Exporting images from Zeiss CZI format to TIF files using ZEN 2.x

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Zeiss Microscopy makes available online a free version of their ZEN 2.x software *(current version is ZEN Black 2.3 Sp1, Zen Blue 2.5)* that users can install on Windows PCs in their labs. The Marley & Life Sciences North facilities will have a copy available since the 64bit download is huge (6.6GB). If you would like to obtain the file directly from Zeiss, see: www.zeiss.com/zen-lite *(Note, you will be asked to provide contact information)* Please see our handout regarding installing the free ZEN software, the installation includes roughly 50 Windows device drivers that are completely unnecessary for users who do not have Zeiss hardware attached to their PC. The handout can be found here: http://microscopy.arizona.edu/learn/printable-materials#instruments

ZEN Black or Blue?

Zeiss' ZEN software comes in two colors, Black (for controlling Zeiss microscopes that use lasers) and Blue (for controlling Zeiss widefield microscopes). The software may look similar, but the feature sets are quite different. The most obvious difference between the two is that the icons and user interface (particularly the bar at the top of the program) of each make use of the colors in the names. According to Zeiss, at some future date these two software programs will be combined, but for now there are two "flavors" of the ZEN software. Black will likely be more familiar most users, since it is the software that is used to control the main campus Zeiss LSM880 microscopes, as well as the Zeiss Elyra S.1 located at the UAHS campus.

The main virtue of Blue for users is that it is the better of the two for exporting images. While it is possible to export CZI files into TIF using Black, Blue is much more flexible. Our Zeiss technical representative has said that we should "always" use ZEN Blue to export images.

During the installation of the ZEN software you will be asked if you want to associate the CZI file extension with Blue or Black. Associating the CZI file with ZEN Blue will ensure that when you double click on a CZI file on your lab or personal computer, ZEN Blue will open. We recommend that you agree to the file association.

Installing ZEN includes a PDF copy of the user guide. While lengthy (1119 pgs for ZEN Blue 2.5), in the appropriate sections the user guide will provide additional detail about how to best use the software. Note: it is possible that some specific functions in the software will require the purchase of a license from Zeiss and therefore will not be available in the Lite version. The ones referenced here are all freely available.

Alternative - Using the FIJI version of ImageJ

Since the Zeiss ZEN Lite software is for MS Windows only, Macintosh users need an alternative way to open CZI files. We recommend the FIJI version of the free image analysis software, ImageJ (https://fiji.sc/, available for MacOS, Windows, Linux). The two key advantages to FIJI are that it includes many pre-installed plugins (including the one that allows users to open CZI files) and it regularly checks to ensure that you are running the latest version. Please note that opening and exporting large image data files *(e.g., Z-stacks, time-lapse data, stitched images)*, can slow down even powerful computers. We recommend that you close down other software before working with large data using FIJI.

- Opening files using Bio-Formats (part of FIJI): http://www.openmicroscopy.org/site/support/bio-formats5.3/users/imagej/load-images.html
- Saving and exporting files from FIJI (ImageJ): http://imagej.net/Saving_and_Exporting

Note: MS Windows users should be aware that FIJI installs differently than most Windows programs. Users (Windows, MacOS, Linux) will also need to install JAVA on their computer. See the simple installation instructions here: http://imagej.net/Fiji/Downloads

Working with CZI files in ZEN Blue 2.5

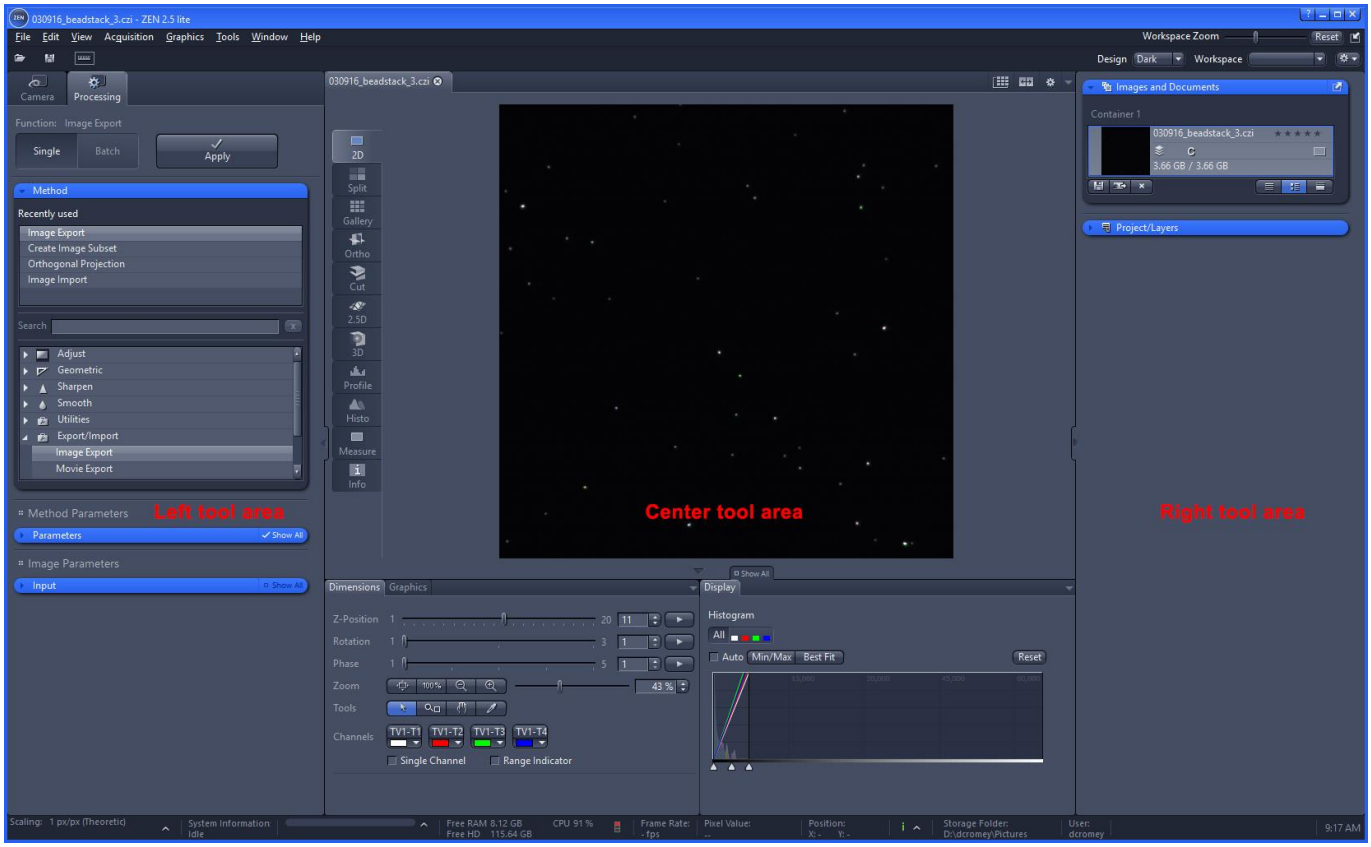
The ZEN user interface has three tool areas (left, center, right). Click the Processing tab (top of the left tool area) to access the available functions such as file export and sub-set functions. The other two tool areas will look similar to ZEN Black, but they are not identical.

Make sure the CZI file that you want to work with is open in the right side tool area. You can do this by using the software's FILE | OPEN dialog or by locating a CZI data file on your disk and double-clicking on the file. If you have multiple files open, you can select one by double clicking on it (this will put a thin blue line around the selected file).

NOTE: CZI files can be quite large. If you are opening your data files from an external hard disk or flash drive, they will open much faster if you use a USB 3.0 device connected to a USB 3.0 port.

If you have the space to copy the files directly to your computer's hard disk drive, the files will open even faster (in most cases).

With the Processing tab open, (using Single) click the Method tool and you can select a large number of functions. This handout will focus on the commonly used *Export/Import > Image Export* and the *Utilities > Create Image Subset* functions. For instructions on using Batch mode (performing functions on multiple CZI files), see section 5.7 of the ZEN 2.5 user guide that was included with the software installation.



More information on the Zeiss CZI file format: https://www.zeiss.com/microscopy/us/products/microscope-software/zen/czi.html

Reducing the number of images to export *(see ZEN Blue 2.5 user guide 5.10.12.7)*

Often a large Z stack or time-lapse will only have a few images that you need or want. Rather than exporting everything, you can create a new CZI file that is a sub-set of the larger file, and then further process or export the sub-set file as needed. You can also use this method (in ZEN Blue) to crop an image to a smaller area.

NOTE: Users of the Zeiss Elyra S.1, or users of the LSM880 who are using the Airyscan, should really only need to export the image data that has been post-processed by ZEN Black. The raw (unprocessed) data from these techniques has very limited use for publication images.

Select the processing tab found in the left tool area. Select and drop down (use the ▶) the blue *Method* menu in the same area. Choose *Utilities* and then from the sub-menu choose *Create Image Subset*. You should now see a *Method Parameters* and *Image Parameters* (input and output) menu in the left tool area.

Create Image Subset – options

- *Channels* – if you have more than one wavelength, you can export all or just some of the channels. To deselect a channel click on the colored box and it will reduce in size to indicate that you do not want that channel to export.
- *Z-position, Time, Block (data set dependent)* – allows you to export a single image, a range of data, several non-contiguous ranges, or at specified intervals (e.g., every 5th z step).
- *Region* – Full gives you the entire screen's worth, while rectangle allows you to crop every image in the stack (or time course) to a smaller size. You will need to use the rectangle drawing tool (lower left of Center Screen Area, Graphics tab) to indicate how the image should be cropped.
- *NOTE: if you open unprocessed Elyra data you will also be able to export specific Rotation or Phase image subsets, however this would only be useful in specific troubleshooting instances.*

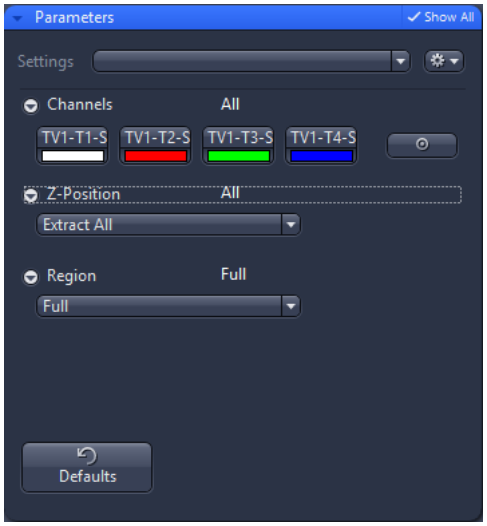


Image Parameters | *Output* gives you a number of flexible options regarding the output name.

Once you have pressed APPLY near the top of the left tool area, you will need to save the sub-set (which will now show up in the file list found in the right tool area) to an appropriate location on disk.

Conserving bit depth information

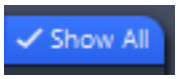
A particularly useful advantage to using the ZEN Blue subset tool is if you want your exported images to retain their full bit depth. Exporting multiple channels to a single TIFF image turns the exported file into a 24bit color image (8 bits of Red, 8 bits of Green, 8 bits of blue). If you have four channels then this is a significant loss of bit depth information, since the original channel images are 16 bit. 16 bit images have over 65,000 grey shades, while the number of grey shades per color (R,G,B) in a 24bit color image is only 256, and if you export 4 channels into a 24bit image you reduce that even more.

If you export each of the channels individually you will have much greater adjustment flexibility using the higher bit depth images. This can be particularly useful if you do not have access to the ZEN software (to open the CZI files) at some later date.

Image Export of CZI files *(see ZEN Blue 2.5 user guide 5.10.13, 7.0.0)*

Select the processing tab found in the left tool area. Select and drop down (use the ▶) the blue *Method* menu in the same area. Choose *Export/Import* and then from the sub-menu choose *Image Export*. You should now see a *Method Parameters* and *Image Parameters* menu in the left tool area.

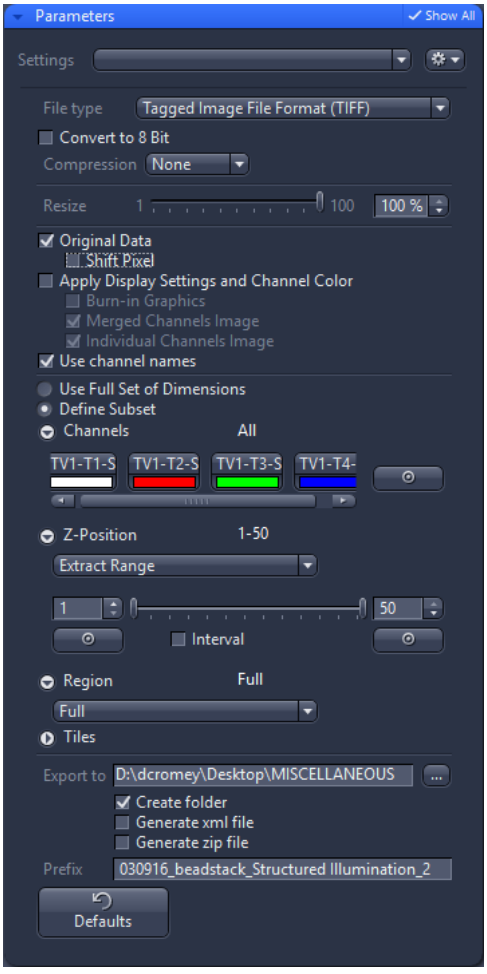
For most file export needs you should choose either *Tagged Image File Format (TIFF)* or *Tiff Format (64Bit) (BigTiff)* from the drop down menu in the *Method Parameters*. Please make sure to have SHOW ALL selected on the upper right corner of the blue parameters bar.



Export options

- JPEG – we strongly discourage the use of JPEG for scientific images.
- BMP – Windows bitmap, not recommended.
- TIFF – Recommended.
- Tiff Format (64Bit) (BigTiff) – May be needed when working with very large files *(e.g., large tiled areas)*.
- PNG – The portable network graphics format can be useful for posting images online as it includes loss-less (non-destructive) file compression and is compatible with all web browsers.
- JPEG XR (WDP) – again, not recommended.
- SUR – as near as we can tell, this file format is primarily useful for people doing surface measurements and metrology, and is probably of no use to biologists.

Exporting to TIFF – options

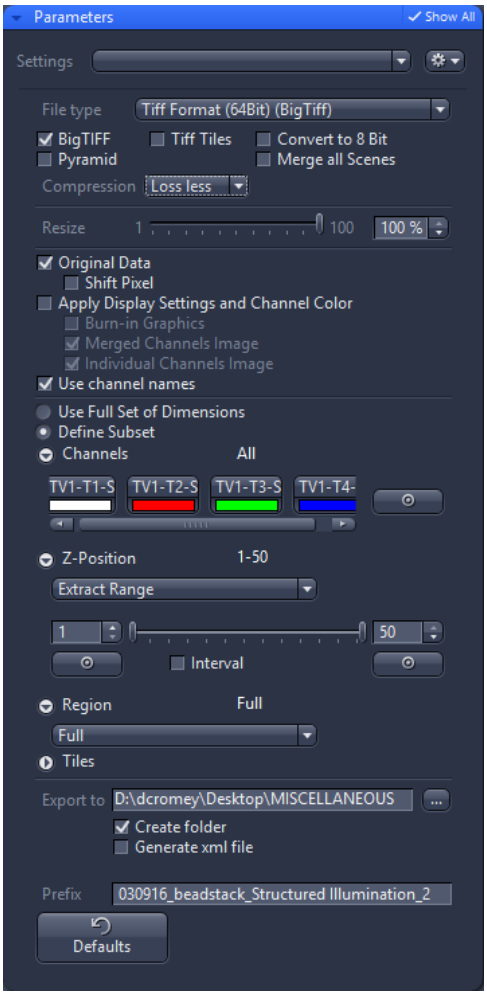


- *Convert to 8 Bit* – Be very careful and aware of this setting. If this is checked it will permanently reduce the number of greyscales in the image. It appears as if the only way you can uncheck this box is to ensure that the "Original Data" box is checked first.
- *Compression* – your choices are None, LZW, or ZIP. If you choose to use compression either choice is non-destructive. The only issue between them is if the software back in your lab is able to open a compressed TIFF file of that type.
- *Resize* – Unless you are exporting a very large tiled (stitched, montage) type image, do not resize your image. You worked hard to get good optical resolution, why throw it away in software?
- *Original Data* – allows you to export to 16bit images (be sure to uncheck "convert to 8 Bit"). The "Shift Pixel" option will upscale 10bit or 12bit images into 16bit, and for most users this should not be used.
- *Apply Display Curve and Channel Color* – Again, a setting to be careful with. If this is "unchecked" you will export the original data, if this is "checked" you will apply the adjustments you made in the center tool area's display histogram. NOTE: it appears that this will need to be checked (temporarily) to be able to select multiple and individual channel images, then uncheck (if you want the raw, unadjusted data).

    - *Burn-in Graphics* – any letters, boxes, arrows, etc. displayed on the image in the center tool area will permanently become part of the exported images.
    - *Merged Channels Image* – combines the channels into a single 24bit color image for each Z stack layer or time point.
    - *Individual Channels Image* – exports the individual channels for each Z stack layer or time point.
- *Use Full Set of Dimensions* – exports everything.
- *Define subset* – you can also define a subset of your data directly within this dialog box. See the discussion above about exporting subsets.
- *Export to* – click the **…** button to select the folder where your exported images will be sent.
- *Prefix* – by default the software will use name of the CZI file as the prefix for the exported file names, adding on channel and/or z slice information.

Exporting to BigTiff – options

- *BigTIFF* – suitable for exported image files that will need to larger than 4GB. For most users this is not necessary.
- *Pyramid* – Takes large, high-resolution images and creates smaller versions so that you can zoom in and out (somewhat similar to how Google Earth works, or if you have ever seen the output from most microscope slide scanners).
- *Tiff Tiles (taken directly from the user guide)*
    - Activated: Generates new rectangle tiles for internal data handling.
    - Deactivated: Combines tiles as stripes for internal data handling.
- *Merge all Scenes – (taken directly from the user guide)* Activated: Generates one image including all scenes. Single scene images will be generated, if the checkbox is deactivated.
- *Compression* – your choices are None, Loss-less or Lossy. If you need a compressed file we recommend the loss-less option (uses LZW). Lossy is a version of JPEG XR and we don't recommend that for science images.
- *Other settings (see the descriptions under the TIFF export)*



The *Image Parameters* window will show the input file you have selected. Once you are satisfied with the configuration of all the options, click the APPLY button near the top of the left tool area to begin your export.

Exporting to Movies *(see ZEN Blue 2.5 user guide 5.10.13.2, 7.2.0)*

Based on recommendations we have seen online, the current "best quality" movie file format would be either MPEG-4 or MOV. The H.264 codec is what is used in Blu-ray disks. Either H.264 or MPEG-4 are considered the state-of-the-art in 2018. See the ZEN Blue 2.5 user guide regarding the need for installing the open source *FFmpeg* application (https://ffmpeg.org/) to use these two codecs.

The other formats are OK, it somewhat depends on your needs and the limitations of the format (and the exporter). Remember that almost every option will use lossy file compression (somewhat similar to JPEG) to make the movie file size more manageable.

Exporting to OME-TIFF *(see ZEN Blue 2.5 user guide 5.10.13.3)*

The Open Microscopy Environment OME-TIFF format is an attempt to standardize the TIFF file format so that it can retain the metadata created by acquiring multi-dimensional data sets (e,g, XYZ, XYTime, XYLambda, etc). See: http://www.openmicroscopy.org/site/products/ome-tiff. This format will allow you to open these specialized TIFFs in other programs for further analysis. The exported images are available as a multipage TIFF file.

To export to OME-TIFF select that method in ZEN (a separate export image option from the one used above) and you will find many similar parameter settings to those under BigTiff.

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