**1.** **SUMMARY:**

The format has been followed.

**2. SUMMARY:** After photobleaching, STORM is used to obtain 3D super-resolution images of the cyanobacterial FtsZ ring.

“STORM” had been expanded as stochastic optical reconstruction microscopy. And STORM is not a trademark or commercial name.

**3. Section 1.1:** Grow the axenic *Prochlorococcus* strain MED4 in the seawater-based Pro99 medium12. Maintain *Prochlorococcus* cultures at 23 °C under the light with an intensity of 35 µmol photons/m2s.

The volume (5 ml) has been updated in section 1.1. Temperature and light intensity had been included in section 1.1. Rotation is not needed; therefore, we do not mention it.

Pro99 medium is not a commercial medium. It is widely used to culture cyanobacteria. The paper we cited describes the recipe of Pro99 medium in detail.

The revised Section 1.1 is this: “Inoculate 1 ml axenic *Prochlorococcus* MED4 to 5 ml of the seawater-based Pro99 medium. Grow *Prochlorococcus* MED4 at 23 °C under the light with an intensity of 35 µmol photons/m2s.”

**4. Section 1.2:** Measure the concentration of the cultures by flow cytometry. When the concentration reaches approximately 108 cell/mL, collect 1 mL log-phase culture into a 1.5 mL tube.

Since the flow cytometry is not the major part of this protocol, we don’t want to introduce too much about the details of flow cytometry. Instead, we’d like to provide the time it takes to reach an appropriate number of cells. And section 1.2 has been combined with section 1.1. Meanwhile, a note has been added.

“1.1 Transfer 1 ml axenic *Prochlorococcus* MED4 to 5 mL of the seawater-based Pro99 medium. Grow the *Prochlorococcus* MED4 at 23 °C under the light with an intensity of 35 µmol photons/m2s. Five days later, collect 1 mL culture into a 1.5 mL tube.

Note: Five days after inoculation, *Prochlorococcus* MED4 will reach the late phase, with approximately 108 cell/ml, which is appropriate for STORM imaging.”

**5. Section 1.4:** Spin down the sample at 13,500 x g for 1 min, then remove the supernatant and re-suspend the cells in 100 µL Pro99 medium 12. Store the sample at 4 °C until immunostaining.

No. The recipe of the Pro99 was described in the paper we cited.

**6. Section 5.1:** Remove the PBS buffer. Add 1 mL freshly prepared permeabilization buffer into the well of washing dish, which contains 0.05% non-ionic detergent-100 (v/v), 10 mM EDTA, 10 mM Tris (pH 8.0) and 0.2 mg/mL lysozyme.

Yes. The sentence has been modified as follows:

“5.1. Remove the PBS buffer using a pipette. Add 1 mL freshly prepared permeabilization buffer into the well of washing dish.”

**7. Section 5.3:** Add 1 mL PBS buffer and place the washing dish on a shaker. Gently shake the washing dish for 5 min, then remove the PBS buffer. Repeat this step three times to wash the coverslip.

The washing dish is shaken on shaker automatically, not manually. The sentence has been modified as follows:

“Add 1 mL PBS buffer and place the washing dish on a shaker which gently shakes the washing dish for 5 min. Then remove the PBS buffer.”

**8. Section 9.2:** Turn on the camera, the LED light and the laser. Open STORM softwares, Rohdea and Luna. Use Rohdea for image acquisition and centroid position determination. Use Luna for sample drifting correction with 1-nm accuracy13.

The Luna and Rohdea have been replaced by generic name “software”. The sentence has been rewritten as follows:

“Turn on the camera, the LED light and the laser. Open STORM software for image acquisition, centroid position determination and sample drifting correction13.”

**9. Section 9.5:** Identify a sample area that contains both cells and fiducial markers. Start Luna to remove sample drifting in real time.

The graphical user interface has been provided in supplemental material (Figure S1). The sentence has been modified as follows:

“9.5. Identify a sample area that contains both cells and fiducial markers. Start the software for sample drifting correction.”

**10. Note:** The ideal sample area needs to have adequate number of cells. Meanwhile, the cells should be separated well to avoid any overlapping cells.

Based on our experience, 10-30 cells per view is an adequate number. The sentence is replaced as follows:

“Note: In general, the ideal sample area contains 10-30 cells. Meanwhile, the cells should be separated well to avoid any overlapping cells.”

**11. Section 9.6:** Acquire one wide-field image as a reference, with camera electron multiplication (EM) gain at 300 and an exposure time at 30 ms (**Figure2A**).

The interface and buttons are provided in supplemental material (Figure S1).

The sentence has been changed as “Acquire one wide-field image as reference with camera electron multiplication (EM) gain at 300 and an exposure time at 30 ms (**Figure 2A**).”

**12. Section 9.7:** Increase the 750-nm excitation laser to a higher power, approximately 4.5 kW/cm2. Once the fluorophores have transitioned into a sparse blinking pattern, acquire one super-resolution image by collecting 10,000 frames at 33 Hz (**Figure2B**).

The interface and buttons are provided in supplemental material (Figure S1). The sentence has been changed as:

“9.7. Increase the 750-nm excitation laser intensity to a higher power, approximately 4.5 kW/cm2. Once the fluorophores have transitioned into a sparse blinking pattern, acquire one super-resolution image by collecting 10,000 frames at 33 Hz (Figure 2B).

**13. Section 10.1:** Use a plugin called “QuickPALM” in ImageJ to reconstruct a three-dimension Color super-resolution image.

The interface and buttons are provided in supplemental material (Figure S2). The “QuickPALM” is an open source plugin. The reference of “QuickPALM” and related website have been cited.

The protocol has been revised as follows:

“10.1. Use a plugin called “QuickPALM” in ImageJ to reconstruct a three-dimension color super-resolution image.”

**14. Section 10.2**: To demonstrate the three-dimension structure in a video, construct a stack of super resolution images with z axis sectioned every 10 nm. Duplicate the stack of one target cell. Use a plugin called “3D Viewer” in ImageJ to generate the 3D image of the cell.

The interface and buttons are provided in supplemental material (Figure S2). The “3D Viewer” is an open source plugin. The reference of “3D Viewer” and related website have been cited.

“10.2. To demonstrate the three-dimension structure in a video, construct a stack of super-resolution images with z axis sectioned every 10 nm using “QuickPALM”. Adjust the brightness of the stacks and Duplicate the stack of one target cell. Use a plugin called “3D Viewer” in ImageJ to generate the 3D image of the cell. Record the rotation of the 3D structure for demonstration purpose.

**15.Movie S1**: Clusters of FtsZ proteins were observed within *Prochlorococcus* MED4 cells.

**Movie S2**: An Incomplete ring of FtsZ proteins was observed within *Prochlorococcus* MED4 cells.

**Movie S3**: A complete ring of FtsZ proteins was observed within *Prochlorococcus* MED4 cells.

**Movie S4**: A double-ring of FtsZ proteins was observed within *Prochlorococcus* MED4 cells.

Noted.

**16. Discussion:**

A summary is added as follows:

“In summary, a proper combination of photobleaching and STORM provides a powerful approach to understand the protein organization in photosynthetic cells in detail, which will further reveal protein dynamics and potential function.”