- File/Does/Contain: w2488 as “RILPL1”
- File/Does/Contain: w3561 as “TMEM55b”
- Hit “update” so Cellprofiler will process the commands and sort the images based on channel.

15 In the **Groups** tab:
- Do you want to group your images? No.

16 Add **MeasureColocalization** module from the “+” sign at the bottom

16.1
- Select images to measure: TMEM55b and RILPL1
- Set threshold as percentage of maximum intensity for the images: 15
- Select where to measure correlation: across entire image
- Run all metrics: No
- Calculate correlation and slope metrics? Yes
- Select “No” for the rest of the colocalization measures

Note

For our purposes we were only interested in the Pearson’s Correlation coefficient.

17 Add the **ExportToSpreadsheet** module from the + at the bottom

17.1
- Select the column delimiter: Tab
- Output file location: choose a folder where you want the images to be saved.

- Add a prefix to file names? Yes. Add date and experiment identifier. Ie. ExM_
- Overwrite existing files without warning? Yes

> Note
>
> This allows you to run the pipeline multiple times without having to individually ask the pipeline to rewrite a file.

- Note: This allows you to run the pipeline multiple times without having to individually ask the pipeline to rewrite a file.
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- Select measurements to export? Yes
- Press button to select measurements: under “Image” select: Correlation, FileName, and PathName
- Calculate the per-image mean values for object measurements? No
- Calculate the per-image median values for object measurements? No
- Calculate the per-image standard deviation values for object measurements? No
- Create GenePattern GCT file? No
- Export all measurement types? No
- Export all measurement types? Yes