

# Olympus FV 3000 operation manual

1. Switch on PC (password olympus)
2. Switch on the main power switch on the cord extender in the Rack. Stage control and microscope controller will switch on automatically.
3. Switch on laser lines which you plan to use. System has 405nm, 445nm, 488nm, 514nm, 561nm, 594 nm and 640 nm cw diode lasers.
4. Turn on the key to activate the lasers. The lasers will blink with green. Then they show white lamp on, they are ready to use.
5. You can add a laser line later when software is running too.
6. Turn on touch PC (the switch is on the back) and wait until it is initialized. Blue and green light should light up on the controller.



7. If you need heating and CO<sub>2</sub>, switch on the cord extender for the Cell Vivo (heating and CO<sub>2</sub> control unit) and start Cell Vivo software. And start heating 1hour in advance minimum
8. Start the FV31S-SW software
9. **Now you can switch on FLIM system (use a FLIM guideline)**
10. Keep resonant scanner unchecked if you do not need it. If you need it, for stability start it 30 minutes before your scans.
11. Keep other settings in the start dialog unchanged (enable XY on)
12. Choose **NO** for stage cleaning
13. Choose objective either in software or on touch PC. The microscope has 1.25x NA 0.04, 4x NA 0.16, 20x NA 0.8, 40x NA 1.4 oil, 30x NA 1.05 Silicon oil and 60x NA 1.3 Silicon oil. If using oil objective, **CHECK THAT YOU USE RIGHT TYPE OF OIL**
14. Check the collar correction on the objective. It should be set to 0.17mm if you use 1.5# cover slips. Note that at 23C and at 37C the correction is different.
15. Use the Camera/Eye button on the touch panel controller to switch between Laser Scanning Mode (LSM, camera symbol) to Ocular view (eye symbol). You will probably need to adjust light intensity.
16. Find your sample using epifluorescence (EPI) or transmission light (DIA). For EPI select Quad filter, on position. 2.
17. For EPI choose 1V for violet excitation (DAPI), 2B for blue excitation (GFP) and 3GR for red excitation (RFP) for LEDs.
18. Open and close shutter when needed
19. Check that COOL LED is on.



20. In software choose in Ocular pannel, and LSM
21. In the "LSM imaging "panel adjust adjust Scan Setting: Scanner Type: Galvo, Oneway mode. Scan speed: 2µs/pix.
22. In the "PMT" panel adjust Scan Setting: use VBF.
23. In the "PMT" panel set channels for your dyes, by pressing the "Dye & Detector select" button. Adjust emission bandwidth if needed.
24. In the "PMT" panel check the confocal aperture. Use AUTO to have it averaged for all channels.
25. Start with low laser power 0.5%, HV 500, offset 0% (max 3%), do not change gain. You can set up two 4 channels in one group plus transmission light channel. Detection regions should not overlap with laser lines.
26. In the "Area Settings" panel select the Scan Size and use the Optimize button to adjust the Zoom for Nyquist-sampling
27. Check for over and underexposure (Hi-Lo).
28. Check bleedthrough, i.e if you see signal from one laser in another channel. If bleedthrough, go to "PMT" panel, select "sequential sc" and the mode "Line" or "Frame" (Frame is faster).
29. Be careful and do not overexpose PMTs. (indicated by red colour in Hi-Lo mode)
30. Set a folder where your images will be saved. Use the D-drive for it and find your AG- folder. The image format will be \*.oir
31. Set averaging/accumulation if you need it.
32. Check if Z series and t-series are off
33. Do your imaging: press **LSM-START**



## Finish your work

1. When finished: close the software.
2. if used heating and CO2 then stop heating and CO2 in the Cell Vivo software and close it. Switch off Cell Vivo switch on the power cord. Close CO2 valves.
3. Copy your data to the data transfer server.
4. **Switch off FLIM system if it was on**
5. On the touch PC screen press OFF, when OLYMPUS is displayed, switch off the touch PC using the switch on the back.
6. Turn the key to deactivate lasers.
7. Switch off all lasers which are on.
8. Switch off the main switch on the power cord in the rack.
9. Remove your sample
10. Clean oil objective with isopropanol and lens paper.
11. Write you name, date and comments in the electronic log book.
12. Switch off the PC.

## For Z-stack:

1. In acquisition choose Z-series on (Z Section panel, bottom right)
2. Find bottom position, register it, then find a top position and register it.
3. Go from the lower position to higher position.
4. Optimize step size accordingly to your needs (Or press the "Optimize" button).

## **ZDC: drift compensation (does not work with 1.25x and 4x)**

1. Check the current position and check NEAR-limit settings: approx. numbers 1800 for cover slips, petri dish 2300, well plate about 3500. (Please verify the values for petri dish and well plate with your vessels again)
2. Press "Apply entered parameters to near limit".
3. Place ZDC dichroic mirror in: **ZDC DM IN**
4. On Touch PC press START for focus search in EPI mode. If it is successful, you hear one beep.
5. Use Single shot mode for multipoint imaging experiments (Z stacks etc) and Continuous mode for long-term time-lapse experiments.
6. At ZDC you cannot change objectives.
7. ZDC does not work well with oil objective, but good with Silicon oil objective.

## **Objectives:**

- 30x Silicon and 60x Silicon are good for live cell imaging: SILICON OIL
- 40x oil is good for fixed samples: NORMAL OIL

## **Shortcuts:**

1. F11 start/stop live
2. F12 start image scan
3. Ctrl and mouse scroll: focusing
4. Double click on the image while live moves the stage

## OIL:



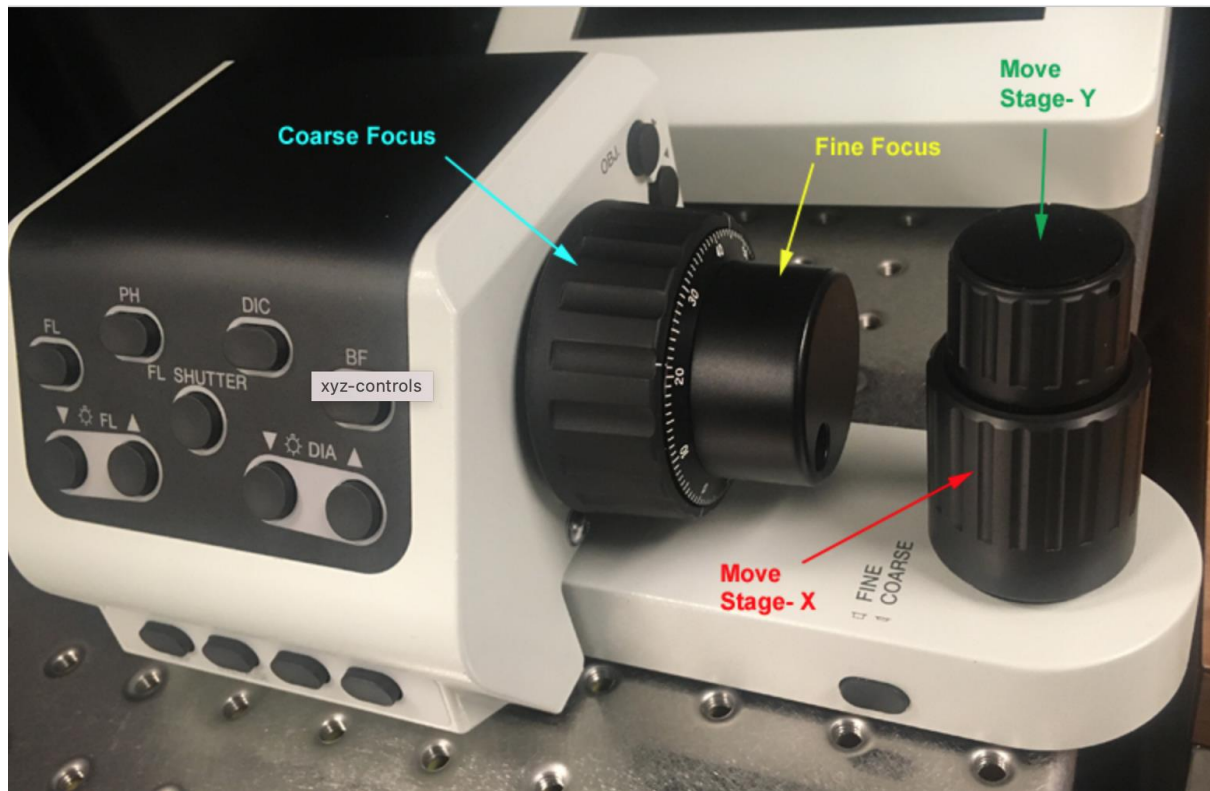
## To use Cell Vivo heating and CO2 controller:

1. If you did not start it before press the button on the power extender under the Cell Vivo controller.
2. Start Cell Vivo software.
3. To heat the incubator set the temperature **H unit 2000** to T you need and press ON.
4. You can log Temperature data.
5. To have stable temperature 1-2 hours heat up time is required.
6. When finished, stop heating
7. Close the software
8. Switch off the controller using the button on the power extender.

## Stage control

Provisionary

<https://uol.de/neurosensorik/forschung/fluorescence-microscopy-service-unit>



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