

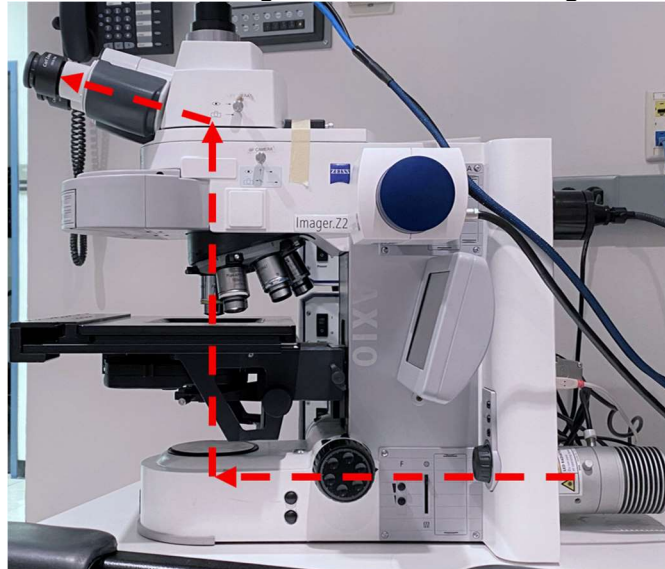
# Microscopy CoRE – Axio Imager.Z2(M) and Z2 – Transmitted Light



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May 2022

The Axio Imager.Z2(M) and Z2 are equipped for Brightfield, Darkfield, Phase Contrast and Differential Interference Contrast (DIC). This guide will explain how to set up each modality.

**Transmitted Light Path of the Axio Imager**



## Prerequisites

1. **Before and after your session, you are responsible for cleaning all objectives.**
  - a. On the touchscreen (see System Overview), press “Load Position” and then press “Set Work Position” so you have enough space to clean the lenses.
  - b. Press “Microscope” to reach the Objective screen. Press on the lens to be cleaned.
  - 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.

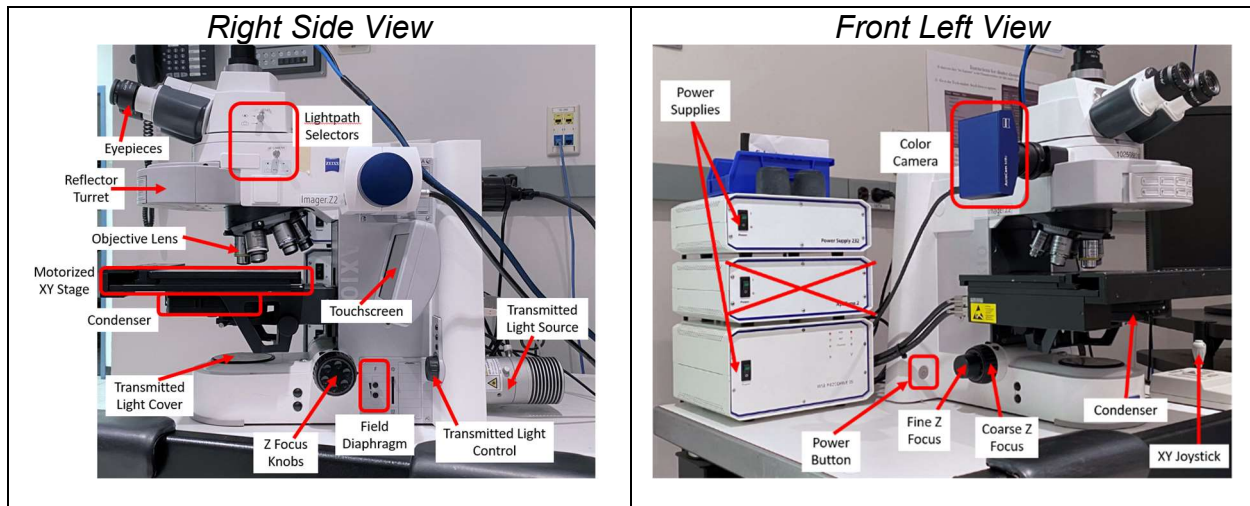
If you cannot get an objective clean, please talk with CoRE staff to have the lenses thoroughly cleaned.

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## System Overview



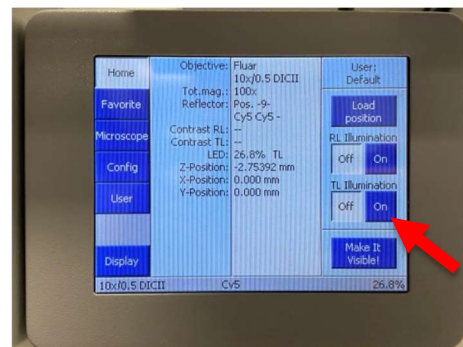
## System Startup – Brightfield

1. Turn on the Power Supply 232. For the Axio Imager.Z2, also turn on the WSB Piezodrive power supply (shown in System Overview, *Front Left View*). *The ApoTome.2 power supply can stay off.*
2. On the left “leg” of the microscope behind the stage, locate the silver Power Button. Press once to start the microscope. (Refer to *Front Left View* System Overview above for correct location.)
3. Remove the rubber Transmitted Light Cover from the base of the microscope.
4. Turn on the computer and log in using the credentials you established during the training session.

## Kohler Illumination

Vital to any transmitted light image is **Köhler Illumination**. Köhler Illumination is required when imaging with a 10x objective or higher and for any transmitted light technique (Brightfield, Darkfield, Phase Contrast, DIC - differential interference contrast).

1. *On the touchscreen* – Tap **On** below **TL Illumination**.



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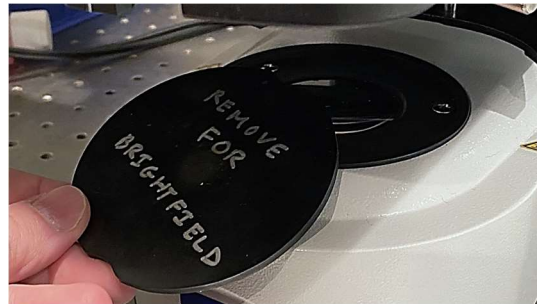
2. On the right “leg” of the microscope, use the **Transmitted Light Control** to adjust lamp intensity. *The lamp is very bright, so setting the lamp to the lowest “orange” range is typical.*



3. On the right side of the microscope behind the Z Focus Knob, gently press and hold the upper button right below “F” (for **Field Diaphragm**) for 3-5 seconds.



4. Remove Transmitted Light Cover from below the condenser.



5. On the condenser, open the **Aperture Diaphragm** fully .



6. Also on the condenser, manually move the **Contrast Ring** to the “H” or “BF” position.



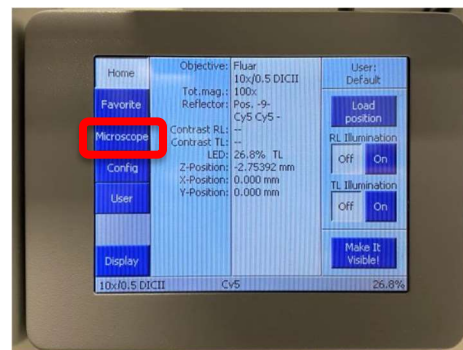
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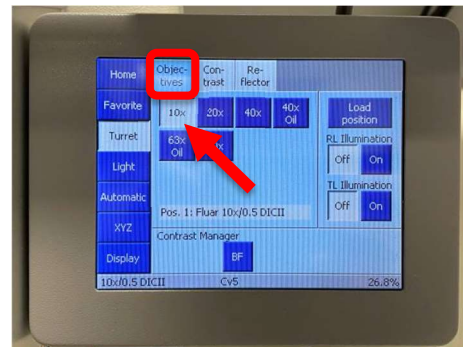
7. Push both **Lightpath Selectors** fully toward the microscope to direct light to the eyepieces.



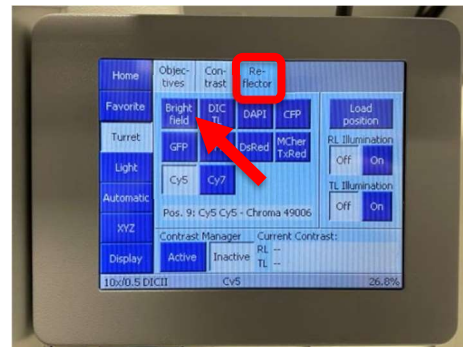
8. *On the touchscreen* – On the left edge, tap **Microscope**.



9. *On the touchscreen* – Along the top edge, press **Objectives**. Tap the **10x** lens to move it to the imaging position.



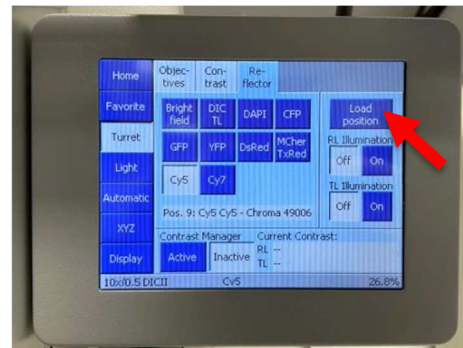
10. *On the touchscreen* – Along the top edge, press **Reflector**. Then, press **Brightfield**.



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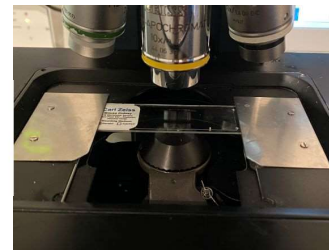
11. *On the touchscreen* – Along the right edge, press **Load Position**. The stage will drop.



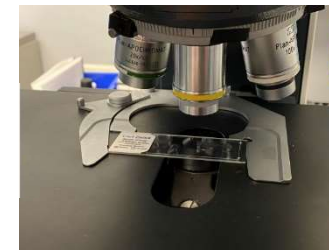
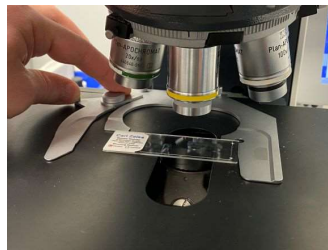
12. Load your slide on to the stage. (*See next page for images.*)

- On the Imager.Z2* – Place the slide coverglass face-up at the lower edge of the stage holder. Slide the sample under the two metal guides until it is flush against the back of the holder. Move the XY joystick briefly in X and Y to ensure the slide is secure.
- On the Imager.Z2M* – Using your left thumb, press the stage clip outward to the left. Mount your slide firmly against the stops in the stage clip area, coverglass face-up. Release the stage clip to clamp your slide in place. Your slide should be flat against the stage. Move the XY joystick briefly in X and Y to ensure the slide is secure.

*Imager.Z2*



*Imager.Z2(M)*



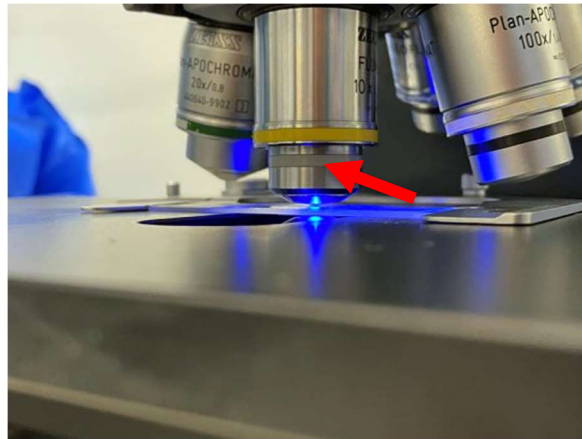
13. *On the touchscreen* – Press the upward-pointing arrow with a line over it to return the stage to the viewing position.

14. Use the **XY joystick** to roughly center your sample under the objective lens. *Tap F1 to toggle fast/slow movement.*

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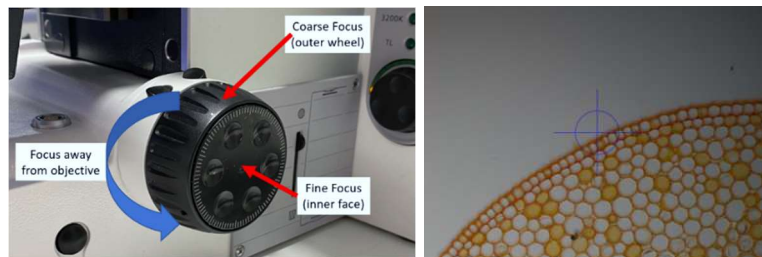
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15. Looking flat across the stage (drop your eyes to the level of the stage), turn the **Z Focus Knobs** away from you (over the top) until the lens is just about to contact the slide. *Go no further or you risk damaging your slide.* Notice the small gap between the lens and slide; that is where to stop.



16. Look through the eyepieces. Set the interpupillary distance between the eyepieces until one circular image is seen.

17. While looking through the eyepieces, turn the Z Focus Knobs toward you (over the top) until your sample comes into focus. Use the XY joystick to locate an area of interest or to move to another area where you can focus.



***Do not touch the Z/sample Focus Knobs for the remainder of this section!***

18. Press and hold the lower “F” button for 3-5 seconds. The image in the eyepieces will get darker.



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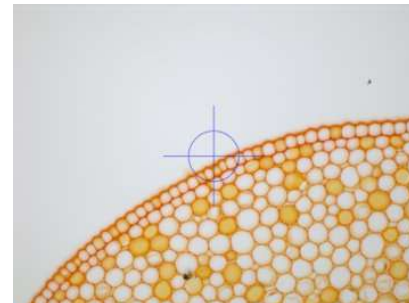
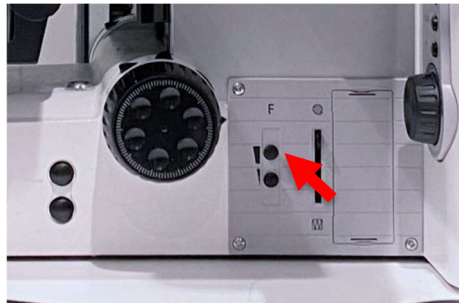
19. Raise and lower the condenser via the knobs below the stage until a sharp hexagon appears in the eyepieces.



20. Using the silver thumbscrews below the condenser, roughly center the bright hexagon in the eyepieces.



21. Tap the upper “F” button until the edge of the hexagon just disappears from the field of view.



22. Adjust the Aperture Diaphragm to roughly “0.4”.



23. *On the touchscreen* – Press **Off** under **TL Illumination**.

24. If a higher magnification is required, switch back on the touchscreen to **Objectives** and press the required magnification.

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If an oil immersion objective is required: Press on the desired lens on the touchscreen. *The stage will drop.*

Use the paddle in the oil immersion bottle to place a single drop of oil on your slide right below the lens. On the touchscreen, press “Done” to bring your sample back into focus.

Double-check focus by briefly turning **TL Illumination** back on. Only minor focus adjustments should be required.



*Do not switch from an oil objective back to a dry/air objective without first cleaning the lens and your slide!*

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**PLEASE NOTE:** When imaging at higher magnifications (40x and above), the imaging field should have even illumination from side to side – no need for shading correction. When imaging at 5x/10x/20x, vignetting (dark corners) may occur. Speak with CoRE Staff about shading corrections if you are unsure if you will need them, and for setup assistance.

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**Your microscope is now set up for Brightfield Illumination.**

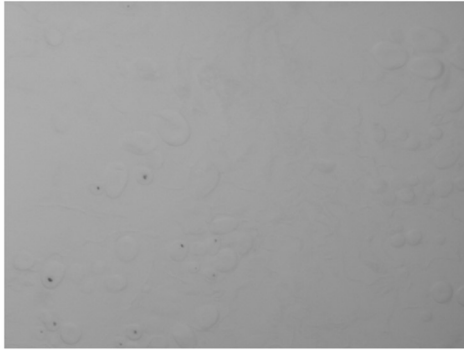


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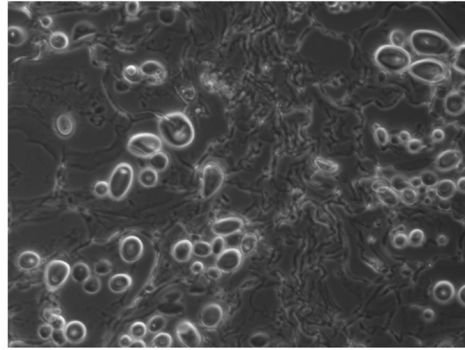
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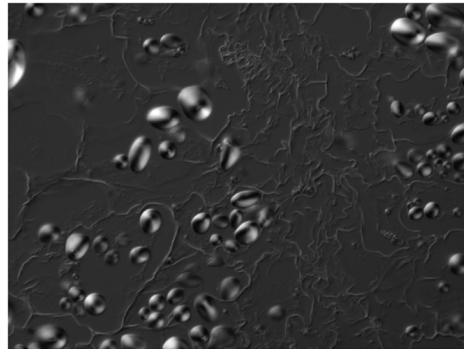
## Phase Contrast and DIC – contrast without staining



Brightfield



Phase  
Contrast



DIC

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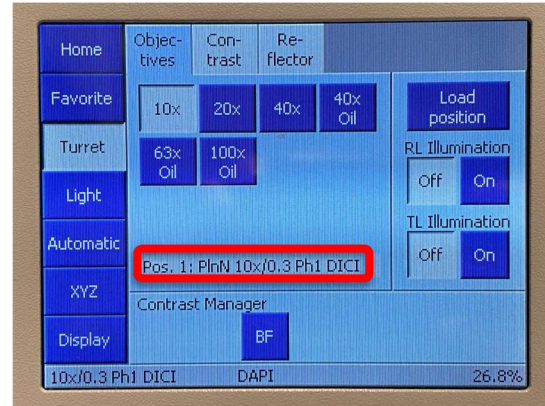
## Phase Contrast

The Core has a small assortment of Phase Contrast objectives available. Inquire via email ahead of your scheduled imaging session.

*Kohler Illumination must be completed for Phase Contrast microscopy to work! Ask CoRE Staff for an appropriate calibration sample.*

1. Via the **Objectives** page on the touchscreen, click on a **Phase Contrast** objective.

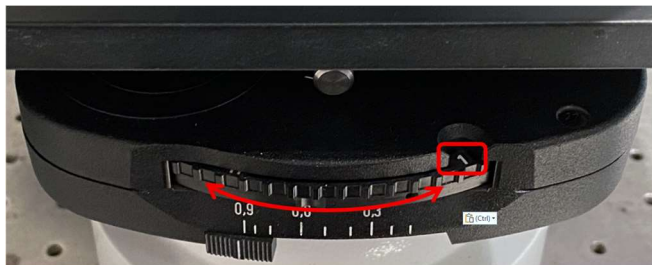
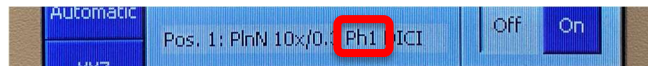
The full name of the lens, highlighted, will show “Ph” in the name.



2. Switch to the **Reflector** tab. Select the “Brightfield” filter cube.
3. Open the **Aperture Diaphragm** fully.



4. Use the number that appears after the “Ph” on the touchscreen to identify which Phase annulus is needed in the condenser. Rotate the condenser’s **Contrast Ring** to the corresponding Arabic number.



*Be sure to use “1, 2 or 3” and NOT “I, II, or III” for Phase contrast!*

5. Increase lamp intensity via the **Transmitted Light Control** for a brighter image.

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6. It should not always be necessary, but it is helpful to remove one eyepiece and look down the empty socket to ensure the light and dark rings for Phase Contrast are aligned over one another. Ask for a Phase Telescope from Core Staff and insert it into the empty eyepiece socket to check this alignment if your Phase Contrast imaging is not very good. (Make sure the Lightpath Selectors direct light to the eyepieces!) Core Staff will assist with the alignment process. Reinstall the eyepiece once complete.



## Differential Interference Contrast

*Kohler Illumination must be completed for DIC microscopy to work! Ask CoRE Staff for an appropriate calibration sample.*

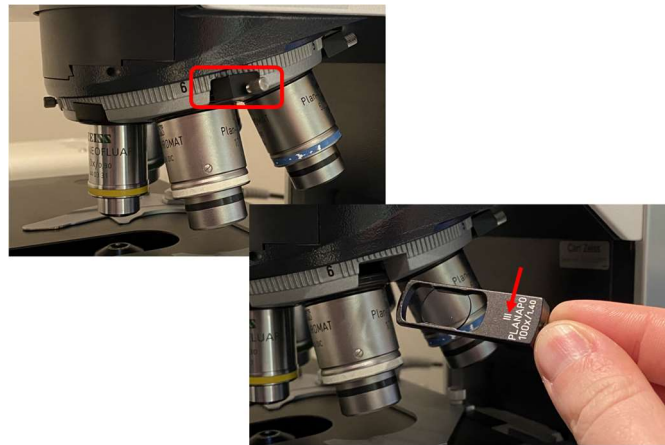
1. On the condenser, adjust the **Aperture Diaphragm** all the way to the left.



2. Some objectives have a DIC slider inserted right above the objective. *If a slider is not installed, no DIC imaging is possible with this lens.*

**Carefully** pull on the silver screw to remove the slider to remove it. Determine which DIC setting – I, II or III – is required for that particular objective.

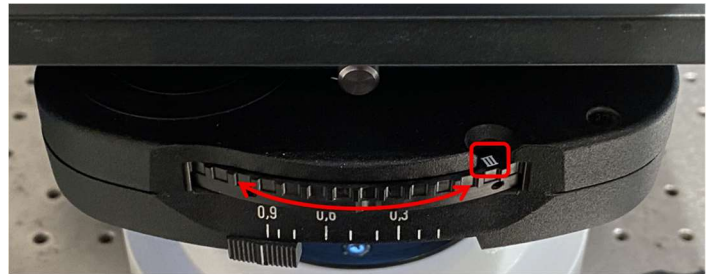
**Carefully** re-insert the DIC slider above the objective; there is only one correct orientation – text facing up. Do not force the slider into place. The slider should easily go all the way in and should “click” into place with little force.



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3. On the condenser, set the **Contrast Ring** to match the DIC number from the slider – use *I, II or III*, not “1, 2 or 3”!



4. On the touchscreen, navigate to **Microscope > Reflector** and select the “DIC” filter cube.
5. Twist the silver screw on the DIC slider back and forth to produce an optimal DIC image for your sample.

The optimal DIC image exhibits good edge contrast for each cell and has a black-to-white gradient across the background.



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## Imaging Setup (Software)

1. On the microscope, pull the Lightpath Selector out for the camera you want to use.

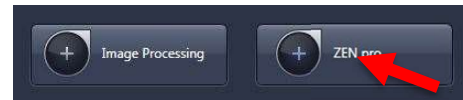
*Brightfield stains require the Color Camera (pull out lower Selector). Phase Contrast and DIC can be imaged on either camera (set Selectors appropriately).*



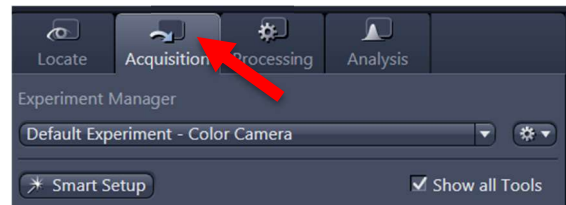
2. On the computer, log into your account using the username and password established for this microscope at your training session.
3. On the desktop, start **ZEN (blue edition)**.



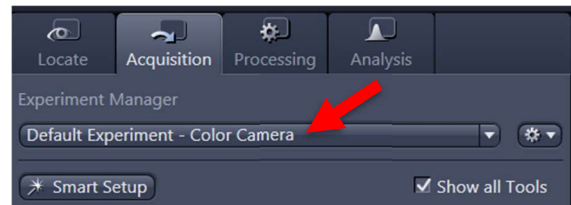
4. Click **ZEN pro** to connect to the microscope.



5. Click **Calibrate Now** when prompted. If your sample was in focus prior to this step, it will not be harmed!
6. Along the top left edge of the software, switch to “Acquisition.”



7. From **Experiment Manager**, select *Default Experiment – Color Camera* under the subheading “Workgroup Documents”.

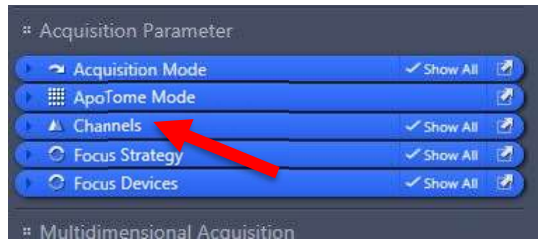


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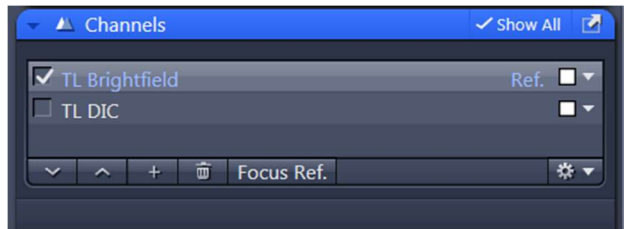


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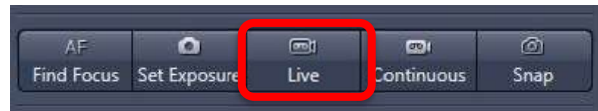
8. Along the left control panel, expand **Channels**.



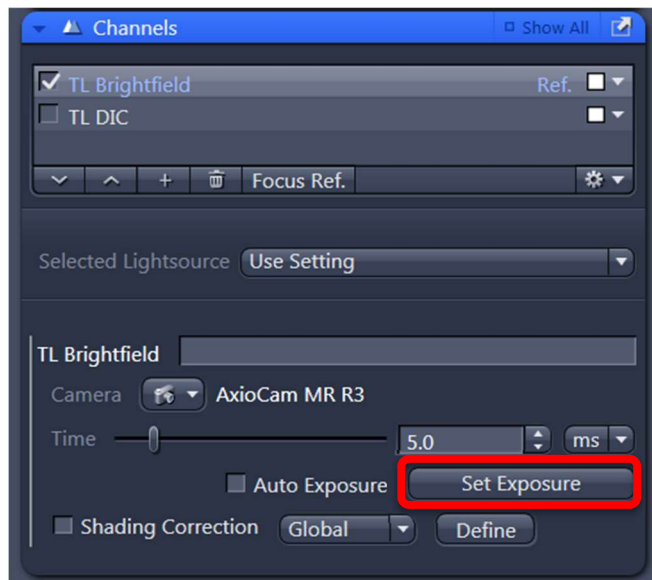
9. Check *either* **Brightfield/Phase** or **DIC**. Remove checkmarks from the other channel.



10. In the Camera Controls, click **Live**. You may be prompted to move a “Manual Component” at this time – the *Lightpath Selector* for the proper camera. Click OK.

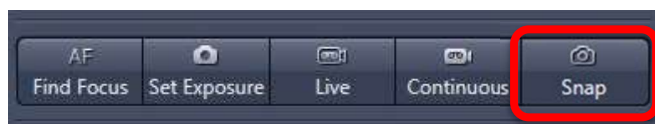


11. Using the microscope **Z Focus Knobs**, refocus the camera image until objects of interest exhibit crisp edges. Use the XY joystick to re-center the region of interest to the camera’s field of view.



12. In **Channels**, click **Set Exposure**. The exposure will be automatically adjusted.

13. In the Camera Controls, click **Snap** to automatically capture the current image field.



14. *To capture more fields*: Re-activate **Live**, move to another area of interest and click “Snap”.

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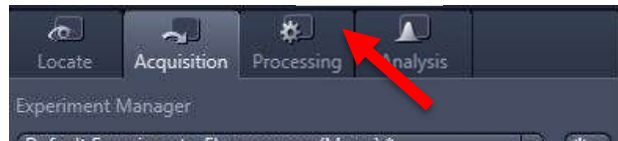


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- As images are captured, go to *File > Save As* (not “Save As with Options”) to preserve the original \*.czi file. This file contains your raw data. CZI’s can be opened and analyzed in many third-party software packages, many of which are available through the CoRE.
  - To save a JPG or TIF image for publication, please use “Image Export” under the Processing tab instead.
  - 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.

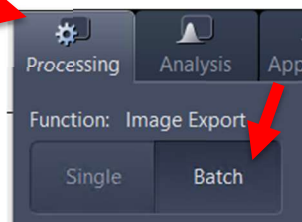
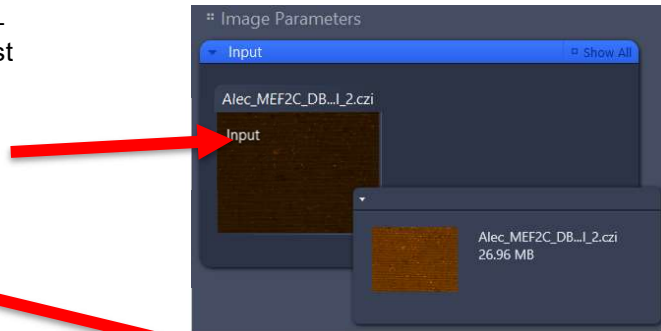
## Image Export

- To convert \*.czi raw files to JPG or TIFF files, move from **Acquisition** to **Processing**.

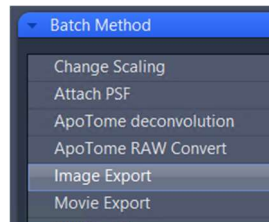


- To export a single image – open the image in ZEN first and load it into **Input**. Under **Method**, search for and click once on **Image Export**. Skip to Step 7.

To batch export – Under **Function**, switch from **Single** to **Batch**.



- Under **Batch Method**, choose **Image Export**. The Parameters window will open.

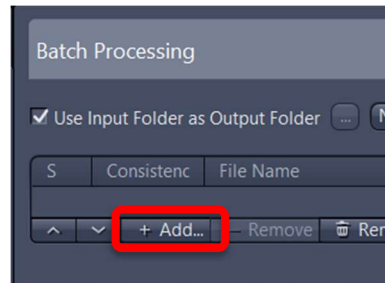


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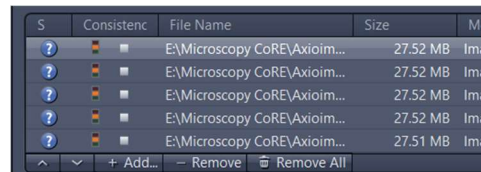
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4. Before setting any Parameters, click “+ Add...” in the central field.

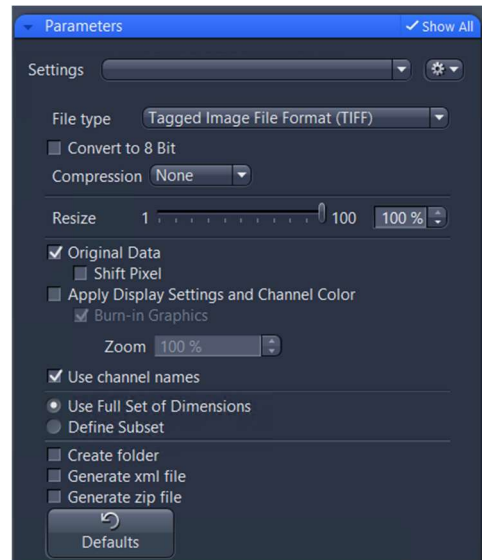


5. Locate, select and load all images you wish to export in a similar manner. *You can hold Shift or Ctrl to select multiple files in the “Add files” dialog box before clicking “Open.”*

6. Click once to highlight the first image in the series.



7. Under **Parameters** at left, choose the file type and preferences for image export. *Check “Show All” to the right of Parameters to reveal the additional options. Recommended parameters are shown at right.*
  - i) Choose “Original Data” if you plan to quantify images later; resulting file extension reads “\_ORG”
  - ii) Choose “Apply Display Settings and Channel Color” if you have manipulated the histogram for a prettier image or added a scale bar that you would like to submit for publication; **DO NOT** quantify data off these images!
  - iii) Select “Use full set of dimensions” unless you prefer to export only subsets of images – e.g. two of four fluorescent channels



**If you are exporting a single image:** After setting Parameters, simply click “Apply” at the top of the Processing tab.

**For batch processing,** continue reading.

8. At top-right of the image list, click **Copy Parameters**.

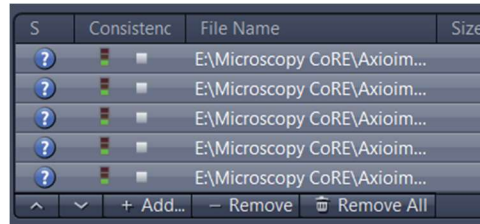




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9. Highlight all images. Use the **Shift** key to select multiple images.



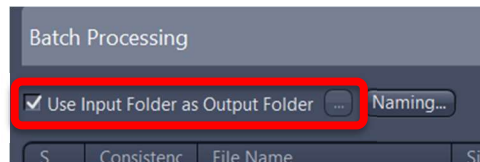
10. Click **Paste Parameters**.



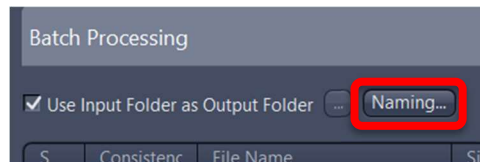
11. Click **Check All**. You should receive little green boxes under the column **Consistency**.



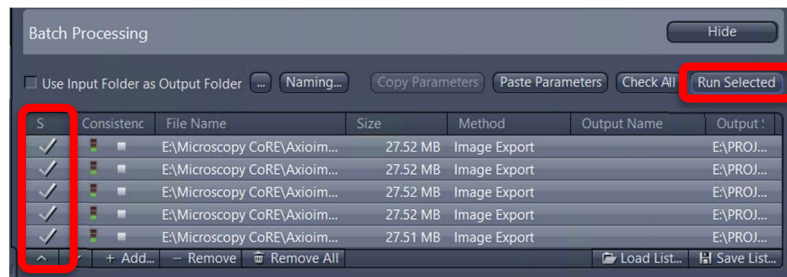
12. By default, all exports will be written to the same folder where the raw image exist. If you wish to change this, **uncheck Use Input Folder as Output Folder** and use the “...” to choose a new location.



13. If you would like to use a specific naming convention for exported images, click **Naming**. Some third-party program algorithms may expect certain filenames; check their instructions for details.



14. When ready to export: Ensure all files to be exported are selected. At top-right, click **Run Selected**. A checkmark should appear to the left of each image name as it is processed.



## System Shutdown

1. Ensure all images are saved in the correct location.
2. On the microscope touchscreen: Tap **Load Position**.

# Microscopy CoRE – Axio Imager.Z2(M) and Z2 – Transmitted Light



[microscopy.core@mssm.edu](mailto:microscopy.core@mssm.edu); 212-241-0400  
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3. Remove your sample from the stage. Gently wipe any oil off your slide with a KimWipe.
4. Clean any oil immersion lenses that were used according to the detailed instructions in the **Prerequisites** section of this document or refer to the cleaning poster on the wall by the microscope.
5. *On the touchscreen* – Return the 10x objective to the imaging position (front).
6. Close the ZEN software and log off or perform a full shutdown. *If another user will come after you, you must at least remember to log off your account but you can leave the microscope on.*
7. Turn off all power supplies (no particular order), most importantly the fluorescence lamp.
8. Replace the dust cover on the microscope.

