Long Lab, Stanford University
http://cmgm.stanford.edu/biology/long/

Using the Nikon Optiphot microscope with DIC

1) Turn on bottom light to fairly high.
2) Push in filter (front and center above lenses) and prism (bar on left). Pull out cubes.
3) DIC lenses: 10x, 20x, 40x, and 100x oil immersion.
Front ring should match the lens.
4) Focus on the bacteria using the 20x or 40x lens. It can help to rotate the prism to darken the field.
5) Close down the field iris (wheel behind the iris) and then focus the condenser (black knob in front of the focus knobs on the left).
6) Open up the field iris most of the way and center the condenser (two silver knobs on either side front of the condenser).
7) Open up the field iris completely.

The prism (shadowing) can be used to make the cells appear flatter to more 3-D (knob at end of the prism). The condenser iris can be used to change lighting/contrast. Increasing the contrast decreases the resolution. The field iris and the specimen are in the same plane of focus. The condenser iris is in another plane which is the same as the filament. The condenser iris cannot be seen in the field, but can be seen if a telescope is put at the eyepiece.

Changing to fluorescence

1) Turn on the power supply for the bulb.
2) Put in the cube for GFP (XF100 or B1E). Pull out the prism and filter.
3) Turn off bottom light.
4) Open shutter.
The lever right in front of the lamp is the field diaphragm.