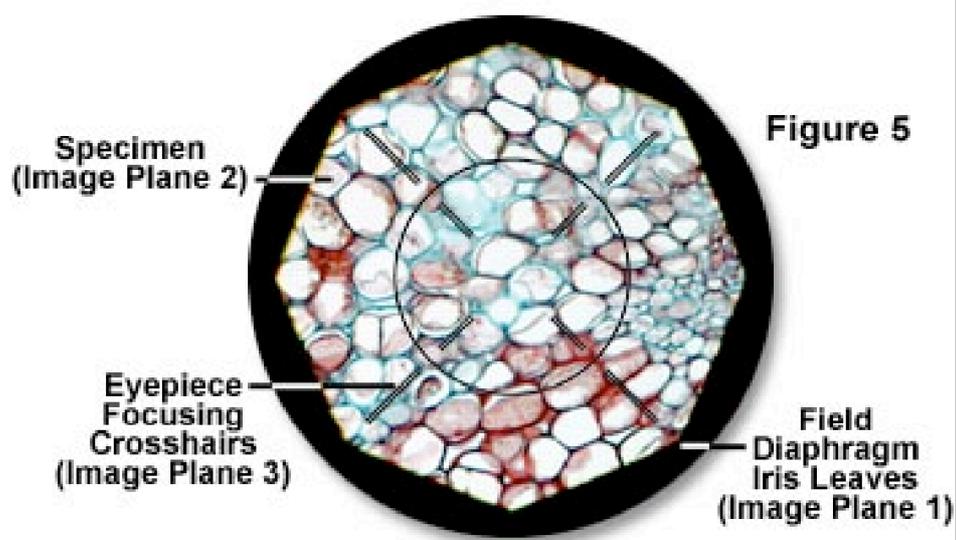
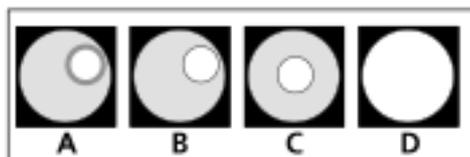


## Alignment for Laser Brightfield Imaging

- Set the LSM software to VIS mode
- Select the 10x objective
- Open both the Aperture Stop and the Field Stop completely (Slide both adjusters towards the front of the microscope)
  - You can tell if they are both Stops are open as the light shining on your sample is brightest
- Adjust the lamp brightness so that it's comfortable to view through the eyepieces
- Focus on your sample
- Close down the Field Stop completely using the Silver Lever on the top right hand side of the condenser
- Adjust the Condenser Focus Knob to move the condenser vertically up as far as possible, then turn the knob the other direction until you see a small bright spot through the eyepieces
- Open up the Field Stop slightly until you see a bright area around 2/3 the size of the Eyepiece Field of View (FOV)
- Adjust the Condenser Focus Knob until the edges of the Field Stop are sharply focused
  - you may get a slight blue or pink halo around the edges of the Field Stop image, find the mid point between colours, but you must have a sharp image of the edge of the Field Stop



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- Open up the Field Stop until the edges of the image are touching the edge of the eyepiece FOV
- Adjust the Condenser Focus Knob so that the edges are sharply focussed
- Center the Field stop image on the FOV using the Condenser Centering Screws
  - You may need to keep adjusting the Field Stop as you center the image so that the edges touch the FOV



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- Open up the Field stop until you can just no longer see the image of the Field stop in the eyepieces
- Now select the Lens you wish to use for imaging, add oil if necessary and refocus on your sample

- Adjust the Aperture Stop to approximately 75% of the Numerical Aperture (N.A) of the lens you are using. The N.A. is written on the lens, for example:
  - 10x/0.45, the lens has an N.A of 0.45
  - 20x/0.75, the lens has an N.A of 0.75
  - 40x/0.75, the lens has an N.A of 0.75
  - 63x/1.4, the lens has an N.A of 1.40
  - 100x/1.4, the lens has an N.A of 1.40
- look through the eyepieces and move the Aperture Stop adjuster back and forth until the image is darkest, this is the closed or “0%” position, Now move the Aperture Stop adjuster the other way until the image is brightest, this is the open or “100%” position.
- Multiply the N.A of your lens by 75%, ie: for a 10x lens,  $0.45 \times 75\% = 33\%$ , so move the aperture stop to the closed or 0% position, then move it back to 1/3 open.
- If you have a lens with a large N.A. such as the 63x/1.4, 75% of 1.4 = 1.05, therefore just open the Aperture stop completely.

