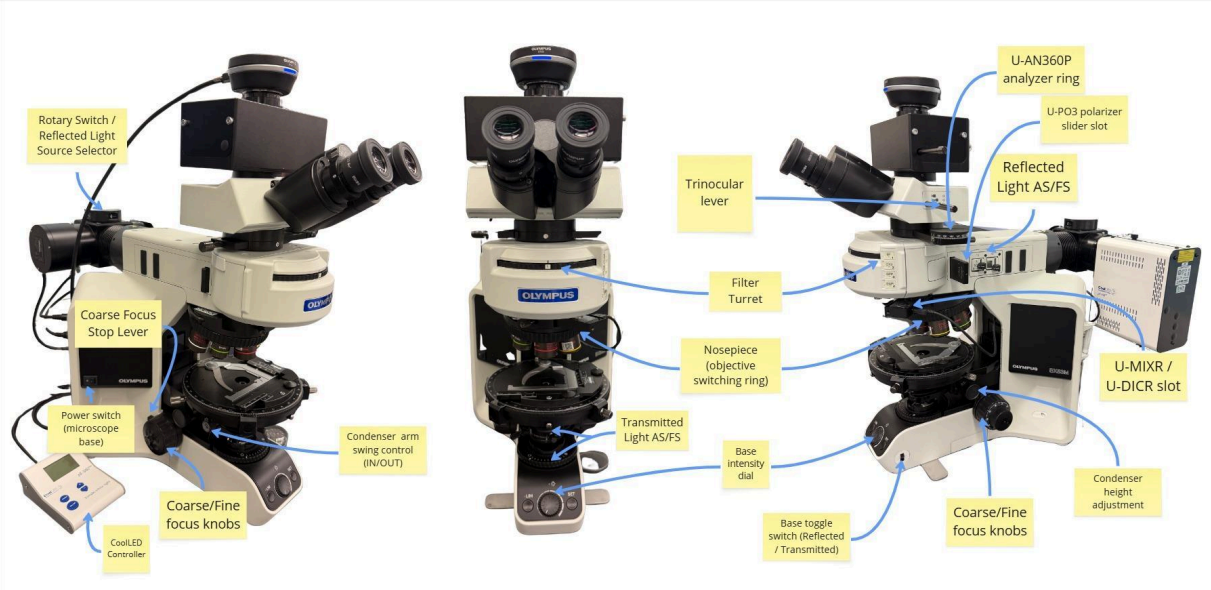
Olympus BX53M Training SOP

Standard Operating Procedure

Table of Contents

Note: We welcome your feedback. This SOP is a living document. If you find a mistake, an inconsistency, a step that is unclear, or anything that was hard to follow, please leave a comment directly on this file in Google Drive. All suggestions are welcome and will help us make this document more useful for everyone.



1 Critical Rules

Read before your first session. These protect the instrument and your samples.

Always start with the stage fully lowered and raise it slowly. Bring the sample up to focus by raising the stage.

Always begin at 5×. Never position a high-magnification objective over an unknown sample. Working distance shrinks sharply at high magnification.

Never touch the objective lenses. Fingerprints and debris cause permanent damage to the optical coatings.

Non-flat samples restrict which objectives you can use safely. If your sample varies in height by more than 0.5 mm, only the **5×** and the **20× long-working-distance** objectives may be used without staff supervision. High-magnification objectives have insufficient working distance and will contact an uneven surface. See Section 2.7 for the full procedure.

Pre-Session Checklist

Before touching any controls, confirm:

- Nosepiece is at the **5× position**

- Stage is in the **lowered position**
- Trinocular lever is set to **camera (100%)**

2 Flow of Operation

The main workflow covers all four imaging modes. At step 2.3, transmitted light users branch directly to Section 3.3. All other modes share a common reflected-light find-focus sequence (steps 2.4–2.5) before their mode-specific setup at step 2.6.

2.1 Start Up

1. Turn on the microscope (power switch on the base).
2. Turn on the computer and log in.
3. Open PRECiV. In the Device Status tab, make sure the right instrument (BX53) is selected in the Device Configuration dropdown. The live image starts automatically.

> **Ready to proceed when:** Live image is visible on screen.

2.2 Load Sample

1. Turn the coarse focus knob to fully lower the stage.
2. Place your sample flat on the stage.
3. Secure the sample if needed (stage clips or an appropriate holder for your sample type).

> **Ready to proceed when:** Sample is flat on the stage and the objective can travel freely above it.

2.3 Select Light Path

Before finding focus, confirm which illumination path your session requires.

Your imaging mode	Do this
Reflected light — brightfield, fluorescence, or polarization	Continue to step 2.4
Transmitted light — imaging through a semi-transparent sample	**Go directly to Section 3.3**; skip steps 2.4 and 2.5; return to step 2.7 when done

> Not sure which you need? **Reflected light** images the surface of a sample from above (opaque or fluorescent samples). **Transmitted light** passes through the sample from below (thin sections, semi-transparent specimens).

2.4 Find Focus (Reflected Modes)

1. Rotate the nosepiece to the **5× objective**; turn until it clicks into position.
2. Rotate the **filter turret** → position **1 (BF)**.

3. Set the **base toggle switch** → reflected icon (light-from-above).
4. Turn the **base intensity dial** to a comfortable brightness (roughly mid-range).
5. While watching the screen, turn the **coarse focus knob** to slowly raise the stage. Stop as soon as the sample comes into view.
6. Switch to the **fine focus knob** to sharpen the image.

> **Warning:** Watch the stage as you raise it. If the objective gets close to the sample before an image appears on screen, lower the stage, reposition the sample, and try again.

> **Ready to proceed when:** Sample is visible and sharp at 5× on screen.

2.5 Optimize Illumination — Köhler Setup (Reflected Modes)

Proper Köhler illumination gives even, glare-free light across the field. For a full explanation of each step, see the Evident Scientific guide at https://evidentscientific.com/en/insights/how-to-align-khler-illumination-in-6-simple-steps. The quick-reference steps for this microscope are below; repeat each time you change objectives or modalities.

> **Note:** This section uses the **reflected-light** FS and AS levers, located on the illuminator arm. This microscope has a second set of levers for transmitted light (on the condenser) — make sure you are using the correct pair. Both sets are labeled in the diagram in Section 4.1.

1. With the sample in focus, close the **FS lever** (on the illuminator arm) completely. The illuminated circle on screen should shrink to a small, sharp-edged disk.
2. If the disk is off-center, use the **illuminator centering screws** to move it to the center of the frame.
3. Open the **FS lever** until the illuminated area just reaches the edges of the camera frame — stop there. Do not open it fully.
4. Set the **AS lever** to align with the **color band** printed on the active objective barrel. The color band indicates the correct aperture range for that objective's numerical aperture. This controls contrast and resolution.
5. Fine-tune brightness with the **base intensity dial**.

> If contrast is poor after setting the color band, close the AS lever slightly. If the image is too dim, open it slightly. See the quick-reference table in Section 4.4.

> **Ready to proceed when:** Field is evenly lit with sharp edges at the frame boundary and no glare or hot spots.

2.6 Mode-Specific Setup

Your sample is in focus and illumination is optimized with reflected light. Now configure for your specific mode.

Your mode	Do this
Reflected brightfield — imaging an opaque surface	Continue to step 2.7; no additional setup needed
Fluorescence — GFP, CY3, DAPI labels	Go to **Section 3.2**, then return to step 2.7
Polarization — birefringent materials	Go to **Section 3.4**, then return to step 2.7

2.7 Change Objective (if needed)

> **Warning: Not sure whether your sample is flat?** Use only the 5× and the 20× long-working-distance objectives until you confirm. See below for the full rules.

Flat samples (height variation $\leq 0.5\,mm$ across the area you are imaging):

1. With the sample in focus at 5×, engage the **coarse focus stop lever** on the focus knob. This sets a hard limit that prevents the stage from being raised further, protecting the objective.
2. Rotate the nosepiece to the target magnification; turn until it clicks. **Do not lower the stage.**
3. Use the **fine focus knob only** to sharpen the image.
4. Repeat steps 2–3 to step up to higher magnifications.
5. Before lowering the stage at the end of your session, **disengage the stop lever first**. Never force the coarse focus knob while the stop is engaged.

Non-flat samples (height variation $> 0.5\,mm$):

> **Warning:** Only the **5**× and the **20**× **long-working-distance** objectives are safe for non-flat samples. High-magnification objectives have very short working distances and will contact the sample before coming into focus. Contact lab staff before using any other objective on a non-flat sample.

> The MPlanFLN objectives are parfocal: after rotating to a new magnification, the sample will stay roughly in view and roughly in focus. Fine adjustment only is needed.

> **Ready to proceed when:** Correct magnification is shown in PRECiV and the image is sharp.

2.8 Capture Image

1. In PRECiV, click **Auto** for a starting exposure, or adjust manually in Observation Settings.
2. Click the **Snapshot** button (camera icon, bottom bar).
3. Confirm the image appears in the PRECiV gallery and is saved to your project folder.

> **Done when:** Image is saved and visible in the gallery.

2.9 Shut Down

1. Turn the coarse focus knob to lower the stage.

2. Remove your sample.
3. Rotate the nosepiece back to the **5× objective**.
4. If the CoolLED pE-300w was used: press **On/Off** on the controller to turn it off, then return the **rotary switch** on the illuminator arm to the reflected LED position.
5. Close PRECiV.
6. Turn off the microscope and computer.
7. Cover the microscope with the dust cover.

> **Ready: Done when:** All lights off, sample removed, software closed.

3 Imaging Modalities

Each modality section is self-contained. Complete the setup steps for your mode, then return to step 2.7 (Change Objective, if needed) and step 2.8 (Capture Image).

3.1 Returning to Reflected Brightfield (BF)

> **Starting a fresh BF session?** You do not need this section. Steps 2.4 and 2.5 already configure reflected brightfield. Use these steps only when switching *back* to reflected BF from fluorescence or another mode mid-session.

To return to reflected BF from another mode:

1. Set the **base toggle switch** → reflected icon.
2. Set the **illuminator dial** → **BF**.
3. Rotate the **filter turret** → position **1 (BF)**.
4. If the CoolLED was active: turn the **rotary switch** on the illuminator arm back to the reflected LED position.
5. Adjust brightness with the **base intensity dial**.
6. Re-run Köhler illumination (step 2.5) if you changed objectives.

> **Ready to proceed when:** Sample surface is visible in even, white reflected light on screen.

3.2 Fluorescence (GFP / CY3 / DAPI)

For imaging fluorescently labeled biological samples. The CoolLED pE-300w provides broad-spectrum white light; the filter cube selects the excitation and emission wavelengths for each label.

> **Set the base intensity dial to minimum before turning on the CoolLED.** Any background light from the built-in LED will wash out the fluorescence signal.

> **Minimize exposure time and CoolLED intensity.** Prolonged illumination bleaches fluorescent labels irreversibly.

> Your sample is already in focus from steps 2.4–2.5. Proceed directly with fluorescence setup below.

Setup:

1. Set the **base intensity dial** → **minimum (0)**.
2. Rotate the **filter turret** to the position matching your fluorescent label:

Your label	Turret position
GFP	Position **3 (GFP)**
CY3	Position **2 (CY3)**
DAPI	Position **4 (DAPI)**

> **Do not look into the eyepieces or directly at the sample when the CoolLED is active.** The CoolLED emits UV light that is invisible to the eye but can cause damage with direct exposure.

1. Turn the **rotary switch** at the back of the illuminator arm to the **CoolLED position** (follow the engraved arrow). This routes CoolLED light into the optical path; the built-in reflected LED is now bypassed.
2. Confirm the **CoolLED pE-300w controller** is on (press the **On/Off** button on the controller box if not already lit).
3. Start at low CoolLED intensity. Increase only until the signal is visible on screen.
4. Adjust exposure in PRECiV: longer exposure gives brighter signal. Stop before areas appear solid white (saturation).

Switching between fluorescence labels:

1. Rotate the filter turret to the next position.
2. Adjust CoolLED intensity and PRECiV exposure as needed; brightness varies between labels.

> **Ready to proceed when:** Fluorescence signal is visible on screen and not saturated. Return to **step 2.7** (Change Objective, if needed) or **step 2.8** (Capture Image).

3.3 Transmitted Light

For semi-transparent or thin samples where structure is visible through the specimen (thin sections, some polymers, transmitted-light biological preparations).

Setup:

1. Swing the condenser (U-POC-2) **IN**; push the swing arm into position below the stage.
2. Set the **base toggle switch** → transmitted icon.

3. Rotate the **filter turret** → position **1 (BF)**.
4. Adjust brightness with the **base intensity dial**.
5. Use the coarse then fine focus knobs to bring the image into focus.

Köhler illumination for transmitted light (quick reference — full guide at https://evidentscientific.com/en/insights/how-to-align-khler-illumination-in-6-simple-steps)**:**

> **Note:** This section uses the **transmitted-light** FS and AS levers — the FS lever is below the condenser in the base, and the AS lever is on the condenser. These are different from the reflected-light levers on the illuminator arm. Both sets are labeled in the diagram in Section 4.1.

1. With the sample in focus (low-magnification objective), close the **transmitted FS lever** (below the condenser, in the base) completely.
2. Adjust the **condenser height** using its focusing ring until the edges of the closed FS are as sharp as the sample features.
3. Center the FS using the **condenser XY adjustment knobs**.
4. Open the **FS lever** until the illuminated circle just reaches the edge of the camera frame — no farther.
5. Set the **condenser AS lever** to the value specified on the active objective barrel.

> **Ready to proceed when:** Sample interior structure is visible through the specimen on screen with even, glare-free illumination. Return to **step 2.7** (Change Objective, if needed) or **step 2.8** (Capture Image).

3.4 Polarization

For imaging birefringent materials: crystals, mineral grains, fibres, and strained polymers. Birefringent materials rotate polarized light and appear bright against a dark background when the polarizer and analyzer are crossed.

Required accessories (confirm both are present before starting):

- **U-PO3 polarizer slider** — inserts into the slot at the front of the illuminator
- **U-AN360P analyzer ring** — mounted on the observation tube above the nosepiece

1. Insert the **U-PO3 polarizer** slider into the illuminator front slot.
2. Confirm the **U-AN360P analyzer** ring is in place on the observation tube.
3. Set the **base toggle switch** → reflected icon.
4. Rotate the **filter turret** → position **1 (BF)**.
5. Slowly rotate the **U-AN360P analyzer ring** while watching the screen. Stop at the position of maximum contrast or darkest background (extinction).

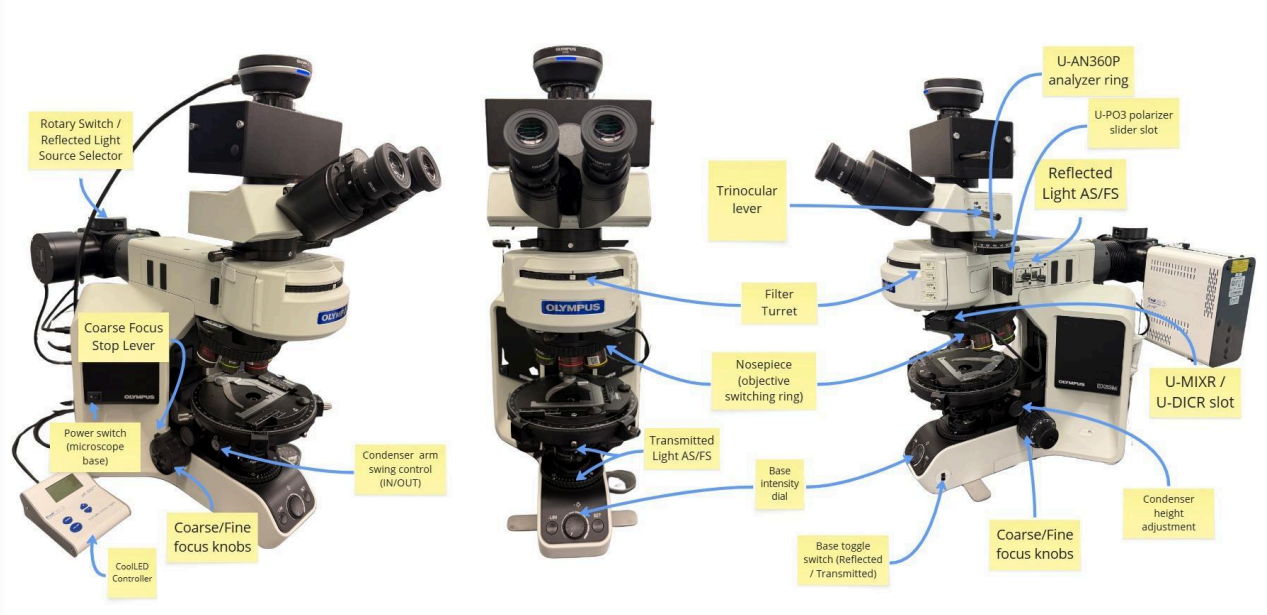
6. Rotate the **stage ring (U-SRP-1-2)** to find the orientation where your sample shows the clearest structure.

> **Ready to proceed when:** Birefringent features are visible against a dark or high-contrast background. Return to **step 2.7** (Change Objective, if needed) or **step 2.8** (Capture Image).

4 Controls Reference

Use this section to identify a control or look up what it does. For step-by-step instructions, see Section 2 and Section 3.

4.1 Instrument Diagram (Labeled Controls)



4.2 Focus Knobs

Located on both sides of the microscope frame.

Knob	Movement	Use for
Coarse focus (outer/larger)	Large increments	Initial approach at 5×; lowering stage at end of session
Fine focus (inner/smaller)	Small increments	Final sharpening at any magnification

4.3 Trinocular Lever

Located on the observation tube. Directs light toward the camera or the eyepieces.

Position	Effect
Camera (100%)	All light goes to the DP28; live image visible in PRECiV. Use this for all imaging.

Position	Effect
Eyepiece	Light directed to eyepieces only; screen goes dark. Not used in this SOP.

> **Note:** If the PRECiV screen is unexpectedly dark, check this lever first. Then check that the correct instrument is selected in the software.

4.4 Illuminator Panel (Reflected Light AS/FS)

Located on the illuminator arm. These controls affect **reflected light modes only** (BF, fluorescence, polarization). Transmitted light has its own equivalent controls on the condenser — see Section 4.7.

Control	What it does
FS lever (positions 1, 3, 5)	Controls the size of the illuminated circle on the sample
AS lever	Controls image contrast and resolution

Where are the AS/FS controls for each mode?

Mode	AS location	FS location
Reflected (BF, Fluo, POL)	Lever on right side of illuminator arm	Lever on illuminator arm (next to AS)
Transmitted	Lever on condenser	Lever below condenser in base

Setting the AS — match to the objective:

The method differs by light path:

- **Reflected light (BF, fluorescence, polarization):** The AS lever on the illuminator arm is marked with color bands. Each objective barrel also has a colored band indicating its numerical aperture range. Set the AS lever to the color that matches your objective's band.
- **Transmitted light:** The AS lever on the condenser is marked with numerical aperture values. Set it to the NA value printed on the active objective barrel.

AS lever quick reference:

Image looks like...	Try this
Features are hard to distinguish, low contrast	Close the AS lever partially
Image is too dim or flat	Open the AS lever
Bright glare ring around the edge of the field	Close the FS lever (not the AS)

4.5 Base Controls

Located on the microscope base.

Control	Location	What it does
Intensity dial	Front-center of base	Sets brightness for whichever built-in LED is active

Control	Location	What it does
Reflected/Transmitted toggle	Right side of base	Selects between reflected LED (light from above) and transmitted LED (light from below)

> **Note:** The reflected and transmitted light sources can also be switched on/off and their brightness adjusted from the **Observation Settings** tab in PRECiV. If a light source appears unexpectedly off at the hardware level, check that it is enabled in the software first.

4.6 Filter Cube Turret

Located on the side of the illuminator body. Rotate to select the configuration for your observation mode.

Position	Label	Use for
	BF	Reflected brightfield; standard mode for imaging opaque surfaces (metals, ceramics, circuit boards). Starting point for every session.
	CY3	Fluorescence; red-emitting labels. Used in immunofluorescence, live-cell imaging, and multicolor staining panels. Common labels: CY3, TRITC, mCherry, Alexa 555.
	GFP	Fluorescence; green-emitting labels. Commonly used with GFP-tagged proteins, FITC staining, and Alexa 488.
	DAPI	Fluorescence; UV-excited, blue emission. Common labels: DAPI, Hoechst 33342.

4.7 Nosepiece & Objectives

The 6-position objective turret. Rotate by hand until each position clicks into place. PRECiV automatically reads which objective is active. See Section 2.6 for objective-switching safety rules based on sample topography.

Position	Objective	Use for
	MPlanFLN 5× BDP	Every session start; finding and navigating sample
	MPlanFLN 10× BDP	Overview of large features
	MPlanFLN 20× BDPL	General imaging
	MPlanFLN 20×	General imaging (alternate)
	MPlanFLN 50× BDP	Fine surface detail
	MPlanFLN 100× BDP	Highest resolution

4.8 Condenser (U-POC-2)

Sits below the stage on a swing arm. Swing **IN** for transmitted light observation; it is required in that mode. Its AS and FS controls are described in Section 4.3. For reflected light work, its position has no effect on the image; swing it out only if it physically obstructs sample loading.

4.9 Light Sources

The two built-in LEDs share one intensity dial. The toggle switch on the right side of the base selects which is active. The CoolLED is a fully separate system used only for fluorescence; it is routed into the optical path via the rotary switch on the back of the illuminator arm.

Source	Brightness control	Select with	Used for
Built-in reflected LED	Base intensity dial	Toggle → reflected icon	BF, DF, DIC, POL
Built-in transmitted LED	Base intensity dial (same dial)	Toggle → transmitted icon	Transmitted light
CoolLED pE-300w	CoolLED controller box	Rotary switch on illuminator arm (then On/Off on controller)	Fluorescence only

> **Note: U-MIXR** is installed in the microscope. It is a motorized fluorescence mirror unit (beam splitter/filter changer) and can be controlled from PRECiV if needed.

CoolLED pE-300w controller:

Button/Display	Function
On/Off	Turns the LED on and off
Mode	Cycles through intensity presets
Up / Down	Fine-adjusts intensity
LCD display	Shows current intensity level

4.10 U-DICR — Differential Interference Contrast (Reflected)

The U-DICR is a DIC prism set for reflected light. It reveals surface topography and subtle height differences on otherwise featureless flat surfaces — useful for semiconductor wafers, polished metals, thin films, and similar materials where standard brightfield shows little contrast. The U-DICR must be physically installed on the microscope before use. Contact lab staff for installation and initial setup.