# Leica DM 6000B - Users' Guide

**Author(s): Mariana Ferreira** **Last Updated – June 2020**

**Purpose**: An automated upright microscope equipped with motorized z-focus, coded 7x nosepiece, transmitted light axis and incident light axis. The Leica DM6000 B provides the main transmitted light contrast methods such as bright field, phase contrast and dark field. The microscope presents a user-friendly interface for fluorescence microscopy introducing Leica's incident light source combined with Fast Filter Wheels for fast change of excitation light, selection of emission light, and individual attenuation of excitation colors. Furthermore, the microscope is equipped with a laser unit (an ablation nitrogen laser-pumped dye laser) emitting invisible ultraviolet (UV) radiation.

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# Safety instructions and contacts

## Safety instructions and first aid procedures

Beam paths are shielded except for a fixed open beam between objective and specimen.

- Never look directly into the beam path.
- Never interfere with the beam path.
- Never change a specimen during acquisition.
- Never change objectives during acquisition.
- Never change filters during acquisition.

Specimens should be sealed before observation under the microscope and handled with care using the appropriate level of protection.

- Leaky or cracked samples must not be examined.
- Any spillages should be cleaned up immediately and the area swabbed with 95% alcohol.
- Glass slides should be disposed of in the contaminated sharps bin – broken fragments of glass should be brushed on to paper and disposed of in a similar fashion.

All biological samples and hazardous waste must be appropriately disposed of by the user. Potential risks associated with the waste material should be addressed by the user in conformity with ITQB-NOVA rules.

## Contacts

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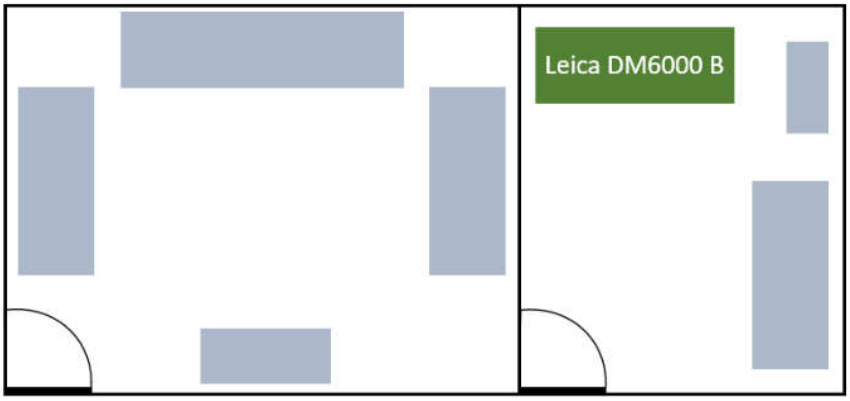
Online information: *http://www.itqb.unl.pt/bic*

# Equipment location, description and technical specifications

Location: Room 5.01 (Bacterial Imaging Cluster), 5th floor, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA)




Manufacturer: Leica

Model: Leica DM 6000B

Year Installed: 2007

Potential Techniques

- Photobleaching
- FRAP
- FRET

Features and Specifications

- Power Supply: Leica CTR6000
- Incident Light Source: Leica EL6000 External Light Source (120W) w/ Fast Filter Wheels for excitation, emission and attenuation
- Leica STP 6000 (SmartTouch Panel)
- Motorized and coded 7x nosepiece
- Magnification Changer (optovar): 1.25x; 1.6x
- Motorized Z Drive
- Manual tube BDT 25+ V100/50/0 (3 beam splitting positions)
- Eyepieces: HC Plan S 10x/25 Br. M

Transmitted Light Techniques

- Dark Field
- Phase Contrast
- Bright Field

Incident Light

- Fluorescence (HXP R 120 W/45C halogen lamp)

Computer

- Type: Desktop
- Brand: Dell™
- Model: Precision T3400
- Features: Intel Core 2 Duo E4600 / 2.4 GHz (Dual Core); 2GB RAM; 160GB HDD
- OS: Windows Vista
- Available Software: **MetaMorph® Version 5.8** (Molecular Devices, LLC)

Monitor

- Brand: Dell™
- Model: E228WFPC

## Objectives

Remember to calibrate your images with the proper pixel size doing montages/size measurements.

| ***Magnification*[1;2]** | **10x** | **40x** | **63x** | **100x** |
|---|---|---|---|---|
| *Leica System* | HC | HCX | HCX | HCX |
| *Class* | PL APO (Apochromats) | PL Fluotar (Semi - Apochromats) | PL APO (Apochromats) | PL APO (Apochromats) |
| *Aperture (A)* | 0.4 | 0.75 | 1.4 | 1.4 |
| *Immersion* | Air | Water | Oil | Oil |
| *Free Working Distance (mm)* | 2.2 | 0.4 | 0.1 | 0.09 |
| *Cover Glass (mm)* | 0.17 | 0.17 | 0.17 | 0.17 |
| *Contrasting Methods* | Phase Contrast (PH1) | Phase Contrast (PH2) | Phase Contrast (PH3) | Phase Contrast (PH3) |
| *Pixel size w/ 1.6x optovar (µm)* | 0.5 | 0.125 | $\frac{8}{100.8}$ | 0.05 |
| *Pixel size w/ 1.25x optovar (µm)* | 0.64 | 0.16 | $\frac{8}{78.75}$ | 0.064 |

[1]Notice that the microscope is equipped with two Eyepieces: HC Plan S 10x/23 Br. M

[2] NA - Numerical Aperture. For more information, follow this link.

## Filter-Sets

| Filter Cube | Excitation Filter | Dichromatic Mirror | Emission Filter | Emission Colour |
|---|---|---|---|---|
| A4; DAPI[1;2] | BP 360/40 | 400 | BP 470/40 | Blue |
| CFP | BP 436/20 | 455 | BP 480/40 | Blue/Cyan |
| L5; GFP[1]; FITC[1;2] | BP 480/40 | 505 | BP 527/30 | Green |
| YFP | BP 500/20 | 515 | BP 535/30 | Green/Yellow |
| CFP-YFP[3] | BP 436/12 - 500/20 | 445 - 515 | BP 467/37 - 545/45 | Blue/Cyan – Green/Yellow |
| CY3; TRITC[1] | BP 545/30 | 565 | BP 610/75 | Orange/Red |
| TX2; FM 4-64[1]; mCherry[1] | BP 560/40 | 595 | BP 645/75 | Red |

[1] Almost equivalent to this filter cube

[2] The filter cube may appear entitled to the name of this filter set in spite of the less known corresponding filter set

[3] Filter set for the FRET technique

BP stands for bandpass filter

**Suggestion for description in "Materials and Methods"**

Images were acquired on a Leica DM 6000B upright microscope equipped with an Andor iXon 885 EMCCD camera and controlled with the MetaMorph V5.8 software, using the 100x 1.4 NA oil immersion objective plus a 1.6x optovar, the fluorescence filter sets GFP + TX2 and Contrast Phase optics.

**Information to be added to the "acknowledgements" section**

This work was partially supported by PPBI - Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122) co-funded by national funds from OE - "Orçamento de Estado" and by european funds from FEDER - "Fundo Europeu de Desenvolvimento Regional".

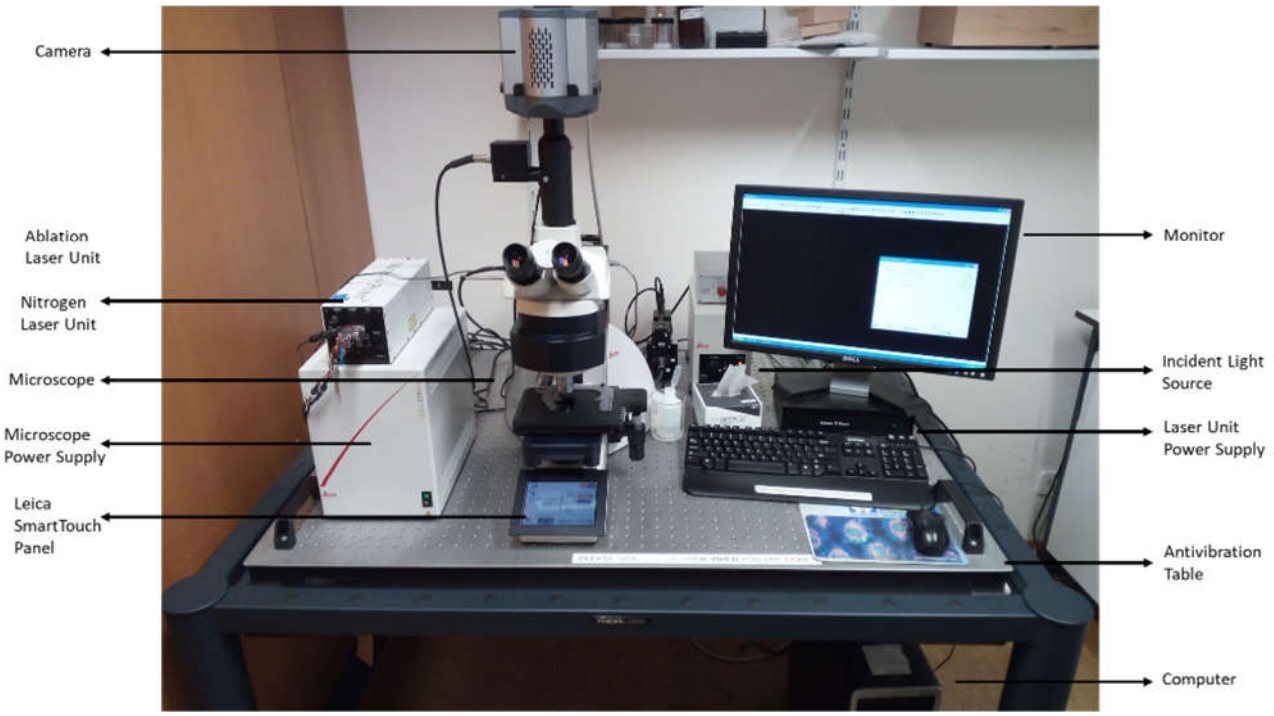
**User pre-requisites, booking and general rules**

- The equipment cannot be used without official training.
- New users must have a clear understanding of the principles of light microscopy and how fluorescence work.
- Before your first use contact Mariana Ferreira to schedule training
- Save all data into your own storage device as we routinely clean the computer's data
- Register your utilization in the logbook
- For all downstream image analysis image analysis use the available workstations with MetaMorph software. Alternatively, you can use FIJI.
- Contact the responsible people in case of any doubt or any issue with the equipment.

Booking is done online via:
https://www.itqb.unl.pt/Internal/modules/equipment/week.php?area=11&room=30

# Instrument startup procedure

## System Overview



**Step by step startup**

1. **Before** you start, if you require fluorescent illumination ensure that the lamp has been off for more than one hour
2. Switch on the devices in the following order:

2.1. Incident Light Source Leica EL 6000 (Lamp) if you need fluorescence


2.2. Microscope's Power Supply Leica CTR6000

2.3. Nitrogen Laser MicroPoint® (right underneath the monitor). **Do not move or touch** the other MicroPoint® box!


2.4. Computer

3. Wait for all components to initialize, once finished the status menu of the Leica SmartTouch will show the current microscope settings
4. On the computer log in into the admin account (User: Admin) by pressing Enter, although the account asks for a password just leave the space blank.
5. Run MetaMorph® Version 5.8 from the desktop
6. Use MetaMorph's toolbar and the Multi-Dimensional Acquisition App to setup the microscope, preventing the alteration of the microscope default settings. You can consult the corresponding Set-Up (See Other Guidelines) for Bright Field, Fluorescence, Darkfield or Phase Contrast optics
7. Check if the stage is not too close to the objective and then place the microscope slide onto the holder
8. If using an oil immersion objective apply a drop of immersion oil on top of the coverslip. **Do not forget** to clean the oil from the objective when finished or when switching to another objective
9. Adjust the BDT25+ tube by pulling out the control bar for camera image acquisition, the middle position for 50/50 view between camera and eyepieces, pushed in for eyepiece view only

10. From now on we strongly advise you to use MetaMorph Software to handle observation and image acquisition (See Single and Multidimensional Acquisition).

# MetaMorph Image Acquisition Guidelines

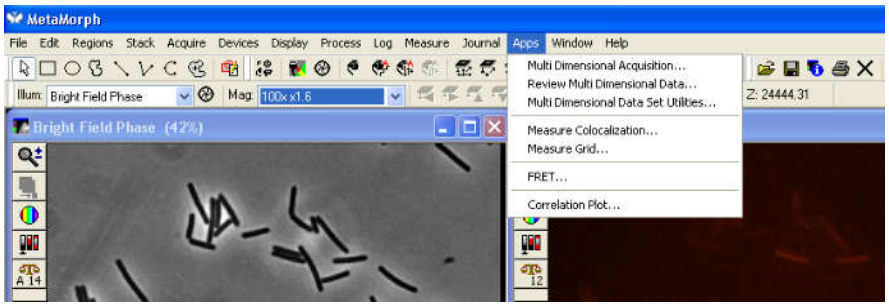
MetaMorph includes 25 toolbars that enable fast access to the more commonly used tools and dialog boxes. Some toolbars contain tools that are used to display several of the dialog boxes from a specific menu, such as the Multi-Dimensional Acquisition.

Images acquisitions are represented on image windows by MetaMorph. Each image window has its own set of Image Window Tools located in a panel on the left side of the image window.

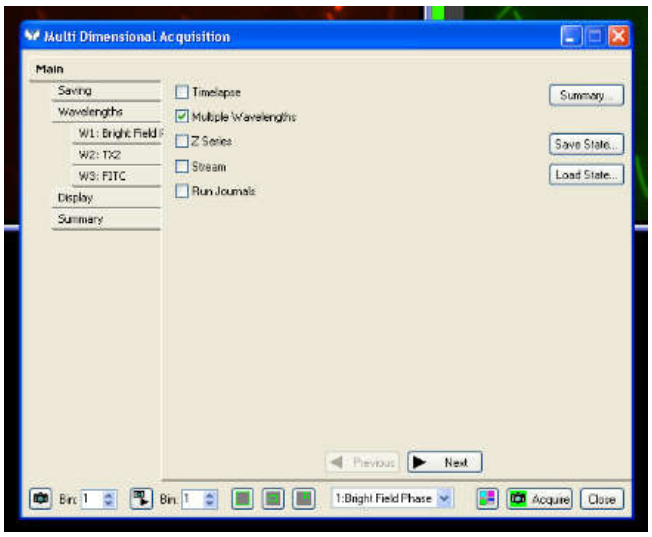
For downstream analysis please use the MetaMorph Offline software available in the computers located at the students' room 5.23 in the 5th floor of ITQB NOVA. For instructions on how to use the MetaMorph Offline software check the MetaMorph Guide available at the BIC website - http://www.itqb.unl.pt/bic.

## Single and Multidimensional Acquisition

1. Go to **Apps** -> **Multi-Dimensional Acquisition**




2. Go to the **Main** tab of the Multi-Dimensional Acquisition window and select **Multiple Wavelengths**, making sure the remainder options are unselected

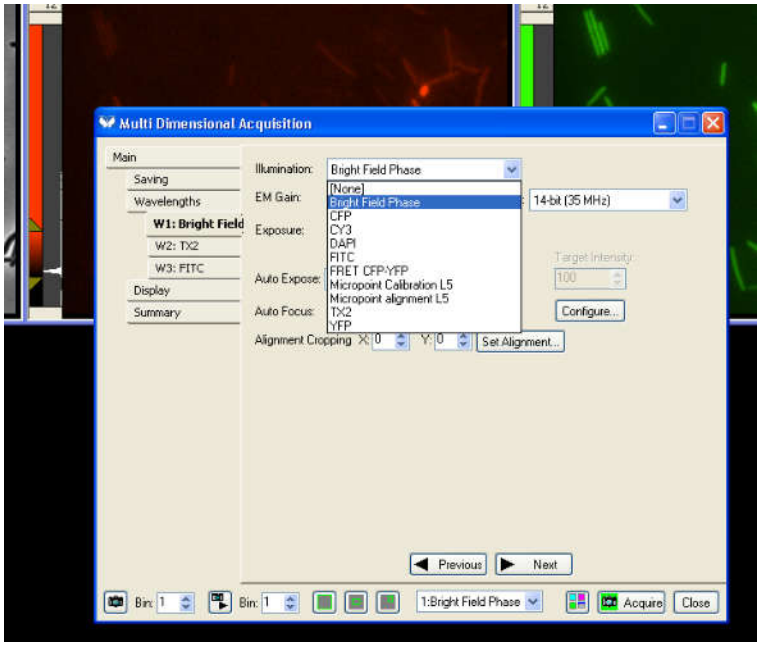



3. Go to the **Saving** tab and select your directory or sub-directory (e.g. new folder within your directory with the current date). If you do not have a directory yet, make your own by following the path: D:\ALL Users*Your Name*

4. MetaMorph will automatically save the images using the name given to the experiment, for sequential acquisitions you can use the hash (#) increment system in the file name. MetaMorph will also add the respective filter/contrast method to the filename in multichannel acquisitions. For example, **ExperimentName#1** with two wavelenghts will yield "ExperimentName#1_ w1BrightField" and "ExperimentName#1_ w2TX2" on the first acquisition. In the next sequence, MetaMorph will automatically increment one number and the name will be "ExperimentName#2_ w1BrightField" and "ExperimentName#2_ w2TX2".

5. Go to the **Wavelengths** tab and select the number of contrast methods and filter sets you want to acquire.

6. For each sub-tab, select the adequate contrast method or filter set in the **Illumination** (See Notes 1). Pay attention to the sequence of the filter sets, as you can bleach the sample (See Notes 2).

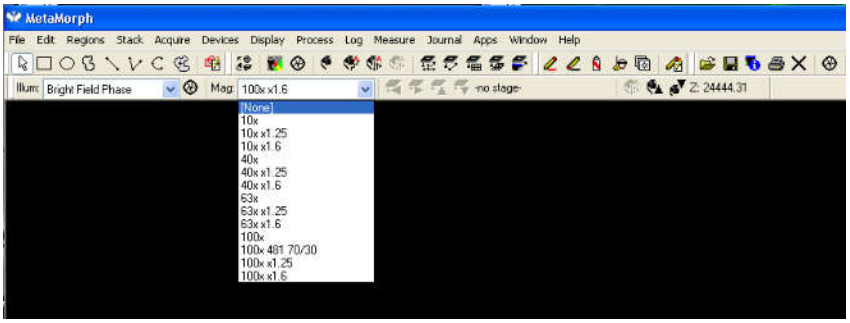



7. Select the adequate **Exposure Time**. Start small (200 ms) and increase if necessary to reduce bleaching (See Notes 3).

8. Focus and visualize the sample using the coaxial pinions, focusing wheel, Leica SmartTouch panel and MetaMorph:

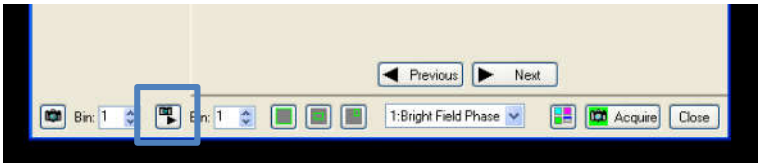
**8.1.** In the MetaMorph toolbar, go to **Illum** and select one of the available contrast methods or filter sets

**8.2.** Yet in the MetaMorph toolbar, go to **Mag** and select an objective/optvar (e.g. 100x x1.6) combination (See Notes **Error! Reference source not found.**).

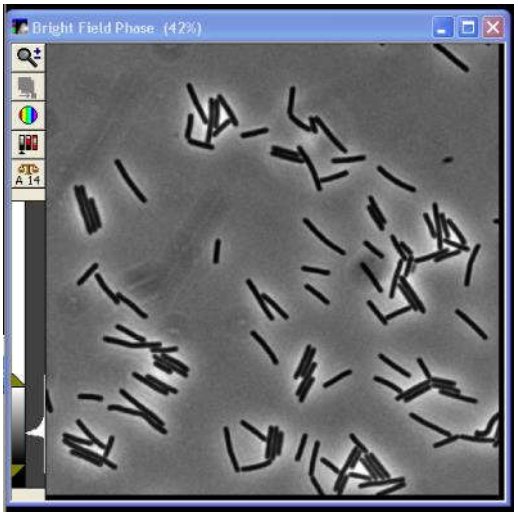



**8.3.** Then, in the Multi-Dimensional Acquisition App select one wavelength sub-tab to focus your sample

**8.4.** Press the Live button in the bottom of the Multi-Dimensional Acquisition window




**8.5.** MetaMorph will open a live image window that should be used to focus the sample.



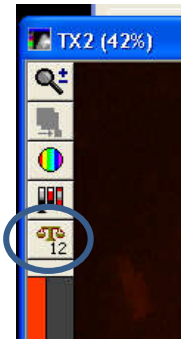
**8.6.** Move the stage over the X and Y axes with the coaxial pinion to move the sample into the light path

**8.7.** Start raising the stage by using the focusing wheel in the **Coarse** mode until the oil touches the objective, or the sample is close (~2mm) for dry objectives. For that, select the **Coarse** button in the navigation menu of the Leica SmartTouch Panel

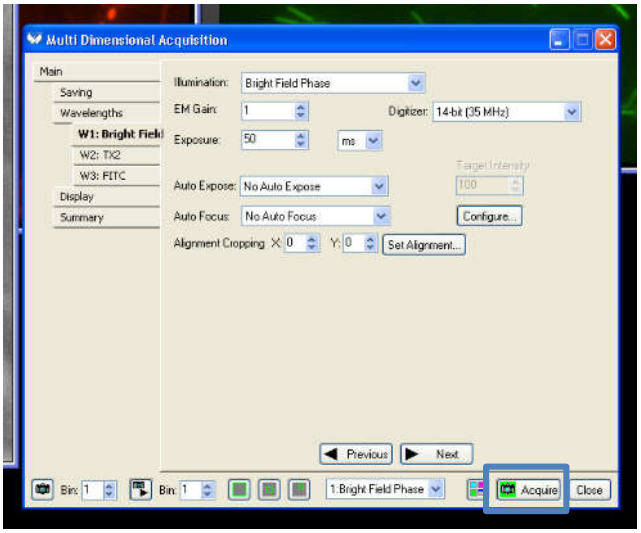

**8.8.** Then, cautiously adjust the Z position of the stage using the focusing wheel in **Fine** mode until a sharp and focused image of the sample you intend to observe. For that, select the **Fine** button in the navigation menu of the Leica SmartTouch Panel

9. **Please notice that the 100x magnification objective has a safety attribute that you must strictly respect. The objective makes a little noise (“click”) when it hits the coverslip. The objective can retract slightly after the “click”, beyond that you may break/scratch the objective. Please be EXTREMELY CAREFUL when focusing your sample.**

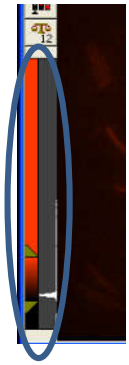
10. Right-click in the **Scale** button in the panel on the left side of the image window and remove the **Auto Scale** option.




11. Click on the **Acquire** button to retrieve images of all contrast methods and filter sets.




12. Use these windows to visualize the distinct multiple contrast methods and filter sets. Adjust the focus and exposure times if the images seem unfocused, blurry, dark, dim or overexposed. Note that each wavelength has a slightly different optimal focus point. Use the scale slider in the panel on the left side of the image window to manually adjust the range of gray levels.



13. Follow the **Final Set Up** instructions after finishing your session.

**Notes**

1. The choice of the fluorescent substrates and dyes will dictate the filter set used for fluorescence microscopy. In our table of filter sets, you can get an overview of the excitation/emission wavelengths supported to outline the experiment
2. Phase contrast or other transmitted light techniques should be acquired first and from live mode (start acquisition while in live mode) because the lamp takes some time to reach maximum intensity and if not given enough time image quality and contrast is lower. Fluorescence images should be acquired in a decreasing wavelength order to reduce photo-bleaching, therefore begin with the long wavelength red fluorophores, then green, and finally the shorter wavelength blues.
3. Start by setting the exposure time to 100 ms, if you cannot see your signal or it is very weak increase the exposure time until acceptable signal levels are shown, if the value is over 1000 ms you should consider increasing the concentration of the fluorophore. If the signal is too strong reduce the exposure time until the signal is no longer saturated, you can also consider reducing the concentration of your fluorophore. According to in-house experiments, typical exposure times are as follows (but may require optimization depending on the microscope configuration): 50–100 ms for DAPI; 100 ms for FM4-64; 50 ms for MTG; the exposure time will depend on the strength of the promoter under study for the $SNAP^{cd}$/$CLIP^{cd}$ (Cassona et al. 2016).

## Other Guidelines

### Bright Field

Used mostly to observe stained or naturally pigmented samples in a bright background, please note the camera is black and white and no color will show on the acquired image.

1. Switch to the Transmitted Light axis (TL) by pushing the **TL/IL** button
2. Select the Bright Field (BF) contrast method by pressing the **BF** button
3. You can adjust the illumination with the **INT** buttons

### Phase Contrast

Light properties are used to make transparent objects show as dark objects in a bright background.

1. Switch to the Transmitted Light axis (TL) by pushing the **TL/IL** button
2. Select the Phase Contrast (PH) method by pressing the **PH** button
3. The available objectives are totally suitable for phase contrast method
4. Note that the microscope automatically selects the correct light ring in the condenser and opens completely the diaphragm aperture without possible adjustment

### Dark Field

Produces brightly illuminated objects on a dark background.

1. Switch to the Transmitted Light axis (TL) by pushing the **TL/IL** button
2. Select the Dark Field (DF) contrast method by pressing the **DF** button
3. Note that the microscope automatically selects the correct light ring in the condenser and opens completely the diaphragm aperture without possible adjustment
4. The maximum objective aperture which may be used for dark field is **0.75**

### Fluorescence

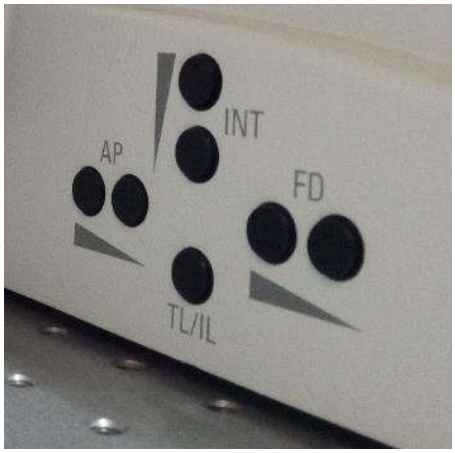
A combination of lamp and filters allow observation of fluorescent samples on a dark background.

1. Switch to the Transmitted Light axis (TL) by pushing the **TL/IL** button. The Leica SmartTouch panel has to indicate the fluorescence contrast method (**FLUO**)
2. The current fluorescence cube is indicated in both Leica SmartTouch panel and MetaMorph environment
3. If you pretend to protect your specimen from fading close the incident light shutter by pressing the **TL-SHUTTER** button
4. See the MetaMorph guidelines to see how to use the ultra-fast internal filter wheel and to select the desired filter-sets

**Combi Mode**

A fast mode to change between fluorescence and the transmitted light techniques above.

1. Select the combi mode by pushing the **COMBI** button
2. The current fluorescence cube is indicated in both Leica SmartTouch panel and MetaMorph environment
3. The illumination settings for both axes can be adjusted separately in the Leica SmartTouch panel
4. To switch between the axes, press the **TL/IL** button and keep up with the Leica SmartTouch panel
5. See the MetaMorph guidelines to see how to use the ultra-fast internal filter wheel and to select the desired filter-sets



## Shutdown procedure

1. **Before** you switch off the devices confirm the next user and if so, ask whether he or she will be using the microscope in the next hour
2. Remove the specimen from the specimen holder
3. Clean the oil immersion objective with Kimtech tissues if it has been used
4. Leave the stage in the lowest position of the Z axis.
5. If there is an interval of more than one hour to the next user, switch off the devices in the following order:
   - **5.1.** Close MetaMorph (When closing the **MetaMorph** software, **WAIT** for the warning message and let the camera cooling down)
   - **5.2.** Nitrogen Laser MicroPoint®
   - **5.3.** Microscope's Power Supply Leica CTR6000
   - **5.4.** Incident Light Source Leica EL 6000 (lamp)
6. Save all data into your own personal storage device or cloud and switch off the computer
7. Sign your registration in the logbook
8. Take everything you brought and keep the room and desk clean for the next user