CZI to TIFF Converter Suite

Utilities to convert CZI images into TIFF images

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Preface

The CZItoTIFF utility suite consists of several applications that serve to convert ZEISS CZI images to TIFF/BigTIFF. There are two main parts to it: CZItoTIFFBatchConverter.exe and CZItoTIFFConverterConsole.exe. Both are described in detail in this document.

This set of utilities allows to convert many different "types" of TIFF/BigTIFF image:

- Tiff or BigTIFF
- Without image pyramid, with multi-page (IFD) pyramid, with single-page (SubIFD) pyramid
- Without attached label & slide preview and with label & slide preview image
- All scenes in final image or each scene separate

The above variations represent 24 different TIFF file "types" or "layouts".
Additionally, for each variation it is possible to specify bit depth, compression type, visual representation and in more advanced use cases, tile size and multi-page sequence.

Requirements and Installation

The utility suite can be placed anywhere on a Windows 7, Windows 8, Windows Server 2008, Windows Server 2012 computer. There is no specific installation required.

The utility consists out of the following list of files and one folder:
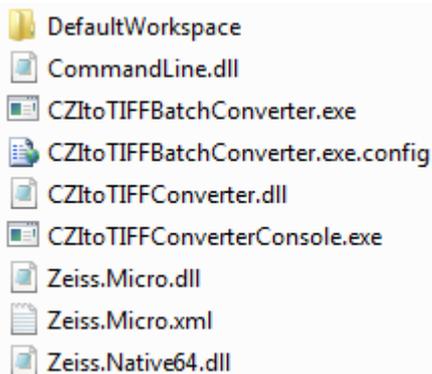
Required additional Software

- Microsoft .NET Framework 4.5
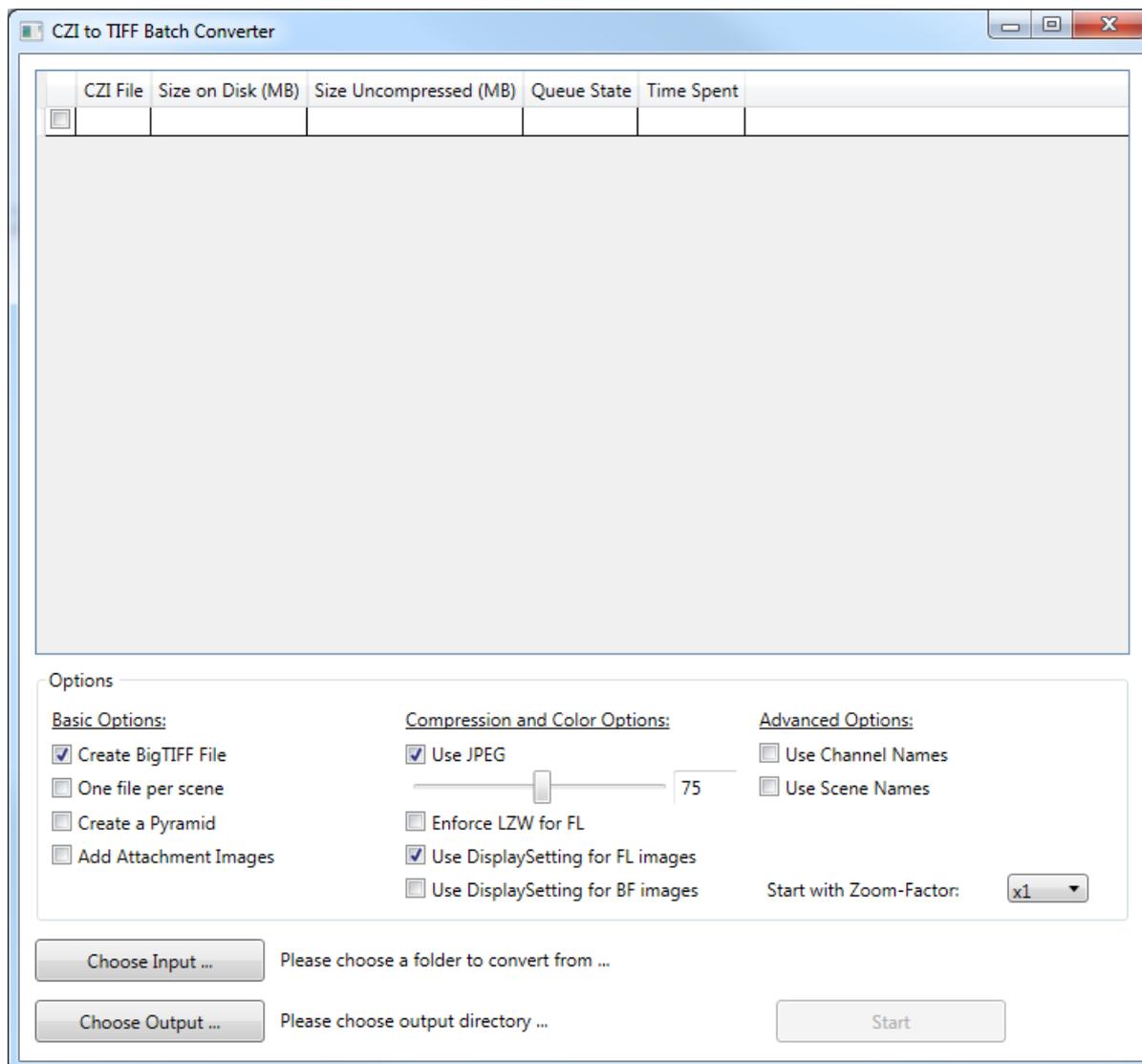- Microsoft Visual C++ 2012 Redistributables

Both are being installed along with a normal ZEN (blue) 2012 installation.

In case it is not desired to have a full ZEN installation on the converter computer, the above listed packages can be downloaded here:

http://www.microsoft.com/en-us/download/confirmation.aspx?id=30679

http://www.microsoft.com/en-us/download/details.aspx?id=30653

CZItoTIFFBatchConverter – A simple Converter Applicaton

General Use

Click button "Choose Input" to select a folder that contains CZI images that shall be converted to TIFF. Click button "Choose Output" to select a folder that will receive the TIF images the converter produces. Once both input folder and output folder have been selected, the button "Start" will become active.
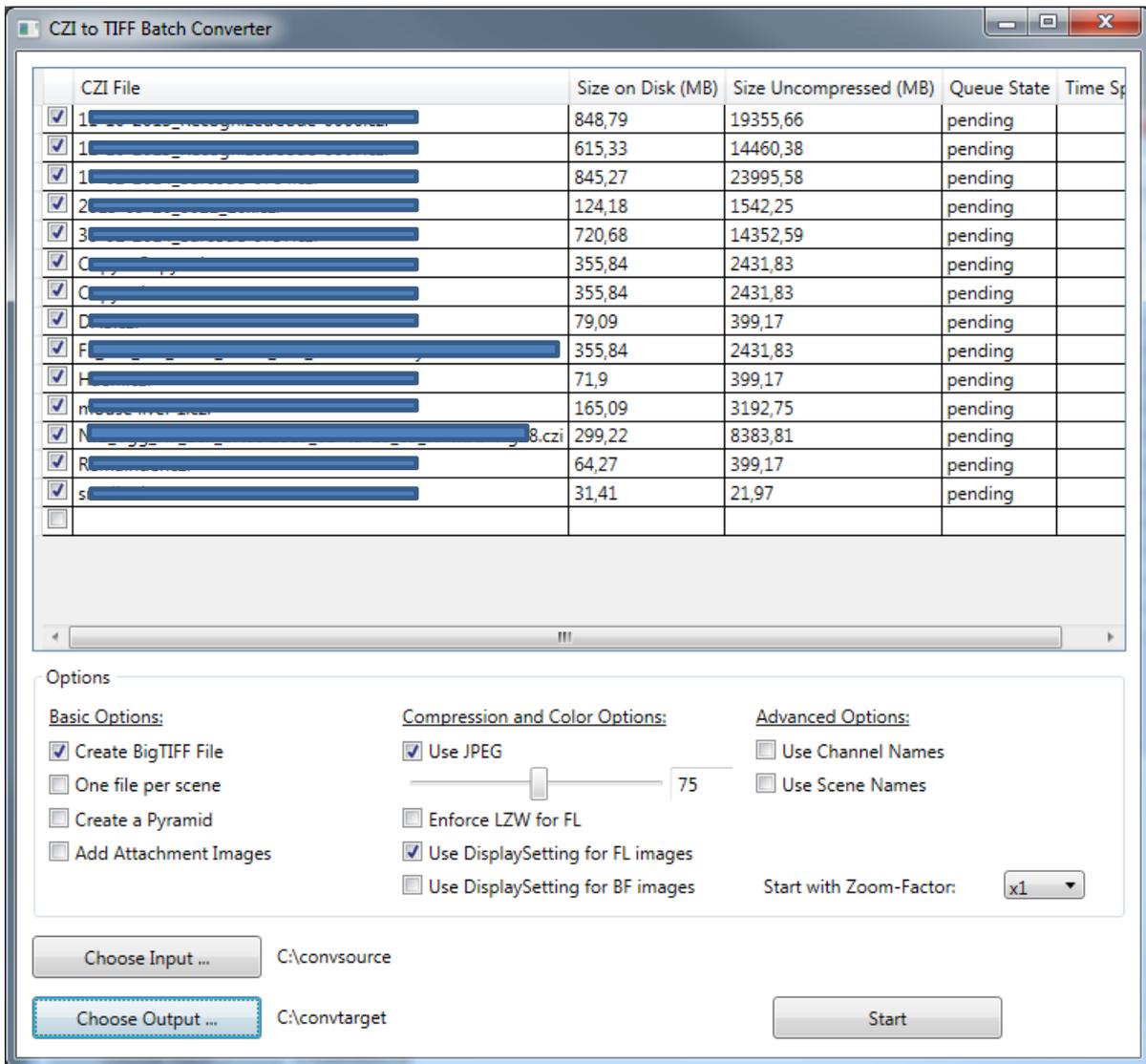
Basic Options

Create BigTIFF File

When checked, the result files will have file format BigTIFF, this allows TIFF images to have a bigger than 4GB file size. However BigTIFF is a fundamentally different file format, so the downstream image processing/viewing applications specifically must list BigTIFF as a supported file format (e.g. 'TIFF/BigTIFF'). Stating TIFF alone is typically not enough.

Important Note: ZEN is not able to consume BigTIFF. ZEN will fail to load a TIFF image that has more than 2GB uncompressed pixel data. This is ca. 26.7k x 26.7k (or 31k x23k @ 4:3 aspect ratio) pixel with 24bit RGB.

One File per Scene

When checked, a CZI image, whenever it has more than one scene will be split into individual scene images. This may become helpful, when not using BigTIFF, because chances are higher that each of the scene images will be less than 4GB in file size.

Create a Pyramid

Some viewing and image processing applications recognize an image pyramid and utilize that to increase load and processing times significantly. When checked, the converter will create an image pyramid where each pyramid level is half of the preceding level (width & height). This benefit comes with typically 1/3 bigger file sizes.

Important Note: ZEN is not aware of TIFF image pyramids.

Add Attachment Images

Some viewing and image processing applications can be made to recognize additional TIFF pages as thumbnails and/or slide preview images. When checked, the converter will add a label and a slide preview iamge (when present). The sequence is: main Image -> optional pyramid -> label -> slide preview.

Compressions and Color Options

Use JPEG and JPEG Quality Slider

When JPEG is selected, the converter will create whenever possible JPEG compressed pixel data. This is the case, when the input image is one of the following

- 24Bit RGB image
- 8 Bit grayscale
- 16 Bit grayscale (e.g. FL channel image) and the checkbox "Use DisplaySetting FL" is activated. Applying a DisplaySetting always will result in a 8 Bit image that can be compressed with the JPEG encoder.
- 48 Bit color image (e.g. brightfield with >8 Bit per RGB channel) and the checkbox "Use DisplaySetting BF" is activated. Applying a DisplaySetting always will result in a 24Bit RGB image.

When JPEG is selected, the specified JPEG quality setting (slider) will be used. The accepted range is 50…99. Lossless JPEG is not supported by this converter because there's no significant benefit over using LZW and lossless JPEG is far less common than lossy JPEG compression.

When JPEG is not selected, all images will be compressed with the lossless LZW encoder.

Enforce LZW for FL

When checked the requested JPEG compression is going to be ignored for fluorescence images. These images then always will be compressed with lossless LZW, regardless if the result image could be compressed with lossy JPEG or not.

Use DisplaySetting for FL

Typical cameras for fluorescence imaging have a very high sensitivity and a very high dynamic range, width valid bit depth exceeding 8 Bit, typically 12,14 or 16Bit. To prevent bleaching and other phototoxic events on a fluorescence sample the big dynamic range is not utilized, as can be seen in the following screen shot:
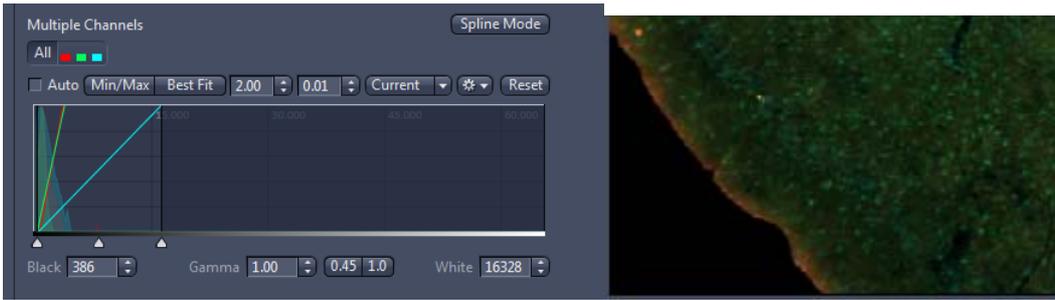
The image is viewable nicely because the DisplaySetting is adjusted accordingly. The image has been captured with a 16Bit camera, using only 12 out of 16Bit. The range of pixel gray values still covers 0 … 4096.

If use DisplaySetting is deactivated for the export of such an image, the DisplaySetting is set to default-linear and then exported.

It is the viewing application's task to adjust its viewing parameters accordingly and make the (contained in full) pixel information visible again.

Use DisplaySetting for BF images
Depending on the target use case it may be advised to activate this option or not.
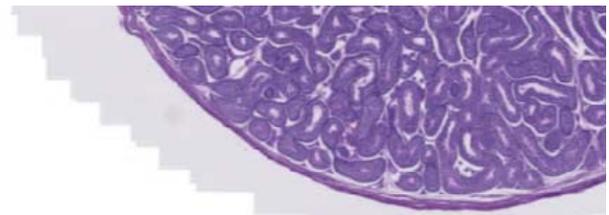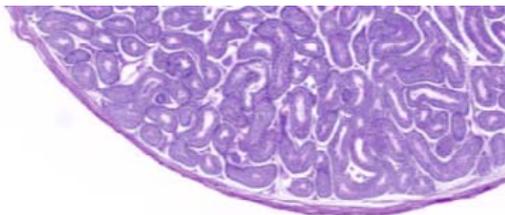
The above two screenshots show a brightfield image with adjusted DisplaySetting (left) and with a linear

DisplaySetting (right). Left is more pleasing to the viewer because the light gray background has been removed. This comes for the cost of reduced dynamic range. It depends on what the downstream applications intend to do with an image. If it is image analysis, then using a linear setting ensures highest precision. When it's print, then eventually applying the DisplaySetting is the better choice. Especially in "print as it is and don't mess around with my colors" printing discussions.

Advanced Options

Use Channel Names

If checked, fluorescence channel images will be named after their fluor being used. This is equivalent to the channel name displayed in ZEN. Single channel images will not be named after their channels.

Use Scene Names

If checked, scene images will be named after their scene names. Images containing only a single scene and/or where scene names have not been used in ZEN, the scene index naming pattern is applied.

Start with Zoom Factor

In situations where it is not required to obtain a full resolution image, this can be adjusted with this selection. The values are downscale factors, 8x translates to 1:8 downscale = 12.5% of original image size. Conversion time significantly is reduced because when creating a pyramid, the converter spends approx. 75% of the time on converting the base layer.

CZItoTIFFConverterConsole – A Versatile CZI to TIFF Converter Utility

CZItoTIFFConverterConsole.exe is the actual conversion tool that is used by the GUI application CZItoTIFFBatchConverter.exe. The GUI application calls the console application for each individual file, using the parameters specified by the user in the GUI.

The console application can be used in two different ways. First method is to convert a single file by providing all necessary parameters via the command line. Second method is providing a XML file that contains control information for the conversion. This XML file can contain either instructions for a single file, very much like the parameters on the command line. Alternatively the XML file can contain a list of conversion instructions.

File Naming Conventions

A CZI image can have more information than a simple, single image plane. Multi-channel images, Z-Stack images, time series. Additionally a CZI can internally be divided into scenes, typically separated by empty space. An example for a multi scene image is a digital slide scan from a ZEISS Axio Scan.Z1 where two individual tissue sections on a slide have been scanned as two separate scenes. For post-processing steps it may be desirable to separate the scenes into individual files.

The converter will generate one file per dimensional unit, be it channel, z-dimension or t-dimension. Scenes are not separated by default. In most cases scenes are the (technical) result of the step "tissue detection" and a user does not want to single out tissue segments that 'by chance' have been detected as individual items. If CZI images containing scenes shall be converted into separate (single) scene TIFF images, the corresponding conversion parameter has to be used. For every dimension (with more than one image plane to it) the converter generates a separate image, following this convention filename-S#-Z#-T#-C#. Numbering will start with '1' padded to the correct number of digits. A time-series with 100 time points will begin with '001'. Scenes and Channels may have a verbal description inside the CZI. If specified, these names will be used. If this is specified and the converter finds the default names in the CZI (e.g. 'ScanRegion0', 'ScanRegion1' or a single channel bright field 'TL-Brightfield') the short form (S#) will be used. Dimensions not present or having only one data point will not be made part of the file name. Therefore a plain single scene brightfield scan 'sample.czi' will become 'sample.tif'.

Command-Line Parameters for CZItoTIFFConverterConsole.exe

To print this on screen use **CZItoTIFFConverterConsole CL** on the command prompt.

CZItoTIFFConverterConsole 1.1.0.0
Copyright © Carl Zeiss Microscopy GmbH 2014
Usage: CZItoTIFFConverterConsole.exe XML -params
CZItoTIFFConverterConsole.exe CL -params
For more help use XML or CL without providing parameters

  -i, --Input   Required. Input CZI image file to be converted.

  -t, --TargetFolder   Required. Output FOLDER for converted files.

  -n, --NoJPEG   (Default: False) Use LZW (lossless) cpompression instead of JPEG (lossy) compression.

  -l, --LZW   (Default: False) Overide option 'n' and enforce LZW (lossless) compression on fluorescence images.

-o, --Original   (Default: False) Applies only to FLUORESCENCE / grayscale images: use original pixel values and do not
                     apply Display Setting. If not set output will be 8 bit.

-c, --ColorLUT   (Default: False) Applies only to BRIGHTFIELD /RGB images: use DisplaySetting. Will result in 24 bit RGB
                     regardless of input's valid bit-depth.

  -b, --BigTIFF   (Default: False) Create a BigTIFF image.

  -s, --SplitScenes   (Default: False) Split scenes into individual images.

  -p, --Pyramid   (Default: False) Create a pyramid.

  -a, --Attachments   (Default: False) Attach label & slide preview to pyramid when available. Required option 'p'.

  -q, --Quality   (Default: 75) JPEG compression quality (only used in combination with option 'j').

  -w, --Writers   (Default: 2) Number of parallel writer processes. Limited by number of physical cores.

  -z, --Zoom   (Default: x1) Zoom factor z for downscale 1:z. Accepts (x1, x2, x4, x8 ... x256)

  -f, --Fluorophores   (Default: False) Use channel name instead of index for image name.

-S, --SceneNames   (Default: False) Use scene name instead of index for image name. If set, overrides 's' option.
                     Ignores default scene names.

  --help   Display this help screen.

Additionally, please check output folder name for trailing '\' at the end.
If present, remove trailing '\' from output folder name.

All parameters that have a default value are optional and are required only in the following cases:
- A true/false parameter should be reversed (e.g. '-b' to create a BigTIFF instead of a classic TIFF image, or '-n' to force LZW compression for brightfield images)
- A different value than the default value is to be used (e.g. '–z x4' to create an image that's been downscaled by factor 4)

XML Parameter files for CZItoTIFFConverterConsole.exe
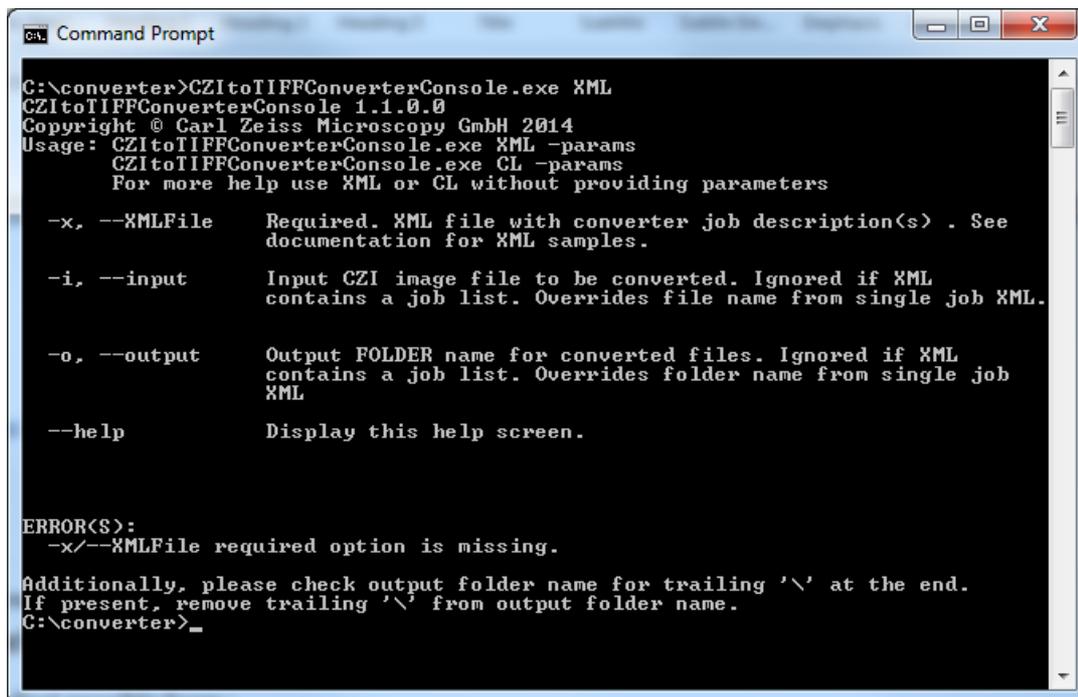
CZItoTIFFConverterConsole 1.1.0.0
Copyright © Carl Zeiss Microscopy GmbH 2014
Usage: CZItoTIFFConverterConsole.exe XML -params
    CZItoTIFFConverterConsole.exe CL -params
    For more help use XML or CL without providing parameters

-x, --XMLFile   Required. XML file with converter job description(s) . See documentation for XML samples.

-i, --input   Input CZI image file to be converted. Ignored if XML contains a job list. Overrides file name from single job
                XML.

-o, --output   Output FOLDER name for converted files. Ignored if XML contains a job list. Overrides folder name from
                single job XML

 --help   Display this help screen.

ERROR(S):
 -x/--XMLFile required option is missing.

Additionally, please check output folder name for trailing '\' at the end.
If present, remove trailing '\' from output folder name.
Two different XML file formats can be used to control the conversion process of a CZI file into TIFF file(s).
One format specifies a single conversion job, the other specifies a list of conversion jobs.

In case the XML file is specifying a single job, the command line parameters 'i' and 'o', if specified, will
replace the values InputFile and OutputFile provided in the XML file. If 'i' and 'o' are omitted, the XML file

must contain values for InputFile and OutputFile. Otherwise an error message will be displayed as output.

Single conversion job, smallest possible form :

```
<?xml version="1.0" encoding="utf-8"?>
<Job xmlns:xsd="http://www.w3.org/2001/XMLSchema"
xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
 <InputFile>c:\testimage\example.czi</InputFile>
 <OutputFolder>c:\testoutput</OutputFolder>
</Job>
```

Note: since parameter *BigTIFF* is not present, the result will be a standard, classic TIFF that is limited to 4GB file size – files produced with this parameter set that are bigger than 4 GB are not readable.

Multiple conversions jobs, smallest possible form:

```
<?xml version="1.0" encoding="utf-8"?>
<JobList xmlns:xsd="http://www.w3.org/2001/XMLSchema"
xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
 <Job>
  <InputFile>c:\testimage\example_1.czi</InputFile>
  <OutputFolder>c:\testoutput</OutputFolder>
 </Job>
 <Job>
  <InputFile>c:\testimage\example_2.czi</InputFile>
  <OutputFolder>c:\testoutput</OutputFolder>
 </Job>
</JobList>
```

Note: since parameter *BigTIFF* is not present, the result will be a standard, classic TIFF that is limited to 4GB file size – files produced with this parameter set that are bigger than 4GB are not readable.

Verbose XML Example

The following XML shows a verbose, almost complete, XML description . The parameters here mirror the parameters that are available on the GUI. Please refer to the comments sections for explanation of each parameter.

```
<Job xmlns:xsd="http://www.w3.org/2001/XMLSchema"
xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
  <InputFile>c:\testimage\example.czi</InputFile>
  <OutputFolder>c:\testoutput</OutputFolder>
  <NoJpeg>false</NoJpeg>
  <LZW_FL>false</LZW_FL>
  <Original_FL>false</Original_FL>
  <Apply_LUT_BF>false</Apply_LUT_BF>
  <BigTIFF>false</BigTIFF>
  <Split_Scenes>false</Split_Scenes>
  <Pyramid>false</Pyramid>
  <Attachments>false</Attachments>
  <Jpeg_Quality>75</Jpeg_Quality>
  <Parallel_Writers>2</Parallel_Writers>
  <Downscale_Factor>x1</Downscale_Factor>
  <Channel_Names>false</Channel_Names>
  <Scene_Names>false</Scene_Names>
</Job>
```

Parameter Description

<Job xmlns:xsd="http://www.w3.org/2001/XMLSchema"
xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
XML root node. Omit the namespace definition in case the container XML is a job list and not a single job.

<InputFile>c:\testimage\example.czi</InputFile>
Input file name. In case it is a single job XML and a file name was provided on the command line, the content of this node is ignored. The file name provided on the command line will be used instead.

<OutputFolder>c:\testoutput</OutputFolder>
Output path where the resulting TIFF images will be placed. The file names are generated automatically. For a simple brightfield image (not having a Z-Stack) the name will change from 'filename.czi' to 'filename.tif'. See separate chapter on naming conventions

<NoJpeg>false</NoJpeg>
If false (default), JPEG is the preferred compression. If set to true even 24bit RGB brightfield images will be stored using LZW compression. Use of parameter BigTIFF is strongly encouraged in that case. If set to true,

all image will be compressed with LZW, even if they could be compressed with JPEG.

<LZW_FL>false</LZW_FL>

If false (default) fluorescence images will be compressed with JPEG compression when possible. This is the case when the DisplaySetting shall be applied. Then resulting image is a 8bit grayscale image that can be compressed with JPEG. When the result image bit depth is greater than 8bit,

<Original_FL>false</Original_FL>

Typically fluorescence images have been acquired with more than 8bit bit depth. 10,12,14,16bit are common. However, it is also typical that the full dynamic range is not utilized.

This has two implications for applications working with such images:

- The application must be able to consume 16bit imagery.
- The application must offer means to adjust the image display in such a way that the images do not appear entirely black because out of 16bit only a small range is actually used (still much more than 8bit's 256 possible values).

Parameter 'Original_FL' with default value 'false' will apply the CZI's internal DisplaySetting and map the pixel data into 8bit, thus allowing applications to work with CZI images even if they can't use the original bit depth. If 'Original_FL' is set to true, the unchanged original pixel values will be transferred into a 16bit TIFF (for original >8bit valid bits). Please note that pixel data with a valid bit depth <16bit may become 'dark' in the output. This is due to the fact that there is no 'n out of 16 valid bits' property in a TIFF image.

<Apply_LUT_BF>false</Apply_LUT_BF>

If false (default) the original 24bit brightfield image data will be transcoded into TIFF. Situations when this parameter could be set to true:

- Image has a (too) dark background which has been compensated via an adjustment of the CZI's DisplaySetting.
- Downstream applications have a very much different gamma setting.

<BigTIFF>false</BigTIFF>

If set to false (default) result TIFF will be a classic TIFF image. If set to true, the result image will be a BigTIFF. TIFF has a 4GB file size limit. BigTIFF does not have this limit. The number of applications that can read BigTIFF is very small compared to applications that can read TIFF. For digital slide CZIs it is recommended to set 'BigTIFF' to 'true'.

<Split_Scenes>false</Split_Scenes>

If set to 'false', the image will be converted as displayed in ZEN without the scene player activated. If set to 'true' every scene (as shown in the scene player mode of ZEN) will be converted into an individual file.

<Pyramid>false</Pyramid>

If set to 'false' (default), no pyramid is calculated in addition to the primary image output. This could result in very long load times with downstream image viewing software. Image processing (analysis) software may not need an image pyramid. If set to 'true', an image pyramid (2^n) is calculated. This adds approximately

33% to the file size. The pyramid is realized as additional pages in the TIFF file. Many digital slide viewing applications recognize the pyramidal character of a TIFF image and will work with the appropriate 'levels' when viewing a TIFF.

\<Attachments>false\</Attachments>

If set to 'false' (default) no additional imagery is added to the TIFF image, even if present in the CZI image. If set to 'true' both possible attachments (label and slide preview) are added to the TIFF.

\<Jpeg_Quality>75\</Jpeg_Quality>

Specifies the JPEG quality. The converter accepts values 50 … 99. Values outside this ranges will be set to the limit they're exceeding. Values below 50 are considered of no use because of the inferior image quality. If omitted, the converter will assume a quality level of 75.

\<Parallel_Writers>2\</Parallel_Writers>

Number of parallel (sub) image conversion processes are utilized. Values exceeding the number of physical cores will be set to number of physical cores. Reduce to 1 when experiencing performance issues. If omitted the default value 2 is used. Applies to parallel processes for ONE image. The converter will convert only one image at a time. Use multiple instances of the application and feed them with their images to convert, if parallel conversion of images is needed. Please observe the potential risk of experiencing disk, memory & network bottlenecks in such a scenario.

\<Downscale_Factor>x1\</Downscale_Factor>

Specifies a start magnification (downscale) as a 2^n value. Possible values are x1, x2, x4, x8, x16, x32, x64, x128 and x256. x1 represents the original size/magnification; x2 is ½ =50% downscale, x4 is ¼ = 25% downscale, x256 is 1/256 downscale. A pyramid, if requested by parameter 'p' will be calculated based on this initial downscale value. Default value , if omitted, is x1 = original image.

\<Channel_Names>false\</Channel_Names>

If set to 'true' ('false' being the default) the converter will name files with their channel names instead of a channel index. For a 2-channel DAPI & FITCI fluorescence image C1 then may become 'DAPI' and C2 'FITC'. The value will be ignored in case of standard, single channel brightfield images. 'TL-Brightfield' is not considered useful information for such images.

\<Scene_Names>false\</Scene_Names>

If set to 'true' (default is 'false') the converter will use the scene names stored in a CZI instead of scene indexes. Value will be ignored, when only the default automatic names are found (i.e. ScanRegion0… ScanRegionN) or there are no scenes (single island of pixels).

Specialized XML Content

The following XML content cannot be provided through the GUI, it is available only via the XML control files.

```
<Pages>
  <Page>x4</Page>
  <Page>x16</Page>
  <Page>Label</Page>
  <Page>SlidePreview</Page>
</Pages>
```

The node <Pages> and its sub node list <Page> allow specifying an individual sequence of TIFF pages. Possible values are the downscale values x1 … x256 and additionally the two key words 'Label' and 'SlidePreview'. If <Pages> is present in a XML file, the parameters for pyramid generation and attachment handling are ignored. Additionally, all pages will be created even if the normal pyramid building process would have stopped at a smaller downscale factor. For the example shown above this would be the case when at x8 the result image already fits into a single tile.

```
<TileSize>
  <Width>1024</Width>
  <Height>768</Height>
</TileSize>
```

In situations where the default tile size 2048 x 2048 is not desired a custom tile size can be specified with the above entry. Width & Height must meet the TIFF tile size requirements, i.e. be a multiple of 16. The converter will adjust the values if necessary. Smallest possible value is 256, provided values smaller than 256 will be set to 256 programmatically.