Alternate procedure for 11.1.3: Observing stages of mitosis in onion root tip squash mount

Work individually on this entire exercise. You are encouraged to share good slides with fellow students, but each person is responsible for preparing and studying their own slides.

In this procedure you will study mitosis in an onion root tip by preparing a squash slide. You may move ahead to Activities 2, 3 or 4 during waiting periods in this slide preparation.

1. Thoroughly clean three microscope slides.
2. Obtain three onion roots by removing each root from the onion at its base (do not cut off the tip alone). Using a scalpel, cut off 2-3 mm of the growing tip (tapered end) of each root and place on separate slides. Discard the remaining section of each root in the trash can.
3. Move each root tip to one end of each slide. Cover each with a few drops of fixative (HCl-EtOH). The fixative kills and fixes cellular structures in whatever activity they were engaged at that moment.
4. After 3-5 minutes in the fixative, the roots will become more opaque (whiter). Place two drops of preservative (HAc-EtOH) on the opposite side of each slide and transfer each root tip to it using a dissecting needle. Keep the root tips covered with preservative for 5-10 minutes.
5. Absorb the fixative using a paper towel. Be very careful to keep it off your skin and clothing, as it contains acid and is caustic.
6. After the root tips have soaked in the preservative for the prescribed amount of time, move each root to the center of the slide and use a paper towel to soak up the preservative.
7. Cover the root tips with three drops of acetoorcein stain for 15-20 minutes, adding more drops of stain periodically to prevent drying.
8. After the staining period, gently lower a coverslip onto the drop of stain containing the root tip. Lay a piece of paper towel over each slide and apply pressure with your thumb directly over the coverslip. This will help spread the cells of the root tip and at the same time absorb excess stain squeezed out from under the coverslip. Tapping FIRMLY on the coverslip over the root tip with the eraser end of a pencil will also aid in spreading the cells. An ideal slide will have no air bubbles.
9. Examine the smudge of cells with the scanning objective to get a general overview. Fewer than 1% of the cells will be in some obvious phase of mitosis. The vast majority of cells will show the typical round, undifferentiated metabolic nucleus of interphase. After examining all three of your preparations, select the best slide for more detailed study.
10. Scan the field of view slowly at low power (100X), moving the slide to view favorable areas of cells until you find some that contain rod-like chromosomes instead of rounded nuclei. DO NOT USE HIGH POWER (400X) FOR SCANNING. Use the diaphragm to keep the light reduced for the best image resolution.
11. When a mitotic cell has been found, center it in the field and switch to high power. After observing it, return to low power and continue to scan. In this manner locate and draw/photograph as many of the stages of mitosis as you can.