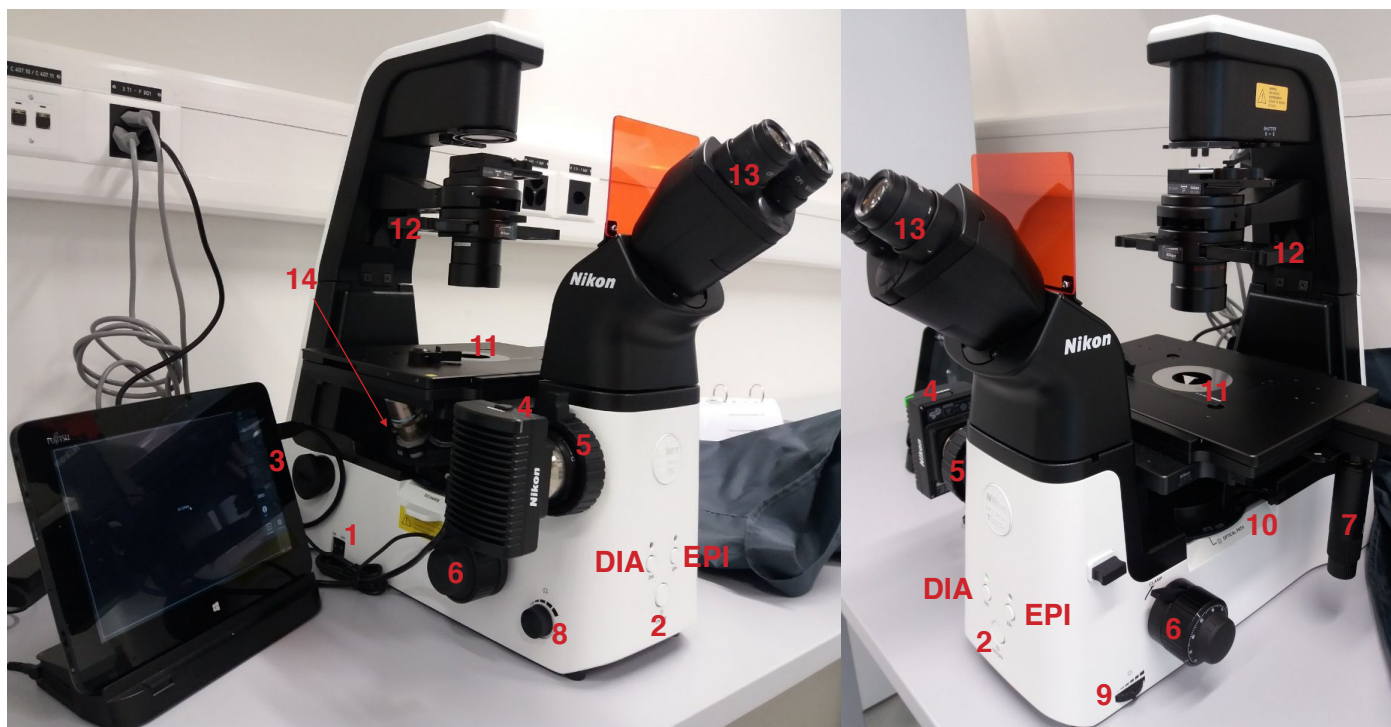


Fluorescence Microscope Protocol

Nikon eclipse Ts2R



1 : microscope switch on/off	8 : light intensity regulator (DIA)
2 : light switch on/off	9 : light intensity regulator (EPI)
3 : tablet switch on/off	10 : optical path (setting of the wavelength)
4 : camera switch on/off	11 : sample stage
5 : shutter eyepiece (up) and camera (down)	12 : phase contrast filters
6 : focus knob (coarse and fine)	13 : eyepiece
7 : sample stage regulator (x-y)	14 : objective lenses (4X, 10X, 20X, 40X)

To use the microscope:

1. Remove the protective cover.
2. Turn on the microscope and camera using the microscope switch (1) and camera switch (4).
3. Turn on the tablet using its switch (3).

To turn off the microscope:

1. Switch off the light (2) and the camera (4).
2. Switch off the microscope (1).
3. Switch off the tablet : Settings > Shut down > Yes.
4. Cover the microscope using its protective cover.

To save the pictures on your USB stick:

1. Plug in your USB stick on the tablet.
2. On the tablet: Menu > Shot Rec > Rec Drive > Ext Mem > choose your USB stick > create a new file if needed > Ok.

Imaging non-fluorescent samples (DIA light)

- Place your sample on the sample stage. If you use a well plate, use the holder and clips designated for well plates.

You don't need to remove the cover of your plate, since the lenses and camera are below the sample.

- Turn on the light (2) and press the DIA button.
- Adjust the phase contrast filter position (12) according to the objective used (see Table 1 below).
- Using the shutter (5), direct the image towards the eyepiece and focus on the sample using the focus knob (6). You can move the sample in the x or y direction using (7).
- To visualize the image with the camera, use the shutter (5). It will display the image on the tablet screen.
- To take a picture, click on "Capture" on the tablet screen.

Objective used:	4X	10X	20X	40X
Corresponding phase contrast filter:	1 st ring (left)	2 nd ring (middle)	2 nd ring (middle)	3 rd (right, no phase contrast)

Table 1: Phase contrast ring according to the objective used.

Imaging fluorescent samples (epifluorescence)

- Place your sample on the sample stage. If you use a well plate, use the holder and clips designated for well plates.

You don't need to remove the cover of your plate, since the lenses and camera are below the sample.

- Choose the wavelength corresponding to your dye excitation wavelength (10).

DAPI staining (blue) : 385nm (position 1)

Calcein staining (green) : 470nm (position 2)

EthD-1 staining (red): 590nm (position 3)

Bright-field : position 4

EthD-1 may be challenging to see using the eyepiece, so first, find the focal plane of your sample using Calcein and then change the optical path (10) for further imaging.

- Using the shutter (5), direct the image towards the eyepiece.
- Turn on the light (2) and press the EPI button.
- Focus on the sample using the focus knob (6). You can move the sample in the x or y direction using (7).
- To visualize the image with the camera, use the shutter (5). It will display the image on the tablet screen.
- To take a picture, click on "Capture" on the tablet screen.

As photobleaching of the dyes occurs upon prolonged light exposure (ambient light and fluorescence-activating light), keep the samples in the dark as much as possible. This can be done by covering your samples with aluminium foil.

The camera will only image the sample using the current optical pathway. To visualize more than one dye on a given area of the sample, you will need to image the sample with each relevant optical pathway, without moving the sample. You will then be able to superimpose the pictures using image processing softwares, such as ImageJ.

To increase the contrast or remove the background fluorescence, you can switch the camera from automatic to manual mode: Menu > Camera > Prog (exp mode) > Manual. You can then change the exposure time of the pictures: Exp time > choose a value between 100us to 30s, and change the gain of the camera: Cam Gain > choose a value between 100 and 6400. Increasing the contrast and/or removing background fluorescence can also be done by further image processing using ImageJ.