Submission ID #: 61563
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18776763

Title: Super-Resolution Live Cell Imaging of Subcellular Structures

Authors and Affiliations: Rajesh Ranjan[1] and Xin Chen[1]

[1]Department of Biology, The Johns Hopkins University

Corresponding Authors:
Rajesh Ranjan
rranjan4@jhu.edu

Xin Chen
xchen32@jhu.edu

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen capture files provided, do not film

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **33**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Rajesh Ranjan**: Our protocol uses regular fluorophore probes and simple specimen preparation techniques that maintain the imaged cells in physiological condition to study highly dynamic cellular process and detailed subcellular structures **[1]**.

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

REQUIRED:

1.2. **Rajesh Ranjan**: This technique allows us to investigate cellular processes at a super-resolution and under physiological conditions for an extended period without sacrificing either the spatial or temporal resolution **[1]**.

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

Protocol

2. Glass Bottom Cell Culture Dish Preparation

2.1. Begin by cutting a 12-14-kilodalton molecular weight cutoff dialysis membrane into small pieces **[1]** and soaking the pieces with 100 microliters of live cell medium for about 5 minutes **[2-TXT]**.

2.1.1. WIDE: Talent cutting membrane

2.1.2. Talent adding membrane to medium, with medium container visible in frame **TEXT: See text for all medium and solution preparation details**

2.2. While the membranes are soaking, cut the outer ring of a 50-milliliter tube into small pieces **[1]** and sterilize the pieces in 70% ethanol **[2]**.

2.2.1. Talent cutting ring

2.2.2. Talent adding ring to ethanol, with ethanol container visible in frame

3. Testes Dissection and Mounting

3.1. For testes dissection and mounting, transfer ten, 2-3-day-old, anesthetized male flies into a dissection dish under a dissecting microscope **[1]** and use fine forceps to remove the testes **[2]**.

3.1.1. WIDE: Talent placing flies into dish **TEXT: Anesthesia: CO_2**

3.1.2. SCOPE: Testes being removed

3.2. When all of the testes have been collected, wash the testes two times in live cell medium **[1]** and use a pipette tip to spread 100-150 microliters of live cell medium to the prepared glass bottom dish **[2]**.

3.2.1. Talent adding medium to dish/testes being washed, with medium container visible in frame. Videographer NOTE: 3.2.1 and 3.2.2 are filmed as a single shot

3.2.2. Medium being spread around dish, with medium container visible in frame.

3.3. Use fine forceps to transfer the fly testes to the center of the dish **[1]** and remove all but about 10 microliters of the medium **[2]**.

3.3.1. Fly testes being moved to center of dish *Videographer: Important step* Videographer NOTE: 3.3.1 and 3.3.2 are filmed as a single shot

3.3.2. Medium being removed *Videographer: Important step*

3.4. Quickly place the pre-wet membrane onto the testes **[1]** and place 2-3 small, plastic weights onto the membrane **[2]**.

3.4.1. Membrane being placed *Videographer: Important/difficult step* Videographer NOTE: 3.4.1 and 3.4.2 are filmed as a single shot

3.4.2. Weight(s) being placed *Videographer: Important/difficult step*

3.5. Immediately add 100-150 microliters of fresh live cell medium **[1]** and place the ring back onto the elevated side of the dish **[2]**.

3.5.1. Talent adding medium to dish, with medium container visible in frame. Videographer NOTE: 3.5.1 and 3.5.2 are filmed as a single shot

3.5.2. Talent placing ring onto dish *Videographer: Difficult step*

3.6. Place the coverslip back onto of the ring **[1]** and swirl a piece of tissue paper in water **[2]** before placing the paper onto the coverslip **[3]**.

3.6.1. Talent placing coverslip onto ring *Videographer: Important step* Videographer NOTE: 3.6.1 to 3.6.3 are filmed as a single shot

3.6.2. Wet paper being swirled *Videographer: Important step*

3.6.3. Talent placing paper onto coverslip *Videographer: Important step*

3.7. Then cover the dish with a lid **[1]** and secure the dish on the stage of a super-resolution microscope **[2]**.

3.7.1. Talent placing lid onto dish

3.7.2. Talent placing dish onto the super-resolution microscope stage. **TEXT: confocal microscope is used for demonstration.** Author NOTE: Instead of using a super-resolution microscope, steps 3.7.2, 4.1 and 4.2 were performed on the confocal microscope for demonstration

4. Live Cell In Situ Male Drosophila Germline Stem Cell (GSC) Imaging

4.1. For germline stem cell imaging, open the imaging software **[1]** and turn on the transmitted light **[2]**.

4.1.1. WIDE: Talent opening imaging software, with monitor visible in frame

4.1.2. Talent turning on light

4.2. Use the 63x objective to focus on the testis tissue **[1]** and turn on the lasers **[2]**.

4.2.1. Talent focusing on tissue

4.2.2. Talent turning on lasers

4.3. Click **Live** to locate germinal stem cells **[1-TXT]**.

4.3.1. SCREEN: Screenshot_1**:** 00:04-00:22 *Video Editor: please speed up* **TEXT: Avoid testes with low fluorescence and/or with niche away from surface**

4.4. Adjust the focus and click **Frame Size** to set the frame size to between 512 x 512 and 1024 x 1024 pixels and **Averaging** to set the frame average to 1 or 2 **[1]**.

4.4.1. SCREEN: screenshot_2: 00:03-00:28 *Video Editor: please speed up*

4.5. Click **Master Gain** to set the electron-multiplying gain to less than 800 and **Lasers** to set the laser power to 1-2% **[1]**.

4.5.1. SCREEN: screenshot_3: 00:03-00:48 *Video Editor: please speed up*

4.6. Zoom in to the region or cell of interest to reduce the image acquisition time and to reduce photobleaching and confirm that the image has been optimally configured before clicking **Start Experiment** to start the time-lapse image capture **[1]**.

4.6.1. SCREEN: screenshot_4: 00:04-00:56 *Video Editor: please speed up*

4.7. If “Airyscan acquisition is not configured optimally” is displayed, click **Optimal** in the frame size, z-stack, and scan area sections and optimize the time interval, number of z-slices, and duration of the time-lapse imaging according to the experimental design and type of specimen **[1]**.

4.7.1. SCREEN: screenshot_5: 00:05-0:40 *Video Editor: please speed up*

4.8. After imaging, select **Processing**, **Batch**, and **Airyscan Processing** and select the images to be processed **[1]**. Then click **Run-Process** to obtain the super-resolution images **[2]**.

4.8.1. SCREEN: screenshot_6: 00:05-00:18 *Video Editor: please speed up*

4.8.2. SCREEN: screenshot_6: 00:21-00:32

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.3., 3.4., 3.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.4., 3.5. Placing the pre-wet membrane onto the testes is most difficult step. Before placing membrane, I quickly remove medium to ensure that testis sticks to the surface.

Results

5. Results: Representative Microtubule Time-Lapse Imaging

5.1. Live cell imaging of *Drosophila* testes expressing alpha-tubulin-GFP (G-F-P) in early-stage germ cells using a spinning disk confocal microscope **[1-TXT]** allows visualization of the asymmetric intensity of GFP signals at two centrosomes as a brighter signal at the mother centrosome **[2]** and a relatively weaker signal at the daughter centrosomes **[3]**.

5.1.1. LAB MEDIA: Figure 2A **TEXT: GFP: green fluorescent protein**
5.1.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize green arrows*
5.1.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize red arrows*

5.2. The difference in brightness is likely reflected by the temporal asymmetry of the microtubule nucleation **[1]**, but the detailed morphology and quantity of the microtubules cannot be resolved using spinning disk confocal microscopy **[2]**.

5.2.1. LAB MEDIA: Figure 2A
5.2.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize white signal in images*

5.3. In contrast, live cell super-resolution imaging allows the visualization and quantification of microtubule morphology and numbers **[1]**.

5.3.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize microtubules in images*

5.4. This improved resolution also reveals patterns of asymmetric microtubule nucleation, elongation, and increased interaction with the nuclear membrane **[1]**.

5.4.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize magenta arrow*

5.5. Live cell imaging also allows the visualization of asymmetric nuclear membrane invagination **[1]** but not of individual microtubules that directly enter the nucleus **[2]**.

5.5.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize Lamin-GFP alpha-tubulin-mCh image column*
5.5.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize orange arrowheads in Lamin image column*

5.6. In contrast, this live cell super-resolution technique allows the direct observation of these events by simultaneous imaging of both microtubules and the nuclear lamina **[1]**.

5.6.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize pink and orange arrows*

5.7. During metaphase, both sister centromeres can be detected as one signal using spinning disc microscopy **[1]**, although the microtubule-centromere attachment cannot be observed **[2]**.

5.7.1. LAB MEDIA: Figure 4A *Video Editor: please emphasize Meta images*
5.7.2. LAB MEDIA: Figure 4A

5.8. In contrast, super-resolution live imaging allows visualization of the microtubule-centromere attachment in early prophase **[1]**.

5.8.1. LAB MEDIA: Figure 4B *Video Editor: please emphasize Early prophase images*

Conclusion

6. Conclusion Interview Statements

6.1. **Rajesh Ranjan**: Be sure to place a membrane over the testes to ensure that they do not move or float during imaging and to optimize the microscope settings to avoid photobleaching and phototoxicity **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.4., 3.5., 4.4.-4.6.)

6.2. **Rajesh Ranjan**: There are many ways that this method can be applied, such as to investigate protein dynamics, lineage tracing, or cellular differentiation processes **[1]**.

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