

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



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May 2022

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 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.

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.

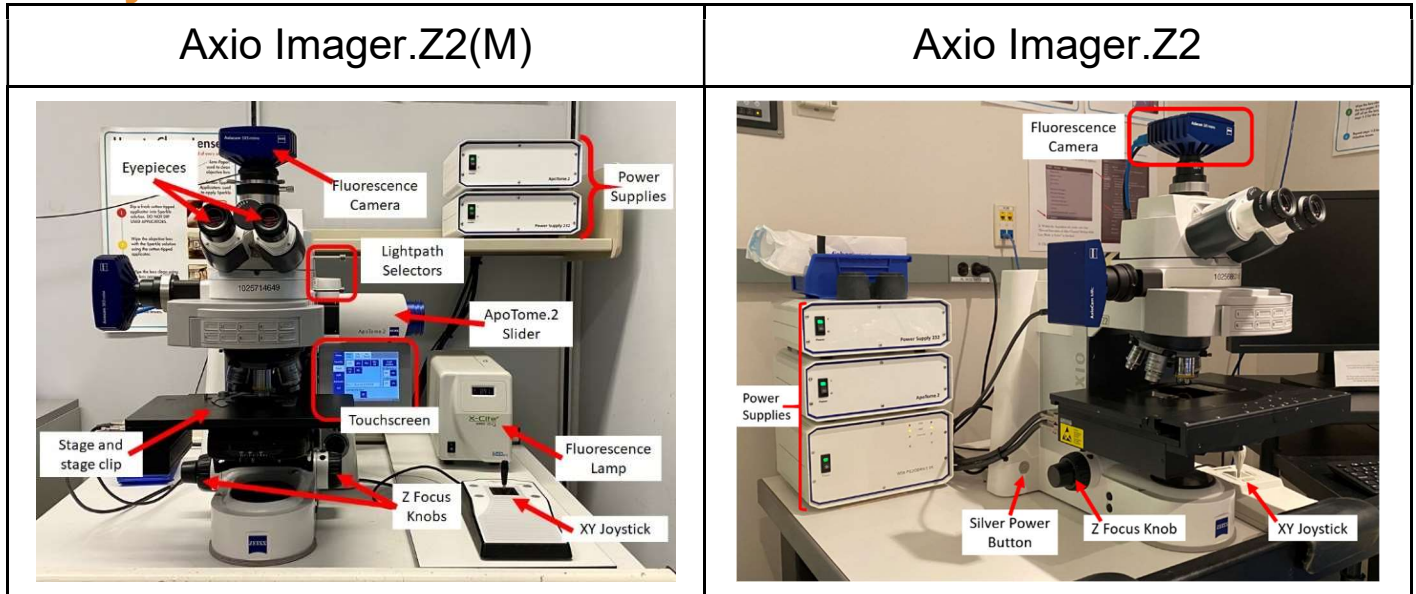
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System Overview



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.

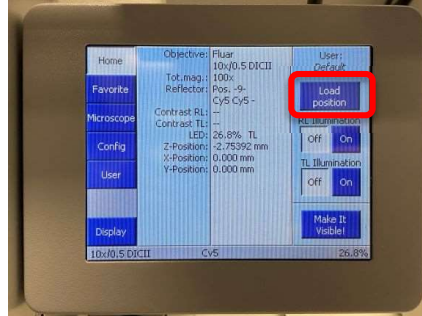
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Fluorescence Setup

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

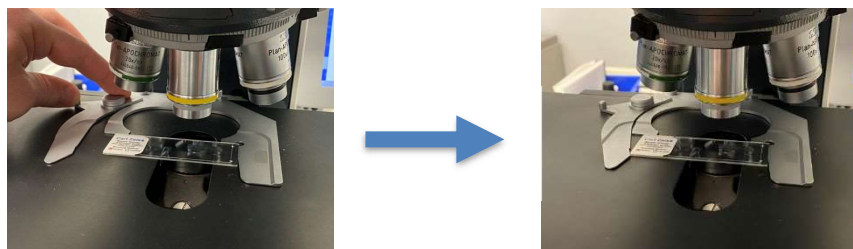


2. Load your slide on to the stage. **IMPORTANT NOTE:** If this is your first time imaging a set of slides, load the slide where you expect the brightest signal first.
 - a) On the *Imager.Z2* – Place the slide coverglass face-up at the lower edge of the stage holder. Slide the sample under the two metal 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)

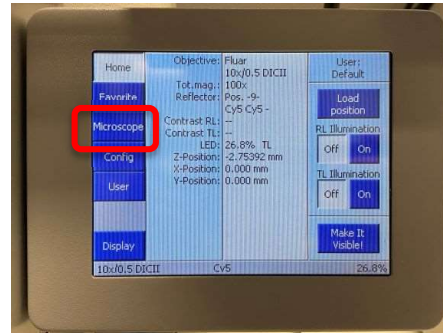


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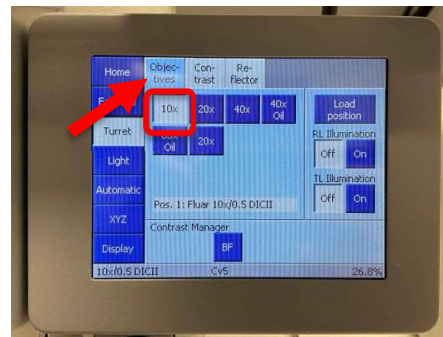
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3. *On the touchscreen:* From the **Home** page, tap **Microscope**.



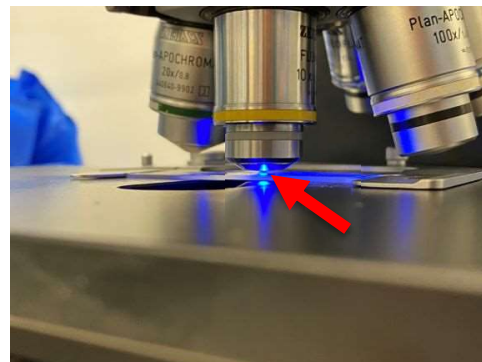
4. *On the touchscreen:* Make sure you are in the **Objective** tab and press **10x**.



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. Take care not to crash the objective lens through your slide! 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.*

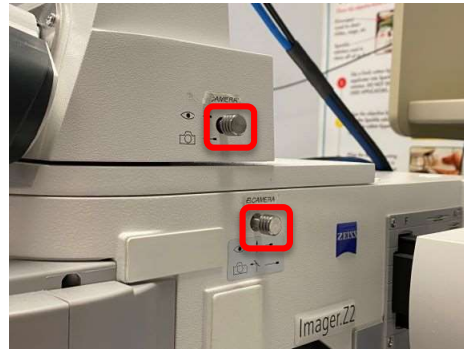


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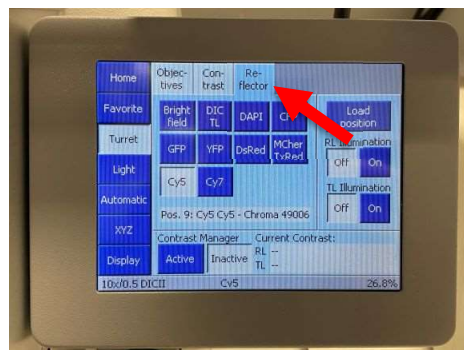
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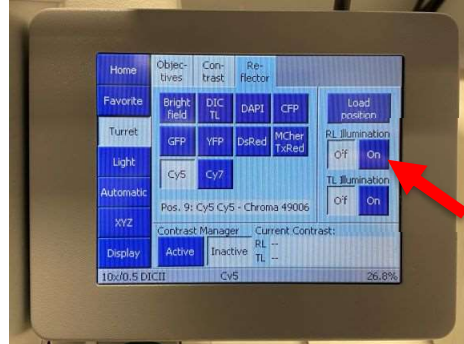
7. Gently push both **Lightpath Selector** fully in toward the microscope. This will direct light to the eyepieces.



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.*

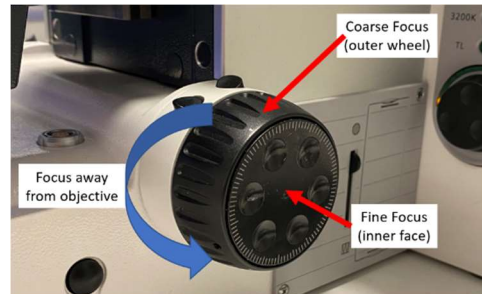


9. *On the touchscreen:* Press **On** for **RL Illumination**. You should now see light on your sample.



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.*

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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.

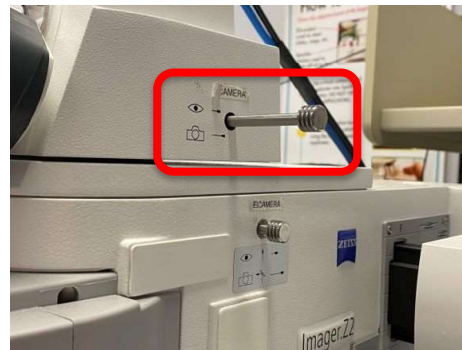
If an oil immersion objective is required: Switching to that lens will drop the stage. Use the paddle in the oil immersion bottle to place a single drop of oil on your slide right below the lens. On the touchscreen, press “Done” to bring your sample back into focus. Double-check focus by briefly turning “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.



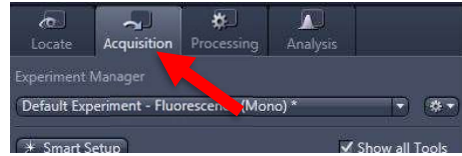
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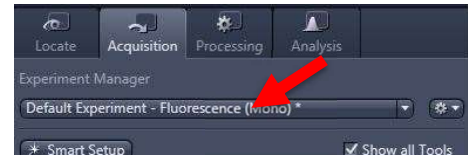
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- Click **Calibrate Now** when prompted. If your sample was in focus prior to this step, it will not be harmed by this step.
- Along the top left edge of the software, switch to “Acquisition.”

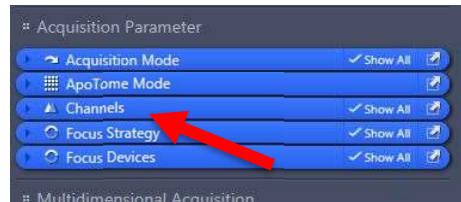


- From **Experiment Manager**, select “Default Experiment – Fluorescence” under the subheading “Workgroup Documents”.

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

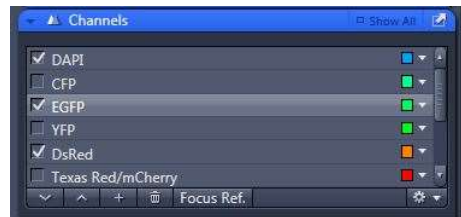


- Along the left control panel, expand **Channels**.



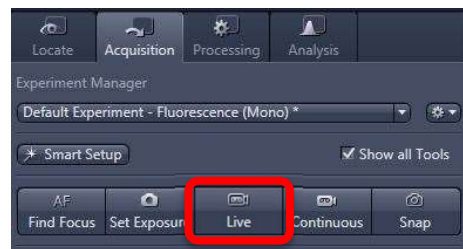
- Add checkmarks to your required fluorescence channels (and DIC channels, as needed). Remove checkmarks from any remaining channels.

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



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

- 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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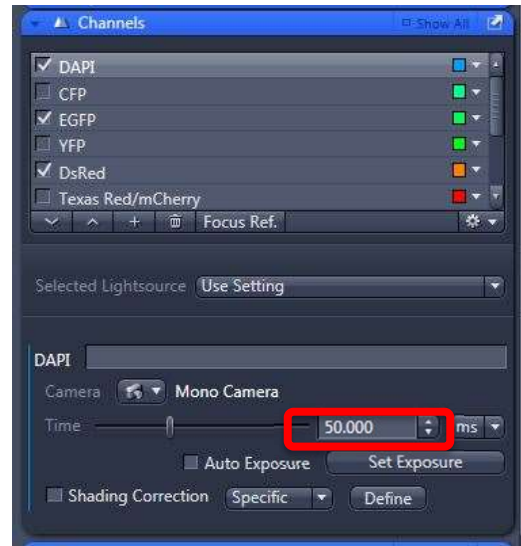
12. Using the microscope **Z Focus Knobs**, refocus the camera image until objects of interest exhibit crisp edges. Use the XY joystick to re-center the region of interest to the camera's field of view.
13. In **Channels**, click **Set Exposure**. The exposure will be automatically set to a proper exposure.

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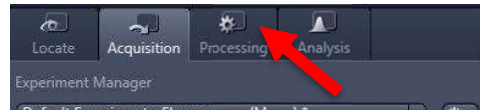
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established. *Changing exposure times will make quantification impossible and data analysis inaccurate.*

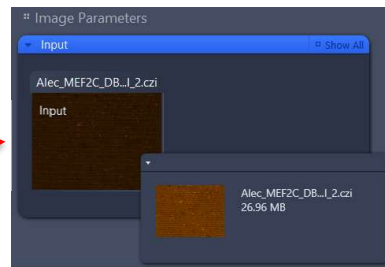
18. To switch slides, press **Load Position** on the microscope touchscreen. After switching slides, press the button at top-left, an up arrow with a line over it, to return to the previous focus position.
19. As images are captured, go to *File > Save As* (not “Save As with Options”) to preserve the 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

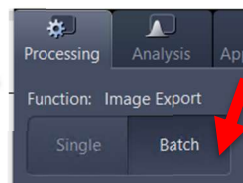
1. To convert *.czi raw files to JPG or TIFF files, move from **Acquisition** to **Processing**.



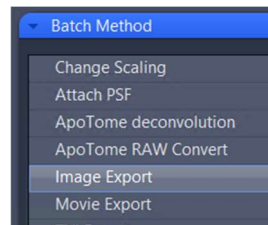
2. To export a single image – open the image in ZEN first and load it into **Input**. Under **Method**, search for and click once on **Image Export**. *Skip to Step 7.*



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



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



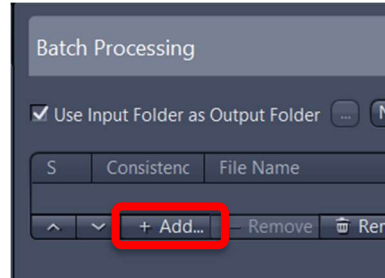
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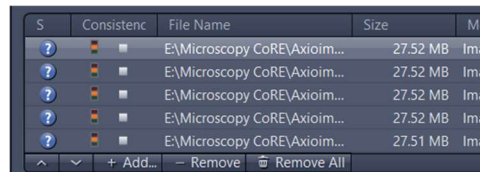
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- Before setting any Parameters, click “+ Add...” in the central field.



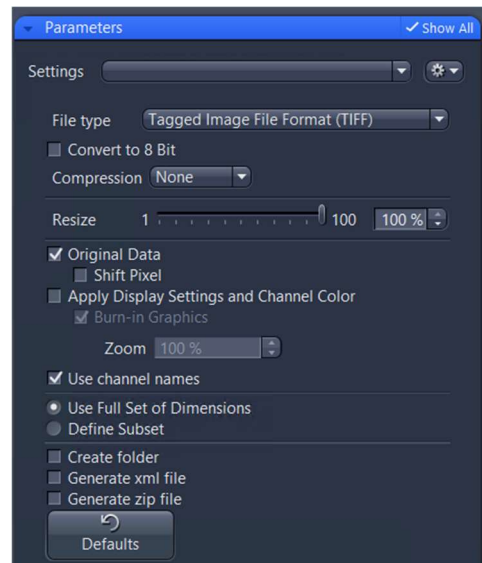
- Locate, select and load all images you wish to export in a similar manner. *You can hold Shift or Ctrl to select multiple files in the “Add files” dialog box before clicking “Open.”*

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

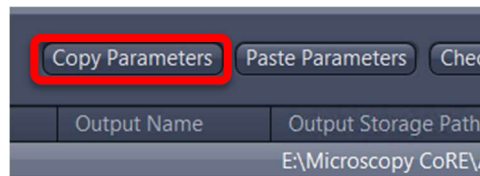


- Under **Parameters** at left, choose the file type and preferences for image export. *Check “Show All” to the right of Parameters to reveal the additional options. Recommended parameters are shown at right.*

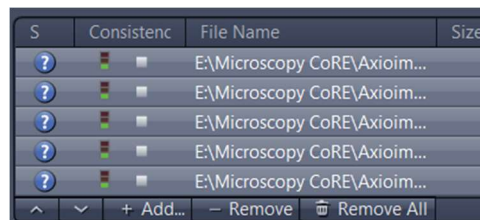
- Choose “Original Data” if you plan to quantify images later; resulting file extension reads “_ORG”
- Choose “Apply Display Settings and Channel Color” if you have manipulated the histogram for a prettier image or added a scale bar that you would like to submit for publication; **DO NOT** quantify data off these images!
- Select “Use full set of dimensions” unless you prefer to export only subsets of images – e.g. two of four fluorescent channels



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



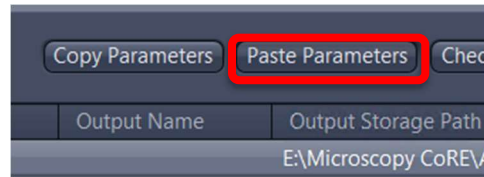
- Highlight all images. *Use the Shift key to select multiple images.*



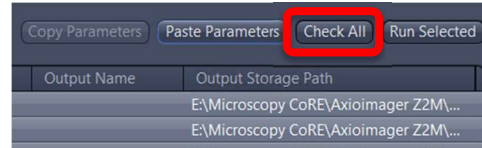
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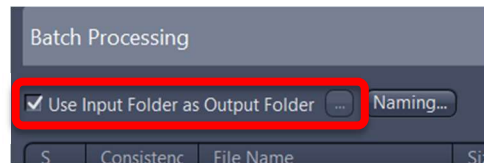
10. Click **Paste Parameters**.



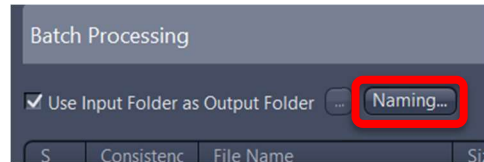
11. Click **Check All**. You should receive little green boxes under the column **Consistency**.



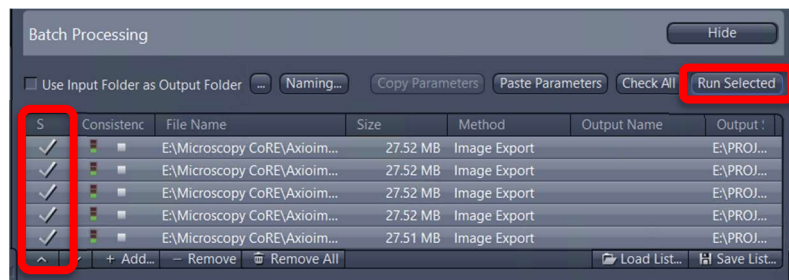
12. By default, all exports will be written to the same folder where the raw image exist. If you wish to change this, **uncheck Use Input Folder as Output Folder** and use the “...” to choose a new location.



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.
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.