

Operating Manual for the Olympus BX51

Brightfield.....	1
Fluorescence.....	3
Phase Contrast.....	5
Nomarski Differential Interference Contrast (DIC).....	7
Simultaneous Fluorescence and Phase Contrast.....	9
Simultaneous Fluorescence and DIC.....	11
Polarized Light.....	13

APPENDIX 1:

Outline Olympus BX51 Microscope.....	15
--------------------------------------	----

Operating Manual for the Olympus BX51

BRIGHTFIELD

1. Remove the microscope cover.
2. Turn on the halogen light switch (1) for transmitted light to "I" (ON).
3. Check the light path. The light path selector knob (2) should be pushed in all the way.
4. Adjust the light intensity using the brightness adjustment knob (3). The numerals to the right of the lamp voltage indicator LEDs (4) indicate the voltage.
5. Adjust the interpupillar distance. While looking through the eyepieces, adjust oculars (5) until the left and right fields of view coincide completely.
6. Make sure the 10x objective (6) is in place.
7. Place the slide with your specimen on the stage (7) and hold it with the specimen holder (8).
8. Find your specimen using the stage controls (9).
9. Focus specimen using fine/course focussing 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 and confirm that the focus is comfortable.
11. If desired switch to the next objective by rotating the nosepiece (12) and focus. Continue until you reach the desired magnification.
12. Establish Koehler illumination:
 - Close 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 if the circle of light is centered in the field of view. If not, use the two 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 in order to achieve the optimum objective performance.

NOTE: Most specimen are usually low in contrast, reducing the diaphragm opening to 70%-80% of the N.A. value of the respective objective will generally provide an image of acceptable quality.

To check the opening;

- a) remove one ocular and look down the tube,
- b) adjust the condenser iris so that 80% of the field is light,
- c) replace the ocular,

or

- a) set condenser aperture iris scale to about 80% of the N.A. value of objective,
- b) example: N.A. of objective is 0.75, set the scale to $0.75 \times 0.8 = 0.6$.

13. Examine specimen and document image if necessary:

- If using 35mm film, use checklist for '*Olympus 35mm Camera*'.
- To obtain still digitized images, use checklist for '*Polaroid Digital Microscope Camera (DMC)*'.
- To obtain still digitized images and digitized movies, use checklist for '*Sony, color Video camera*'.

14. When finished:

- Lower the stage by turning the focus knob (**10**) towards you and remove the slide with your specimen from the stage (**7**).
- Turn the nosepiece (**12**) back until the 10x objective is into place.
- If the 100x objective was used, clean the lens carefully with **lens paper ONLY (do not use Kimwipes)**.
- Turn the light intensity down using the brightness adjustment knob (**3**).
- Turn off the halogen light switch (**1**) to "0" (OFF).
- Cover the microscope.
- Leave the room CLEAN and TIDY!

FLUORESCENCE

1. Remove the microscope cover.
2. To turn on the mercury bulb, set the main switch **(18)** on the Power Supply Unit to “I”(ON). The light indicator **(19)** on the Power Supply Unit should go ON. Wait 5 to 10 minutes for the mercury bulb to stabilize. The mercury bulb should stay on for at least 15 minutes before turning off.
3. Setup microscope for brightfield imaging (see instructions on ‘*Brightfield*’ page 1, # 2-12).
4. Engage the fluorescence mirror unit **(17)** until the proper filter cube is in place:
 - WU – #1, dichroic mirror DM400, excitation filter BP330-385, barrier filter BA420
 - WB – #2, dichroic mirror DM500, excitation filter BP450-480, barrier filter BA515
 - WG – #3, dichroic mirror DM570, excitation filter BP510-550, barrier filter BA590
5. Turn the light intensity down all the way using the brightness adjustment knob **(3)**.
6. To allow light from the mercury bulb to reach the specimen, slide the shutter knob **(20)** to position marked ○.
7. To improve image contrast and to prevent color fading of fluorescent light in other part than observed region, pull out the field iris diaphragm knob **(21)** so that the image of the field iris diaphragm just circumscribes the field of view.
8. To help adjusting the brightness of you the observed image and to improve the contrast, pull out the aperture iris diaphragm knob **(22)**, the aperture iris diaphragm will get smaller.
9. Examine specimen and document image if necessary:
 - If using 35mm film, use checklist for ‘*Olympus 35mm Camera*’.
 - To obtain still digitized images, use checklist for ‘*Polaroid Digital Microscope Camera (DMC)*’.
 - To obtain still digitized images and digitized movies, use checklist for ‘*Sony, color Video camera*’.
10. To avoid bleaching, slide the shutter knob **(20)** to the position marked ● whenever you are not observing the specimen.
11. When finished:
 - Slide the shutter knob **(20)** to the position marked ●.
 - If mercury bulb has burned for at least 15 minutes, turn off the mercury bulb by setting the main switch **(18)** on the Power Supply Unit to “O”(OFF). The light indicator **(19)** on the Power Supply Unit should go out.
NOTE: The mercury bulb needs to cool down for at least 15 minutes before turning back on.
 - Lower the stage by turning the focus knob **(10)** towards you and remove the slide with your specimen from the stage **(7)**.
 - Turn the nosepiece **(12)** back until the 10x objective is into place.
 - If the 100x objective was used, clean the lens carefully with **lens paper ONLY (do not use Kimwhipes)**.
 - Push the field iris diaphragm knob **(21)** and the aperture iris diaphragm knob **(22)** all the way back into the microscope.
 - Engage the fluorescence mirror unit **(17)** back to BF (brightfield) - #6
 - Cover the microscope when the mercury bulb is cooled down (15 minutes after it was turned off).
 - Leave the room CLEAN and TIDY!

▼ **Examples of fluorochromes to be used with selected filter cube.**

<u>Filter cube</u>	<u>Fluorochromes</u>
WU - #1	Autofluorescence observation DAPI: DNA staining Hoechst 33258, 33342: Chromosome
WB - #2	FITC: Fluorescent antibody Acridine orange: DNA, RNA Auramine: Tubercle bacillus EGFP, S65T, RSGFP
WG - #3	Rhodamine, TRITC: Fluorescent antibody Propidium iodide: DNA RFP

PHASE CONTRAST

1. Remove microscope cover.
2. Turn on the halogen light switch (1) for transmitted light to “I” (ON).
3. Check the light path. The light path selector knob (2) should be pushed in all the way.
4. Adjust the light intensity using the brightness adjustment knob (3). The numerals to the right of the lamp voltage indicator LEDs (4) indicate the voltage.
5. Adjust the interpupillar distance. While looking through the eyepieces, adjust oculars (5) until the left and right fields of view coincide completely.
6. Make sure the 10x phase objective (6) is in place (phase objective has a ‘Ph’ engraved on it).
7. Place the slide with your specimen on the stage (7) and hold it with the specimen holder (8).
8. Find your specimen using the stage controls (9).
9. Focus specimen using fine/course focussing 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 and confirm that the focus is comfortable.
11. If desired switch to the next phase objective by rotating the nosepiece (12) and focus.
12. Focus the light on the specimen:
 - Close 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 if the circle of light is centered in the field of view. If not, use the two 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.
22. Open the condenser aperture iris diaphragm (16) all the way.
23. Rotate the condenser turret (23) to engage the phase contrast ring until the visible marking matches the ‘Ph’ mark on the respective objective.
24. While looking through the oculars, increase the intensity (3) until you can see clearly.
25. Remove one of the oculars and look down the tube. A bright and a dark ring can be seen. If the rings do not exactly overlap (most likely they will), use the optical element centering screws (24) to center the phase contrast ring so that the bright ring overlaps the dark ring within the field of view.
26. Replace the ocular.
27. Examine specimen and document image if necessary:
 - If using 35mm film, use checklist for ‘*Olympus 35mm Camera*’.
 - To obtain still digitized images, use checklist for ‘*Polaroid Digital Microscope Camera (DMC)*’.
 - To obtain still digitized images and digitized movies, use checklist for ‘*Sony, color Video camera*’.

28. When finished:

- Rotate the condenser turret (**23**) until the BF (brightfield) mark is visible.
- Lower the stage by turning the focus knob (**10**) towards you and remove the slide with your specimen from the stage (**7**).
- Turn the nosepiece (**12**) back until the 10x objective for brightfield imaging is into place.
- Turn the light intensity down using the brightness adjustment knob (**3**).
- Turn off the halogen light switch (**1**) to "0" (OFF).
- Cover the microscope.
- Leave the room CLEAN and TIDY!

NOMARSKI DIFFERENTIAL INTERFERENCE CONTRAST (DIC)

1. Remove the microscope cover.
2. Turn on the halogen light switch **(1)** for transmitted light to “I” (ON).
3. Check the light path. The light path selector knob **(2)** should be pushed in all the way.
4. Adjust the light intensity using the brightness adjustment knob **(3)**. The numerals to the right of the lamp voltage indicator LEDs **(4)** indicate the voltage.
5. Adjust the interpupillar distance. While looking through the eyepieces, adjust oculars **(5)** until the left and right fields of view coincide completely.
6. Make sure the 10x objective **(6)** is in place.
7. Place the slide with your specimen on the stage **(7)** and hold it with the specimen holder **(8)**.
8. Find your specimen using the stage controls **(9)**.
9. Focus specimen using fine/course focussing 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 and confirm that the focus is comfortable.
11. If desired switch to the next objective by rotating the nosepiece **(12)** and focus. Continue until you reach the desired magnification.
12. Focus the light on the specimen:
 - Close 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 if the circle of light is centered in the field of view. If not, use the two 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.
13. Check if the condenser turret **(23)** is set to BF (brightfield) observation (with no optical elements engaged).
14. Engage the reflected light analyser (U-AN) **(24)**, positioned in the slot above the fluorescence mirror unit **(17)**, into the light path.
15. Push the polarizer **(25)** into the light path.
16. Loosen the smaller knob on the polarizer and rotate the polarizer rotation knob (big knob on polarizer) to the position where the field of view is perfectly dark, then tighten the polarizer clamping knob (smaller knob on polarizer) lightly.
17. Engage the transmitted light DIC prism slider **(26)**, positioned on the nosepiece **(12)**, into the light path and tighten the clamping knob lightly.
18. Rotate the condenser turret **(23)** and engage the DIC prism that matches the objective in use.
19. Stopping down the aperture iris diaphragm somewhat may increase the contrast.
20. Rotate the prism control knob on the DIC slider **(26)** to adjust the contrast of the background color as discussed on the next page.

21. Examine specimen and document image if necessary:

- If using 35mm film, use checklist for '*Olympus 35mm Camera*'.
- To obtain still digitized images, use checklist for '*Polaroid Digital Microscope Camera (DMC)*'.
- To obtain still digitized images and digitized movies, use checklist for '*Sony, color Video camera*'.

22. When finished:

- Lower the stage by turning the focus knob (**10**) towards you and remove the slide with your specimen from the stage (**7**).
- Turn the nosepiece (**12**) back until the 10x objective is into place.
- If the 100x objective was used, clean the lens carefully with **lens paper ONLY (do not use Kimwipes)**.
- Rotate the condenser turret (**23**) until the BF (brightfield) mark is visible.
- Loosen the clamping knob from the DIC prism slider and disengage the transmitted light DIC prism slider (**26**) from the light path.
- Pull the polarizer (**25**) out of the light path.
- Pull the reflected light analyser (U-AN) (**24**) out of the light path.
- Turn the light intensity down using the brightness adjustment knob (**3**).
- Turn off the halogen light switch (**1**) to "0" (OFF).
- Cover the microscope.
- Leave the room CLEAN and TIDY!

The prism control knob of the DIC slider.

Rotate the prism control knob of the DIC slider to obtain the background interference color that can achieve maximum contrast according to the specimen under observation.

- If the background color is black, darkfield-like observation can be performed.
- If the background color is gray, a 3D-like image with maximum contrast with gray sensitive gray can be obtained.
- If the background color is sensitive magenta, even a minor optical retardation is observed as a color change.

NOTE: Care should be taken to keep the specimen surface clean, as even a small amount of contamination on the surface may show up due to the sensitivity of the DIC method.

SIMULTANEOUS FLUORESCENCE AND PHASE CONTRAST

1. Remove the microscope cover.
2. To turn on the mercury bulb, set the main switch (**18**) on the Power Supply Unit to “I”(ON). The light indicator (**19**) on the Power Supply Unit should go ON. Wait 5 to 10 minutes for the mercury bulb to stabilize. The mercury bulb should stay on for at least 15 minutes before turning off.
3. Setup the microscope for Phase Contrast imaging (see instructions on ‘*Phase contrast*’ page 5, #2-26).
4. Engage the fluorescence mirror unit (**17**) until the proper filter cube is in place:
 - WU – #1, dichroic mirror DM400, excitation filter BP330-385, barrier filter BA420
 - WB – #2, dichroic mirror DM500, excitation filter BP450-480, barrier filter BA515
 - WG – #3, dichroic mirror DM570, excitation filter BP510-550, barrier filter BA590
5. To allow light from the mercury bulb to reach the specimen, slide the shutter knob (**20**) to position marked ○.
6. Adjust the transmitted light for the best balance of fluorescence and phase contrast brightness using the brightness adjustment knob (**3**).
7. To improve image contrast and to prevent color fading of fluorescent light in other part than observed region, pull out the field iris diaphragm knob (**21**) so that the image of the field iris diaphragm just circumscribes the field of view.
8. To help adjusting the brightness of you the observed image and to improve the contrast, pull out the aperture iris diaphragm knob (**22**), the aperture iris diaphragm will get smaller.
9. Examine specimen and document image if necessary:
 - If using 35mm film, use checklist for ‘*Olympus 35mm Camera*’.
 - To obtain still digitized images, use checklist for ‘*Polaroid Digital Microscope Camera (DMC)*’.
 - To obtain still digitized images and digitized movies, use checklist for ‘*Sony, color Video camera*’.
10. To avoid bleaching, slide the shutter knob (**20**) to the position marked ● whenever you are not observing the specimen.
11. When finished:
 - Slide the shutter knob (**20**) to the position marked ●.
 - If mercury bulb has burned for at least 15 minutes, turn off the mercury bulb by setting the main switch (**18**) on the Power Supply Unit to “O”(OFF). The light indicator (**19**) on the Power Supply Unit should go out.
NOTE: The mercury bulb needs to cool down for at least 15 minutes before turning back on.
 - Lower the stage by turning the focus knob (**10**) towards you and remove the slide with your specimen from the stage (**7**).
 - Turn the nosepiece (**12**) back until the 10x objective for brightfield imaging is into place.
 - Push the field iris diaphragm knob (**21**) and the aperture iris diaphragm knob (**22**) al the way back into the microscope.
 - Engage the fluorescence mirror unit (**17**) back to BF (brightfield) - #6
 - Rotate the condenser turret (**23**) until the BF (brightfield) mark is visible.
 - Turn the light intensity down using the brightness adjustment knob (**3**).

- Turn off the halogen light switch (**1**) to “0” (OFF).
- Cover the microscope when the mercury bulb is cooled down (15 minutes after it was turned off).
- Leave the room CLEAN and TIDY!

SIMULTANEOUS FLUORESCENCE AND DIFFERENTIAL INTERFERENCE CONTRAST

1. Remove the microscope cover.
2. To turn on the mercury bulb, set the main switch (18) on the Power Supply Unit to "I"(ON). The light indicator (19) on the Power Supply Unit should go ON. Wait 5 to 10 minutes for the mercury bulb to stabilize. The mercury bulb should stay on for at least 15 minutes before turning off.
3. Setup the microscope for Differential Interference Contrast (DIC) imaging (see instructions on 'DIC' page 7, #2-20).
4. Engage the fluorescence mirror unit (17) until the proper filter cube is in place:
 - WU – #1, dichroic mirror DM400, excitation filter BP330-385, barrier filter BA420
 - WB – #2, dichroic mirror DM500, excitation filter BP450-480, barrier filter BA515
 - WG – #3, dichroic mirror DM570, excitation filter BP510-550, barrier filter BA590
5. To allow light from the mercury bulb to reach the specimen, slide the shutter knob (20) to position marked ○.
6. Adjust the transmitted light for the best balance of fluorescence and DIC brightness using the brightness adjustment knob (3).
7. To improve image contrast and to prevent color fading of fluorescent light in other part than observed region, pull out the field iris diaphragm knob (21) so that the image of the field iris diaphragm just circumscribes the field of view.
8. To help adjusting the brightness of you the observed image and to improve the contrast, pull out the aperture iris diaphragm knob (22), the aperture iris diaphragm will get smaller.
9. Examine specimen and document image if necessary:
 - If using 35mm film, use checklist for '*Olympus 35mm Camera*'.
 - To obtain still digitized images, use checklist for '*Polaroid Digital Microscope Camera (DMC)*'.
 - To obtain still digitized images and digitized movies, use checklist for '*Sony, color Video camera*'.
10. To avoid bleaching, slide the shutter knob (20) to the position marked ● whenever you are not observing the specimen.
11. When finished:
 - Slide the shutter knob (20) to the position marked ●.
 - If mercury bulb has burned for at least 15 minutes, turn off the mercury bulb by setting the main switch (18) on the Power Supply Unit to "O"(OFF). The light indicator (19) on the Power Supply Unit should go out.
NOTE: The mercury bulb needs to cool down for at least 15 minutes before turning back on.
 - Lower the stage by turning the focus knob (10) towards you and remove the slide with your specimen from the stage (7).
 - Turn the nosepiece (12) back until the 10x objective is into place.
 - If the 100x objective was used, clean the lens carefully with **lens paper ONLY (do not use Kimwhipes)**.
 - Push the field iris diaphragm knob (21) and the aperture iris diaphragm knob (22) all the way back into the microscope.
 - Engage the fluorescence mirror unit (17) back to BF (brightfield) - #6

- Rotate the condenser turret (**23**) until the BF (brightfield) mark is visible.
- Loosen the clamping knob from the DIC prism slider and disengage the transmitted light DIC prism slider (**26**) from the light path.
- Pull the polarizer (**25**) out of the light path.
- Pull the reflected light analyser (U-AN) (**24**) out of the light path.
- Turn the light intensity down using the brightness adjustment knob (**3**).
- Turn off the halogen light switch (**1**) to "0" (OFF).
- Cover the microscope when the mercury bulb is cooled down (15 minutes after it was turned off).
- Leave the room CLEAN and TIDY!

POLARIZED LIGHT

1. Remove the microscope cover.
2. Turn on the halogen light switch (1) for transmitted light to “I” (ON).
3. Check the light path. The light path selector knob (2) should be pushed in all the way.
4. Adjust the light intensity using the brightness adjustment knob (3). The numerals to the right of the lamp voltage indicator LEDs (4) indicate the voltage.
5. Adjust the interpupillar distance. While looking through the eyepieces, adjust oculars (5) until the left and right fields of view coincide completely.
6. Make sure the 10x objective (6) is in place.
7. Place the slide with your specimen on the stage (7) and hold it with the specimen holder (8).
8. Find your specimen using the stage controls (9).
9. Focus specimen using fine/course focussing 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 and confirm that the focus is comfortable.
11. If desired switch to the next objective by rotating the nosepiece (12) and focus. Continue until you reach the desired magnification.
12. Focus the light on the specimen:
 - Close 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 if the circle of light is centered in the field of view. If not, use the two 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.
13. Check if the condenser turret (23) is set to BF (brightfield) observation (with no optical elements engaged).
14. Engage the reflected light analyser (U-AN) (24), positioned in the slot above the fluorescence mirror unit (17), into the light path.
15. Push the polarizer (25) into the light path.
16. Loosen the smaller knob on the polarizer and rotate the polarizer rotation knob (big knob on polarizer) to the position where the field of view is perfectly dark, then tighten the polarizer clamping knob (smaller knob on polarizer) lightly.
17. Examine specimen and document image if necessary:
 - If using 35mm film, use checklist for ‘*Olympus 35mm Camera*’.
 - To obtain still digitized images, use checklist for ‘*Polaroid Digital Microscope Camera (DMC)*’.
 - To obtain still digitized images and digitized movies, use checklist for ‘*Sony, color Video camera*’.

18. When finished:

- Lower the stage by turning the focus knob (**10**) towards you and remove the slide with your specimen from the stage (**7**).
- Turn the nosepiece (**12**) back until the 10x objective is into place.
- If the 100x objective was used, clean the lens carefully with **lens paper ONLY (do not use Kimwipes)**.
- Pull the polarizer (**25**) out of the light path.
- Pull the reflected light analyser (U-AN) (**24**) out of the light path.
- Turn the light intensity down using the brightness adjustment knob (**3**).
- Turn off the halogen light switch (**1**) to "0" (OFF).
- Cover the microscope.
- Leave the room CLEAN and TIDY!

APPENDIX 1:

Outline Olympus BX51 Microscope

