Olympus BX51 Microscope

(May 2014)

BX51 Microscope

Bright field halogen lamp ; reflected (top) or transmitted (bottom) illumination
Fluorescence mercury lamp
Filter positions
1. HQ:RDil (red)
2. DAPI (340-380 Ex; 400 dichroic; 435-485 Em)
3. DIC
4. bright field
5. dark field
6. Qdot hxCM (red/blue)

Objective	Magnification	Immersion	N.A.	Working Distance	Filters
M Plan Fluorite	2.5X	Dry	0.08	10.7	bright field only (w/polarizer)
UM Plan Fluorite	5X	Dry	0.15	12.0	all
UM Plan Fluorite	10X	Dry	0.30	11.0	all
LM Plan Fluorite	20X	Dry	0.40	12.0	all
LM Plan Fluorite	50X	Dry	0.50	10.6	all
LM Plan Fluorite	100X	Dry	0.80	3.4	all (available upon request)

Filters
Wheel Position 1 – HQ:Rdil *red*
Position 2 – DAPI, fluorescence filter for fluorophores, nuclear stain
Position 3 – DIC Nomarski Differential Contrast filter
Position 4 – BF, bright field *(for all white light)*
Position 5 – DF, dark field filter
Position 6 – Qdot hxCM *red/blue*

Cooke SensiCAM Camera

Black and White camera

ImagePro Plus Software

Capture single images (8-bit or 12-bit) or movies (.avi files)
Image adjustments (gamma, saturation, background subtraction, etc.)
Measure distances
Add labels or scale bars
Color adjustments and false color

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QUICK GUIDE

(Bold numbers are shown on the microscope diagram).

1. Log in to CORAL.
2. Turn on the halogen lamp **(1)**.
3. Select transmitted (bottom) light **(4)** for transparent samples or reflected (top) light.
4. Turn on the camera for pictures or measurements (switch atop the blue camera).
5. ONLY IF using fluorescence, turn on the mercury lamp **(18).**
6. Set the light path for the halogen or mercury lamp **(30).**
7. Choose the filter wheel setting **(17)** (NOT BF for UV light or you will damage your eyes).
8. Choose the objective **(6).**
9. Load sample on the stage.
10. Focus **(10)** and adjust illumination **(3).**

If transmitting fluorescence

Slide the filter wheel to position 2. **The position 4 (BF) filter will expose your eyes to UV light.**
Use the 25% ND filter **(31)** to reduce the UV intensity.
Close the shutter **(20)** to protect sample from excess UV light.

For pictures *(See page 9 for details).*

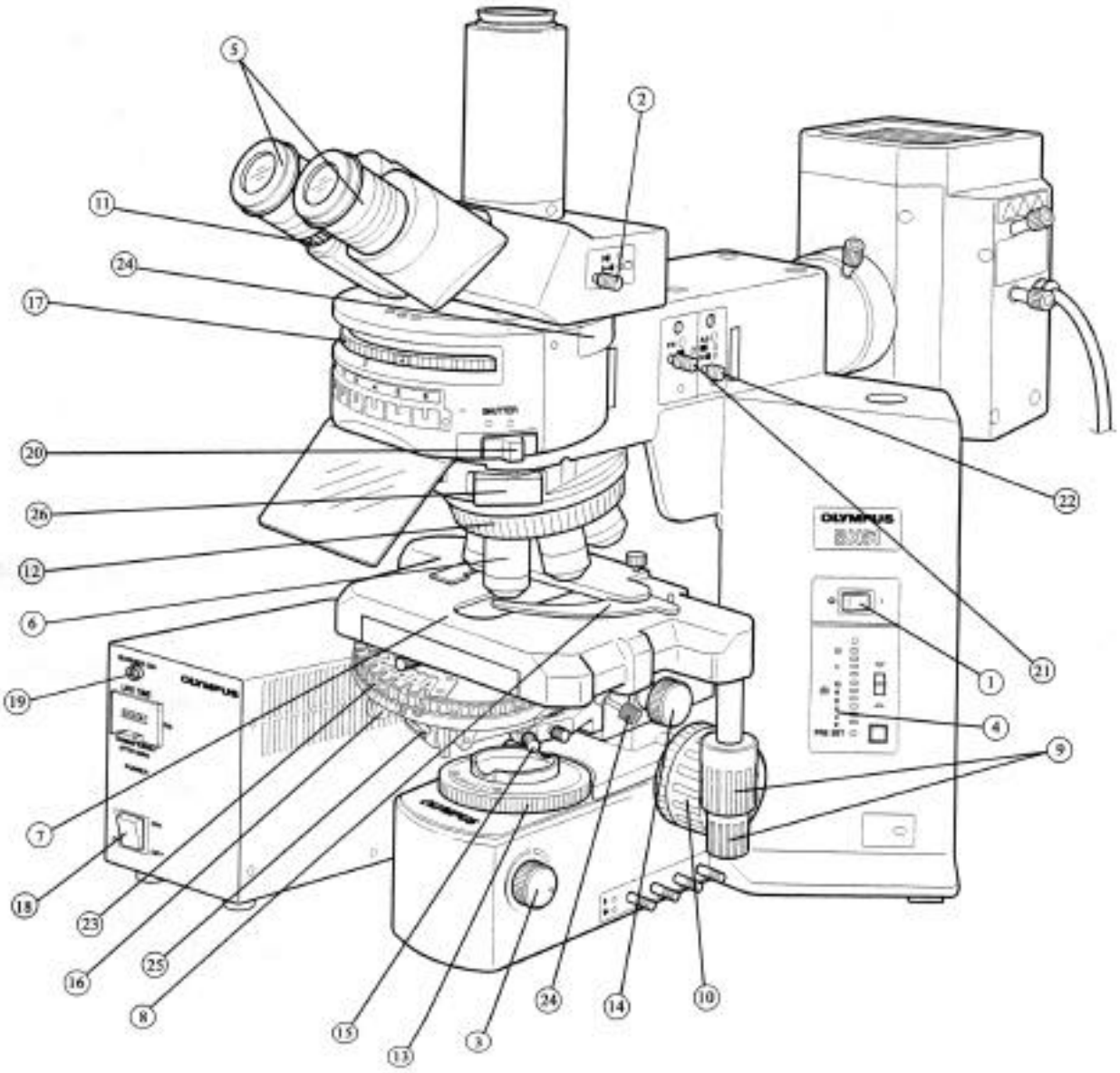
Send light to the camera with the camera/eyepiece splitter knob **(2).**
Open Image Pro Plus software with the desktop icon.
Open an acquisition window.
Select the SensiCAM Cooke Driver v3.6.
Set bit acquisition depth.
Set file saving options.
Set single, interval or time lapse capture modes.
Set spatial calibration to match the objective that is being used.
Set exposure time or use auto exposure.
Open a live preview window.
Focus, adjust exposure time or lighting, adjust stage position.
Stop the preview and capture the image (or movie).
Save the image.

For measurements

Verify that the spatial calibration matches the objective.
Measure the distance or feature.
To burn the notations into the image, select the camera icon and save the new file.
To burn a scale bar into the image, use the caliper button and the 'Marker' button. Save the new file.

Shut down Steps
Always turn off the camera.
Always turn off the lamps.
Swing the 2.5X objective in place.
Transfer your images.

WHERE'S THAT KNOB?



WHAT DOES THAT KNOB DO?

(1) White (halogen) light power switch – turns on both transmitted and reflected light.
(2) Eyepiece/Camera light path selector – sends light to eyepieces, camera or both.
(3) Brightness adjustment – controls intensity of transmitted and reflected light.
(4) Light intensity and toggle switches –The toggle selects the lower (transmitted) or upper (reflected) light source. The 'PRESET' button gives maximum illumination.
(5) Eyepieces – can adjust the interpupillary distance and focus. The left eyepiece has a micrometer.
(6) Objectives
(7) Stage with steel plates for magnetic probe arms for fluidic devices. Remove the glass stage to use magnetic probes.
(8) Stage clip. *Ask Beth to put it on.*
(9) Stage controls *(actually on left side of stage).*
(10) Fine and Coarse focus. *Each unit is 1 micron in stage height adjustment.*
(11) Diopter ring – for focusing the ocular
(12) Rotating nosepiece – for selecting objectives
(13) Field iris diaphragm for transmitted light – for establishing Koehler illumination
(14) Condenser height adjustment - for establishing Koehler illumination
(15) Condenser centering screws - for establishing Koehler illumination
(16) Condenser aperture iris diaphragm - for establishing Koehler illumination
(17) Filter wheel holding mirrors and filters for reflected light
(18) Mercury lamp power switch – to turn the fluorescence light on and off.
(19) Mercury lamp power indicator – red when the mercury bulb is ON.
(20) Shutter knob –shutter for reflected light (white or fluorescent) to protect sample from excess UV light.
(21) Field iris diaphragm – restricts the diameter of the beam of light entering the objective (excludes extraneous light and improves image contrast). Also slows fading in other parts of the sample.
(22) Aperture iris diaphragm – adjusts the brightness of the observed image and improves contrast. Use to fine-tune after using the ND filters.
(23) Not on this model
(24 top) - condenser screw. Not on this model
(24 bottom) – reflected light analyzer. Not on this model.
(25) Polarizer
(26) DIC prism slide filter – adjusts the contrast when using DIC
(30) Light path selector – selects halogen lamp and or mercury lamp for reflected light.
(31) Buttons for built-in filters

ND6 decreases excitation light intensity to 6% (fluorescence applications)
ND25 decreases excitation light intensity to 25% (fluorescence applications)
LBD color conversion filter to simulate daylight (BF, image capture)
OP dunno

(32) Slide filters for reflected light

U-25FR, frost slide filter - to eliminate uneven illumination
U-25ND25, ND slide filter - to reduce the excitation light intensity.

(33) Rear halogen lamp housing – source of the reflected white light.

BRIGHTFIELD Transmitted light

White light transmits through a transparent sample from underneath.

1. Turn on the white (halogen) light switch (**1**) for transmitted light to "|" (ON).
2. Engage the bright field (BF) mirror unit (**17**).
3. Adjust the light path. The light path selector knob (**2**) should be pushed in all the way.
4. Select transmitted light (lower position) on the switch by the brightness indicator (**4**).
5. Adjust the light intensity using the brightness adjustment knob (**3**). The numerals by the lamp voltage indicator (**4**) indicate the voltage. Deselect the "PRESET" button (not illuminated green) to avoid maximum illumination.
6. Adjust the interpupillary distance. While looking through the eyepieces, adjust the eyepieces (**5**) until the left and right fields of view coincide.
7. Swing the 2.5x objective (**6**) in place. Mount the specimen on the stage **(7).**
8. Find the specimen using the stage controls (**9**).
9. Focus on the specimen using fine/course focusing knobs (**10**).
10. Adjust the diopter:
    - Close your left eye and focus on the specimen using the fine focus knob.
    - Close your right eye and focus on the specimen using the diopter ring (**11**) on the left ocular.
    - Open both eyes to see if the focus is correct.
11. If needed, switch to the next objective by rotating the nosepiece (**12**) and focus. Continue to the desired magnification.
12. Establish Koehler illumination:
    - Close the field iris diaphragm (**13**) until you can see the edges.
    - Focus the image of the field iris diaphragm by raising or lowering the condenser using the condenser height adjustment knob (**14**).
    - Check whether the light is centered in the field of view. If not, use the condenser centering screws (**15**) to move the field iris diaphragm image to the center of the field of view.
    - Open the field iris diaphragm until its image circumscribes the field of view.
    - Match the opening of the condenser aperture iris diaphragm (**16**) with the N.A. of the objective in use to achieve the optimum objective performance.
13. Examine specimen and take a picture, if needed *(See page 9 for details).*
14. When finished:
    - Lower the stage by turning the focus knob **(10)** and remove the specimen.
    - Turn the nosepiece (**12**) until the 2.5x objective is into place.
    - Lower the light intensity to zero using the brightness adjustment knob (**3**)
    - Turn the halogen light switch (**1**) to "O" (OFF).
    - Return any slide filters **(26) (31) (32)** to their disengaged positions.

BRIGHT FIELD Reflected light

White light is shone onto a reflective sample from above.

1. Turn the halogen bulb switch (**1**) for reflected light to "|" (ON). Engage the bright field (BF) filter (**17**).

2. Adjust the light path.
   - The light path selector knob (**2**) should be pushed in all the way.
   - The knob on the reflected light splitter (**30**) should be pushed in all the way.
   - The shutter knob **(20)** should be slid to the position marked "○" (OPEN).

3. Select reflected light (upper position) on the switch by the brightness indicator (**4**).
4. Adjust the light intensity using the brightness adjustment knob (**3**). The light intensity scale (**4**) indicates the lamp voltage. Deselect the "PRESET" button (not illuminated green) to avoid maximum illumination.
5. Adjust the interpupillary distance. While looking through the eyepieces, adjust the eyepieces (**5**) until the left and right fields of view coincide.
6. Swing the 2.5x objective (**6**) in place.
7. Place the specimen on the stage (**7**).
8. Find the specimen using the stage controls (**9**).
9. Focus on the specimen using fine/course focusing knobs (**10**).
10. Adjust the diopter:
    - Close your left eye and focus on the specimen using the fine focus knob.
    - Close your right eye and focus on the specimen using the diopter ring (**11**) on the left eyepiece.
    - Open both eyes and confirm that it is in focus.
11. Switch to the next objective by rotating the nosepiece (**12**) and focus. Continue until you reach the desired magnification.
12. Establish Koehler illumination:
    - Close the field iris diaphragm (**13**) until you can see the edges.
    - Focus the image of the field iris diaphragm by raising or lowering the condenser using the condenser height adjustment knob (**14**).
    - Check whether the light is centered in the field of view. If not, use the condenser centering screws (**15**) to move the field iris diaphragm image to the center of the field of view.
    - Open the field iris diaphragm until its image circumscribes the field of view.
    - Match the opening of the condenser aperture iris diaphragm (**16**) with the N.A. of the objective in use to achieve the optimum objective performance.
13. Examine specimen and take a picture, if needed. *(See page 9 for details).*
14. When finished:
    - Lower the stage by turning the focus knob **(10)** and remove the specimen.
    - Turn the nosepiece (**12**) until the 2.5x objective is into place.
    - Lower the light intensity to zero using the brightness adjustment knob (**3**)
    - Turn the halogen light switch (**1**) to "O" (OFF).
    - Return any slide filters **(26) (31) (32)** to their disengaged positions.

FLUORESCENCE (Transmitted light)

NOTE: The mercury bulb is fragile. It needs to be cool for 30 minutes before being turned on again. It needs to stay on for at least 15 minutes.

1. Switch on the mercury lamp by setting the switch **(18)** to "I" (ON). The indicator **(19)** should light up.
   The bulb will stabilize in 5 -10 minutes.

2. Set the microscope for bright field imaging ('*Bright field reflected light*' page 2, steps 2 – 12.)
   Use the reflected light splitter knob **(30)** to switch between white light and UV light lamps.

3. Turn the filter wheel **(17)** to select a filter cube:
   position # 1. HQ:RDil (red)
   2. DAPI (340-380 Ex; 400 dichroic; 435-485 Em)
   3. DIC
   4. Bright field (BF)
   5. Dark field
   6. Qdot hxCM (red/blue)

4. When using the filter cubes, turn the light intensity down all the way **(3)**.

5. To avoid photobleaching, close the shutter **(20)** "●" (CLOSED) when not acquiring an image.

6. To improve image contrast and to prevent photobleaching other parts of the specimen, pull out the field iris diaphragm knob **(21)** so that the image of the field iris diaphragm just circumscribes the field of view.

7. To adjust the brightness and to improve contrast, pull out the aperture iris diaphragm knob **(22)**, the diaphragm will be smaller.

8. Examine specimen and take a picture, if needed. *(See page 9 for details).*

9. When finished:
   - Slide the shutter knob **(20)** to "●" (CLOSED).
   - Push the knob on the reflected light splitter (**30**) in all the way.
   - If mercury bulb has burned for at least 15 minutes, turn it off by setting the main switch **(18)** to "O".
   - Lower the stage by turning the focus knob **(10)** and remove the specimen.
   - Turn the nosepiece (**12**) back until the 10x objective is into place.
   - Push the field iris diaphragm knob **(21)** and the aperture iris diaphragm knob **(22)** all the way in.
   - Turn the filter wheel **(17)** to BF (bright field).
   - Return any slide filters **(26) (31) (32)** to their disengaged positions.

Image acquisition Cooke SensiCAM B/W camera; driver v3.6 & ImagePro Plus software; v6.2

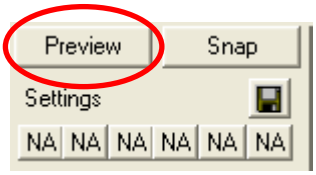
1. Turn on the blue SensiCAM camera (switch at the top of the camera). Wait until the blinking light turns green.

2. Slide the light path selector knob **(2)** to share the image with the camera (eye and camera position).

3. Open ImagePro Plus and adjust settings.

-Log on the computer as CNF USER (no password).
-Double-click on the "**USE this ImagePro Plus**" desktop icon.
-Select Acquire: Video/Digital.
If a window to select an "Analog Simulation Image File" appears, click "Cancel"
-A window titled 'Analog Simulation' appears. Use the Current Driver pull down menu to select the "SensiCAM Cooke Driver (3.6)" BE SURE THE DRIVER REMAINS SELECTED.
-Set the Capture Depth to 8-bit mono in the pull down menu.
-Set the preview (pvw) and acquisition (acq) areas (Full is 1376x1040).

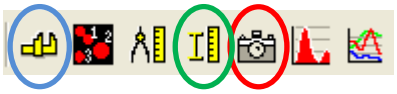
4. Open a live image **Preview button**.



-Adjust focus and lighting **(3)** on the microscope.
-Adjust camera exposure.
-Set how the image file is to be saved using the Image Tab.
-For multiple images/movies, check the 'Enable Multiple Images Capture' box, and set controls.

5. Capture the image.
-Stop the live Preview using the 'Stop' button.
-Click the 'Snap' button, and an image file will appear.
-Save the image. If it was taken in 12-bit mono, the computer can't open it. You could convert it using File>Batch Conversion and select the source file and the destination folder and file type (TIFF is best).

6. Take measurements in the saved image.



-Select the objective (spatial calibration). Click the caliper button on the menu bar, and select the objective that is in use.
-Click the measure Distance button on the menu bar to open a 'Measure Distance' pop up window.
-Click and drag to measure the feature (in microns).
-To save the annotation, click the camera button on the menu bar. A new file is created. Save it as a TIFF.

7. To insert a scale bar, click the caliper button on the menu bar, and double-check that the correct objective is selected.
-Select the 'Marker' button in the pop-up window.
-A 'Spatial calibration Marker' pop-up window appears.
- Choose color, marker length, and non-destructive mode.
- Change the positions by double-clicking on the bar or the label BEFORE closing the message window.
-To save the scale bar, click the camera button on the menu bar. A new file is created. Save it.

8. Take the images on a flash drive or transfer them to the **Lab_xfer icon**.
Files are automatically deleted in 7 days.

9. Close the ImagePro Plus software.

10. Leave the computer on.

Troubleshooting

1. White (bright field) light isn't making it to the sample.

CAUSE & REMEDY

Lamp not on.

Turn on the lamp **(1)** and look to see if both bulbs come on. One lamp is under the stage **(13)** and the other is in the rear lamp housing **(33).**

Turn on the mercury lamp **(18)** (if it has been off for at least 30 minutes). Wait 5-10 minutes for the bulb to stabilize.

Wrong light source or incorrect light path.

Select the ocular path **(2)** to allow the sample to be seen in the oculars.

Select reflected (top-side) or transmitted (underneath) white light with switch **(4).**

Select reflected white light vs. fluorescent light with slide knob **(30).**

Open the shutter **(20)** for reflected light.

Intensity is low.

Turn **(3)** up the intensity of light source.

Disengage the ND filters **(32).**

Open the field iris diaphragm **(13).**

Open the aperture iris diaphragm **(22)** and field iris diaphragm **(21).**

Select BF, DF or DIC mirrors **(17)** when using white light.

2. PROBLEM: White light (bright field) is too intense.

CAUSE & REMEDY

"Preset' **(4)** is pushed in which automatically turns on the maximum light voltage.

Deselect the button (green light will go off).

Intensity is turned up too high.

Dial **(3)** it down.

3. PROBLEM: Fluorescent light is not hitting sample correctly.

CAUSE & REMEDY

Lamp not on.

Turn on the mercury lamp **(18)** (if it has been off for at least 30 minutes).

Wait 5- 10 minutes for the bulb to stabilize.

Wrong light source or incorrect light path.

Select the eyepiece path **(2)** to allow the sample to be seen in the oculars.

Select reflected light (top-side) switch **(4).**

Select fluorescent light with slide knob **(30).**

Open the shutter **(20)** for reflected light.

4. PROBLEM: Can't capture a digital image.

CAUSE & REMEDY

Camera is not turned on or stabilized yet

Turn on the camera **(34)** at the black power switch and wait 2 minutes.

Incorrect light path for the camera to access the sample.

Select the eyepiece path setting **(2)** to allow the sample to be seen by the camera.

Wrong driver is selected for software image capture.

Select the Cooke SensiCAM Driver. Select Acquire: Video/Digital from the menu.

Click the 'Setup' tab in the window, and select the correct driver from the pull-down menu.