How to use the NDD

N.B. The ND detectors are very sensitive and so imaging must be done under red lights.

1. The ND detector is only appropriate for use with the two-photon laser to excite the fluorophores.
2. In the Configuration window select the NDD tab.
3. You will now see a simple schematic. Using the Config option you should either choose the NDD red or NDD green configuration depending upon the fluorophore(s) present.
4. You must then manually turn the turret wheel on the microscope to 5 and open the slide shutter (the one also used to allow mercury light to illuminate the sample) between the NDD and objective.
5. Any emitted light is now being sent to the NDD through the appropriate filter set.
6. The process of obtaining and optimising an image is now identical to that for using any other imaging configuration on this microscope.
7. It is possible to set up two channels for NDD allowing you to image both red and green fluorophores sequentially. However, due to the requirement to manually turn the turret and open the shutter it is not really possible to obtain both NDD and de-scanned images at the same time. If these are required you will need to obtain the images separately and then overlay them.

How to overlay images

1. Collect or open the images of the same area that you wish to overlay
2. Go to the Main Menu bar and select “Processes”
3. You should now see the option to “copy”
4. Select this and a new window will open with two boxes. Select one of the images that you wish to overlay into each of these boxes.
5. The menu next to the picture in the **bottom** box should contain a dropdown box containing the options Ch2, Ch3 and New. Select NEW to avoid overwriting one of your original pictures.
6. Click “Apply” and you should see a new image consisting of the two pictures overlaid. You can then treat this as a normal 2 channel image.
7. If you wish to add a third channel simply repeat the process using the 2 channel image as one of the images to copy.