Olympus Inverted microscope IX 83 Instruction

1. **Turn on microscope**
   1) Turn on main switch of mercury lamp X-Cite
   2) Turn on main switch of control box IX3-CBH
   3) Turn on main switch of control box BX3-SSU
   4) Turn on main switch of touch panel controller
   5) Turn on shutter controller (black box), make sure the switch is in auto position
   6) Turn on PC and open Cellsens software

2. **Load sample**
   1) Use the knob above the condenser to move the condenser up
   2) Load sample on the sample stage
   3) Lower the condenser by the knob to the end position

3. **Imaging:**
   1) Choose imaging mode on the software: BF, DIC, TRITC, FITC, DAPI, etc. (If you use DIC imaging, you have to push in the DIC filter on the bottom of the stage)
   2) Choose the object lens on the software: 4X, 10X, 20X, 40X, 60X
   3) Click on **live** icon button
   4) Adjust the exposure by moving the slider to right until your image appears properly exposed
      a. The exposure normally can be set as **auto** in bright field or DIC image mode and as **manual** in fluorescent image mode
   5) Using controller U-MCZ coarse and fine focus knob to focus the sample by eyepieces or on the computer screen
   6) Move the sample by XY controller to find interesting area
   7) Focus your image to the camera using the fine focus if needed
   8) Normalize background:
      a. For Bright field images: on the top right toolbar, click the white dropper icon
      b. For fluorescent images: on the top right toolbar, click the black dropper icon
      c. Then select an area of 'background' on your image slide by drawing a small box encompassing only the background. This

sets the write or black balance for your image and reduces background noise.

8) Click the **Snapshot** icon button to capture an image
   a. Save your image under the **user** folder on the desktop or in your personal device (if you have scale bar on your images, when you save the images as .tiff, the images will have two layers; when you save the images as .jpg, the images will only have one layer)

4. **Movie recording**
   1) Select Movie recording
   2) Click **movie** icon button to capture a movie
   3) Save your movie .avi file under the user folder on the desktop or in your personal device

5. **Process set up**
   1) On the right set of the software screen, select process management window
   2) Set up channel selection
   3) Set up Z stack condition if needed
   4) Set up XY stage capture condition if needed
   5) Set up time-lapse capture condition if needed

6. **Experiment set up**
   1) On the right set of the software screen, select experiment management window
   2) Open the saved experiment or establish a new experiment by clicking on **new**
   3) Set up image acquisition
   4) Set up Z stack condition if needed
   5) Set up stage loop if needed
   6) Set up time laps loop if needed
   7) Set up transmitted or reflected shutter if needed
   8) Set up reflected lamp if needed
   9) Save the experiment if needed

7. **Turn off the microscope**
   1) Save all your files
   2) Close Cellsens software
   3) Turn off shutter controller (black box)

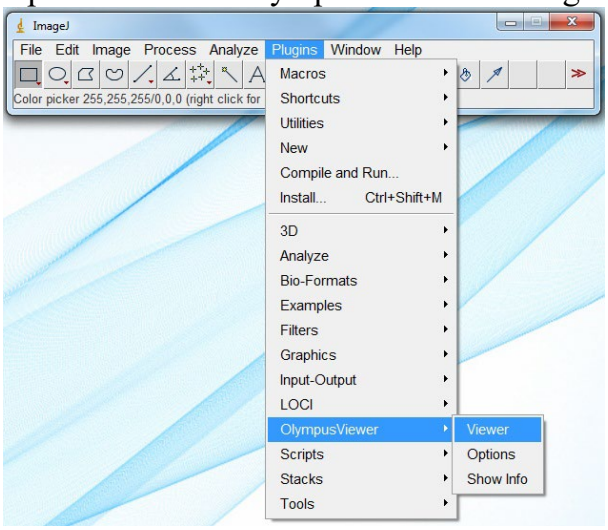
4) Turn off touch panel controller on the screen and its main switch on the back
5) Turn off main switch of control box BX3-SSU
6) Turn off main switch of control box IX3-CBH
7) Turn off main switch of mercury lamp X-Cite
8) Sign in log book

Note: The experiment or process file will automatic saved under E:/user/temp as .vsi file. To view the images in the experiment or process file. The .vsi file can be open in ImageJ by following instruction http://imagej.net/OlympusImageJPlugin.
Or open the .vsi file in Cellsens software, then under file, select export to export all the images into a folder as .tiff files.

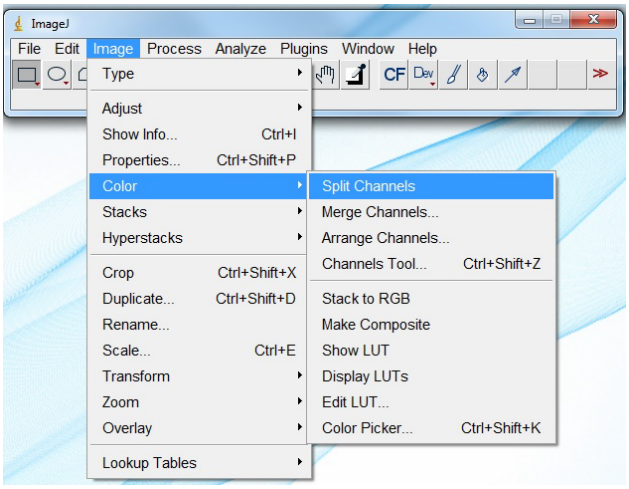
How to open .vis file images in ImageJ in correct color:

1. Download and install Olympus viewer plugin from http://imagej.net/OlympusImageJPlugin

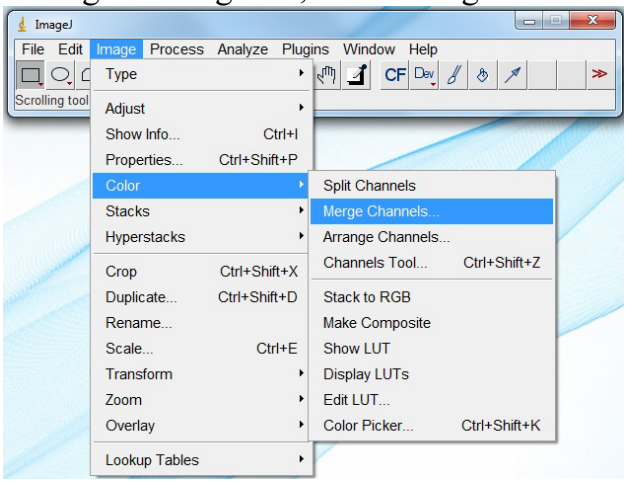
2. Open .vis file in Olympus viewer in ImageJ



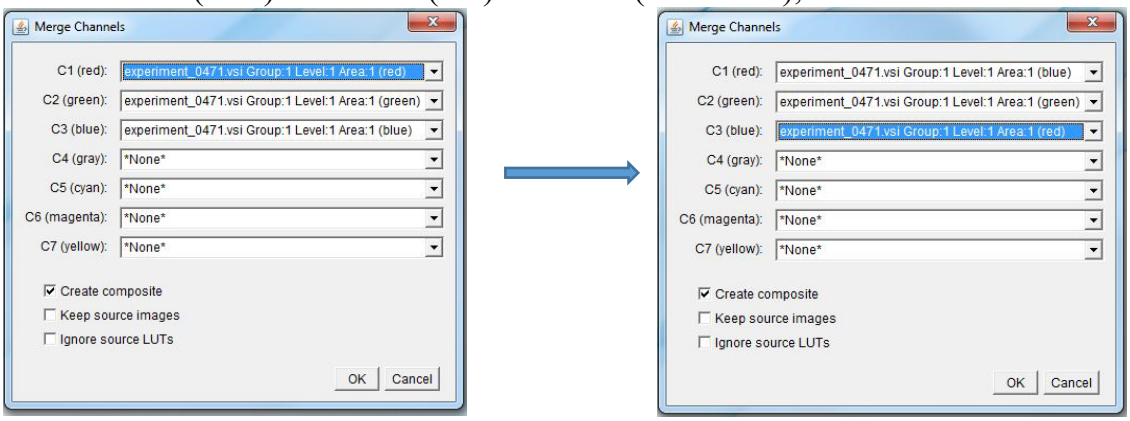
3. Go to Image tab, select Split Channels



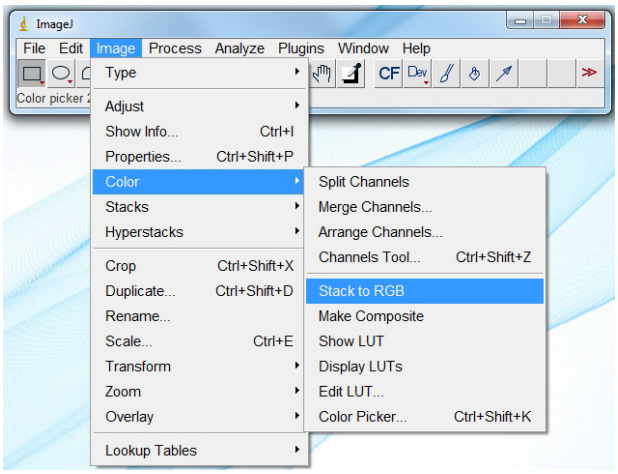
4. Then go to Image tab, select Merge Channels



5. Switch C1 to (blue) and C3 to (red) channels (see below), then click OK.



6. Final step, go to Color, select stack to RGB. At this point, the images should be in the correct color. Then save the new correct color image file.


ImageJ
File Edit Image Process Analyze Plugins Window Help
Color picker
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Split Channels
Merge Channels...
Arrange Channels...
Channels Tool... Ctrl+Shift+Z
Stack to RGB
Make Composite
Show LUT
Display LUTs
Edit LUT...
Color Picker... Ctrl+Shift+K