# 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 controller and brightfield light source.
   * No need to turn on switches on the power extension bar.
3. Remove the microscope cover.
4. Turn on microscope ② by pressing the power button located on the left side of the microscope.
5. Turn on the computer ③ and log in with "**User**".
6. **Only if needed**, turn on the fluorescence light source ④ with the wall socket switch.

Setting the microscope

7. Put the specimen in **upside down** position (i.e. cover slip at the bottom) onto the stage with appropriate adaptor.

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.
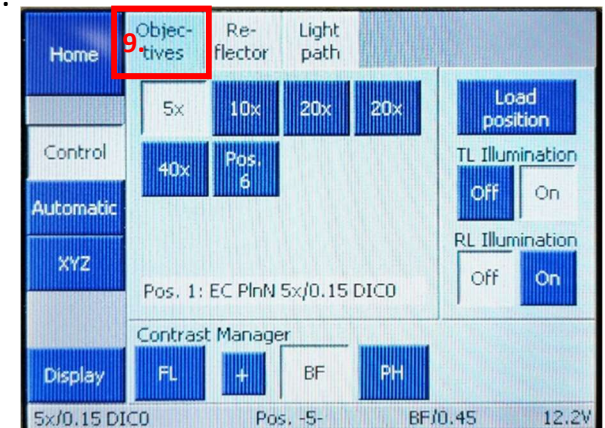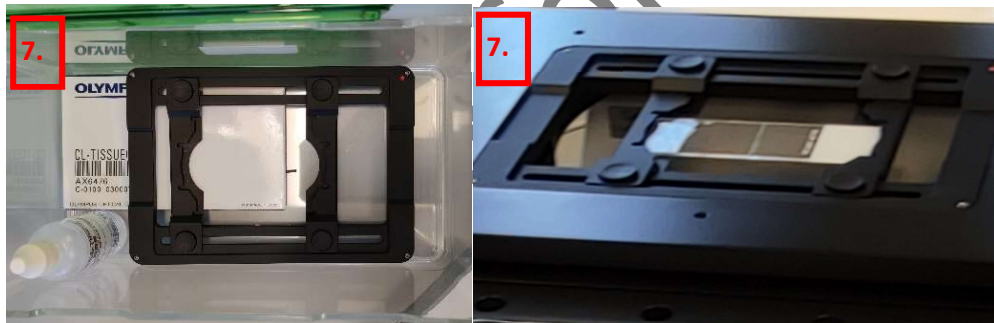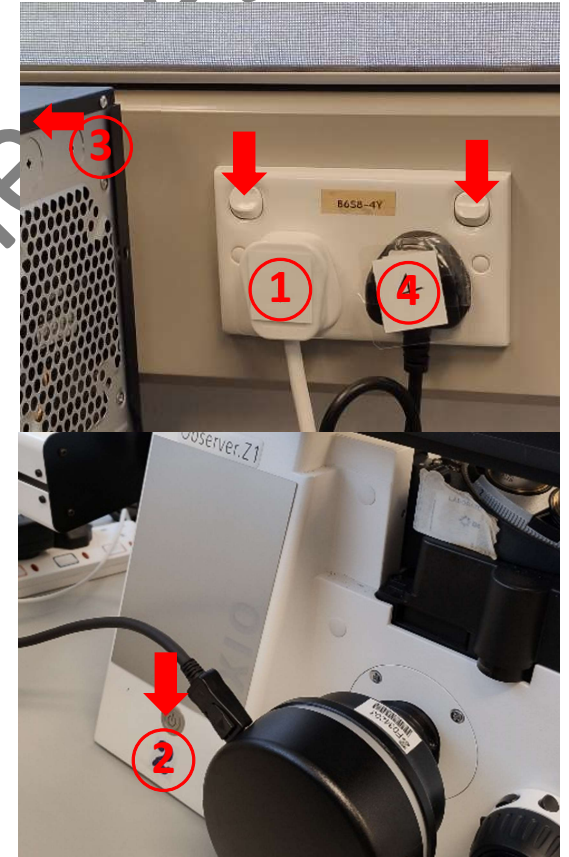
For Brightfield,

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 pressing ◑ 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.
    * By default, the intensity is 7% for each channel.
23. Adjust focus as needed by turning the knobs.
24. Turn **off** the light source when is not in use.
25. Cover your sample with the black cover if needed.
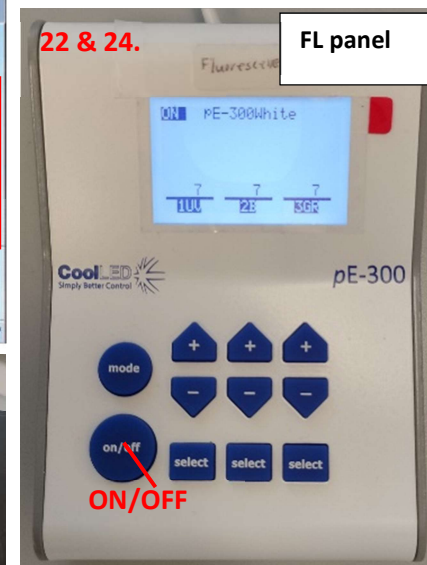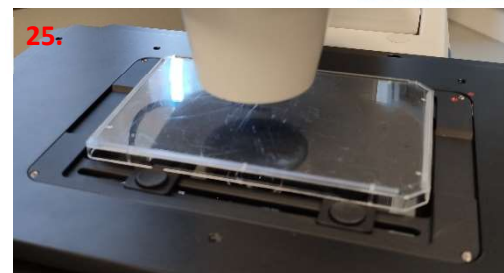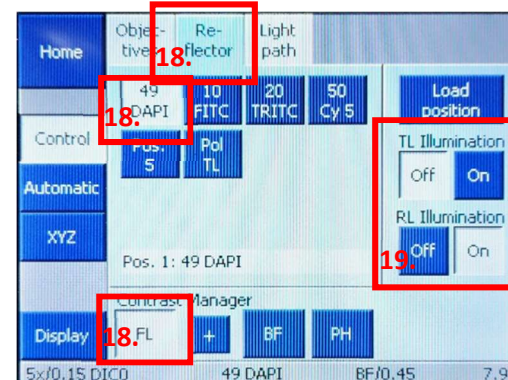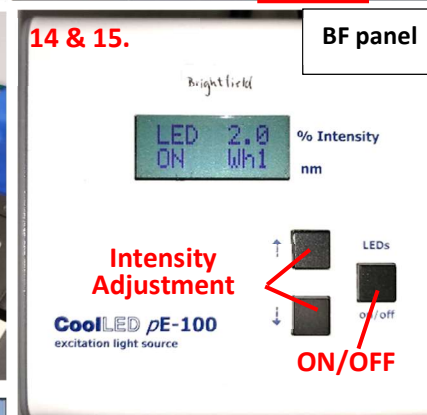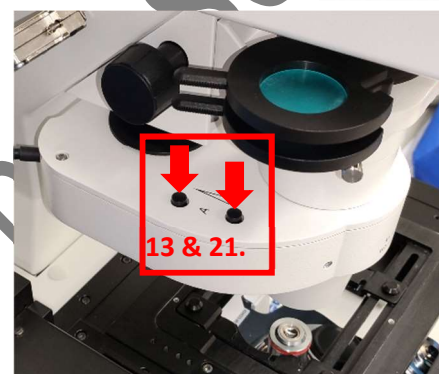
Acquiring Live Image and snapshot

26. Find the field of view (FOV) with eyepiece

27. Switch observing mode to "**Sideport L**" by touching [Sideport L] in "**Light Path**" of the microscope display screen.

28. Launch the "**cellSens Standard**" software [cS] on desktop.

29. Select "**CPOS**" tab on the upper-right corner.

Acquisition / Measure / **CPOS** / Layout ▼ — **29.**

🐞 5x0.15 🐞 10x0.45 🐞 20xLD0.4 🐞 20x0.8 🐞 40xLD0.6 🐞 2.5x0.12 | 🔅 Reflected | 🐝 BF 🐝 DAPI 🐝 FITC 🐝 TRITC 🐝 Cy5
30. — **30.**

Camera Control

30. 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 proper scale bar.

31. Select the "**Acquisition Setting**" button 🎁 to change saving name/location.

32. Press Camera Control.

33. Adjust the exposure time (manual/automatic) on the right column.

34. Press the "**Live**" button to acquire live image.

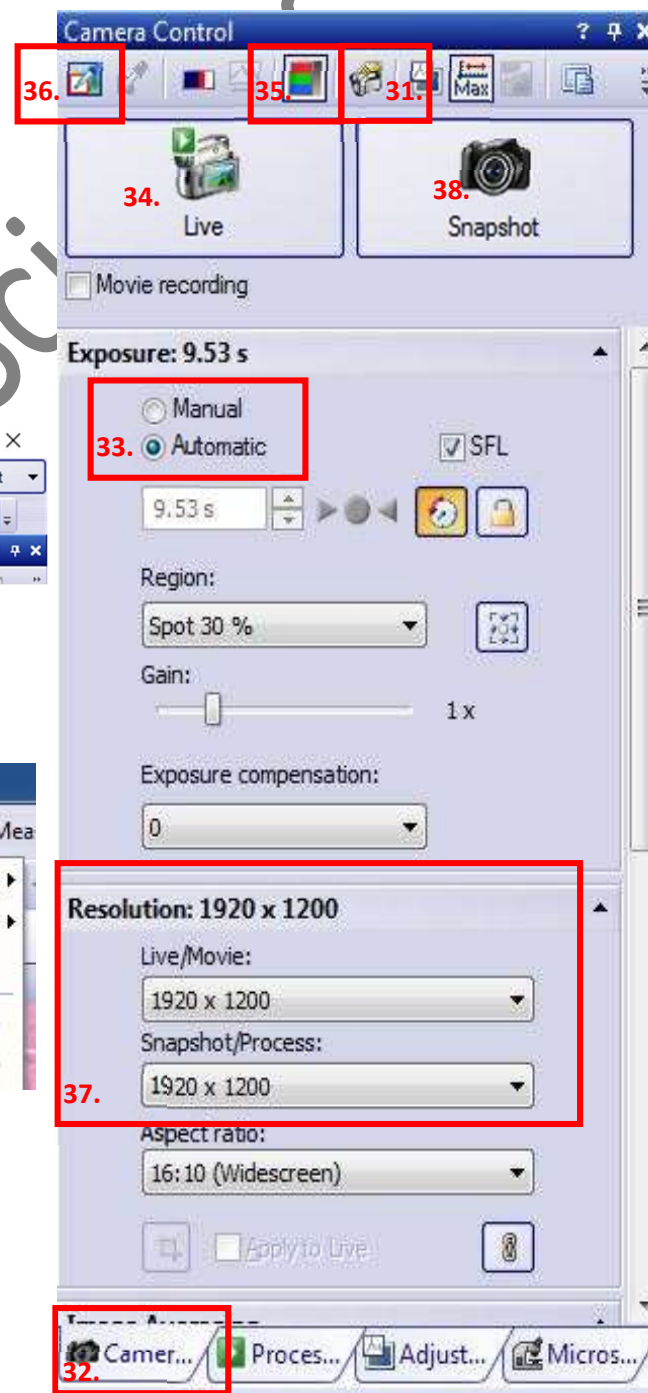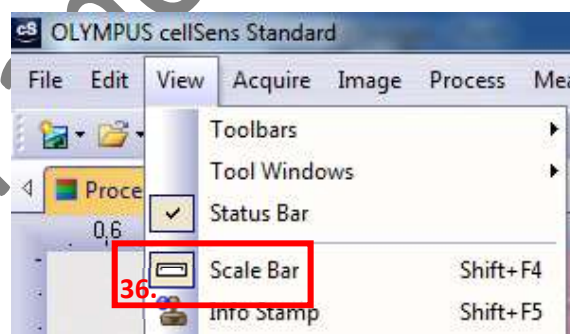35. Select **RGB mode** 🟥 instead of **B&W** ⬛.

36. Set white balance by pressing 🖼 and drag to select a blank region.

37. Select the desired resolution of image.

38. 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 "**E:\User\Scale bars**"

cS OLYMPUS cellSens Standard

File Edit View Acquire Image Process Mea

- Toolbars ▶
- Tool Windows ▶
- ✓ Status Bar
- **36.** ☐ Scale Bar   Shift+F4
-     🖼 Info Stamp   Shift+F5

Camera Control ? 📌 ✕

36. 🖼 ⬛🎞 **35.** 🟥 🎁 **31.** 📏 Max

☐ Movie recording

Exposure: 9.53 s ▲

33. ○ Manual ◉ Automatic   ☑ SFL

9.53 s ▲▼ ▶ ● ◀ 🧭 🔒

Region:
Spot 30 % ▼   [⚙]

Gain:
━━━▮━━━   1 x

Exposure compensation:
0 ▼

Resolution: 1920 x 1200 ▲

Live/Movie:
1920 x 1200 ▼

Snapshot/Process:
37. 1920 x 1200 ▼

Aspect ratio:
16:10 (Widescreen) ▼

🔲 ☐ Apply to Live [🔋]

34. Live   **38. Snapshot**

🎥 Camer... / 📊 Proces... / 📈 Adjust... / 🔬 Micros...
32.

3

Manual Stitching

39. Check the correct setting for the objective and mode of imaging.
40. Press "**Process Manager**".

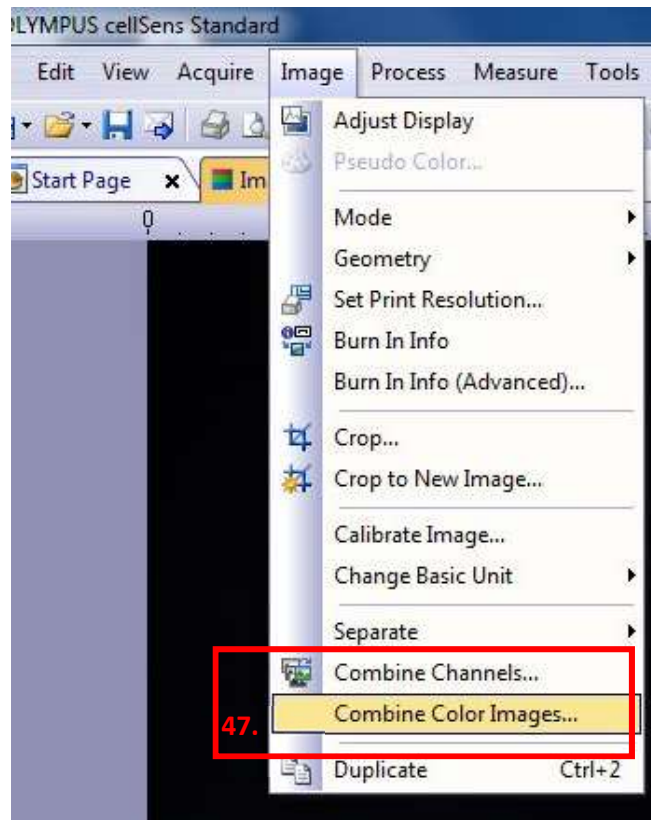41. Select "**Manual Process**" > "Instant MIA" .
42. Move to a position **WITH** objects (*cellSens* **CANNOT** stitch area with blank only).
43. Click "**Start**" to acquire.
44. Move the stage **MANUALLY** by the stage controller to acquire desired range of image.
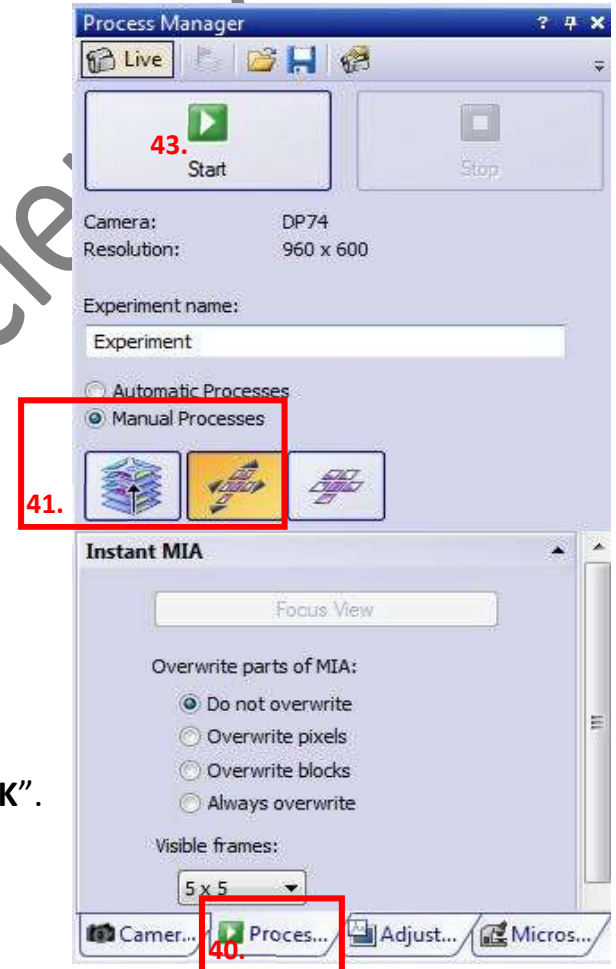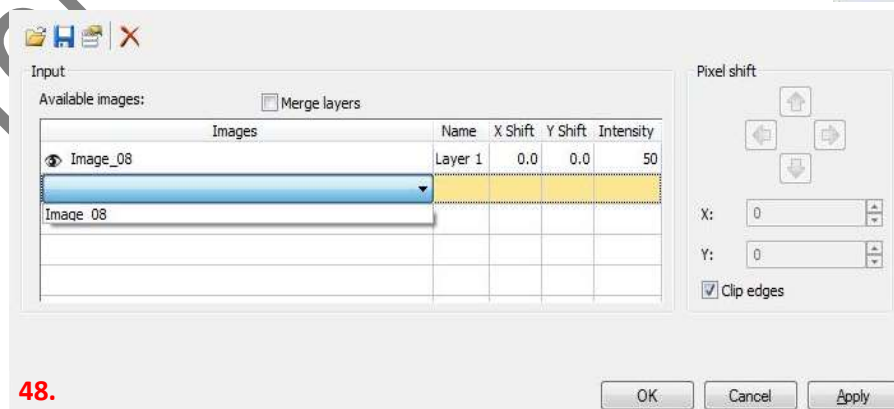45. Press "**Stop**" to finish.

Combining Multiple Images

46. Open all images that need to be merged.
47. Click Image > Combine Color image.
48. Select the corresponding images and press "**OK**".

Data Saving

49. To save the image, press ![save] on the top bar.

50. Save in "**E:\User\ (PI) \ (Name) \ (image.tif/jpg)** "
    * Fluorescence image.**tif** might show black in Windows, however it can be opened in imaging software (e.g. imageJ).
    * Scale Bar can be saved as overlay in **.jpg** file.

51. To open existing image ![folder], press and choose from the corresponding folder.

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

Turning off the system

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