FV1000 Confocal Laser Scanning Microscope Operation Instruction

Part I: Turn on microscope, set up sample and scanning channel

1. Turn on the microscope according to the sequence.

2. Double click icon ![FV icon] to open the software, then click OK (no password).

3. Click on button [Escape] on acquisition setting window to lower the sample stage, load sample slide, then click button [Escape] again to up the stage.

4. Select the objective lens on the hand switch panel, then open the transmitted light shutter by click button [icon] on image acquisition control window.


5. Move sample by the joystick. Use the focus knob on either side of the microscope to focus sample. You can click on button [Fine] on acquisition setting window to choose coarse or fine focus. click button [icon] again to close the transmitted light shutter.

6. To check the sample under fluorescent light, click on button [icon] on image acquisition control window to open the fluorescence light shutter, then select the fluorescent light wavelength on the hand switch panel. After focus sample, close the fluorescent light shutter by click on button [icon] again.

7. Before start scanning, click button [icon] on the image acquisition control window to open Dye list window. To select a Dye, double click on the Dye

name, the Dye will appear on the assigned Dye window area. To unselect a Dye, double click on the Dye needs to be removed. When finished, click on Apply button, the selected Dyes will appear in different channels. Then close Dye list window.

Part II: start scanning

8. To start scanning, click button .

9. If there are more than one channel image scanning, get multi-channel view in live scanning window by click button , then choose .

10. Adjust scanning image by using the tab in each channel, normally on starting, set Gain value as 1, Offset value as 0. Adjusting HV value to increase or decrease scanning image intensity (normally the best range for HV is 500-800). Increasing Gain value will increase scanning image intensity; increasing Offset will reduce noise in the background. (Beware of increase Gain will increase noise in the image background and increase Offset with reduce information on the image). If HV is out of the 500-700 range for good imaging, can adjusting the laser intensity to bring the HV back to the 500-700 range.
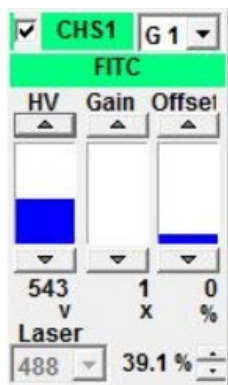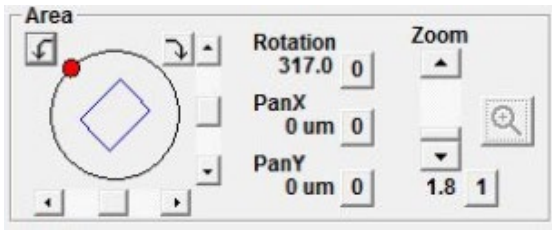

11. Adjusting the scanning speed by using the sliding bar

Adjusting the scanning image pixel setting by using

Adjusting zoom, image rotation and image small movement (only work when zoom is not 1) by using


Part III: XY image acquisition

12. When ready to take image, click button . Then adjusting scanning speed to slower speed, click button to start acquire image.
13. When the image acquisition finished, a 2D view window will pop up with the acquired image.
14. If there are more than one channel image acquired, make sure the 2D view window is in single view mode .

15. Select which channel image show up by click on channel button to select the corresponding channel.
16. Save image: right click on the image, select **save as** to save image as .oib or .oif file which can only open in the Fluoview software. .oib or .oif file will save all the image acquisition setting too; Select **export** to save each channel's image as a .tiff file (no combine channel image). Select **save display** to save whichever image showed in the 2D view window as a .tiff file.

Part III: XYZ image acquisition

17. Follow step 8-11 to start scanning, click , then click on or until reach the sample upper limit or the sanning image just dissapeared, click button "set" under "start"; Then click on or until reach the sample lower limit or the the sanning image just dissapeared, click button "set" under "end".
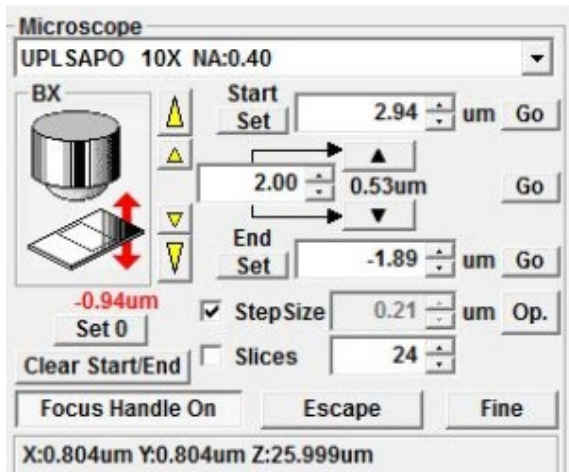
18. Enter the step size, select slower scanning speed

19. Click button [Depth], then click [XY LZ] to acquire stack image.

20. When finished click on [Series Done], the a 2D view window will pop up with the acquired image.

21. If there are more than one channel image acquired, make sure the 2D view window is in single view mode [□].

22. Click button [⊕], then click [Z↕] to show the projection view of the acquired stack image.

23. Select which channel image show up by click on channel button [1 / 2] to select the corresponding channel.

24. Save image: right click on the image, select **save as** to save image as .oib or .oif file which can only open in the Fluoview software. .oib or .oif file will save all the image acquisition setting too; Select **export** to save every step image of the selected channels as a .tiff file in a folder (no combine channel image). Select **save display** to save whichever image showed in the 2D view window as a .tiff file.

25. When finished, turn off microscope according to the sequence.