

Submission ID #: 71307

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21748683>

Title: Quantitative Live Cell Imaging of Nuclear Shape and Chromatin Dynamics During Development and Environmental Stress in *Arabidopsis thaliana* Root

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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**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**
- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
- 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: 39

Number of Shots: 56

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Joh Demura-Devore:** Our research investigates genes and mechanisms that enable long-term root recovery from osmotic stress to fortify crops against drought.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Joh Demura-Devore:** Although developed for Arabidopsis roots, this protocol can be adapted to systems with fluorescent protein marker lines.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Joh Demura-Devore:** This protocol enables tracking and quantification of fluorescently labeled protein movement over time in Arabidopsis roots.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Joh Demura-Devore:** The most important challenge in this protocol is obtaining high-quality images, as photobleaching and sample drift can make tracking difficult.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Joh Demura-Devore:** Future studies should investigate the mechanisms underlying the reduced chromatin movement observed in our study.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

2. Live Imaging of Nuclear Envelope and Chromatin Dynamics in *A. thaliana* Roots

Demonstrator: Joh Demura-Devore

- 2.1. Obtain the growth plates containing *Arabidopsis thaliana* seedlings prepared for imaging [1-TXT].
 - 2.1.1. WIDE: Talent walking into the room with a tray of growth plates. **TEXT: WIP1-GFP x CENH3-mRFP** *Videographer: Please obtain CU shot as well for this shot.*
- 2.2. Pipette 60 microliters of pure water onto a glass slide [1].
 - 2.2.1. Talent pipetting 60 microliters of pure water onto a glass slide.
- 2.3. Lay 3 six-day-old *A. thaliana* seedling roots into the water [1].
 - 2.3.1. CLOSE-UP: Talent positioning 3 six-day-old *Arabidopsis thaliana* seedling roots in the water on the glass slide.
- 2.4. Place a 22-millimeter × 22-millimeter number 1.5 coverslip over the roots without covering the leaves [1].
 - 2.4.1. CLOSE-UP: Shot of placing the coverslip over the roots while ensuring the leaves remain uncovered.
- 2.5. In Confocal microscope settings, set the laser exposure time to 1000 milliseconds [1].
 - 2.5.1. SCREEN: Show the confocal microscope acquisition settings and enter 1000 milliseconds as the laser exposure time. **Note: The videographer has filmed the SC in section 2.**
- 2.6. In the laser combiner settings, set the 488-nanometer laser to 50% for green fluorescent protein or GFP (*G-F-P*) imaging [1]. Set the 561-nanometer laser to 50% for red fluorescent protein or RFP (*R-F-P*) imaging [2]. Image the sample using a 20× objective lens without optical zoom [3].
 - 2.6.1. SCREEN: Show the laser combiner panel and the 488 nanometer laser power being adjusted to 50% for GFP. **Videographer's Note: 2.6.1 and 2.6.2 were combined.**
 - 2.6.2. SCREEN: Show the laser combiner panel and the 561 nanometer laser power being adjusted to 50% for RFP.

- 2.6.3. SCREEN: Show the objective selection and acquisition settings being configured with a 20× objective lens and no optical zoom.
- 2.7. Set the Z-slice step size to 0.3 micrometers [1].
 - 2.7.1. SCREEN: Show the Z-stack settings and step size of 0.3 micrometers being entered.
- 2.8. Set the imaging interval to 1 minute [1] and the total imaging duration to 10 minutes [2].
 - 2.8.1. SCREEN: Show the time-lapse settings and enter an interval of 1 minute. Videographer's Note: 2.8.1 and 2.8.2 were combined.
 - 2.8.2. SCREEN: Show the time-lapse settings and enter a total duration of 10 minutes.
- 2.9. Locate the roots [1] and select up to 20 Z-slices [2]. Capture 16-bit images [3].
 - 2.9.1. SCOPE: Show the live microscope view as the roots are located within the sample. Note: The videographer has filmed the scope shot.
 - 2.9.2. SCREEN: Show the 20 Z-slices being selected. Videographer's Note: 2.9.2 and 2.9.3 were combined
 - 2.9.3. SCREEN: Show the 16-bit image settings being enabled.
- 2.10. Start the time-lapse acquisition and save the time-lapse as a TIFF file using lossless compression [1].
 - 2.10.1. SCREEN: Show the acquisition software and click the **Start Timelapse** button to begin recording and select TIFF format with lossless compression enabled before saving the file.
- 2.11. Move the acquired data to a computer for analysis [1].
 - 2.11.1. Talent transferring the acquired imaging data to a computer for subsequent analysis.

3. Chromatin Tracking and Analysis

- 3.1. Open the ImageJ (*Image-J*) or Fiji software [1].
 - 3.1.1. SCREEN: 71307_screenshot_1.mp4: 00:04-00:11
- 3.2. Click and drag the .vsi (*V-S-I*) file onto the main ImageJ or Fiji application window [1].
 - 3.2.1. SCREEN: 71307_screenshot_1.mp4: 00:23-00:26
- 3.3. In the **Bio-Formats Import Options** prompt, click **OK** in the bottom right [1].
 - 3.3.1. SCREEN: 71307_screenshot_1.mp4: 00:26-00:33

- 3.4. Then, in the **Bio-Formats Series Options** prompt, ensure **Series 1** is selected [1]. Click **OK** in the bottom left [2].
- 3.4.1. SCREEN: 71307_screenshot_1.mp4: 00:33-00:36
- 3.4.2. SCREEN: 71307_screenshot_1.mp4: 00:36-00:39
- 3.5. Once the image has loaded, split the signals by selecting **Image** [1]. Select **Color** and choose **Split Channels** [2]. View the separate windows generated for GFP-labeled nuclei and RFP-labeled chromatin [3].
- 3.5.1. SCREEN: 71307_screenshot_1.mp4: 00:58-01:07
- 3.5.2. SCREEN: 71307_screenshot_1.mp4: 01:08-01:11
- 3.5.3. SCREEN: 71307_screenshot_1.mp4: 01:12-01:13
- 3.6. Determine which of the 2 image windows corresponds to chromatin [1].
- 3.6.1. SCREEN: 71307_screenshot_1.mp4: 01:14-01:20
- 3.7. After clicking the chromatin image window, apply the Z-project operation by selecting **Image**, choosing **Stacks**, and then clicking **Z Project** from the main ImageJ window [1].
- 3.7.1. SCREEN: 71307_screenshot_1.mp4: 01:24-01:31
- 3.8. Change the projection type to **Max Intensity** [1]. Ensure that **All Time Frames** is active and click **OK** to generate the projection [2].
- 3.8.1. SCREEN: 71307_screenshot_1.mp4: 01:32-01:38
- 3.8.2. SCREEN: 71307_screenshot_1.mp4: 01:32-01:38 Notes: 3.8.1 and 3.8.2 were combined. Show the SC only once.
- 3.9. Open the contrast menu by pressing **Ctrl+Shift+C** [1] or by selecting **Image** and choosing **Adjust**, and then clicking **Brightness or Contrast** from the main ImageJ window [2].
- 3.9.1. Close-up shot of pressing **Ctrl+Shift+C**
- 3.9.2. SCREEN: 71307_screenshot_1.mp4: 01:51-01:56
- 3.10. Slide the **Minimum** bar to the left until the chromatin is clearly visible [1].
- 3.10.1. SCREEN: 71307_screenshot_1.mp4: 01:57-02:08
- 3.11. Navigate to the **Plugins**, click the down arrow in the **Plugins** menu to scroll to the **tracking** option [1].
- 3.11.1. SCREEN: 71307_screenshot_1.mp4: 02:09-02:15
- 3.12. Select **Tracking** and click **TrackMate** [1].
- 3.12.1. SCREEN: 71307_screenshot_1.mp4: 02:16-02:18
- 3.13. Confirm that the calibration settings are accurate and click **Next** [1].
- 3.13.1. SCREEN: 71307_screenshot_1.mp4: 02:19-02:25

3.14. Select Thresholding Detector and click Next [1].

3.14.1. SCREEN: 71307_screenshot_1.mp4: 02:26-02:30

3.15. Move to the last frame of the time-lapse on the image window [1].

3.15.1. SCREEN: 71307_screenshot_1.mp4: 02:31-02:35

3.16. Click **Auto** next to the intensity threshold and click **Preview** [1].

3.16.1. SCREEN: 71307_screenshot_1.mp4: 02:36-02:39

3.17. Adjust the intensity threshold and click **Preview** [1]. Repeat the adjustment and preview process until all chromatin is included with minimal noise, and click **Next** [2].

3.17.1. SCREEN: 71307_screenshot_1.mp4: 02:40-02:46

3.17.2. SCREEN: 71307_screenshot_1.mp4: 02:47-02:49

3.18. Allow the initial thresholding detection to complete and click **Next** [1].

3.18.1. SCREEN: 71307_screenshot_1.mp4: 02:50-02:52

3.19. Manually adjust the threshold until only chromatin is visible and click **Next** [1].

3.19.1. SCREEN: 71307_screenshot_1.mp4: 02:53-03:09

3.20. ~~Ignore the spot filtering page~~ Adjust the fluorescence image display and click **Next** [1].

3.20.1. SCREEN: 71307_screenshot_1.mp4: 03:10-03:21

3.21. Select Simple LAP Tracker and click **Next** [1].

3.21.1. SCREEN: 71307_screenshot_1.mp4: 03:22-03:26

3.22. Set the **linking max distance** to **5 microns** [1], **gap-closing max distance** to **15 microns** [2], **gap-closing maximum frame gap** to **1**, and click **Next** [3].

3.22.1. SCREEN: 71307_screenshot_1.mp4: 03:27-03:31 **Note: 3.22.1, 3.22.2, 3.22.3 were combined. Show the SC only once.**

3.22.2. SCREEN: 71307_screenshot_1.mp4: 03:27-03:31

3.22.3. SCREEN: 71307_screenshot_1.mp4: 03:27-03:31

3.23. Allow tracking to complete [1]. Observe the tracks displayed in the original image window and click **Next** [2].

3.23.1. SCREEN: 71307_screenshot_1.mp4: 03:32-03:34 **Note: 3.23.1 and 3.23.2 were combined. Show the SC only once.**

3.23.2. SCREEN: 71307_screenshot_1.mp4: 03:32-03:34

3.24. Press the **green + (green-plus)** button in the bottom left corner to create a filter [1]. Select **Number of Spots in Track** mode and click **Auto** [2].

3.24.1. SCREEN: 71307_screenshot_1.mp4: 03:35-03:38

3.24.2. SCREEN: 71307_screenshot_1.mp4: 03:39-03:51

3.25. Adjust the threshold slider, ensure the filter mode is set to Above, and then click **Next [1]**.

3.25.1. SCREEN: 71307_screenshot_1.mp4: 03:52-04:04

3.26. Click the **Tracks** button at the bottom of the display options page **[1]**. Select **Export to CSV [2]**.

3.26.1. SCREEN: 71307_screenshot_1.mp4: 04:05-04:11

3.26.2. SCREEN: 71307_screenshot_1.mp4: 04:12-04:17

3.27. Ignore the built-in plotting page and click **Next [1]**.

3.27.1. SCREEN: 71307_screenshot_1.mp4: 04:18-04:23

3.28. Review the TrackMate analysis results, click **Capture Overlay**, and then choose **Execute [1]**. Save the video by selecting **File** and clicking **Save As**, and then choosing **GIF [2]**.

3.28.1. SCREEN: 71307_screenshot_1.mp4: 04:24-04:37

3.28.2. SCREEN: 71307_screenshot_1.mp4: 04:38-04:56

Results

4. Results

- 4.1. Salt-treated root cells appeared stressed [1], and centromeric foci appeared larger than in control conditions [2].
 - 4.1.1. LAB MEDIA: Figure 8. *Video editor: Emphasize the right image labeled Salt.*
 - 4.1.2. LAB MEDIA: Figure 8. *Video editor: Emphasize the magenta dots in both the images labeled salt and control.*
- 4.2. Chromatin speed was significantly higher in control conditions [1] than in 100 millimolar sodium chloride-treated conditions [2].
 - 4.2.1. LAB MEDIA: Figure 9. *Video editor: Emphasize the Control blue plot.*
 - 4.2.2. LAB MEDIA: Figure 9. *Video editor: Emphasize the Salt pink plot.*

Pronunciation guides:

1. Arabidopsis
Pronunciation link: <https://www.merriam-webster.com/dictionary/Arabidopsis>
IPA: /ˌer.ə.bəˈdɑːp.sɪs/
Phonetic Spelling: air·uh·buh·DAH·p·sis
2. thaliana
Pronunciation link: <https://www.howtopronounce.com/thaliana>
IPA: /ˌθɑːliˈɑːnə/
Phonetic Spelling: thah·lee·AH·nuh
3. seedlings
Pronunciation link: <https://www.merriam-webster.com/dictionary/seedling>
IPA: /ˈsiːd.lɪŋ/
Phonetic Spelling: SEED·ling
4. microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪ.kroʊ.liː.tər/
Phonetic Spelling: MY·kroh·lee·ter
5. coverslip
Pronunciation link: <https://www.merriam-webster.com/dictionary/coverslip>
IPA: /ˈkʌv.əˌslɪp/
Phonetic Spelling: KUV·er·slip
6. Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>

IPA: /kən'foʊ.kəl/

Phonetic Spelling: kuhn·FOH·kuhl

7. fluorescent

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>

IPA: /flʊ'res.ənt/, /flɒ:'res.ənt/

Phonetic Spelling: floor·ESS·ent

8. objective

Pronunciation link: <https://www.merriam-webster.com/dictionary/objective>

IPA: /əb'dʒek.tɪv/

Phonetic Spelling: ub·JEK·tiv

9. optical

Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>

IPA: /'ɑ:p.tɪ.kəl/

Phonetic Spelling: AHP·tih·kuhl

10. micrometers

Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>

IPA: /maɪ'krɑ:.mə.tə/

Phonetic Spelling: my·KRAH·muh·ter

11. acquisition

Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>

IPA: /,æk.wə'zɪʃ.ən/

Phonetic Spelling: ak·wuh·ZIH·shuhn

12. TIFF

Pronunciation link: <https://www.merriam-webster.com/dictionary/TIFF>

IPA: /tɪf/

Phonetic Spelling: tiff

13. ImageJ

Pronunciation link: <https://imagej.net/ij/>

IPA: /'ɪm.ɪdʒ dʒeɪ/

Phonetic Spelling: IM·ij·JAY

14. Fiji

Pronunciation link: <https://www.merriam-webster.com/dictionary/Fiji>

IPA: /'fi:.dʒi/

Phonetic Spelling: FEE·jee

15. Bio-Formats

Pronunciation link: <https://www.openmicroscopy.org/bio-formats/>

IPA: /'baɪ.ʊʊ 'fɔ:r.mæts/

Phonetic Spelling: BY·oh FOR·mats

16. chromatin

Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatin>

IPA: /'krɒʊ.mə.tɪn/

Phonetic Spelling: KROH·muh·tin

17. nuclei
Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleus>
IPA: /'nu:.kli.əɪ/
Phonetic Spelling: NOO·klee·eye
18. projection
Pronunciation link: <https://www.merriam-webster.com/dictionary/projection>
IPA: /prə'dʒek.ʃən/
Phonetic Spelling: pruh·JEK·shuhn
19. intensity
Pronunciation link: <https://www.merriam-webster.com/dictionary/intensity>
IPA: /ɪn'ten.sə.ti/
Phonetic Spelling: in·TEN·suh·tee
20. Plugins
Pronunciation link: <https://www.merriam-webster.com/dictionary/plugin>
IPA: /'plʌɡ,ɪn/
Phonetic Spelling: PLUG·in
21. TrackMate
Pronunciation link: <https://imagej.net/plugins/trackmate/>
IPA: /'træk.mert/
Phonetic Spelling: TRAK·mate
22. calibration
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibration>
IPA: /,kæl.ə'breɪ.ʃən/
Phonetic Spelling: kal·uh·BRAY·shuhn
23. Thresholding
Pronunciation link: <https://www.merriam-webster.com/dictionary/threshold>
IPA: /'θref,həʊl.dɪŋ/
Phonetic Spelling: THRESH·hohl·ding
24. detector
Pronunciation link: <https://www.merriam-webster.com/dictionary/detector>
IPA: /dɪ'tek.tə/
Phonetic Spelling: dih·TEK·ter
25. fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flɒ'res.əns/, /flɒ:'res.əns/
Phonetic Spelling: floor·ESS·uhns
26. microns
Pronunciation link: <https://www.merriam-webster.com/dictionary/micron>
IPA: /'maɪ.krɑ:n/
Phonetic Spelling: MY·kron
27. centromeric
Pronunciation link: <https://www.howtopronounce.com/centromeric>

IPA: /ˌsɛn.trəˈmɛr.ɪk/

Phonetic Spelling: sen·truh·MAIR·ik

28. foci

Pronunciation link: <https://www.merriam-webster.com/dictionary/focus>

IPA: /ˈfoʊ.sai/

Phonetic Spelling: FOH·syə

29. sodium

Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium>

IPA: /ˈsoʊ.di.əm/

Phonetic Spelling: SOH·dee·uhm

30. chloride

Pronunciation link: <https://www.merriam-webster.com/dictionary/chloride>

IPA: /ˈklɔːr.aɪd/

Phonetic Spelling: KLOR·ide