Updated 02.08.2016

RULES FOR BIOMICROSCOPES

When something goes wrong, or you want to send EMAIL TO the microscope users, send it to:

su-biomicroscope-users@lists.sabanciuniv.edu

We have two fluorescent microscopes, both have signup sheets, you must use these signup sheets. The BX60 microscope on the left takes slides. The IX70 microscope on the right takes tissue culture plates (you can also view slides on this).

1. You must SIGN IN BEFORE you use the machine (name, date, time you turned on the machine)

2. You must SIGN OUT AFTER you use the machine (time you turned off the machine)

3. The power supply for the fluorescent light bulbs have reset buttons. DO NOT RESET. The fluorescent light emitting mercury bulb has a life of approximately 200 hours. The timer on the power supply tells us how much we've used the light bulb. There are pipette tips stuck in the reset buttons, DO NOT TOUCH THESE PIPET TIPS (even if you need to use them to scratch your ear).

4. Fluorescent light bulb is sensitive; it will have to warm up for 5-10 min to give the best light. Once the light is on, it must stay on for AT LEAST 15 min before being turned off. If you are not going to use fluorescent light in your experiment, do not turn on the fluorescent light power. DO NOT turn off the power supply for 15 min after turning it on. When you turn OFF the power supply, you must turn it on again only after 10-15 min. There is mercury in the light bulb, which needs to warm up/cool down during/after an experiment. Prematurely turning on/off the light bulb increases the risk of it exploding and contaminating the whole room with mercury vapor, resulting in a slow and painful death for the occupants of the room. Prematurely turning on/off the light bulb also decreases the life of the lamp!

5. Do not remove the objectives. Each objective is calibrated to the housing in the objective holder.

6. Do not touch the knobs with pink stickers; these knobs will not change anything (that will affect your experiment-other than screwing it up).

7. The camera is locked into a position that makes it in focus at the same time as the eyepiece, DO NOT try to change the focus of the camera.

8. For the inverted microscope, visible light comes from the top and fluorescent light comes from the bottom. For phase contrast microscopy, use the lenses with GREEN letters (with green stickers). We have 3 phase contrast lenses:
10X (yellow band with green letters),
20X (green band with green letters),
40X (blue band with green letters),
These must be matched with the filters in front of the visible light source such that:
10X (PhC- setting 3),
20X (PhCU- setting 1),
40X (Ph2- setting 2),
For non-DIC light and fluorescent microscopy we have two lenses:
40X (must match DP40 filter) and 100X (must match DP100 filter)

Updated 02.08.2016

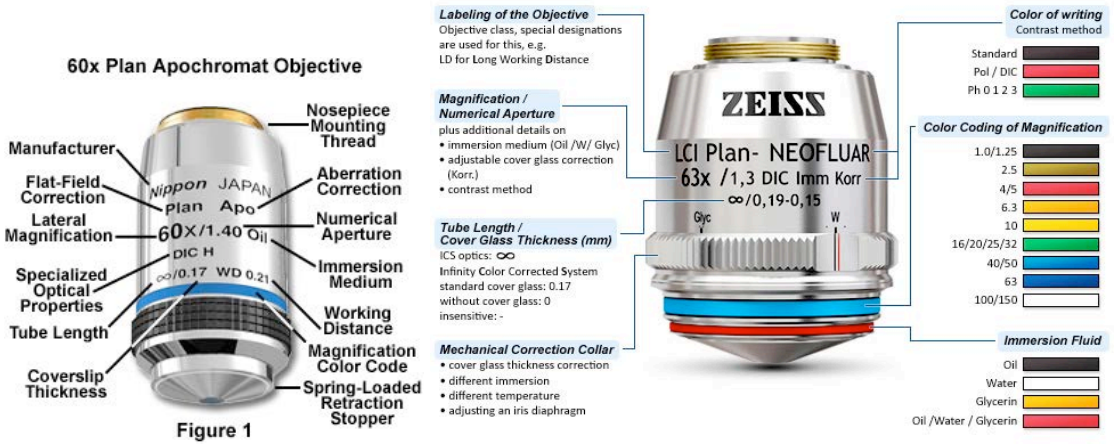


Figure 1

The objectives turn in the counter-clockwise direction. They go from low magnification (10X) to high mag (up to 100X). NEVER EVER EVER UNSCREW AN OBJECTIVE OUT OF ITS POSITION! they are NOT interchangable!

Objectives for the IX70 Inverted Microscope (color of lettering/color of band):
CPlanFL 10X/0.30 PhC (green/yellow) use for phase contrast (green sticker)
UPlanFL 10X/0.30 (white/yellow)
LCPlanFL 20X/0.40 Ph1 (green/green) use for phase contrast
LUCPLFLN 40X/0.60 (∞/0-2/FN22) (blue with yellow sticker) 2.7-4 mm long working distance (for tissue culture plates and 2mm thick slides)
LCPlanFL 40X/0.60 (white/blue)

Objectives for the BX60 Upright Microscope:
UPlanFL 10X/0.4 (white/yellow)
UPlanApo 20X/0.7 inf/0.17 (white/green)
UPlanApo 40X/0.85 (white/blue)
PlanApoN 60X/1.42 Oil inf/0.17/FN26.5 UIS2 BFP1 (white/blue)
UPlanApo 100X/1.35 Oil IRIS (white/white)

Extra Objectives: (these are in storage please let me know if you need to use it)
UPlanFL 40X/0.75 Ph2 (green/blue) use for phase contrast
PlanApo N 60X/1.42 Oil inf/0.17FN26.5 (white/blue) Identical to the one on the microscope.
UPlanFL 10X/0.30 Ph1 (green/yellow)

9. The BX60 microscope has a 60 X and 100X oil lens, you need to add oil on top of it so that it makes contact with the slide. When finished viewing a sample, you MUST clean the lens with ethanol (if there is no ethanol left in the room, stop your experiment, go out of the room, get ethanol from the lab and come back, while cursing yourself for not thinking of this before you started the experiment). You must wipe the oil lens clean of the oil EVERY TIME you view/change a sample, do not wait for the end of the experiment!

10. NEVER put oil onto the 10x, 20x and 40x lenses. Oil is ONLY for the 60X and 100x lens! You must clean the oil off the lens after you use it EVERY TIME with ethanol and distilled water!

11. If you cannot see an image from the eyepiece/camera even though your sample is in place, do not panic! There is a push-in dial on the right of the eyepiece, with 3 settings. This allows you to switch the image from the eyepiece to the camera, or to view both from the eyepiece and the camera.

12. When the 10X lens is in focus, you may move up to a higher magnification lens. You will find

that if the 10X lens is in focus, the 20X, 40X, 100X lenses will also be close to their focus. It is much easier to focus with 10X than 100X, starting from an unknown focus.

13. Focus on your samples using DIC light (visible), NOT fluorescent light. Once you are in focus, you may open the fluorescent light shutter. Always minimize the fluorescent light exposure to your samples to minimize fading. Close the fluorescent shutter immediately after viewing/photographing your samples.

14. Cameras! We have two cameras. You turn on the cameras by turning on the computers. The old spot camera has been removed. Please ask me if you would like to use this old camera. The IX70 inverted microscope has a black and white (B&W) new camera (XM-10IR) for fluorescent pictures. This camera can take pictures in the infrared range (IR). You can switch between the eyepiece and this camera with the black knob on the right of the microscope above the focus knob. Currently when you focus using the eyepiece, the camera will be slightly out of focus and vice versa. This will be fixed soon. The BX60 upright microscope has a DP72 camera attached to it. The camera has a black and white (B&W) and color setting. Keep this in (B&W), the camera measures the intensity of photons coming from the sample. It cannot differentiate between colors; the color you see on the monitor is artificially added by the software (pseudo-coloring). The software tells you how much light came into the camera from the sample and you tell it to color it red or green or whatever color you want. The human brain likes to see green for GFP and red for RFP, but there is no “information” about what wavelength light came into the camera in the color we see on the monitor.

15. There are various filter cubes that limit the wavelength of light coming from the fluorescent light source and hitting the sample (excitation filter), and light emanating from the sample and hitting the eyepiece/camera (emission filter). There is a third filter called a dichroic filter. All three filters are placed in a cube that sits in a round housing on the bottom of the stage in the IX70 inverted microscope (and on top of the stage in the upright BX60 microscope). Each housing can hold up to 4 cubes. We have cubes that allow us to excite/emit at UV wavelengths, at green wavelengths and at red wavelengths.

16. Dust is a microscope’s worst enemy. Always cover the microscopes with the plastic tarp after use. You need to wait 10-15 minutes until the light source cools down so it does not melt the plastic.

Updated 02.08.2016

FILTERS

BX60 has the following filters:

Setting	Filter	Use
WIBA	UMWIBA	(blue excitation) for EGFP, EYFP, FITC
NU	UMNU	(UV excitation) for DAPI, Hoechst 44258
WIG	U-MWIG3	(green excitation) for Cy3 7AAD PI TRITC
NIB	UMNIB3	(blue excitation) for EGFP, EYFP, FITC

IX70 has the following filters:

Setting	Filter	Use
O	Qmax-Blue	for DAPI
EMPTY	Qmax-Red	(green excitation) for visualizing J-Red
	Omega XF130-2	(blue excitation) for ECFP (these two filters will be used interchangably) ask me if you want to use this filter.
WIG	UMWIG3	(green exc.) for Cy3, 7AAD, PI, TRITC
WIBA	U-MWIBA3	(blue excitation) for EGFP, EYFP, FITC this has less bleed-through than UMNIB3!

Filter	Dichroic	Emitter	Exciter
UMNU	400	420	360-370
UMNIB3	505	510	470-495
UMWIBA3	505	510-550	460-495
UMWIG3	570	575	530-550
Qmax-Red	580LP	600-650	530-570
Qmax-Blue	410LP	420-480	355-405
Omega XF130-2	445LP	480LP	430-450

Quick summary of steps to take when using the microscope:

1.signup
2. turn on visible light power (switch towards the right/rear of the microscopes)
3. turn on fluorescent light power
4. turn on computer/camera power (DP camera turns on automatically when the software is opened on the computer (no power switch)
5. move the stage down
6. switch to 10X magnification lens (this ensures that a high magnification lens-which is longer-does not get scratched by the slide.
7. put your slide/TC dish onto the stage
8. make sure the light is going to the eyepiece and/or the camera, adjust the eye piece push-in dial.
9. turn on the visible light, press the button with the green light to temporarily turn off the light hitting your sample.
10. make sure the fluorescent light diaphram and the visibe light diaphram is open
11. make sure the correct fluorescent cube is in place. For exciting GFP, FITC etc, you need to see BLUE light coming out of the lens, for seeing RFP, Cy3 etc, you need to see red light.
12. Save images as TIFF files at 1360x1024 resolution.
13. Save your data on an external drive/ CD they will periodically be deleted from the hard disk.
14. Return the lens setting to the 10X lens, so the next person does not scratch the 20X, 40X or 100X lens when they put their slide/plate on the stage.
15. Turn off power supplies
16. Sign off
17. Leave and come back 10 minutes later to replace the dust cover after the power supply cools down.
18. LOCK THE DOOR, replace the key to its place.

Updated 02.08.2016

New Rules for the Zeiss Axiovert A1 inverted microscope with Colibri LED light source.

ONLY for this Colibri light source, you do NOT need to wait 15min. before and after using the microscope.

There are 5 **objectives** on this microscope:
A-Plan5X/0.12 (see note below)*
A-Plan10X/0.25 Ph1
LD A-Plan20X/0.35 Ph1
LD A-Plan40X/0.55 Ph1
LD A-Plan63X/0.65 Ph1
A-Plan 40X/0.65 infinity/0.17

ALL OF THESE OBJECTIVES ARE AIR OBJECTIVES.
THIS MEANS ABSOLUTELY NO OIL ON THIS MICROSCOPE!!!!!!

*we removed the 5X objective and put a second 40X objective instead. If you need to use the 5X please let me know. The second 40X objective should give better images with coverslips. The 40X objective shown on the left is optimum for coverslips (0.17mm) the 40X objective on the right and all the other objectives are optimum for petri dishes.

The objectives with Ph1 means that you can use the phase ring on the top part with the visible light LED with these objectives to get a 3D brightfield picture of your cells.
You do not need to turn on the power of the microscope on the left side, IF you will not take brightfield images, you can only turn on the power for the Colibri lightsource for fluorescence.

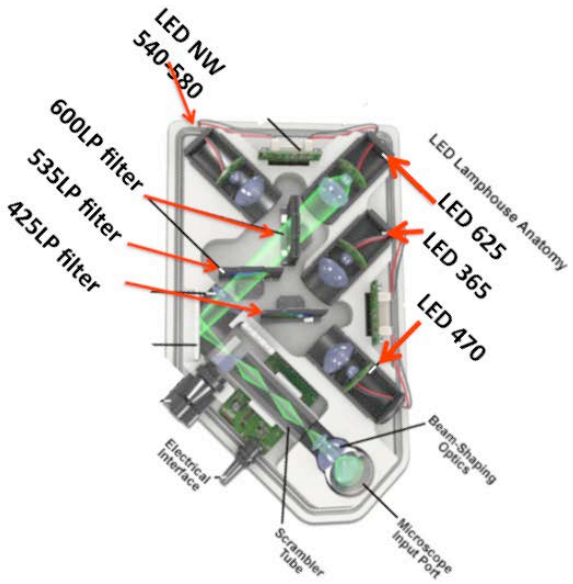
LD means long working distance. This microscope is designed to use tissue culture plates, with plastic bottoms that are 1mm thick. This means that if you want to look at slides, you should image your slides with the slide side facing the objective and not the coverslip side facing the objective!

Note that this is different than the two Olympus microscopes we have and the Zeiss confocal microscope we have. Those 3 other microscopes have objectives that work best with size 1.5 glass coverslips (thickness 0.17mm- 170 micrometers).

This microscope has a Colibri LED **lightsource** which has the following LED lamps:

Position 1 LED 365nm (goes through 425LP and 535LP filters, reflected and reflected)
Position 2 LED 470nm (goes through 425LP and 535LP filters, transmitted and reflected)
Position 3 Neutral White 540-580nm (goes through 600LP and 535LP filters, transmitted and reflected)
Position 4 LED 625nm (goes through 600LP and 535LP filters, transmitted and transmitted)

Updated 02.08.2016



For DAPI turn on LED380 and set Filterset 49
For GFP, Alexa488 turn on LED470 and set Filterset 38
For DsRED, dTomato, Alexa555 turn on LED-NeutWhite and set Filterset 43
For Cy5, Alexa647 turn on LED625 and set Filterset 50
For mCherry, mitotrackerRed LED 590nm or LED-Neutralwhite(540-580nm) set Filterset 60HE
For eCFP or turn on LED445 and set Filterset 60HE
For eYFP or turn on LED590 and set Filterset 60HE

FOR ADVANCED USERS: see me for these LED lamps:
LED 445nm
LED 590nm

********interchangeable filters:
LED590 and Neutralwhite (540-580)
LED445 and LED470

Also this microscope has the following excitation emission dichroic filters:
Filterset 49 for DAPI, Hoechst 33258
(Excitation 365LP; dichroic 395LP; emission 445/50 BP)

Filterset 38 for FITC, GFP, CFP, Alexa488,
(Excitation 470/40 BP; dichroic 495LP; emission 525/50 BP)

Filterset 43 for Cy3, PE, DsRED, dTomato, Alexa555, Nile Red
(Excitation 545/25 BP; dichroic 570LP; emission 605/70 BP)

Filterset 50 for Cy5, Alexa647, APC, faint mCherry!
(Excitation 640/30 BP; dichroic 660LP; emission 690/50 BP)

FOR ADVANCED USERS: see me for these filters:

Filterset 60HE Excitation 545/25 BP; dichroic 570LP; emission 605/70 BP
excitation 1,2,3 BP 445/25, 510/15, 588/27

Updated 02.08.2016

dichroic 460, 520,605
emission 480,546,615

Excitation BP588/27
Excitation BP510/15

Visit this site for more info:
https://www.micro-shop.zeiss.com/?s=420211745f283e&l=en&p=us&f=f&a=f&o=