

# Microscopy CoRE – Leica DMI8, Fluorescence Operation



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May 2022

The Leica DMI8 widefield microscope is a versatile fixed- and live-cell imaging platform for unstained samples as well as brightfield- and fluorescently-labeled samples. Featuring fast tiling acquisition, a multi-LED fluorescence light source for speedier imaging, and an optional stage-top incubator, this microscope has a lot of flexibility to accommodate many experiments.

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## Prerequisites

### 1. Before and after your session, you are responsible for cleaning all objectives.

- a. Tilt the transmitted light arm (above the stage) gently backward to access the lenses.
- b. On the touchscreen (see System Overview), press the Objectives tab. Press on the lens to be cleaned (twice if switching between oil and dry lenses).
- c. **For Dry Lenses:**
  - i. Tear off one sheet of lens paper and remove two cotton swabs from the package.
  - ii. Soak the cotton tip of one swab with cleaning solution. Gently rub the cotton tip in a circular motion over the objective lens. Throw this swab out.
  - iii. Place the lens paper, unfolded, over the objective. Using the second dry swab, use the cotton tip end to gently press the lens paper to the lens. Gently rub in a circular motion.
- d. **For Oil Immersion Lenses:**
  - i. Tear off one sheet of lens paper.
  - ii. Fold the lens paper twice, once in half widthwise and once in half lengthwise.
  - iii. Wet one corner of the folded lens paper with cleaning solution.
  - iv. Place the wet corner over the objective lens. Use your pointer finger to gently press the paper to the lens. Gently rub the lens in a circular motion.
  - v. Move your pointer finger to a dry area of the same lens paper. Now gently press this dry area to the lens to dry it off. Move in a circular motion.

If for any lens you notice that the lens paper is shiny after cleaning, repeat the appropriate cleaning process until the paper is no longer shiny. **Use fresh lens paper and cotton swabs each time – do not reuse supplies.**

If you cannot get an objective clean, please talk with CoRE staff to have the lenses thoroughly cleaned.

- ### 2. Before imaging, be certain that any mountants or sealants are completely dry. This can take at least 24 hours from when the slide is prepared. Refer to the manufacturer's instructions.
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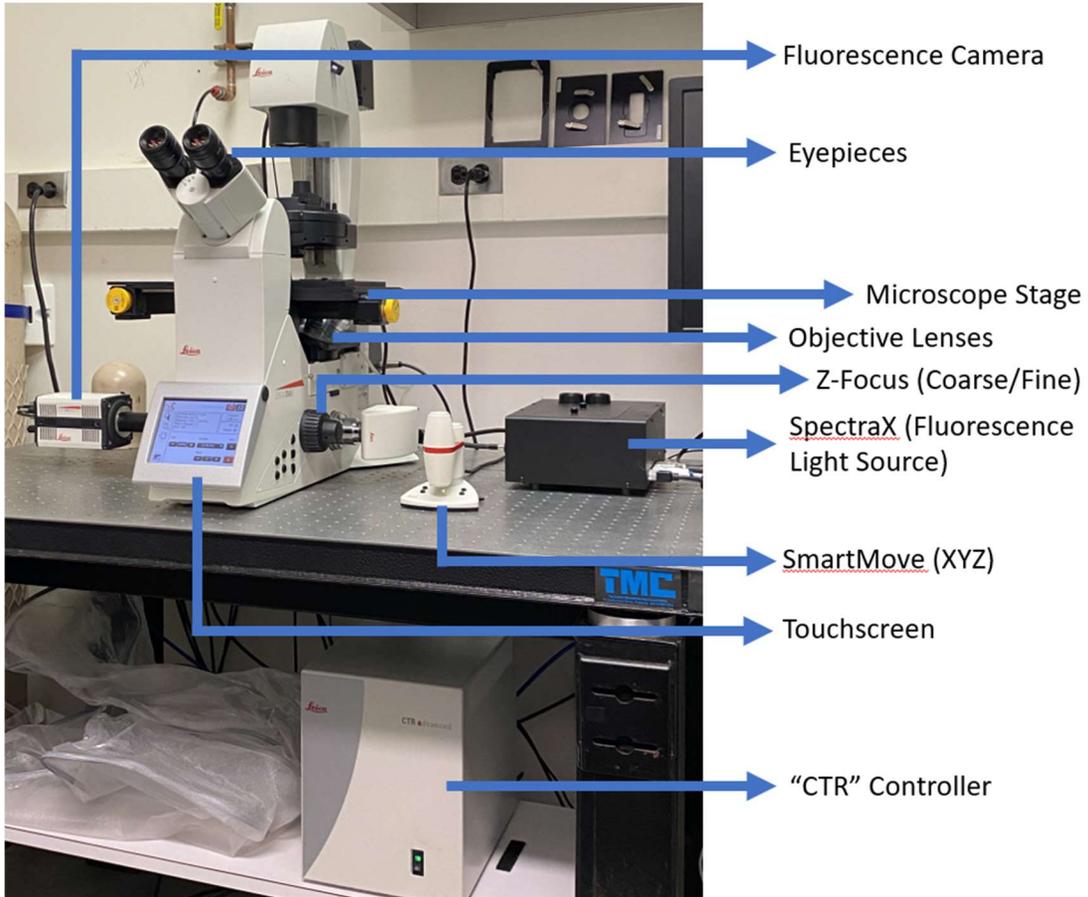


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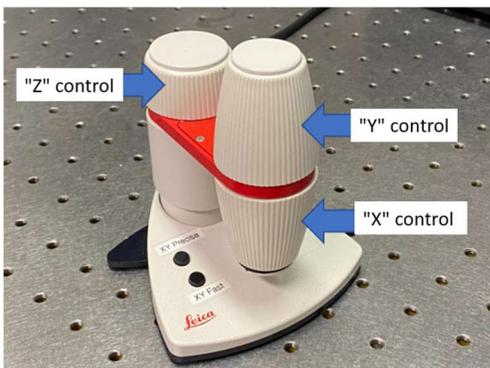
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## System Overview

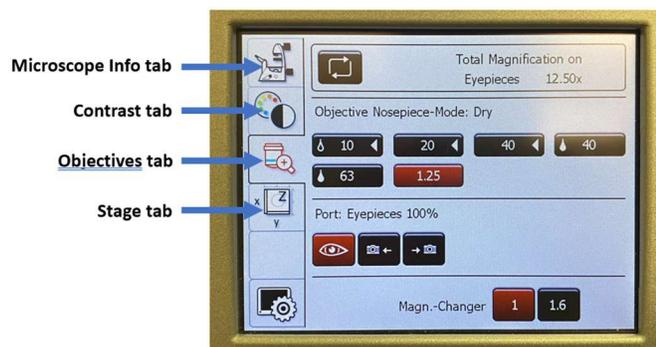
### Microscope Detail



SmartMove XYZ Controller



Microscope Touchscreen



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## System Setup – Fluorescence

1. Turn on the CTR Controller below the microscope.
2. Turn on the **fluorescence camera** (left camera port, gray toggle switch to “ON”).
3. Turn on the **SpectraX fluorescence light source** (black box; yellow toggle switch UP).
4. Turn on the computer workstation. Log onto the workstation using the credentials established at the training session.
5. Tilt the **Transmitted Light Arm** backwards (see below) to access lenses for cleaning as well as for sample insertion.
6. Insert the appropriate stage insert and ensure it is flush with the stage surface. *Ask Core Staff for assistance with swapping if necessary.*
7. After cleaning lenses, insert your sample on to the stage. *Check: Is your coverglass oriented in the correct direction?*
8. Tilt the Transmitted Light Arm back into position (forward).

## Switch to Oil Immersion Lenses

1. Remove slide from microscope stage.

*Tilt the Transmitted Light Arm to the rear and remove your slide.*



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- From “Magnification” screen in LAS X or Objectives tab on touchscreen, click on an oil immersion lens (denoted by oil drop symbol to left of the magnification).

*Tap twice on objective on touchscreen.  
Click OK on prompt if switching from software.*



- Unscrew lid with paddle from oil immersion bottle.



- Place drop of oil on coverglass side of slide in the approximate location of observation.



- Carefully invert slide and mount it back on the microscope stage.
- Return to imaging.

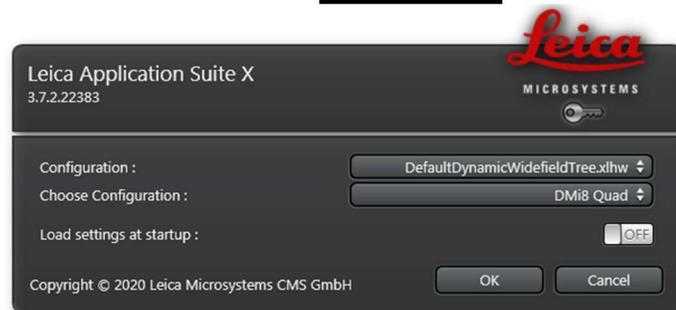
***ALWAYS REMEMBER to wipe oil immersion off slides before switching from oil immersion lenses back to air/dry lenses, or your air/dry lenses will not work!***

## Fluorescence – Software Setup

1. Start **LAS X** from the desktop.



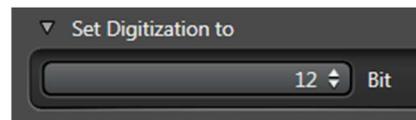
2. Ensure the log-on screen looks the same as shown.



3. Once the software starts, switch to **Configuration**.



4. Change **Set Digitization to** to 12- or 16-bit (depth).



5. Switch back to **Acquire**.



6. In the **Channels** field, use the drop-down to select:

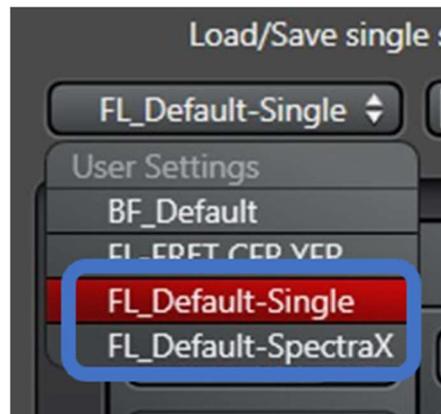
“FL\_Default-Single”

OR

“FL\_Default-SpectraX.”

*“Single” will provide better spectral separation/minimize crosstalk whereas “SpectraX” will allow for faster imaging.*

*These experiments default to 200ms exposure times, no binning, and 50% LED power. Change values as needed.*



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- Remove unnecessary channels by clicking on the channel and using the “-” button.

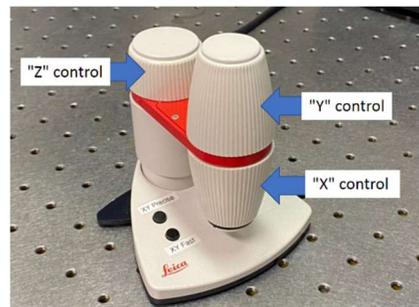


- Under Magnification, choose the required objective lens.

*Start with lower magnifications and work your way up.*



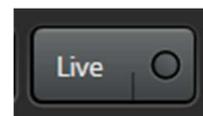
- Use the XYZ SmartMove controller to roughly center your sample over the lens.



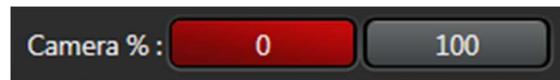
- Select a fluorescent channel in the software. Click once to highlight it (it will turn red).



- In the bottom left corner, click **Live**.

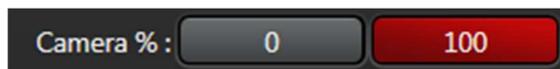


- Under Illumination Settings, change “Camera %” to 0% to direct light to the eyepieces.



- Looking through the eyepieces, center and focus on the sample using the SmartMove controller.

- In the software, click “Camera %” to 100%.



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15. To the left of the image, switch to **Over-/Underexposure** mode.



16. Adjust the “Exposure [ms]” (Image) and the LED power (light path) as needed. Consider using *Binning* to improve the *Signal-to-Noise Ratio*.

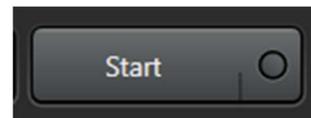
Use the *Over-/Underexposure* button to the left of the image to minimize sensor saturation (too many blue pixels). Click this button twice more to return to the pseudo-color view.



17. Click on the next fluorescent channel and repeat Step 16.

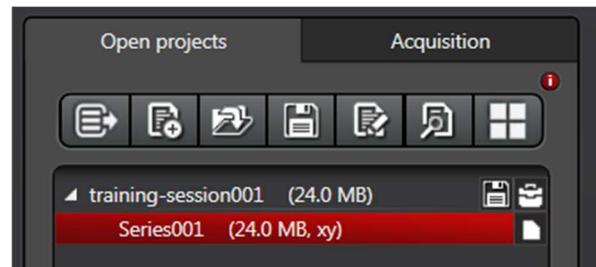


18. When all imaging parameters are optimized, click **Start** to capture the multicolor image.



19. Switch to **Open Projects** and save images.

**Remember the Data Policy** – no USB drives or portable hard drives. Save all images to the HIVE / “Y” Drive > User Data for remote retrieval.



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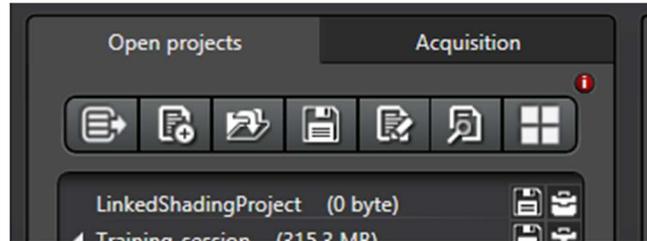


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1. Switch to **Open Projects** and save your images. *Rename image files in this window.*

**All images must be saved to the “Y:” drive, also called the HIVE.** When saving, be sure you have run the MapDrive utility on the desktop. Save all images under the resulting network location, “MicroscopyPublic” either under “Temp” or your lab folder under “PROJECTS.” *Do not save locally to the PC – images will be promptly deleted. Do not connect a USB drive to any CoRE instrument to remove your files.*



2. Close out of software (“X” in top right corner).
3. Shut down PC from the **Start Menu**.
4. Clean objective lenses. Turn off microscope (“CTR” controller).
5. Replace the dust cover over the microscope.
6. Turn off the room light and close the sliding door.