Zeiss Axioplan2 Plane Polarized Light Protocol

1) **Microscope control**

   - Take note of the microscope light path:
     A. Light source
     B. Direction of light through microscope
     C. Focus knob
     D. Neutral Density Filters
     E. Field Diaphragm: used in **Kohler illumination** (see poster for details)
     F. Lower Polarizer: variable setting, used for **Plane Polarized light**
     G. Condenser: Brightfield setting (I/H)
     H. Slide
     I. Lens
     J. \( \lambda/4 \) plate: used for Circularly Polarized light.
     K. Upper Polarizer: used for **Plane Polarized light**
     L. Camera/Eyepiece slider: note the locking positions of the slider and remember to **be delicate!** The slider pulls glass.

2) **Brightfield**

   - Under the Hardware Panel, select the **Brightfield** illumination.
   - Under the Hardware Panel, select the 10x lens.
   - Set the **Neutral Density filters**. The starting point settings are:
     - Rear: 0.4
     - Front: 12 (note: that this can vary)
   - Select the I/H condenser filter
   - Gently push the camera/eyepiece slider **all the way in** to send the light to the eyepiece.
   - Use the stage movement wand and focus knobs to **focus and center your specimen** on a region of interest.
   - Set up **Kohler Illumination**; you can refer to the poster for help. This step is **critical and necessary** for uniform field illumination in imaging and must be done every time you switch lenses.
   - With one hand on the focus knob and the other on the condenser iris control buttons, **set the iris** for your desired level of brightness, contrast, and morphological detail.
     - Higher Lens NA -> larger condenser aperture
     - Lower Lens NA -> smaller condenser aperture

Sample Brightfield image:
(lamb hoof sample courtesy of Virginia Gillespie)
3) **Polarized Light**

- Shift the **Upper Polarizer** and **Lower Polarizer** into the light path.
  - Upper Polarizer:

- Lower Polarizer:

  While viewing the specimen through the eyepieces, vary the angle of the **Lower Polarizer** to achieve extinction. (note: you may need to increase the amount of light to the specimen via the **Neutral Density filters**.)
4) **Imaging**

- **Pull the camera/eyepiece slider out** to send light to camera.
- **Take this time to set the exposure time and white balance:**
  - Click **Live** at the top of the screen to view a live camera feed of the specimen.
  - At the bottom of the **Live** window, click **Exposure** to calculate the exposure time.
  - On the Hardware panel, click the **Interactive...** button:
  - Click an area that should represent the background in your image.
  - The image may have slightly darkened. You can click **Exposure** again to calculate a new exposure time.
- **Once you have finished,** click **Snap** along the top menu bar to acquire your image.
- **Click the Save As button** to save the image in your directory within the specified User Data folder.