

Submission ID #: 71566

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21827678>

Title: Mapping Microglial Parameters Software (MMPS): An Open Source, User-Friendly Tool for Quantitative Microglia Morphology Analysis

Authors and Affiliations:

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Author Questionnaire

1. **Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**
3. **Filming location:** Will the filming need to take place in multiple locations? **No**
4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 18

Number of Shots: 55

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Dominick Cicala**: Our research uses Mapping Microglial Parameters Software, or MMPS, to study how ferroptosis alters microglial subpopulations following stroke.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Dominick Cicala**: MMPS addresses key bottlenecks in microglia morphology analysis, bypassing the need for cumbersome software and specialized coding skills.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Dominick Cicala**: This protocol allows users to study intricate microglial branching, morphological homogeneity, and structural complexity.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Dominick Cicala**: Acquiring high-resolution immunofluorescence images is essential for accurate microglial characterization using MMPS.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Dominick Cicala**: Future studies can utilize this protocol to characterize how ferroptosis inhibitors reshape microglial morphology in neurological disease models.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. **Dominick Cicala, Graduate Assistant, West Virginia University:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Dominick Cicala, Graduate Assistant, West Virginia University:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at West Virginia University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

Note: Except shot 2.1.1, all are SCREEN shots

2. MMPS Setup and Rat Microglial Cell Image Preparation

Demonstrator: Dominick Cicala

2.1. To begin, open the GitHub repository on the computer [1] and download the Mapping Microglial Parameters Software, or MMPS (*M-M-P-S*) standalone package [2]. In the Downloads folder, double click to launch the **Archive Utility** to automatically extract the compressed folder [3]. Double click the extracted MMPS application icon to verify [4] and open the MMPS package [5].

2.1.1. WIDE: Talent opening the GitHub repository on a computer. ***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. LAB MEDIA: 71566_2.1.2_Screen_Recording.mov. 00:00:00–00:00:08. ***Video Editor:** Please emphasize clicking on the 1st option “MMPS-v1.00-macos.zip and show the download happening on top right*

2.1.3. LAB MEDIA: 71566_2.1.3_Screen_Recording.mov. 00:00:00–00:00:08.

2.1.4. LAB MEDIA: 71566_2.1.3_Screen_Recording.mov. 00:00:09–00:00:19.

2.1.5. LAB MEDIA: 71566_2.1.3_Screen_Recording.mov. 00:00:24–00:00:26.

2.2. Click on **Select the Image Folder** option [1] and navigate through the computer's directory to locate the project files [2]. Grant folder access permissions [3] and choose the **Image Storage** directory [4]. Next, click **Select Output Folder** [5], then choose the output directory [6].

2.2.1. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:00–00:00:02. ***Video Editor:** Please emphasize clicking the “Select the Image folder” button on the top left.*

2.2.2. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:03–00:00:08

2.2.3. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:09–00:00:11

2.2.4. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:12–00:00:16

2.2.5. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:17–00:00:19. ***Video Editor:** Please emphasize clicking the “Select Output Folder” button on the top left below the “Select the Image folder” button.*

- 2.2.6. LAB MEDIA: 71566_2.2_Screen_Recording.mov. 00:00:20–00:00:23
- 2.3. Select the image to be processed and then choose **Image Labeling [1]**. Assign animal identifiers and treatment groups to the image and save the inputs **[2]**.
- 2.3.1. LAB MEDIA: 71566_2.3-and-2.4_Screen_Recording.mov. 00:00:00–00:00:05
- 2.3.2. LAB MEDIA: 71566_2.3-and-2.4_Screen_Recording.mov. 00:00:06–00:00:16
- 2.4. To calculate the pixel size of the image, select **Calibrate from scale bar [1]**. Select the beginning and end points of the scale bar **[2]** and enter the known scale-bar distance in micrometers and click **Apply [3]**.
- 2.4.1. LAB MEDIA: 71566_2.3-and-2.4_Screen_Recording.mov. 00:00:17–00:00:20. *Video Editor: Please emphasize clicking the 'Calibrate from scale bar' option on the left side.*
- 2.4.2. LAB MEDIA: 71566_2.3-and-2.4_Screen_Recording.mov. 00:00:21–00:00:25
- 2.4.3. LAB MEDIA: 71566_2.3-and-2.4_Screen_Recording.mov. 00:00:26–00:00:31.
- 2.5. Select the fluorescence channel containing the microglial signal **[1]**. Select images for processing by checking the box next to each image name and process them individually if background fluorescence differs between images **[2]**. Review the **Original, Preview, Processed**, and **Masks** tabs to compare image-processing stages **[3]**.
- 2.5.1. LAB MEDIA: 71566_2.5_Screen_Recording.mov. 00:00:00–00:00:07. *Video Editor: Please emphasize the arrow movement in the screen capture at all timestamps to highlight the action.*
- 2.5.2. LAB MEDIA: 71566_2.5_Screen_Recording.mov. 00:00:07–00:00:09
- 2.5.3. LAB MEDIA: 71566_2.5_Screen_Recording.mov. 00:00:09–00:00:14

3. Image Processing and Soma Outlining

- 3.1. Select **Preview Current Image** to adjust additional processing settings **[1]**. For background subtraction, set the rolling-ball values to 5 and 150 pixels **[2]**. To reduce illumination artifacts, use a denoising value of 3 **[3]** and set the sharpening value to 1 and 2 to improve edge definition **[4]**.
- 3.1.1. LAB MEDIA: 71566_3.1_Screen_Recording.mov. 00:00:00 – 00:00:08
- 3.1.2. LAB MEDIA: 71566_3.1_Screen_Recording.mov. 00:00:31 – 00:00:39
- 3.1.3. LAB MEDIA: 71566_3.1_Screen_Recording.mov. 00:00:39 – 00:00:41
- 3.1.4. LAB MEDIA: 71566_3.1_Screen_Recording.mov. 00:00:41 – 00:00:45
- 3.2. After optimization of image-processing settings, click on **Process Selected Images [1]**.
- 3.2.1. LAB MEDIA: 71566_3.2_Screen_Recording.mov. 00:00:00 – 00:00:09

- 3.3. Next, select **Pick Somas All Images [1]**. Click on **Show Color C** for RGB (*R-G-B*) composite view **[2]**. Select **Channels**, then deselect any unused channels if the image appears distorted in color **[3]**.

3.3.1. LAB MEDIA: 71566_3.3_Screen_Recording.mov. 00:00:00 – 00:00:03

3.3.2. LAB MEDIA: 71566_3.3_Screen_Recording.mov. 00:00:05 – 00:00:08

3.3.3. LAB MEDIA: 71566_3.3_Screen_Recording.mov. 00:00:12 – 00:00:22

- 3.4. Click on **Display Adjustments** to adjust the channel brightness in the RGB view **[1]**. Select microglial somas displaying DAPI (*dah-pee*) and ionized calcium binding adaptor molecule-1, or Iba1 (*I-B-A-one*) colocalization **[2]**. Remove incorrectly selected soma using the **Backspace** or **Delete** key **[3]**. Press **Enter** or **Return** to proceed to the next image **[4]**.

3.4.1. LAB MEDIA: 71566_3.4_Screen_Recording.mov. 00:00:00 – 00:00:08

3.4.2. LAB MEDIA: 71566_3.4_Screen_Recording.mov. 00:00:12 – 00:00:19

3.4.3. LAB MEDIA: 71566_3.4_Screen_Recording.mov. 00:00:20 – 00:00:24

3.4.4. LAB MEDIA: 71566_3.4_Screen_Recording.mov. 00:00:24 – 00:00:28

- 3.5. Select **Outline Somas All** after soma selection is complete **[1]**. Outline the soma perimeter of each selected microglial cell with at least 8 outline points, excluding the cellular processes **[2]**. Remove incorrectly placed outline points using **Undo Last Point**, **Delete**, or **Backspace** **[3]**.

3.5.1. LAB MEDIA: 71566_3.5-and-3.6.1_Screen_Recording.mov.00:00:00 – 00:00:02

3.5.2. LAB MEDIA: 71566_3.5-and-3.6.1_Screen_Recording.mov.00:00:46 – 00:00:52

3.5.3. LAB MEDIA: 71566_3.5-and-3.6.1_Screen_Recording.mov.00:00:31 – 00:00:35

- 3.6. Press **Enter**, **Return**, double-click, or right-click to finalize each soma outline **[1]**. Click **Generate All Masks** after completion of soma outlining **[2]**.

3.6.1. LAB MEDIA: 71566_3.5-and-3.6.1_Screen_Recording.mov.00:01:08 – 00:01:11

3.6.2. LAB MEDIA: 71566_3.6.3_Screen_Recording.mov. 00:00:00 – 00:00:04. *Video Editor: Please emphasize the arrow clicking on "Generate all Masks" at 00:00:01.*

- 3.7. In Mask Generation settings, adjust minimum intensity to reduce background pixels and balance background suppression and branch preservation **[1]**.

3.7.1. LAB MEDIA: 71566_3.7_Screen_Recording.mov. 00:00:00 – 00:00:08; 00:00:51 – 00:00:55

4. Mask Validation and Morphological Parameter Calculation

- 4.1. Select **QA (Q-A) All Masks** to begin mask quality assurance and review all masks

presented from largest to smallest areas [1]. Compare each mask to the corresponding microglia using the **Processed** and **Mask** tabs [3].

4.1.1. LAB MEDIA: 71566_4.1_Screen_Recording.mov. 00:00:00–00:00:13 *Video Editor: Please emphasize the arrow clicking on “QA All Masks” at 00:00:01*

4.1.2. LAB MEDIA: 71566_4.1_Screen_Recording.mov. 00:00:14–00:00:20

4.2. To accept masks, use the **A** keyboard shortcut [1]. To reject masks, use the **R** keyboard shortcut [2]. To undo an acceptance or rejection, press **B** [3]. Use keyboard arrow keys to navigate through mask sizes [4]. Select the largest acceptable mask that accurately captures microglial branching without including neighboring cells, excessive background, image borders, or scale bars [5].

4.2.1. LAB MEDIA: 71566_4.2_Screen_Recording.mov. 00:00:00 – 00:00:04

4.2.2. LAB MEDIA: 71566_4.2_Screen_Recording.mov. 00:00:05 – 00:00:08

4.2.3. LAB MEDIA: 71566_4.2_Screen_Recording.mov. 00:00:09 – 00:00:11

4.2.4. LAB MEDIA: 71566_4.2_Screen_Recording.mov. 00:00:11 – 00:00:15

4.2.5. LAB MEDIA: 71566_4.2_Screen_Recording.mov. 00:00:16 – 00:00:30

4.3. If most are inaccurate, use the **Clear All Masks** option to regenerate the masks with a different threshold value [1]. Next, select **Calculate Simple Characteristics** to calculate various parameters for accepted masks, then click OK [2].

4.3.1. LAB MEDIA: 71566_4.3_Screen_Recording.mov. 00:00:00 – 00:00:06 *Video Editor: Please emphasize the arrow hovering over “Clear All Masks”*

4.3.2. LAB MEDIA: 71566_4.3_Screen_Recording.mov. 00:00:07 – 00:00:12

4.4. For additional analysis, download **FractalAnalysis_ImageJ.py** (*Fractal-Analysis-Image-J-dot-P-Y*), **Sholl.py** (*Sholl-dot-P-Y*) and **SkeletonAnalysisImageJ.py** (*Skeleton-Analysis-Image-J-dot-P-Y*) plug-ins [1]. For fractal and hull analyses, open and run FractalAnalysis_ImageJ.py as a Fiji plugin, then enter the output directory location [2]. Input the pixel size and select the **largest mask only** option to analyze the largest accepted mask per cell [3].

4.4.1. LAB MEDIA: 71566_4.4_Screen_Recording.mov. 00:00:00 – 00:00:20

4.4.2. LAB MEDIA: 71566_4.4_Screen_Recording.mov. 00:00:21 – 00:00:42

4.4.3. LAB MEDIA: 71566_4.4_Screen_Recording.mov. 00:00:43 – 00:00:55

4.5. For Sholl analysis, open **Sholl.py** as a Fiji plugin [1]. Enter the output-directory location, desired pixel size, step size, select **Use soma radius as start radius** or enter a fixed soma radius value if MMPS-derived soma radii are not used [2].

4.5.1. LAB MEDIA: 71566_4.5_Screen_Recording.mov. 00:00:02 – 00:00:06; 00:00:11 – 00:00:15

4.5.2. LAB MEDIA: 71566_4.5_Screen_Recording.mov. 00:00:12 – 00:00:35

- 4.6. Open **SkeletonAnalysisImageJ.py** as a Fiji plugin for skeleton analysis [1]. Browse and enter the paths for the masks directory, output directory, and pixel size when prompted [2]. Upon finishing, export all plugin-generated results as comma-separated values files into the specific output directories [3].

4.6.1. LAB MEDIA: 71566_4.6.1-2_Screen_Recording.mov. 00:00:00–00:00:09

4.6.2. LAB MEDIA: 71566_4.6.1-2_Screen_Recording.mov. 00:00:10–00:00:16;
00:00:34 – 00:00:39

4.6.3. LAB MEDIA: 71566_4.6.3_Screen_Recording.mov. 00:00:01–00:00:07;
00:00:19–00:00:22; 00:00:19–00:00:22; 00:00:32–00:00:38; 00:00:48–00:00:50

Results

5. Results

- 5.1. Validation study of MMPS using a lipopolysaccharide, or LPS (*L-P-S*)-induced rodent neuroinflammation model, showed that LPS administration significantly decreased average centroid distance and cell perimeter compared with vehicle-treated controls [1]. No significant differences were observed in mask area [2], roundness [3], or eccentricity between treatment groups [4].
 - 5.1.1. LAB MEDIA: Figure 3A and 3B. *Video Editor: Please emphasize that the red box is lower than the blue box in both the graphs and highlight the “*” mark on top of the graphs.*
 - 5.1.2. LAB MEDIA: Figure 3C. *Video Editor: Please emphasize that the red and blue boxes and highlight the value “p=0.254” on top of the graph.*
 - 5.1.3. LAB MEDIA: Figure 3D. *Video Editor: Please emphasize that the red and blue boxes and highlight the value “p=0.070” on top of the graph.*
 - 5.1.4. LAB MEDIA: Figure 3E. *Video Editor: Please emphasize that the red and blue boxes and highlight the value “p=0.084” on top of the graph.*
- 5.2. The soma area was significantly increased in LPS-treated animals compared with vehicle controls consistent with activation-associated morphological remodeling of glial cells [1]. Further downstream advanced morphological analyses demonstrated only a weak correlation between microglia roundness and perimeter in vehicle-treated rats [2] and a strong correlation in LPS-treated rats, suggesting the association of increased microglial activation with reduced roundness and perimeter [3].
 - 5.2.1. LAB MEDIA: Figure 3F. *Video Editor: Please emphasize that the red box is higher than the blue box and highlight the “***” mark on top of the graph.*
 - 5.2.2. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the value “R²=0.17” on top of the graph.*
 - 5.2.3. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the value “R²=0.64” on top of the graph.*
- 5.3. Additionally, the mask area demonstrated strong positive correlations with average centroid distance in both vehicle [1] and LPS-treated groups consistent with changes in branching linked to microglial activation [2].
 - 5.3.1. LAB MEDIA: LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the value “R²=0.71” on top of the graph.*
 - 5.3.2. LAB MEDIA: LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the value “R²=0.88” on top of the graph.*

Pronunciation Guide:

1. Microglial
Pronunciation link: <https://www.merriam-webster.com/dictionary/microglial>
IPA: /ˌmaɪkroʊˈɡliːəl/
Phonetic Spelling: my·kroh·glee·uhl
2. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/, /flɔːˈresəns/
Phonetic Spelling: floo·reh·suhns
3. Calibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibrate>
IPA: /ˈkæləˌbreɪt/
Phonetic Spelling: kal·uh·brayt
4. Micrometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkroːmɛtər/
Phonetic Spelling: my·krah·muh·ter
5. Denoising
Pronunciation link: <https://www.merriam-webster.com/dictionary/denise>
IPA: /diːˈnoɪz/
Phonetic Spelling: dee·noyz
6. Illumination
Pronunciation link: <https://www.merriam-webster.com/dictionary/illumination>
IPA: /ɪˌluːməˈneɪʃən/
Phonetic Spelling: ih·loo·muh·nay·shuhn
7. Colocalization
Pronunciation link: <https://www.howtopronounce.com/colocalization>
IPA: /ˌkoʊˌloʊkəlaɪˈzeɪʃən/
Phonetic Spelling: koh·loh·kuh·ly·zay·shuhn
8. Ionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/ionized>
IPA: /ˈaɪəˌnaɪzd/
Phonetic Spelling: eye·uh·nyzd
9. Adaptor
Pronunciation link: <https://www.merriam-webster.com/dictionary/adaptor>
IPA: /əˈdæptər/
Phonetic Spelling: uh·dap·ter

10. Perimeter
Pronunciation link: <https://www.merriam-webster.com/dictionary/perimeter>
IPA: /pəˈrɪmətər/
Phonetic Spelling: puh·rim·uh·ter
11. Fractal
Pronunciation link: <https://www.merriam-webster.com/dictionary/fractal>
IPA: /ˈfræktəl/
Phonetic Spelling: frak·tuhl
12. Skeleton
Pronunciation link: <https://www.merriam-webster.com/dictionary/skeleton>
IPA: /ˈskelətən/
Phonetic Spelling: skel·uh·tuhn
13. Morphological
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphological>
IPA: /ˌmɔːrfəˈlɑːdʒɪkəl/
Phonetic Spelling: mor·fuh·loj·ih·kuhl
14. Microglia
Pronunciation link: <https://www.merriam-webster.com/dictionary/microglia>
IPA: /maɪˈkraːɡliə/
Phonetic Spelling: my·krah·glee·uh
15. Fiji
Pronunciation link: <https://www.merriam-webster.com/dictionary/Fiji>
IPA: /ˈfiːdʒiː/
Phonetic Spelling: fee·jee
16. Pixel
Pronunciation link: <https://www.merriam-webster.com/dictionary/pixel>
IPA: /ˈpɪksəl/
Phonetic Spelling: pik·suhl
17. Threshold
Pronunciation link: <https://www.merriam-webster.com/dictionary/threshold>
IPA: /ˈθreʃˌhoʊld/
Phonetic Spelling: thresh·hohld
18. Parameter
Pronunciation link: <https://www.merriam-webster.com/dictionary/parameter>
IPA: /pəˈræmətər/
Phonetic Spelling: puh·ram·uh·ter
19. Repository
Pronunciation link: <https://www.merriam-webster.com/dictionary/repository>

IPA: /rɪˈpɑːzəˌtɔːri/

Phonetic Spelling: rih·pah·zuh·tor·ee

20. Sharpening

Pronunciation link: <https://www.merriam-webster.com/dictionary/sharpen>

IPA: /ˈʃɑːrpən/

Phonetic Spelling: shar·puhn