

Submission ID #: 71930

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21938698>

Title: Measuring the Effects of Pharmacological Compounds on Mitochondrial Morphology in Mouse Aging Models

Authors and Affiliations:

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **08/12/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 26

Number of Shots: 37

Interviews

Authors: *Answers are slightly edited in accordance with journal guidelines. Please note that the questions will not appear on screen. Please answer in **stand-alone full sentence with sufficient context**.*

INTRODUCTION:

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

- 1.1. **Wayne Mitchell:** Our research focuses on determining the impacts of different pharmacological compounds on mitochondrial morphology in aging mouse models.
 - 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. **Cecília G. de Magalhães:** Existing methods either do not work well with tissue immunofluorescence images or require extensive coding knowledge; this protocol overcomes these limitations.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

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

- 1.3. **Wayne Mitchell:** The most important consideration is accurately segmenting mitochondria, which requires finesse and is crucial for successfully performing this protocol.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**Are there any additional methods or analyses that can be performed following this procedure?**~~

- 1.4. **Cecília G. de Magalhães:** Following this procedure, these methods can be generalized to other tissue types and potentially other organelles as well.

- 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. **Wayne Mitchell**: Future studies could develop methods to quantitatively measure and classify mitochondrial cristae structures in mouse tissues using super-resolution microscopy.

- 1.5.1. 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 Brigham and Women's Hospital and Harvard Medical School

Protocol

2. Airyscan Image Acquisition and Processing

Demonstrator: Wayne Mitchell

NOTE: The screen recordings provided are the best the authors can obtain in their system

2.1. Retrieve immunofluorescent-stained and mounted tissue sections from the freezer [1].

2.1.1. WIDE: Talent arranging the immunofluorescently stained tissue sections on the laboratory bench. [slides2.1NEW.mov 00:01-00:15](#)

NOTE to video editor: Please zoom into the left half of the screen for steps 2.2 to 2.10 and highlight or zoom into the areas where cursor is clicking for all the shots

2.2. To set up the acquisition on the ZEISS Airyscan2 point scanning LSM 980 (*L-S-M-nine-eighty*) confocal, add a single **Airyscan SR** (*Airyscan-S-R*) track [1]. Select the appropriate fluorophore [2].

2.2.1. SCREEN: airyscan.mp4 00:01-00:05

2.2.2. SCREEN: airyscan.mp4 00:05-00:13

2.3. Select the 10-inch plate [1] and the appropriate emission bandpass filters [2].

2.3.1. SCREEN: airyscan.mp4 00:13-00:17

2.3.2. SCREEN: airyscan.mp4 00:19-00:22-00:28-00:31

2.4. Enable the appropriate lasers [1]. Set the **Nyquist sampling criterion** to 2 by clicking **SR** [2]. Adjust the pixel size to 0.05 micrometers [3].

2.4.1. SCREEN: airyscan.mp4 00:36-00:39

2.4.2. SCREEN: airyscan.mp4 00:44-00:46

2.4.3. SCREEN: airyscan.mp4 00:50-00:52

NOTE to video editor: Please zoom into the left half of the screen for steps 2.2 to 2.10 and highlight or zoom into the areas where cursor is clicking for all the shots

2.5. Click **Live** and select the live alignment feature to align the Airyscan detector [1]. Once the intensity is localized at the center of the detector icon and the detector turns green, select **Stop** [2].

2.5.1. SCREEN: airyscan.mp4 00:55-1:00

2.5.2. SCREEN: airyscan.mp4 01:00-1:09

NOTE to video editor: Please zoom into the left half of the screen for steps 2.2 to 2.10 and highlight or zoom into the areas where cursor is clicking for all the shots

2.6. Click **Live** again [1]. Adjust the laser intensity until a strong signal is obtained without saturated pixels [2]. Click **Stop** once the settings are optimized [3].

2.6.1. SCREEN: airyscan.mp4 01:14-01:16

2.6.2. SCREEN: airyscan.mp4 01:16-01:40

2.6.3. SCREEN: airyscan.mp4 01:40-01:43

2.7. To perform data acquisition, click **Z-Stack** and verify that the step size is set to 0.1 micrometers [1].

2.7.1. SCREEN: airyscan.mp4 01:46-01:54

2.8. Click **Live** and set the lower and upper bounds of the z-stack [1].

2.8.1. SCREEN: airyscan.mp4 01:56-02:00 and 02:05-02:11

2.9. Click **Start Experiment** [1].

2.9.1. SCREEN: airyscan.mp4 02:15-02:18

NOTE to video editor: Please zoom into the left half of the screen for steps 2.2 to 2.10 and highlight or zoom into the areas where cursor is clicking for all the shots

NOTE to video editor: Please zoom into the lower mid part of the screen for steps 2.10 and 2.11 and highlight or zoom into the areas where cursor is clicking for all the shots

2.10. After collecting the z-stack, open the **Airyscan** tab underneath the image [1], select the **current image 3D**, and click on **Create Processed Images** ~~while maintaining consistent Wiener filter denoising parameters across replicates and treatment groups~~ [2].

Author's NOTE: Delete the second part of VO

2.10.1. SCREEN: airyscan.mp4 04:52-04:55

2.10.2. SCREEN: airyscan.mp4 04:55-05:04

2.11. Save the processed Airyscan z-stack [1].

2.11.1. SCREEN: airyscan.mp4 05:15-05:22

3. Image Segmentation and Quantitative Analysis Using Arivis and RStudio

NOTE: The screen recordings provided are the best the authors can obtain in their system

3.1. Convert Airyscan-processed .czi (*C-Z-I*) files to .sis (*S-I-S*) format using the arivis conversion tool [1].

3.1.1. SCREEN: arivis.mp4 00:06: 00:10 and 00:20-00:28

NOTE to video editor: Please zoom into the screen and highlight the areas where the cursor is clicking and doing some action

3.2. To set up and optimize the pipeline, open a representative image in the software and set the Tom20 (*tom-twenty*) channels as grey and auto-adjust the brightness and contrast [1]. Select the Tom20 immunofluorescence channel as the input for analysis [2].

3.2.1. SCREEN: arivis.mp4 00:32-00:46

3.2.2. SCREEN: arivis.mp4 00:50-00:54

3.3. Apply a denoising step before segmentation [1].

3.3.1. SCREEN: arivis.mp4 00:57-01:20 *Video editor: Please speed up*

3.4. Segment individual mitochondria using the **Blob Finder** algorithm [1].

3.4.1. SCREEN: arivis.mp4 01:52-01:55

3.5. Adjust the segmentation parameters, including the object size and sensitivity threshold [1]. Visually inspect the overlap between the detected objects and the denoised image to ensure accurate segmentation [2].

3.5.1. SCREEN: arivis.mp4 02:08-02:13

3.5.2. SCREEN: arivis.mp4 02:41-02:45 and 02:51-02:54 and 05:48-06:02-06:07

3.6. To perform feature extraction and quality control, extract the volume, surface area, and sphericity for each detected object [1].

3.6.1. SCREEN: arivis.mp4 05:38-05:46

3.7. Export the segmented objects as TIFF files [1].

3.7.1. SCREEN: arivis.mp4 06:18-06:40

3.8. Open the exported TIFF files in FIJI [1]. Overlay them onto the original image to inspect the segmentation accuracy [2].

3.8.1. SCREEN: arivis.mp4 06:49-06:52 and 07:49-07:51

3.8.2. SCREEN: arivis.mp4 08:34-08:40 and 08:50-08:54

3.9. Export the quantitative measurements as individual spreadsheet files for each sample [1].

3.9.1. SCREEN: arivis.mp4 09:10-09:25

3.10. Then, import the optimized pipeline [1].

3.10.1. SCREEN: arivis.mp4 10:32-10:43 and 10:56-11:00

3.10.2. Apply the pipeline to additional images from all experimental groups [1].

3.10.3. SCREEN: arivis.mp4 11:00-11:12

3.11. Apply the finalized pipeline in batch mode to all images in the study [1].

3.11.1. SCREEN: arivis.mp4 11:51-11:57

Results

4. Results

- 4.1. Representative optical slices illustrated the image processing workflow for both Control [1] and Treated samples [2].
 - 4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the row labelled 'CONTROL' showing the sequence of images from 'Tom20' to 'Den. + Segm.'*
 - 4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the row labelled 'TREATED' showing the sequence of images from 'Tom20' to 'Den. + Segm.'*
- 4.2. After particle enhancement denoising, the background signal was substantially reduced [1], and the remaining fluorescence was confined to discrete, well-defined structures [2].
 - 4.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the image labelled 'Denoising' in the CONTROL row, showing the darker background around the fluorescent structures.*
 - 4.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the image labelled 'Denoising' in the TREATED row, showing the darker background around the fluorescent structures.*
- 4.3. Detected objects co-localized precisely with the denoised Tom20 fluorescence signal in Control samples [1] and Treated samples [2].
 - 4.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the 'Den. + Segm.' inset in the row labeled 'CONTROL' with its zoomed-in box.*
 - 4.3.2. LAB MEDIA: Figure 2. *Video editor: Highlight the 'Den. + Segm.' inset in the row labelled 'TREATED' with its zoomed-in box.*
- 4.4. High-quality segmentation was consistently achieved across both Control [1] and Treated samples [2].
 - 4.4.1. LAB MEDIA: Figure 2. *Video editor: Highlight the complete row labelled 'CONTROL'.*
 - 4.4.2. LAB MEDIA: Figure 2. *Video editor: Highlight the complete row labelled 'TREATED'.*
- 4.5. Segmented mitochondria were measured for mitochondrial volume [1], sphericity [2], and surface area [3].
 - 4.5.1. LAB MEDIA: Figure 3. *Video editor: Highlight the graph titled 'Volume'.*

- 4.5.2. LAB MEDIA: Figure 3. *Video editor: Highlight the graph titled 'Sphericity'.*
- 4.5.3. LAB MEDIA: Figure 3. *Video editor: Highlight the graph titled 'Surface Area'.*
- 4.6. Treated samples exhibited significantly higher mitochondrial volume [1] and surface area [2] than Control samples [3].
 - 4.6.1. LAB MEDIA: Figure 3. *Video editor: Highlight the red 'Treated' box in the graph titled 'Volume'.*
 - 4.6.2. LAB MEDIA: Figure 3. *Video editor: Highlight the red 'Treated' box in the graph titled 'Surface Area'.*
 - 4.6.3. LAB MEDIA: Figure 3. *Video editor: Show the graph titled 'Volume' and 'Surface Area'*
- 4.7. The elbow method determined the optimal number of clusters for k-means clustering [1].
 - 4.7.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the line plot titled 'Elbow method – optimal k'.*
- 4.8. Random subsamples of mitochondria from the Control and Treated groups were plotted using uniform manifold approximation and projection and colored by mitochondrial subtype [1] and treatment group [2].
 - 4.8.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the left plot titled 'Mitochondrial subtypes — UMAP' and the legend labelled 'Subtype'.*
 - 4.8.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the right plot titled 'Experimental groups — UMAP' and the legend labelled 'Group'.*
- 4.9. The proportions of each mitochondrial subtype were plotted for the Control [1] and Treated groups [2].
 - 4.9.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the stacked bar labelled 'Control'.*
 - 4.9.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the stacked bar labelled 'Treated'.*
- 4.10. The Treatment group contained fewer fragmented mitochondria [1], but showed no significant change in the proportion of elongated mitochondria [2] or networked mitochondria [3].
 - 4.10.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the brown box plots labelled treated above 'Fragmented'.*
 - 4.10.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the brown box plots labelled treated above 'Elongated'.*
 - 4.10.3. LAB MEDIA: Figure 4C. *Video editor: Highlight the brown box plots labelled treated above 'Networked'.*