

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)



[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400

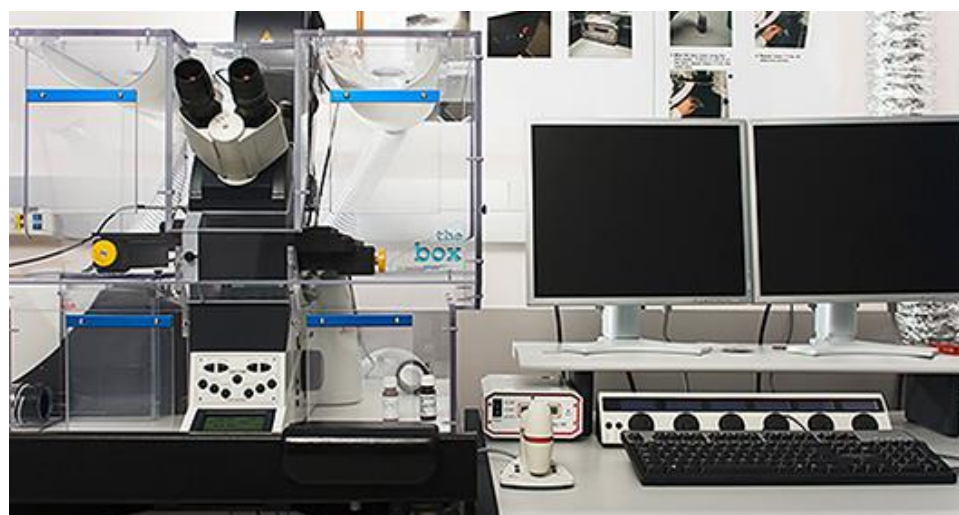
December 2020

The Leica SP5 DMI is a confocal inverted imaging platform for fixed- and live-cell studies. This system features seven laser excitation lines. The system also uses a motorized stage for multi-position imaging; 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 Overview



## System Startup

1. At right, below the computer desk, flip on the three switches in sequence from left to right.

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
December 2020

2. Next to the switches, turn the laser key clockwise to ON.
3. Turn on the fluorescence lamp, located between the microscope and the screen.
4. Log on to the computer using your credentials that you set up during training.

## Basic Fluorescence Setup

1. On the microscope, gently tilt the transmitted light arm to the rear until it stops.
2. 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. Squeeze the two holders together around your slide to secure it. Ensure the slide is sitting flat in the holder.
  - b. If you are using well plates, gently slide the well plate into the holder.
3. Gently tilt the transmitted light arm back into place over the stage and center your specimen over the objective lens using the XY controls of the SmartMove.

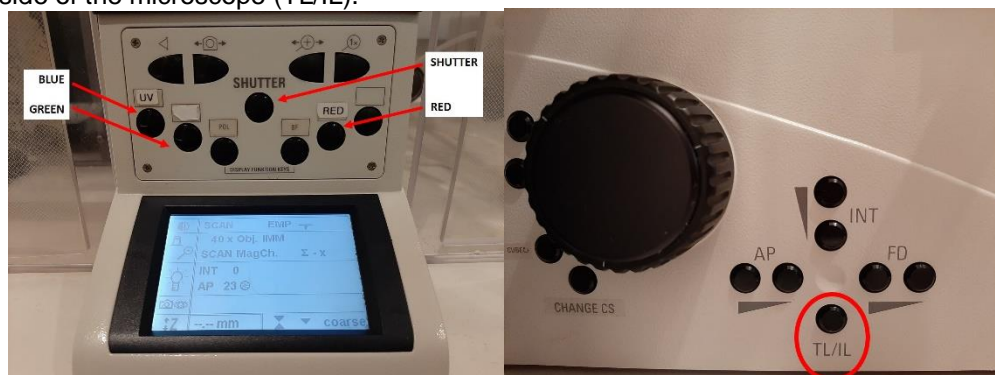
Stage movement knob: Y-Axis  
Stage movement knob: X-Axis  
Fine stage movement button  
Coarse stage movement button



4. Switch lens to 10x. Use the buttons on the right side of the microscope to switch between lenses.



5. *On the microscope* – Press the buttons to choose the widefield filter of interest: blue, green or red. To switch between reflected (fluorescence) and transmitted light, use the button located on the left side of the microscope (TL/IL).



6. *On the FL lamp* – Press the **On/Off** button to open the internal shutter.

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
December 2020

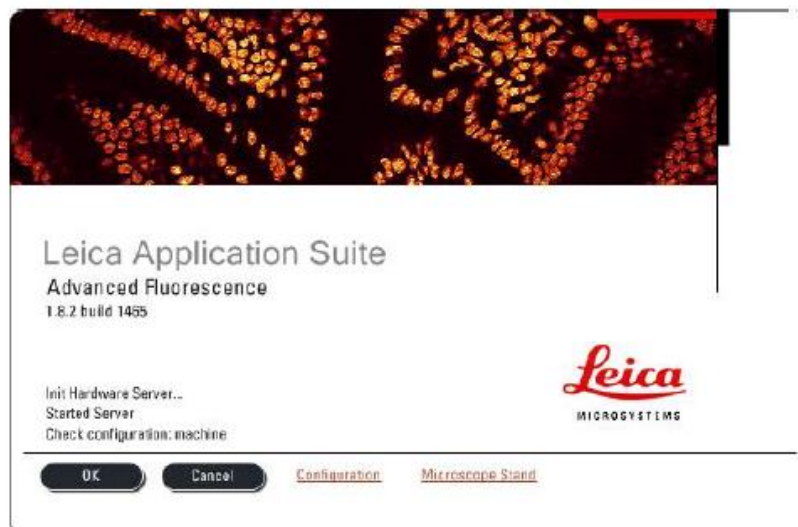
7. *On the microscope* – Press the **Shutter** button to illuminate the sample.
8. Use both hands to set the correct interpupillary distance: when you look through the eyepieces, you should see one image circle instead of two.
9. Use the XYZ SmartMove controller to center and focus on your sample.



10. *On the microscope* – Press 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.*
11. When initial observation is complete, press the **Shutter** button on the microscope.
12. To switch to an oil immersion lens – Move manually or choose the correct lens on the software. *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 paddle to the front lens of the objective. Replace your sample and refocus.*

## Software Startup

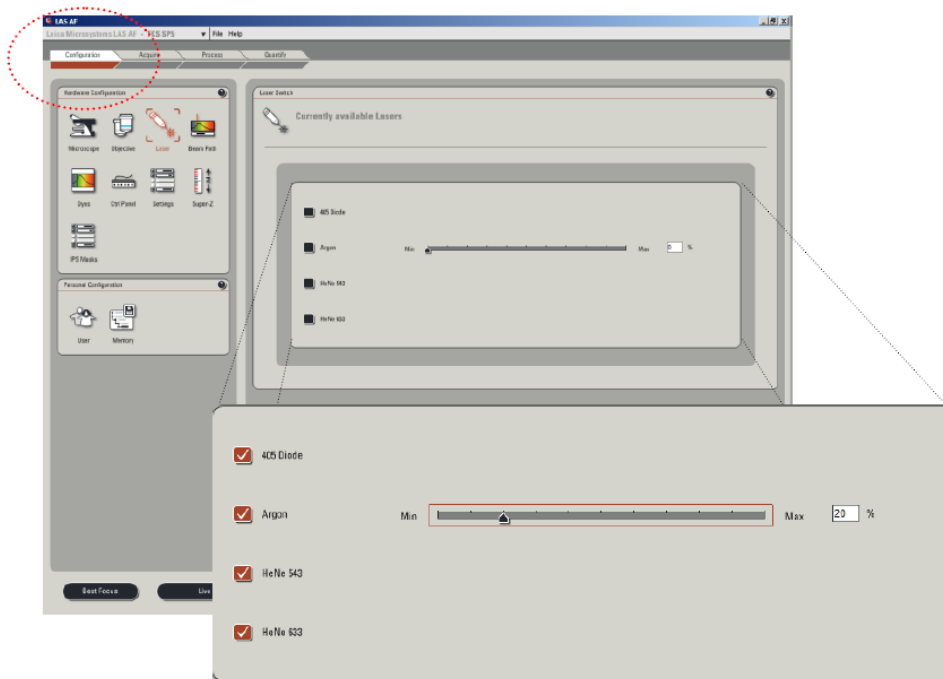
1. On the desktop, launch **LAS AF**.  
(add snapshot)
2. When prompted, click OK to initialize the system.



3. Switch to **Configuration** along the top edge.
4. Click on **Laser** in the Hardware Configuration panel. *Only activate lasers that will be needed for that experiment – leave others turned off. If using Argon laser, set the percentage to 20.*

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
December 2020



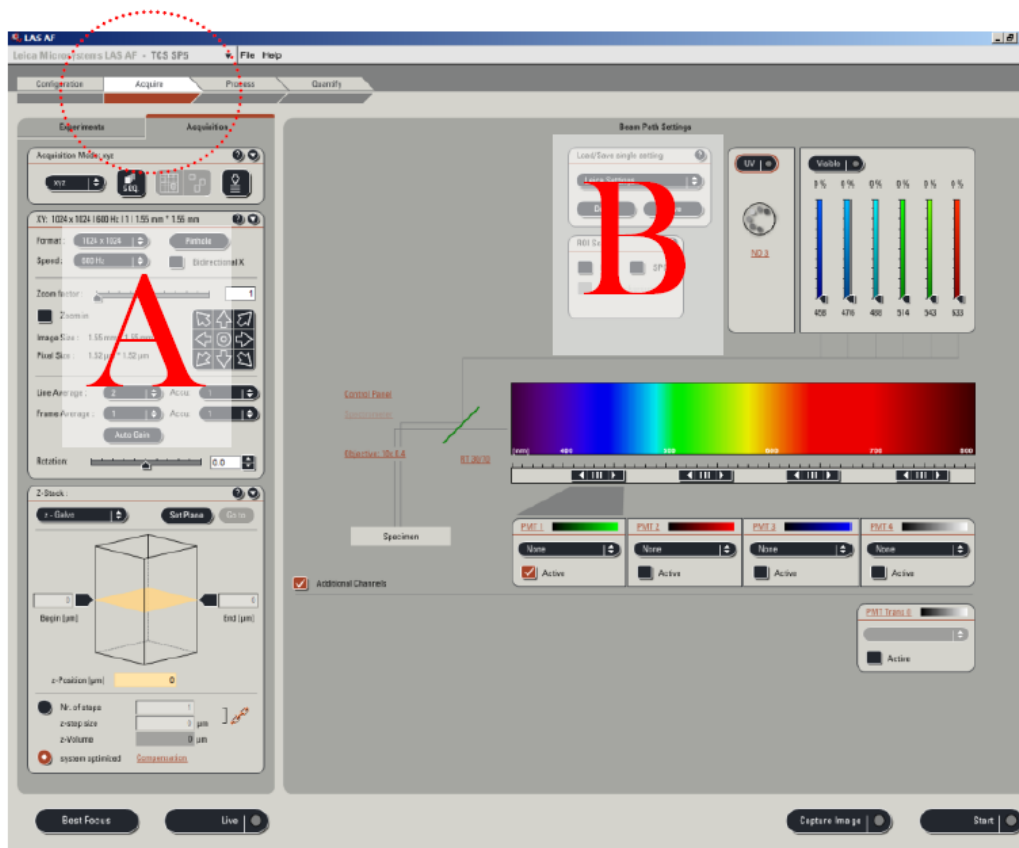
Laser Line	Excitation Wavelengths	Recommended Percentage	Compatible Fluorophores ( <i>limited list; many more options available</i> )
405 Diode	405nm	N/A	DAPI, Hoechst
Argon	458, 476, 488, 514nm	20%	CFP, AF488, EGFP, FITC, YFP
HeNe 568	568nm	N/A	AF568, AF594, Rhodamine, Texas Red
HeNe 633	633nm	N/A	AF633, AF647, Cy5

## Imaging Setup (Software)

1. Switch back from Configuration to the **Acquire** screen.

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

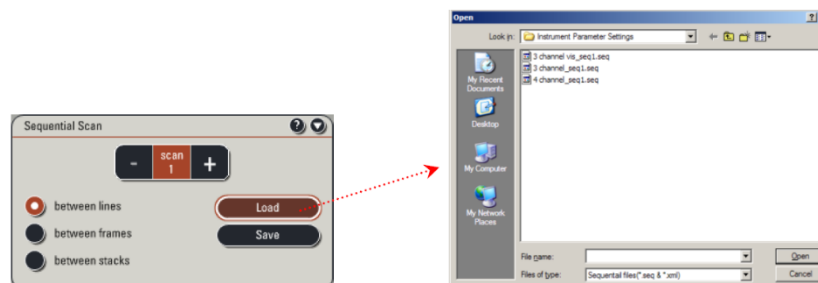
[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
December 2020



- In Panel A above: set **Format** to 1024 x 1024 and **Speed** to 600 Hz (click More and type 600)
- We have built some imaging settings that fit most of the needs of our users. To load these settings activate the “**SEQ**” sequential scan menu under **Acquisition**.



- In **Sequential Scan** menu, click Load.



- In **Y:\Leica SP5 DMI\Configurations**, you will find different configuration settings:

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)



[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400

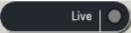

December 2020

DAPI-A488-A568.seq	3 color sequential acquisition (blue, green, red)
DAPI-A488-A568-A647.seq	4 color sequential acquisition (blue, green, red, far red)
DAPI-A488-A568-A647HyD.seq	4 color sequential acquisition (blue, green, red, far red) - uses HyD detector for far-red channel
DAPI-A488-A568HyD.seq	3 color sequential acquisition (blue, green, red) - uses HyD detector for red channel
DAPIHyD-A488HyD-A568HyD-A647HyD.seq	4 color sequential acquisition (blue, green, red, far red) - uses the same HyD detector for all the channels

## When to use a PMT or HyD

- HyDs are lower-noise detectors and therefore recommended for weaker signals. Use a HyD over a PMT with higher Smart Gain to avoid increasing background noise in an image.
  - PMT are more suitable for stronger signals (good signal to noise ratio).
6. In the Sequential Scan menu, each sequence corresponds to one channel acquisition. Using the (-) button, you can delete any channel that you do not need. Click once on the sequence you want to delete first, then click “-“.

## Image Acquisition

1. In Sequential Scan, click once on the channel you want to use to begin.
2. In the bottom left of the screen, click  to start your preview scan.
3. Click the **Quick LUT** button  to toggle through the three available pseudo-color options:
  - a. Current channel with pseudo-color applied (default)
  - b. Glow Scale (Over/Underexposure)
  - c. Grayscale
4. Select **Glow Scale**. The image will display the following colors based on pixel intensity:
  - a. Green – absolute black; expected result for background areas
  - b. Shades of Red – properly-exposed image areas
  - c. Blue – overexposed; reduce laser intensity or detector sensitivity before capturing your final image
5. Set your gain:



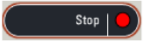
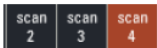
- a. Using the **Smart Gain** dial (“A”), adjust the gain until you see saturation (blue pixels).
- b. Use the Z Position knob (“C”) to locate the brightest focal plane.



# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400

December 2020

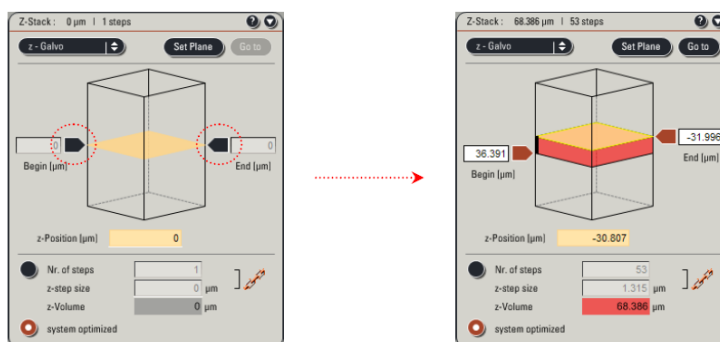
- c. Readjust the Smart Gain to minimize the amount of blue pixels until you have very few or none left in your image. *Your final Smart Gain range must be between 400-800 V for quantifiable results.*
6. If using a PMT, adjust the **Smart Offset** (“B”) counterclockwise until you see a background mostly saturated by green pixels (absolute black). Then, increase the Offset (clockwise) to minimize the amount of green pixels until you have only a few left in your image.
7. Click  in the bottom left to stop the Live view.
8. If different channels are being acquired, click on the next sequence  and repeat steps 1-6 for each channel.





---

**PRO TIP:** If you are not happy with the image area currently in view and wish to move the specimen, temporarily change the Format back to 512x512. Use the XY controls of the SmartMove to locate a new region of interest. Then, be sure to reset the Format to 1024x1024 before final imaging.

---

9. If a fluorophore’s signal is quite weak, you are using a PMT, and you have already adjusted the Smart Gain up to 800V, increases in **Line Accumulation** and/or laser power might be needed.
10. Adjust format/speed again if needed. Additionally, line/frame average can be increased to reduce background.
11. Click **Start** to capture your image (all channels). Click **Capture Image** to acquire only the active sequence.
12. Z-Stack acquisition:
  - a. Expand the Z-Stack menu and ensure that you have selected **z-Galvo**.



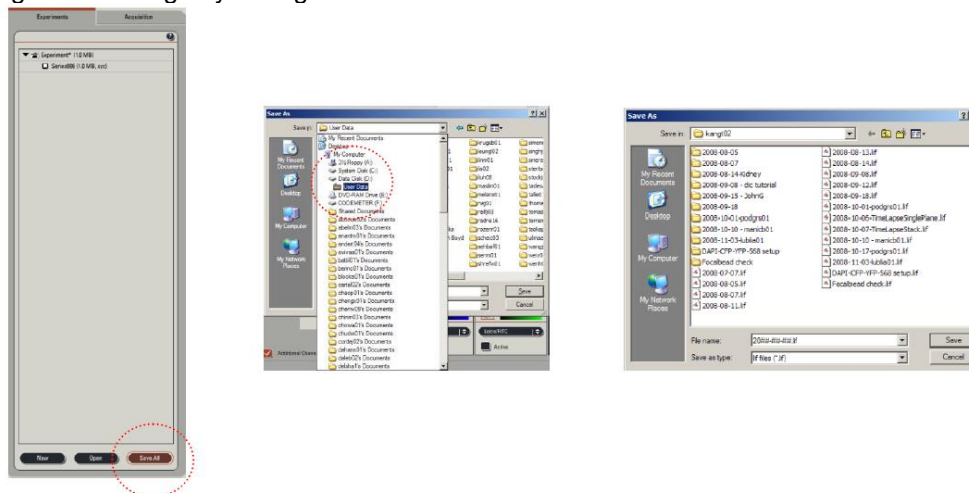
- b. Click **Live** and set your stack limits
  - c. Use the **Z Position** knob (C on step 4) to set the focal plane towards the bottom of your specimen (counterclockwise) and click the left arrow  so that it’s orange .
  - d. Use the **Z Position** knob to set the focal plane towards the top of you specimen (clockwise) and click the right arrow  so that it’s orange .
  - e. Click **Stop**. The z-stack panel should appear similar to the right image above.
  - f. Note the stack size (z-Volume) and number of steps. The software selects System Optimized by default (Nyquist sampling) for postprocessing techniques like deconvolution or 3D reconstruction.
    - i. If accurate 3D reconstruction or analysis is not required (for example: only MIP is required), select “Nr. of steps” and either decrease the number of steps or increase the Z-step size to capture fewer images.
  - g. Click **Start** to begin the scan.
13. To save your image/s, click on **Experiments** (tab next to **Acquisition**, at top left).

# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)

[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400

December 2020

14. To rename the images, right click on the image name and select **Rename**.
15. Click **Save All** to save your images. Make sure you save the file again after acquiring more images or making any changes.



16. The software stores all the images in one database file (.lif). Please save your files in your folder within your User Data folder in the **Y:\ drive (HIVE server)**.

## Export Images

ImageJ/FIJI can read \*.lif files. Simply drag the file on to the ImageJ bar and the BioFormats plugin will read the contained images. You can choose to open all the files or select just the ones you want to open at that time.

To export images for publications: In LAS AF, select the image or images you want to export on the “Experiments” tab and right-click 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 and so on. It is recommended that you always save the raw \*.lif files when exporting.

## Apply previous imaging settings

To reuse the same settings from a previous image, open the \*.lif file that contains the image. Click once on the image from which you want to recall settings. Under the image list, click **Apply**. This reads the metadata of the file and copies the information to the Acquisition window.

## System Shutdown

1. Save all your images/projects.
2. Switch to Configuration along the top edge.



# Microscopy CoRE – Leica SP5 DMI Basic Operation (Confocal)



[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
December 2020

3. Click on **Laser Config** and uncheck all lasers to turn them off.
4. Close the software. It will take a few minutes to shut down.
5. Turn off the computer. *If there is another user coming after you, sign out instead of shutting down and do not follow the next steps.*
6. Turn off the fluorescence lamp.
7. Turn off the first and second switches (looking from left-to-right), and turn off the Laser key. DO NOT switch off the third switch yet.
8. If using the Argon laser, wait until the fan (cooling system) stops. Use this time to clean oil off objectives and wipe off your slides.
9. When the Argon fan turns off, flip the third switch to the Off position.