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Title: Live Cell Imaging of Microtubule Cytoskeleton and Micromechanical Manipulation of the *Arabidopsis* Shoot Apical Meristem

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Author Questionnaire:

1. Microscopy: Does your protocol involve video microscopy? Y

2. Does your protocol demonstrate software usage? Y

3. Which steps from the protocol section below are the most visually important?

3.3., 4.1., 6.3., 6.4.

4. What is the single most difficult aspect of this procedure and what do you do to ensure success

3.3. Dissection of SAM requires a bit practice.

6.3. SAM ablation under a microscope manually could be a bit challenging. Relax. Laying the weight of fingers not holding the needle on the box might help to better control.

5. Will the filming need to take place in multiple locations (greater than walking distance)? N

Section - Introduction

Videographer: Interviewee Headshots are required. Take a headshot for each interviewee.

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length and clarity.**

    1.1. **Arun Sampathkumar**: This protocol facilitates the monitoring of microtubule cytoskeleton behaviors to assess how molecules that regulate organism growth respond to changes in mechanical force **[1]**.

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

    1.2. **Yang Wang**: Although this method requires little sophistication, it provides a robust, quantitative readout of the differences in mechanical responses between different genotypes and conditions **[1]**.

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

Section - Protocol

2. Plant Growth

2.1. Begin by growing *Arabidopsis* seeds expressing microtubule binding domains fused with green fluorescent protein in soil at 20 and 6 degrees Celsius under long day conditions for 1 week **[1-TXT]**.

2.1.1. WIDE: Talent planting seeds **TEXT: Long day: 16 h day/8 h night**

2.2. After germination, transfer the seedlings into new pots with sufficient growth space to allow robust vegetative growth **[1]** and place the plants at 20 and 16 degrees Celsius under short day conditions **[2-TXT]**.

2.2.1. Talent transferring seedling to pot

2.2.2. Talent placing pot(s) under short day conditions **TEXT: Short day: 8 h day /16 h night**

2.3. After 3-5 weeks, transfer the plants back to long day conditions at the same temperature until the plants bolt **[1-TXT]**, allowing the inflorescence to grow up to 2-5 centimeters long **[2]**.

2.3.1. Talent placing plants under long day conditions **TEXT: Bolting occurs 2-3 wks**

2.3.2. Shot of 2-5 cm inflorescence

3. Shoot Apical Meristem (SAM) Dissection

3.1. For shoot apical meristem dissection, cut the inflorescence **[1]** and use sharp forceps to peel the flowers at the base of the peduncles until it is difficult to see the peduncles with the naked eye to remove the older flower buds **[2]**.

3.1.1. WIDE: Talent cutting inflorescence

3.1.2. Flower being peeled

3.2. Use the forceps to create a slit in the agarose in a dissecting dish **[1-TXT]** and plant the inflorescence base into the thick agar **[2]**.

3.2.1. Slit being created **TEXT: See text for dish preparation details**

3.2.2. Inflorescence base being planted

3.3. Place the dish under a dissecting microscope **[1]** and use the forceps to push each flower bud down until the shoot apical meristem is visible **[2-TXT]**.

3.3.1. Talent placing dish under microscope

3.3.2. SCOPE: Flower bud being pushed down *Videographer: Important/difficult step* **TEXT: Remove buds from oldest to youngest stage**

3.4. Starting with the oldest flower bud, push with the forceps to remove the flower bud from the plant **[1]**.

3.4.1. SCOPE: SAM bud being pushed down

3.5. The shoot apical meristem is usually exposed when older flowers up to stage 6 to 7 are removed **[1]**.

3.5.1. SCOPE: Shot of exposed SAM

4. Cultured SAM Transfer and Growth

4.1. As soon as the shoot apical meristem is exposed, plant the freshly dissected sample in growth medium in an ethanol-sterilized rectangular plastic hinged culture box **[1-TXT]** with the shoot apical meristem just exposed above the medium surface **[2]**.

4.1.1. WIDE: Talent planting sample in box *Videographer: Important step* **TEXT: See text for medium preparation details**

4.1.2. Shot of SAM just exposed above medium surface *Videographer: Important step*

4.2. Add a few drops of sterile deionized water to the edges of the culture box **[1-TXT]** and close the lid to maintain the humidity inside the box **[2]**.

4.2.1. Water being added to edge of box **TEXT: Ensure water does not cover SAM**

4.2.2. Talent closing lid

4.3. Wrap the box with micropore tape **[1]** and place the growth box under long or continuous day conditions at 22 degrees Celsius for 12-24 hours **[2]**.

4.3.1. Talent wrapping box

4.3.2. Talent placing box at 22 °C

5. SAM Imaging

5.1. When the plant has recovered from the dissection procedure, cover the sample with sterile deionized water **[1]** and check for air bubbles under the dissecting microscope **[2-TXT]**.

5.1.1. WIDE: Talent covering sample w/ water

5.1.2. SCOPE: Shot of sample w/o bubbles OR Bubbles being removed **TEXT: Forcefully spray water with 1-mL pipette to remove bubbles**

5.2. Transfer the culture box to an upright confocal microscope stage **[1]** and select the 40- or 60x water dipping lens **[2]**.

5.2.1. Talent placing box onto microscope stage

5.2.2. Talent selecting water dipping lens

5.3. Lower the objective into the water **[1]** and check for air bubbles on the front lens of the object **[2]**.

5.3.1. Objective being lowered

5.3.2. ECU: Shot of front lens and air bubbles

5.4. To remove bubbles, lower the stage **[1]** and gently wipe the lens with an optical tissue **[2]**.

5.4.1. Stage being lowered

5.4.2. Lens being wiped

5.5. Then use a Pasteur pipette to add a small volume of water to the front lens of the objective **[1]** before re-immersing the lens in the water **[2]**.

5.5.1. Water being added to lens

5.5.2. Lens being immersed

5.6. Next, use the GFP (G-F-P) filter and epi-illumination module of the confocal microscope to adjust the XY controller to locate the sample **[1-TXT]**.

5.6.1. Talent adjusting XY controller/locating sample **TEXT: GFP: green fluorescent protein**

5.7. Adjusting the position of the oculars, position the shoot apical meristem directly under the light source **[1]** and focus along the Z-axis until the apex is located **[2]**.

5.7.1. Talent adjusting ocular position

5.7.2. Apex being located

5.8. Use a laser capable of exciting GFP to illuminate the sample **[1]** and adjust the optical zoom of the microscope so that the entire shoot apical meristem and the stage 1 floral primordia are in the field of view **[2]**.

5.8.1. SCREEN: imaging settings: 00:50-01:08 *Video Editor: please speed up*

5.8.2. SCREEN: imaging settings: 01:09-01:21

5.9. Then adjust the power of the laser output and the gain settings to obtain an optimal signal-to-noise ratio **[1]**.

5.9.1. SCREEN: imaging settings: 01:35-02:14 *Video Editor: please speed up*

5.10. After allowing the sample to settle for 2-5 minutes, acquire confocal Z stacks of the sample at 0.25-0.5 micrometer Z slice intervals at an approximately 0.3-micrometer pixel size-resolution **[1]**.

5.10.1. SCREEN: imaging settings: 02:18-02:54 *Video Editor: please speed up* **TEXT: Do not acquire >10 min**

5.11. At the end of the acquisition, immediately remove the water **[1]** and transfer the culture box back to the growth chamber **[2]**.

5.11.1. Talent removing water

5.11.2. Talent placing box into growth chamber

6. Micromechanical SAM Perturbation and Data Analysis

6.1. For micromechanical shoot apical meristem perturbation, acquire pre-ablation image stacks of the cortical microtubule organization as just demonstrated **[1]** and decant the water from the culture box into a culture dish **[2]**.

6.1.1. WIDE: Talent at microscope, imaging SAM

6.1.2. Talent decanting water

6.2. Transfer the shoot apical meristem under a dissecting microscope **[1]** and slowly approach the shoot apical meristem with a clean 0.4- x 20-millimeter syringe needle **[2-TXT]**.

6.2.1. Talent placing SAM under microscope

6.2.2. SCOPE: SAM being approached **TEXT: Breath-holding and handling needle w/ relaxed grip helps avoid shaking**

6.3. Briefly contact the periphery of the dome of the shoot apical meristem with the needle tip to confirm that the ablation has been completed **[1]** and refill the culture dish with sterile deionized water **[2]**.

6.3.1. SCOPE: Dome being contacted *Videographer: Important/difficult step*

6.3.2. Talent refilling culture dish

6.4. Then add 10 micrograms/milliliter of propidium iodide to the dish **[1]** and immediately acquire image stacks as demonstrated every 2 hours for 6 hours **[2]**, returning the culture dish to the incubation between each time point **[3-TXT]**.

6.4.1. Talent adding PI to dish, with PI container visible in frame *Videographer: Important step*

6.4.2. Talent at computer acquiring images, with monitor visible in frame 00:02-00:42 *Video Editor: please speed up*

6.4.3. Talent placing culture dish into incubator **TEXT: Refresh water and wrap dish with micropore tape to prevent desiccation as necessary**

6.5. For data analysis, generate surface projections of the image stacks using an appropriate image analysis software program **[1]** and perform extraction of the cortical microtubule anisotropy using the FibrilTool macro in FIJI **[2]**.

6.5.1. Talent at computer, generating surface projections, with monitor visible in frame

6.5.2. SCREEN: FibrilTool anisotropy analysis: 00:05-01:42 *Video Editor: please speed up*

Section – Results

7. Results: Representative Cortical Microtubule Anisotropy Quantification and Mechanical Ablation in *Arabidopsis* SAM

7.1. Here typical projection images obtained from microtubule binding domain-GFP lines **[1]** with cells at the center of the dome containing disorganized cortical microtubules **[2]**, cells at the periphery having a circumferential distribution **[3]**, and boundary domain cells containing cortical microtubules aligned parallel to the cell's long axis can be observed **[4]**.

7.1.1. LAB MEDIA: Figures 1A and 1B
7.1.2. LAB MEDIA: Figures 1A and 1B *Video Editor: please emphasize yellow area of Central domain image in Figure 1B*
7.1.3. LAB MEDIA: Figures 1A and 1B *Video Editor: please emphasize peripheral cells in Figure 1A Surface projection image*
7.1.4. LAB MEDIA: Figures 1A and 1B *Video Editor: please emphasize yellow cells in Boundary domain image in Figure 1B*

7.2. Time lapse imaging shows cortical microtubule alignment changing from a highly disordered array **[1]** to a more organized array within 6 hours of ablation **[2]**.

7.2.1. LAB MEDIA: Figures 1C and 1D top row of images *Video Editor: please emphasize Pre Ablation and 0 hrs images*
7.2.2. LAB MEDIA: Figures 1C and 1D top row of images *Video Editor: please emphasize 2 hrs – 6 hrs images in Figure 1C and top row of images in Figure 1D*

7.3. Tensors could be superimposed on the cortical microtubule images **[1]** and the extracted information could be represented by plotting the mean anisotropy over time **[2]**.

7.3.1. LAB MEDIA: Figures 1D bottom row of images *Video Editor: please emphasize yellow rectangles w/ red lines*
7.3.2. LAB MEDIA: Figures 1D bottom row of images and Figure 1E images

Section - Conclusion

8. Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

8.1. **Yang Wang**: When adjusting the laser output power and gain settings, ensure a clear observation of the filaments and avoid overexposure, as saturated signals can bias the tensor direction during the analysis **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (5.9.)

8.2. **Arun Sampathkumar**: Atomic force microscopy can also be performed to assess how mechanical perturbation can impact the actual physical properties of cell surfaces **[1]**.

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