Microscopy CoRE – Axio Imager.Z2(M) and Z2 – Fluorescence Operation

microscopy.core@mssm.edu; 212-241-0400
June 2024

The ZEISS Axio Imager.Z2(M) and Z2 are widefield microscopes powered by ZEISS' ZEN blue software. They feature a variety of magnifications, fluorescent filter sets and software capabilities.

Prerequisites

1. **Before and after your session, you are responsible for cleaning <u>all</u> 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. <u>For Dry Lenses:</u>
        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. <u>For Oil Immersion Lenses:</u>
        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 <u>completely dry</u>.** This can take at least 24 hours from when the slide is prepared. Refer to the manufacturer's instructions.

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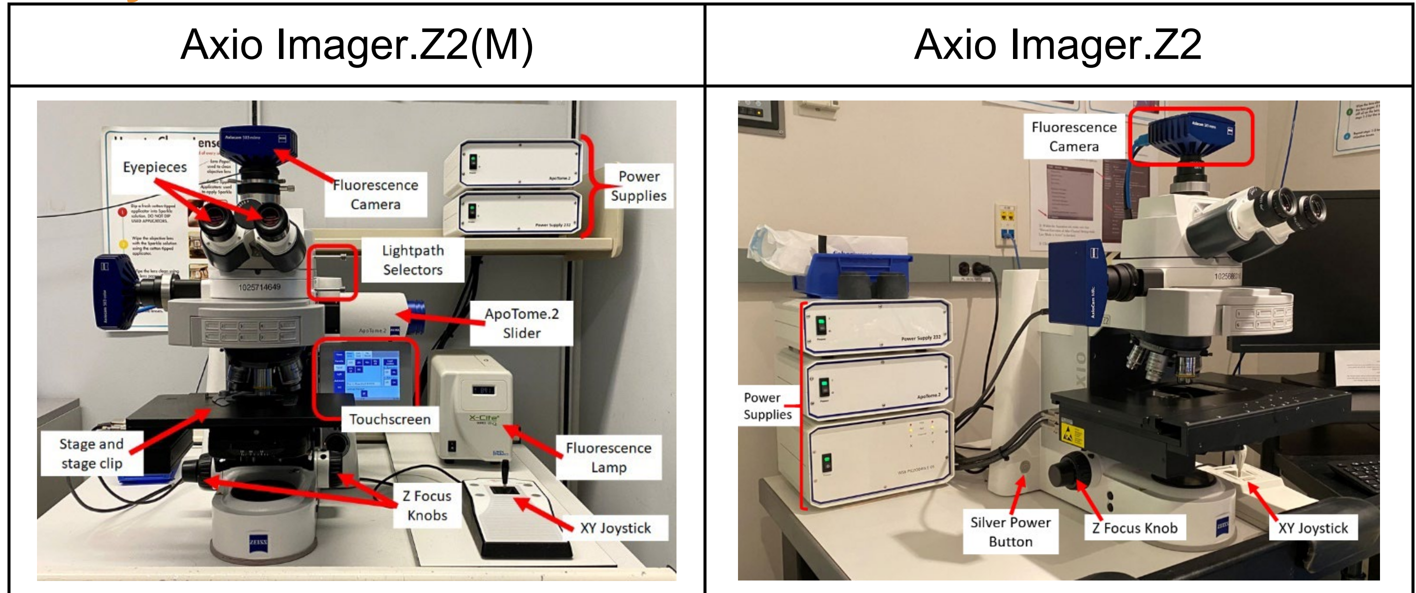
System Overview



System Startup - Fluorescence

1. Remove the blue dust cover from the microscope. Set it aside.

2. Locate the white control boxes for the microscope: on the shelf above the Z2(M), to the left of the Z2. Turn on the “Power Supply 232,” then “ApoTome.2” (if using).
    a. For the Z2, also turn on the stage controller “WSB Piezodrive.”

3. On the left “leg” of the microscope behind the stage, locate the silver button. Press once to start the microscope. (Refer to “Z2” System Overview above for correct location.)

4. Turn on the fluorescence lamp: to the left of the microscope for the Z2(M), on the lower shelf of the computer table for the Z2.

5. Locate the computer (bottom shelf of the computer table). Press the power button.

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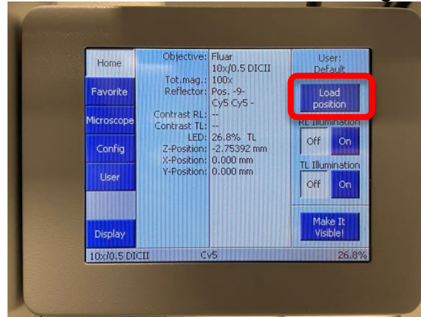
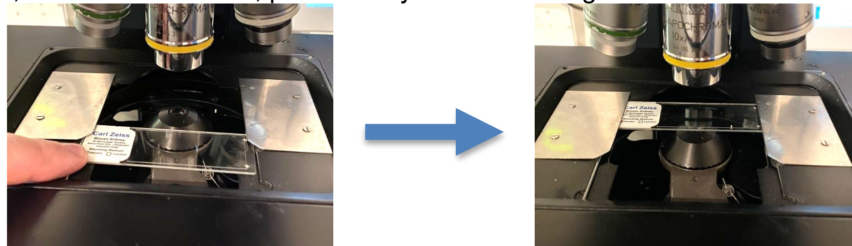
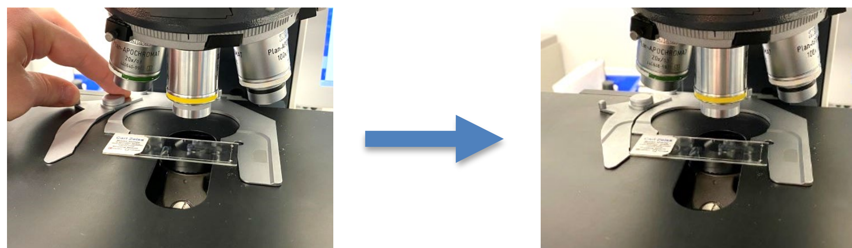
Fluorescence Setup

1. *On the touchscreen*: Press **Load Position** to lower the stage.

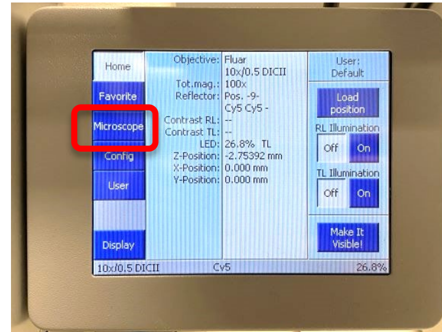


2. Load your slide on to the stage. *IMPORTANT NOTE: If this is your first time imaging a set of slides, load the slide where you expect the <u>brightest</u> signal first.*

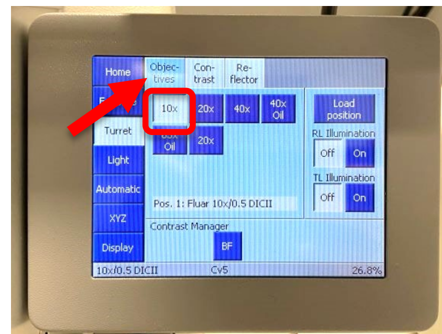
    a) *On the Imager.Z2* – Place the slide coverglass face-up at the lower edge of the stage holder. Slide the sample under the two metal guides until it is flush against the back of the holder.
    b) *On the Imager.Z2M* – Using your left thumb, press the stage clip outward to the left. Mount your slide 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. When complete, on the touchscreen, press the symbol of a triangle with a line over it.

Imager.Z2	
Imager.Z2(M)	

3. *On the touchscreen*: From the **Home** page, tap **Microscope**.

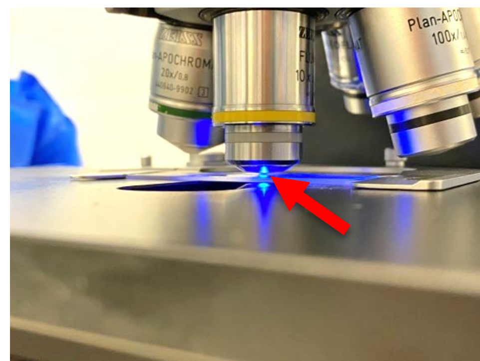


4. *On the touchscreen*: Make sure you are in the **Objective** tab and press **10x**.



5. Using the XY joystick, roughly center your sample underneath the objective lens. *Press **F1** on the upper-right corner of the joystick to change stage speed.*

6. Place your eyes level with the surface of the stage. Raise the stage by rotating the Z Focus Knobs over the top, away from you. <u>Take care not to crash the objective lens through your slide!</u> Raise the stage until the objective is about to touch the slide and go no further. *Note the small spacing between the objective and the slide in the image below*.



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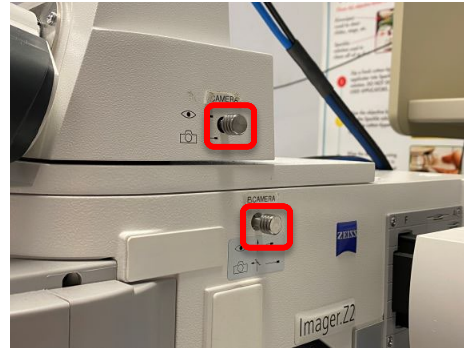
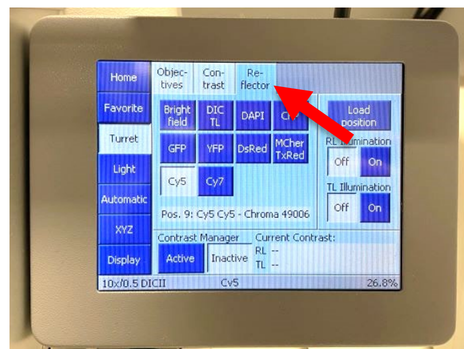
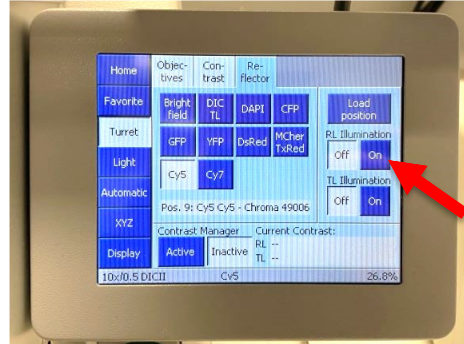
7. Gently push both **Lightpath Selector** fully in toward the microscope. This will direct light to the eyepieces.



8. *On the touchscreen*: Along the top, switch to **Reflector**. Select the filter set that matches one of your sample's fluorophores. *In this example, the "Cy5" filter cube is currently in use.*

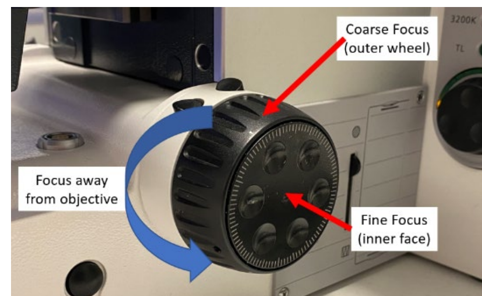


9. *On the touchscreen*: Press **On** for **RL Illumination**. You should now see light on your sample.



10. Look through the eyepieces. Adjust the interpupillary spacing of the eyepieces using both hands until one circular field of view is visible through them.

11. Rotate the coarse (outer) **Z Focus Knobs** toward you (over the top). Stop when your sample comes into perfect focus. *If coarse focus is moving too quickly, use the fine focus knob instead*. If your region of interest is not well-centered under the lens, use the XY joystick to move the sample.



12. *On the touchscreen*: Tap other fluorescent filters under the **Reflector** tab to examine staining quality of any remaining fluorophores. *If staining is poor, consider stopping, returning to your lab, and examining your staining protocol for optimization. New samples might be necessary.*

13. Once the initial examination is acceptable, return to the touchscreen and press **Off** under **RL Illumination**. *This step will keep your fluorescent signal from quenching.*

14. If a higher magnification is required, switch back on the touchscreen to "Objective" and press the required magnification.

If an oil immersion objective is required: Switching to that lens will drop the stage. 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 "RL 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!*

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 may occur at the periphery. Speak with CoRE Staff about shading corrections if you are unsure if you will need them, and for setup assistance.

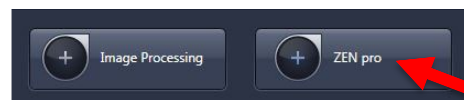
Imaging Setup (Software)

1. On the microscope, pull the top **Lightpath Selector** fully outward.

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**. The microscope will initialize.

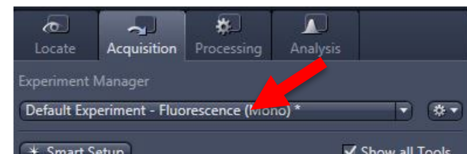


5. Click **Calibrate Now** when prompted. If your sample was in focus prior to this step, it will not be harmed by this step.

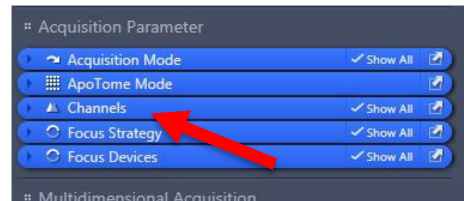
6. Along the top left edge of the software, switch to "Acquisition."

7. From **Experiment Manager**, select "*Default Experiment – Fluorescence*" under the subheading "Workgroup Documents".

   To recover previous acquisition settings instead, re-open an older image and click "Reuse" underneath the image, in Dimensions.

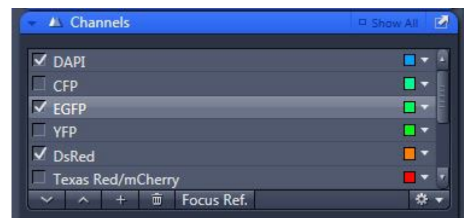


8. Along the left control panel, expand **Channels**.



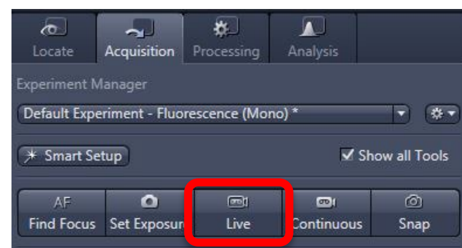
9. Add checkmarks to your required fluorescence channels (and DIC channels, as needed). Remove checkmarks from any remaining channels.

In this example, DAPI, EGFP and DsRed will be captured.



10. Click once on any of the fluorescence channels that will be imaged.

11. In the Camera Controls, click **Live**. Monochromatic light should once again hit the stage and the Live image will appear in the center of the software. *You may be prompted to move a "Manual Component" at this time. Click OK.*



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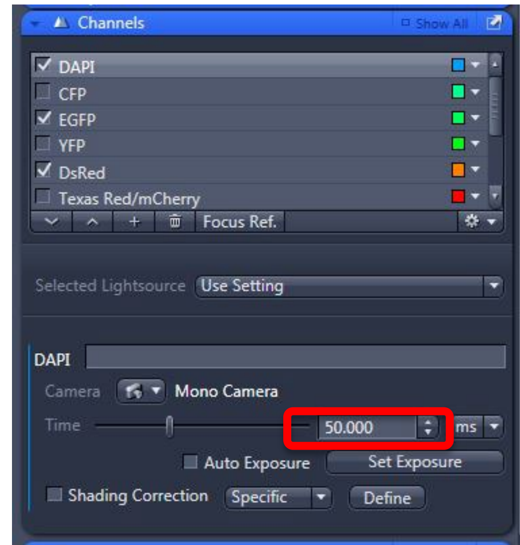
12. Using the microscope **Z Focus Knobs**, refocus the camera image until objects of interest exhibit crisp edges. Use the XY joystick to re-center the region of interest to the camera's field of view.



13. In **Channels**, click **Set Exposure**. The exposure will be automatically set to a proper exposure.

IMPORTANT NOTES ABOUT EXPOSURE:
Exposures are set per fluorescence channel on your brightest fluorescence signals. Depending on your experiment, your brightest signals for each fluorophore may be on one slide, or they may be on multiple slides. Change slides if you need to before setting the relevant exposure time.

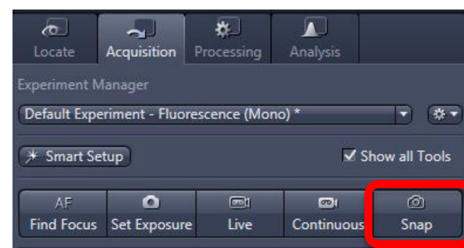
For tissue samples, first move to the area where you expect the brightest signal for that fluorophore.

Sometimes artifacts are present in the current field of view – stray hairs, edges of mounting media. If you see these artifacts in your current image, move to an adjacent area.

14. In **Channels**, click on the next fluorophore. Click **Set Exposure** again.
    a) Different channels may be in "perfect" focus at different focal planes. *If focus correction is necessary, please speak with CoRE staff for guidance on how to set this up.*

15. Repeat Step 14 for any additional fluorophores. When you are done, click **Stop** (where the "Live" button had been) to close the fluorescent shutter.

16. In the Camera Controls, click **Snap** to automatically capture a single, multi-color image.



17. *To capture more sample fields:* In **Channels**, click back on the first fluorophore and click **Live**. Using the Live Image (or the eyepieces, pushing in the top Lightpath Selector), move to another area of interest and click "Snap" to capture more fields.
    a) Single-fluorophore positive controls should be used to establish exposure times for each channel. It is best practice to NOT adjust exposure times once they are

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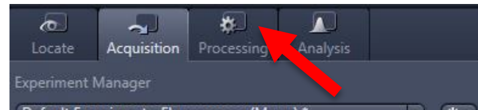
established. *Changing exposure times will make quantification impossible and data analysis inaccurate.*

18. To switch slides, press **Load Position** on the microscope touchscreen. After switching slides, press the button at top-left, an up arrow with a line over it, to return to the previous focus position.
19. 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.
    a) *To save a JPG or TIF image for publication, please use "Image Export" under the Processing tab instead.*
    b) **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.*

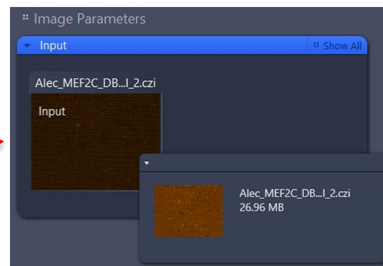
Image Export

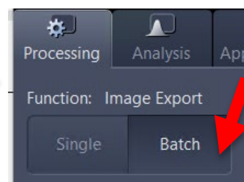
1. To convert *.czi raw files to JPG or TIFF files, move from **Acquisition** to **Processing**.



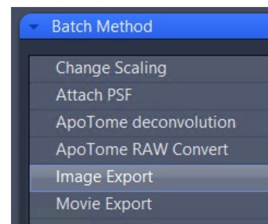
2. *To export a single image* – open the image in ZEN first and load it into **Input**. Under **Method**, search for and click once on **Image Export**. *Skip to Step 7.*

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





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



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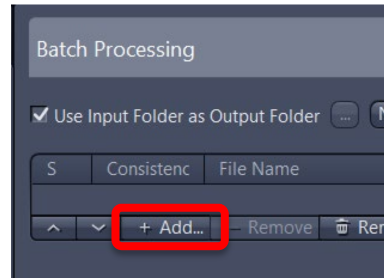
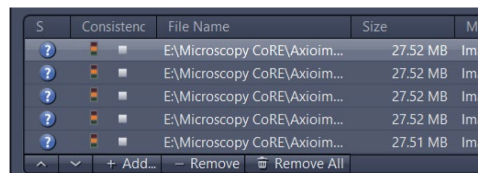
4. Before setting any Parameters, click "+ Add…" in the central field.

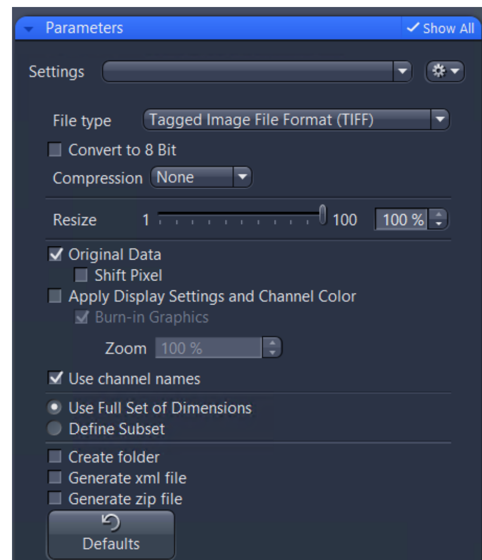


5. Locate, select and load all images you wish to export in a similar manner. *You can hold Shift or Ctrl to select multiple files in the "Add files" dialog box before clicking "Open."*

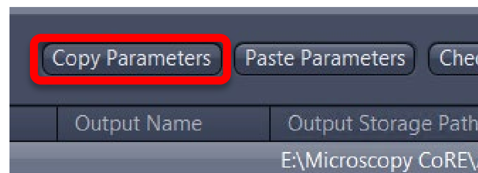
6. Click once to highlight the first image in the series.



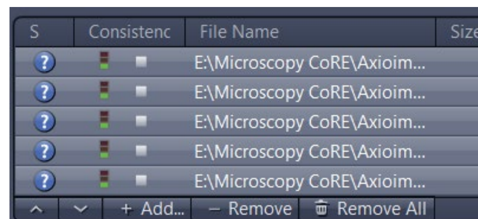
7. Under **Parameters** at left, choose the file type and preferences for image export. *Check "Show All" to the right of Parameters to reveal the additional options. Recommended parameters are shown at right.*
   i) *Choose "Original Data" if you plan to quantify images later; resulting file extension 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*



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



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



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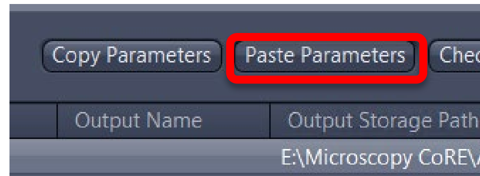
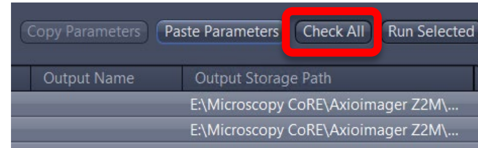
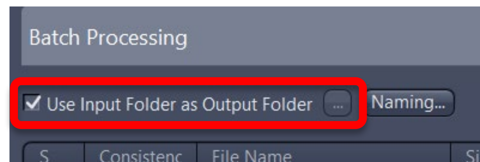
10. Click **Paste Parameters**.



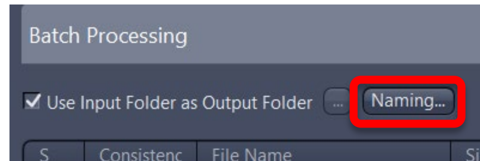
11. Click **Check All**. You should receive little green boxes under the column **Consistency**.



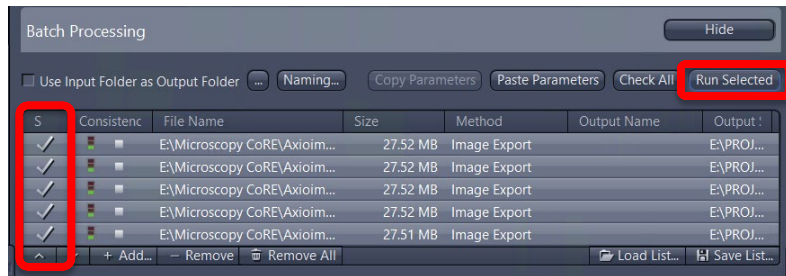
12. By default, all exports will be written to the same folder where the raw image exist. If you wish to change this, **uncheck Use Input Folder as Output Folder** and use the "…" to choose a new location.



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.



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System Shutdown

1. Ensure all images are in the correct location on the HIVE. Shut down the software and PC, or log off if another user will start imaging right away.
2. On the microscope touchscreen: Tap "Load Position."
3. Remove your sample from the stage by holding back the stage clip. 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. Turn off all power supplies (no particular order), most importantly the fluorescence lamp.
7. Replace the dust cover on the microscope.

Tips for Opal Dyes

Use the following filters based on the Opal dyes in the sample:

Opal 480 – "Aqua"
Opal 520 – "Narrow Green"
Opal 540 – (not recommended)
Opal 570 – "Orange" or "Gold"
Opal 620 – "Red"
Opal 650 OR 670 – "Far Red"
Opal 690 – (not recommended)
Opal 780 – "FISH IR"

Good panel: 480, 520, 570, 620, 650/670, 780
Or any combination of the above. Selection is based on how well Opal spectra match our existing FISH filter sets.

Single-color controls are still highly recommended here.

Filters must be installed by the CoRE prior to imaging, and removed at the end of your session. Please book all reservations only within business hours (Monday-Friday, 9AM-5PM).

These filter selections are available under the Experiment Manager drop-down. Select "Default Experiment – FISH" to use these filters.

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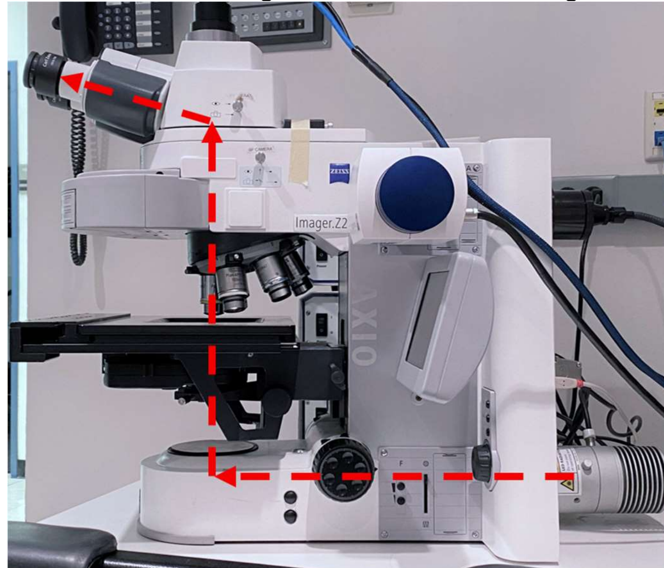
The Axio Imager.Z2(M) and Z2 are equipped for Brightfield, Darkfield, Phase Contrast and Differential Interference Contrast (DIC). This guide will explain how to set up each modality.

Transmitted Light Path of the Axio Imager



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   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.

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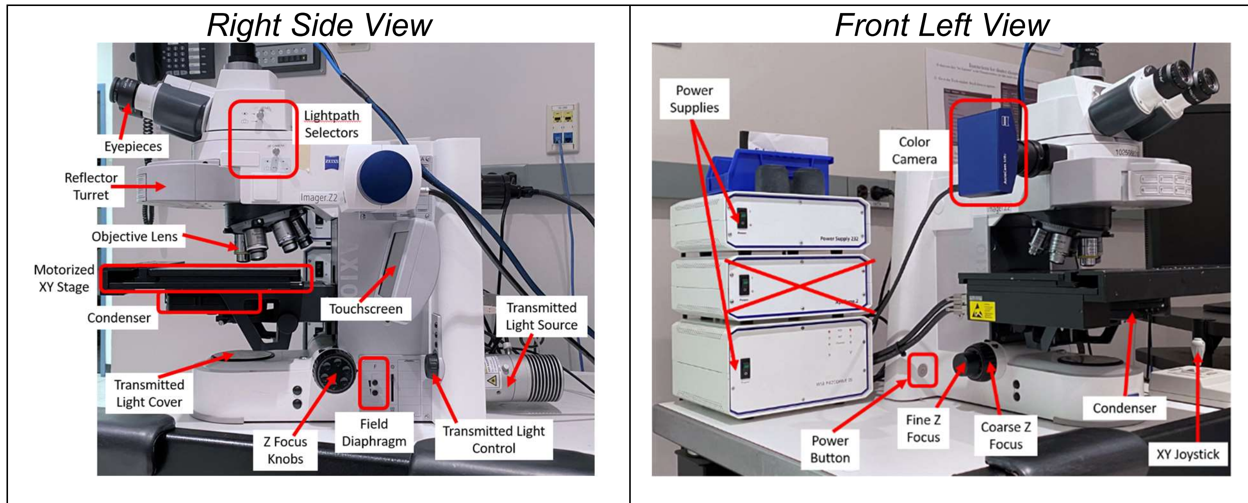
System Overview



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.)

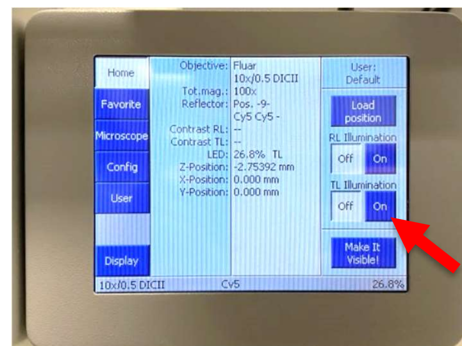
3. Remove the rubber Transmitted Light Cover from the base of the microscope.

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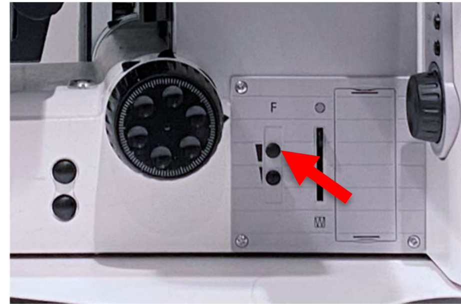
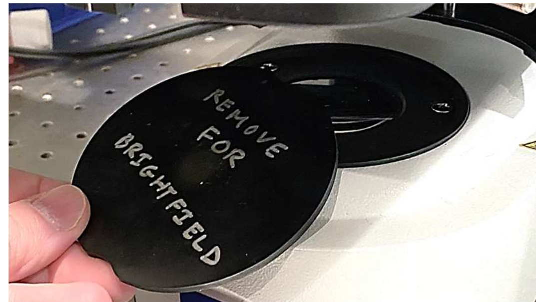
2. On the right "leg" of the microscope, use the **Transmitted Light Control** to adjust lamp intensity. *The lamp is very bright, so setting the lamp to the lowest "orange" range is typical*.

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.

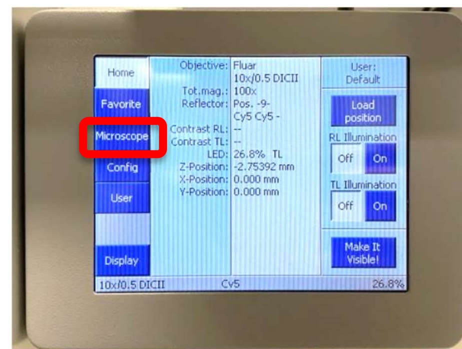


5. On the condenser, open the **Aperture Diaphragm** fully .

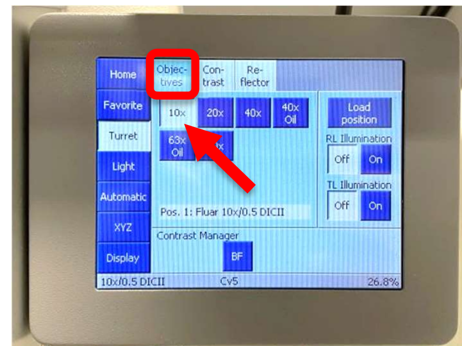
6. Also on the condenser, manually move the **Contrast Ring** to the "H" or "BF" position.

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

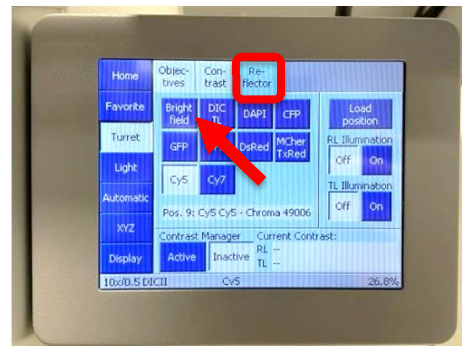
8. *On the touchscreen* – On the left edge, tap **Microscope**.



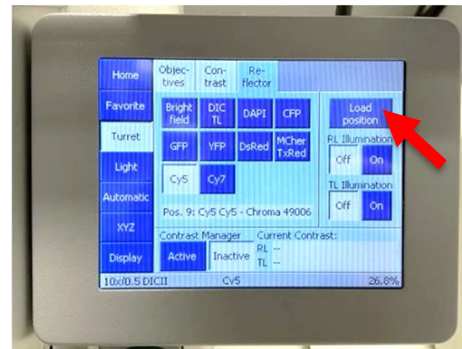
9. *On the touchscreen* – Along the top edge, press **Objectives**. Tap the **10x** lens to move it to the imaging position.



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

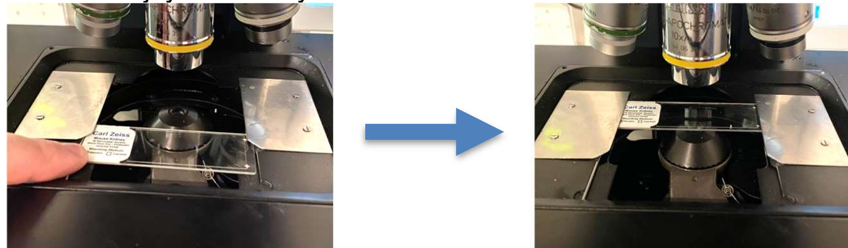


11. *On the touchscreen* – Along the right edge, press **Load Position**. The stage will drop.

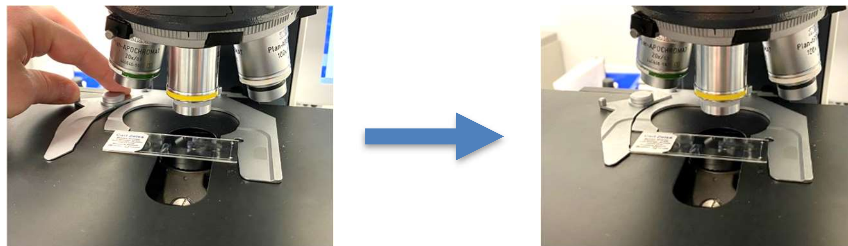


12. Load your slide on to the stage. *(See next page for images.)*
    a. *On the Imager.Z2* – Place the slide coverglass face-up at the lower edge of the stage holder. Slide the sample under the two metal guides until it is flush against the back of the holder. Move the XY joystick briefly in X and Y to ensure the slide is secure.
    b. *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



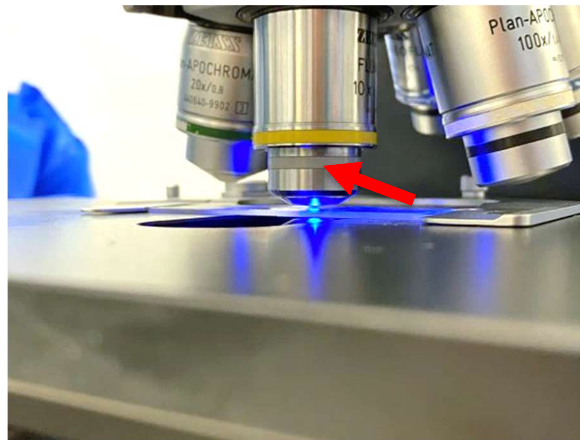
Imager.Z2(M)



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.*

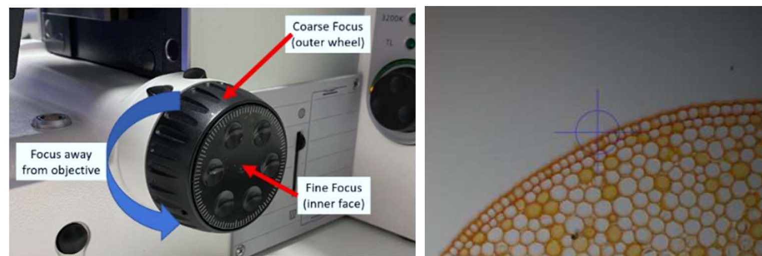
15. Looking flat across the stage (drop your eyes to the level of the stage), turn the **Z Focus Knobs** away from you (over the top) until the lens is just about to contact the slide. *Go no further or you risk damaging your slide.* Notice the small gap between the lens and slide; that is where to stop.



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!

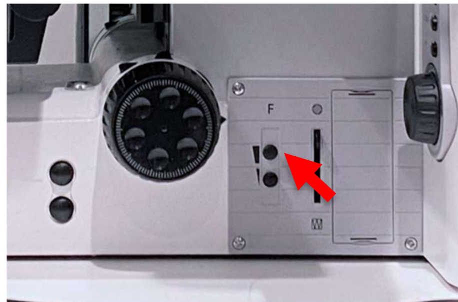


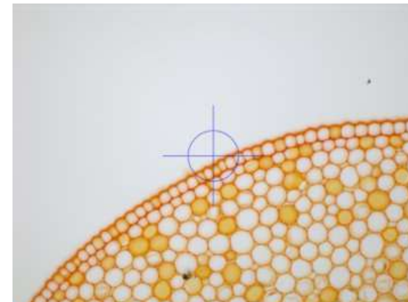
18. Press and hold the lower "F" button for 3-5 seconds. The image in the eyepieces will get darker.

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.

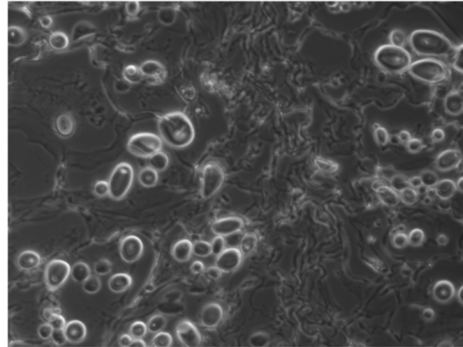
Do not switch from an oil objective back to a dry/air objective without first cleaning the lens and your slide!

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.

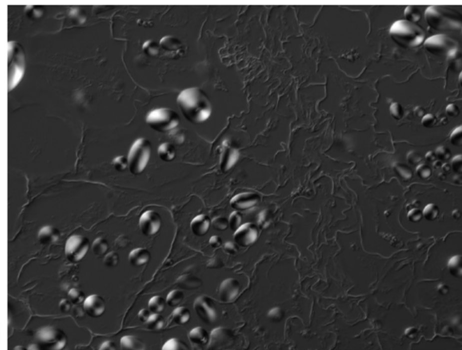
Your microscope is now set up for Brightfield Illumination.

Phase Contrast and DIC – contrast without staining

Brightfield



Phase Contrast



DIC

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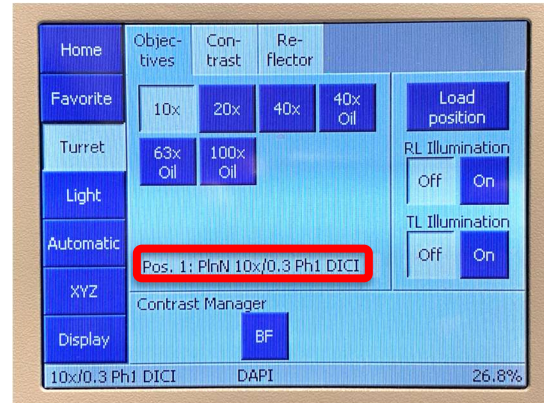
Phase Contrast

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.

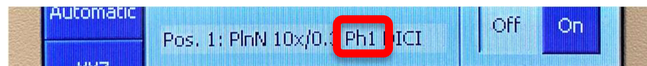
   The full name of the lens, highlighted, will show “Ph” in the name.

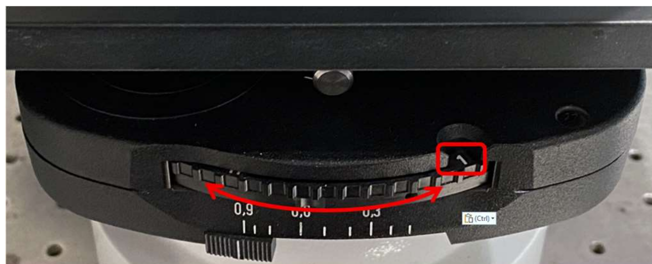


2. Switch to the **Reflector** tab. Select the “Brightfield” filter cube.
3. Open the **Aperture Diaphragm** fully.

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.

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

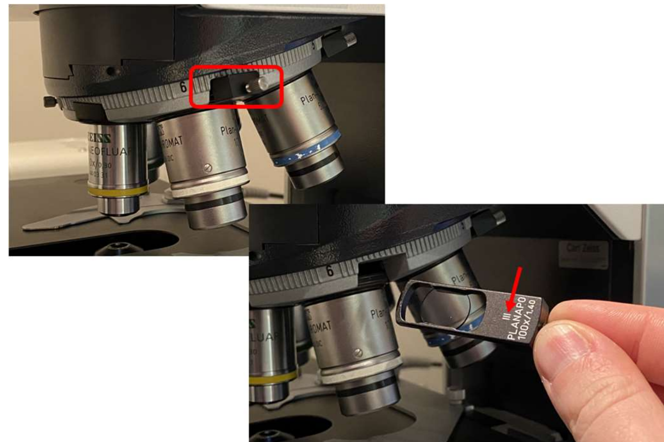
Kohler Illumination must be completed for DIC microscopy to work! Ask CoRE Staff for an appropriate calibration sample.

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

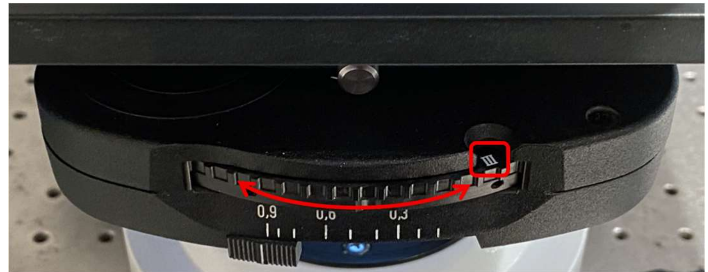
2. Some objectives have a DIC slider inserted right above the objective. *If a slider is not installed, no DIC imaging is possible with this lens.*

   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. <u>Do not force the slider into place</u>. The slider should easily go all the way in and should “click” into place with little force.



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)

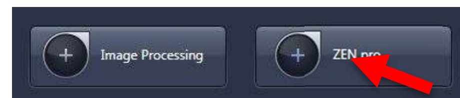
1. On the microscope, pull the Lightpath Selector out for the camera you want to use.

   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.

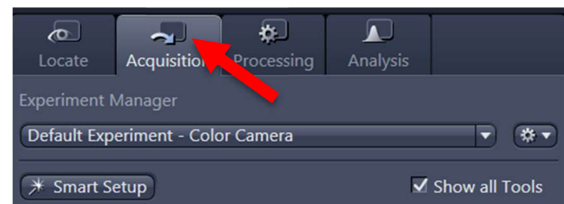
3. On the desktop, start **ZEN (blue edition)**.

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

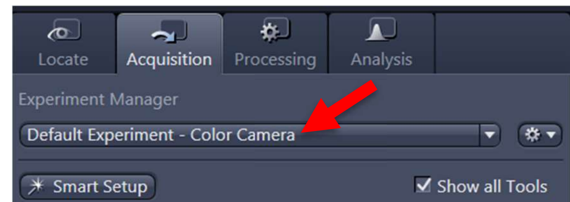


5. Click **Calibrate Now** when prompted. <u>If your sample was in focus prior to this step, it will not be harmed!</u>

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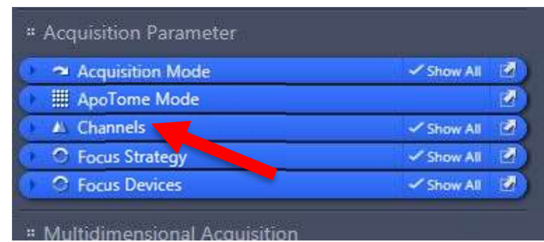
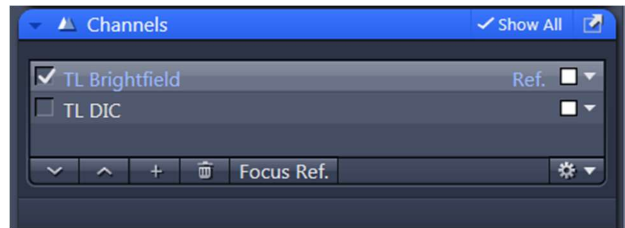
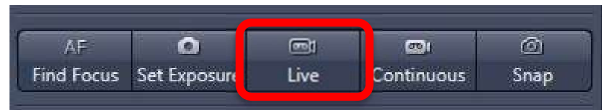
8. Along the left control panel, expand **Channels**.



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

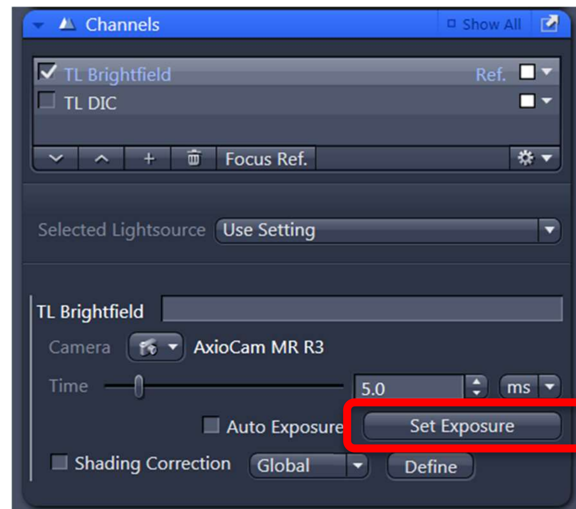


10. In the Camera Controls, click **Live**. *You may be prompted to move a "Manual Component" at this time – the Lightpath Selector for the proper camera. Click OK.*



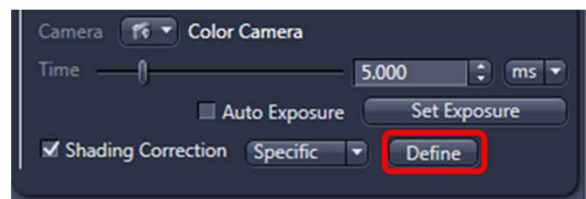
11. Using the microscope **Z Focus Knobs**, refocus the camera image until objects of interest exhibit crisp edges. Use the XY joystick to re-center the region of interest to the camera's field of view.

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



13. A Shading Correction must be performed for transmitted light imaging (Brightfield/Phase Contrast/DIC).

    First, move off the sample to a blank area of your slide (no sample, no dirt in the field).
    Turn the fine focus knob one-half turn toward you (defocus a little).
    Under **Channels,** click **Define** which is to the right of **Shading Correction**.



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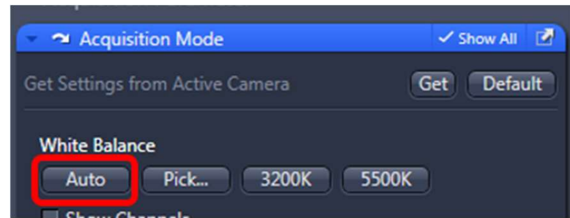
14. **For Brightfield stains**: When using the Color Camera to capture H&E and similar staining, a **White Balance** must be performed.

    While still off the sample and a little out of focus, open **Acquisition Mode**. Under **White Balance**, click **Auto**.



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

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

17. 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.
    a. *To save a JPG or TIF image for publication, please use "Image Export" under the Processing tab instead.*
    b. **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.*

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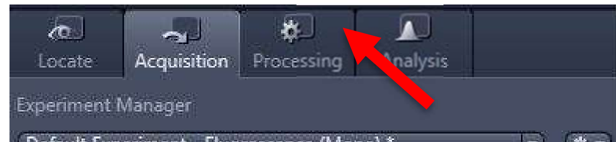
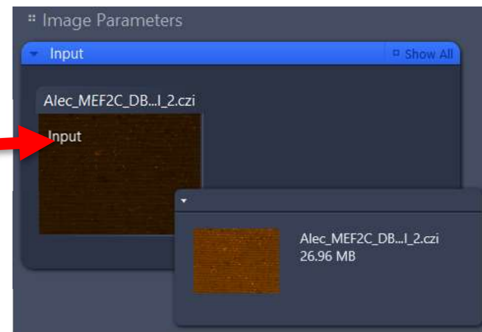
Image Export

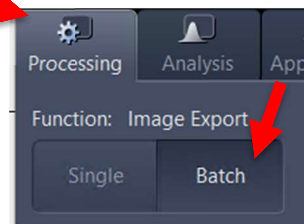
1. To convert *.czi raw files to JPG or TIFF files, move from **Acquisition** to **Processing**.



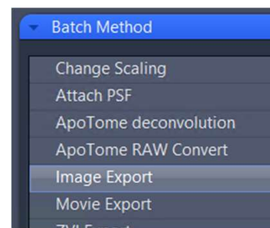
2. *To export a single image* – open the image in ZEN first and load it into **Input**. Under **Method**, search for and click once on **Image Export**. *Skip to Step 7.*

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

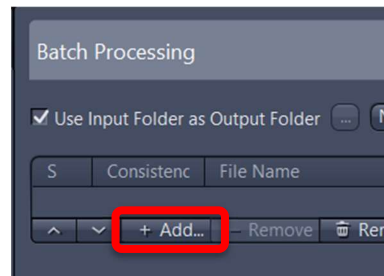




3. Under **Batch Method**, choose **Image Export**. The Parameters window will open.

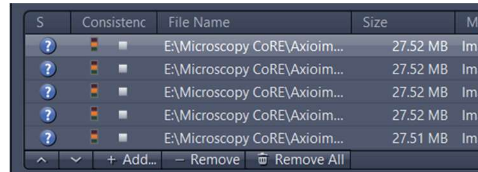


4. Before setting any Parameters, click "+ Add…" in the central field.

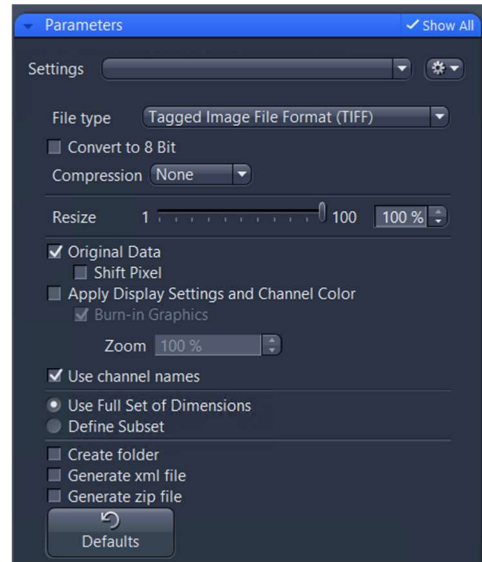


5. Locate, select and load all images you wish to export in a similar manner. *You can hold Shift or Ctrl to select multiple files in the "Add files" dialog box before clicking "Open."*

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*

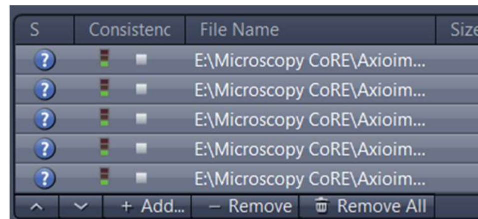


<u>If you are exporting a single image</u>: After setting Parameters, simply click "Apply" at the top of the Processing tab.

<u>For batch processing</u>, continue reading.

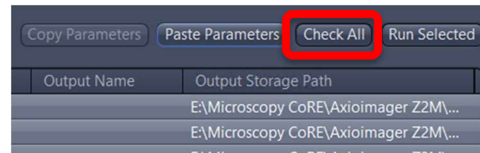
8. At top-right of the image list, click **Copy Parameters**.

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

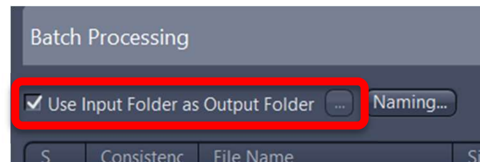


10. Click **Paste Parameters**.

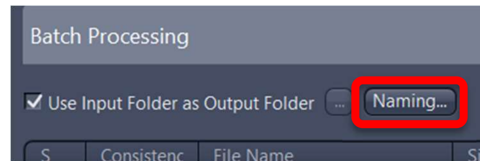
11. Click **Check All**. You should receive little green boxes under the column **Consistency**.



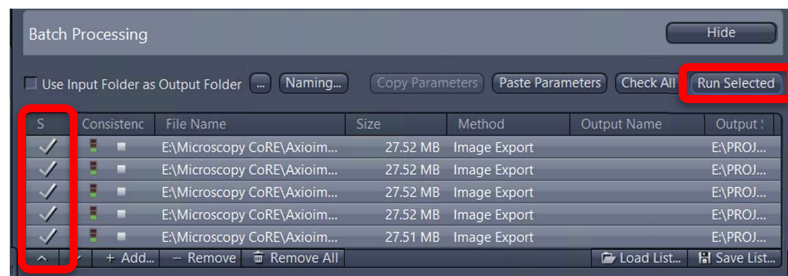
12. By default, all exports will be written to the same folder where the raw image exist. If you wish to change this, **uncheck Use Input Folder as Output Folder** and use the "…" to choose a new location.



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**.
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.

Instrument	**Axio Imager.Z2 (mot XY)**	License number 1384994013
SN	**3523000975**	
SW Version	**ZEN blue pro 3.5**	

	Magnification/NA	Immersion	Coverglass	Manufacturer PN	Working Distance
Objectives	1) Fluar 10x/0.5	air/dry	0.17mm ("#1.5") coverglass		
	2) PlanApo 20x/0.8	air/dry	0.17mm ("#1.5") coverglass		
	3) EC PlanNeoFluar 40x/0.75	air/dry	0.17mm ("#1.5") coverglass		
	4) EC PlanNeoFluar 40x/1.3	oil	0.17mm ("#1.5") coverglass		
	5) PlanApo 63x/1.4	oil	0.17mm ("#1.5") coverglass		
	6) PlanApo 100x/1.4	oil	0.17mm ("#1.5") coverglass		
	EC PlanNeoFluar 5x/0.16 (on request)	air/dry	(negligible)		

Filters	(standard config)	Excitation	Beamsplitter	Emission	Manufacturer PN
	1) none	N/A			N/A
	2) DIC	N/A			(Zeiss)
	3) Cy7	675-750	760	765-855	Chroma 49007
	4) Cy5	590-650	660	665-740	Chroma 49006
	5) mCherry/TexasRed	540-580	585	595-670	Chroma 49008
	6) DsRed	530-560	570	590-650	Chroma 49005
	7) YFP	490-510	515	520-550	Chroma 49003
	8) GFP	450-490	495	500-550	Chroma 49002
	9) CFP	426-446	455	460-500	Chroma 49001
	10) DAPI	325-375	400	435-485	Chroma 49000

OPTIONAL FILTERS	Excitation	Beamsplitter	Emission	Manufacturer PN
"Aqua FISH"	426-446	455	465-495	Chroma 49302
"Gold FISH"	441-451	556	560-583	Chroma 49304
"Red FISH"	568-593	600	610-640	Chroma 49306
"Orange"	528-549	556	560-591	Chroma 49309
"Narrow Green"	480-500	505	515-535	Chroma 49312
"Far Red"	620-640	647	652-682	Chroma 49307
FISH IR	720-760	770	765-855	Chroma - custom filter

Cameras	Axiocam 503 mono	monochrome	3MP	38FPS @ full frame	Pixel size: 4.54um X 4.54um	Wavelength capture range: 400-850nm
	Axiocam 503 color	color	3MP	38FPS @ full frame	Pixel size: 4.54um X 4.54um	Wavelength capture range: 400-700nm

Techniques Available
- TL Brightfield
- TL DIC
- Fluorescence - WF
- Fluorescence - ApoTome.2 optical sectioning
- SW - Tiling/Stitching, Time Lapse, Z-stack

Compatible with: standard slides (26x76mm)