Submission ID #: 69268
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21126788

Title: Assessment of Mitochondrial Fission/Fusion Dynamics in Kidney Proximal Tubular Cells

Authors and Affiliations:
Vikky Awasthi, Sulaiman Qamar Sharief, Meriem Bkhache, Aasthika Das, Rihab Bouchareb

Lewis Katz Medical School at Temple University

Corresponding Authors:
Rihab Bouchareb rihab.bouchareb@temple.edu
Email Addresses for All Authors:
Vikky Awasthi fnu.vikky@temple.edu
Sulaiman Qamar Sharief sulaiman.sharief@temple.edu
Meriem Bkhache meriem.bkhache@temple.edu
Aasthika Das aasthika.das@temple.edu
Rihab Bouchareb rihab.bouchareb@temple.edu

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: 17
Number of Shots: 38

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Rihab Bouchareb:** Our lab develops innovative methods to isolate efficient, dynamically active mitochondria for transplantation aimed at restoring function in injured organs.

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

~~What are the current experimental challenges?~~

1.2. **Aasthika Das:** Current challenges include tracking transplanted mitochondria without staining while accurately assessing their integration, functionality, and dynamic behavior within recipient cells.

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

CONCLUSION:

~~What significant findings have you established in your field?~~

1.3. **Vikky Awasthi:** Using cells expression fluorescent photo-switchable mitochondria helped us track mitochondria and assess their dynamics in recipient cells.

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

~~How will your findings advance research in your field?~~

1.4. **Sulaiman Qamar:** Live-cell mitochondrial morphology measurement reveals real-time cell health; gene tagging and photoswitchable proteins enhance accuracy and measurement robustness.

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

~~What questions will future research focus on?~~

1.5. **Meriem Bkhache:** Future research will explore cellular machineries, both mitochondrial and cytoplasmic, that coordinate membrane remodeling to shape tubular, spherical, or circular mitochondria.

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.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Dr Rihab Bouchareb, Assistant Professor, Temple University**: (authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Dr Rihab Bouchareb, Assistant Professor, Temple University**: (authors will present their testimonial statements live)

Ethics Title Card

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

2. Imaging and Quantitative Analysis of Mitochondrial Fusion in Cisplatin-Treated Cells

~~**Demonstrator:**~~

Note: Demonstrator's name(s) were not provided

2.1. To begin, seed kidney proximal tubular cells in glass-bottom culture plates **[1-TXT]**. Incubate the cells overnight to allow for adherence and growth **[2]**. The next day, add cisplatin to the experimental plate to a final concentration of 2 micromolar **[3-TXT]**.

2.1.1. WIDE: Talent pipetting cell suspension into multiple wells of a glass-bottom culture plate. **TXT: Seeding density: 1 - 5 × 10^5 cells/well depending on experimental requirements**

2.1.2. Shot of the culture plate placed inside the incubator.

2.1.3. Talent pipetting cisplatin into the well of a culture plate inside a biosafety cabinet. **TXT: Ensure 0.004% final concentration of DMSO**

2.2. Next, configure the lasers of a confocal microscope to 405 nanometers, 488 nanometers for unconverted Dendra2 excitation, and 561 nanometers **[1]**. Turn on the lasers and allow approximately 10 minutes for stabilization **[2]**.

2.2.1. SCREEN: 69268_screenshot_000 00:00-00:09

2.2.2. SCREEN: 69268_screenshot_000 00:10-00:24

2.3. Place the culture disc in the incubation chamber **[1]**. Add water around the chamber and set the temperature to 37 degrees Celsius, ensuring the supply of carbon dioxide is maintained **[2]**. Allow the incubation chamber to equilibrate before imaging **[3]**.

2.3.1. Talent carefully placing a culture disc inside the incubation chamber.

2.3.2. Close-up of the water tray being filled and the control panel showing 37 degrees Celsius and CO_2 indicator.

2.3.3. Shot of the incubation chamber closed with timer or indicator light on.

2.4. Visualize the cells using higher magnification and resolution for mitochondrial imaging **[1]**. Open the drop-down menu in the top-left corner and select the **FRAP** *(frapp)* options **[2]**.

2.4.1. SCREEN: 69268_screenshot_A 00:10-00:27

2.4.2. SCREEN: 69268_screenshot_B 00:00-00:07

2.5. Using LAS X *(Las-Ex)* software, define the region of interest where photobleaching will be performed **[1]**. Set lasers to 405 nanometers at 0.00, 488 nanometers at 0.20, and 561 nanometers at 0.20, and ensure the cells are in focus **[2].** ~~Then acquire a series of pre-bleach images at low laser intensity to establish baseline fluorescence intensity by **[3]**.~~

2.5.1. SCREEN: 69628_screenshot_1 00:25-00:46

2.5.2. SCREEN: 69628_screenshot_1 00:50-01:10

~~2.5.3. SCREEN: Pre-bleach image acquisition is being done.~~
NOTE: Shot deleted by authors~~-~~

2.6. Now apply a high-intensity laser pulse to bleach the region of interest by setting FRAP lasers to 405 nanometers at 4.00, 488 nanometers at 0.00, and 561 nanometers at 0.00 **[1]**. ~~Set the pre-bleach to 5.140 seconds for 59 iterations and define the post-bleach timing **[2]**.~~

2.6.1. SCREEN: 69628_screenshot_1 01:10-01:24

2.6.2. ~~SCREEN: The pre-bleach settings are being defined~~.
NOTE: Shot deleted by authors

2.7. For post-bleach imaging, continue to acquire images at low laser intensity for 10 minutes using FRAP lasers at 405 nanometers at 0.00, 488 nanometers at 0.50, and 561 nanometers at 0.50 **[1]**.

2.7.1. SCREEN: 69628_Screenshot _2 00:00-00:21

2.8. To profile the merged green and red mitochondria, first install the RGB profiler plugin **[1]**. Open ImageJ software **[2]**, then from the **File** menu, locate and open the image file to analyse **[3]**.

2.8.1. SCREEN: 69268_Screenshot_3 00:00-00:16

2.8.2. SCREEN: 69628_Screenshot _4 00:10-00:16

2.8.3. SCREEN: 69628_Screenshot _5 00:08-00:16, 00:25-00:26

2.9. Select the **color mode composite**, check the **Auto Scale** option, and open the video or image stack to analyze **[1]**. Draw a rectangular area over the image **[2]** and right-click to select **Duplicate [3]**.

2.9.1. SCREEN: 69628 _Screenshot _5 00:17-00:25, 00:28-00:35

2.9.2. SCREEN: 69628 _Screenshot _5 00:39-00:42

2.9.3. SCREEN: 69628 _Screenshot _5 00:43-00:46

2.10. Select the **duplicate hyper stack** option to duplicate all the image stacks **[1]**. Select the image and choose **RGB** in the **Image** dropdown menu To convert the duplicated stack to RGB format **[2]**.

2.10.1. SCREEN: 69628 _Screenshot _5 00:46-00:53

2.10.2. SCREEN: 69628 _Screenshot _6 00:01-00:15, 00:23-00:28

2.11. Duplicate the RGB image and select only one frame **[1]**. If the mitochondria appear curved, draw a line along their length **[2]**, then go to the **Edit** menu, choose **Selection**, and click **Straighten [3]**.

2.11.1. SCREEN: 69628_ Screenshot _7 00:00-00:12

2.11.2. SCREEN: 69628_Screenshot _8 00:00-00:04

2.11.3. SCREEN: 69628_Screenshot _8 00:05-00:08

2.12. Now choose a title for the straightened image and set the line width to 20 pixels **[1]**. Draw a line along the straightened mitochondrion to align it properly **[2]** and open the **RGB profiler plugin [3]**.

2.12.1. SCREEN: 69628_Screenshot _8 00:09-00:13

2.12.2. SCREEN: 69628_Screenshot _8 00:14-00:25

2.12.3. SCREEN: 69628_Screenshot _8 00:32-00:38

2.13. Once activated, the plugin will generate a plot showing red and green fluorescence intensities along the drawn line **[1]**. The height of each peak will be directly proportional to the fluorescence intensity **[2-TXT]**.

2.13.1. SCREEN: 69628_Screenshot _8 00:39-00:45

2.13.2. SCREEN: 69628_Screenshot _8 00:46-00:51

TXT: Verify that each florescence is localized to adjacent mitochondria

~~2.14. Before mitochondrial fusion, confirm that red fluorescence is localized to one mitochondrion, and green fluorescence is localized to an adjacent one **[1].** Verify that the corresponding intensity plot displays two distinct peaks with minimal overlap **[2].**~~

~~2.14.1. SCOPE/SCREEN: 69628_Screenshot _8 00:53-00:51~~

~~Mitochondrial pair with clearly separated red and green fluorescence are being seen.~~

~~**AUTHORS: Please use the cursor to show the clearly differentiated colors**~~

~~2.14.2. SCREEN: 69628_Screenshot _8 00:41-00:51~~

~~Intensity plot showing distinct red and green peaks.~~

NOTE: Converted to on-screen text

2.15. After fusion, confirm that the red fluorescence has spread into the adjacent green mitochondrion indicating content mixing **[1]**. Verify that the intensity plot now shows overlapping red and green peaks **[2]**.

2.15.1. SCOPE/SCREEN: 69628_Screenshot _9 00:00-00:08

2.15.2. SCREEN: 69628_Screenshot _9 00:09-00:19

2.16. To assess fusion, select healthy mitochondria and photobleached mitochondria for fusion in live cell imaging **[1]**. Monitor the change in red to green fluorescence ratio over time to indicate fusion events **[2]**.

2.16.1. SCOPE/SCREEN: 69628_Screenshot _10 00:10-00:26
Field showing selection of intact and bleached mitochondria.

2.16.2. SCREEN: 69628_Screenshot _11 00:30-00:50
Time-series graph displaying the ratio shift of red to green fluorescence over time.

Results

3. Results

3.1. Control cells showed elongated mitochondria with yellow matrix, indicating mitochondrial fusion of the red and green matrices **[1]**. Cisplatin-treated cells exhibited fragmented mitochondria with reduced overlap of red and green signals, suggesting impaired fusion **[2]**.

3.1.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the upper row of time-lapse images labeled "Control"*

3.1.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the lower row of time-lapse images labeled "Cisplatin Treated"*

3.2. Fluorescence recovery in the photobleached region declined over time in control cells, indicating active mitochondrial fusion **[1]**. Cells treated with cisplatin maintained a nearly stable fluorescence ratio, indicating impaired mitochondrial fusion **[2]**.

3.2.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the downward-sloping blue line representing "Control"*

3.2.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the red line labeled "Cisplatin treated"*

3.3. The expression of CLRS-1 *(C-L-R-S-One)* was significantly reduced in cisplatin-treated cells compared to controls **[1]**. The expression of NRF1 *(N-R-F-One)* was significantly lower in cisplatin-treated cells than in controls **[2]**.

3.3.1. LAB MEDIA: Figure 6C. *Video editor: Highlight the first bar graph labeled "CLRS-1" and highlight the lower red bar (cisplatin treated)*

3.3.2. LAB MEDIA: Figure 6C. *Video editor: Highlight the second bar graph labeled "NRF1" and highlight the shorter red bar (cisplatin treated)*

Pronunciation Guide:

 Mitochondrial
Pronunciation link: https://www.merriam-webster.com/dictionary/mitochondrial
IPA: /ˌmaɪtəˈkɑːndriəl/
Phonetic Spelling: my-tuh-kon-dree-uhl
 Fission
Pronunciation link: https://www.merriam-webster.com/dictionary/fission
IPA: /ˈfɪʃən/
Phonetic Spelling: fih-shun
 Fusion
Pronunciation link: https://www.merriam-webster.com/dictionary/fusion
IPA: /ˈfjuːʒən/
Phonetic Spelling: fyoo-zhun
 Proximal
Pronunciation link: https://www.merriam-webster.com/dictionary/proximal
IPA: /ˈprɑːksɪməl/
Phonetic Spelling: prok-sih-muhl
 Tubular
Pronunciation link: https://www.merriam-webster.com/dictionary/tubular
IPA: /ˈtjuːbjələr/, /ˈtuːbjələr/
Phonetic Spelling: tyoo-byuh-ler / too-byuh-ler
 Cisplatin
Pronunciation link: https://www.merriam-webster.com/dictionary/cisplatin
IPA: /ˈsɪsˌplætən/
Phonetic Spelling: sis-plah-tin
 Micromolar
Pronunciation link: https://www.howtoppronounce.com/micromolar
IPA: /ˌmaɪkroʊˈmoʊlər/
Phonetic Spelling: my-kroh-moh-ler
 Confocal
Pronunciation link: https://www.merriam-webster.com/dictionary/confocal
IPA: /kɑːnˈfoʊkəl/
Phonetic Spelling: kon-foh-kuhl
 Nanometer
Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
IPA: /ˈnænəˌmiːtər/
Phonetic Spelling: nan-uh-mee-ter
 Dendra2
Pronunciation link: No confirmed link found
IPA: /ˈdɛndrə tuː/
Phonetic Spelling: den-druh-too

 FRAP (Fluorescence Recovery After Photobleaching)
Pronunciation link: No confirmed link found
IPA: /fræp/
Phonetic Spelling: frap
 Photobleaching
Pronunciation link: No confirmed link found
IPA: /ˌfoʊtoʊˈbliːtʃɪŋ/
Phonetic Spelling: foh-toh-blee-ching
 Fluorescence
Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
IPA: /flʊˈrɛsəns/, /flɔːˈrɛsəns/
Phonetic Spelling: floo-reh-sense / floor-eh-sense
 Cytoplasmic
Pronunciation link: https://www.merriam-webster.com/dictionary/cytoplasmic
IPA: /ˌsaɪtoʊˈplæzmɪk/
Phonetic Spelling: sy-toh-plaz-mik