

CONDENSED OPERATING INSTRUCTIONS FOR THE KTL TILL-PHOTONICS FAST WIDE-FIELD LIVE-CELL IMAGING FACILITY

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The running costs of the imaging apparatus are charged from users by the hour based on reservations in the electronic booking system. The fee is 9.91 €/hour.

The light source provides an excitation wavelength range from 320 nm to 680 nm, with an approximate bandwidth of 15 nm. Filter sets are available for the following fluorochromes/fluorochrome combinations:

CFP

GFP

YFP

CFP/YFP

GFP/dsRed

Dehydroergosterol (DHE)=fluorescent cholesterol analogue

Dehydroergosterol/GFP

Pyrene excimer

Pyrene excimer/GFP

In addition, the microscope is equipped with a beam splitter which allows simultaneous recording of two channels. This system also makes it possible to carry out fluorescence resonance energy transfer (FRET) experiments. The filter sets available for the beam splitter application are CFP/YFP and GFP/dsRed.

The objective lenses available for oil immersion are:

40x and 60x

In addition, there is a 40 x water immersion lense.

NOTE! Make sure that you never contaminate the water immersion lense with oil!!!

Basic operation

1. Start the temperature regulation on the day before the experiment (or latest 2 h before) – the Solent Scientific box on top of the temperature controlled chamber)
2. When beginning the experiment, switch all power switches off
3. Start lamp (the large box, main switch in the left-hand rear corner; the lamp is started from the black button in the front panel)
4. Start camera (on the left side of the microscope, button in the middle, between the wires)
5. Restart temperature control
6. Turn on the switch of the through-light, the right-hand rear corner of the microscope
7. Turn on the monitors
8. Turn on the computer
9. Log on as “administrator”, no password required, logon to
10. Start the program Vision 4
11. Apply a drop of oil on oil immersion lense or water on the water immersion lense; Put your cell dish on top, add the CO2 box and close the lid of the box.

12. Open the CO₂ bottle (5% CO₂ in air, gas mixture) valve if you are using a bicarbonate-buffered medium that requires CO₂.
13. Open the needle valve, the running pressure has to be below 0.7
14. Turn on the CO₂ from the Solent Scientific box (purge time about ½ for the small CO₂ box)
Flow: Adjust the gas streaming after the purge is finished, about 1 bubble at a time in the water (on the right at the bottom of the chamber). NOTE! When you open the chamber, always switch off the CO₂!!!
15. On the PC:
 - Grab settings
 - Reset camera settings
 - image type: fluorescence
 - wavelength: for instance 480 (GFP)
 - switch on: light enters microscope
 - switch off: light is off
 - horizontal: 2
 - vertical: 2
16. Push on the through-light switch on the left front
17. Select the appropriate filter: Roll the filter wheel within the chamber. When necessary, change filter wheel:
 - objective lense in the low position
 - specimen table as far back as it goes
 - pull filter wheel out, directly to the right
 - insert the other filter wheel
 BE CAREFUL! This may require use of some force.
18. Finding cells
 - the wheel on the right hand side in the “eye” position
 - form the program snapshot: on (fluorescence is switched on)
 - when nice cell is found: switch off

Beginning the recording

19. Turn the wheel on the right-hand side to “SP” position, when you start recording
20. Live: Focus from the microscope when necessary; stop
21. File; open workspace
D:/vision workspace templates:gfp.vws
Select for example ready-made program 480 10’’ loop 250 x protocol >repeat 250 times
Repeat 250
Cycle time 30 000 ms (2 h)
Fluorescence 480nm> exposure time 20-40 –100 ms; (for example 10-20 ms for a 2 h program)
Video on: arrow down and to the side-icon on the top (execute protocol)
Finishing: Either “break” or when the program has come to an end
22. Fluorescence 480 nm double click
From the right-hand side; overlay and A-from the top panel Save workspace as
Watching the video: manual – you can adjust brightness; adjustment of speed from bottom right
23. Saving files: While you record save the files on :/E. After you have finished transfer the file to external mass memory device (:/H)
or

log out

log on to the network using your personal profile and save your files to BBU-server

Mapping the BBU server

Map network drive (or start, run)

\\BBU-NASA.ktl.fi

“Connent using a different user name”

Type in BBU username and password (username begins with ltdk\BBU-...)

Go to BBU-Root and the filespace of your organization and group there.

24. Closing down:

Switch off all power switches and the CO2 supply.

Clean any spilled oil/water from the microscope and clean the objectives.