

Microscopy CoRE – Leica DMI8 Brightfield

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May 2022



The inverted Leica is a fully automated research microscope from Leica that runs on the LAS X software platform. For brightfield imaging, it features a color camera, automated tiling (Navigator) for capturing large tissue areas, controls to establish proper Kohler illumination, and a live-cell stage-top chamber.

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 to reach the Objective screen. 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 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.

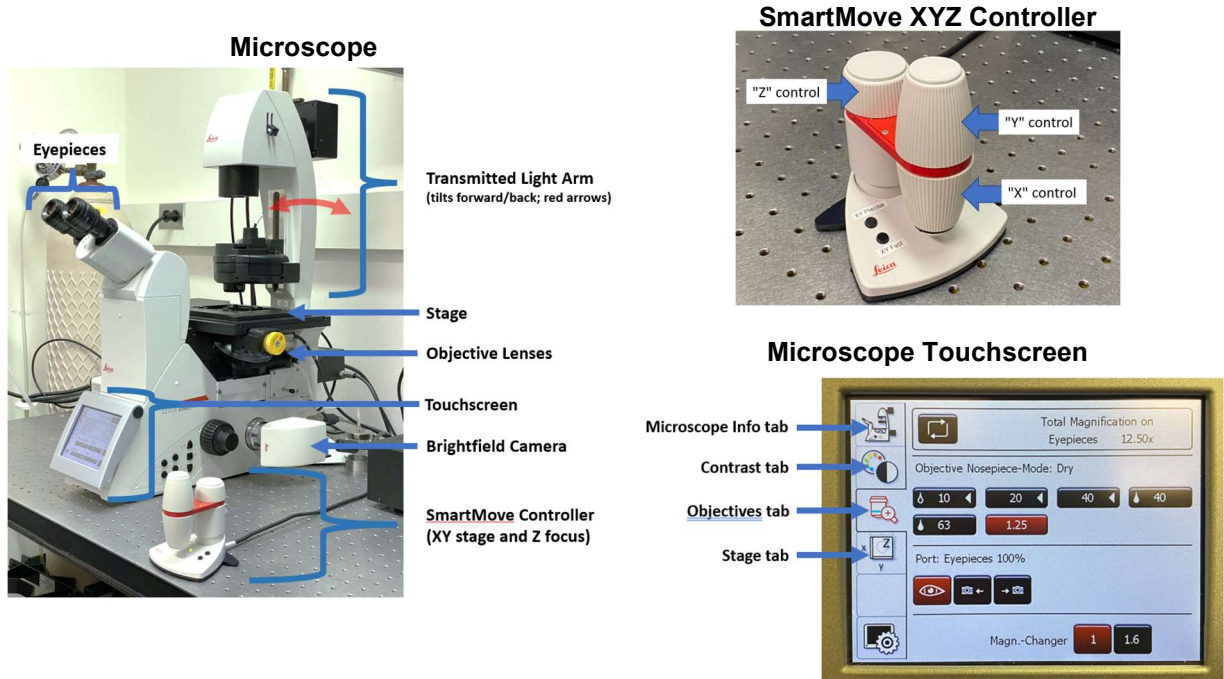
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System Overview



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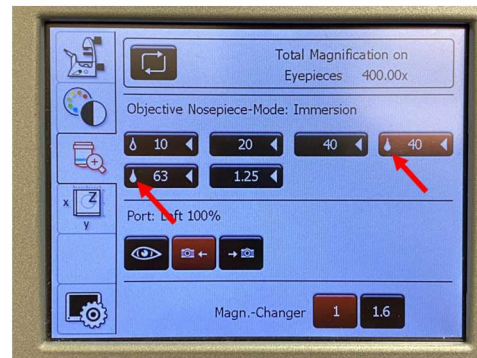
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- From **Magnification** screen in LAS X or **Objectives** tab on touchscreen, switch to oil immersion lens (denoted by oil drop symbol to left of the magnification).

*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.

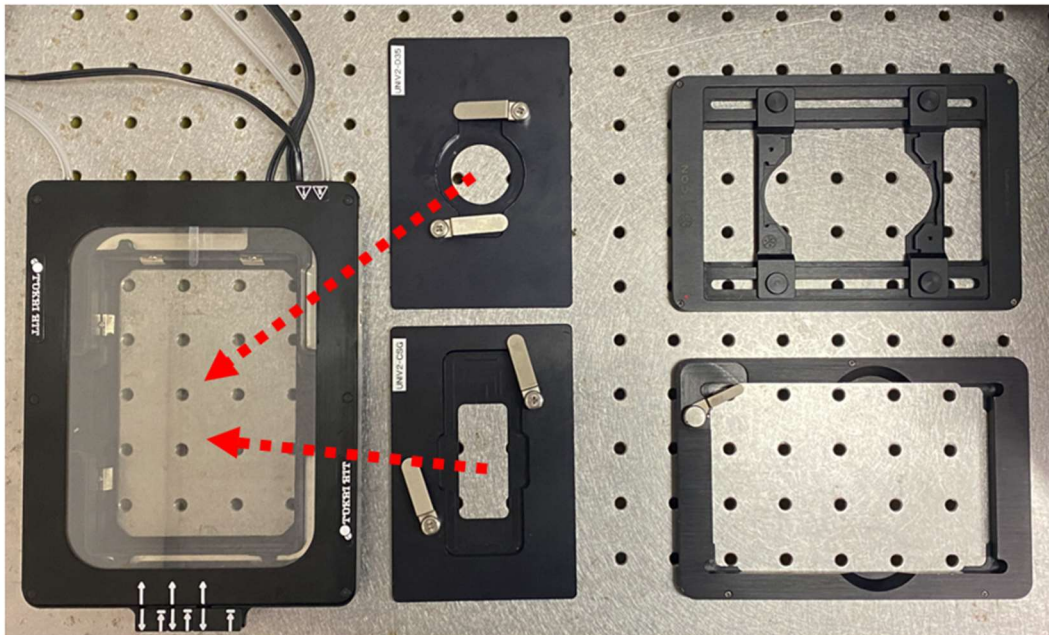


- Carefully invert slide and mount it back on the microscope stage.
- Return to imaging.

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!

System Setup - Brightfield

1. Remove the dust cover and set aside.
2. Turn on the “CTR” Controller beneath the microscope.
3. Turn on the computer. Log in using the credentials established during training.
4. Tilt the Transmitted Light Arm gently backward until it hits the stop.
5. Insert the appropriate sample holder into the scanning stage. Refer to the image below for the correct holder. *If you need assistance choosing or inserting the holder, please speak with Core Staff located in the main office.*



TokaiHit live-cell
chamber
Well-plate holder

TokaiHit adapters:
35mm dish (top),
chamber slide (bottom)

Fixed slides, 35mm dishes
(top)
Fixed plates (bottom)

SPECIAL NOTE: *If you are using dishes or multi-well plates with thicknesses other than “coverglass thickness” (0.17mm), please alert Core Staff who can determine if objective correction collars need to be changed. This is important for optimal image quality!*

6. Insert your sample into the sample holder. *Check: Is your slide’s coverglass face-down toward the objective lens?*
7. Tilt the Transmitted Light Arm gently forward.

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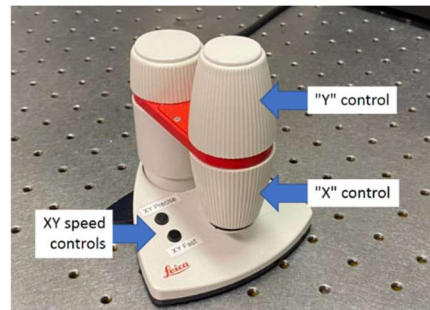
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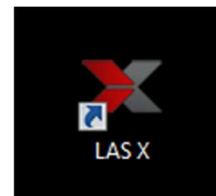
Brightfield Software Setup

1. Using the SmartMove controller, roughly center your sample over the objective lens using the X and Y controls located toward the front.



2. On the computer desktop, start **LAS X**.

The software will give a few errors – you may ignore them.



3. Ensure the log-on window matches what is shown at right.

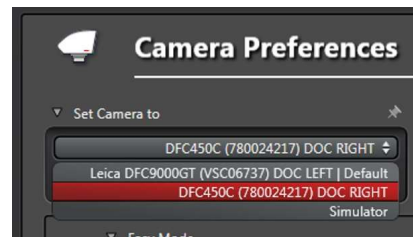


4. Click **Configuration** at the top of the software.



5. Under *Camera > Set Camera to*, choose the option **DFC450C (DOC RIGHT)**.

After selecting the camera, ensure both options for “Image Flip” are unchecked.



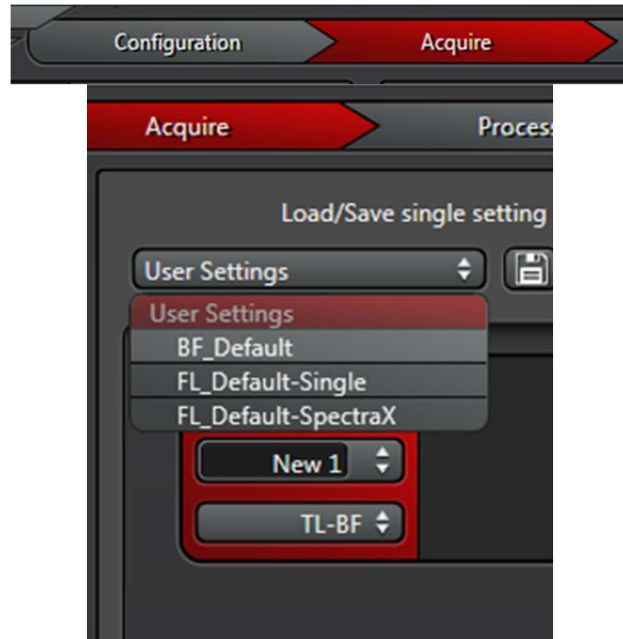
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6. Return to **Acquire**.
7. From the **User Settings** drop down, choose **BF_Default**.



8. "BF_Default" is for Brightfield imaging. For Phase Contrast or Differential Interference Contrast (DIC), use the drop down menu in the **Channels** field to switch imaging modes.



9. For **Magnification**, select the 10x or 20x objective to start. (For slightly more magnification, switch the Magnification Changer from "1" to "1.6".)



To switch to oil immersion lenses: See section, "Switch to Oil Immersion Lenses."

SPECIAL NOTE: *The microscope retains lamp intensity as well as aperture and field diaphragm settings based on the current objective; there should be little reason for adjustment. But, adjust as needed.*

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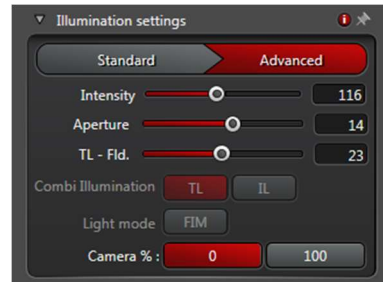
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10. In the bottom left corner of LAS X, click **Live**.



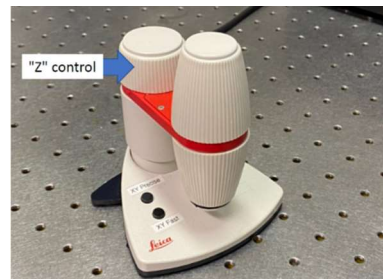
Press this same button to turn off Live.

11. Under **Illumination Settings**, direct light to eyepieces – click “0” for **Camera %**.

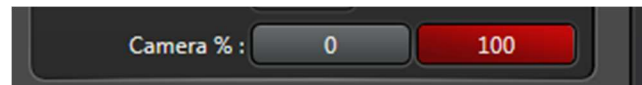


Adjust the interpupillary distance between the eyepieces until one circular image field is observed.

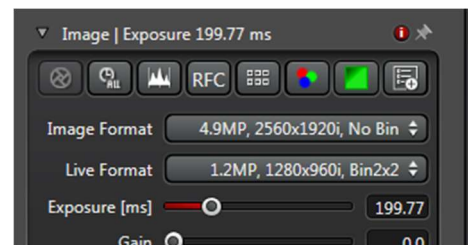
12. While looking through the eyepieces, use the SmartMove Z-control to focus on your sample. Use the XY controls to more accurately center your sample as well.



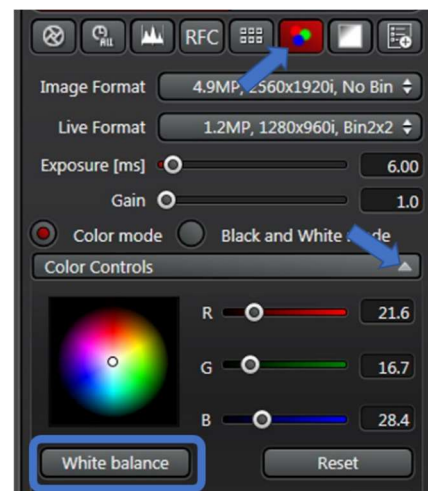
13. Return light to camera (**Camera %** to “100”). Adjust **Exposure (ms)** under **Image** as needed.



Slight refocusing for the camera may be necessary.



14. **WHITE BALANCE:** Move off your sample to a blank area of your slide – no sample, no dust, no scratches. *Make sure your slide is clean!* Rotate the focus (Z-axis) control half a turn in either direction to defocus the image. In **Image**, expand **Color Controls**. Click “White balance.”



The result of this step should be a neutral white Live background.

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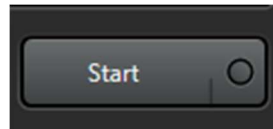
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15. Return to your sample and focus using the SmartMove XYZ controls.

16. Click **Start** to capture the current image field.



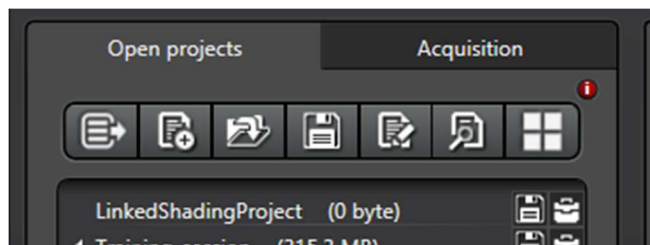
17. Reposition your sample to a new region of interest and continue to image.

NOTE: Changing exposure may become necessary when you change objective lenses. White balance can be left alone within an imaging session after being set.

System Shutdown

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.