

Wide-Field Microscopy Session 3: Leica

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Background

Bright-field microscopy: The bright-field microscope is probably the best-known form of light microscopes. With a conventional bright-field microscope, light from an incandescent source is aimed toward a lens beneath the stage (called the condenser), through the specimen, through an objective lens, and to the eye through a second magnifying lens (the ocular or eyepiece). The image is formed due to the light-absorbing properties of the imaged objects.

Köhler illumination: When using transmitted light for microscopy it is important that the light path is set up properly. The method for doing this is called Köhler illumination after August Köhler, the man who invented it. In this method two aperture iris diaphragms are used to set the light path so that the specimen is illuminated evenly with a minimum of glare.

White balance: The human eye readily adapts to changing illumination conditions in order to identify a white object as white even when the surrounding intensity and color temperature fluctuate. In contrast, digital cameras require careful scrutiny and adjustment of the red, green, and blue (RGB) signal amplitudes in order to produce similar results.

Histological staining: When stained tissue sections are inspected under a microscope, differences in cell morphology can be detected. One of the most common staining procedures is a mixture of hematoxylin and eosin (HE). Hematoxylin colors the cell nuclei blue, eosin colors the cytoplasm pink.

Immunohistochemical staining (IHC): Method of localizing proteins in tissue sections exploiting the principle of antibodies binding specifically to antigens in biological tissues. The antibodies are bound to specific enzymes whose substrate properties are used to create a visible stain.

Polarized light microscopy: In this method, polarized light is used; it consists of light waves which all feature the same direction of vibration, i.e. which are linearly polarized. This very “ordered” light is generated by a polarizer which filters out a privileged plane from the statistical confusion of vibration directions prevailing in natural light. It is an important fact that two filters of this type do not let any light pass when they are arranged one behind the other in the beam path at an angle of 90° to each other. The first filter sorts out the vibration directions in such a way that the second filter cannot let them pass.

When illuminated, many specimens turn the vibration direction of the polarized light out of the plane produced by the polarizer. Such specimens are mainly birefringent materials, in which the refractive index depends on the vibration direction of the incident light. This is mainly the case with crystals, such as starch and minerals, but also with polymers. If such materials are viewed under the polarization microscope between the crossed polarizer and the analyzer, bright areas can be seen in the image because light is partially transmitted by the analyzer.


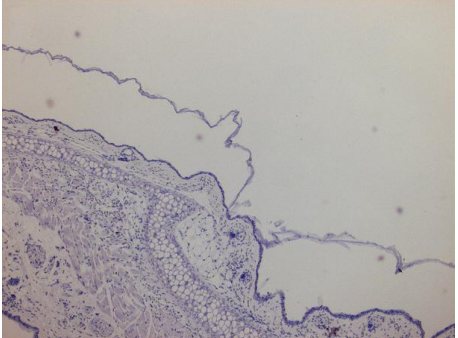


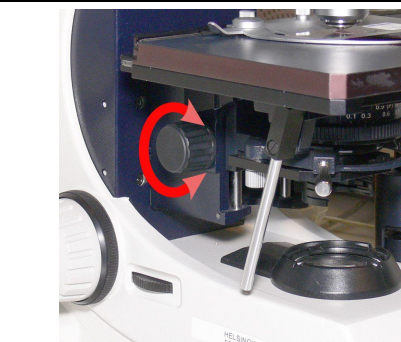
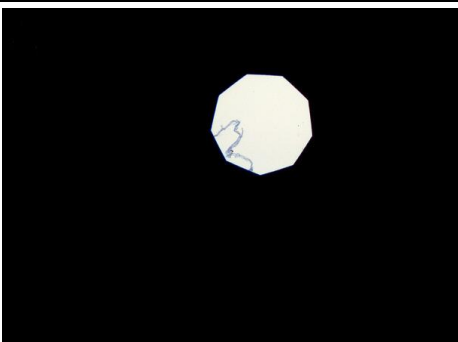
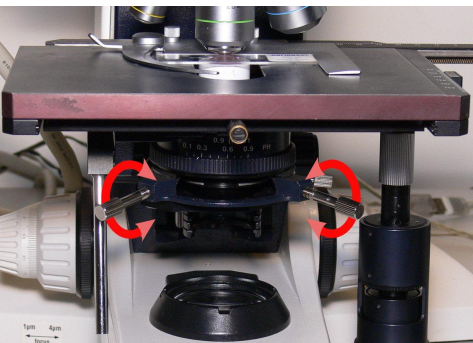
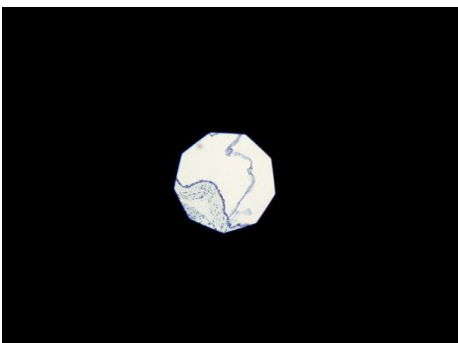
Instrument and material used

- Leica DB LB research microscope
- HE-stained specimen
- IHC-stained specimen
- Specimen with polyethylene particles

Work instructions

Köhler illumination

Adjust the Köhler illumination according to the following instructions with the HE sample.

<p>Use 10x objective and focus on sample</p>	 A photograph of a microscope's objective turret. The 10x objective lens is highlighted with a red square. Below the turret, there are labels for '10x', '40x', and '100x' with arrows indicating the direction of rotation.	 A micrograph showing a cross-section of tissue stained with hematoxylin and eosin (H&E). The tissue shows various cellular structures and layers.
<p>Close the luminous-field diaphragm by turning the adjustment wheel clockwise</p>	 A close-up photograph of the luminous-field diaphragm adjustment wheel. The wheel is highlighted with a red circle, and a red arrow indicates it should be turned clockwise.	 A micrograph showing the luminous-field diaphragm closed, resulting in a dark field with a bright, circular spot of light in the center.
<p>Move the condenser up or down, so that the diaphragm edges are in focus.</p>	 A photograph of the microscope's condenser assembly. A red arrow points to the condenser adjustment wheel, indicating it should be turned to move the condenser up or down.	 A micrograph showing the diaphragm edges in focus, appearing as a bright, circular spot with a dark, irregular border.
<p>Center the diaphragm in the viewfield by rotating the condenser adjusting screws.</p>	 A photograph of the microscope's condenser assembly. Two red arrows point to the condenser adjusting screws, indicating they should be rotated to center the diaphragm.	 A micrograph showing the diaphragm centered in the viewfield, appearing as a bright, circular spot with a dark, irregular border.

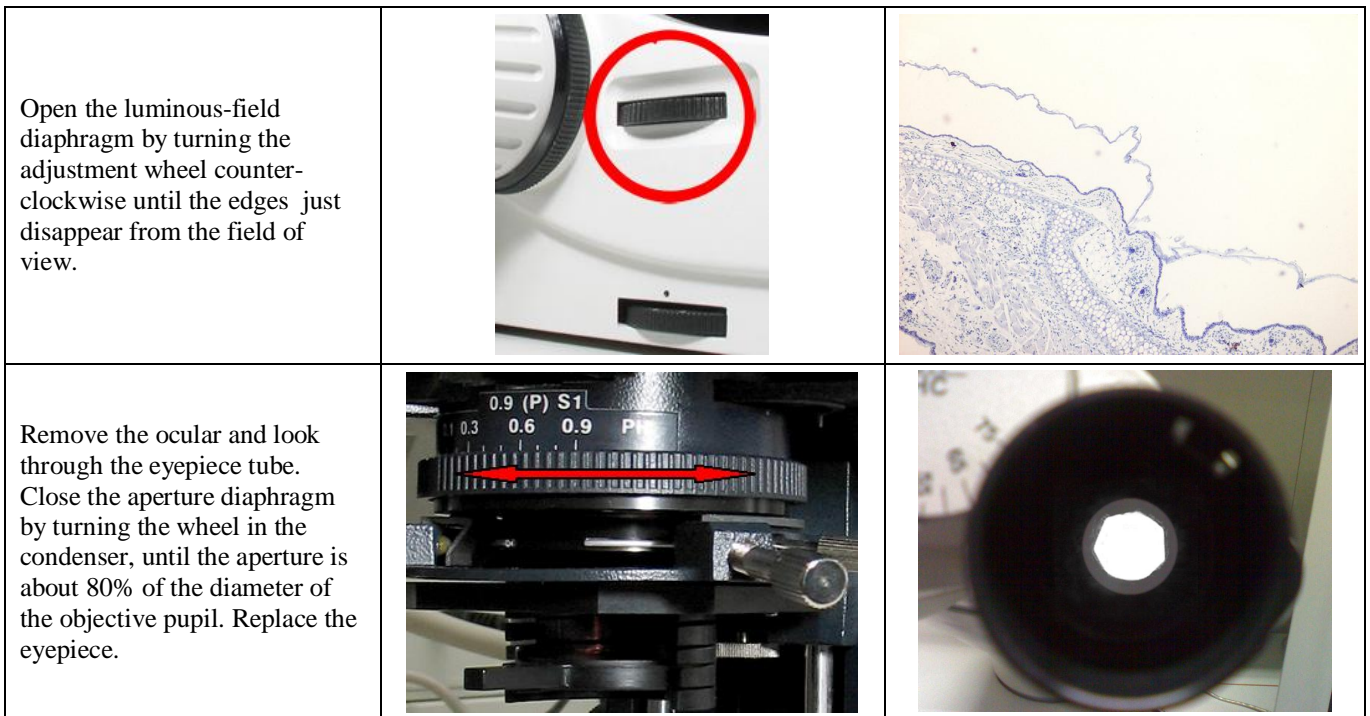


Image acquisition

An Olympus DP50 color CCD camera is attached on the top of the microscope. It is controlled by a Mac computer and Studio Lite software.

- Press Live image button (green triangle in Figure 1) in the Viewfinder window and then focus and move the sample with the help of the image on the computer screen.

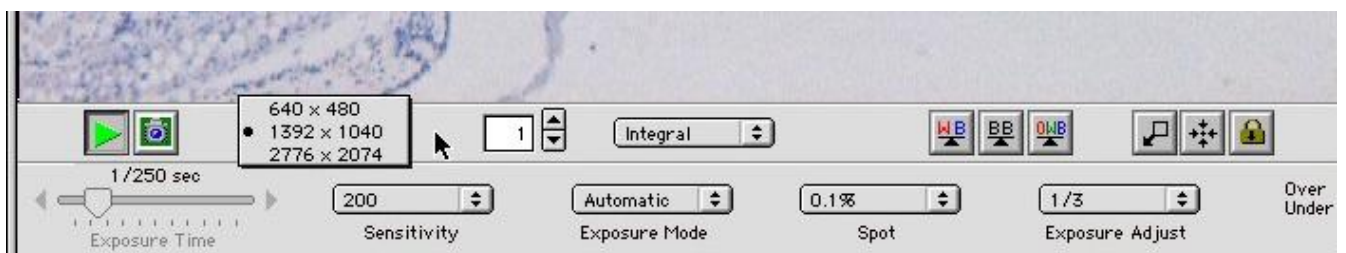


Figure 1: Adjustments in Viewfinder window

For this exercise the program is set up to automatically adjust exposure times etc.

White balance

By pressing the WB button and then clicking on an area in the live image that should be white (i.e. background) the program will automatically adjust the white point of an image.

To acquire the final image click the camera icon (next to the green triangle). This image can then be saved on the hard disk, external file server, or USB stick. Take a picture of both the HE and IHC samples with appropriate white balance adjustments.

Polarization contrast

For polarization contrast microscopy, an analyzer filter is inserted in the light path and a polarizer filter in a holder below the condenser. The polarizer is placed with the handle label side upwards. The analyzer is on the light path when pushed all the way in.

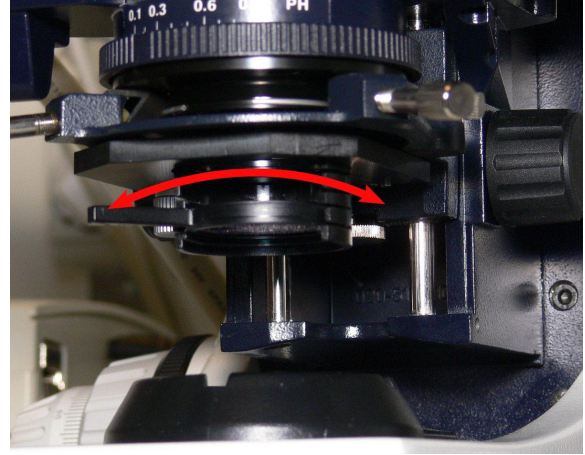
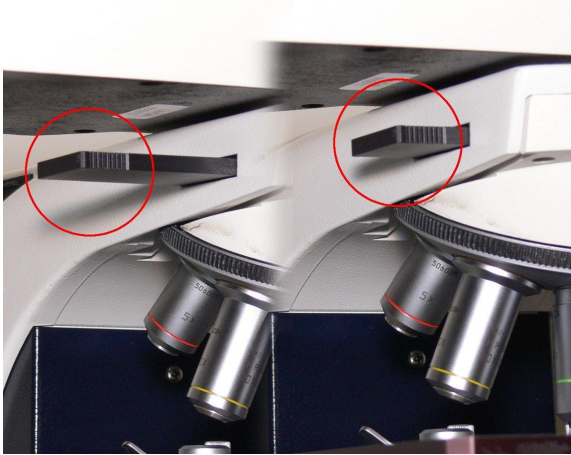


Figure 2: Polarizers

To cross the polarizers, turn the round polarizer until the maximum extinction position is reached i.e. the view in the oculars is as dark as possible. As the polyethylene particles in the sample polarize light differently from the rest of the sample you should now be able to see small bright particles against a black background.

Links

<http://micro.magnet.fsu.edu/>

http://en.wikipedia.org/wiki/Bright_field_microscopy