Chemistry Teaching Lab

Polarizing Microscope

(CEWEI LWT350LPT)

Key Features

- Single- and orthogonal-polarized light, and conoscopic observations for birefringent substances
- Coaxial design for coarse and fine focus
- Rotating stage with a 360° equal division scale
- 360° rotating angle between polarizer and analyzer
- High-quality microphotography using CaptureV2.4 software under 100% light transmission
- A precise temperature platform CEWEI CW400N

Standard Operating Procedure (SOP)

This guide provides basic instructions for acquiring polarizing microscopy images using the CEWEI LWT350LPT polarizing microscope coupled with the CaptureV2.4 software.

Time reservation

1. Authorized users can reserve polarizing microscope instrument from the following link:https://outlook.office365.com/owa/calendar/DKUDNASChemistrylab2@mscloud.duke.edu/bookings/s/NJkeEG1ILE6VGAjiXg6-oA2
2. Reservations should be made at least 24 hours in advance. Reservations should be canceled as soon as possible when they are no longer needed.

Lighting

1. Turn on the power switch at the bottom left of the instrument.
2. Choose a reflected or transmitted light mode for the illuminator by turning on the “up” or “down” switch at the bottom left of the instrument and pulling in or out the transmission/reflection switching lever.
3. Adjust the brightness knob at the bottom right of the instrument to the desired brightness. Avoid adjusting the lighting to its strongest state as this significantly reduces the lifespan of the bulb working at full load. Minimize the light brightness before switching light modes or turning off the power switch.

Focusing and switching objective lens

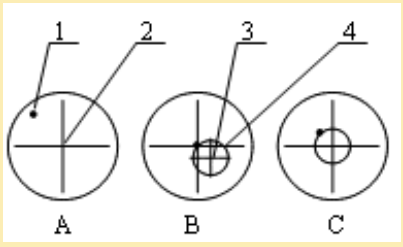
1. When observing a specimen, start with the low-power objective lens.

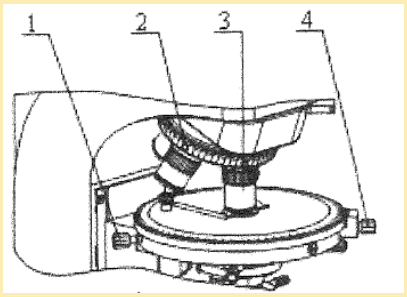
2. Adjust coarse focus knobs located on both sides of the frame to raise the specimen stage so the specimen approaches the objective lens.
3. Lower the specimen stages until the specimen image is seen.
4. Adjust coaxial fine-focus knobs to optimize the clarity until the image is clear.
5. Switch another objective lens with the higher power of magnifications by rotating the anti-skid turntable. The specimen image almost stays in focus due to the design of parfocalled objectives. DO NOT pinch the objective lens directly to avoid contaminating or knocking off the lens.

Centering between the stage and objective lens *(Optional)*

Under normal circumstances, DO NOT try the centering step for aligning the center of the stage and objective lens.

1. View the specimen with a reticle eyepiece and a 10X objective.
2. After focusing clearly, locate a target point (see “A”) and move the specimen to make the target point coincide with the center of the field of view or at the crosshair focus of the eyepiece.
3. Rotate the specimen stage. Meanwhile, check if the target point rotates in a circular trajectory around a certain spot (see “B”), as the optical axis of the objective lens deviates from the rotation center of the stage.
4. If so, align the center of the stage by adjusting the stage-centering screws (Labeled as “1”) so that the center of the trajectory is close to the center of the field of view (see “C”).



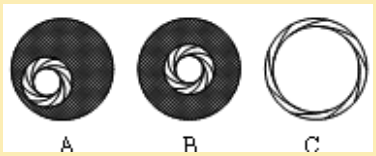


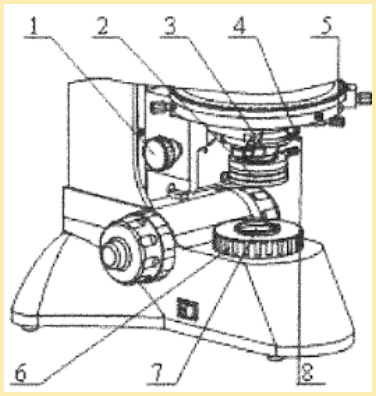
5. If other objective lenses are not in the center, adjust the objective lenses to the center of the optical axis with the objective-centering screw (Labeled as “2”).
6. Tighten the stage locking screw (Labeled as “4”).

Centering the condenser *(Optional)*

Under normal circumstances, DO NOT try the following condenser adjustment.

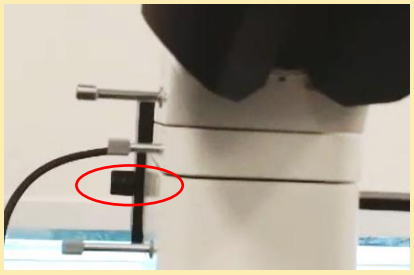
1. With a 10X objective lens, reduce the size of the aperture diaphragm (labeled as “8”) and lower the condenser through the condenser lifting handwheel (labeled as “1”) to observe the diaphragm image.
2. Check if the aperture image is unclear and not centered on the field of view (see “A”).
3. If so, adjust the condenser lifting handwheel to clarify the image. Adjust two condenser centering screws (labeled as “4”) to center the condenser (see “B”).
4. Increase the aperture to be slightly larger than the field of view (see “C”).
5. Raise the condenser to a normal viewing state.





Orthogonal polarized light observation

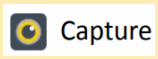
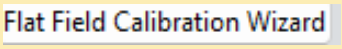
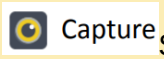
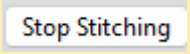
1. Since the polarizer is always in the optical path, the instrument is in a single-polarized light condition.
2. After adjusting the image clearly, insert the analyzer and rotate it to ensure the scale reaches the "0" position. The polarizer scale also must be in the “0” position.
3. In this case, the polarizer and analyzer filters reach an orthogonal state. The polarizer’s polarization direction is in the east-west direction, and the analyzer’s is in the north-south direction.
4. Lower the condenser through the condenser lifting handwheel when using 10X and lower-power objective lenses.
5. Raise the condenser to the highest point when using 25X and higher power objective lenses.
6. If needed, a gypsum test plate, a ¼λ mica plate, or a quartz wedge is inserted into the compensator slot. The optical compensator produces a specific optical path difference to increase the contrast and quality of the image.

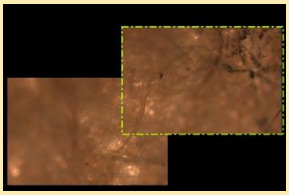


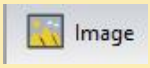
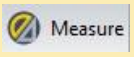
Conoscopic observations

1. Use 25X and higher-power objective lenses and put the instrument in orthogonal polarized light conditions.
2. Pull out the Bertrand lens switching lever to put the instrument in the working state of conoscopic observation.
3. Observe the interference Figures.

Microphotography by CaptureV2.4 software

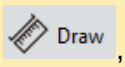
1. Pull out the camera switching lever  .
2. Double-click to open the CaptureV2.4 software  on the desktop.
3. Click  on the top Status Bar. If needed, adjust parameters under tabs of [Resolution], [Binning], [Exposure Control], [Bit of Depth], [White Balance (WB)], [Image Adjust], and [Histogram]. A middle range of resolution is suggested to optimize the acquisition speed for video recording. If flatting the images and reducing defects of color patches in the images, click on  under the “Image Adjust” tab.
4. Adjust parameters under the tab of “File Save”. The file name adopts a format of “custom + time-stamp”. Input the custom name and choose the time-stamp naming format. Select image formats (JPG\TIF\PNG\DICOM) and save paths for the files.
5. Capture a live previewing image by clicking  . The captured image is displayed in the Preview Window on the right. If the Preview Window is invisible, click the control button  at the right edge.
6. Record videos by clicking on  under the [Video Record] tab of  Status Bar.
7. If needed, click the tab [Image Stitching(Live)] to combine individual images with adjoining positions on the sample into a stitched image with a large field of view. Click on  , slowly move the sample (no more than ¾ away from the former position), and then stop. The navigation bar appears green in the stitching window. Repeat the next move until the live-stitched image meets with expectations. Click on  to end stitching. The stitched image is displayed in the Preview Window.



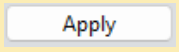
8. If needed, post-process images by clicking  Status Bar to adjust parameters under tabs of [Image Adjust], [Fluorescence], [Advanced computational imaging], [Binaryzation], [Smooth], and [Filter/Extract/Inverse Color].
9. Add a scale bar to switch the number of pixels into practical distance by selecting the calibration scale in the [Calibration Table] at the bottom panel under the  Status Bar. Calibration scales for 10X, 20X, and 40X objective lenses have been created so far.

Measure Data | Calibration Table | Class Counting | Automatic Counting Table | Automatic Counting Statistics

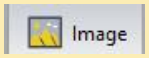
	Current	Name	Length	TotalPixel	Unit	Unit/Pixel	Resolution
3	☑	10x	1000.00	2047.42	μm	0.49	2736x1824
4	☐	20x	400.00	1624.36	μm	0.25	2736x1824
5	☐	40x	180.00	1493.95	μm	0.12	2736x1824

(Optional) If a new calibration scale for other objective lenses is needed, put a ruler on the specimen stage. Click the [Calibration] tab. After clicking 

, a reticle appears in the Window. Draw a straight line parallel along the line on the ruler image. The blank of the number of pixels is automatically filled in. Create a name of the calibration scale, fill in the real distance, select the measuring unit in 

, and click 

to scale the measuring unit.

10. Click  under the [Measure Tool] tab. Drag the scale bar to a proper position. Adjust the format of the scale bar (e.g., line, text, and border) under the [Scale Property] tab. Click  to inscribe the scale bar on the image.
11. Select the image or video in the Preview Window. Click 

on the top Status Bar. Click on the icon  on the top right corner, input file name and formats and choose the save path in the following pop-up window.

Copy data

Transfer your data from this processing computer to your computer using the attached USB flash drive. Make sure to format the USB flash drive afterward.

Users are responsible for their data. All data saved on the processing computer must be transferred promptly to their computer.

Logout procedure

1. Remove your sample.
2. Turn off the heating platform.
3. Minimize the light brightness of the illuminator. Turn off the power switch.
4. Turn off the software and the computer.
5. Clean up the working areas.
6. Cover the microscope using the plastic cover bags.
7. Sign the logbook, including Date, Name/PI, Nucleus, Start Time, and End Time.
8. Take your samples with you when you leave.

Location

IB 2078

Requirement

- Avoid dust, vibration, acid, alkali, or steam.
- DO NOT disassemble any parts of the instrument at will.

Policy for Sharing

- Approval by PI
- Pass training
- Sign agreement
- Online booking system

Contact

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