Jan 09, 2026

An ImageJ/FIJI Preprocessing Workflow for Multi-Series Confocal Microscopy Datasets Prior To CellProfiler Analysis

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8xjzdv2w/v1

Aaran Vijayakumaran[1,2], Suzanne Pfeffer[1,2]

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

Team Alessi

Suzanne R Pfeffer
Stanford University School of Medicine

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl8xjzdv2w/v1

Protocol Citation: Aaran Vijayakumaran, Suzanne Pfeffer 2026. An ImageJ/FIJI Preprocessing Workflow for Multi-Series Confocal Microscopy Datasets Prior To CellProfiler Analysis. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.5jyl8xjzdv2w/v1

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

Created: January 09, 2026

Last Modified: January 09, 2026

Protocol Integer ID: 238352

Keywords: cellprofiler analysis the overall aim, cellprofiler analysis, tiff file, cellprofiler, using cellprofiler, intensity projection, intensity projections for user, independent image dataset, step imagej, container of independent image dataset, dimensional image, fiji preprocessing workflow, embedded dataset, imagej, standalone ome

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

Abstract

The overall aim of this workflow is to generate three-slice maximum-intensity projections (MIPs) in an effort to preserve weak, spatially localized signals that can be missed when projecting across the entire Z-stack. For this purpose, a two-step ImageJ/FIJI workflow was developed.

In the first step, a multi-series .czi file is treated as a container of independent image datasets, and each embedded dataset is extracted and exported as a standalone OME-TIFF file, preserving all acquired channels as full Z-stacks. In the second step, these per-dataset stacks are processed to generate three-slice maximum-intensity projections for user-defined channels. The resulting two-dimensional images are saved with a consistent naming scheme, enabling straightforward downstream grouping and high-throughput quantitative analysis using CellProfiler.

Method Summary

1 Prompt the user to select a multi-series .czi input file

0.2 Prompt the user to select an output directory

0.3 Import the multi-series .czi file using Bio-Formats

0.4 Separate the embedded datasets (series) within the file

0.5 Export each dataset as an individual OME-TIFF file containing all channels

0.6 Prompt the user to select the folder containing the per-dataset stacks generated in Script A

0.7 Open each dataset as a hyperstack and read its dimensions

0.8 Process the Z-stack in consecutive groups of three slices

0.9 For each Z-block, extract user-defined channels

0.10 Generate a maximum-intensity projection for each channel and save as a 2D TIFF image

Procedure

1 Drag and drop the **Script A** macro into ImageJ/Fiji (or run via Plugins → Macros → Run...)

2 Select the multi-series .czi file when prompted

3 Select an output folder when prompted

4 The macro automatically exports each embedded image dataset as a separate OME-TIFF file like this: ‘Series cindexe_AllChannels.ome.tiff’

5 No additional parameters need to be set or modified

6 Close Script A

7 Drag and drop the **Script B** macro into ImageJ/Fiji (or run via Plugins → Macros → Run...)

8 Select the folder containing the per-dataset OME-TIFF stacks (these are the output files generated by Script A)

9 For each input stack, the macro automatically creates a subfolder named after the input file (excluding the file extension) [Series_cindexe_AllChannels]

10 Each dataset is processed independently as follows:
The image is opened as a hyperstack
The Z-stack is processed in consecutive groups of three slices
For each set of three slices, a maximum-intensity projection (MIP) is generated for each user-defined channel

11 Projected images are saved as 2D TIFF files using the naming convention: ‘MIP_cstartSlicee_cchannelNamee.tif’, where cstartSlicee indicates the first slice of the three-slice group.

User-Editable Settings

12 Line 48-51: which channels are exported and what they are named.
saveChannelMIP(1, "DARFF32");
saveChannelMIP(2, "pSNCA");
saveChannelMIP(3, "DAPI");
saveChannelMIP(4, "CHAT");
How to edit:
Replace the channel index (first argument) to match your acquisition order

Replace the channel name (second argument) to match the label you want in the output filename
Example: To change to “DAPI and GFP”, replace the code above with this:
saveChannelMIP(1, "DAPI");
saveChannelMIP(2, "GFP");

13 Line 39: Projection Type
This code is currently for maximum intensity projection on Z-stacks. This can be changed to
Average intensity (projection=[Average Intensity]) or
sumslices (projection=[Sum Slices])

14 Line 25 - 29: Number of Z-slices per projection
Three-slice grouping is controlled by the Z-loop step size and the stop calculation.
How to edit:
For example, for 5-slice projections (rather than 3):
change z += 3 to z += 5 (line 25)
and stop = z + 2 to stop = z + 4 (line 28)

Protocol references

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.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012 Jun 28;9(7):676-82. doi: 10.1038/nmeth.2019. PMID: 22743772; PMCID: PMC3855844.

Acknowledgements

Procedure:

1. Drag and drop the **Script A** macro into ImageJ/Fiji (or run via Plugins → Macros → Run...)
2. Select the multi-series .czi file when prompted
3. Select an output folder when prompted
4. The macro automatically exports each embedded image dataset as a separate OME-TIFF file like this: 'Series □cindex□e_AllChannels.ome.tiff'

No additional parameters need to be set or modified

5. Close Script A
6. Drag and drop the **Script B** macro into ImageJ/Fiji (or run via Plugins → Macros → Run...)
7. Select the folder containing the per-dataset OME-TIFF stacks (these are the output files generated by Script A)
8. For each input stack, the macro automatically creates a subfolder named after the input file (excluding the file extension) [Series_□cindex□e_AllChannels]
9. Each dataset is processed independently as follows:
   a. The image is opened as a hyperstack
   b. The Z-stack is processed in consecutive groups of three slices
   c. For each set of three slices, a maximum-intensity projection (MIP) is generated for each user-defined channel

10. Projected images are saved as 2D TIFF files using the naming convention: 'MIP_□cstartSlice□e_□cchannelName□e.tif', where □cstartSlice□e indicates the first slice of the three-slice group.

User-Editable Settings:

Line 48-51: which channels are exported and what they are named.

- `saveChannelMIP(1, "DARFF32");`
- `saveChannelMIP(2, "pSNCA");`
- `saveChannelMIP(3, "DAPI");`
- `saveChannelMIP(4, "CHAT");`

How to edit:

- Replace the channel index (first argument) to match your acquisition order
- Replace the channel name (second argument) to match the label you want in the output filename

Example: To change to "DAPI and GFP", replace the code above with this:

- `saveChannelMIP(1, "DAPI");`
- `saveChannelMIP(2, "GFP");`

Line 39: Projection Type

This code is currently for maximum intensity projection on Z-stacks. This can be changed to

- Average intensity (projection=[Average Intensity]) or
- sumslices (projection=[Sum Slices])

Line 25 - 29: Number of Z-slices per projection

Three-slice grouping is controlled by the Z-loop step size and the stop calculation.

How to edit:

- For example, for 5-slice projections (rather than 3):
  - change z += 3 to z += 5 (line 25)
  - and stop = z + 2 to stop = z + 4 (line 28).