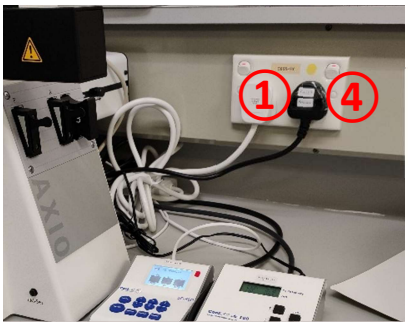
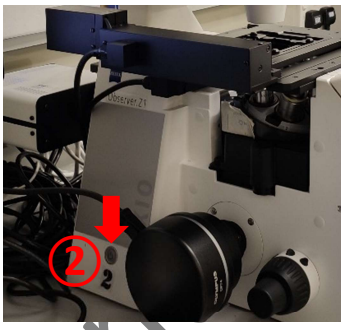
Color Imaging Microscope
Standard Operation Protocol

Turning on the system

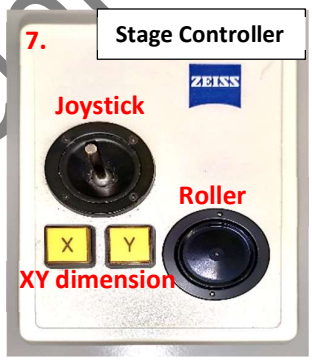


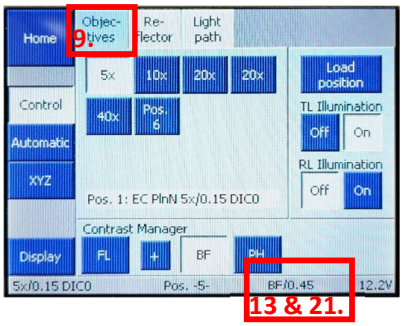


1. Sign on the log sheet before switching on system.
2. Switch on wall socket switch for the power extension ① to turn on the microscope, stage controller and brightfield light source.
   * No need to turn on switches on the individual boxes
3. Turn on microscope by pressing the power button located on the left side of the microscope ②.
4. Turn on the computer ③ and log in with "**User**".
5. **Only if needed**, turn on the fluorescence light source ④ with the wall socket switch.

Setting the microscope

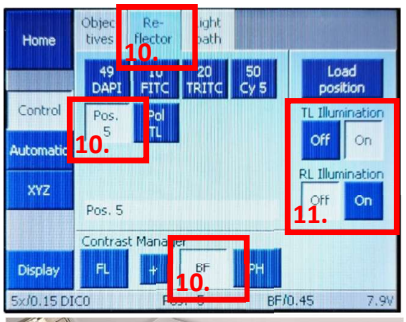
6. Put the specimen in **upside down** position (i.e. cover slip at the bottom) on the stage
7. Adjust the position with the stage controller. Press X/Y for moving dimension before rolling.
8. Touch the "**Microscope**" icon on the left side of the microscope display screen.



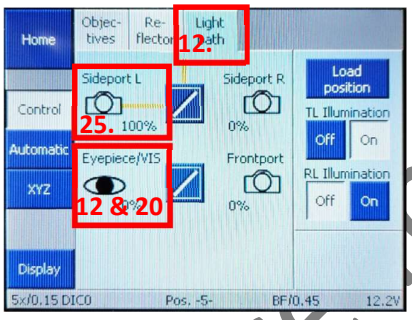


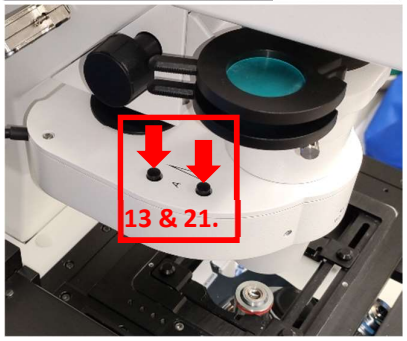
9. Select desired objectives "***5X/10X/20X long distance[LD]/20X/40X***" on the "***Objectives***" tab

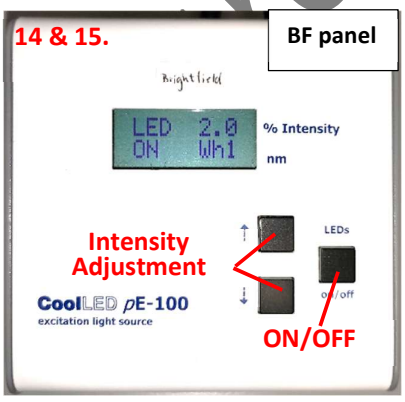
For Brightfield,



10. In the "**Reflector**" tab of the "***Control***" page, select "***BF***" and "**Pos. 5**".
11. Check that "**TL illumination**" is "**On**" and "**RL Illumination**" is "**Off**".
12. Switch observing mode to "***Eyepiece***" by touching  in "***Light Path***" tab.
13. Adjust the condenser aperture for every objective to-be-used to "**0.45**" by pressing the **up** or **down** arrow on the condenser.
14. Turn on brightfield light source by pressing the "***ON/OFF***" button on the BF panel.
15. Adjust the intensity by pressing **up** or **down** arrow.
16. Adjust focus as needed by turning the knobs.
17. Turn **off** the light source when is not in use.

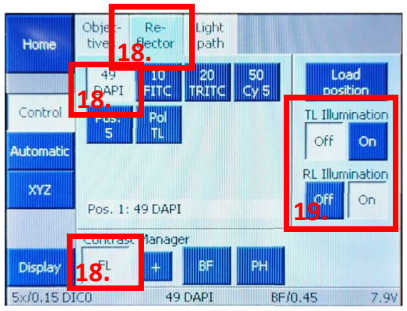




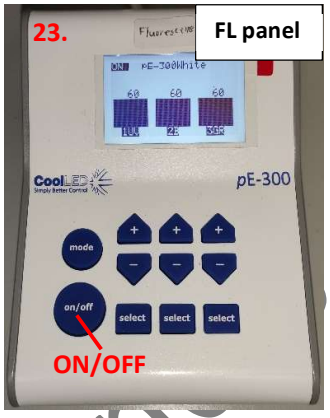


For Fluorescence,

18. In the "**Reflector**" tab of the "***Control***" page, select "***FL***" and the corresponding filter "***DAPI/ FITC/TRITC/Cy5***".
19. Check that "**TL illumination**" is "**Off**" and "**RL Illumination**" is "**On**".
20. Switch observing mode to "***Eyepiece***" by touching  in "***Light Path***" tab.



21. Adjust the condenser aperture for every objective to-be-used to "**0.45**" by pressing the **up** or **down** arrow on the condenser.
22. Turn on the fluorescence light source by pressing the "***ON/OFF***" button on the FL panel.
    * Do not change the setting of the three channels. By default the intensity is 60% for each channel.
23. Adjust focus as needed by turning the knobs.
24. Turn **off** the light source when is not in use.

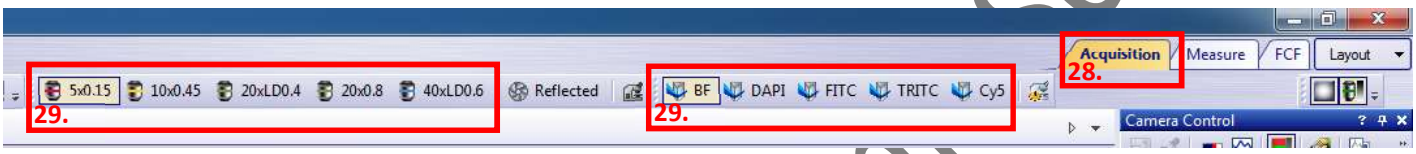


Acquiring Live Image and snapshot

25. Find the field of view (FOV) with eyepiece
26. Switch observing mode to "***Sideport L***" by touching 

in "***Light Path***" of the microscope display screen.
27. Launch the "*cellSens Standard*" software 

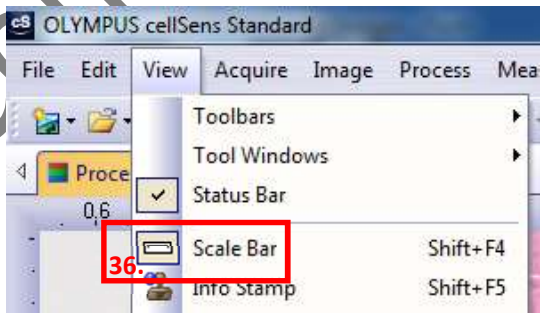
on desktop.
28. Select "Acquisition" tab on the upper-right corner.

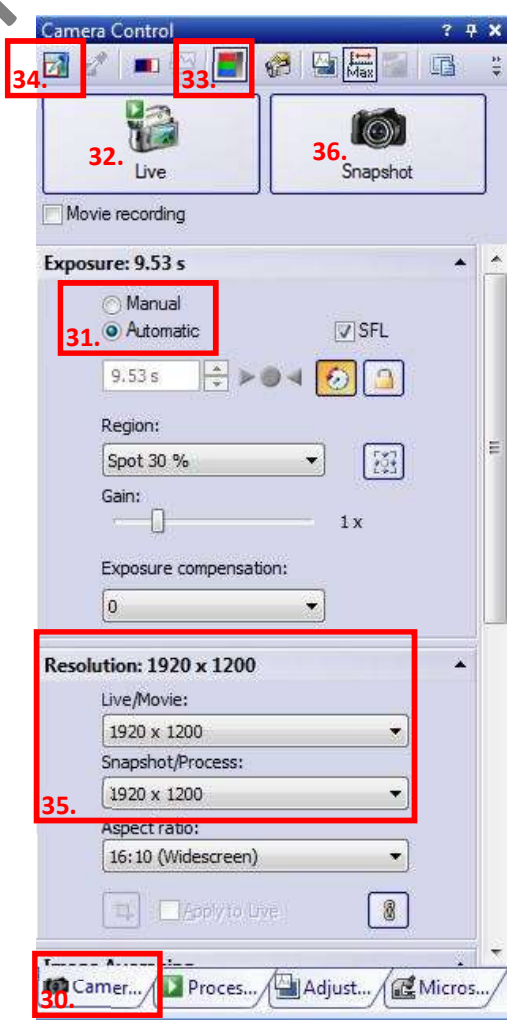


29. Choose the corresponding objective and mode of imaging "**BF/ *DAPI/ FITC/TRITC/Cy5***" in the software.
    *The microscope is not connected to the computer, so remember to select correct objective **MANUALLY** for better image quality.
30. Press Camera Control.
31. Adjust the exposure time (manual/automatic) on the right column.
32. Press the "*Live*" button 

to acquire live image.
33. Select RGB mode 
instead of B&W 
.
34. Set white balance by pressing 
and drag to select a blank region.
35. Select the desired resolution of image.
36. Go to a region of interest (ROI), press the "*Snapshot*" button 

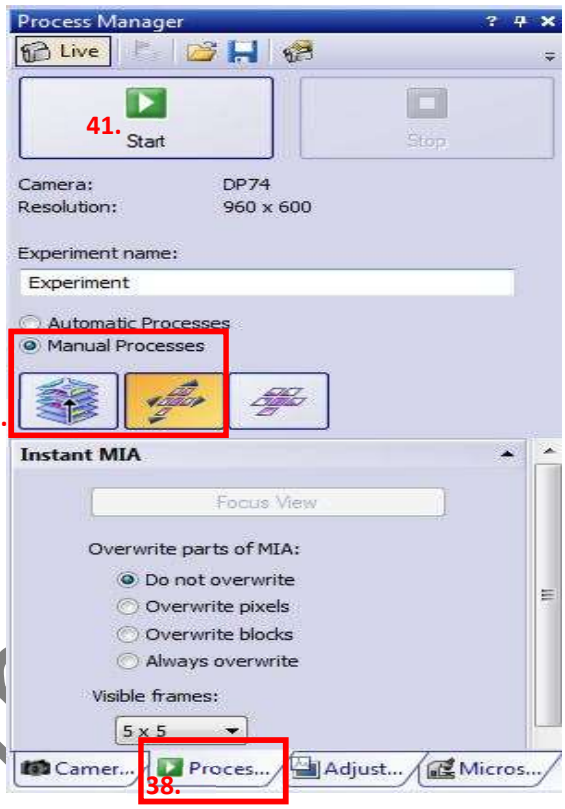
to acquire image
    * Scale bar can be set under "View" > "Scale Bar".
    * Scale bar for each objective is saved in "C:\User\FCF USER\Desktop\User\Scale bars"





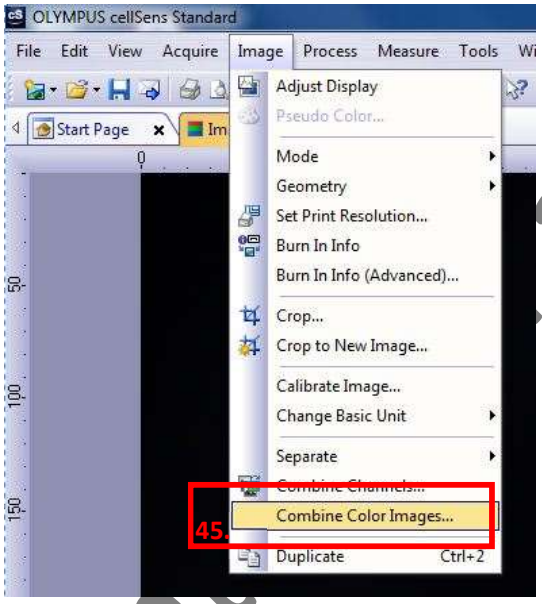
Manual Stitching

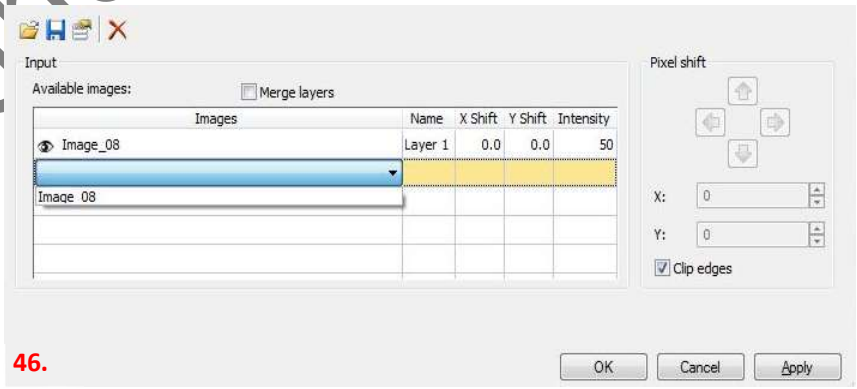
37. Check the correct setting for the objective and mode of imaging.
38. Press "*Process Manager*".
39. Select "*Manual Process*" > "Instant MIA"  .
40. Move to a position **WITH** objects (*cellSens* **CANNOT** stitch area with blank only).
41. Click "*Start*" to acquire.
42. Move the stage **MANUALLY** by the stage controller (rolling is preferred) to acquire full slide (or desired range of) image.
43. Press "*Stop*" to finish.



Combining Multiple Images

44. Open all images that needed to be merged.
45. Click Image > Combine Color image.
46. Select the corresponding images and press "*OK*".





Data Saving

47. To save the image, press  on the top bar.
48. Save in Desktop > User > PI > Name > image.tif/jpg
    * Fluorescence image.**tif** might show black in window, however it can be opened in imaging software (e.g. imageJ).
    * Scale Bar can be saved as overlay in **.jpg** file.
49. To open existing image  , press and choose from the corresponding folder.

Data Transfer (for details, please refer to Q & A section on FCF website)

50. Map to MEDVPN2.
51. Connect to FCF server with HKU portal account.
52. Upload files onto the server.
53. Disconnect from FCF server.
54. Disconnect MEDVPN2.

Turning off the system

55. Exit the software. Remember to save all wanted images before exit.
56. Turn off the computer ③.
57. Select "*5x*" objective.
58. Press "*Load Position*" on the microscope display screen to lower the nosepiece.
59. Turn off microscope ②.
60. Switch off the wall socket ①.
    * Do **NOT** turn off individual switches on the boxes.
61. Turn off the fluorescence light source ④ if turned on.
62. Cover back the microscope with the cover.
63. Sign on the log sheet before departure.