LSM 980 + Airyscan 2: User Quick Guide

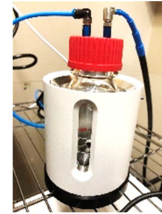
ZEN 3.3 (Blue Edition)

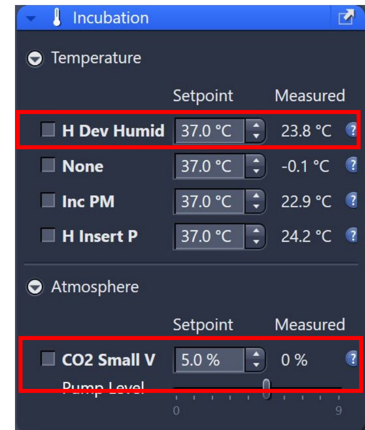
Starting Up the System

1. Turn on the **MAIN SWITCH** on the laser rack to the left side of the air table (**1**).
2. Turn on the **COMPONENTS** (**2**) switch on the small remote box.
3. Turn on the **PC power button** (**3**).
4. Once the PC boots up, the "ZEISS" Windows account should automatically log in.

If Performing Live Cell Imaging (Temperature, CO_2, Humidity):

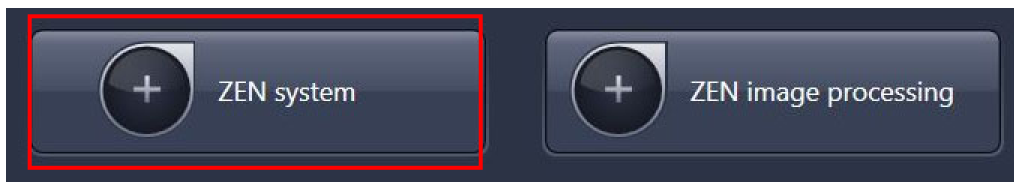
- Check that the humidity bottle has a suitable amount of demineralized water. If low, carefully unscrew the bottom glass bottle and fill midway. (*Avoid tap water, which leaves heavy deposits*.)
- Once ZEN launches (see below), activate the required incubation controls in the right side tool area (in the "Incubation" window). This includes options for heating, CO2, and humidity control.
- If using stage-surround heating, activate the "Heating XL" checkbox and wait ~20 minutes for temperature to equilibrate.



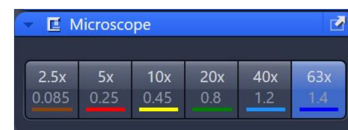


Launching the ZEN 3.3 Software

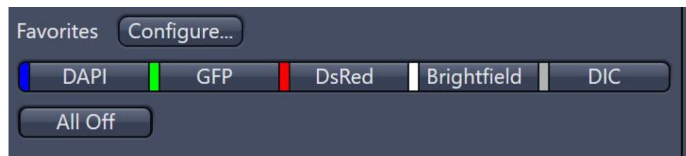
1. Double-click on the ZEN 3.3 (Blue) software icon on the desktop.
2. Select the "ZEN System" option to initialize the hardware. (*Alternatively, the "Image Processing" mode can be used if only offline data viewing is required*.)



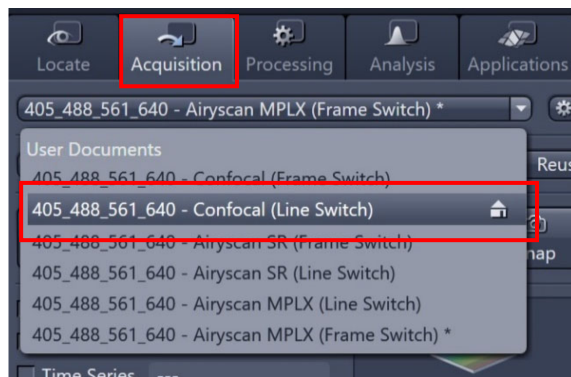
Setting Up the Microscope; Locating Specimens



1. Click the “Locate” tab to permit access to the binoculars.
2. Select the appropriate objective for examining the slide/dish.
3. If using water/oil immersion (40x, 63x), add a drop of water/oil directly to the objective front lens. *Never use water/oil on air objectives (e.g. – 10x, 20x), and clean residual water/condensation/oil on slides as necessary*.
4. Place samples on the stage with coverlsip facing downwards.
5. Use the stage XY joystick to center the objective front lens over an area of interest.
6. To view the specimen with brightfield contrast (transmitted light), click the “Brightfield” preset button found in the “Locate” tab.
7. To view the specimen with fluorescence contrast, click on the suitable “DAPI,” “GFP,” or “DsRed” preset buttons.



Loading an Experiment for Routine Confocal Scanning

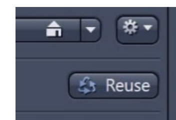


Method #1

In the “Acquisition” tab, use the drop-down box to select a pre-defined configuration with an appropriate combination of laser lines required. The “**405_488_561_640 – Confocal (Line Switch)**” experiment is an excellent default starting point for up to 4 colors that require very little adjustment of filter stringency.

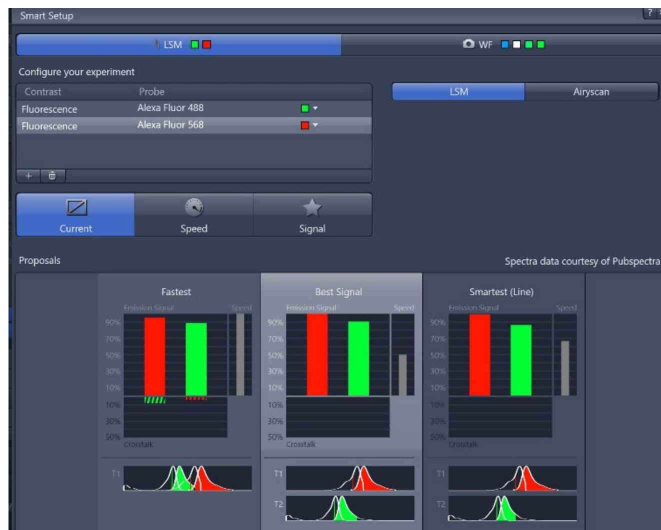
Method #2

Open any previous image (saved with the .CZI file extension) with similar channels; click the “Reuse” button to repopulate all settings from the image.



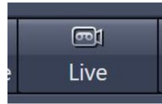
Method #3

Use the “Smart Setup” feature to initialize a stepwise wizard for adding specific fluorophores to the current experiment. The light path will be adjusted based on the given combination, with multiple proposals given to optimize speed, filter flexibility, or a compromise of both.

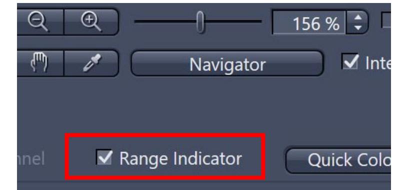


Adjusting Channel Sensitivity ("Channels" Window)

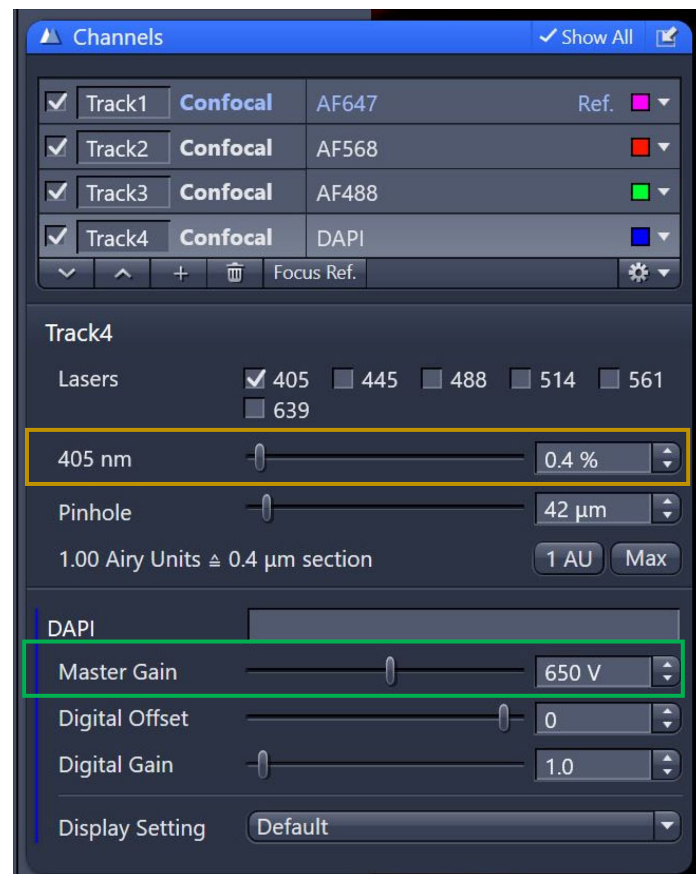
1. Highlight a single track in the "Channels" window to view the available parameters associated with that wavelength.



2. Select the "Live" button to start a fast XY scan.
3. Activate the Range Indicator checkbox to toggle the scanned image into a display mode where red pixels indicate saturation (maximum intensity reached) and blue pixels represent a zero value (minimum intensity).



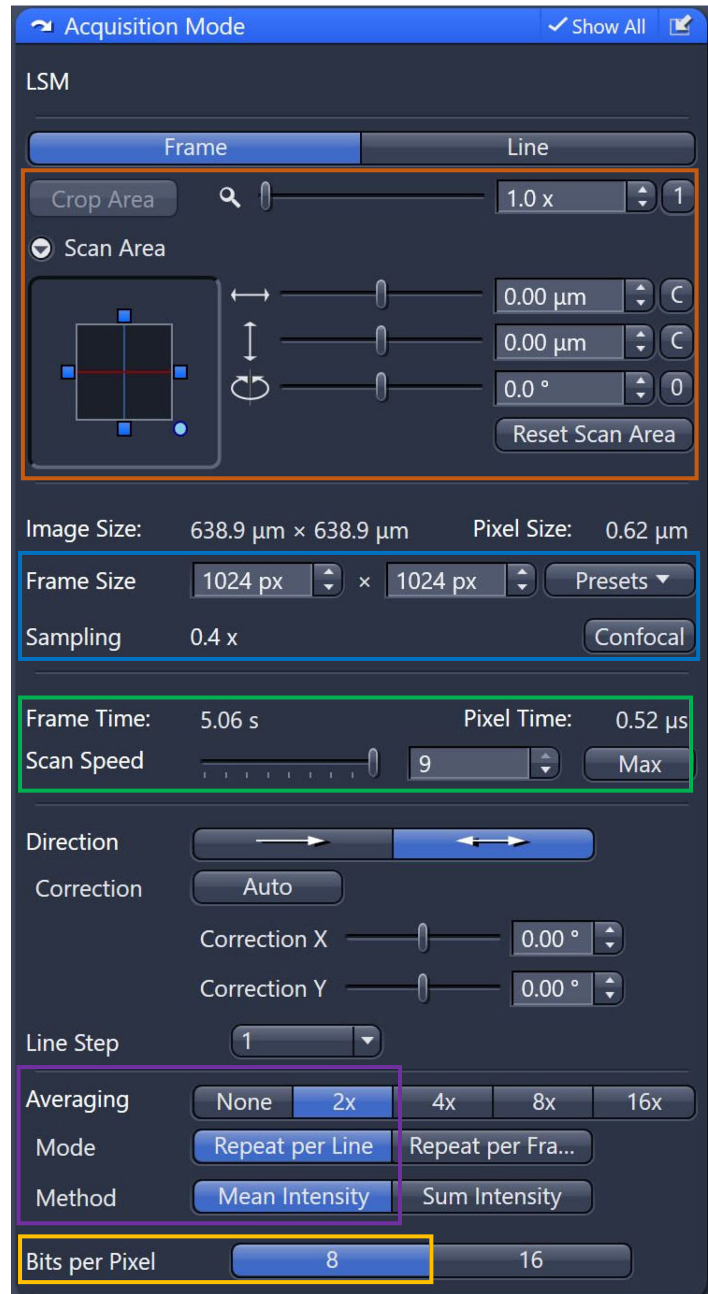
4. Set the pinhole slider to 1 AU (Airy Units); this will maximize the optical sectioning capability for the given objective lens.
5. To fine-tune the image sensitivity parameters:

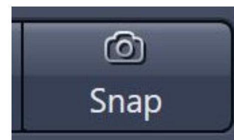


   a. Increase the Master Gain until a relatively small amount of red pixels (indicating saturation of the detector) appear in the image. Try to avoid values above 850 V.
   b. Increase the Laser Power if the detector gain cannot achieve the desired intensity. Values between 1 – 3% are relatively safe starting points.
   c. Adjust the focus with the fine adjustment knob to identify the brightest or preferred Z-position; re-adjust the gain and laser power again if necessary.
   d. *Optional* – decrease the Digital Offset to reduce background signal. Caution should be used here, as true signal may be eliminated and sensitivity is not altered.
   e. *Optional* – increase the Digital Gain to amplify all signal and background, thereby stretching the histogram. Again, sensitivity is not directly altered with this function.
6. Repeat step #5 for additional highlighted tracks/channels as needed.

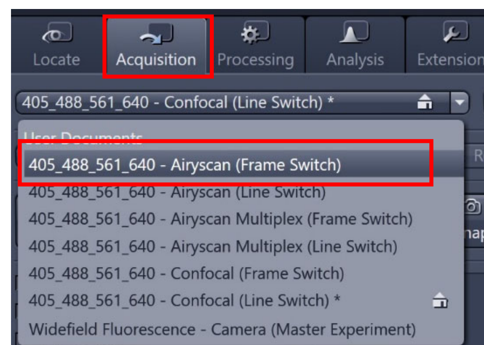
Adjusting Scanning Parameters ("Acquisition Mode" Window)

1. In the "Acquisition Mode" window, select a suitable Area and Zoom setting to capture an area of interest. (Alternatively, use the Crop button after a "Live" image has been stopped to adjust the desired field of view.)
2. Select a Frame Size to represent the desired area per pixel. While a preset value of 1024 x 1024 is a widely-used starting point, the Confocal button will calculate the best possible diffraction-limited sampling for the given wavelength and objective lens.
3. Adjust the Scan Speed slider to yield a sensible scan time. A setting of 6 – 8 is usually safe, though noisier samples may benefit from a slower setting .
4. Select a value for Averaging, which can improve image quality via successive scans with identical settings. A small amount of averaging (2 or 4) is typically more effective at improving signal-to-noise ratios than a slower scan speed.
5. Select a dynamic range (8, 16 bit) with the Bits per Pixel setting. 8 bits is often sufficient, but more regimented image quantification may benefit from a 16 bit setting. Note that file sizes will jump significantly with higher bit depths.
6. Click the Snap button to acquire a single (or multi-channel) frame.



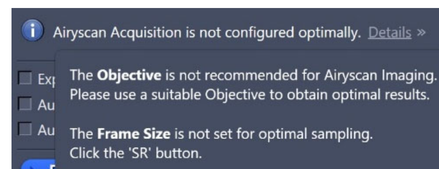


Setting Up an Airyscan Experiment

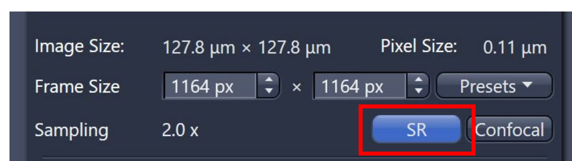


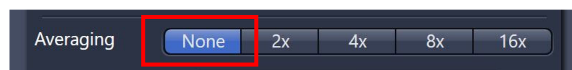
1. Move to an Airyscan-optimized objective (typically 63x/1.4 oil) and ready the specimen.
2. Load either a preset configuration – such as "**405_488_561_640 – Airyscan (Frame Switch)**" – or use the "Smart Setup" feature, select the Airyscan mode, and add fluorophores.
3. Consider adjusting the scan area > 1.7x, as larger empty areas will yield longer scanning time.
4. Highlight a track in the "Channel" window, and click the "Live" button.
5. Adjust the Master Gain and Laser Power (with the help of the Range Indicator tool) to minimize saturation. (*See earlier section, 'Adjusting Channel Sensitivity'*) Extra care should be used in this mode to minimize grossly saturated pixels. As before, avoid use of gains above 850 V. Note that the Pinhole and Digital Offset are not adjustable in this mode.

6. Click the Airyscan detector view button along the bottom toolbar; confirm that the hexagonal detector view is centered and aligned.
7. Repeat step #5 for additional tracks/channels as needed.
8. Check the optimal settings (*e.g.* – Frame Size, Scan Area, objective lens) as instructed in the experiment warning(s).

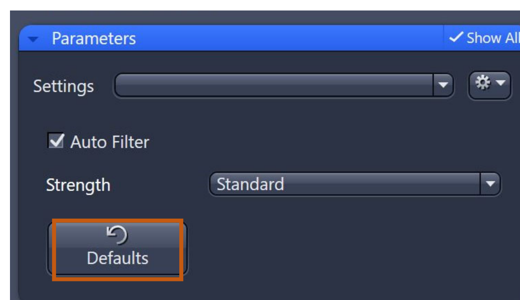


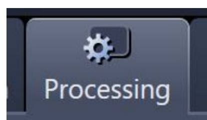
   a. Using a superresolution-limited pixel count ("SR") is critical for maximizing the capabilities of the Airyscan concept. Note that these values may be significantly higher than standard confocal resolution experiments.
   b. The benefits of Averaging are less critical to routine operation of the Airyscan. To keep scan times feasible, consider starting with 1x Averaging values.
   c. Raw Airyscan data is efficiently collected with 8-bit data depths; Airyscan processing always yields 16-bit image results.

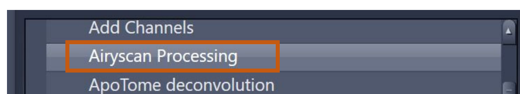




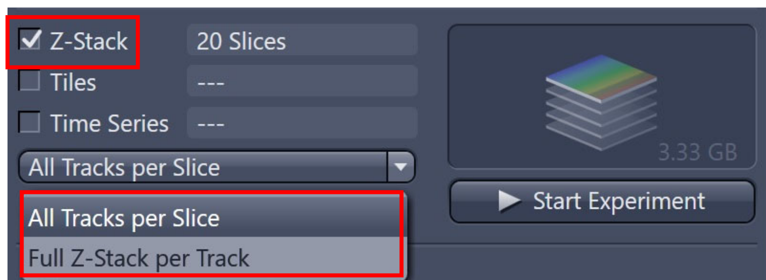
9. Click the "Snap" button and wait for the scanning to complete.
10. After the scan, navigate to the "Processing" tab and select Airyscan Processing in the "Methods" window. Apply the setting to generate the pixel-reassigned and deconvolved result.



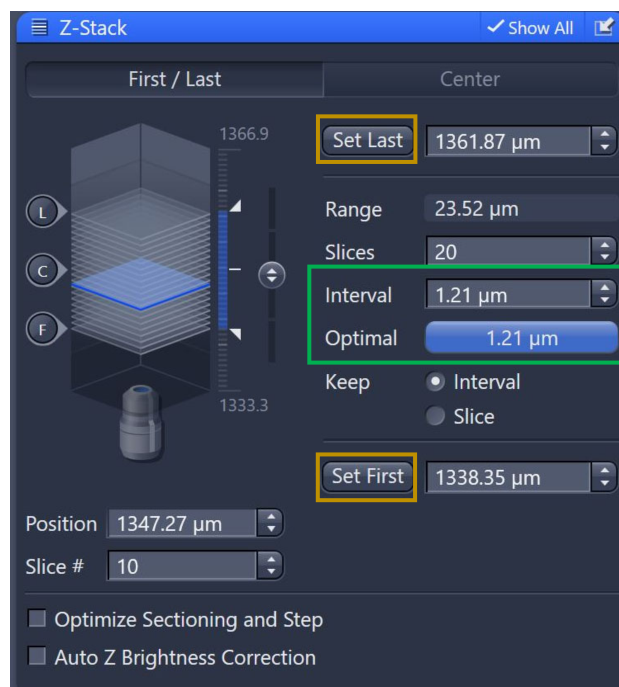




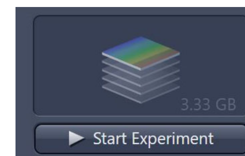
Setting Up and Acquiring a Z-Stack



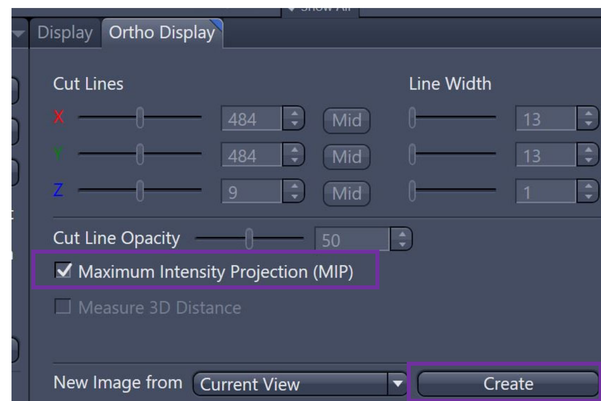
1. Activate the **Z-Stack** checkbox in the main experiment tool area.
2. Select a preferred scanning mode (*e.g.* – "All Tracks per Slice," "Full Z-stack per Track") in the drop-down box. The "All Tracks per Slice" mode is the default, but may be slower for experiments that use multiple filter changes.
3. Expand the Z-Stack control window.
4. Highlight a channel that exhibits signal throughout the desired sample volume, then click the "Live" button.
   a. Use the focus knob to locate one end of the specimen, then click the Set First button.
   b. Focus to another end of the specimen, then click the Set Last button. This will mark the total range of the volume. (*The relative orientation of the sample does not matter, as the system will always move the focus drive against gravity during acquisition.*)
   c. Stop the "Live" scan.
   d. Set the Interval to define a step size during the Z-stack. For the best 3D reconstruction, consider using the value of the Optimal button. This will yield a sampling step size that is 50% of the optical section thickness of each plane.



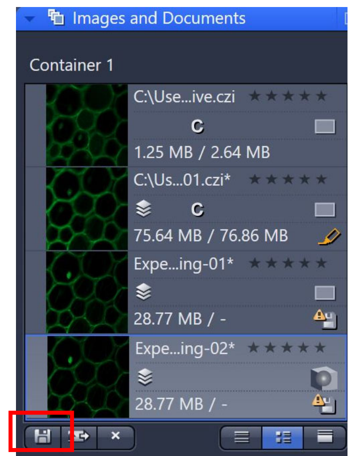
5. Click on the Start Experiment button to begin the recording of the Z-Stack scanning. Save the resulting .CZI file.



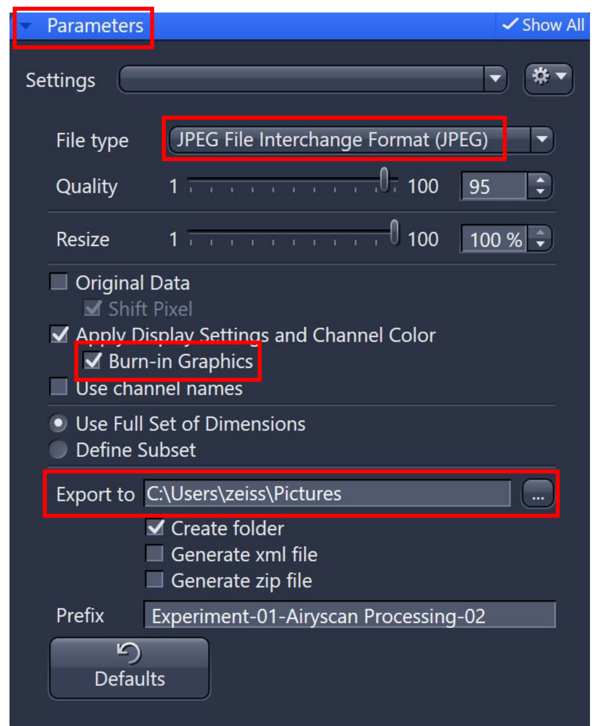
6. *Optional* – to create a Maximum Intensity Projection image from the stack, navigate to the Ortho tab, which shows a cross-sectional view of the Z-Stack. At the bottom, toggle the "Maximum Intensity Projection" checkbox and wait for the image to process. If a separate image is needed, click the "Create" button at the bottom and select one or more of the axes shown.
7. *Optional* – to view the sample in the rendering/visualization engine, click the 3D tab on the image. Use the mouse to modify the viewing angle as needed. Snapshots of renders can be generated using the "Create Image" button in the bottom tool tray.



Storing and Exporting Data



1. To save an acquired or processed image, click on that image in the right tools area to highlight it. Click the Save button (disk icon).
2. Create or choose a folder in the D:\ drive of the PC. Enter a file name and <u>save as a .CZI format</u>. Data should not be saved onto the C:\ of the system!
3. After saving a copy of the .CZI data (which retains all hardware information used during the experiment), images can be <u>exported</u> to various formats as needed.
    a. To export, go to the menu entry **File → Export → Export**.
    b. In the "Parameters" window, choose the file type and any relevant compression settings.
    c. If scale bars do not need to be exported, the "Burn-in Graphics" checkbox can be deselected.
    d. Double-check a destination path.
    e. Click the "Apply" button at the top.



Turning Off the System

1. Clean off oil from any immersion objectives using lens paper only.
   a. First gently wick up the oil on the objective with a piece of lens paper.
   b. Moisten a fresh piece of lens paper with the Lens Cleaner solution; wipe the objective front lens in a slow circular motion.
   c. Lightly dry off the Lens Cleaner solution with an additional piece of lens paper.
2. Save all needed data.
3. **If performing live cell imaging, untick any active controllers running in the right side "Incubation" window of ZEN.**
4. Close the ZEN software and **shut down the PC** (**3**) if the system is not being used soon.
5. Shut off the **COMPONENTS** (**2**) switch and the **MAIN SWITCH** (**1**) knob.