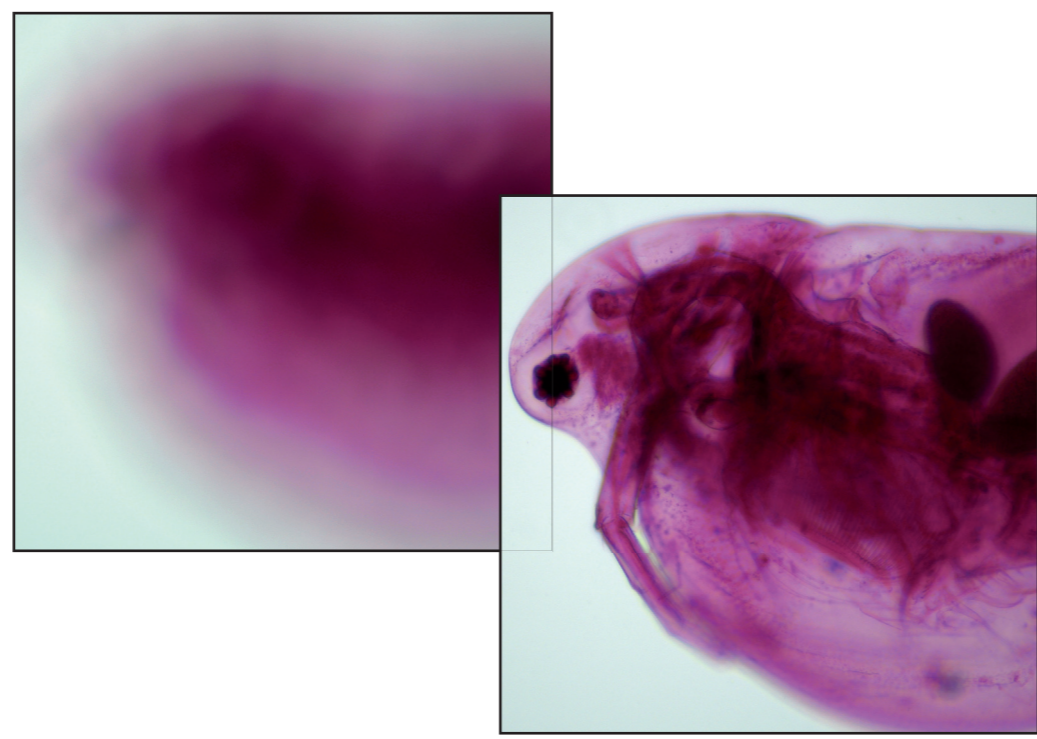
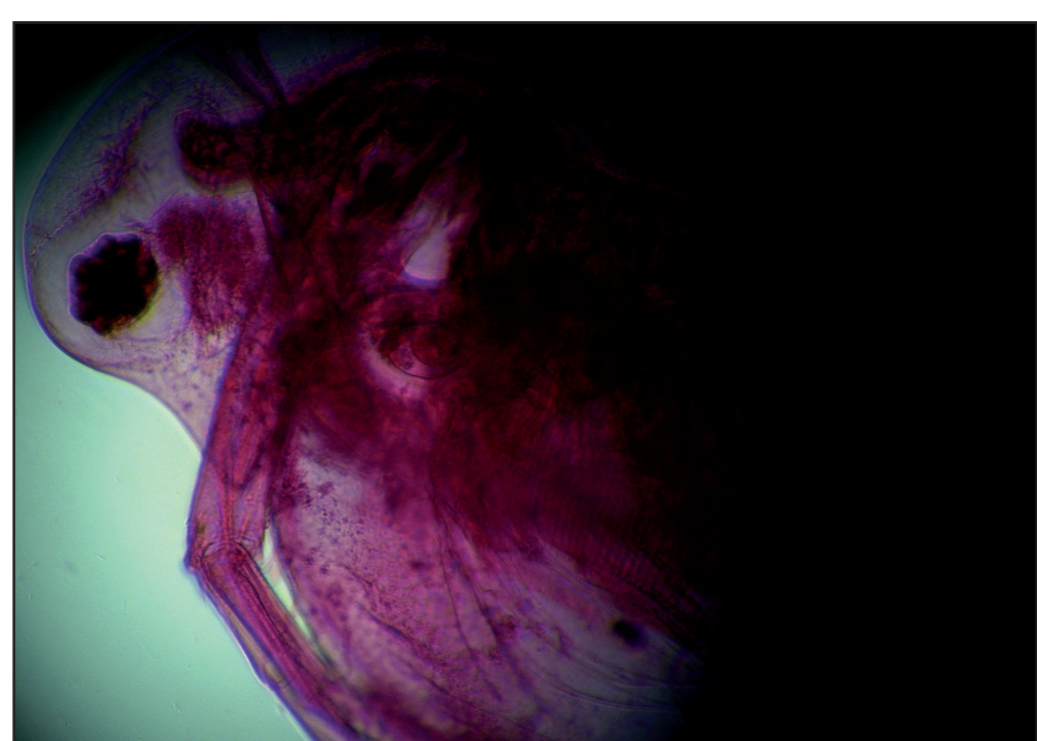


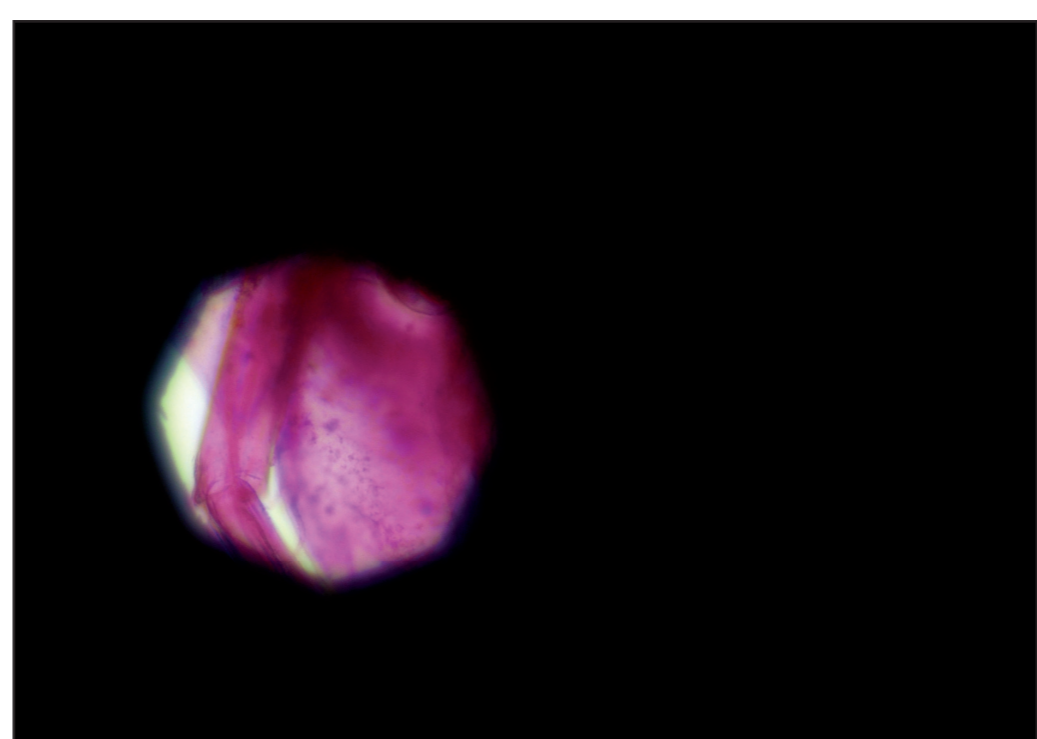
Köhler Illumination & Phase Contrast Alignment on the Nikon Ti2



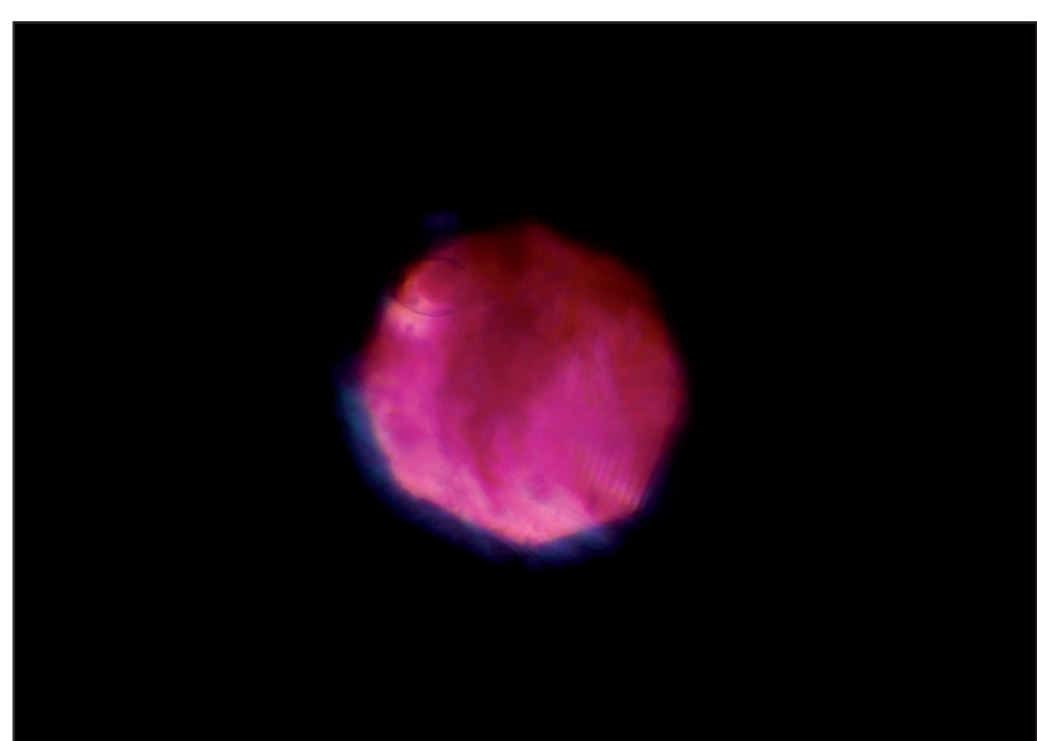
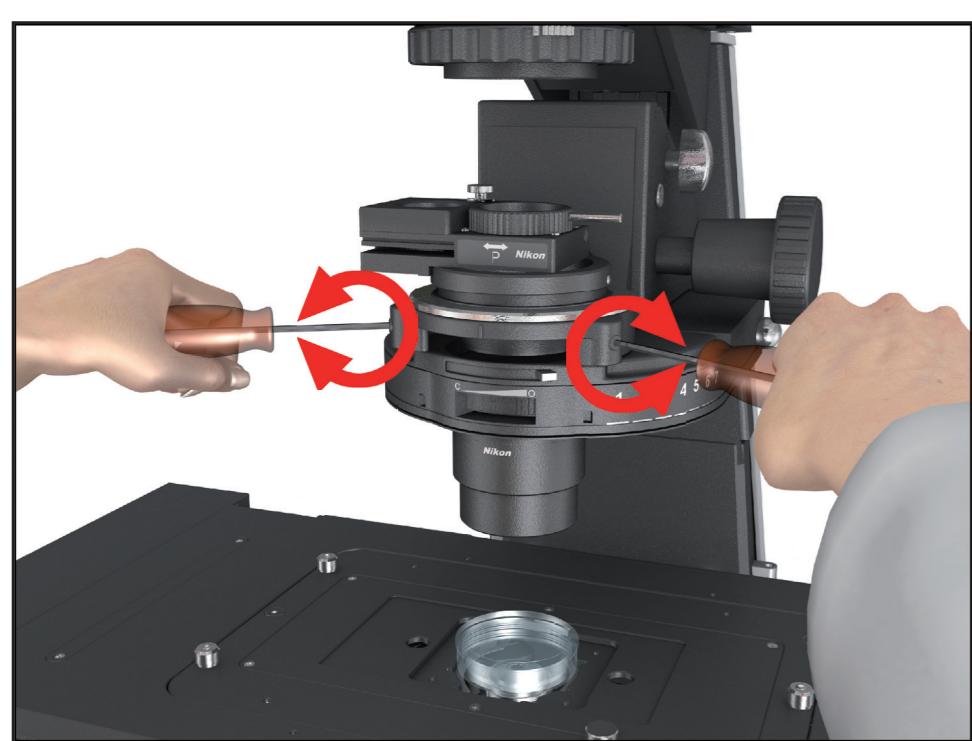
1 Focus on Sample – Set condenser turret to empty brightfield position, remove all other filters and prisms from light path, and fully open field and aperture diaphragms. Use the focus knob to bring the sample into focus.



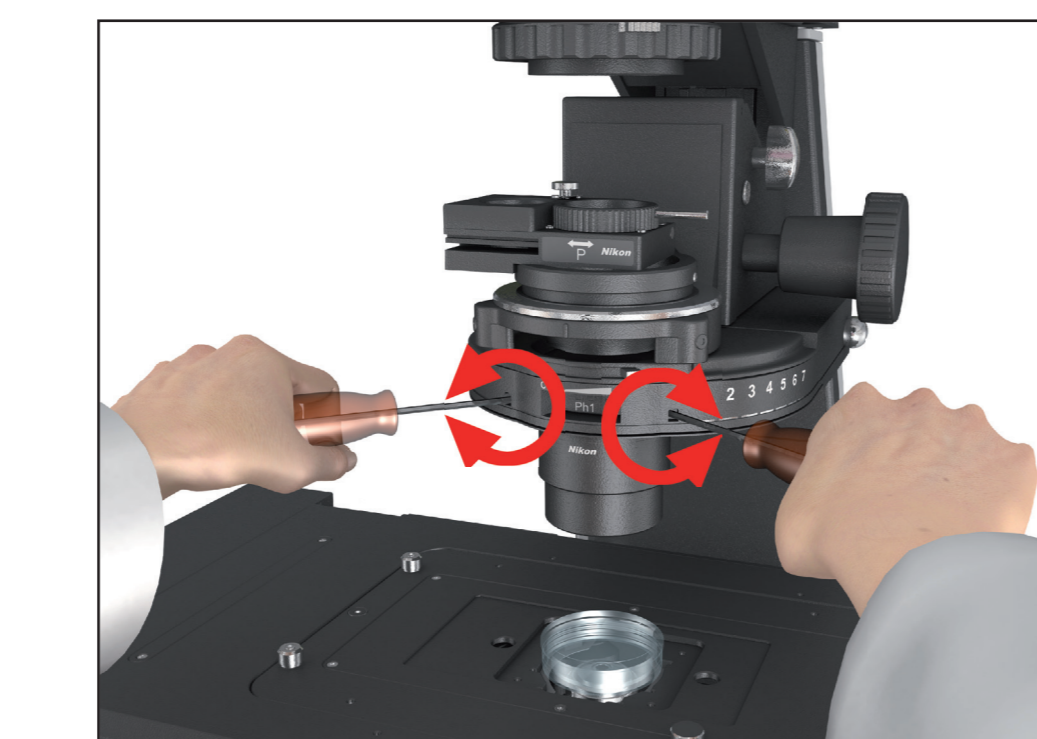
2 Close Field Diaphragm – Once closed, the out-of-focus image of the field diaphragm should be somewhat visible within the field of view.



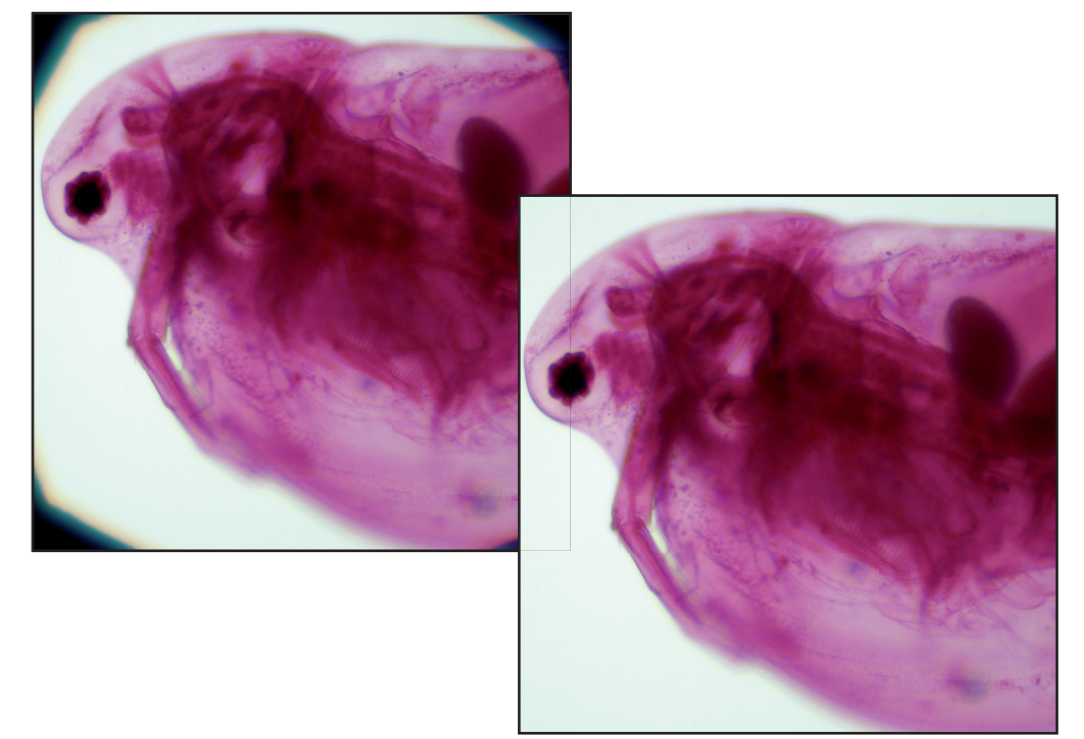
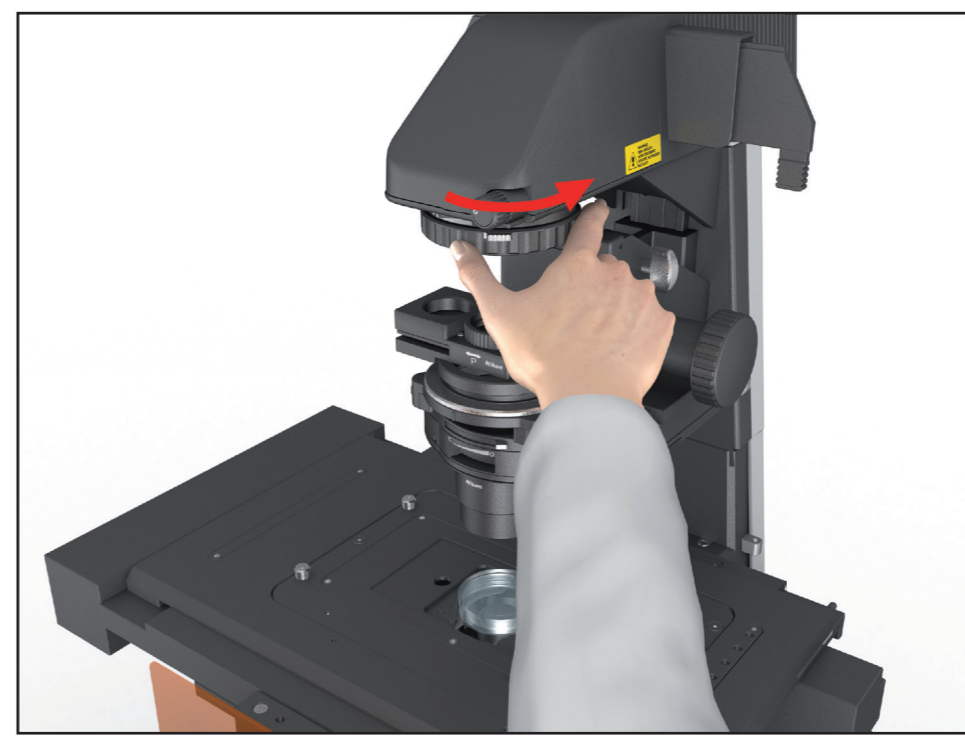
3 Focus Image of Field Diaphragm – While keeping the sample in focus, rotate the condenser focusing knob as illustrated to bring the edges of the field diaphragm into focus.



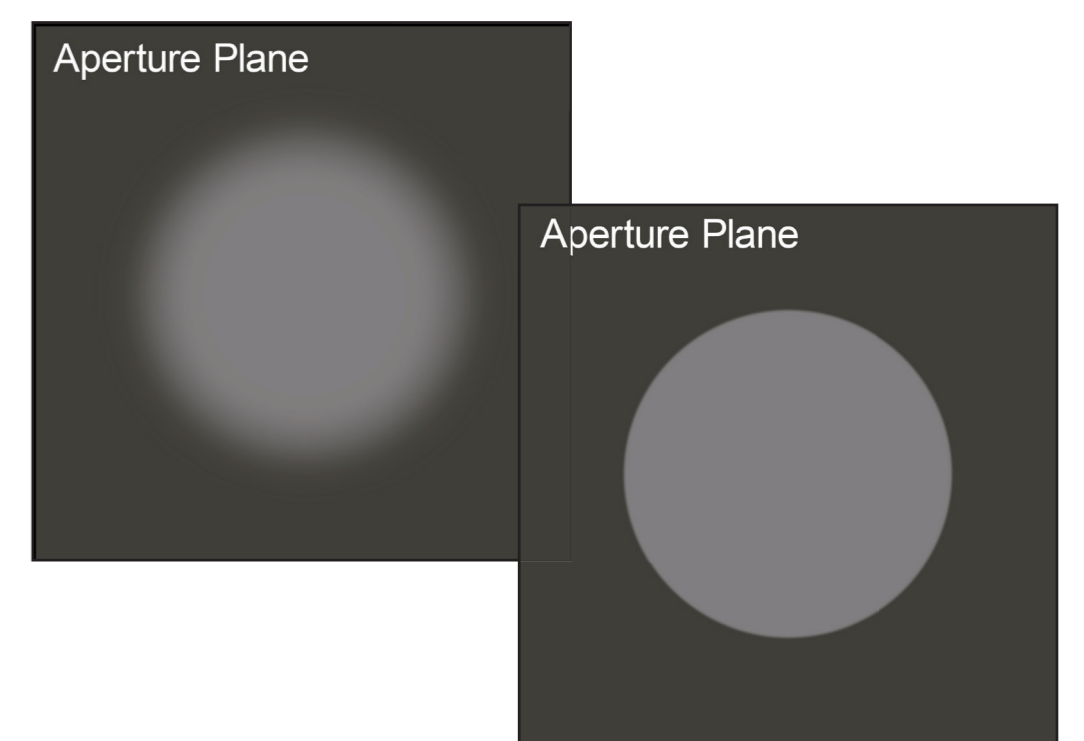
4 Center Image of Field Diaphragm – Insert a pair of 2 mm hex keys into the slots as illustrated and rotate to center the image of the field diaphragm.



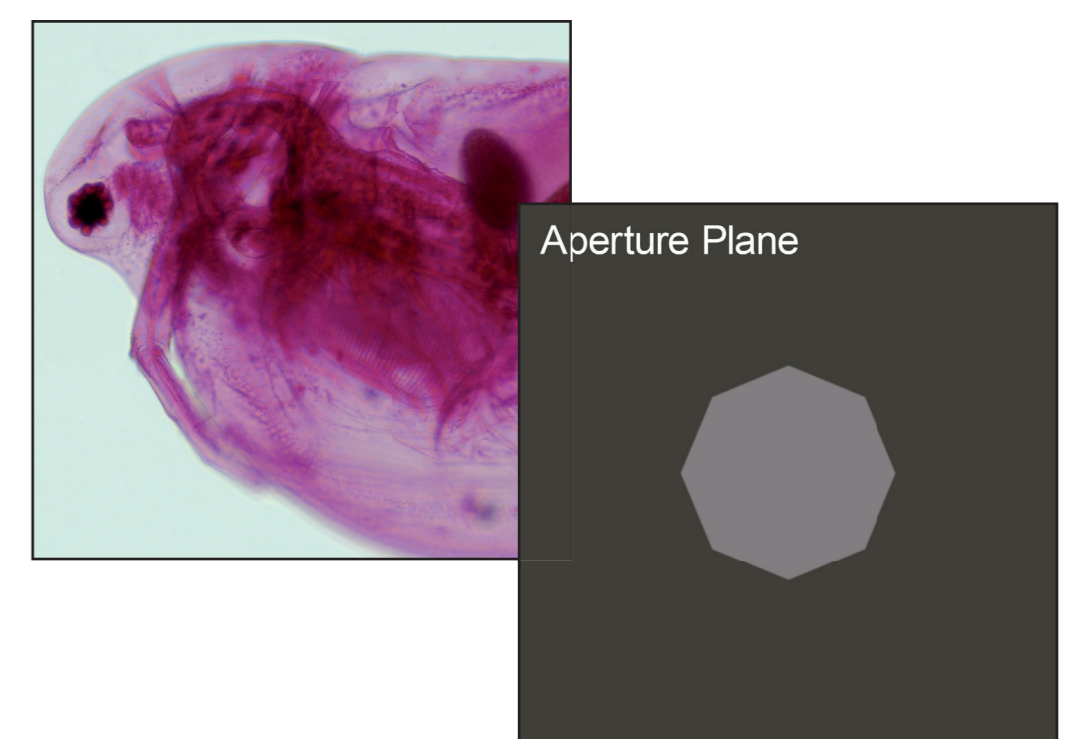
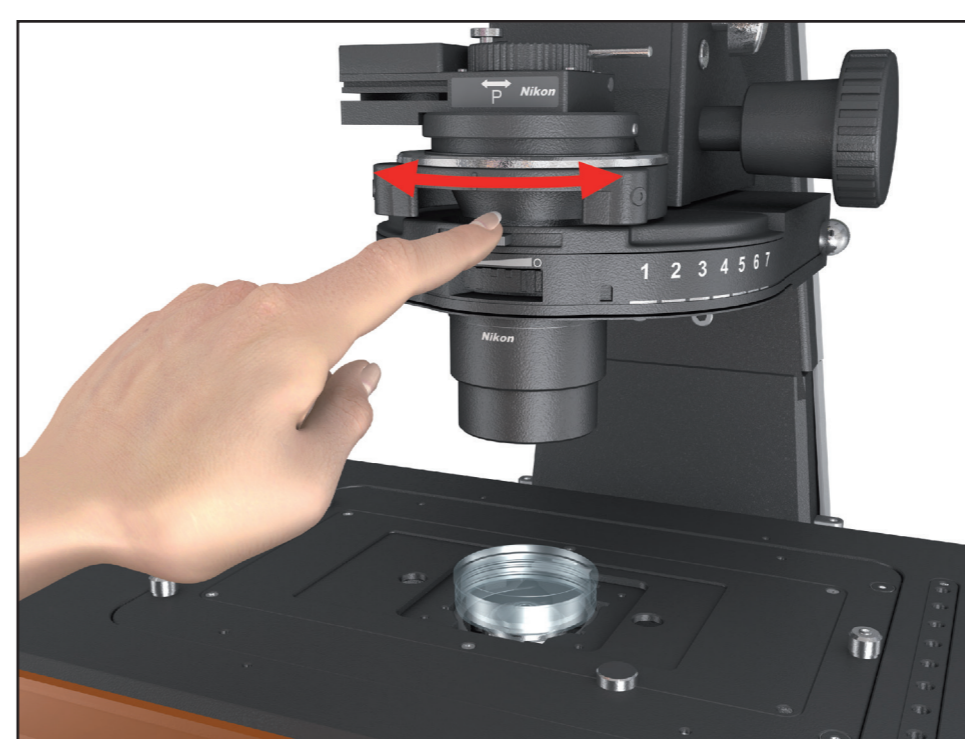
9 OPTIONAL: Center Phase Annulus – Insert a pair of 2 mm hex keys into the illustrated slots and rotate to center the image of the condenser phase annulus (light gray ring) within the image of the fixed-position phase annulus etched into the objective (black ring). The Bertrand lens may now be removed and the system is ready for phase contrast imaging.



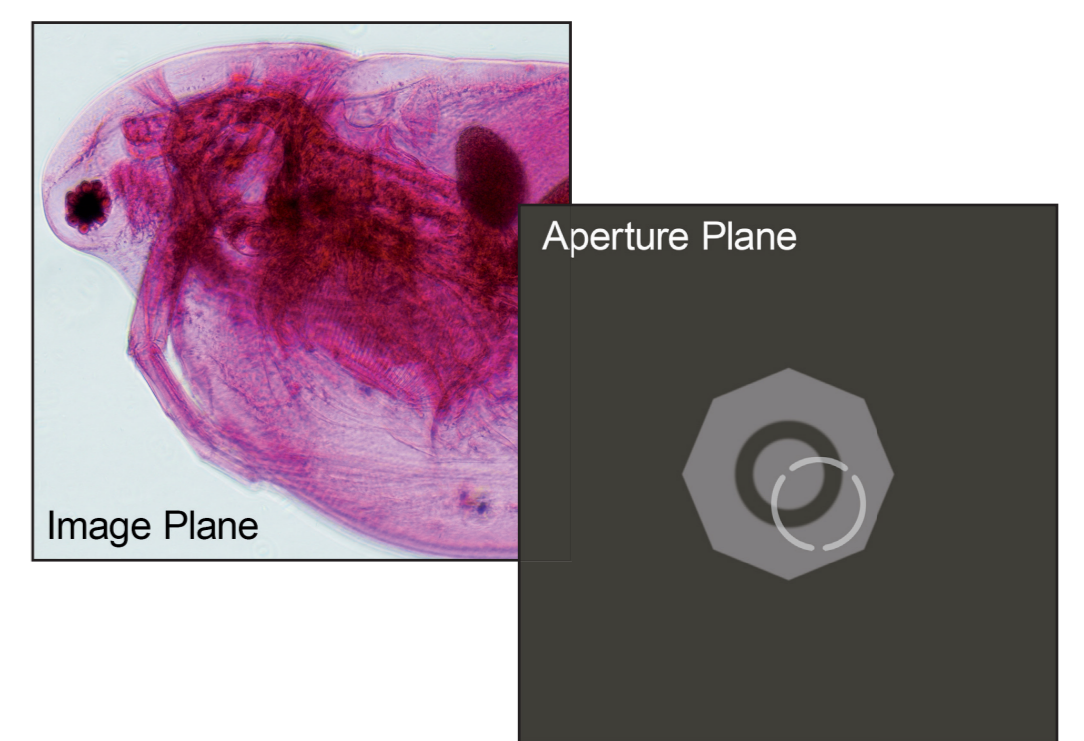
5 Open the Field Diaphragm – Once open, the edges should extend just past the desired field of view. Fine centration of field diaphragm may be performed while mostly (but not fully) opened.



6 Visualize Back Aperture – Insert Bertrand Lens into light path by rotating illustrated knob (a). Focus on edges of back aperture using smaller internal knob (b).



7 Adjust Condenser Aperture Diaphragm – The aperture diaphragm should be closed until its image fills about 70% of the back aperture.



8 OPTIONAL: Toggle Phase Annulus into Light Path – Use the illustrated switch to toggle appropriate condenser phase annulus into the light path. (PhL, Ph1, Ph2, or Ph3).

