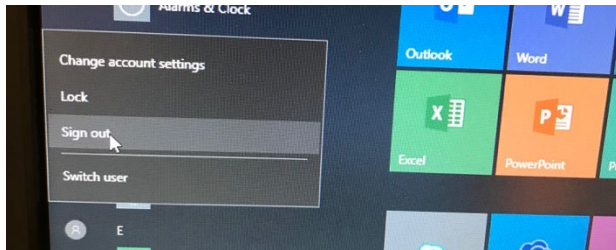# Olympus IX70 (CTAC) (ICBR Cytometry)

User Manual

Location: LG-164, MBI

Login, find "cell and tissue" block, find IX70, and start the timer; Sign out

Find the microscope

Turn on florescence lamp, make sure the light output is 100%

Turn on the camera

Turn on the bright field of the microscope (in the back)

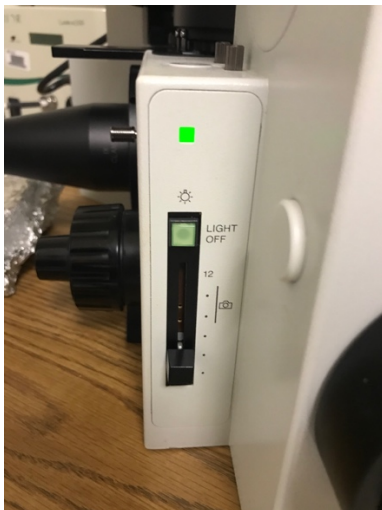Turn on the computer and open the software (Q-capture); check the camera name (Retiga 4000R)

Use the "eye" model to identify your samples under the microscope

Play with different "filters"; for GFP use the blue light

The control panel of bright light: switch and intensity

Once you identified your sample, change to the "camera" (SP) mode

Click "live image" on the software, refocus your sample

While the light is on (shuttle is open), play with different **parameters**; check the **saturation**! Make sure there is no red spot on your screen

The one used for **blue light** is: 1X1 Bin (best resolution); 8-bit mono; Exposure **90 millionseconds**; Gain 4; Offset 0 (default); Gamma 1.00 (default); usually change one parameter (exposure time), and keep the other consistent. **Using CONSISTENT parameters under a specific filter (bright field/blue light)**

Once everything is okay, take a picture (capture); then **close the shutter (especially for blue light) avoid bleaching the GFP signal**

Take another picture under the **bright field**; **keep the same focus!** 1X1 Bin (best resolution); 8-bit mono; Exposure **15 millionseconds**; Gain 4; Offset 0 (default); Gamma 1.00 (default). **Using CONSISTENT parameters under a specific filter (bright field/blue light)**

For the picture take under **blue light;** add **green** color on the original capture image (right click the image); then click "Adjust" ➔ "Composite"; "Add" the original capture; "Display" the parameters; change to: **B(brightness): 62; C(contrast): 60; G 50; BL: 0; WL: 255 (keep same parameters for all comparable images!)**; click "Apply"; then a composited color picture will be created; save it as **tiff** format (click the big Q at the right-left corner). Save the original capture image as well!
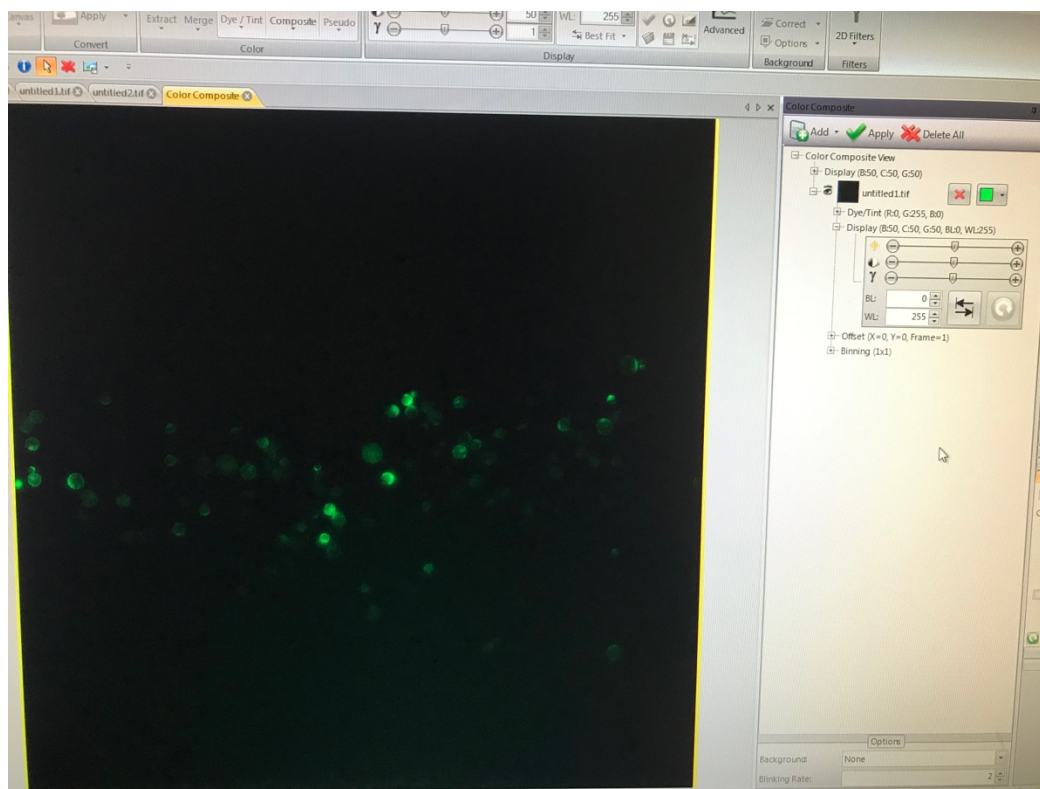
For the picture take under **bright field**; click "Adjust" ➔ "Composite"; "Add" the original capture; "Display" the parameters; change to: **B(brightness): 64; C(contrast): 68; G 50; BL: 0; WL: 255 (keep same parameters for all comparable images!)**; click "Apply"; then a composited monochrome picture will be created; save

it as **tiff** format (click the big Q at the right-left corner). Save the original capture image as well!

For each sample, save four images:

20190718_Tpo_AtCas9_40h(1)_blue_comp.tif

20190718_Tpo_AtCas9_40h(1)_blue_raw.tif

20190718_Tpo_AtCas9_40h(1)_white_comp.tif

20190718_Tpo_AtCas9_40h(1)_white_raw.tif

The directory to save images is: USER DATA ➔ Soltis ➔ Shan; copy all images from the computer to a flash drive.

Once the sample is done, close all tabs from that sample

Switch off all lights and camera; turn off the computer; stop the timer, and sign out your account at the front desk computer

QUESTION:

How to add a scale bar? Scale bar folder; full dimension (2048 X 2048)

Right click on the picture? Fine tune; best fit