## Setting up Kohler Illumination with the condenser on a Zeiss Axioplan scope (Scope 2 in room 310)

- 1) Turn on the PC and the microscope, set the bulb power level to 3.
- 2) Start the Zen software.
- 3) IMPORTANT: Only change objectives with the buttons under the focus wheel.
- 4) Select the 10x objective (yellow) using the buttons below the right side focus wheel.
- 5) Place a slide on the stage and focus your sample.
- 6) Rotate the condenser contrast selector wheel to Brightfield (marked H or H/DICII or BF).
- 7) Set the condenser aperture wheel to about 0.3 (if a number is marked) or about 3 quarters to the right.
- 8) Make sure the swing out condenser lens is engaged when using the 10x or higher magnification objectives.











for 10x 20x 40x 63x

- 9) Move the condenser close to your slide (about 1mm below), using the condenser focus wheel. There is one on each side of the scope.
- 10) Close the field stop aperture with the control wheel and focus the octagonal aperture image by moving the condenser up and down with the condenser focus wheel. Then align the condenser with the silver alignment screws so it is in the middle of the image.

Field stop aperture not focused and misaligned





11) Now open the field aperture so your whole image is illuminated.
12) Finally, set the condenser aperture to about 75% of the NA of the objective used (i.e 10x/ NA0.3, so aperture just below 0.3).
Closing the aperture increases contrast and darkens the image, opening the aperture increased resolution (not that you'd notice) and reduces contrast.



13) If your condenser aperture wheel doesn't have numbers you need to remove an eyepiece and look down the tube. Open the aperture wheel (move to the left) until you see the full size circle down the tube. The move the aperture wheel right until the hexagon is about 75% the size of the full size circle.



14) When switching objectives, you can open or close the field aperture to just illuminate your field of view (not so important) and you must adjust the condenser aperture for the NA of your objective (very important). The condenser aperture controls contrast, adjust to suit your sample, or set to ~75% of the NA.

## Condenser not focused (Dust on image, uneven illumination)

Contrast too high – condenser closed too much (some artefacts)





Contrast too low – Condenser open too far Just about perfect – You can adjust the contrast to suit your staining.

15) You're all set. Just remember to set the white balance in the software, and adjust subtract background (in the 'General' tab of the live image properties window) if needed.