# Widefield Microscopy

**Goals:**

- Understand the light path organization in a widefield microscope
- Adjust microscope to achieve Köhler illumination
- View fluorescent samples in epi-fluorescence mode
- View birefringent samples using polarization optics

**1. Brightfield Imaging (Köhler Illumination)**

- Block fluorescence light with intensity slider (on top part of microscope)
- Turn on HBO lamp (microscope on/off button on the right side)
- Set condenser turret to brightfield (H/DIC)
- Set fluorescence cube slider to brightfield
- Tilt polarizer below turret out of the light path
- Make sure the polarizer above the objective (analyser), marked 43 36 05, is in the "out" position
- For all objectives with magnification 10x or higher, the front lens of the condenser should be in the light path (it is another lens that can be tilted into or out of the light path by moving a small lever and it reads "0.9" on it.).
- Adjust objective to focus on sample
- Set field diaphragm to half the size and adjust height of condenser lens for field diaphragm aperture to be in focus and adjust centering with the screws
- Open field diaphragm

**Notes:** There is no adjustable condenser diaphragm.
This procedure must be repeated for every new objective.

**2. Epi-Fluorescence**

- Block the transmission light from the HBO by placing the cap on the field diaphragm.
- Choose a dichroic filter (either marked Red or Green).
- Turn on the fluorescence light (Xenon).
- There is a slider in the path for the xenon light with two neutral density filters, one empty slot and one position to block all light to adjust intensity. Set to one of the open positions.
- Adjust diaphragm aperture with stick near neutral density filter slider.
- With diaphragm half-closed, center using two screws.

**3. Polarization Imaging**

- Set Köhler illumination for Brightfield (see 1).
- Slide polarizer (marked 43 36 05) above microscope objective into place.
- Tilt polarizer below condenser turret into the light path.
- The orientation of the (linear) polarization of the incoming tranmission light can be adjusted by rotating the polarizer below condenser (it can be adjusted between 0˚ and 90˚).
- With no sample, when both polarizers (above and below the objective) are aligned the intensity is maximal, whereas when their polarization is orthogonal intensity is minimal.
- Samples that are birefringent (e.g. collagen) will change the polarization of the incoming light thereby changing the contrast.

**Note:** the stage itself can be slightly tilted as well.

**Condenser turret notation:**
1: Phase 1 10x
2: Phase 2 20x-25x
H/DIC: Brightfield or differential interference contrast
(hell=bright in German)
DIC: differential interference contrast