FINAL SCRIPT: APPROVED FOR FILMING

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Title: Live Imaging Characterization of Centromere Movements During Male Meiotic Prophase in *Arabidopsis thaliana*

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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? **YES**

   NO, we can not record but we have a camera port

   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**.

   Our stereomicroscope Nikon SMZ800 has a camera port that we use, connected to a screen, to give demonstrations to students. I can send you a picture if you need.

   2.4
   2.5
   2.6
   2.7

   Videographer: Please film SCOPE shots using the scope kit

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? **YES**

   200 meters

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. **NO**

Current Protocol Length
Number of Steps: 22
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. **Laurence Cromer:** Our research explores plant meiosis, aiming to uncover molecular mechanisms guiding homologous chromosome recognition, alignment, and pairing during meiotic progression.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll: 2.4*~~

~~What are the current experimental challenges?~~

1.2. **Laurence Cromer:** The challenge is visualizing chromosome movement in meiotic prophase, requiring live tissue imaging with high resolution and speed to track trajectories.

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. **Laurence Cromer:** This protocol enabled first-time quantification of meiotic chromosome movement in Arabidopsis, revealing dramatic centromere dynamics during zygotene and pachytene.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll: Figure 5*~~

~~What new scientific questions have your results paved the way for?~~

1.4. **Laurence Cromer:** This protocol enables studying genomic region movements during meiotic prophase and can be adapted for application in other plant species.

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. **Laurence Cromer:** This work enables exploration of chromosome movement–recombination interplay, deepening understanding of mechanisms driving genetic diversity during plant gamete formation

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B roll: 4.4*~~

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

Protocol

2. Dissecting Anthers and Slide Preparation

Demonstrator: Laurence Cromer

2.1. To begin, ~~prepare a glass slide.~~ remove the protective film of the spacer on one side and place it at the center of a glass slide then remove the second protective film **[1]**. Deposit 8 microliters of tap water into the center of the spacer **[2]**.

ADDED SHOT: EXTRA 2.1.1B peel off first side of the spacer

2.1.1. WIDE: Talent placing a spacer at the center of a glass slide and peeling off protective films from both sides.

2.1.2. Talent pipetting 8 microliters of tap water into the spacer cavity.

2.2. Using forceps, pick an inflorescence from one of the main stems **[1]**. Place the inflorescence on a new slide positioned under a stereomicroscope fitted with a ruler **[2]**. Select flower buds that measure approximately 0.5 millimeters in length **[3]**.

2.2.1. Talent using forceps to pick an inflorescence from the stem. AUTHOR'S NOTE: Use the last take

2.2.2. Talent placing the inflorescence on a glass slide beneath a stereomicroscope equipped with a ruler.

2.2.3. SCOPE: View through the stereomicroscope showing selection of flower buds measuring about 0.5 millimeters.

Videographer: Please film SCOPE shots using the scope kit

2.3. Using sharp needles, gently open the buds by pulling apart the sepals and petals to retain only the anthers **[1-TXT]**.

2.3.1. SCOPE: Talent using sharp needles to open flower buds and separate sepals and petals. **TXT: Avoid damaging the anthers or letting them dry**
AUTHOR'S NOTE: use take 3, and take 4 for result

2.4. Transfer the anther bunches delicately into the water in the spacer cavity immediately after removing the sepals and petals to prevent dehydration **[1]**. Deposit several anther bunches inside the spacer cavity **[2]**.

2.4.1. SCOPE: Talent transferring the freshly isolated anther bunches into the water-

filled spacer cavity.

2.4.2. SCOPE: Close-up shot of multiple anther bunches deposited inside the cavity. AUTHOR'S NOTE: Shot with 2.4.1

2.5. To cover the slide with a coverslip while avoiding air bubbles, add 8 microliters of water onto a coverslip **[1]**, then carefully invert the coverslip with the drop of water facing the spacer cavity **[2]**. Ensure the anther bunches are immersed in a total of 16 microliters of water and that the coverslip adheres well to the spacer **[4]**.

2.5.1. Talent pipetting 8 microliters of water onto a coverslip.

2.5.2. Talent carefully turning the coverslip over and placing it onto the spacer cavity. AUTHOR'S NOTE: Shot with 2.5.1

2.5.3. Close-up shot showing the coverslip fully sealed to the spacer with anthers immersed in water. AUTHOR'S NOTE: slated 2.5.1 so check the file name

3. Confocal Microscopy Time-Lapse Observation – Imaging Setup

Demonstrator: Laurence Cromer

3.1. Set up the microscope by exciting GFP at 488 nanometers **[1]** and detecting it with a hybrid detector between 494 nanometers and 547 nanometers **[2]**.

3.1.1. SCREEN: 68569_screenshot_3-1-1.MKV 00:02-00:20

3.1.2. SCREEN: 68569_screenshot_3-1-2.MKV 00:00-00:14

3.2. Then, localize the anther using brightfield illumination **[1]**. Using the brightfield channel, determine the meiotic stage based on the shape of the cells **[2]**.

3.2.1. SCREEN: 68569_screenshot_3-2-1.MKV 00:03-00:18

3.2.2. SCREEN: 68569_screenshot_3-2-2Diplo.MKV 00:00-00:09;
68569_screenshot_3-2-2Lepto.MKV 00:02-00:05,
68569_screenshot_3-2-2ZygoPachy.MKV 00:09-00:17

3.3. Visualize the anther on the software and use the RFP signal as an additional indicator of the meiotic stage of the cells **[1]**.

3.3.1. SCREEN:68569_screenshot_3-3-1.MKV 00:00-00:12

3.4. To perform continuous acquisitions, set the time interval to zero in the **Time** section **[1-TXT]**. Then, use the spot module to automatically track spots **[2]**.

3.4.1. SCREEN: 68569_screenshot_3-4-1part1.MKV 00:00-00:20;
TXT: Acquisition time: 16 slices in 6 - 8s; Total duration: 2 - 20 min

3.4.2. SCREEN: 68569_screenshot_3-4-2.MKV 00:04-00:16

4. Image Analysis – Centromere Movement Tracking

4.1. Choose the green source channel and set the XY diameter to 1 micrometer for the GFP-CENH3 *(Cen-H-Three)*-marked centromere **[1-TXT]**. ~~Perform object detection to identify all objects in the image **[2].**~~

4.1.1. SCREEN:68569_screenshot_4-1-1.MKV 00:00-00:19
TXT: Perform object detection to identify all objects

~~4.1.2. SCREEN:Display object detection identifying all centromere marked objects in the image. Screenhot include in Screenshot4-1-1~~

4.2. Then, modify the quality of detection based on signal intensity using the graph at the bottom of the control window **[1]**.

4.2.1. SCREEN:68569_screenshot_4-2-1.MKV 00:00-00:19

4.3. Use the auto-regressive motion tracking algorithm to trace centromere trajectories and visualize all trajectories to easily distinguish meiocytes from somatic tissues **[1]**.

4.3.1. SCREEN: 68569_screenshot_4-3-1.MKV 00:00-00:24

4.4. Next, use the Spot module to track spots over time on the selected nucleus **[1].** Define a Region of Interest by selecting the option **Segment only a Region of Interest** and perform spot tracking within it **[2]**.

4.4.1. SCREEN: 68569_screenshot_4-4-1.MKV 00:00-00:15

4.4.2. SCREEN:68569_screenshot_4-4-2.MKV 00:00-00:29

4.5. Select a Region of Interest that properly includes the nucleus in all three dimensions and over time **[1-TXT]**.

4.5.1. SCREEN: 68569_screenshot_4-5-1.MKV 00:12-00:25
TXT: Perform centromere detection & tracking using the same parameters as whole anther

4.6. In a lineage plot, use the software's 3D viewer to visualize all trajectories. Remove the trajectories outside the analyzed meiocyte by selecting them and pressing the **Delete** key on the keyboard **[1]**.

4.6.1. SCREEN: 68569_screenshot_4-6-1.MKV 00:00-00:32

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4.7. In **Edit** mode, delete or add an object, ensuring that the object is added in the correct plane **[1]**. In **Edit Tracks** mode, manually connect the new objects on the lineage plot

after thoroughly analyzing the cell in 3D **[2]**.

4.7.1. SCREEN: 68569_screenshot_4-7-1.MKV 00:30-00:47

4.7.2. SCREEN: 68569_screenshot_4-7-2.MKV 00:20-00:47

5. Data Analysis

Demonstrator: Sandrine Lefranc

5.1. Compute the instantaneous speed at each time step to monitor variations in movement velocity over time **[1]**. Calculate the turning angle as the change in direction of movement between consecutive time steps to reveal trajectory patterns **[2]**. Store the result together with the instantaneous speed **[3]**.

5.1.1. SCREEN: 68569_screenshot_5-1-1bis.MKV 00:00-00:17, 00:46-00:48

5.1.2. SCREEN: 68569_screenshot_5-1-2.MKV 00:00-00:18

5.1.3. SCREEN: 68569_screenshot_5-1-3.MKV 00:04-00:18

5.2. Compute the outreach ratio using celltrackR *(Cell-Track-R)* to measure how far a tracked object moves relative to its starting position, indicating exploratory behavior **[1-TXT]**.

5.2.1. SCREEN: 68569_screenshot_5-2-1.MKV 00:00-00:17
TXT: Store the result as results/dataframes/metrics-celltrackr.tsv

5.3. Compute the average speed for each track to summarize movement characteristics across the observation period **[1]**. Calculate the average turning angle per track to analyze movement directionality and behavioral patterns and store the result **[2]**.

5.3.1. SCREEN: 68569_screenshot_5-3-1.MKV 00:00-00:15

5.3.2. SCREEN: 68569_screenshot_5-3-2.MKV 00:00-00:17

5.4. Next, compute the centroid size at each timestep to measure the spatial dispersion of centromeres around their average position **[1]**. Use variations in centroid size over time to evaluate global consistency across centromere tracks and store the result **[2]**.

5.4.1. SCREEN: 68569_screenshot_5-4-1.MKV 00:00-00:17

5.4.2. SCREEN: 68569_screenshot_5-4-2.MKV 00:00-00:21

5.5. Compute the speed cross-correlation to analyze relationships between the movement dynamics of different tracked objects, revealing potential coordinated

behaviors **[1-TXT]**.

5.5.1. SCREEN: 68569_screenshot_5-5-1.MKV 00:00-00:20
TXT: Store the result as results/dataframes/metrics-correlations.tsv

5.6. Then, compute the mean squared displacement for each track to quantify the average squared distance traveled by centromeres over a given time interval **[1]**.

5.6.1. SCREEN: 68569_screenshot_5-6-1.MKV 00:00-00:20

Results

6. Results

6.1. Meiotic centromeres exhibited a significantly higher average speed of 109.35 nanometers per second compared to somatic centromeres **[1].**

6.1.1. LAB MEDIA: Figure 5A. V*ideo editor: Highlight the taller violin plot labeled "M" on the left, showing higher average speed values.*

6.2. Meiotic centromeres showed a broader range and higher normalized outreach ratio compared to somatic centromeres **[1].** The turning angle distribution differed between meiotic and somatic centromeres **[2].**

6.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the wider violin plot labeled "Meiotic" on the left, showing a broader distribution*.

6.2.2. LAB MEDIA: Figure 5C.

6.3. The mean square displacement of meiotic centromeres increased over time, while somatic centromeres remained largely static **[1].**

6.3.1. LAB MEDIA: Figure 5D. *Video editor: Highlight the upward trend line labeled "wt-m", indicating increased movement over time.*

Pronunciation Guide:

1. **Arabidopsis thaliana**
   Pronunciation link: https://www.merriam-webster.com/dictionary/Arabidopsis
   IPA: /ˌær.ə.boʊˈdɒp.sɪs θæ.liˈæn.ə/
   Phonetic Spelling: a·ruh·boh·dop·sis tha·lee·a·nuh
2. **centromere**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centromere
   IPA: /ˈsɛn.trəˌmɪr/
   Phonetic Spelling: sen·truh·meer
3. **meiosis / meiotic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/meiosis
   IPA: /maɪˈoʊ.sɪs/
   Phonetic Spelling: my·oh·sis

Pronunciation link: https://www.merriam-webster.com/dictionary/meiotic
IPA: /maɪˈɑː.tɪk/
Phonetic Spelling: my·aa·tik

4. **prophase**
   Pronunciation link: https://www.merriam-webster.com/dictionary/prophase
   IPA: /ˈproʊˌfeɪz/
   Phonetic Spelling: proh·fayz
5. **inflorescence**
   Pronunciation link: https://www.merriam-webster.com/dictionary/inflorescence
   IPA: /ˌɪn.fləˈrɛs.əns/
   Phonetic Spelling: in·fluh·reh·suhns
6. **anther**
   Pronunciation link: https://www.merriam-webster.com/dictionary/anther
   IPA: /ˈæn.θər/
   Phonetic Spelling: an·ther
7. **sepal**
   Pronunciation link: https://www.merriam-webster.com/dictionary/sepal
   IPA: /ˈsiː.pəl/
   Phonetic Spelling: see·puhl
8. **petal**
   Pronunciation link: https://www.merriam-webster.com/dictionary/petal
   IPA: /ˈpɛt.əl/
   Phonetic Spelling: peh·tuhl
9. **microliter**
   Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
   IPA: /ˈmaɪ.krəˌliː.tər/
   Phonetic Spelling: my·kruh·lee·ter

10. **stereomicroscope**
    Pronunciation link: No confirmed link found
    IPA: /ˌstɛr.i.oʊˈmaɪ.krəˌskoʊp/
    Phonetic Spelling: stair·ee·oh·my·kruh·skoap
11. **GFP (Green Fluorescent Protein)**
    Pronunciation link: No confirmed link found
    IPA: /ˌdʒiːˌɛfˈpiː/
    Phonetic Spelling: gee·ef·pee
12. **nanometer**
    Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
    IPA: /ˈnæn.əˌmiː.tər/
    Phonetic Spelling: nan·uh·mee·ter
13. **hybrid detector**
    Pronunciation link: https://www.merriam-webster.com/dictionary/hybrid
    IPA (hybrid): /ˈhaɪ.brɪd/
    Phonetic Spelling: hy·brid
14. **brightfield**
    Pronunciation link: No confirmed link found
    IPA: /ˈbraɪtˌfiːld/
    Phonetic Spelling: bryt·feeld
15. **RFP (Red Fluorescent Protein)**
    Pronunciation link: No confirmed link found
    IPA: /ˌɑːrˌɛfˈpiː/
    Phonetic Spelling: ar·ef·pee
16. **meiocyte**
    Pronunciation link: No confirmed link found
    IPA: /ˈmaɪ.oʊˌsaɪt/
    Phonetic Spelling: my·oh·syte
17. **nucleus**
    Pronunciation link: https://www.merriam-webster.com/dictionary/nucleus
    IPA: /ˈnuː.kli.əs/
    Phonetic Spelling: noo·klee·us
18. **algorithm**
    Pronunciation link: https://www.merriam-webster.com/dictionary/algorithm
    IPA: /ˈæl.gəˌrɪð.əm/
    Phonetic Spelling: al·guh·rih·thum
19. **trajectory**
    Pronunciation link: https://www.merriam-webster.com/dictionary/trajectory
    IPA: /trəˈdʒɛk.təˌri/
    Phonetic Spelling: truh·jek·tuh·ree
20. **centroid**
    Pronunciation link: https://www.merriam-webster.com/dictionary/centroid

IPA: /ˈsɛnˌtrɔɪd/
Phonetic Spelling: sen·troid

21. **correlation**
Pronunciation link: https://www.merriam-webster.com/dictionary/correlation
IPA: /ˌkɔːr.əˈleɪ.ʃən/
Phonetic Spelling: kor·uh·lay·shun
22. **displacement (in scientific context)**
Pronunciation link: https://www.merriam-webster.com/dictionary/displacement
IPA: /dɪsˈpleɪs.mənt/
Phonetic Spelling: dis·play·smuhnt
23. **zygote / zygotene**
Pronunciation link (zygote): https://www.merriam-webster.com/dictionary/zygote
IPA: /ˈzaɪˌgoʊt/
Phonetic Spelling: zy·goht

Pronunciation link (zygotene): No confirmed link found
IPA: /ˈzaɪ.goʊˌtiːn/
Phonetic Spelling: zy·goh·teen

24. pachytene
Pronunciation link: No confirmed link found
IPA: /ˈpæk.ɪˌtiːn/
Phonetic Spelling: pa·kih·teen
25. **somatic**
Pronunciation link: https://www.merriam-webster.com/dictionary/somatic
IPA: /soʊˈmæt.ɪk/
Phonetic Spelling: soh·ma·tik
26. CENH3 (Cen-H-Three)
Pronunciation link: No confirmed link found
IPA: /ˌsiː.iːˈɛnˈeɪtʃˈθriː/
Phonetic Spelling: see·ee·en·aych·three
27. **micrometer (µm)**
Pronunciation link: https://www.merriam-webster.com/dictionary/micrometer
IPA: /maɪˈkrɑː.mɪ.tər/
Phonetic Spelling: my·krah·mih·ter