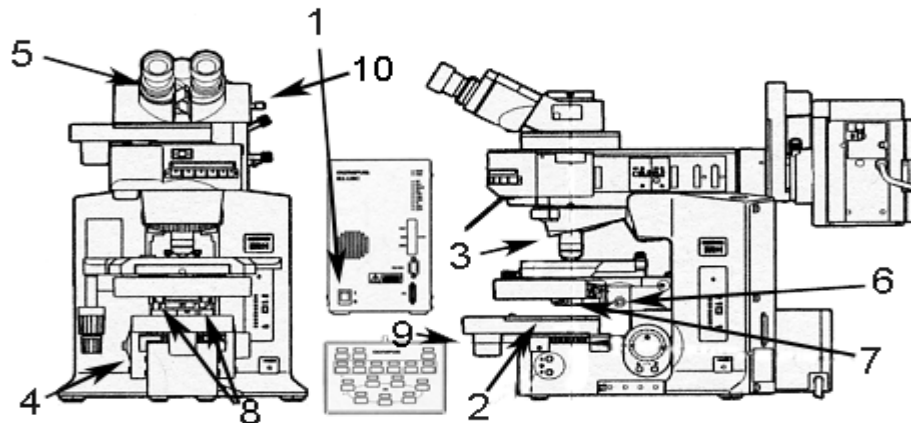


## **BX-61: Brightfield Instruction /Continue to scroll for Fluorescent Instructions**



**Schematic of Olympus BX-61.**

### **Starting up:**

1. Turn on Olympus microscope power box (left of microscope) with toggle switch on the left side of the base (1).
2. Turn on Prior Proscan II stage control.
3. Turn on the computer and log in with your HawkID.
4. Open cellSens Dimension software. You should be at the start page.
5. Choose the [Acquisition] tab. From here, click on the [Microscope Control] tab.
6. Click `[Escape]`, this will lower the stage. Place a slide on the stage, go back to the working position by clicking `[Return]`.
7. Choose the brightfield mode (BF).
8. Move the slider on the right side of the binocs (10) to give adequate light to the eyepiece.
9. Select the 4x lens from the software. Make sure that the condenser lens is out of the light path (7). Find your sample in the eyepiece and focus.

### **Adjusting binoculars for strain-free illumination:**

10. Choose the 10x lens from the upper tool bar. Place the condenser lens in the light path (7). Find your sample and focus.
11. Close your left eye. Does the sample still appear focused? If not, focus the sample with the focus knob.
10. Close your right eye. Focus the sample for this eye using the adjustment ring on the left eyepiece (5).
11. Adjust the interpupillary distance of eyepieces, until you see **one, in-focus** image.

### **Aligning for Koehler Illumination:**

12. Close the field diaphragm by turning the ring counter clockwise (2).

13. Focus the aperture edges by raising or lowering the condenser lens. You will need to use the Allen wrench in the screw hole to the right side of the condenser assembly (6).
14. Open the field diaphragm and center aperture with the Allen wrench inserted into the small centering screw holes (8) at either side the lower part of the condenser assembly.
15. Continue to open the field diaphragm so that it is just outside the field of view and the entire edge disappears all at once. Do not open any further.
16. Set the iris to correspond to the numerical aperture (NA) of the lens. This can be adjusted with the sliding lever at the front of the stage (9). Use the top setting if the condenser lens is not in the beam path. Use the bottom scale if the condenser lens is used.

### Using the cellSens software:



17. Move the slider on the right side of the binocs (10) to give adequate light to the camera.
18. Generate an image by selecting **LIVE** in the [Camera Control] tab.
19. Set the background:
 

In the **brightfield mode** if the image color on the screen does not match the color visible through the eyepiece, select white balance by clicking on the eyedropper with the white background and drag the cursor to the image. The cursor will appear as an eyedropper. Move the eyedropper to an area of the slide where there is no tissue. Hold down the left mouse button and expand to the reference area. Release mouse button.
20. Freeze the image by clicking the camera icon labeled **SNAP**.
21. Select the [Image] menu from the top toolbar. Choose [Burn In Info] and answer [yes]. This embeds the scale bar into the image.
22. Save the image to a thumb drive or your healthcare account.

\*\*\*\*\*IMAGES LEFT ON THE HARD DRIVE WILL BE DELETED WITHOUT NOTICE\*\*\*\*\*


### Collecting Tiled Images:

#### Automated Tiling:

23. Mark the corners of the area you wish to scan with a Sharpie.
24. Make sure the shading correction is selected. The shading is represented by the icon  from the top tool bar.
25. Choose the [Processing] tab.
26. Click on [Automatic Process Coordinates] icon.
27. Make sure the [Overwrite acquired positions] and the [Ignore Z axis settings] are selected.
28. Choose the middle icon  with the 1-2 square. You should have a prompt to move to the top left-hand corner of the area you wish to collect. Move the joystick to the upper left-hand Sharpie mark. Click on the prompt. You will receive a second prompt to move to the lower right-hand corner of your collection area. Find the corresponding mark and click.
29. Click on the [start>] button.
30. Select the [Image] menu from the top toolbar. Choose [Burn In Info] and answer [yes]. This embeds the scale bar into the image.
31. Save the image to a thumb drive or your healthcare account.

#### Manual Tiling:

32. Mark the corners of the area you wish to scan with a Sharpie.

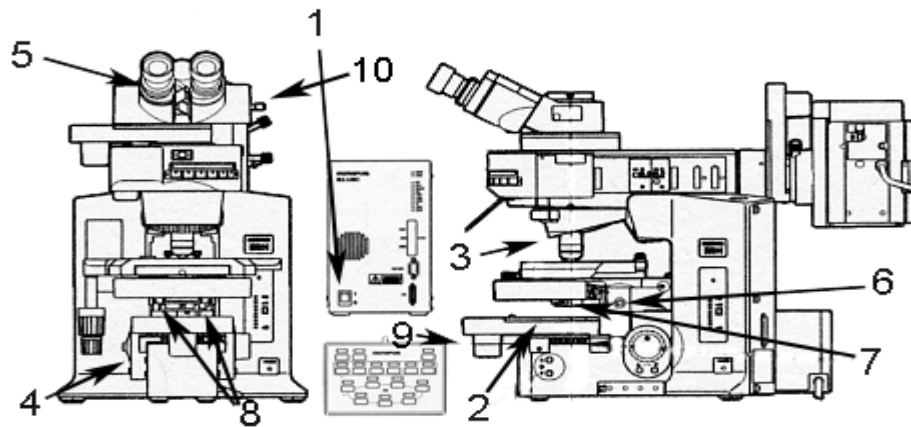
33. Make sure the shading correction is selected. The shading is represented by the icon  from the top tool bar.
34. Choose the [Processing] tab.
35. Click on [Manual MIA].
36. Press start.
37. Use the arrows to move the stage collecting the area of interest. Click **STOP** when you have the image you want.
38. Save the image to a thumb drive or your healthcare account.

### **Cleaning lenses:**

Oil lenses should always be cleaned after usage and occasionally even dry lenses accidentally come in contact with an oil-saturated coverslip. The lenses in question are very expensive and easily damaged. It is essential that proper procedure is observed when cleaning these lenses.

1. Use ethanol, Sparkle, or a commercial lens cleaner.
2. Use only lens paper or cotton-tipped applicators (DO NOT USE: Kim-wipes, paper towels or steel-wool)
3. Saturate a clean applicator with solution.
4. Touch the wet applicator to the center of the lens and wipe, spiraling outwards.
5. Repeat with a clean, dry applicator.

## **BX-61: Fluorescent Imaging**



**Schematic of Olympus BX-61.**

### **Starting up:**

1. Turn on Olympus microscope power box (BX-UCB, left of microscope) with toggle switch on the left side of the base (1).
2. Turn on Prior Proscan II stage control.
3. Turn on the computer and log in with your HawkID.
4. Turn on the halogen lamp (Prior Lumen200). The Halogen lamp should be on for at least 5 minutes before turning it off.
5. Open cellSens Dimension software. You should be at the start page.
6. Click on the [Microscope Control] tab.
7. Click  , this will lower the stage. Place a slide on the stage, go back to the working position by clicking  .
8. Choose the Brightfield mode (BF).
9. Move the slider on the right side of the binocs (10) to give adequate light to the eyepiece.
10. Select the 4x lens from the software. Make sure that the condenser lens is out of the light path (7). Find your sample in the eyepiece and focus.

### **Adjusting binoculars for strain-free illumination:**

11. Choose the 10x lens from the upper tool bar. Place the condenser lens in the light path (7). Find your sample and focus.
12. Close your left eye. Does the sample still appear focused? If not, focus the sample with the focus knob.
13. Close your right eye. Focus the sample for this eye using the adjustment ring on the left eye piece (5).
14. Adjust the interpupillary distance of eyepieces, until you see **one, in-focus** image.

### Using the cellSens software:

15. Change magnification to 20x.
16. Select the filter that corresponds to your fluorophore.  
DAPI = UV range  
GFP = ~488nm  
TXRED = ~568nm  
CY5 = Far Red range
17. Move the slider on the right side of the binocs (10) to give adequate light to the camera.
18. Generate an image by selecting **LIVE**
19. Click on the [SFL] box.
20. If the background color on the screen does is not sufficiently black, select black balance by clicking on the eyedropper with the black background. Drag the cursor to the image. The cursor will appear as an eyedropper. Move the eyedropper to an area of the slide where there is no signal. Click at this spot.
21. Select [Manual] exposure. Adjust image with the slider until the desired brightness is achieved.
22. Freeze the image by clicking the camera icon labeled **SNAP**
23. Select the [Image] menu from the top toolbar. Choose [Burn In Info] and answer [yes]. This embeds the scale bar into the image.
24. Save the image to a thumb drive or your healthcare account.

### Cleaning lenses:

Oil lenses should always be cleaned after usage and occasionally even dry lenses accidentally come in contact with an oil-saturated coverslip. The lenses in question are very expensive and easily damaged. It is essential that proper procedure is observed when cleaning these lenses.

1. Use ethanol, Sparkle, or a commercial lens cleaner.
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3. Saturate a clean applicator with solution.
4. Touch the wet applicator to the center of the lens and wipe, spiraling outwards.
5. Repeat with a clean, dry applicator.