Chapter 16

Using Live-Cell Markers in Maize to Analyze Cell Division Orientation and Timing

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Abstract

Recently developed live-cell markers provide an opportunity to explore the dynamics and localization of proteins in maize, an important crop and model for monocot development. A step-by-step method is outlined for observing and analyzing the process of division in maize cells. The steps include plant growth conditions, sample preparation, time-lapse setup, and calculation of division rates.

Key words Maize, Mitosis, Plant, Live-cell imaging, Microtubules, Cell division, Fluorescent proteins, Confocal, Microscopy

1 Introduction

Live-cell imaging in plant cells is used to study many dynamic processes including meristem growth [1–3], development [4], root hair growth [5], organelle movement [6], and microtubule and actin dynamics [7–11]. Live-cell imaging has also provided insight into the mechanisms of cell division in plants [12–16]. The recent development of stably transformed maize lines expressing fluorescently tagged proteins makes it possible to answer questions regarding the dynamics of cell division and other processes [17, 18]. Maize is an ideal model system for this type of study because its relatively large cells divide within the framework of other cells while growing in a precisely defined developmental gradient.

Live plant cell imaging performed to understand the process of division has primarily used *Arabidopsis thaliana*, *Tradescantia virginiana*, and tobacco-cultured cells. Reliable, stable transformation of *A. thaliana* [19] has resulted in the fusion of many proteins of interest to fluorescent proteins with subsequent examination of protein dynamics. A more challenging technique that has also yielded dynamic information is microinjection of fluorescently labeled proteins and subsequent live-cell imaging in the spiderwort

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T. virginiana [20–22]. Another powerful model used to answer questions about cell division is the cultured tobacco cell line bright yellow 2 (BY-2). BY-2 cells are easy to transform [23], synchronize [24], and observe in vitro on the microscope [25–29]. *Physcomitrella patens* recently emerged as a model for live cell imaging [8, 30, 31] which will likely soon be expanded to explore the dynamics of division.

Maize has lagged as a model system for live plant cell imaging because there were very few available maize lines expressing proteins fused to fluorescent proteins. A notable exception to the lack of live cell imaging is the analysis of chromosome movement during meiosis, which does not require fluorescent proteins [32]. Although bombardment of maize leaf cells has been used to transiently express fluorescent proteins, bombardment has the disadvantage of transforming a small number of cells. Moreover, it is a damaging process that requires lengthy in vitro culture of isolated tissues, which may alter protein dynamics or localization [33]. Stable transgenic lines expressing a variety of proteins fused to fluorescent proteins circumvent some of the problems with transient maize transformation, allowing live-cell imaging of dynamic processes.

Maize is an excellent model system for development and cell biology in monocots because its leaves develop progressively and reproducibly. This reproducible developmental gradient in the leaves is referred to as the "base to tip gradient" [34, 35]. Near the base of the leaf, cells divide symmetrically. Further from the base, some cells divide asymmetrically to differentiate and to establish specialized cell types [36]. Finally, towards the tip of the leaf, cells expand rapidly. This reproducibility allows direct comparisons to be made regarding distinct developmental stages, including a recent explosion in large-scale "-omics" analysis [37–43]. The recent influx of genomics resources together with the developmental gradient and live-cell imaging tools will synergize to improve our understanding of monocot biology, potentially impacting next-generation crop production.

To explore the subcellular localization and dynamics of many processes, including cell division, stable transformed maize lines expressing live-cell markers have been created [17, 18]. Like many other land plant cells, maize cells form typical dividing structures in symmetric and asymmetric divisions [44–47]. A preprophase band is formed before mitosis and is thought to predict the future site of the new cell wall [48]. The preprophase band is a ring of microtubules, microfilaments and a large number of other proteins that usually forms at the cell cortex around the nucleus [12]. The preprophase band disassembles during metaphase while the spindle forms [49]. The spindle is an organized antiparallel array of microtubules that moves chromosomes during anaphase. During telophase, the plant-specific structure called the phragmoplast

forms. The phragmoplast is composed of microfilaments and an antiparallel array of microtubules that serve as tracks for the transport of cell-wall components to the newly forming cell plate. The cell plate is the nascent cell wall, and eventually it expands outwards to the cortex to complete cytokinesis [50, 51].

The method described here provides a protocol for live-cell imaging of actively dividing maize cells using YFP-TUBULIN as an example. In it, plant growth conditions are outlined, as well as sample preparation for microscopy, setting up the time lapse, and finally analyzing the results. Recognition of common problems, such as sample damage and movement, is discussed and solutions presented.

2 Materials

2.1 Greenhouse Materials

1. Maize seeds that produce plants expressing one or more live-cell markers such as YFP-TUBULIN [18]. Full list available at (http://maize.jcvi.org/cellgenomics/index.php).
2. Soil.
3. Slow release fertilizer.
4. Calcium-magnesium supplementary fertilizer (e.g., Peters Excel Cal-Mag Special 15-5-15 water-soluble fertilizer).
5. 10 cm square pots in 15 pot flats.
6. 4 g/L glufosinate-ammonium in 0.1 % Tween 20.
7. Large 7.5 L ("2 gallon") pots.
8. Cotton applicators.
9. LED lights or other supplemental lighting. Light intensity should be ~230 $\mu E/m^2/s$ at a height of 1 m. A detailed protocol for greenhouse growing conditions can be found at agron-www.agron.iastate.edu/ptf/protocol/Greenhouse%20Protocol.pdf.

2.2 Microscopy Materials

1. Confocal microscope system. Either a spinning disk or a point scanning confocal system can be used together with an inverted microscope, an EM-CCD camera, and appropriate lasers and filters for imaging various fluorescently labeled proteins. Various microscopes have benefits and drawbacks (*see* **Note 1**).
2. Airstream incubator or thermostat.
3. Mid-range infrared thermometer.
4. Glass cover slips.
5. Rose chamber for holding the sample still during long time lapse. Rose chambers or alternatives can be used to stabilize the sample within the field of view (*see* **Note 2**).

6. Vacuum grease loaded into a 10 cm^3 needle-less syringe.
7. Water.
8. 200 μL pipette and tips.
9. Digital calipers.
10. Straight scalpel blade.
11. Forceps.
12. Software for running the microscope (e.g., Micromanager 1.4 https://www.micro-manager.org/).

2.3 Data Analysis Materials

1. ImageJ or FIJI (a regularly updated and modified version of ImageJ available at http://fiji.sc/Fiji).
2. Statistical package to import and analyze data.

3 Methods

3.1 Plant Growth

Temperature and lighting conditions will affect the growth rate and cell division rate of maize. Consistent growth conditions, consistent plant age, and tissue type are required for any comparative quantitative analyses.

The following steps list a reproducible method of growing plants.

1. Plant seeds ~2 cm deep in pre-wetted, pre-fertilized soil in 10 cm square pots loaded into a 15-pot flat for easy transport.
2. Germinate seedlings in standard long-day greenhouse conditions. The conditions are 16 h light at 25 °C, 8 h dark at 21 °C with supplemental lighting provided by LED or high-pressure sodium and metal halide lamps.
3. Water plants when the soil is dry, likely three times a week.
4. Fertilize with the Cal-Mag fertilizer once a week.
5. After 1 week of growth, use a permanent marker to mark the second or third leaf, and then apply 4 g/L glufosinate-ammonium in 0.1 % Tween to the marked location using a cotton-tipped applicator.
6. Two to three days after herbicide application, score resistant and sensitive plants (*see* **Note 3**).
7. Transplant resistant plants into 2-gallon pots.
8. Grow for a total of 3–5 weeks after planting. Plants should have at least seven visible leaves at this stage of growth. Do not use diseased or poorly growing plants. Figure 1a shows acceptable growth for a 4-week-old plant and describes the parts of the plants discussed in this paper (*see* **Note 4**).

9. Figure 1b indicates the part of the plant dissected to observe many dividing cells (described in more detail below in sample preparation for microscopy and *see* **Note 4**).

3.2 Sample Preparation for Microscopy

1. Set the thermostat. Alternatively, turn on the airstream incubator and point it onto the stage for at least 10 min prior to placing the sample (Fig. 2a). Determine what setting will provide the correct temperature before use. I used 21 °C (*see* **Note 5**).
2. Use a mid-range-infrared thermometer to measure the temperature using the laser to guide the thermometer to stage right next to the sample (Fig. 2b).
3. Place a clean glass cover slip on the lower half of the Rose chamber (Fig. 3a).
4. Prepare a glass cover slip for the sample by applying a thin film of vacuum grease in a circle with ~1 cm diameter (*see* **Note 6**).
5. Add a ~100 mL drop of water inside the vacuum grease circle with a pipette (*see* **Note 7**).

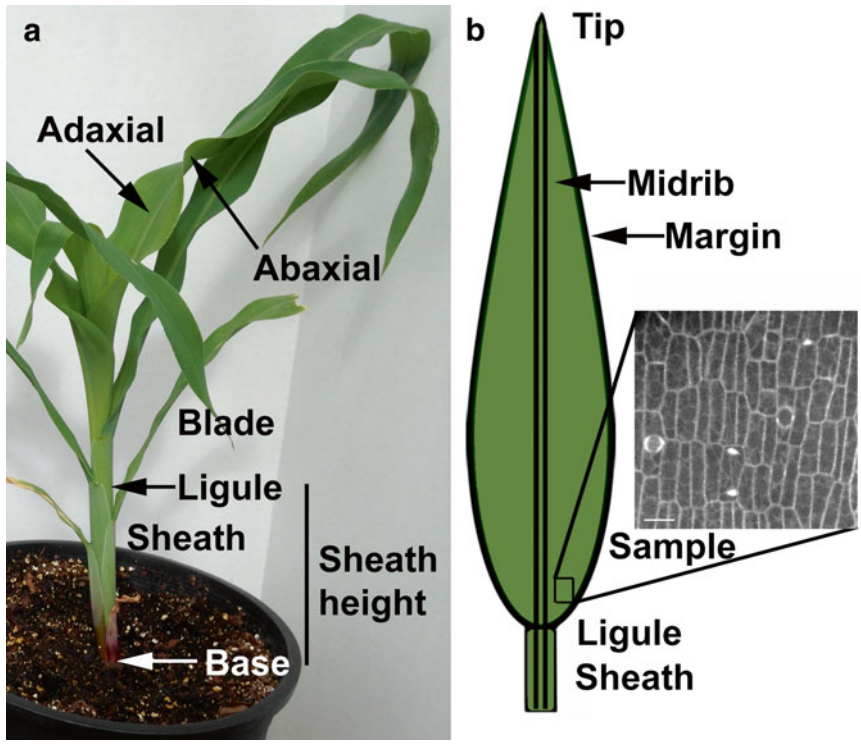

Fig. 1 Representative example of a maize plant and descriptions of the parts of the leaf used in this method. (**a**) A 28-day-old plant grown in standard greenhouse conditions with relevant parts and descriptors of the plant indicated. (**b**) Schematic of a plant leaf, with more descriptors, as well as a micrograph of a young maize leaf expressing YFP-TUBULIN. Bar is 50 μm

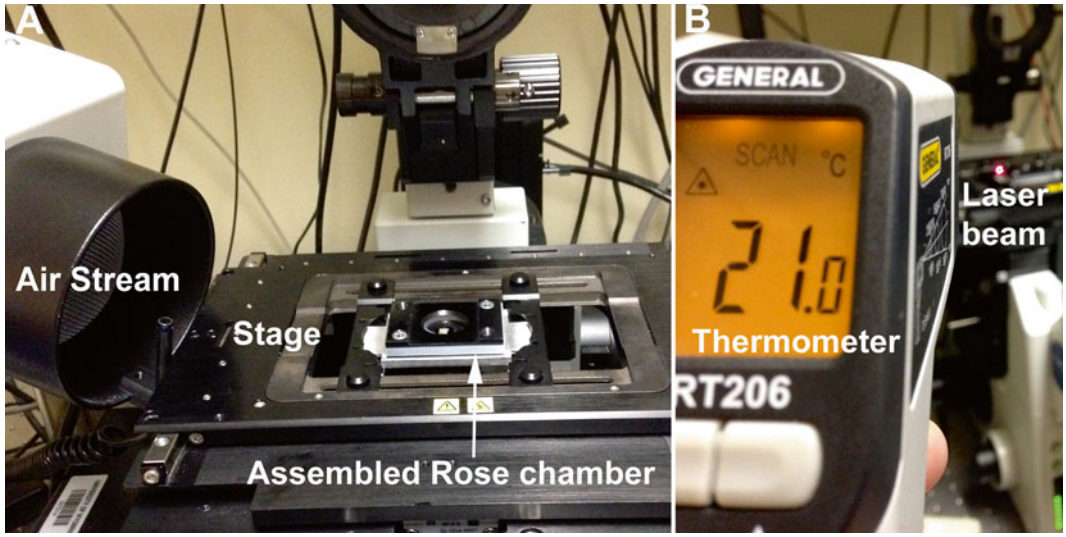

Fig. 2 Temperature control. (**a**) Photograph of the inverted stage with the airstream pointing toward the sample. The Rose chamber is assembled on the microscope. (**b**) The infrared thermometer is shown with the laser readout pointed toward the sample on the microscope

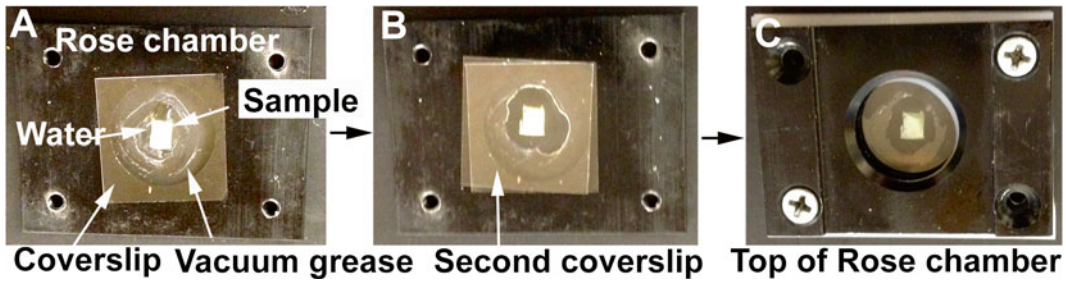

Fig. 3 The Rose chamber holds the sample steady during time-lapse imaging. This figure shows the steps needed to assemble it. (**a**) The bottom part of the rose chamber is shown with the cover slip, vacuum grease, water and sample mounted. (**b**) The second cover slip is carefully mounted on top of the sample, spreading the water evenly. (**c**) The top of the Rose chamber is screwed into place, and the sample is ready to be loaded onto the holder and the stage

6. After 3–5 weeks of growth, harvest plants for microscopy. Refer to Subheading 3.1 for growth conditions and Subheading 3.1, **step 8** for acceptable plants to harvest.
7. Cut off the above-ground portion of the maize plant, at the base of the sheath, leaving behind the roots (Fig. 1b).
8. Sequentially peel away the outer leaves until the sheath height is less than 3 mm. Sheath height can be measured with digital calipers or a ruler.
9. Excise a ~0.2–1.0 cm^2 leaf piece just above the ligule and between the midrib and the margin (Fig. 1b) using a straight scalpel blade.

10. Carefully peel the excised portion from the rest of the leaf, holding the sample by the edge with forceps. Avoid touching the tissue directly.
11. Mount the adaxial side down in the water droplet towards the objective when the rose chamber is fully assembled (*see* Figs. 1a and 4 and **Note 4**).
12. If there are air bubbles (*see* Fig. 5d), carefully remove your sample from the water and set it down again (*see* **Note 8**).
13. Gently place another cover slip on top of the sample and bottom cover slip (Fig. 3b).
14. Adjust the sample so that it is in the middle of the Rose chamber (*see* **Note 9**).
15. Place the top half of the Rose chamber, including the silicone sandwich, onto the top cover slip, and carefully screw it down (Fig. 3c).
16. Load the rose chamber into the holder and mount it on the microscope stage after adding the correct immersion liquid for the objective (*see* **Notes 10** and **11**).
17. Figure 2a shows a Rose chamber loaded onto the stage and Fig. 4 shows the schematics of the Rose chamber and its final orientation relative to the objective.

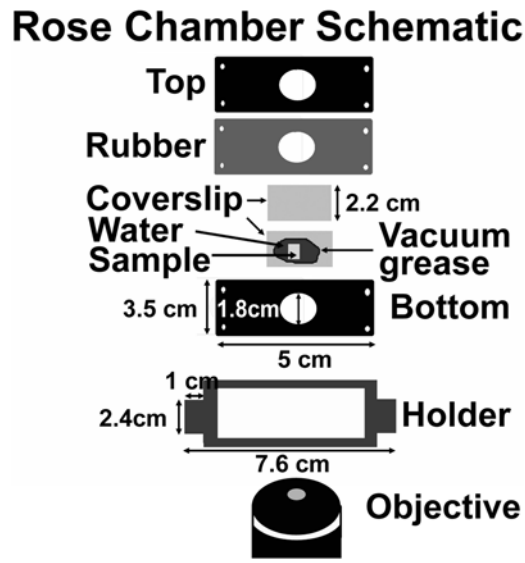

Fig. 4 Schematic of the Rose chamber with parts and measurements labeled. All pieces but the metal holder will eventually be screwed together to gently sandwich the sample and stabilize it during time-lapse imaging. The cover slip at the bottom of the Rose chamber will come into contact with the immersion liquid and the objective

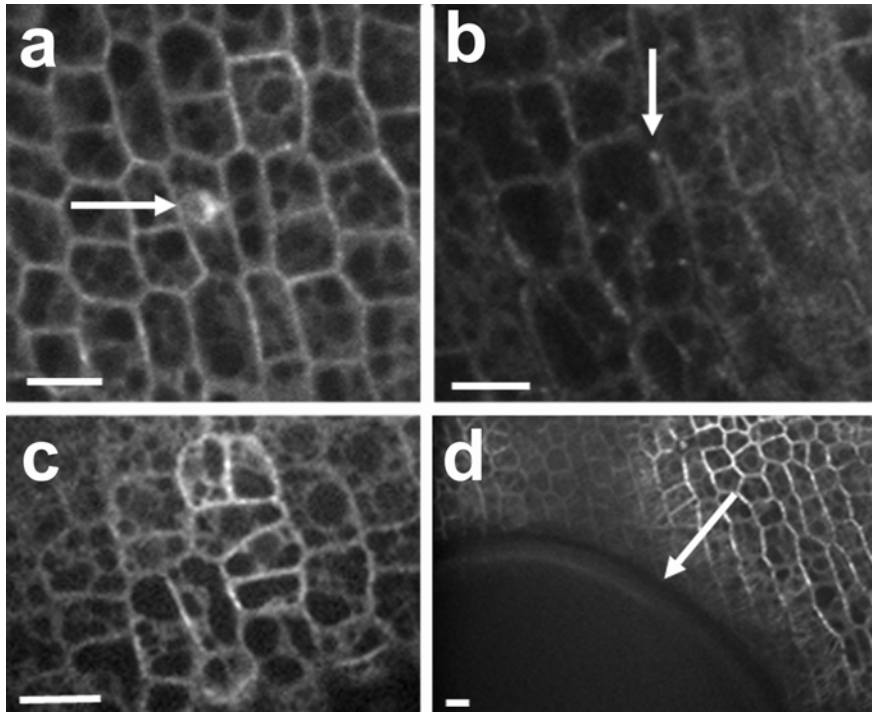

Fig. 5 Examples of cells expressing YFP-TUBULIN that are damaged and an air bubble. (**a**) Aberrant division structure indicated by an *arrow*. (**b**) Abnormal microtubule structure that looks like a dot indicated by an *arrow*. (**c**) Example of cells with a lot of small vacuoles. (**d**) Air bubble indicated by an *arrow*. Bar is 20 μm

3.3 Microscopy

1. Start the computer, microscope, camera, focus and filter controller, laser launch, etc. Consult a microscope manual or knowledgeable person for details on its operation.
2. Find the epidermal cell layer using bright field. Avoid taking images near the cut sites.
3. Switch to the camera view to start observing the fluorescent proteins in the cells. If cells are damaged or there is an air bubble, prepare a new sample. Examples of damaged cells are shown in Fig. 5 (*see* **Note 8**).
4. Adjust the exposure time, EM gain, and laser attenuation (if necessary) so that the illuminated sample has high dynamic range (*see* **Note 12**).
5. If the goal of the time lapse is to capture the entire process of division, prioritize capturing cells in late prophase at the start of the time lapse. Figure 6, time 0, shows an example of a cell in late prophase with a disassembling preprophase band. More examples of cells in late prophase are shown in Fig. 7 (*see* **Note 13**).
6. Use the multi-acquisition tool to set up your time lapse. Specify Z stack positions, exposure time, EM gain, and amount of

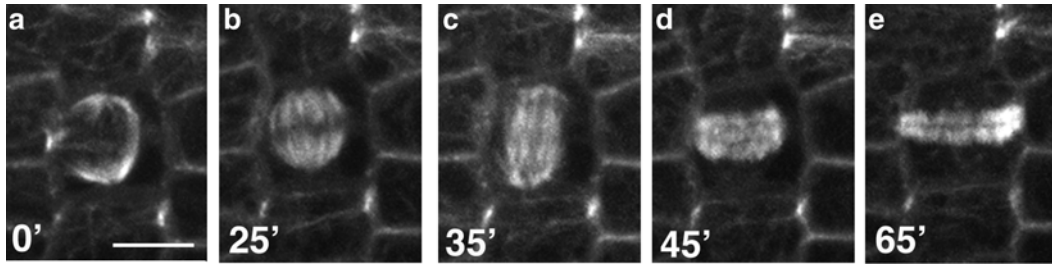

Fig. 6 Time lapse of a cell progressing from late prophase to the end of telophase and examples of cells in late prophase. Microtubules are labeled with YFP-TUBULIN. Time is indicated in the *bottom left-hand side* in minutes. (**a**) Late prophase. Note that the preprophase band has almost fully disassembled. (**b**) The bipolar spindle is formed in metaphase. (**c**) The anaphase spindle elongates to separate the chromosomes. This is the shortest stage in the cell cycle and generally takes 10–15 min. (**d**) A phragmoplast, an antiparallel set of microtubule arrays, forms in telophase. The cell wall materials are transported along the microtubule tracks towards the phragmoplast midline. (**e**) The phragmoplast expands outwards towards the cortex to complete cytokinesis. Bar is 10 μm

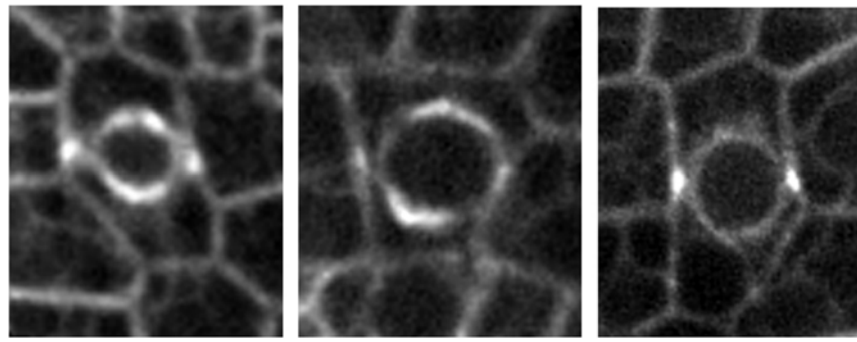

Fig. 7 Three examples of cells transitioning between prophase and metaphase. Note the prominent microtubule accumulations around the nuclear envelope

time between image collection. For observing mitosis in maize, 5-min time lapse is a good compromise between photobleaching and loss of temporal resolution (*see* **Note 14**).

7. Choose the "save as function" to save time-lapse images as they are produced into a single folder so that images are not lost in case of power outages (*see* **Note 15**).
8. Start the time lapse.
9. Adjust for sample movement, especially in the first 20 min. The most frequent trouble is slow drift in one direction. If there is a lot of drift, the sample is incorrectly positioned on the cover slip and a new sample should be loaded.
10. Check the time lapse for sample movement every hour. Compare the start of the time lapse with the new positions, and readjust your sample if the *x*, *y*, or *z* planes shift during the time-lapse.

11. Stop the time lapse after the cells are finished dividing or 5 h, whichever occurs first. Empirically, 5 h was the maximum amount of time that division progressed with this sample type at this temperature.

3.4 Data Analysis

1. Open stacks in the image analysis program Fiji or ImageJ.
2. Make maximum projections of the Z stacks: Process: Batch:Macro. Then specify input (the folder with the Z stacks) and output files (a new folder to put the max projections) with output format [Tiff]. Use this macro: run ("Z Project…", "start = 1 stop = 10 projection = [Max intensity]") (*see* **Note 16**).
3. Open the maximum projections as an image sequence (File:Import:Image sequence) and scroll through the file.
4. If the sample drifts a lot during the time-lapse, perform a correction by loading Plugin:Registration:StackReg or Plugin:Registration:Register Virtual Stack Slices and choose "Translation" (*see* **Note 17**).
5. Use the timestamps on original Z stacks to calculate the timing of division stages. Figure 5 illustrates the microtubule structures to look for at various stages of the cell cycle, and illustrates one potential way of indicating time.
6. Measure the amount of time needed to progress from one stage to another. These transitions are very clear when time-lapse images are viewed as a movie. The time can be analyzed in by calculating (the time between frames) × (number of frames cells are in a particular stage) = total time cells are in that state. An alternative method, if the stacks are not generated in 5-min intervals, is to compare the time-stamps on the images as they were originally saved.
7. Scroll through the image sequence focused on one mitotic structure. The spindle microtubules will start accumulating before the preprophase band disassembles. Preprophase band disassembly is observed as a loss of fluorescence at the cortex.
8. Next, the spindle, a football-shaped structure, coalesces perpendicular to the final division site and then expands during anaphase.
9. A sharp transition occurs from the orientation of the spindle to the orientation of the phragmoplast. The spindle is generally perpendicular to the final division site, while the phragmoplast is generally aligned with the final division site. This visually striking morphological change is usually obvious from one frame to the next.
10. The phragmoplast expands towards the cortex, sometimes contacting one side before the other [52]. Viewed from the side, it looks like two microtubule-containing disks with the

midline containing very few microtubules. As the phragmoplast expands, the interior microtubules may disassemble. Viewed from the top the phragmoplast looks like an expanding torus.

11. Finally, the phragmoplast reaches the cortex and starts to disassemble. Once it is completely disassembled, stop the time lapse and measure the complete time of all the steps.
12. Import the time values into Microsoft Excel or another spreadsheet program. Usually, the long and unpredictable amount of time that cells spend in preprophase/prophase means that gathering this type of data is more difficult. If cells do not progress, they may be damaged (Fig. 5 and *see* **Note 8**).
13. Other measurements can also be made such as the rate of phragmoplast expansion or the rotation and movement of the spindle. These are not discussed further in this method.

4 Notes

1. *Microscope selection*

   A confocal microscope must be used to avoid out of plane fluorescence with intact plant tissues. Either a laser scanning microscope or a spinning disk microscope can be used. The benefits of using a spinning disk microscope are speed and reduced photo-damage to the specimen. For fluorescent proteins that photo-bleach rapidly, a spinning disk microscope with a sensitive camera is essential. If the fluorescently tagged protein is abundant and does not photo-bleach easily, such as YFP-TUBULIN, it is possible to use a point-scanning microscope for time-lapse imaging. If necessary, the images can also be binned to reduce both photo-damage and the time required to gather each individual image.

2. *The Rose chamber*

   The Rose chamber as it is used here prevents movement of the sample by applying enough pressure to flatten a curved sample, but not too much to damage the cells. There is no commercially available option for purchase of a Rose chamber, but it is straightforward for a machine shop to make one. It consists of a metal holder, two pieces of metal and one piece of silicone or rubber with a width of 5 cm × 3.5 cm with a hole cut in the middle of both 1.8 cm diameter, four aligned screw holes, illustrated with dimensions shown in Fig. 4. An inexpensive alternative to the Rose Chamber is a glass slide sealed with VALAP (This is a 1:1:1 mixture by weight of paraffin, lanolin and Vaseline, doi:10.1101/pdb.rec12380*Cold Spring Harb Protoc 2010.*). VALAP, a solid at room temperature, is

gently heated on a hot plate to liquefy. VALAP provides a strong and biologically inert attachment between the slide and the cover slip. Do not use Vaseline or vacuum grease unless the sample is thin because the cover slip may slip during imaging.

3. *PCR to distinguish segregating transgenes*

   When multiple transgenes are segregating, it is helpful to confirm the genotype using a PCR based method, particularly when the plants express fluorescent proteins only in dividing tissue. A general method for maize DNA extraction and PCR can be found at http://rasmussenlab.weebly.com/protocols.html.

4. *Age and type ofmaizematerial suitable for imaging cell division by time lapse.*

   Overall, it is important to observe similar samples from one experiment to the next so that the data can be combined in the quantitative analyses. This is also why temperature control (*see* **Note 5**) and growth conditions are vital to the success of the experiment. Several parts of the plant are actively dividing including the base of the leaf, the meristem, the young sheath material and the root tips. Maize primary roots are thick, and cutting them appropriately for stable time-lapse imaging can be difficult. Young blade or sheath tissue is preferred because it is flat, has mostly undeveloped chloroplasts (and therefore little to no autofluorescence), and has a high proportion of dividing cells. If asymmetric divisions are preferred, older leaf tissue should be used. Either the adaxial or abaxial side of the leaf can be used. If juvenile leaves are preferred, either because the fluorescent protein is better expressed or the developmental stage is more ideal for the experiment, plants ~2 weeks old can be harvested.

5. *Temperature control*

   The temperature must be the same between experiments if comparisons are going to be made between samples. The main reason to keep the temperature then same is that microtubule dynamics (and other protein dynamics) are different at different temperatures [53]. Other methods that can be used to control temperature are a thermostat-regulated room or a heated chamber for microscopy.

6. *Vacuum grease*

   Vacuum grease is recommended to form the small well between the two cover slips because it has the correct viscosity and is chemically inert. It is important that a thin, consistent layer is applied for optimal time-lapse microscopy. If the layer is too thin or if it is spread unevenly, the sample will slip during time lapse (*see* **Note 8** on sample slipping). Other materials

that can be used are Vaseline and VALAP. Vaseline will work in a pinch, but it may not adequately protect the sample during mounting because it is much softer than vacuum grease. VALAP is tricky to apply in a thin layer because it solidifies very quickly.

7. *Water*

   Use water to mount maize samples. Other materials, such as mineral oil or phosphate buffered saline, will damage the cells and prevent timely progression of mitosis. Other materials, such as perfluorocarbons [54], may be better for imaging, but I have not had an opportunity to use them.

8. *Avoid sample damage and air bubbles*

   Damage and air bubbles both cause significant trouble for time-lapse imaging. Avoid smashing, squashing, or otherwise disturbing the sample. If the sample is damaged, take a new slice from the plant, which should be wrapped in moist paper towels to preserve it for a few hours if necessary. The most obvious signs that cells are damaged are a lack of organized cortical interphase microtubule array in nondividing cells, cells with many small vacuoles, cells that fail to progress in the cell cycle or cells with abnormal mitotic arrays (*see* Fig. 5). Air bubbles will cause your sample to slip out of focus and may alter the rate of division. If the sample has an air bubble, carefully take the sample out, and set up a fresh cover slip, vacuum grease, and water to place the sample.

9. *Sample placement*

   Placing the sample in the middle of the Rose chamber is very important because if it is not correctly centered, the objective may run into the side of the chamber. This has the potential to scratch the objective, disturb correct Z stack imaging, and make the sample slip.

10. *Objective selection*

    For semi-high-throughput imaging, the lowest objective that still allows unambiguous identification of mitotic structures should be used. I used a 20× objective for this purpose. For producing a time-lapse figure, a higher objective (such as 40 or 60×) should be used to more clearly illustrate the mitotic figures. An alternative, should it be feasible with your microscope setup, is to use *x*,*y* motorization to move between multiple different samples during time-lapse.

11. *Immersion liquid*

    The correct immersion liquid must be used on your objective, but what type of objective is best for time-lapse imaging? Some objectives do not require any immersion liquid but use air instead. Unfortunately, the refractive index of air is very different than plant cells. The benefit of using an oil objective is

that the immersion oil will not evaporate during imaging. However, it reduces the quality of imaging because the refractive index of immersion oil is not very similar to plant cells. Water has a closer refractive index to your sample, and will produce a more beautiful micrograph. However, water objectives are not ideal for long time lapse because the water can evaporate within 1 h of time-lapse imaging. Glycerin or silicone oil may provide the best compromise between a good refractive index and slower evaporation but I do not have experience with either of these materials. Perfluorocarbon immersion liquids can also be used.

12. *Laser power*

    Keep laser power low to minimize photo-damage to the cells. Instead of increasing laser power, consider increasing EM gain or exposure times. Empirical testing may be done to confirm that the settings do not cause photo-damage before long time lapses are performed. Use a sample to test for photo-damage by collecting the total number of images the time lapse will acquire during one session. This can be done in micromanager using the "burst" function. After imaging, measure fluorescence loss and observe whether damage occurs.

13. *Cells in lateprophase*

    Since the amount of time a cell spends in preprophase can be quite long (>3 h), it is best to focus the sample such that a cell in late prophase is in the middle of the field of view. These cells are characterized by an obvious accumulation of microtubules at both poles of the imminent spindle, but still have a preprophase band (Fig. 7).

14. *Selecting appropriate image collection intervals*

    One goal of time-lapse imaging is good temporal resolution but another more important goal is prevention of photo-damage. Short time intervals (e.g., 2 min) can increase the risk of photo-damage to the cells, which impedes division. Long time intervals (e.g., 15 min) can result in data loss. For example, anaphase, the shortest stage of the cell cycle (~10 min) will not be observed within 15-min intervals. A decent time compromise is 5-min intervals for dividing cells, but this will need to be optimized depending on the type of sample and temperature (*see* **Note 5**). An alternative way to minimize photo-damage is to capture a single image or a short Z stack, such as 4–1 μm intervals at each time point instead of a full Z stack (covering the top through the bottom of the cells usually at 1 or 1.5 μm stacks for 10–20 separate Z stacks).

15. *Automatic saves*

    The "save as" function saves images as they are produced, showing an accurate timestamp on each Z stack. The save as

function also protects against loss of already acquired images during power failures or other malfunctions that can occur during time-lapse imaging.

16. *Set the Z stack so that the maximum projection will provide a clear image*

    Maximum projection, a post-acquisition method of visualizing the Z stacks compressed into one image, can be used to more clearly distinguish structure types and changes in mitotic structures. Sometimes the maximum projection to produce the clearest image will not be the entire Z stack, particularly if the sample is thick.

17. *Automatic corrections inImageJor FIJI*

    Two plug-in programs in ImageJ/FIJI can be used to adjust for sample movement during imaging: Stack Reg and Registration:Register Virtual Stack Slices. Both can automatically correct the slow drift caused by plant growth or movement in one direction. However, if large manual adjustments are performed during time-lapse imaging, these programs may not be able to correct the image. Manually correct large shifts in ImageJ, and then run StackReg or Register Virtual Stack Slices.

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References

1. Reddy GV, Roy-Chowdhury A (2009) Live-imaging and image processing of shoot apical meristems of *Arabidopsis thaliana*. Methods Mol Biol 553:305–316
2. Hamant O, Das P, Burian A (2014) Time-lapse imaging of developing meristems using confocal laser scanning microscope. Methods Mol Biol 1080:111–119
3. Heisler MG, Ohno C (2014) Live-imaging of the Arabidopsis inflorescence meristem. Methods Mol Biol 1110:431–440
4. Sappl PG, Heisler MG (2013) Live-imaging of plant development: latest approaches. Curr Opin Plant Biol 16(1):33–40
5. Grierson C et al (2014) Root hairs. In: Arabidopsis book, vol 12. American Society of Plant Biologists, Rockville, MD
6. Kong SG, Wada M (2014) Recent advances in understanding the molecular mechanism of chloroplast photorelocation movement. Biochim Biophys Acta 1837(4):522–530
7. Ehrhardt DW, Shaw SL (2006) Microtubule dynamics and organization in the plant cortical array. Annu Rev Plant Biol 57:859–875
8. Furt F et al (2013) Apical myosin XI anticipates F-actin during polarized growth of *Physcomitrella patens* cells. Plant J 73(3): 417–428

9. Fishel EA, Dixit R (2013) Role of nucleation in cortical microtubule array organization: variations on a theme. Plant J 75(2):270–277
10. Shaw SL, Ehrhardt DW (2013) Smaller, faster, brighter: advances in optical imaging of living plant cells. Annu Rev Plant Biol 64:351–375
11. Henty-Ridilla JL et al (2013) Actin dynamics in the cortical array of plant cells. Curr Opin Plant Biol 16(6):678–687
12. Rasmussen CG, Wright AJ, Muller S (2013) The role of the cytoskeleton and associated proteins in determination of the plant cell division plane. Plant J 75(2):258–269
13. Rasmussen CG, Sun B, Smith LG (2011) Tangled localization at the cortical division site of plant cells occurs by several mechanisms. J Cell Sci 124(Pt 2):270–279
14. Spinner L et al (2013) A protein phosphatase 2A complex spatially controls plant cell division. Nat Commun 4:1863
15. Van Damme D et al (2011) Adaptin-like protein TPLATE and clathrin recruitment during plant somatic cytokinesis occurs via two distinct pathways. Proc Natl Acad Sci U S A 108(2):615–620
16. Lipka E et al (2014) The phragmoplast-orienting kinesin-12 class proteins translate the positional information of the preprophase band to establish the cortical division zone in *Arabidopsis thaliana*. Plant Cell 26:2617
17. Wu Q et al (2013) Fluorescent protein marker lines in maize: generation and applications. Int J Dev Biol 57(6-8):535–543
18. Mohanty A et al (2009) Advancing cell biology and functional genomics in maize using fluorescent protein-tagged lines. Plant Physiol 149(2):601–605
19. Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16(6):735–743
20. Cleary AL et al (1992) Microtubule and F-actin dynamics at the division site in living Tradescantia stamen hair cells. J Cell Sci 103(4):977–988
21. Wang H et al (2003) Modifying plant growth and development using the CDK inhibitor ICK1. Cell Biol Int 27(3):297–299
22. Cleary AL (2001) Plasma membrane-cell wall connections: roles in mitosis and cytokinesis revealed by plasmolysis of *Tradescantia virginiana* leaf epidermal cells. Protoplasma 215(1-4):21–34
23. An G (1985) High efficiency transformation of cultured tobacco cells. Plant Physiol 79(2): 568–570
24. Kumagai-Sano F et al (2006) Cell cycle synchronization of tobacco BY-2 cells. Nat Protoc 1(6):2621–2627
25. Murata T et al (2013) Mechanism of microtubule array expansion in the cytokinetic phragmoplast. Nat Commun 4
26. Van Damme D et al (2004) Molecular dissection of plant cytokinesis and phragmoplast structure: a survey of GFP-tagged proteins. Plant J 40(3):386–398
27. Buschmann H et al (2011) Cytoskeletal dynamics in interphase, mitosis and cytokinesis analysed through Agrobacterium-mediated transient transformation of tobacco BY-2 cells. New Phytol 190(1):258–267
28. Higaki T et al (2006) Actin microfilaments regulate vacuolar structures and dynamics: dual observation of actin microfilaments and vacuolar membrane in living tobacco BY-2 Cells. Plant Cell Physiol 47(7):839–852
29. Kojo KH et al (2013) Roles of cortical actin microfilament patterning in division plane orientation in plants. Plant Cell Physiol 54(9):1491–1503. doi:10.1093/pcp/pct093
30. Vidali L, Bezanilla M (2012) *Physcomitrella patens*: a model for tip cell growth and differentiation. Curr Opin Plant Biol 15(6): 625–631
31. Spinner L et al (2010) The function of TONNEAU1 in moss reveals ancient mechanisms of division plane specification and cell elongation in land plants. Development 137(16):2733–2742
32. Sheehan MJ, Dawe RK, Pawlowski WP (2013) Live imaging of chromosome dynamics. Methods Mol Biol 990:79–92
33. Kirienko DR, Luo A, Sylvester AW (2012) Reliable transient transformation of intact maize leaf cells for functional genomics and experimental study. Plant Physiol 159(4): 1309–1318
34. Sharman BC (1942) Developmental anatomy of the shoot of *Zea mays L.* Ann Bot 6: 245–284
35. Sylvester AW, Cande WZ, Freeling M (1990) Division and differentiation during normal and *liguleless-1* maize leaf development. Development 110(3):985–1000
36. Rasmussen CG, Humphries JA, Smith LG (2011) Determination of symmetric and asymmetric division planes in plant cells. Annu Rev Plant Biol 62:387–409
37. Facette MR et al (2013) Parallel proteomic and phosphoproteomic analyses of successive stages of maize leaf development. Plant Cell 25(8): 2798–2812

38. Ponnala L et al (2014) Correlation of mRNA and protein abundance in the developing maize leaf. Plant J 78(3):424–440
39. Kajala K et al (2014) Omics and modelling approaches for understanding regulation of asymmetric cell divisions in Arabidopsis and other angiosperm plants. Ann Bot 113(7):1083
40. Candaele J et al (2014) Differential methylation during maize leaf growth targets developmentally regulated genes. Plant Physiol 164(3): 1350–1364
41. Zhang Z et al (2013) Developmental distribution of the plasma membrane-enriched proteome in the maize primary root growth zone. Front Plant Sci 4:33
42. Nelissen H et al (2012) A local maximum in gibberellin levels regulates maize leaf growth by spatial control of cell division. Curr Biol 22(13):1183–1187
43. Li P et al (2010) The developmental dynamics of the maize leaf transcriptome. Nat Genet 42(12):1060–1067
44. Cleary AL, Smith LG (1998) The Tangled1 gene is required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. Plant Cell 10(11):1875–1888
45. Adamakis ID, Panteris E, Eleftheriou EP (2014) The nitrate reductase inhibitor, tungsten, disrupts actin microfilaments in *Zea mays L.* Protoplasma 251(3):567–574
46. Panteris E, Apostolakos P, Galatis B (2006) Cytoskeletal asymmetry in *Zea mays* subsidiary cell mother cells: a monopolar prophase microtubule half-spindle anchors the nucleus to its polar position. Cell Motil Cytoskeleton 63(11):696–709
47. Wright AJ, Gallagher K, Smith LG (2009) discordia1 and alternative discordia1 function redundantly at the cortical division site to promote preprophase band formation and orient division planes in maize. Plant Cell 21(1):234–247
48. Pickett-Heaps JD, Northcote DH (1966) Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. J Cell Sci 1(1):109–120
49. Ambrose JC, Cyr R (2008) Mitotic spindle organization by the preprophase band. Mol Plant 1(6):950–960
50. Jurgens G (2005) Cytokinesis in higher plants. Annu Rev Plant Biol 56:281–299
51. Jurgens G (2005) Plant cytokinesis: fission by fusion. Trends Cell Biol 15(5):277–283
52. Cutler SR, Ehrhardt DW (2002) Polarized cytokinesis in vacuolate cells of Arabidopsis. Proc Natl Acad Sci U S A 99(5):2812–2817
53. Kawamura E, Wasteneys GO (2008) MOR1, the *Arabidopsis thaliana* homologue of Xenopus MAP215, promotes rapid growth and shrinkage, and suppresses the pausing of microtubules in vivo. J Cell Sci 121(24):4114–4123
54. Littlejohn GR et al (2014) An update: improvements in imaging perfluorocarbon-mounted plant leaves with implications for studies of plant pathology, physiology, development and cell biology. Front Plant Sci 5:140