Rules for bright field and DIC microscopy:

1. Köhler illumination
   - Switch on the light source
   - Focus sample and then move out from sample area
   - Close down field stop to get octagonal shape
   - Move condenser to see sharp edges of this octagonal shape
   - Put this octagonal shape in a middle of view area
   - Open field stop far enough to not see black colour
2. Set the light
   - Try to use 3200K for pictures
   - Decrease the light intensity by 2 filter rings sitting on right side of microscope (close to micro/macro screw)
   - Check aperture setting
   - Choose technique you want to use on condenser ring (bright field - H/I, DIC – I, II or III)
   - Check settings on filter ring over objective, in case of DIC choose position 8, in case of bright field choose position 7
3. Epifluorescence:
   - Switch on source of fluorescent light
   - Choose correct filter according to list below
   - **Do not forget to switch light off!!!**
4. Troubleshooting
   - If you see yellow / green / blue light in the microscope, check filter ring over objectives
   - If view is disturb by some dark object, check all filters position, most probably it is not left "in a middle position"
   - If you see your sample correctly in a microscope but on a computer it looks yellow/ pinkish, play with white balance in camera settings
   - If you see some stable, old "burn out" sample or any view on a monitor which you don't see in a microscope – contact Marta

Microscope position	Filter set		Usage
1	#DAPI	Exc. BP 365 B. splitter FT 395 Em. LP 397	Fluorescence **DAPI**
2	#9	Exc. BP 450-490 B. splitter FT 510 Em. LP 515	Fluorescence **GFP/YFP** broadband filter
3	#15	Exc. BP 546+/-12 B. splitter FT 580 Em. LP 590	Fluorescence **RFP** broadband filter
4	#46	Exc. BP 500+/-20 B. splitter FT 515 Em. LP 535+/-30	Fluorescence **GFP, Alexa 488, FITC etc.**
5	#47	Exc. BP 436+/-20 B. splitter FT 455 Em. LP 480+/-40	Fluorescence **CFP**
6	#38	Exc. BP 470+/-40 B. splitter FT 515 Em. LP 525+/-50	Fluorescence **CFP+GFP**
7	empty		**Bright field pic.**
8	DIC	Analyzer (polarization filter DIC)	**DIC**

DIC

Condenser filters:

- 10x/0,3 ----> **I**
- 20x/0,5 ----> **II**
- 40x/0,3 ----> **II**
- >40x ----> **III**

Plus filter ring position "8" DIC

Phase contrast

- Ph1 ----> 10x and 20x
- Ph2 ----> 40x
- Ph3 ----> 100x