Simple user guide for the KTL (MLO) Leica TCS SP1 confocal microscope/ Vesa Olkkonen Aug 9, 2005

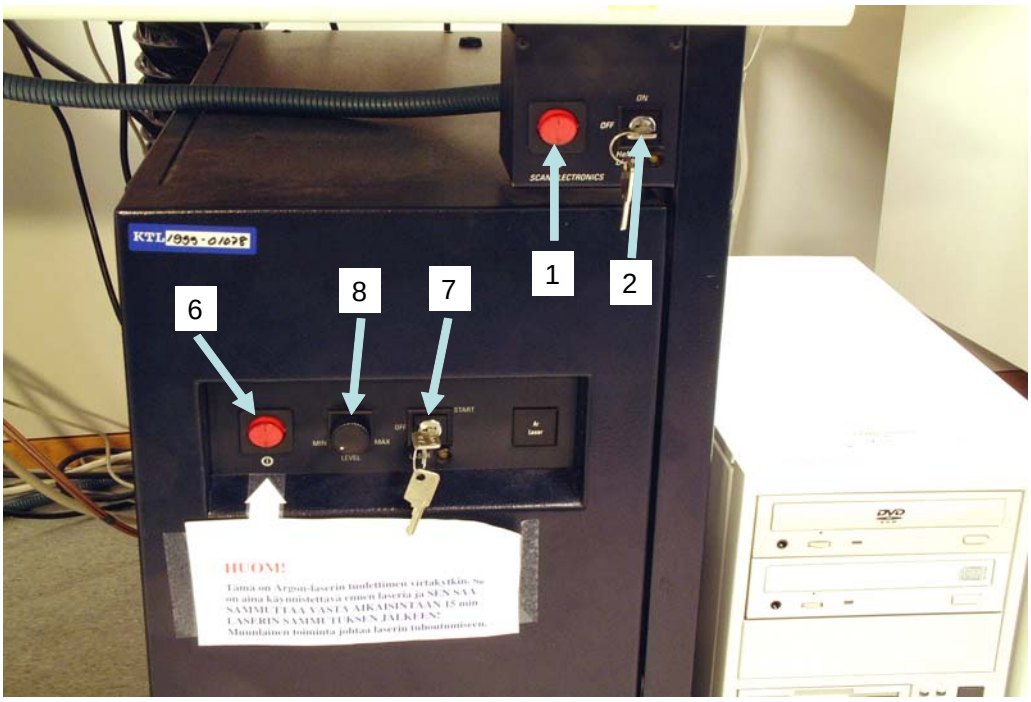
From users from outside the KTL we charge 20.63 € per hour to cover the running and laser renewal costs. This is done based on reservations in the electronic booking system. For this purpose the users have to fill in a research agreement form which also contains the billing address- contact Vesa Olkkonen, room A309b, tel. 4744 8286, e-mail vesa.olkkonen@ktl.fi

The available laser excitation lines:

Argon: 458, 476, 488 and 514 nm
Melles Griot diod laser: 561 nm
He/Ne: 633 nm

Note!

Before start-up, check log book for any recent problems. Make your switch-on time entries to the log book. Make sure that you only turn on those lasers that you need for your specific purpose. Check you fluorochrome excitation and emission spectrum against the available laser excitation lines listed above.



Start-up:

-turn on the PC and the 2 monitors
-turn on scan electronics (1)
-turn on the HeNe laser (Cy5 etc excitation peaks) using the key (2)
-turn on the Melles Griot diod laser (TRITC, Texas Red, Alexa568 etc) using the key (3) on the small control unit on the table, and press the green on button (4) – two yellow leds are lit, the upper one blinks –when it stops blinking, the laser is on
-turn on the power source of the Argon laser (6)
-turn on the Argon laser (7) (the key works like the ignition in a car, turn it to the right, let the key return to center position, the yellow light indicated that the laser is on)(FITC, GFP, Alexa488 etc. excitation peaks)
The IF microscope:
-start the power source of the Mercury lamp (on the floor)
-turn on the power of the microscope itself
-adjust Argon laser power (the black knob to the left of the laser ignition key) to 11 o'clock position (8)

Shut down:

Log off and shut down the PC
Switch off:

-the monitors
-scan electronics (1)
-HeNa laser from the key (2)
-the Melles Griot diod laser by pressing the red off button (5)(you can also switch the key to 0 position, but this is not necessary)

-the Argon laser from the key (7) (before this turn the laser power to minimun from the black knob (8) beside the key

If you have used an oil immersion objective lense (40x, 63x or 100x), clean the lense first with dry lense paper, then with one soaked in the objective rinsing liquid, and then dry it with fresh lense paper.

NOTE! Never switch off the power of the Argon laser unit (6) before the laser, since also the cooling fan of the laser is under this power switch! Leave the power on for 15 min after the laser is off. **Failing to obey this order can lead to destruction of the laser! This also leads to cancellation of your user rights! Be careful!**

-the power of the IF microscope and the Mercury lamp

-after turning off the Mercury lamp, it has to cool down for at least 30 min before restart!

NOTE!

Always write the information on your session in the user log book, including the time you switched everything off, how long you used the system, and the reading on the display on the Mercury lamp power source.

If you are not using your booked time, you are responsible that the apparatus is appropriately switched off, not the previous user. Also, please inform the next user that she/he can have the system earlier if you do not need you booked time.

Acquiring images

KTL users: Log on the KTL domain with your own user id and password. Then map network drive \\BBU-NASA.ktl.fi to be able to store images there under your group's division. To map this drive you need a BBU user ID and password.
External users: Log on the local domain (CONSTELLATION) as TCS_user. No password required. Then map network drive to the NASA server at BBU as specified above, using your own BBU username and password.

Start the LCS software (if this is the 1st time choose *Company profile*, thereafter *personal profile*)

On the left monitor you see the window for operating the system and on the right the previewer. If there are no buttons on the left edge of the previewer window use the right click button of the mouse and select *left buttons*.

Put your sample in the microscope, find a nice field and focus (if you do this before starting LCS, the focus will disappear)

Press *Acquire* – this gives you the appropriate buttons you need now

From the lower left corner buttons, choose

-*Obj* – selection of the lens (this has to be selected correctly, otherwise the size information accompanying your images will be wrong; also confocality of images depends on the correct choice here)
-*Mode* (xyz is normal xy scan)
-*Format* (1024 gives you the highest resolution)
-(scan) *speed*, we normally use medium
-*beam* – open this window, you need this to control the excitation peaks and the emission windows for getting optimal images
-select a scanning program from the right hand menu of the *beam* window, by double-clicking. You see now what excitation peak(s) and emission window(s) the system will use. You can also choose colours (look-up tables) for the channels from this window.

Start scanning with *continuous*, look at the image in the preview window on the right

Adjust photomultiplier gain from the round black knobs, leftmost is PMT1 (FITC/GFP/Alexa488 channel, the next one PMT2 (TRITC/Texas red/Alexa568), and the third PMT3 (Cy-5 channel), until you have an image with nice intensity.

Adjust Z position from the rightmost black knob (*Zpos*) to find the nicest Z level within your sample

Check that the pinhole value in the numerical display window is 1, this means that you have optimal confocality of images. You can adjust the pinhole using the black knob labelled *pinhole*. If you for some reason want to collect a "thicker" image, you can click on the *pinhole* button and select manually a desired pinhole size or widen the pinhole using the black knob.

Stop scan and select an *average* value, e.g. 4-8. This makes you an image that is averaged over several scans – you get improved signal to noise ratio and a nicer image for publication. Scan the averages image using *single scan* (now it stops after the desired number of scans).

If you are recording only one channel, choose *single* from the left buttons of the preview window. Now the image fills the entire window; If you are recording 2-3 channels, choose *tiled* – it shows all channels simultaneously as smaller images.

If you want to see an overlay of the channels, choose *ovl* from the left buttons; it shows you an overlay in the 3rd or 4th small window.

Zoom: By clicking on *zoom*, you can choose a fixed zoom value; *others* allow you to adjust the zoom manually to any value; the *Z.in* (zoom-in) button (you can bring this to your profile from *tools, customized, basic scan*) allows you to select a square area from a scanned field on the screen using the mouse and zoom into that specific region; when you press *continuous* again, the selected area now fills the entire field.

You can move the field in your sample using the arrows in the middle.

Sequential scanning of 2-3 channels: The best way to avoid channel cross-talk problems is to scan the channels separately, still getting them into the same file. There are 2 ways of doing this:

1. The slow but proper way: Scan your specimen first with one program and adjust everything (PMT, Offset, averaging). Save these setting from the *Save* button of the *Beam* window. Give the file a name. The settings will go under the *Users* menu. Do the same for the 2nd (and 3rd) channel settings. Activate the *Seq* window and *Add* or drag the saved settings into the *Seq* window. In the end, start sequential scanning from the *Series* button (*Continuous* and *Single* scan buttons will be inactive). The channels will now be scanned separately one after each other with the settings you have saved.
2. The fast way: *Add* or drag the desired scan programs from the right hand menu and into *Seq* scan window. Having focused on a nice view with a single or double channel scanning programme, set *average* at 10 and start seq scan using the *series* button. The system will scan the channels individually. During the 10 averagings of each channel you can now adjust the PMT gain to an optimal value and at the same time record an averaged view of each channel.

Scale bar: you get a scale bar in your image by ticking *scale* in a window on the left.

Saving images

Save images by *file*, *save as* commands. NOTE! Do not store any files on the hard disks of CONSTELLATION. The primary location for image files in the BBU NASA server (for mapping this path see *Acquiring images* above). As you make scans, they will be collected temporarily to the desktop in an *experiment* folder. When you save as, the entire experiment will always be saved. Therefore, before saving, remove poor images using right button of the mouse and *delete*. And, after each recorded and saved image select *new*; it gives you a new experiment and a new preview window (you can close the old one). If you have forgotten to open a new experiment with *new*, you should before saving remove all the old images from your experiment with right mouse button, *delete*. When you exit LCS, you lose all images/experiments on the desktop that you have not saved!

NOTE! You will get from a single scanned channel image 3 files: a lei one which is required for the LCS software to be able to open the image; a txt one that contains the information on the image (size, PMT gain values, pinhole value etc), and a tif one which contains the actual image. The tif file can be opened with any graphics software for further use.

If you have made an overlay, this will not be saved unless you bring this to the experiment on the desktop: activate the overlay by clicking on it with left button, then right button of the mouse: SEND TO, EXPERIMENT, SELECTION (RAW). Now you have it as a tif image in you experiment. The same can be done using SEND TO, EXPERIMENT, SNAPSHOT, which saves any possible modifications on the image. BUT: at the moment the display controller of the confocal PC does not allow snapshooting the scale bar!

Other useful functions

Z-scanning

Get yourself an *Ysel* button from *tools, customize, hardware.*
When you have an xy scan in the preview window, select *mode xzy*, then press *Ysel*. This gives you a red horizontal line which you can move within your image using the rightmost black knob (*Zpos*) to select a line from which you want a Z scan to be made. And then start scan with *continuous* or *single scan* if you want to average.

Making stacks of images

Scan your sample with *continuous*. Use *Zpos* black knob to go first to one end of your sample (top or bottom) and click *Begin* (or *End*). Then go to the other extremity and click the other one. The stop scanning and click on the small *series* button. This will show you how thick your sample is. Now you can select the number of Z planes you want to record from the *sect* button. NOTE! By selecting from this window *optimised* you get the maximal number of sections that it makes sense (from an optical point-of-view) to make from your sample. You can then use this number or select manually a lower one. Select an *average* value (to be taken from each section), and start scan from *series*. When it is done the scanner stops. You can see all the sections simultaneously if you select *Gall.* (gallery) from the left buttons of the preview window.

Eliminating overexposure

Select *glowover* from the look-up table in the beam window. Scan *continuous*ly and adjust PMT gain until you no longer see extensive blue areas within your image (blue indicates over scale). Now go back to normal (e.g. red or green) look-up table, *average, single scan*, and save the image.

Quantifying fluorescence in a limited area of your sample

Scan an image, or load one from memory. Select *quantify, histogram, and poly* (polygon). Now go to your image and draw an area (ROI, region of interest) with the mouse. You will see in the window appearing the histogram of fluorescence intensities and the numerical values on your region of interest; these can also be exported. Using the *sel* button in the *quantify* window you can activate the ROI which allows you to move it or stretch it depending on how you grab it with the mouse. You can also quantify fluorescence on a linear line or within regions of other geometric shape. *Clear* removes all ROIs you have drawn in the image.