



### ZEN Blue for lab and personal computers

At the University of Arizona, most users will need to request assistance from IT personnel to install ZEN Blue lite on a university-controlled computer. Unfortunately, Zeiss has made downloading<sup>i</sup> and installing the most recent version of ZEN Blue lite (3.7) a complex and involved process (*possibly due to compliance with the European GDPR privacy regulations*). The 3.4 version is the last one that can be installed from a single file. We have a copy of ZEN Blue lite version 3.4 available for our users in a BOX folder. Simply request access and we will send you a link to the BOX folder.

The ZEN Blue on many of our Core facility microscope computers is version 2.x (older). This is because typically after an instrument is installed, the version number of the installed software does not change (*with allowance for necessary patches and hot fixes*). There should not be a significant difference in the functions described below.

### Why ZEN Black and 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. Zeiss' future plans will have all their instruments running ZEN Blue.



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

Installing ZEN includes a PDF copy of the software manual for your reference. Note: it is possible that some specific functions will be mentioned in the document that require the purchase of a license from Zeiss and therefore will not be available in the free version. The functions referenced here all work without additional licensing costs.

### Alternative 1 - Using the FIJI version of ImageJ

Since the Zeiss ZEN software is for MS Windows only, Macintosh users may feel the need for an alternative way to open CZI files. We recommend the FIJI version of the free image analysis software, ImageJ (*available at <https://fiji.sc/>, for MacOS, Windows, Linux*). The two key advantages to FIJI are that it includes many pre-installed plugins (*including the Bio-Formats plugin that allows users to open CZI files*) and FIJI 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 exit other software before working with large data using FIJI.** The RII Imaging Cores – Optical has powerful workstations available for users working with large datasets.

- Opening files using Bio-Formats (part of FIJI): <https://bio-formats.readthedocs.io/en/stable/users/imagej/load-images.html>
- Saving and exporting files from FIJI (ImageJ): <https://imagej.net/formats/#saving-and-exporting>

**Note:** MS Windows users should be aware that FIJI installs differently than most Windows programs. See the simple installation instructions here: <http://imagej.net/Fiji/Downloads>

## Alternative 2 – Use OMERO to manage your CZI data

OMERO is client/server software developed by the Open Microscopy Environment which includes the BioFormats plugin. The University of Arizona has an instance of OMERO available for research labs that want to manage their image data in a secure online data store. Importing data into OMERO removes the need for having other software to open a proprietary image data file format, the data can be viewed (after logging in) in a web browser. To learn more about OMERO at UArizona see: <https://datascience.arizona.edu/analytics-powerhouse/omero> If you have questions, you can also email: [RII-OMERO@arizona.edu](mailto:RII-OMERO@arizona.edu)

## Working with CZI files in ZEN Blue 3.x

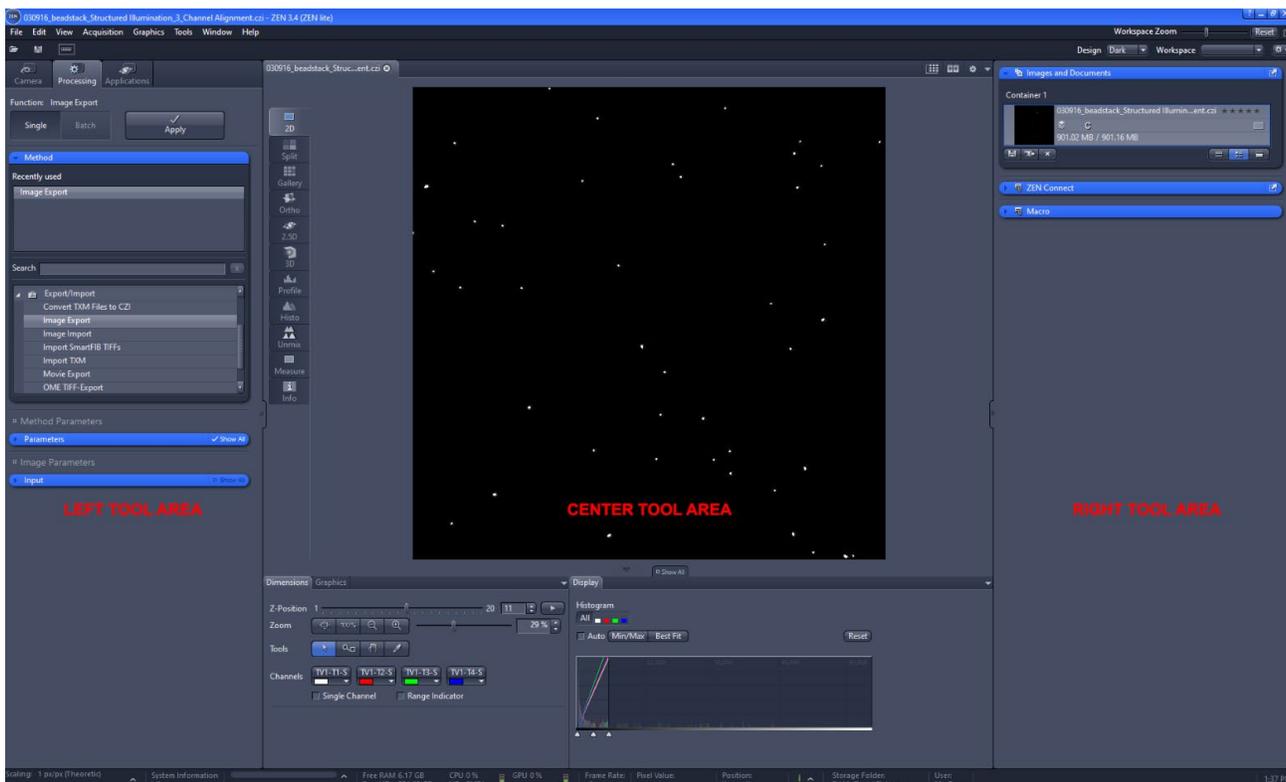
The ZEN user interface has three tool areas (left, center, right). Click the Processing tab (top of the left tool area) to access the available software functions such as file export and subset functions. The other two tool areas will look similar to ZEN Black, but they are not identical.

Make sure the CZI file that you want to work with is open in the right side tool area (Images and Documents). 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 in ZEN, 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).

To work with a single image at a time (Single Function), make sure that you are in the Processing tab. 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. To apply these tools to an entire folder of images, see the batch processing section below.



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 (ZEN Blue 3.7 manual 4.10.9.13.15)

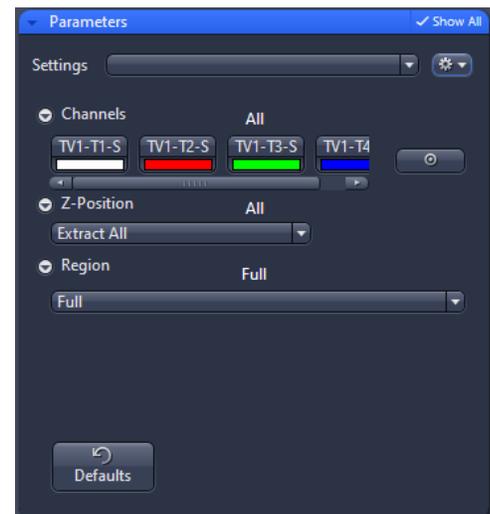
Often a large Z stack or time-lapse will have only a few images that you need or want. Rather than exporting everything, you can create a new CZI file that is a subset of the larger file, and then you can further process or export the newly created subset file as needed. You can also use this method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

To subset the data from a single CZI file (Single Function), make sure that you are in the Processing tab. 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 the *Method Parameters* and *Image Parameters* (input and output) menu boxes in the left tool area. If you do not see these, use the ► to expand the window.

**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.

### 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, Scene (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 5<sup>th</sup> z step, one of several location/scenes).
- *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 may only be useful in specific troubleshooting instances.*



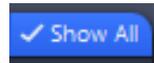
*Image Parameters / Output* gives you a number of flexible options regarding the output name.

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.

### Image Export of CZI files (ZEN Blue 3.7 manual 4.11)

To export the data from a single CZI file (Single Function), make sure that you are in the Processing tab. 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 the *Method Parameters* and *Image Parameters* menu in the left tool area. If you do not see these, use the ► to expand the window. Click & drag your image from the “Images and Documents” on the right pane over into the *Image Parameters > Input* window.

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 (maximum file size is 2GB)
- 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 other image handling software 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 and proper Nyquist sampling of that 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 before converting them down to 8bit, and for most users this should not be used. *NOTE: This “might” be appropriate for users of the 14bit camera on the Axio Observer 7 w/ Apotome III system, but we have not tested this yet.*

- *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. If you use the adjustments in ZEN to change the image (such as, to remove unwanted background fluorescence), you will need to track your

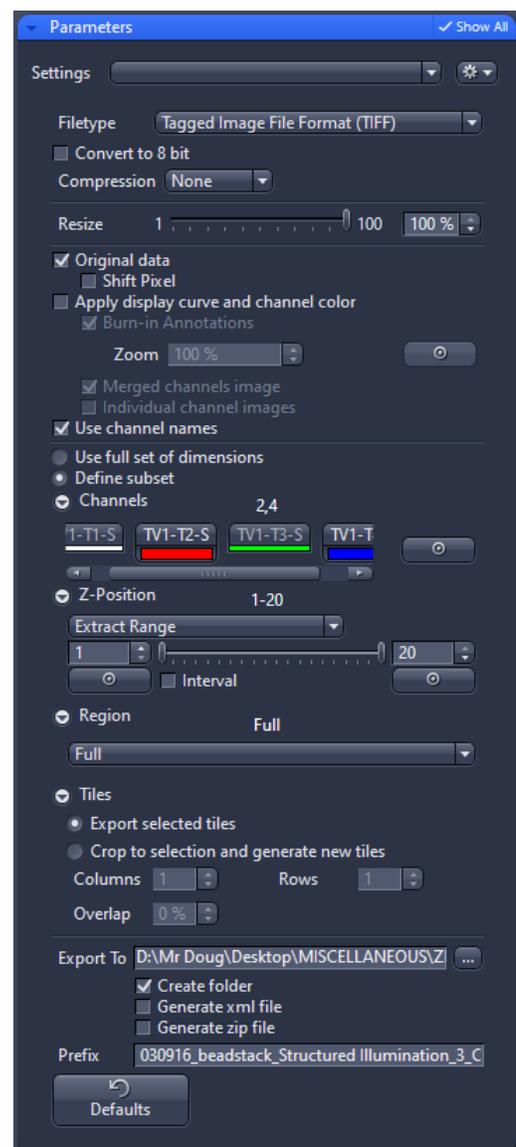


image processing steps and declare them when you publish the data.

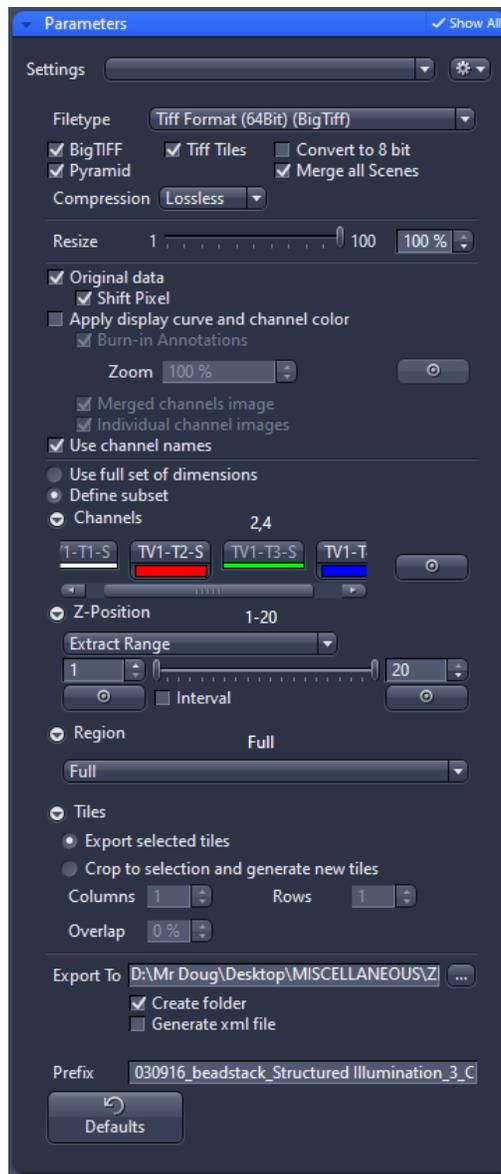
*NOTE – to access the check boxes listed below you will need to temporarily check the “Apply Display Curve and Channel Color” box, select what you want and then uncheck the “Apply Display Curve and Channel Color” box.*

- *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.
  - You can have the exported images Create a folder for each CZI file, Generate an XML file (exports the CZI file’s metadata), or save all the exported data as a single ZIP (compressed) file.
- *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 be larger than 2GB. 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 some 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)

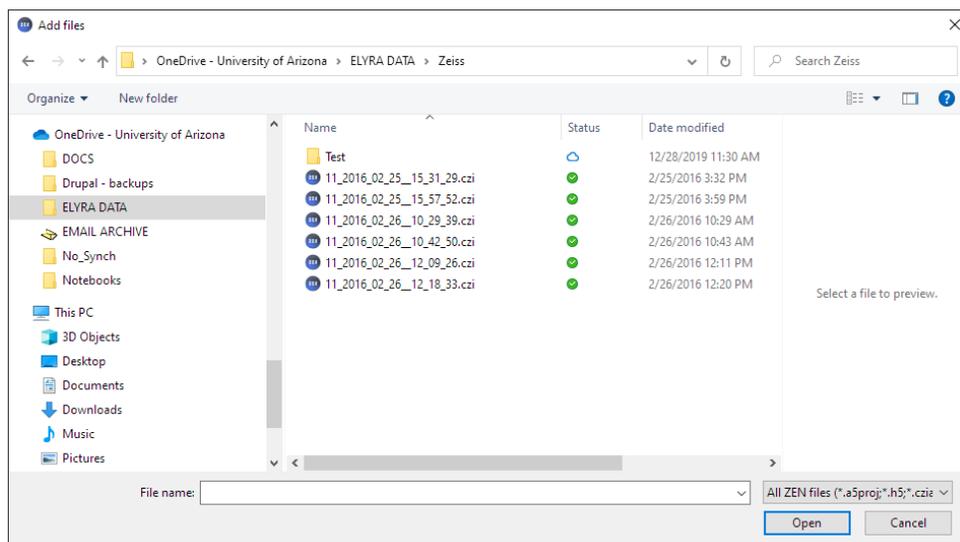
The *Image Parameters* window will show the input file you have selected. Once you are satisfied with the configuration of all the options, click the APPLY button near the top of the left tool area to begin your export.



## Batch processing (ZEN 3.7 manual section 4.10.4)

*Prerequisite - You already have a folder with several CZI-images to be exported as a new image type.*

1. Select the *Processing* tab. Click on the *Batch* button.
2. Open the *Method* window and select the entry *Image Export*.
3. In the *Method Parameters* section, open the *Parameters* tool.
4. In the *Batch Processing* tool, click on the *Add* button. Using the *Add Files* directory window, select the folder, mark all images to be exported, and click on *Open*.



5. Activate the checkbox *Use Input Folder as Output Folder* to save the exported images in the folder of the original images.
6. Click on one image in the list and configure the export settings using the *Parameters* tool (see above). As an example, you might choose to export to TIF format for all z-stack levels and channels. This setting is (at the moment) only valid for the selected image, but can be copied to further images in the list with identical dimensions (*e.g., do not apply a z-stack export to single plan time lapse image data*).
7. Click on the *Copy Parameters* button. Select the image with identical dimensions in the list and click on the *Paste Parameters* button.
8. Continue with the other images of the list. Use *Copy Parameters* and *Paste Parameters* or define the export settings for each image individually in the *Parameters* tool.
9. Click on the *Check All* button. All images in the list will be tagged with a green marker, if they have the correct setting.



10. Click on the *Apply* button.

All exported images in the list will be checked and are exported in separate folders in the Input folder.

### **Exporting to Movies** (*ZEN Blue 2.3 manual 5.9.13.2*)

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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. To save movies in the MOV format you will need to download the open source *FFmpeg* application (<https://ffmpeg.org/>). Copy the downloaded *ffmpeg.exe* into the same folder where ZEN.exe is located (note, this may require IT assistance).

The other movie 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 comments below*)

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The Open Microscopy Environment OME-TIFF format is an open source 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: <https://docs.openmicroscopy.org/ome-model/6.0.0/index.html#ome-tiff>

While ZEN can export to OME-TIFF, there have been reported issues regarding the loss of metadata (information about how the image was acquired) using this option in ZEN. Since all we can offer our users is an older software version (ZEN Blue 3.4), it is unlikely that this issue has been fixed (it was reported in ZEN 3.0-3.5).

If you are importing data into the UA’s OMERO server (or any other software that uses BioFormats, such as ImageJ/FIJI), it can read CZI files directly, so there’s no need to convert them to OME-TIFF.

One possible reason to convert a CZI file to OME-TIFF is because the image data is a large area tile scan and the OME format is pyramidal, whereas CZI is not. One easy option is that you can bulk convert CZI files to OME-TIFF using the NGFF-converter software found here: <https://www.glencoesoftware.com/products/ngff-converter/>

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<sup>i</sup> Access to the most recent version of ZEN Blue lite <https://www.zeiss.com/microscopy/en/products/software/zeiss-zen-lite.html>

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