LEICA DM IRE2 MICROSCOPE MANUAL

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This manual prepared by Tony Brown.

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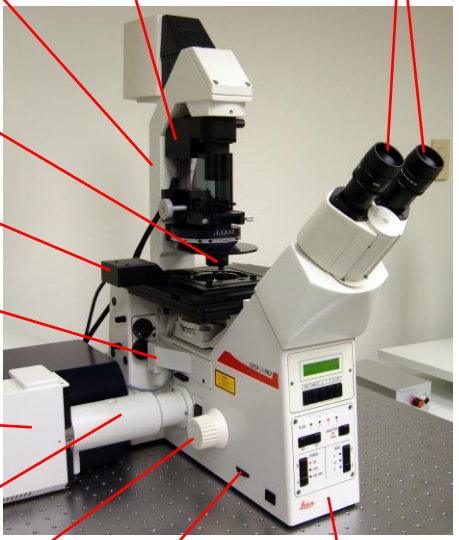
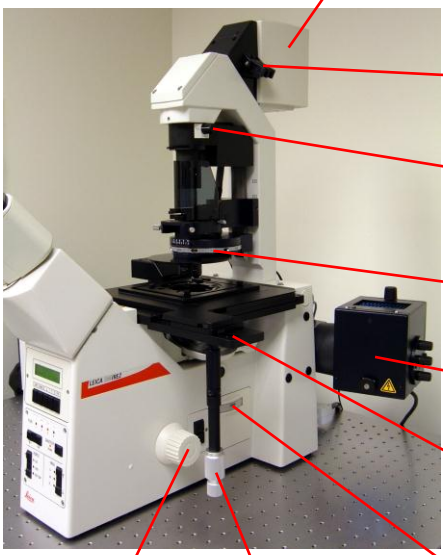
Getting to know the microscope

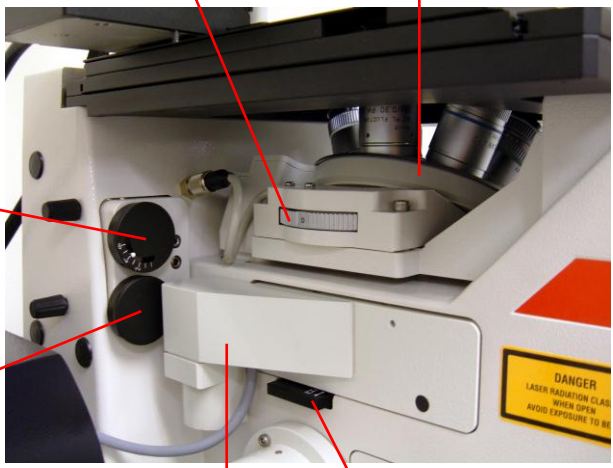
VIEW FROM LEFT



VIEW FROM RIGHT



OBJECTIVE TURRET (DETAILED VIEW)



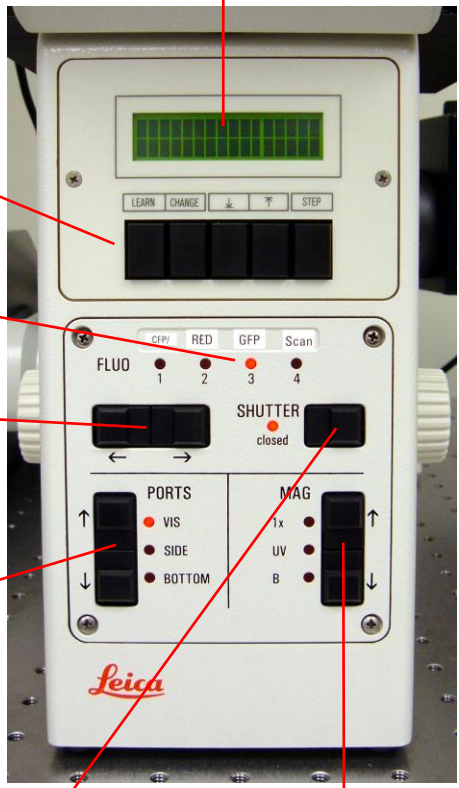
OBJECTIVE PRISM TURRET POSITIONS

Objective turret position	Description	Matching objectives
BF	Empty (for bright field or phase contrast)	
C	DIC objective prism	20x multi-immersion
D	DIC objective prism	63x water immersion 100x oil immersion
E	DIC objective prism	40x oil immersion 63x oil immersion

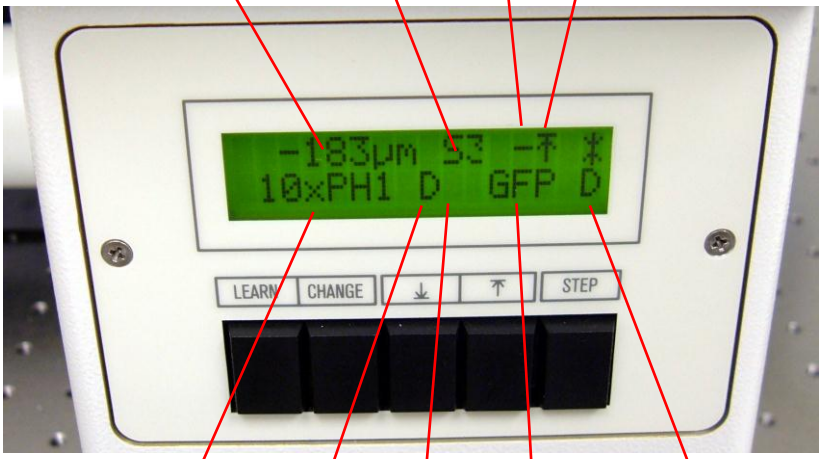
CONDENSER TURRET POSITIONS

Condenser turret position	Description	Matching objectives
BF	Empty (for bright field)	
PH1	phase ring	10x dry
PH2	phase ring	40x dry
20	DIC condenser prism	20x mulit-immersion
40/63	DIC condenser prism	40x oil immersion 63x water immersion
63/100	DIC condenser prism	63x oil immersion 100x oil immersion

Microscope control panel



LCD display panel



Indicates position of objective relative to **upper limit**

Indicates current **step size** setting for focusing

↓ arrow indicates **lower limit** has been set

↑ arrow indicates **upper limit** has been set

Indicates current objective

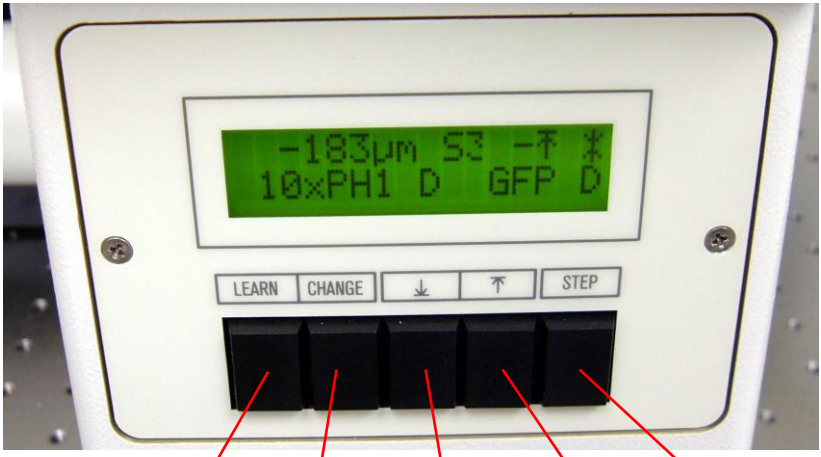
Indicates **desired** DIC objective prism

Indicates **actual** DIC objective prism in light path

Indicates current filter cube

D= dry mode
I= immersion mode

LCD display panel buttons



Learn mode **(DO NOT USE!)**

Toggle between **voltage readout** and **objective readout**

Set **lower limit** of travel for objective **(DO NOT USE!)**

Set **upper limit** of travel for objective **(DO NOT USE!)**

Adjust **step size** for coarseness of focusing

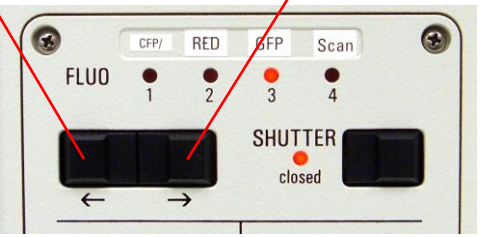
Do not press the LEARN button (this will enter you into learn mode, which you must not play with). If you accidentally press this button, you will see the word “EXIT “ flashing. Press the LEARN button again to exit the learn mode.

Changing filters

Use the motorized fluorescent filter cube changer on the microscope control panel:

Press left button to rotate filter cube turret left

Press right button to rotate filter cube turret right



Red light shows current filter cube selection

The filter cube turret contains three filters plus an empty slot

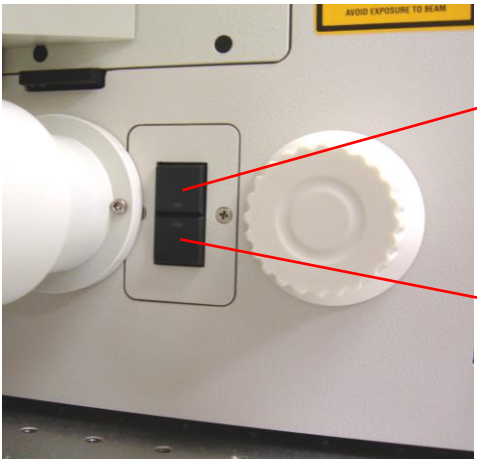
- **GFP** : cube for green fluorochromes
- **RED** : cube for red fluorochromes
- **CFP/** : cube for far red fluorochromes
  or cube for CFP (see Paula for details)
- **SCAN** : empty slot

Notes:

- The cube in the **CFP/** position is normally the far red cube, suitable for fluorochromes such as TOTO-3 and Cy5
- The **SCAN** position is used for confocal imaging and for transmitted light observation through the eyepieces

Changing objectives

Use the **objective turret control buttons** on the left side of the microscope



Press upper key to **increase** magnification

Press lower key to **decrease** magnification

Current objective indicated on **LCD display panel**

To switch between dry and immersion objectives

The standard objectives on our microscope are grouped into three blocks or modes:

100x oil immersion
63x oil immersion
40x oil immersion
] oil immersion mode

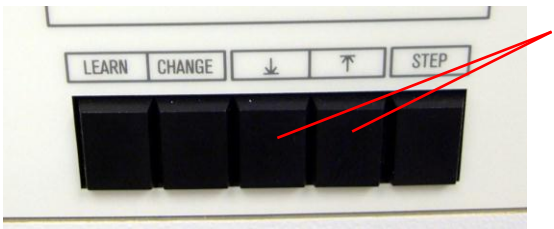
20x oil/water/glycerol immersion] multi immersion mode

40x dry
10x dry
] dry mode

To reduce the chance of immersion medium getting on to a dry objective or the chance of mixing of different immersion media, the microscope will not allow you to move freely between these modes using the objective turret control buttons.

To switch from one mode to another:

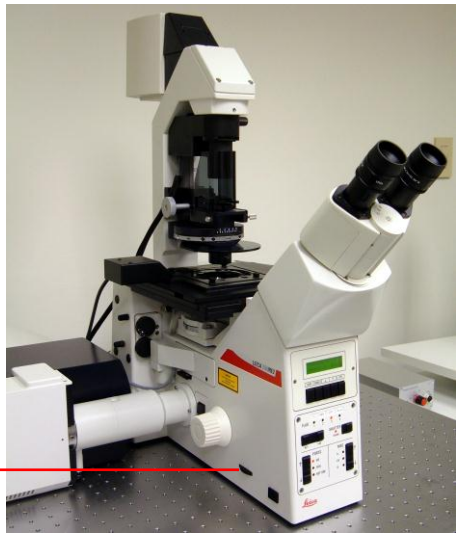
- simultaneously press the “upper limit” and “lower limit” buttons on the microscope control panel
- the words “CHANGE OBJECTIVE” flash on the LCD display panel
- now you can change the objective using the **objective turret control buttons**



Press these two buttons simultaneously

Adjust halogen lamp brightness

Use the dial on the front left side of the microscope stand near the base



The lamp voltage will display automatically on the microscope control panel display when the intensity dial is adjusted

To **switch off** transmitted light illumination adjust lamp intensity to 2.5V then continue rotating dial beyond this point

0V on control panel display indicates illumination is off

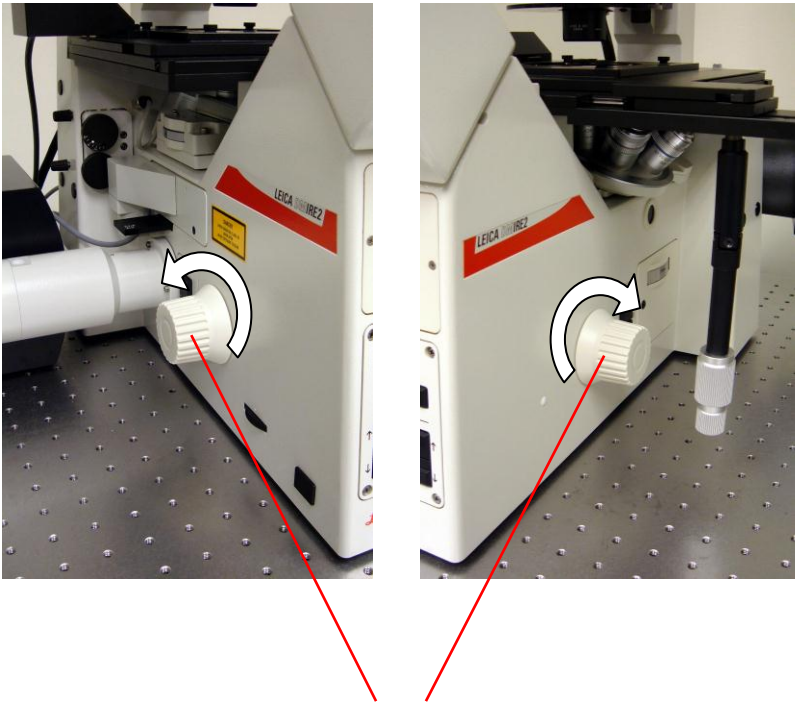
To **switch on** transmitted light illumination, rotate briefly in the opposite direction

To focus using the focusing knobs

One way to focus is using the **focusing knobs** located on the left and right side of the microscope.

Turning the knob so that your thumb moves **away** from you focuses **down**

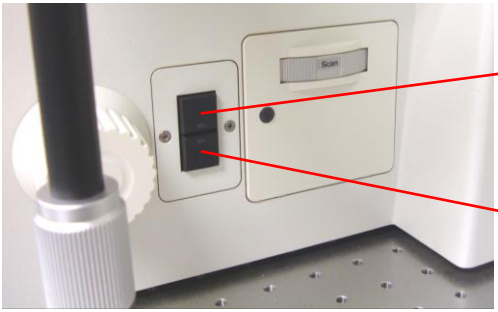
Turning the knob so that your thumb moves **toward** from you focuses **up**



Focusing knobs
(turn in direction of arrows to focus objective down)

Using the focusing buttons

Another way to focus is to use the **focusing buttons l**ocated on the right side of the microscope



Note about upper limits:

If an **upper limit** is set for the objective (see LCD display panel) then the objective will not move above that limit if you are focusing using the **focusing buttons**.

The only way to focus above the upper limit is to use the **focusing knobs**

This is a **safety feature to prevent accidental damage of the objective** when focusing using the focusing buttons.

Changing the coarseness of the focusing

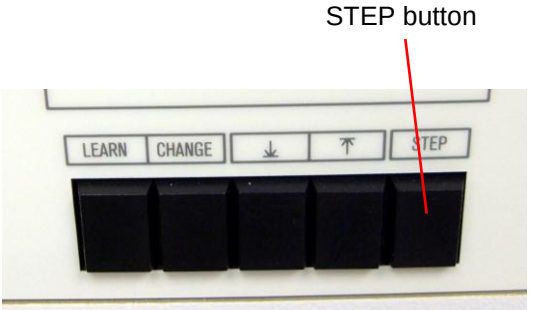
The focusing is electronic and has five settings:

Setting	Step size
S0	Fine
S1	Medium fine
S2	Medium coarse
S3	Coarse

You can use any step size with any objective, but when you first select an objective the **default step size** will be as follows:

Objective	Setting
63,100x	S0
40x	S1
20x	S2
10x	S3

Press **STEP button** to switch between S0, S1, S2 and S3



Eyepieces

Adjusting interpupillary distance:

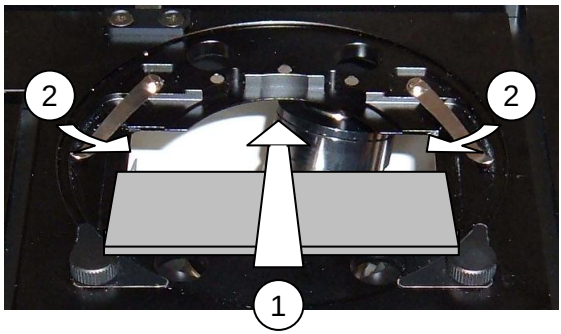
- Adjust eyepieces to match interpupillary distance by moving the eyepieces closer together or further apart

Adjusting parfocality

- Focus on specimen using electronic focusing controls
- Close right eye and adjust the left eyepiece so that the image appears in focus to your left eye
- Close the left eye and adjust the right eyepiece so that the image appears in focus to your right eye
- The eyepieces are now parfocal

Placing a slide on the Z-galvo stage

- To avoid touching or damaging the objective, lower the objective turret all the way using the **focusing buttons**



- Position the slide holder clips as shown above
- Insert slide into the holder in a front-to-back motion (1)
- Slide clips inward onto slide to secure the slide (2)

Transmitted light detector selection knob

For any transmitted light observation (bright field, DIC, phase contrast), this knob should be in the **vis** position.

Bright field observation

Bright field observation means observation with transmitted light using no contrast enhancement method (e.g. no phase contrast or DIC)

For bright field observation:

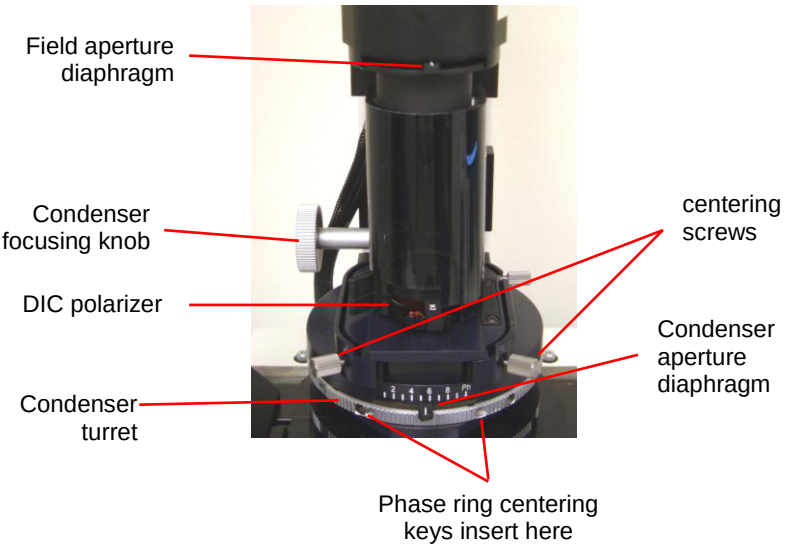
- rotate **condenser turret** to BF (empty) position
- rotate **objective prism turret** to BF (empty) position
- switch **filter cube turret** to SCAN (empty) position

Condenser turret in bright field (BF) position

Koehler illumination

Koehler illumination is essential to obtain good transmitted light images

- Select objective
- Open the **condenser aperture diaphragm** (move lever to the right)
- Focus on specimen
- Close the **field aperture diaphragm** (move lever to left)
- Focus condenser using **condenser focusing knob** until the image of the aperture is sharp
- If necessary center the field diaphragm in the field of view using the two **centering screws** located on the front of the condenser
- Open **field aperture diaphragm** until it just disappears from the field of view
- Close down the **condenser aperture diaphragm** until the desired contrast is achieved



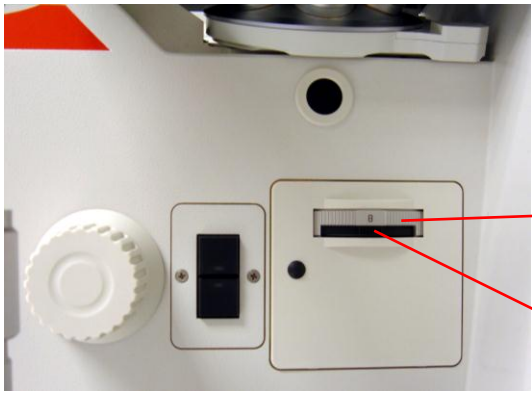
Phase contrast

For phase contrast, you need:

- a phase contrast objective
- a matching phase ring in the condenser turret

To set up phase contrast:

- Select a phase contrast objective (i.e. either of the dry objectives)
- rotate **objective prism turret** to BF (empty) position
- Rotate **condenser turret** to select phase ring
  PH1 for 10x
  PH2 for 40x
- Focus on specimen
- Insert the **Bertrand lens** into the optical path using by rotating the **tube lens module** from “SCAN” to “B”
- Focus on the phase ring using the Bertrand lens **focus slider** on the tube lens module
- If condenser phase ring (dark ring) is not centered on the objective phase ring (light ring), center it using the insertable phase ring centering keys **(see Paula for instructions)**
- Set Koehler illumination



Differential interference contrast (DIC)

For DIC, you need:

- A **DIC objective** (i.e. any of the immersion objectives)
- A **polarizer** above the condenser (no need to insert this – it is kept in the light path at all times)
- A condenser prism in the **condenser turret**
- An **analyzer** beneath the objective prism turret
- An objective prism in the **objective prism turret**

To set up DIC:

- Select a DIC objective (any of the immersion objectives)
- The objective DIC prism required for that objective is displayed on the **microscope control panel**
- Manually rotate **condenser turret** to select the appropriate objective DIC prism

Objective	Condenser prism	Objective prism
100x oil	63/100	D
63x oil	63/100	E
63x water	40/63	D
40x	40/63	E
20x multi-immersion	20	C

- Manually rotate **objective prism turret** to select objective prism
- Insert **analyzer** into light path

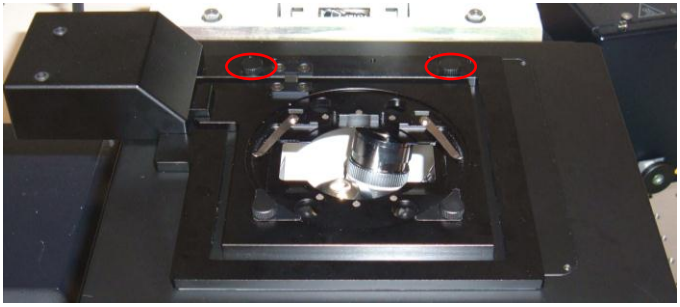
Switching stage holders

The microscope has two stage holders:

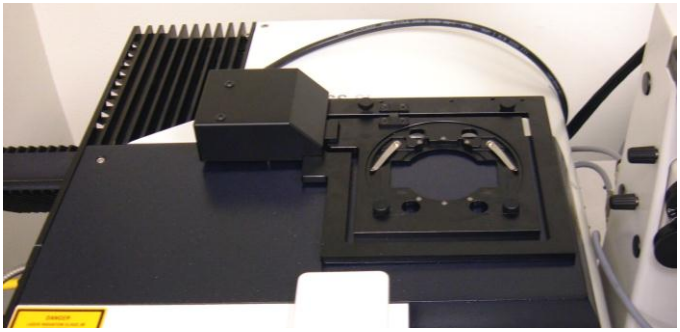
- **Z- galvo stage** (permits rapid and precise movement in Z axis for acquisition of Z stacks)
- **Universal stage holder** (holds a wider range of dishes)

To remove Z-galvo stage:

- Unscrew the two thumbail screws (circled in red):



- Place stage (still attached to the cable) on top of the laser scan head as shown:



Take care not to touch condenser lens surface when removing and replacing the stages!

Using immersion objectives

OIL immersion:

- Clean coverslips before using immersion objectives
- Apply oil to the objective or to the coverslip before placing your slide on the stage
- Use only LEICA IMMERSION OIL
- Use the minimum amount of oil necessary
- If you use too much oil it may run down the side of the objective and damage the optics!

To apply oil to an objective:

- dip oil applicator in immersion oil bottle
- allow excess to drain off
- apply oil to objective by touching to the metal next to the lens
- DO NOT TOUCH THE LENS DIRECTLY!

WATER immersion:

The 63x water immersion objective (not normally installed on the microscope) uses water rather than oil as the immersion medium.

Contact Paula for instructions on using this objective.

Cleaning objectives

This microscope is equipped with the highest quality objectives, with a total value exceeding $30,000!

You must exercise great care to preserve these objectives

To clean oil off immersion objectives:

- Remove specimen from stage
- Blot (not wipe!) off excess oil using lens tissue
- Use a fresh area of the lens tissue for each blot
- Repeat blotting until no more oil comes off onto the lens tissue
- Wipe the metal housing around the lens using lens tissue
- It typically takes 2 or 3 sheets of lens tissue to properly clean a single objective

What to do if you accidentally get oil on a dry objective:

- Blot off excess with lens tissue
- Then contact Paula, who will clean the lens with special cleaning fluid (do not attempt this yourself!)
- Lens tissue alone will not get all the oil off the lens and this will interfere with the optics

Applying oil to objective without removing slide

FOR ADVANCED USERS ONLY!

You can use the following procedure if you are observing your slide and you wish to switch to an oil immersion objective without removing the slide from the stage holder.

- Focus objective turret all the way down using the **focus down button**
- In the LCS software, click on obj button and select the objective diametrically opposite in the objective turret from the objective you want to apply oil to (i.e. **3 positions away from the current objective**)

Objective you want to use	Objective in opposite position
100x oil	20x multi-immersion
63x oil	40x dry
40x oil	10x dry
20x multi-immersion	100x oil
40x dry	63x oil
10x dry	40x oil

- This will rotate the objective that you want to apply oil to so that it is accessible from the right of the microscope
- Apply oil to the objective
- In the LCS software, click on obj button and select the objective that you applied oil to
- This will rotate the objective back into the light path
- Focus up on your specimen

Specimen preparation

Some general tips:

- Select fluorochromes that are optimally excited by the confocal laser lines
- For multiple labeling, the less overlap between the excitation and emission spectra the better
- Always use #1.5 coverslips
- Mount coverslip to slide securely and seal with nail enamel or mounting medium that solidifies
- Do not observe on the microscope until the nail enamel or mounting medium has hardened!
- Secure the slide with the clips when acquiring Z series

DOs and DON'Ts

DOs

- Use only lens tissue to clean objectives
- Use Kimwipes to clean coverslips and slides

DONTs

- Never touch any optical surface with anything other than lens tissue
- Never clean the objectives with any kind of solvent
- Never use Kimwipes to clean objectives or any other optics
- Never use any liquid cleaners or solvents to clean the microscope optics or any part of the microscope!

When to ask for Paula's assistance

- Changing mercury or halogen bulbs
- Aligning mercury and halogen lamps
- Adding or removing objectives
- Adding or removing filter cubes
- Using the 63x water immersion objective
- Cleaning oil off 10x and 40x dry objectives
- Using the Leica objective warmer
- Using the Bioptechs heated chamber
- Any other time you are unsure what you are doing!

Extras

Setting	Step size	Objective
S0	Finest	63,100x
S1		40x
S2		20x
S3		10x
SC	Coarsest	

Note about the coarsest focusing setting (SC):

Press both upper and lower focus keys simultaneously to switch to the coarsest focusing setting (SC)

Press both focus keys simultaneously to switch back to fine focusing setting (S0-S3)