Mitosis of *Vicia faba*

The first lab is to introduce you to techniques in slide preparation that at first may seem to be frustrating but are necessary to develop your own methods that you will serve you for a long time. My experience has been that the students with the most difficulty in the first two-labs find the final project a piece of cake. A little bit of forethought into what type of material you are dealing with and what you are trying to accomplish goes along way in the cytogenetics lab.

Dividing cells of young seedling plants are located in the shoot and root apical meristem. These areas are very easy to dissect out of freshly germinated healthy material .Collecting root tips is one of the easiest methods of checking the chromosome number.

The general procedure of the squash or smear technique for root tips involves six steps:

1) Germination of seeds to obtain young root tips
2) Pretreatment of the tips to arrest cells at metaphase and enhance chromosome observation
3) Fixation to preserve the material
4) Hydrolysis to soften cell walls and make the root tip malleable
5) Maceration, staining and slide preparation
6) Observation and documentation

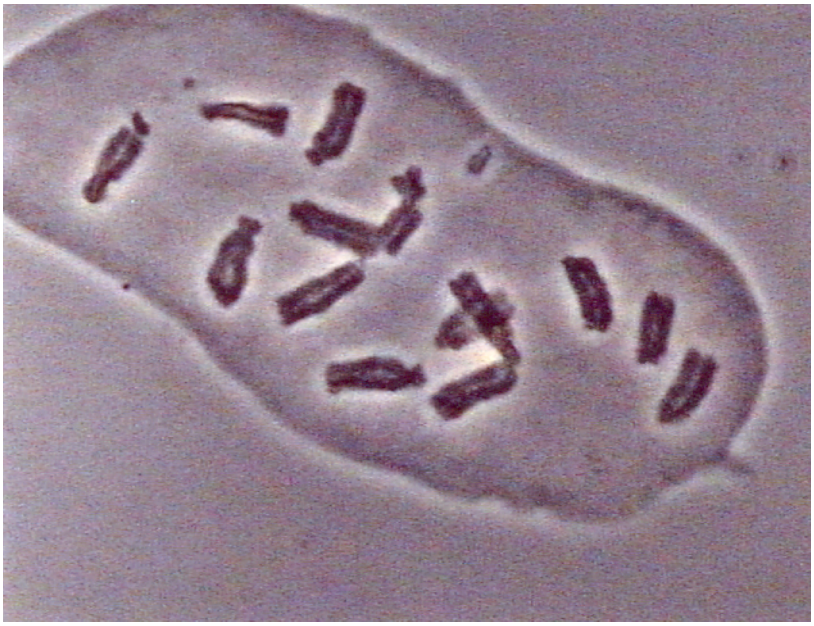

Figure 1 Metaphase in Vicia faba 2n=11, Magnification 260X

Procedure

Figure 2 Materials required for preparing root tip squashes. From top left clockwise: microscope slides; prepared and stained root tips; acetocarmine stain; alcohol lamp; matches; forceps; razor blade; cover slips.

Germination and pre-treatment:

Faba bean seeds typically range between 55 to 75 grams per 100 seeds. For a class of 27 students (plus 3 for working up the procedure), germinate about 150 seeds = 90 gm.

Vicia faba seeds are soaked in warm water for 3-4 hours before planting in moistened vermiculite. When roots are 2-3 cm long (usually about 4 days after planting), the last 1 cm of root is taken off and placed in ice water over night at 4 ºC (pre-treatment). Warning! It is critical that the roots not be allowed to freeze. A good way to test a refrigerator is to put a container of water in overnight to see whether or not the water freezes. Freezing will ruin the nuclei.

Fixation: Roots are transferred to Farmers Fixative (see appendix 3) at 4º C overnight.

Storage: Roots are stored in 70% ethanol 4 ºC.

Hydrolysis: Three or four root tips should be placed in a clean vial with a 1 N HCl solution at 60 ºC for 8-10 minutes. Length of hydrolysis and concentration of HCl will vary for different species.

Staining and Slide Prep: Select a root tip and blot it on a paper towel.

Place the root tip on a clean microscope slide and check for the root tip, it should be slightly pointed

and appear opaque. A dissecting microscope is handy if you're not sure. Place a drop of the prepared aceto-carmine on the tip and dissect the smallest bit of the root tip that you can (1-2 mm).

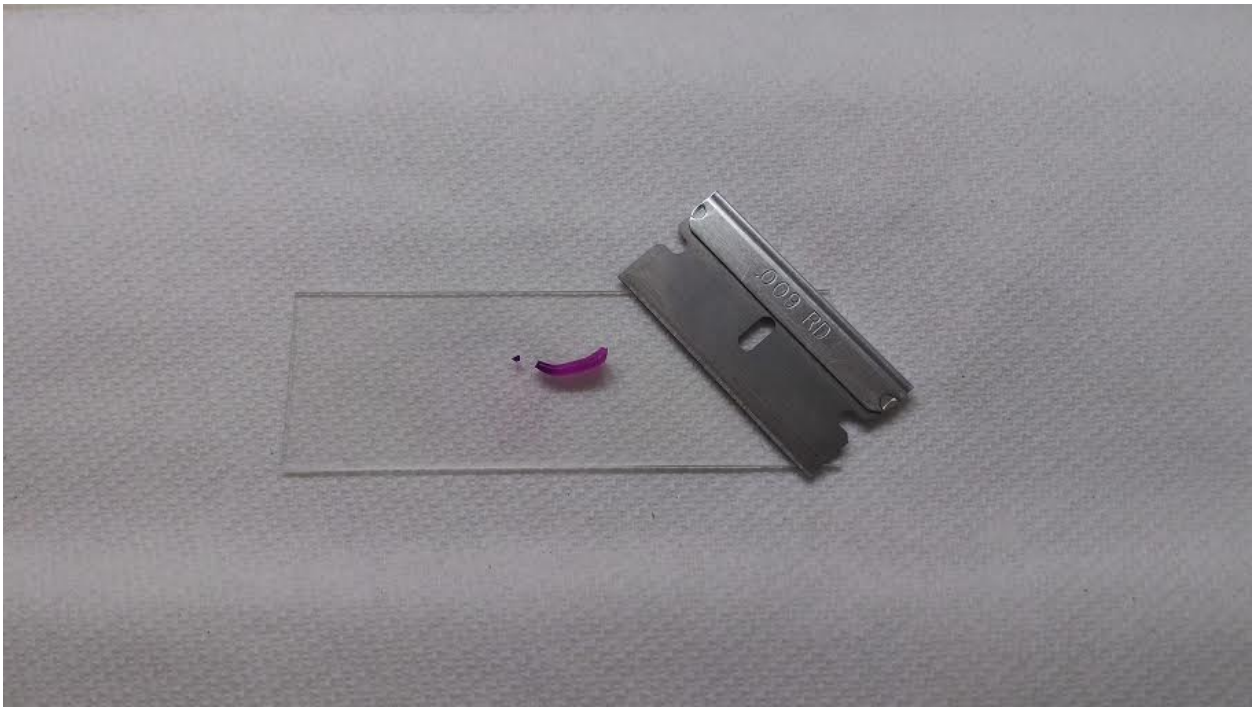

Figure 3 Using a razor blade, cut off a 2-3 mm section from the root tip.

Chop it in half if possible, the smaller the section you prepare here the better the chance of getting a single layer of cells to observe.

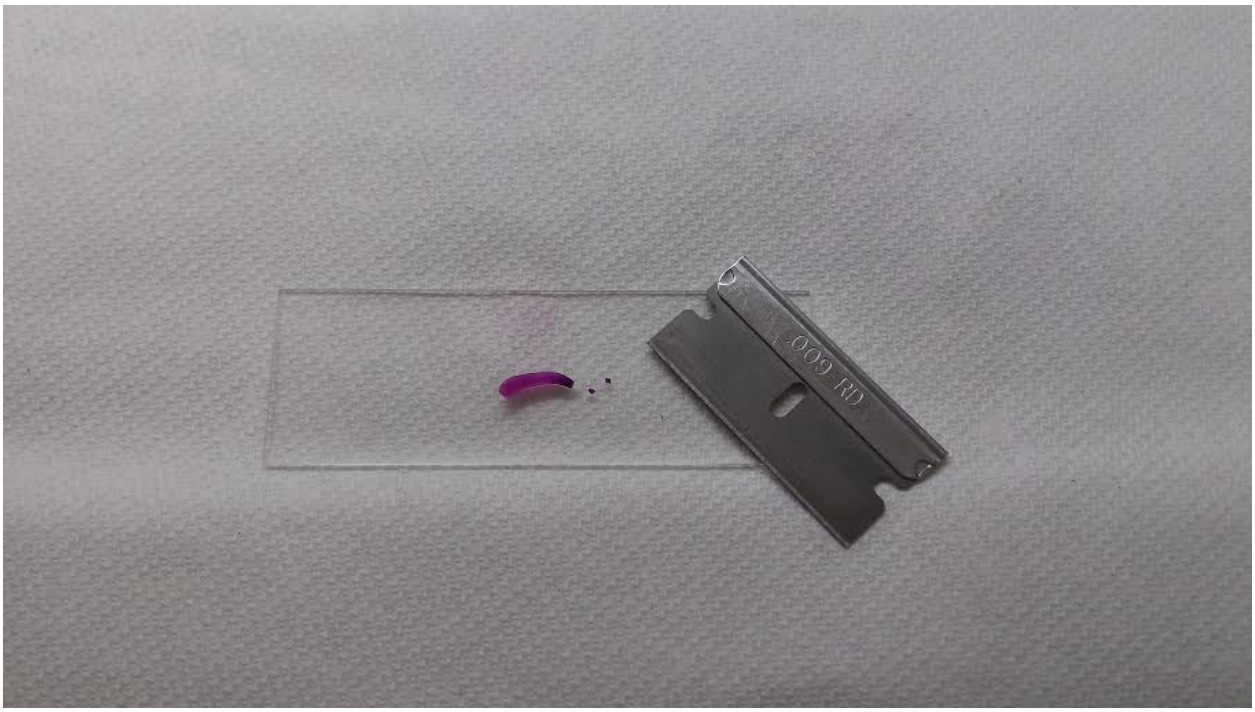

Figure 4: Cut the root tip in half longitudinally.

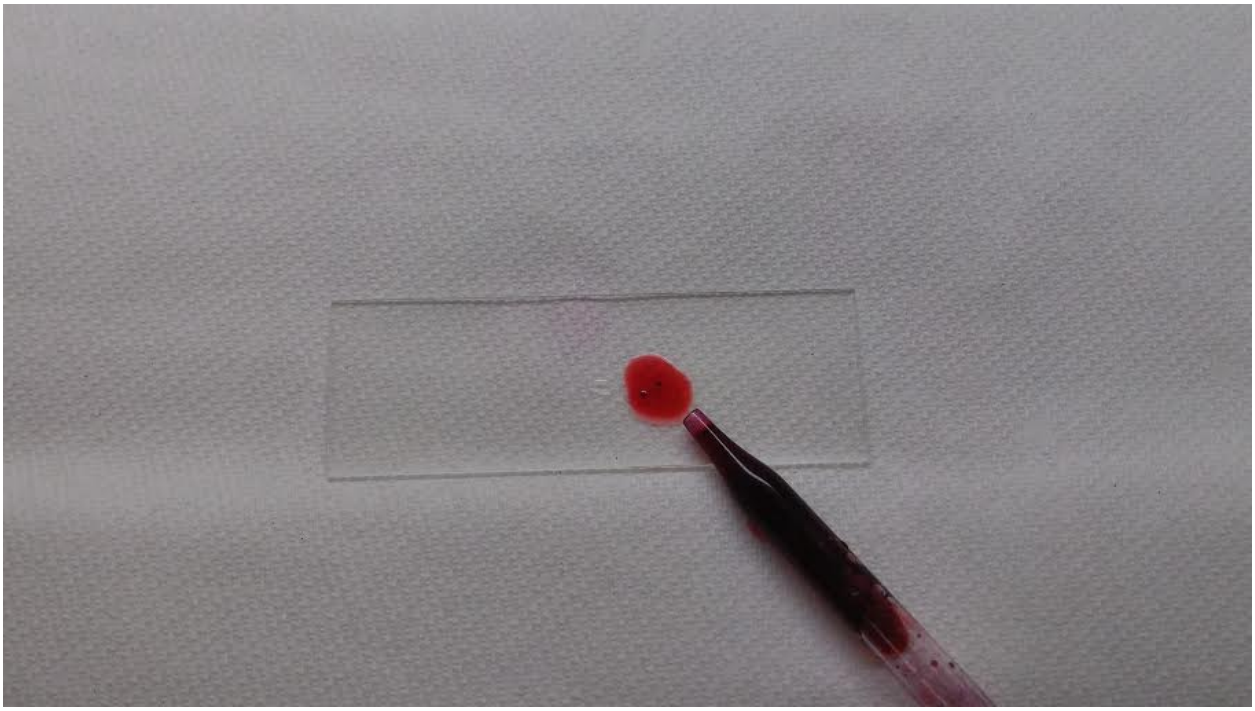

Figure 5: Place a single drop of acetocarmine stain on the root fragments.

Place a cover slip on the slide at an angle, slowly lowering it onto the stain will prevent bubbles from forming.

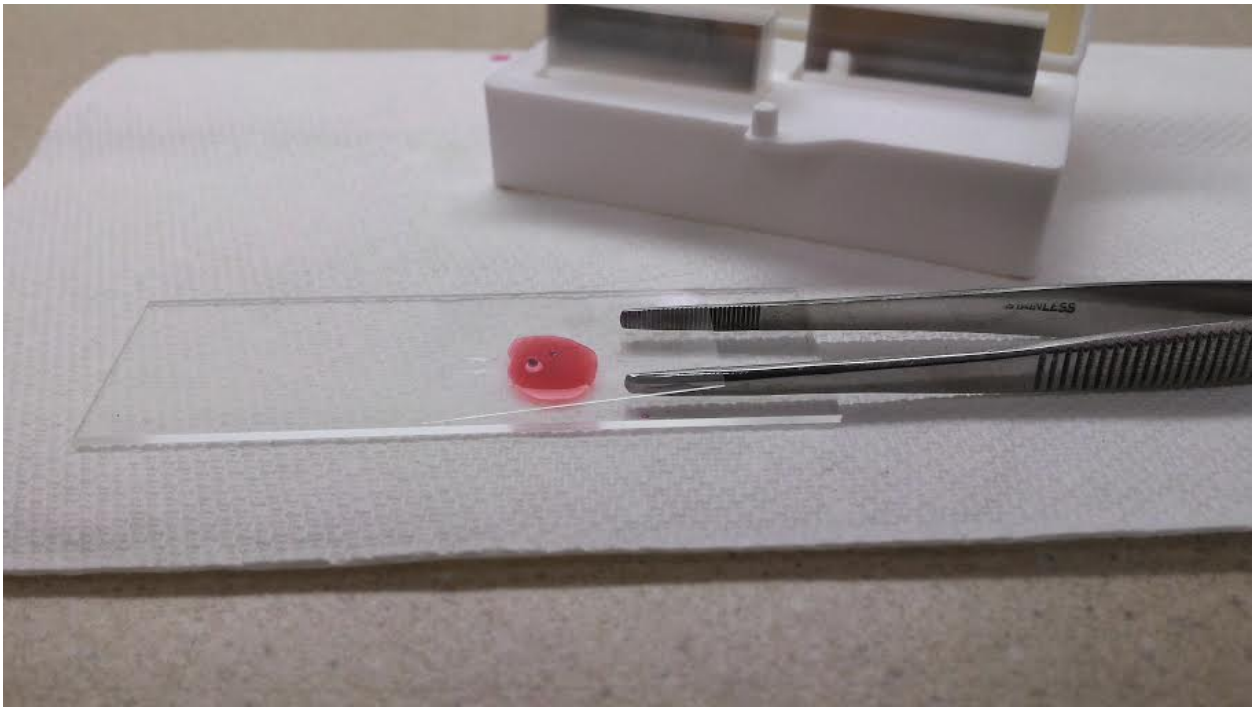

Figure 6: Carefully lower the coverslip on the stain and root.

Hold one edge of the cover slip down with a paper towel and tap on the area of the root tip with the blunt end of a dissecting needle or the eraser end of a pencil. Apply enough pressure to squash the root tip out under the cover slip, but if you break the cover slip it will allow oil to seep in when you attempt to use the oil immersion objective of the drawing microscope.

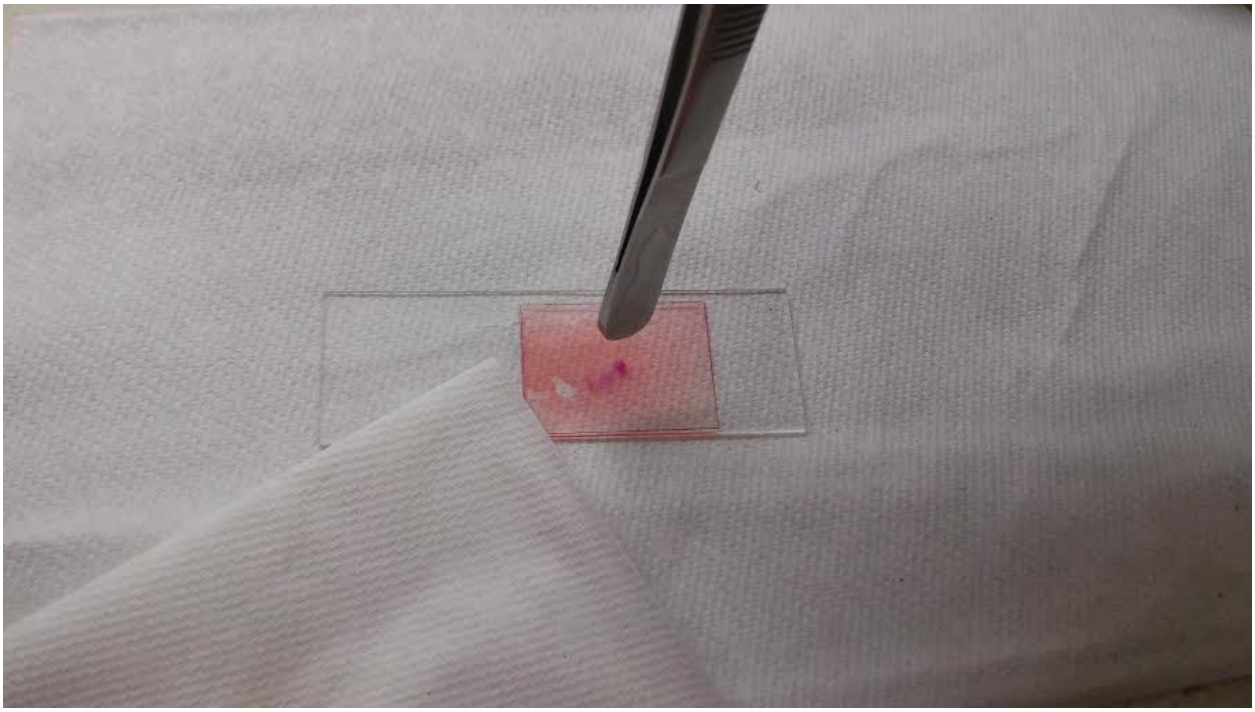

Figure 7: Hold the corner of the coverslip with a paper towel to soak up extra stain. Tap on the root tissue with the back of your forceps to disperse the tissue.

Use the alcohol burner to heat the slide slightly and then tap the cover slip down again to get an even flatter preparation. By the time you are finished your root tip should be barely visible on the slide.

Figure 8: Pass the microscope slide 8-10 times over an alcohol lamp. DO NOT hold the slide over the flame too long or the stain will boil off.

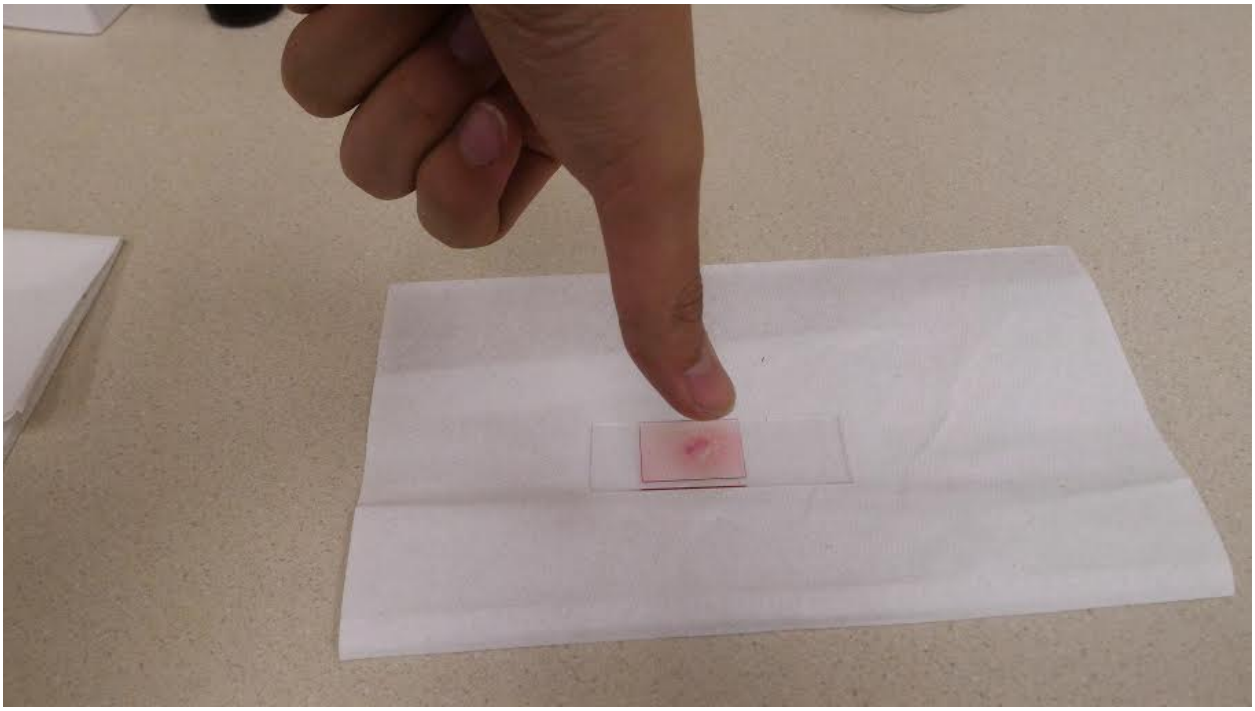

Figure 9: Once cooled, use your thumb to press the coverslip to flatten the remaining root tissue.

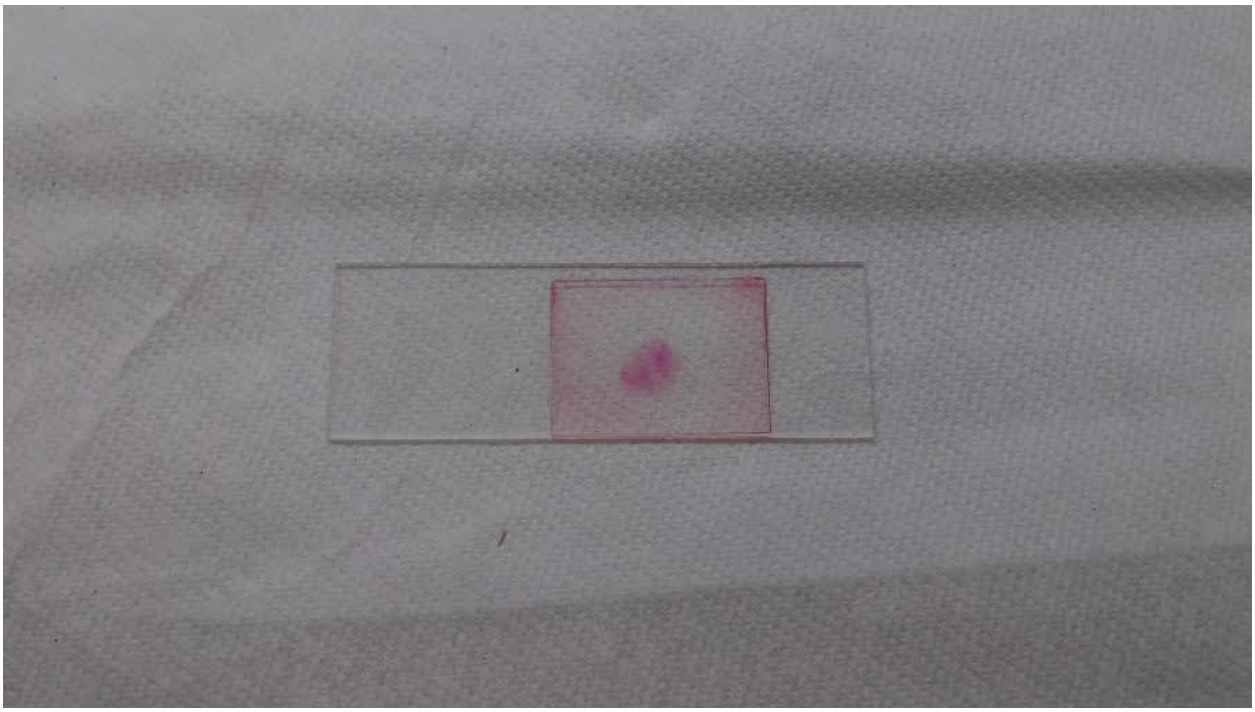

Figure 10: If properly prepared, you will not see any intact root tissue left. The root tissue should appear as a smear. You will likely observe chromosomes along the periphery of the smear.

Observe: Place your slide on the microscope at 10X power and locate the dividing cells. Remember the root apical meristem is located slightly behind the root tip, protected by a layer of cells that slough off as the root pushes itself through the soil. The cells should be small and cube shaped with a well-defined nucleus. Scan at 10X for any cells in mitosis, move to the 40X objective (phase 2) to check the exact stage. By the end of the period you should be able to identify prophase, metaphase, anaphase and telophase. Once you have found good cells, prepare your slide for transfer to the photo-microscope. Use a slide transfer method described in appendix 2 and seal the slide with Cytoseal™ 60 (Fisher Scientific 23244257).

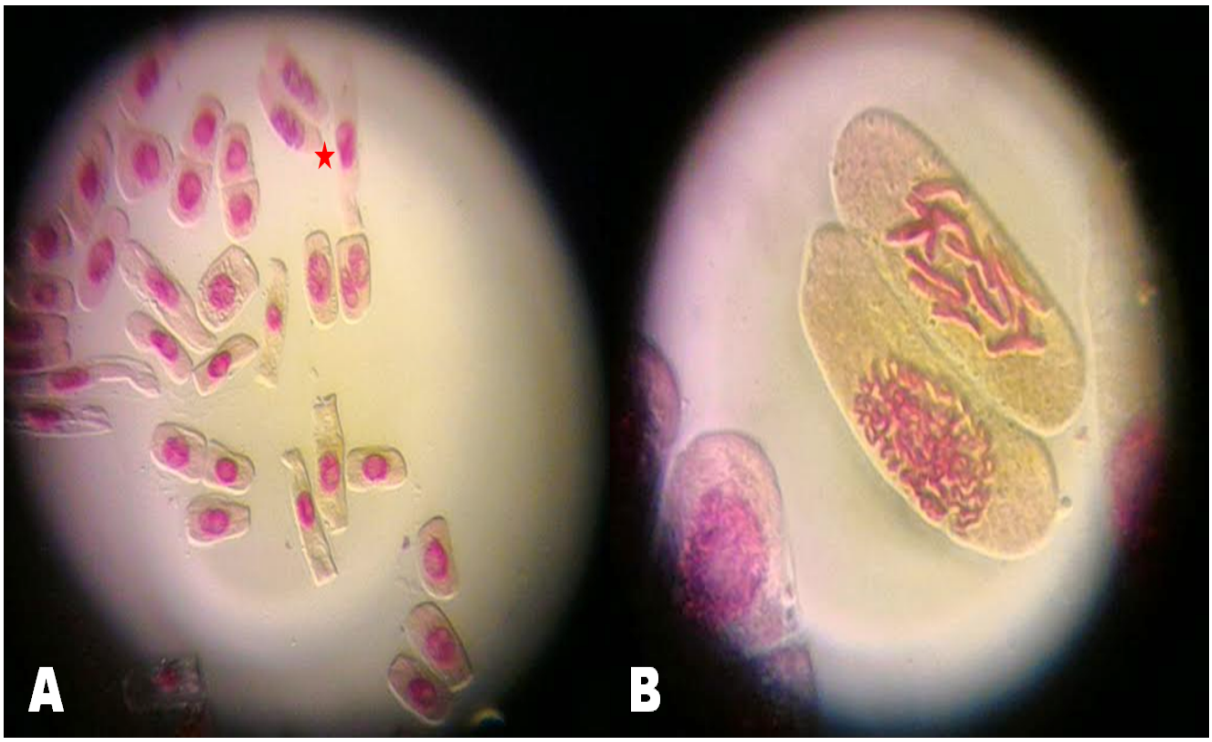

Figure 11 Vicia faba chromosomes observed in root tips at (A) 400x and (B) 1000x magnification.