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Title: Visualizing Yeast Organelles with Fluorescent Protein Markers

Authors and Affiliations:

Chang-Yue Liu[1], Jing Zhu[1], Zhiping Xie*[1].

[1]State Key Laboratory of Microbial Metabolism & Joint International Research Laboratory of Metabolic & Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University

☑ All author names and affiliations are correct.

Corresponding Authors:
Zhiping Xie (zxie@sjtu.edu.cn)

Email Addresses for All Authors:
Chang-Yue Liu (631381932@qq.com)
Jing Zhu (zhujingbiology@sjtu.edu.cn)
Zhiping Xie (zxie@sjtu.edu.cn)

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? **N**o

If **Yes**, can you record movies/images using your own microscope camera?
Enter Yes or No.

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

Enter make and model of microscope.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Y**es

If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? 100 meters, within the same building.

To ensure that your script can be filmed in one day, the Protocol section is restricted to **55 shots** (shots are the 3-digit numbers like 2.1.1, 2.1.2…etc)

Current Protocol Length

Number of Steps: 19
Number of Shots: 28

Introduction

1. Introductory Interview Statements

Videographer: Obtain headshots for all authors.

Your answers to these questions will become author interview statements, which authors will memorize and then deliver on camera.

- Enter the **full name** of the author who will deliver the statement.
- If possible, each author should deliver **no more than two statements**.
- Fill out **both** required statements, **one** optional statement may also be selected.
- Answer in full sentences, in a style suitable for being spoken aloud.
- Limit the length of each statement to **30 words or fewer**.
- Answers will be edited for length, clarity, and consistency with journal style guidelines.

REQUIRED: Why is your protocol significant? *OR* What key questions can this method help answer?

1.1. **LIU, Chang-Yue:** This protocol provides detailed instructions to observe organelles and to identify protein subcellular localization in yeast.

REQUIRED: What is the main advantage of this technique?

1.2. **LIU, Chang-Yue:** It provides a starting point for researchers entering from other research fields to explore live cell imaging of yeast organelles.

OPTIONAL: Do the implications of this technique extend toward the therapy (or diagnosis) of a particular disease, disability, or challenge? How so?

1.3. Enter author name**:** Click here if you choose this question. Please write in a style that you will be comfortable memorizing and speaking aloud. Limit length to 30 or fewer words.

OPTIONAL: Are there any specific areas of research that this method could provide insight into? *OR* Can this method be applied to any other systems?

1.4. Enter author name**:** Click here if you choose this question. Please write in a style that you will be comfortable memorizing and speaking aloud. Limit length to 30 or fewer words.

OPTIONAL: How would you expect an individual who has never performed this technique to struggle? Do you have any advice to offer to somebody who is trying this technique for the first time?

Introduction of Demonstrator on Camera

Complete this statement **ONLY** if any of the individuals who will be demonstrating the procedure on camera will not be delivering an Introductory Interview Statement.

1.5. Enter name of author who will introduce demonstrator: Demonstrating the procedure will be Click here to enter name of demonstrator(s)., a Click here to enter demonstrator job title. from my laboratory. Include additional demonstrators as needed.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

Please review this section to make sure that it accurately describes your protocol. Use **Track Changes** when making edits or revisions.

- The one-digit numbers represent **sections** of the video. The text will appear onscreen.
- The two-digit numbers (e.g. 2.1., 2.2.) represent **steps** of your protocol. The text will be recorded by a professional voiceover talent.
- The three-digit numbers (e.g. 2.1.1., 2.2.2.) represent the **shots** that our videographer will capture at your lab.
- To ensure that your protocol can be **filmed in one day**, the protocol is restricted to **25 steps** and/or **55 shots**.

Please use this draft script to help you prepare for filming day.

- Filming should take no more than 10 minutes per step. If a step will take more than 10 minutes, prepare the product from that step in advance.

2. Fluorescence Microscopy: General Procedures and Single Time-Point Imaging

2.1. Start by diluting yeast culture to approximately 0.2 optical density of yeast culture at 600 nanometer using a fresh medium **[1]**. Continue culture till optical density reaches approximately between 0.8 to 1 **[2]**.

2.1.1. Talent diluting yeast culture using a fresh medium.

2.1.2. Talent putting yeast cells for culture.

2.1.3. Talent measuring OD600 on a spectrophotometer to ascertain that the value is within range. Note: In real life, after dilution it takes several hours for yeast to grow. For demonstration purpose, we can have a sample ready for use without further waiting.

2.2. Put a cover glass on top of tissue paper on a flat surface **[1]**, spread 5 microliters of 1 milligram per milliliter concanavalin A on the top side of the cover glass, and wait for 5 minutes **[2]**.

2.2.1. Talent putting a cover glass on top of a tissue paper.

2.2.2. Talent spreading concanavalin A to cover glass.

2.3. Combine cover glass with a supporting glass slide, with the yeast cells sandwiched in-between; press with appropriate force to secure the attachment **[1]**. Mount the sample slide on the microscope stage **[2]**.

2.3.1. Talent combining cover glass with a supporting glass slide and yeast cells in between.

2.3.2. Talent mounting the sample on the microscope stage.

2.4. Locate and focus on a patch of yeast cells using differential interference contrast or phase contrast illumination **[1]**.

2.4.1. SCOPE: Talent locating patches of yeast cells. Authors: Do you want to show it live or provide video screen videos for this shot? Answer: Maybe a combination of both? Live demo of operating the focusing nob and stage control, plus screen capture of microscope image feed.

2.5. Manually configure three sets of parameters to collect image z-stacks: z-sectioning, imaging channels, and exposure parameters. For z-sectioning, collect slices at 0.5 micrometers stepping for 15 slices **[1]**.

2.5.1. SCREEN: Talent setting step size and layers.

2.6. Select **DIC** or **PC** for cell contours and appropriate fluorescence channels as needed. Set excitation light intensity and exposure time as appropriate **[1]**. For z-sectioning, set to collect slices at 0.5 µm stepping for 15 slices **[2]**. Authors: Would you like the voice over artist to pronounce 'DIC' as 'D-I-C' and 'PC' as 'P-C'? If not, please provide pronunciation guide. Answer: We prefer to say the full name of the acronyms in speech, i.e., "differential interference contrast" and "phase contrast".

2.6.1. SCREEN: Talent selecting appropriate fluorescence channel and setting excitation light intensity and exposure time.

Authors: Please identify if this shot requires screen captures. If yes, create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: **https://www.jove.com/account/file-uploader?src=19473078** Answer: Yes, it is a screen capture.

2.6.2. SCREEN: Talent selecting z-sectioning parameters.

2.7. Use the same imaging settings for all samples to be compared. Save the data in 16-bit multichannel stack format. Move to completely different area for next image stack collection **[1]**.

2.7.1. SCREEN: Talent saving the data.

Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: **https://www.jove.com/account/file-uploader?src=19473078**

3. Time-Lapse Imaging

3.1. For Sample preparation, coat a 35-millimeter glass-bottom dish with 1 milligram per milliliter concanavalin A and wait 5 minutes **[1]**. Add 1.5mL of yeast liquid culture to the

dish and wait for 5 minutes to allow the yeast cells to settle to the glass surface **[2]**. Note: We updated the steps according to the final version of the text.

3.1.1. Talent coating the glass bottom dish.

3.1.2. Talent adding yeast liquid culture to the dish.

3.2. Using a pipette, aspirate the liquid medium from the edge of the dish **[1]**, then gently rinse the patch of yeast sediment with about 1mL of fresh medium to remove insecurely attached cells **[2]**.

3.2.1. Talent aspirating the liquid medium from edge of the dish.

3.2.2. Talent rinsing the patch of yeast sediment.

3.3. Repeat the rinsing 2 to 3 times. Aspirate with a pipette **[1]**, then gently add 2mL of fresh culture media **[2]**. Collect slices at 0.5 micrometer stepping for 15 slices **[3]**.

3.3.1. Talent adding fresh culture media.

3.3.2. Talent mounting the sample on the microscope stage.

3.4. Select imaging channels as needed. Use minimal excitation light intensity and exposure time to discern the subcellular structure under investigation **[1]**. Set the timing intervals appropriate for the biological process being investigated **[2]**.

3.4.1. SCREEN: Talent selecting imaging channels, setting excitation light intensity and exposure time. Talent selecting z-sectioning parameters

3.4.2. SCREEN: Talent setting appropriate time interval.

4. Visualization of Image Stacks and Assessment of Integration Copy Number

4.1. In Fiji, open a couple of z-stack images. Drag the z-position in each stack to the mid-section. Go to **Image>Adjust>Brightness/Contrast** and click on **Reset [1].**

4.1.1. SCREEN: Z-stack image being opened, and z-position is being adjusted followed by brightness reset

4.2. In the **Brightness/Contrast** window, use the scroll bars to change two parameters, **Minimum** and **Maximum values**, to discern the structure under investigation clearly **[1].**

4.2.1. SCREEN: Minimum and maximum values of brightness is being set.

4.3. In the **Brightness/Contrast** window, click on **Set** and select **Propagate to all other open Images** checkbox in the popup window **Set Display Range [1].**

4.3.1. SCREEN: In 'Brightness/Contrast' window- 'Set' is being clicked, flowed by 'Propagate to all other open images' and 'Set display range'

4.4. For multichannel images, pick image display settings for each channel. Go to **Image> Color> Channels Tool,** select the **Greyscale** mode in the popup window **Channels [1].**

4.4.1. SCREEN: Image display settings are being picked by clicking on 'Image-Color-Channels Tool' and selecting 'Greyscale mode'.

4.5. Go through each channel, pick the proper **Brightness/Contrast** for the channel, and write down the settings **[1]**. With the **Brightness/ Contrast** adjusted, go back to the **Channels** window, and change to **Composite** mode for simultaneous visualization of multiple channels **[2]**.

4.5.1. SCREEN: Brightness/Contrast for each channel is being set.

4.5.2. SCREEN: Composite mode for channel window is being selected.

4.6. Once the same **Brightness/Contrast** settings are applied across all opened images, compare the image intensities across samples to infer plasmid integration number **[1]**.

4.6.1. SCREEN: Image intensities across samples is being compared.

4.7. Examine images from eight transformants for each strain, ensuring to use the same **Brightness/Contrast** settings. Re-streak and save three or more single copy transformants for subsequent study **[1]**.

4.7.1. Talent re-streak transformants to new plates. Note: Re-streaking is an experimental step. Alternatively, we can probably just have a voice over, with an image of re-streaked plates.

4.8. Export images for presentation. Go to **Image> Duplicate** to make a copy of the image of interest and give it a name of choice **[1]**. Go to **Image > Type > 8-bit** to convert the duplicate copy from 16-bit to 8-bit and save it as needed **[2]**.

4.8.1. SCREEN: Images of interest are being duplicated and renamed.

4.8.2. SCREEN: Images are being converted to 8-bit and saved.

Protocol Script Questions

Authors: Please use the **step and shot numbers from the script above** (not step numbers from the manuscript) when answering the questions below. Do not include steps that will be screen-captured and do not list entire sections.

1) Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps (steps are indicated with the 2-digit numbers, like 2.1, 2.2, etc.).

   2.2, 2.3, 2.4, 3.1, 3.2, 3.3.

2) If a dissection or stereo microscope is required for your protocol, please list all shots that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

   N/A.

Results

Please review this section to make sure that it accurately reflects your findings.

- Use **Track Changes** when making edits or revisions.
- If you would like the video to include different results, please revise this section.
- When revising, please keep the length of the voiceover below 200 words. Current word count: 138. (Voiceover is the text that follows the two-digit numbers)
- Please note that the video cannot include voiceover without an accompanying visual.

5. Results: Imaging Yeast Organelles Using Protein Markers

5.1. Several organelles have their distinct morphological features, thus are easy to recognize without extensive comparison with other organelle markers. **[1]**.

5.1.1. LAB MEDIA: Figure 3 A-B

5.2. Transformants with single-copy integration can be screened when verifying their markers expression by fluorescence microscopy. The fluorescence signal in transformants carrying multiple copies is much brighter than with a single copy **[1]**.

5.2.1. LAB MEDIA: Figure 2 B

5.3. Membrane dynamics investigation using Time-lapse imaging demonstrated frequent formation and consumption of autophagosomes in starved yeast cells labeled with GFP-Atg8 **[1]**. Authors: How would you like the voice-over artist to pronounce 'GFP-Atg8'? Answer: Letter by letter, as "G-F-P-pause-A-T-G-eight".

5.3.1. LAB MEDIA: Figure 4

5.4. Time Lapse imaging showed that different instances of autophagosome biogenesis can occur independently at different subcellular locations in a single yeast cell over time **[1].**

5.4.1. LAB MEDIA: Figure 4 Authors: Would you like the video editor to highlight any certain part in this figure for this VO? Answer: We have several series of arrows, each color following a particular dot over time. Is it possible to animate the arrows with some pop-up or glowing effect, series by series, to draw attention to the tracking over time?

5.5. A cross comparison with co-expressed red organelle makers, it is observed that Sft1 partially colocalizes with early Golgi and late Golgi/early endosome markers, which provide important clues for understanding Sft1 function **[1]**. Authors: How would you like the voice-over artist to pronounce 'Sft 1' and 'Golgi'? Answer: "Sft1" can be pronounced as "S-F-T-one". "Golgi" can be pronounced as "/ˈgɔːldʒi/"; this is the name of its discoverer.

5.5.1. LAB MEDIA: Figure 5

Conclusion

6. Conclusion Interview Statements

Below are prompts for interview statements that can be used to further emphasize the significance of your protocol.

- Answer **one** or **two** of the prompts below.
- Limit the statements to **30 words**.
- Answer the questions in full sentences; you will need to memorize and deliver the interview statements during filming.
- Indicate the **full name** of the author who will deliver each statement.

What is the most important thing to remember when attempting this procedure? Please indicate the steps (*e.g.*, 2.4., 2.5.) in the Protocol section of the script that this advice applies to.

6.1. **LIU, Chang-Yue:** (2.5, 3.4) Be sure to choose imaging settings manually, and write down the settings in a lab note.

Following this procedure, what other methods can be performed? What questions would these additional methods answer?

1.1. Enter author name**:** Click here to answer. Please use language that you will be comfortable memorizing and speaking aloud. Limit length to 30 or fewer words.

After its development, did this technique pave the way for researchers to explore new questions within a specific scientific field? If so, how?

1.2. Enter author name**:** Click here to answer. Please use language that you will be comfortable memorizing and speaking aloud. Limit length to 30 or fewer words.

Thank you for addressing our questions. We will incorporate your answers and suggestions, and send you the final script before your filming day. You will also receive detailed preparation instructions in the email accompanying the final script.