Exporting images from Zeiss CZI format to TIF files using ZEN 2.3

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Zeiss Microscopy makes available online a free version of their ZEN 2.x software that users can install on Windows PCs in their labs. The Marley & Life Sciences North facilities will have a copy available since the download is quite large (800MB). 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 a number of Windows device drivers that are completely unnecessary for users who do not have Zeiss hardware attached to their PC.

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 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 a future date these somewhat similar software programs will be combined, but for now there are two "flavors" of the ZEN software with different feature sets. 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 Lite 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, ZEN Blue will open. We recommend that you agree to the file association.

Installing ZEN includes a PDF copy of the user manual. While lengthy (665 pgs), in the appropriate sections the manual will provide additional detail about how to best use the software. Note: it is possible that some specific functions in the software 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.

- 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/Downloads

Working with CZI files in ZEN Blue

The software's user interface has three tool areas (left, center, right). Click the <u>Processing tab</u> (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 <u>not</u> identical.

Make sure the CZI file that you want to work with is open in the right 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 <u>much</u> faster if you use a USB 3.0 device connected to a USB 3.0 port.

With the Processing tab open, (using Single) click the <u>Method</u> 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 5.6 of the ZEN 2.3 manual that was included with the software installation.
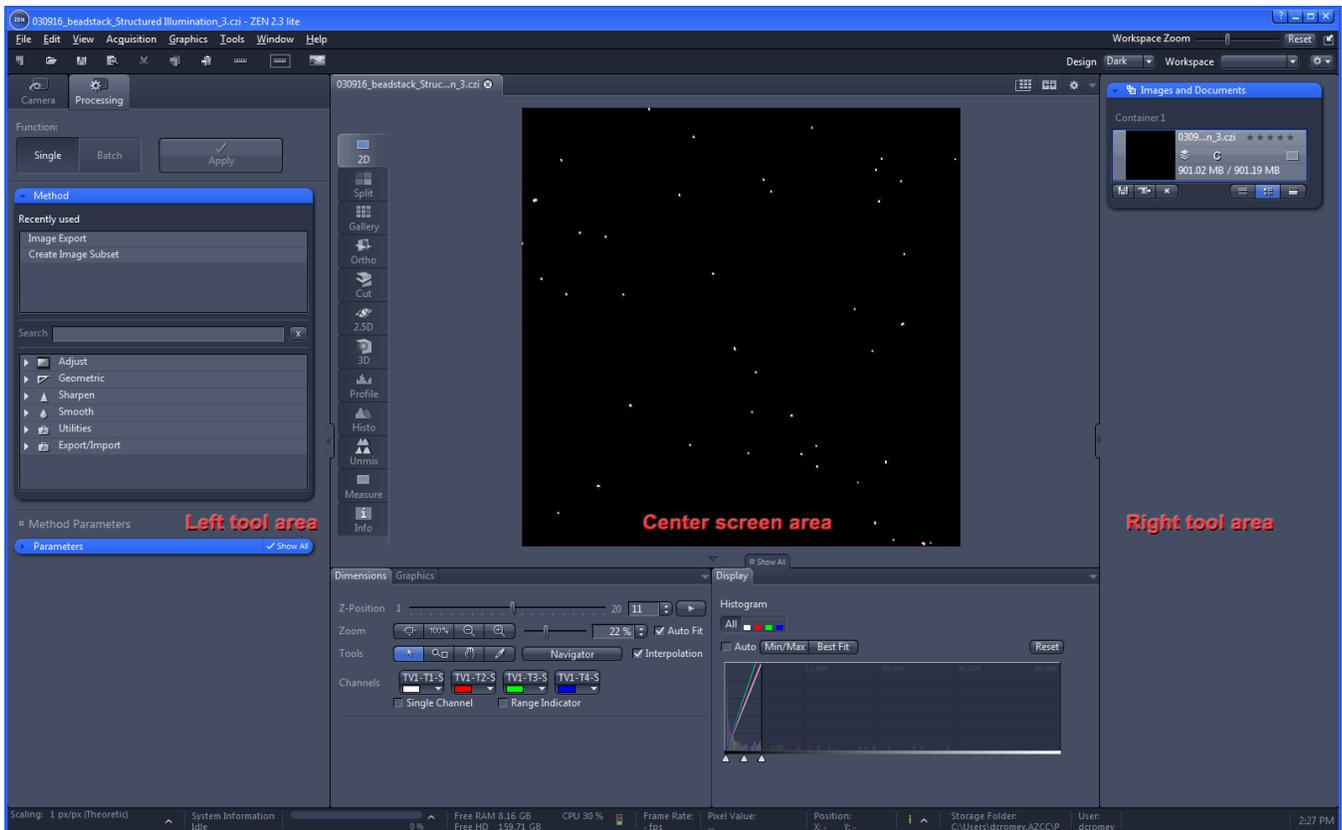

More information on the Zeiss CZI file format:
https://www.zeiss.com/microscopy/us/products/microscope-software/zen/czi.html

Image Export of CZI files *(see ZEN manual 5.9.13)*

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*.

- JPEG – we strongly discourage the use of JPEG for scientific images.
- BMP – Windows bitmap, not recommended.
- TIFF – choosing TIFF will automatically change the bit depth (number of grey shades) in each channel from 16 bit (65,536) to 8 bit (256). This might be useful for preparing images for publication, but is not as useful for other types of analysis or post-processing.
- Tiff Format (64Bit) (BigTiff) – This will create large files, but you will keep the full bit depth of the original images.
- 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* is not optional. This will permanently reduce the number of greyscales in the image.
- *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 will 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?
- *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.
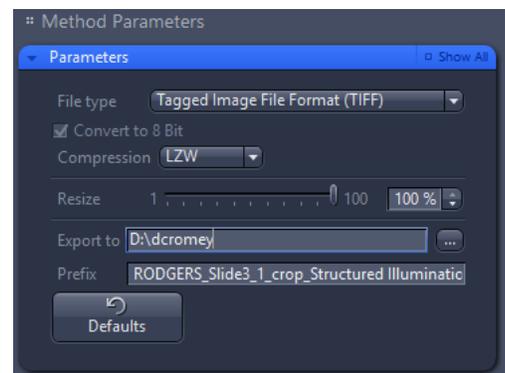
Exporting to BigTiff – options
- *BigTIF* – suitable for exported image files that will need to larger than 4GB. For most users this is not necessary.
- *Pyramid* – Takes large, high resolution images and creates smaller versions so that you can zoom in and out (somewhat similar to how Google Earth works, or if you have ever seen the output from most microscope slide scanners).
- *Tiff Tiles* – Not sure what this does?
- *Merge all Scenes* – Not sure what this does either?
- *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.
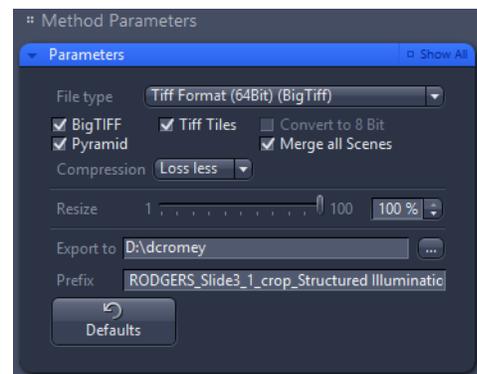
- *Resize* – Unless you are exporting a very large tiled (stitched, montage) type image, do not resize your image. You worked hard to get good optical resolution, why throw it away in software?
- *Export to* – click the **…** button to select the folder where your exported images will be sent.
- *Prefix* – 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.

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

Exporting to Movies *(see ZEN manual 5.9.13.2)*

Based on recommendations we have seen, 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 and either that or MPEG-4 are considered the state-of-the-art in early 2017. See the ZEN manual regarding the need for installing the open source *FFmpeg* application 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 (frequently a variation on the JPEG discrete cosine function) file compression to make the movie file size more manageable.

Exporting to OME-TIFF *(see ZEN manual 5.9.13.3)*

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.

Reducing the number of images to export *(see ZEN manual 5.9.12.7)*

Sometimes 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.

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.
- *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 to a smaller size.

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 (shows up in the file list of the right tool area) to an appropriate location on disk.