Analysis of cell protrusions using Fiji

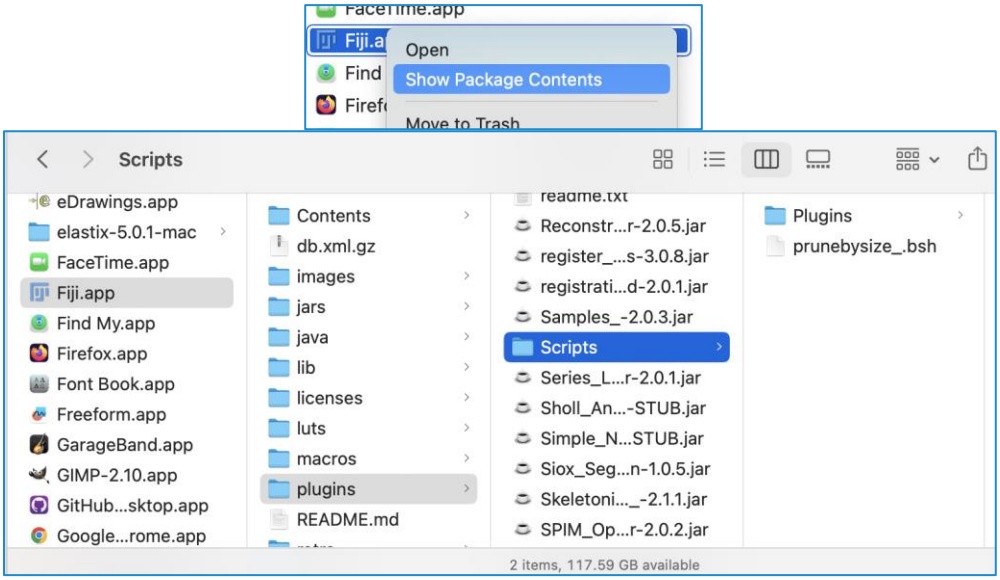
Overview

- This protocol can be used to assess protrusion length in 2D fluorescence images of cells.
- Unlike traditional neurite tracing, this method does not require cells to be well separated, and does not require protrusions to be traceable to a cell body. The protrusion segments are detected and measured individually.
- The number of cell bodies in the image is also counted o assist with normalization to cell number.
- **Limitations**:
    - If protrusions cross, the length will be underestimated, because each segment up to the intersection is measured separately.
    - Cell bodies are included in the skeleton, leading to overestimation of protrusion length. However, they contribute a relatively small proportion of overall length in normal cells.

Setup

Fiji and custom scripts are needed for the image analysis.

1. Download and install **Fiji** from https://imagej.net/software/fiji/ .
2. Download two custom scripts from https://github.com/CUIMC-Confocal/zhiguozhang :
    2.1. ***analyze_protrusions.ijm***
    2.2. ***prunebysize.bsh***
3. Move the script **prunebysize_.bsh** into Fiji's *plugins/scripts* folder. To see this folder on Mac, right-click Fiji.app > *Show Package Contents*.

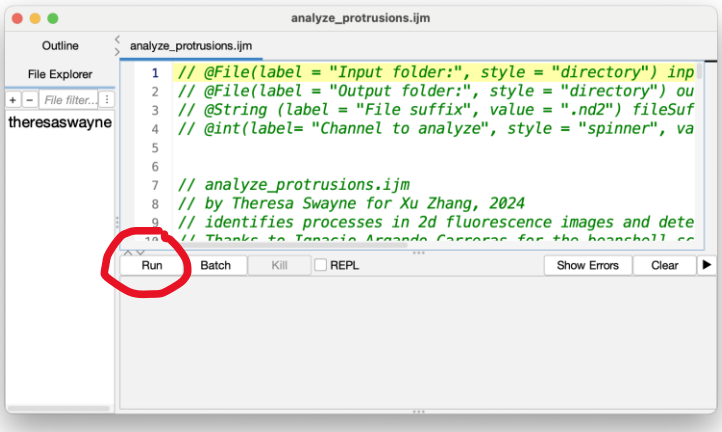


Data preparation

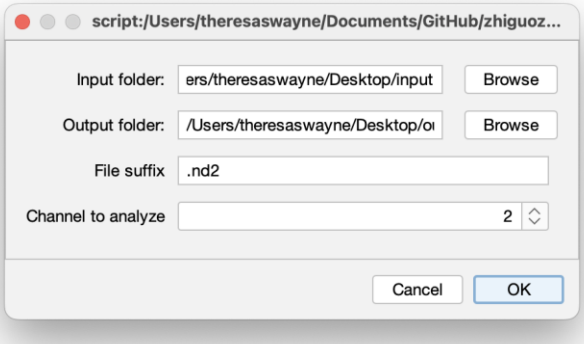
- Requirements/assumptions
  - Input images must be **2D** (not Z stack); they can be max projections of a Z stack.
  - Images must be **single timepoints**.
  - Images can be multichannel. You can specify which channel is used for the analysis.
  - Images can be any format that Fiji can read, including microscope formats such as .ND2.
  - The protocol was developed for images with a **pixel size of 0.37 µm**.
- Batch processing
  - The script will process all the images within a folder. Place all of the images you want to analyze in a single folder.

Image analysis

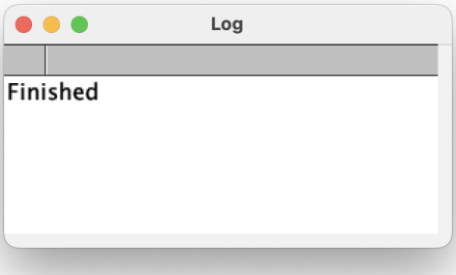
1. In Fiji, open the script ***analyze_protrusions.ijm***
2. In the Script Editor window, click **Run**.



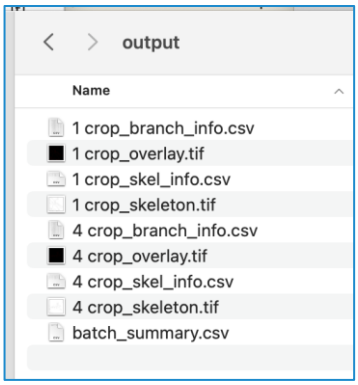
3. Set the input and output folders, the file suffix, and the channel to analyze, then click OK.



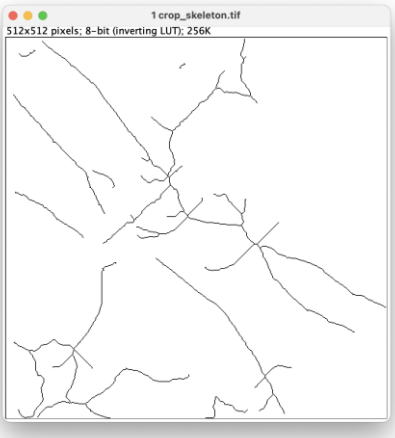
4. When all images have been processed, the Fiji Log will show "Finished”:

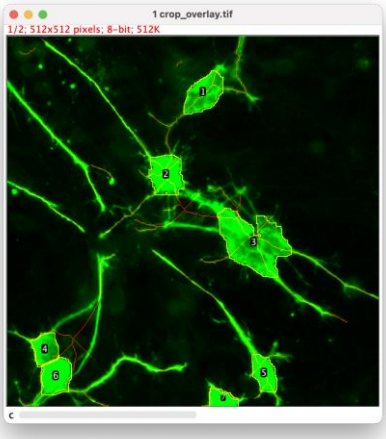


5. Check the designated output folder. It should contain 2 output images and 2 data tables for each input image, and a **batch summary**.



5.1. The output images are the **skeleton** (detected protrusions) and an **overlay** of the original fluorescence with the detected cell bodies and skeleton. These may be useful for visually validating the results.





5.2 The data tables for each image are the "skeleton info" and the "branch info."

- The **branch info** contains the important length information about the individual protrusion segments. The Branch Length is used to calculate the results in the batch summary.
- The **skeleton info** relates to distinct skeletons in the image, and is not really informative if the cell processes overlap.

1 crop_branch_info.csv

Skeleton ID	Branch length	V1 x	V1 y
1	88.296	9	76
1	69.983	63	139
1	27.556	113	200
1	16.899	132	236

1 crop_skel_info.csv

	# Branches	# Junctions	# End-point voxels	# Junction voxels	# Slab voxels
1	9	4	2	8	166
2	23	11	6	17	678
3	4	2	1	3	20
4	50	24	19	52	1443
5	2	1	1	1	97

6. The **batch summary** contains the derived data for the whole folder (one row per input image).
   - 6.1. **Total Length** is the sum of all skeleton segments in the image
   - 6.2. **Number of cells** is the number of detected cell bodies
   - 6.3. **Normalized length** is total length divided by the number of cells.
   - 6.4. **Median branch length** is the median length of all skeleton segments in the image.

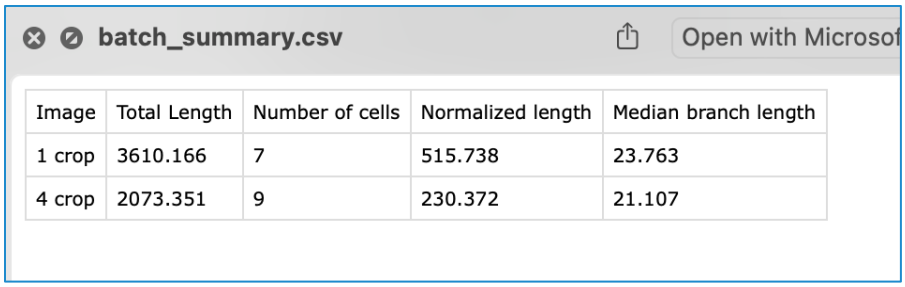

batch_summary.csv Open with Microsof

Image	Total Length	Number of cells	Normalized length	Median branch length
1 crop	3610.166	7	515.738	23.763
4 crop	2073.351	9	230.372	21.107

Technical details

- Cell counts are obtained by applying the default ImageJ threshold algorithm and applying binary Open and Watershed operations, then filtering by size.
- Protrusions are detected by a multi-step process:
  - Local (adaptive) Phansalkar thresholding to enhance dim processes
  - Frangi vesselness filter to enhance thin linear objects
  - Global percentile thresholding to detect the enhanced processes and cell bodies
  - Filtering to remove more circular objects
- The resulting objects, representing processes and their attached cell bodies, are skeletonized (thinned repeatedly until they are 1 pixel wide). This allows analysis of the protrusions as a network of segments (documentation: Analyze Skeleton).
  - The detected skeletons are "pruned" to remove the smallest segments (which are usually artifacts).
  - The pruned skeletons are analyzed to generate the values in the summary.

References: please cite these in your papers

- **Fiji**: Schindelin et al, "Fiji: an open-source platform for biological-image analysis," *Nature Methods* 2012, doi:10.1038/nmeth.2019.
- **Analyze Skeleton:** Arganda-Carreras, I., et al. (2010). 3D reconstruction of histological sections: Application to mammary gland tissue. *Microscopy Research and Technique*, 73(11), 1019–1029. doi:10.1002/jemt.20829
- **The CSMSR**. **Suggested text for acknowledgement:**
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