

# Microscopy CoRE – Leica TCS SP8 (Icahn 13-71) Basic Operation



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The Leica TCS SP8 is an advanced confocal inverted imaging platform for fixed- and live-cell studies. This system features seven laser excitation lines; a motorized stage for multi-position imaging; an advanced detection system for spectral unmixing; and a full incubation setup for long-term research.

## Prerequisites

- 1. Before and after your session, you are responsible for cleaning the objectives.**
  - a. Immersion lenses - Use lens paper and lens cleaner, located in the blue container, to wipe off any oil.
    - i. On the touchscreen (image on next page), press the lens control menu and switch to an immersion lens.
    - ii. Tear off one sheet of lens paper from the packet. Wet a small area of lens paper with the cleaner (pink liquid). **Do not use KimWipes** to clean any microscope optics.
    - iii. With your pointer finger, gently press the wet area to the front lens of the lens and wipe in a circular motion 1 or 2 times.
    - iv. Switch to a dry area of the same paper and gently wipe 1-2 times in a circular motion.
    - v. Repeat as necessary until the lens is clean. Use fresh lens paper and cleaner for each lens; use fresh paper as well if you must wipe off a lens more than once.
  - b. Dry/Air lens - Using dry lens paper, gently wipe (do not press into) the front lens of the objective using one circular swipe. Examine the paper for oil (shiny smears). If oil is noted, please talk with CoRE staff to have the lenses thoroughly cleaned. Do not attempt to clean dry/air lenses yourself.
- 2. Before imaging, be certain that any mountants or sealants are completely dry.** This typically takes at least 24 hours from the time the slide is prepared. Refer also to manufacturer's instructions.

## System Startup

1. At right, below the computer desk, flip on the three switches in sequence from left to right.
2. Next to the switches, turn the laser key clockwise to the ON position.
3. Turn the fluorescence lamp, located to the left of the monitors, on if it does not come on automatically.
4. Log on to the computer using your credentials that you set up during the training.

## Basic Fluorescence Setup

1. On the microscope, slide open the two front incubation chamber doors as well as the two top covers surrounding the transmitted light arm.
2. Gently tilt the transmitted light arm to the rear until it stops.
3. There are two sample holders available for this system. *The slide holder is typically installed. If you need the well plate holder, please alert Core staff when booking the instrument. **Do not push too hard on either sample holder due to their delicate nature.***
  - a. If you are using slides, place the slide coverglass face down in the center of the slide holder. Move the slide clips over the slide to secure it in place.
  - b. If you are using well plates, gently slide the well plate into the holder. There is a rubber plug along the top edge that will hold your plate securely in place.
4. Gently tilt the transmitted light arm back into place over the stage and center your specimen using the XY controls over the objective lens. Close all doors to the incubation chamber.

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5. *On the touchscreen* – Along the left edge, tap the Lens Control tab (objective lens with a “+” symbol).
6. *On the touchscreen* – Tap the 10x objective (twice if it does not move right away), and under Port, tap the picture of the eye.



7. *On the touchscreen* – Along the left edge, tap the Filter Control (colorwheel/black-white wheel).
8. *On the touchscreen* – Along the bottom edge, select one of the three available widefield filters – DAPI (blue), FITC (green), or RHOD (red).
9. *On the touchscreen* – Tap IL Shutter. Fluorescent light should now appear on the stage.



10. Use both hands to set the correct interpupillary distance: when you look through the eyepieces, you should see one image circle instead of two.
11. Use the XYZ controller to center and focus on your sample.



12. *On the touchscreen* – Tap either of the two remaining widefield filters to ensure there is signal and specificity for all fluorophores used in your sample.
  - a. *If your sample does not appear to be exhibiting fluorescent signal where you would expect, or as brightly as you may expect, this may be a good time to return to the lab to review your sample preparation methods to see if anything can be adjusted for a better result.*
13. When initial observation is complete, tap IL Shutter again to turn off the fluorescence lamp.
14. To switch to an oil immersion lens – Tap on the Lens Control tab. Tap on the required magnification. *The oil immersion lens will swing into place. Carefully tilt the transmitted light arm back and remove your sample as per Steps 1 and 2. Apply one drop of immersion oil using the*

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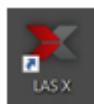


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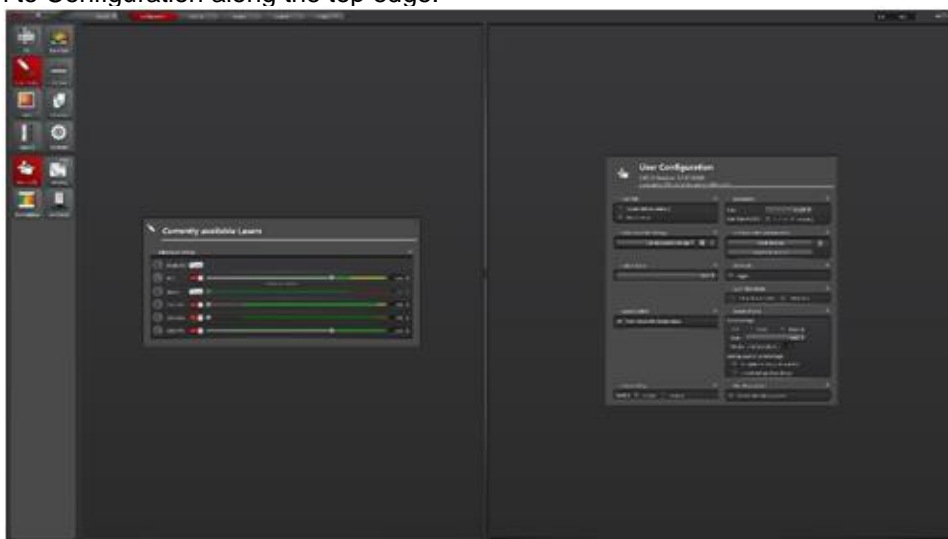
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*paddle to the front lens of the objective. Replace your sample, tap on the objective name again on the touchscreen (it will be blinking) and refocus.*

## Software Startup



1. On the desktop, launch LAS X.
2. When prompted, set the options as follows and click OK: **get screenshot of launch window**
3. Switch to Configuration along the top edge.



4. Click on Lasers. Set the following percentages for activated laser lines. *Only activate lasers that will be needed for that experiment – leave others turned off.*

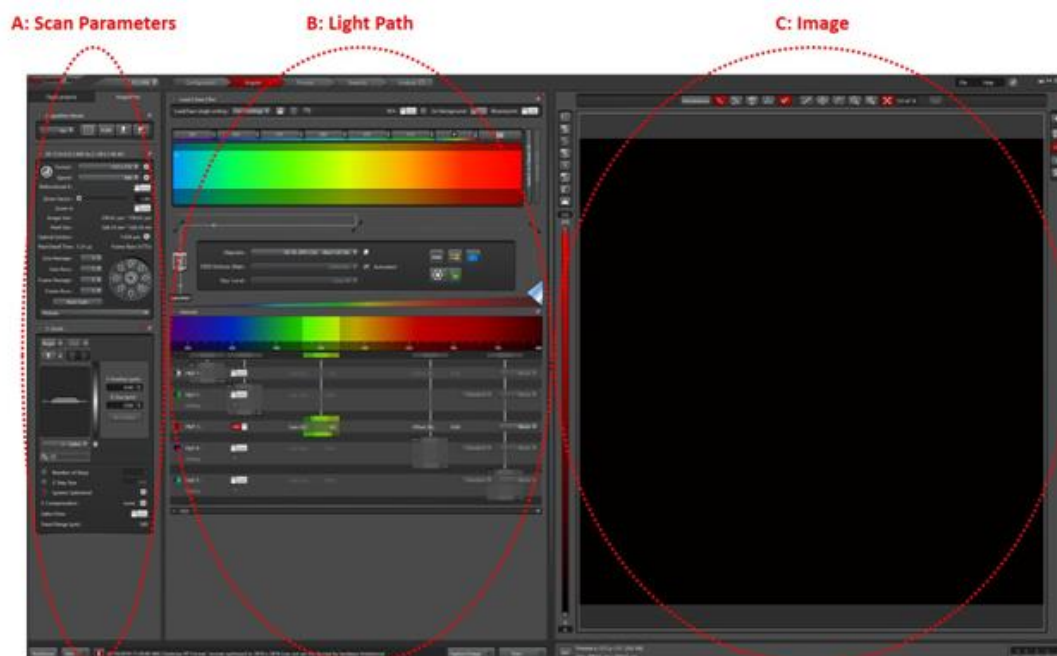
Laser Line	Excitation Wavelengths	Recommended Percentage	Compatible Fluorophores (limited list; many more options available)
405nm	405nm	N/A	DAPI, Hoescht
Argon	458, 488, 514	20%	CFP, AF488, EGFP, FITC, YFP
560nm	-	20%	AF568, DsRed
594nm	-	20%	AF594, mCherry
633nm	-	20%	AF633, AF647, Cy5

## Imaging Setup (Software)

1. Switch back from Configuration to the Acquisition screen.

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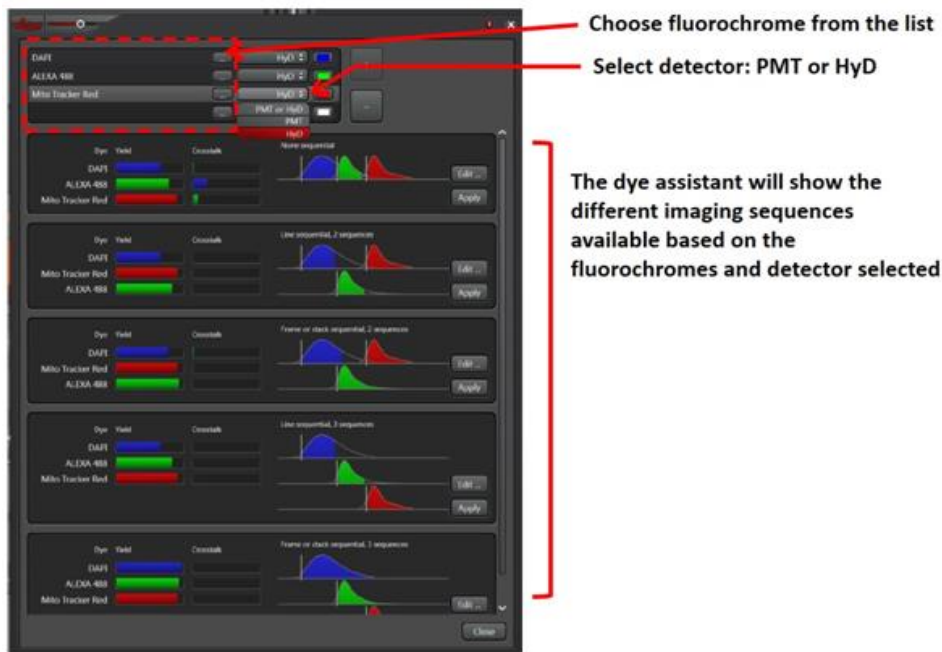
2. In Scan parameters: set Format to 1024 x 1024 and Speed to 600 Hz
3. In Light Path: use the dye assistant to set up the imaging parameters for your fluorophores



Click on Dye Assistant to choose the imaging settings for your fluorochrome/s

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## \*\*When to use a PMT or HyD:

- HyDs are lower noise detectors and therefore recommended for weaker signals.
- PMT are more suitable for stronger signals (good signal to noise ratio)

## \*\*Choosing the acquisition mode:

- **None sequential:** fastest mode for acquisition. All fluorophores are detected at the same time using different detectors. Since all the lasers needed to excite the fluorophores are “on” at the same time, this can lead to signal crosstalk or bleed-through (detection of two different fluorophores in the same channel).
- **Line sequential:** to avoid crosstalk, the excitation-detection of the different fluorophores can be alternated. In this case, the excitation-detection of the different fluorophores switched between lines until the whole frame is scanned. This mode is optimal for scanning fast events since the switch between sequences is very fast. In this mode, the hardware settings (detector position, averaging, accumulation...) need to be the same for all sequences need to be the same to allow the fast switching.
- **Frame sequential:** similar to line sequential but the switch between sequences is done after acquiring the whole frame. For this mode, hardware settings can be adjusted individually for each channel giving more flexibility for the detection of each fluorophore.

When imaging on sequential mode: All lasers should be always on (red button) but lowered to 0% during the sequences that are not needed.

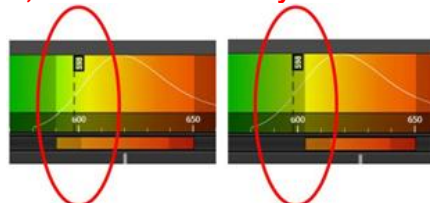
In the example below, lasers 405 and 594 are set at 0% in the sequence for A488 detection

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**CAUTION: MAKE SURE THAT THE EXCITATION LIGHT IS NOT BEING DETECTED BY THE PHOTODETECTOR, SPECIALLY THE HyD. IT CAN DAMAGE THE SYSTEM**



**DO NOT** extend the detector over the excitation laser

**Always** keep the excitation laser **outside** the detection range

## Image Acquisition

1. In the bottom left of the screen, click “Live” to start your preview scan.
2. Click the Quick LUT button  to toggle through the three available pseudocolor options: (1) current channel's color, (2) Glow Scale (Over/Underexposure), and (3) grayscale.
3. Select the Glow Scale, the image color will be red/orange/yellow and may have patches of blue or green. Blue denotes saturation while green indicated absolute black.
4. Set your gain:



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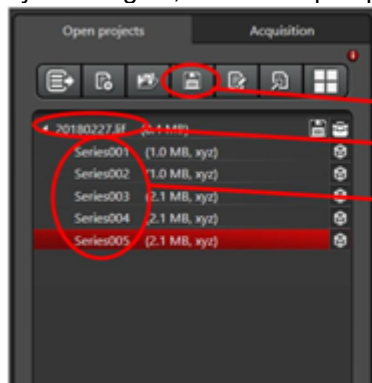
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- Using the Smart Gain knob (A), adjust the gain until you see saturation (blue pixels)
  - Use the z-position knob (C) to find your brightest focal plane in your specimen of interest
  - Readjust the gain to minimize the amount of blue pixels until you have a few left in your image
- If using a PMT, set your offset: using the Smart Offset knob (B), adjust the offset until you see green pixels (absolute black) then readjust the offset to minimize the amount of green pixels until you have a few left in your image
  - Click “Stop” in the bottom left (“Live” button) to stop your live scan
  - If different channels are being acquired. Click on the next sequence and repeat steps 1-6 for each channel.



- If signal is still low, adjustments on “Line accumulation” and laser power might be needed.
- Adjust format/speed again if needed. Additionally, line/frame average can be increased to reduce background.
- Click “Start” (bottom) to capture your image (all the channels). Click “Capture image” to only acquire the active sequence.
- To save your image/s, click on “Open projects” (tab next to “Acquisition” parameters):



- save all open projects
- project name – click on the text to rename
- image name – click on the text to rename

- The software stores all the images in a database file (.lif). please save your folder within the User Data folder in the Y:\ drive (HIVE server).

## Export images

ImageJ/Fiji can read lif files. Just drag the lif file on the ImageJ bar and the bioformats plugin will read the images. You can choose to open all the files or just select from the list of images.

In LAS X, select the image or images you want to export on the “Open projects” tab and click the right button on the mouse. From all the list of options, choose “Export” – “as Tiff”. Select the options you want to export: choose directory, overlay channels, micron scale...

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## Apply previous imaging settings

To reuse the same settings from a previous image, open the lif file that contains the image. Click the right button on the mouse and select “Apply image settings”. This reads the metadata of the file and copies the information on the acquisition window.