

Zeiss Efficient Navigation (ZEN) **Blue Edition** Standard Operation Protocol

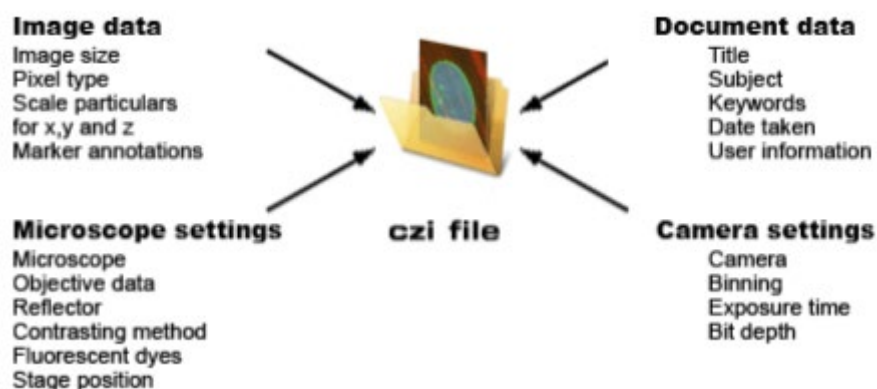
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B. Overview

ZEN 2.3 (blue edition) is a modular image acquisition, processing and analysis software for imaging systems by Zeiss.

ZEN accepts a special file format called *.czi (Carl Zeiss Image). The *.czi image format saves a lot of additional data, for example the date of acquisition, microscope settings, exposure values, size and scale details, contrast procedures which were used. Also all annotations and measured values are saved with the file.



ZEN Blue has been installed onto two workstations in Faculty Core Facility. Please see below for different modules installed.

Computer Name	Software	Special Modules installed
ELYRA offline	ZEN 2.3 (Blue Edition)	<ul style="list-style-type: none"> - Airyscan - Measurement - Image Analysis - Extended Focus - Colocalization
ZEN offline	ZEN desk 2012 (Blue Edition)	<ul style="list-style-type: none"> - Physiology - Measurement - Image Analysis - Extended Focus

C. Start up

Double click on the program icon on desktop.

Or

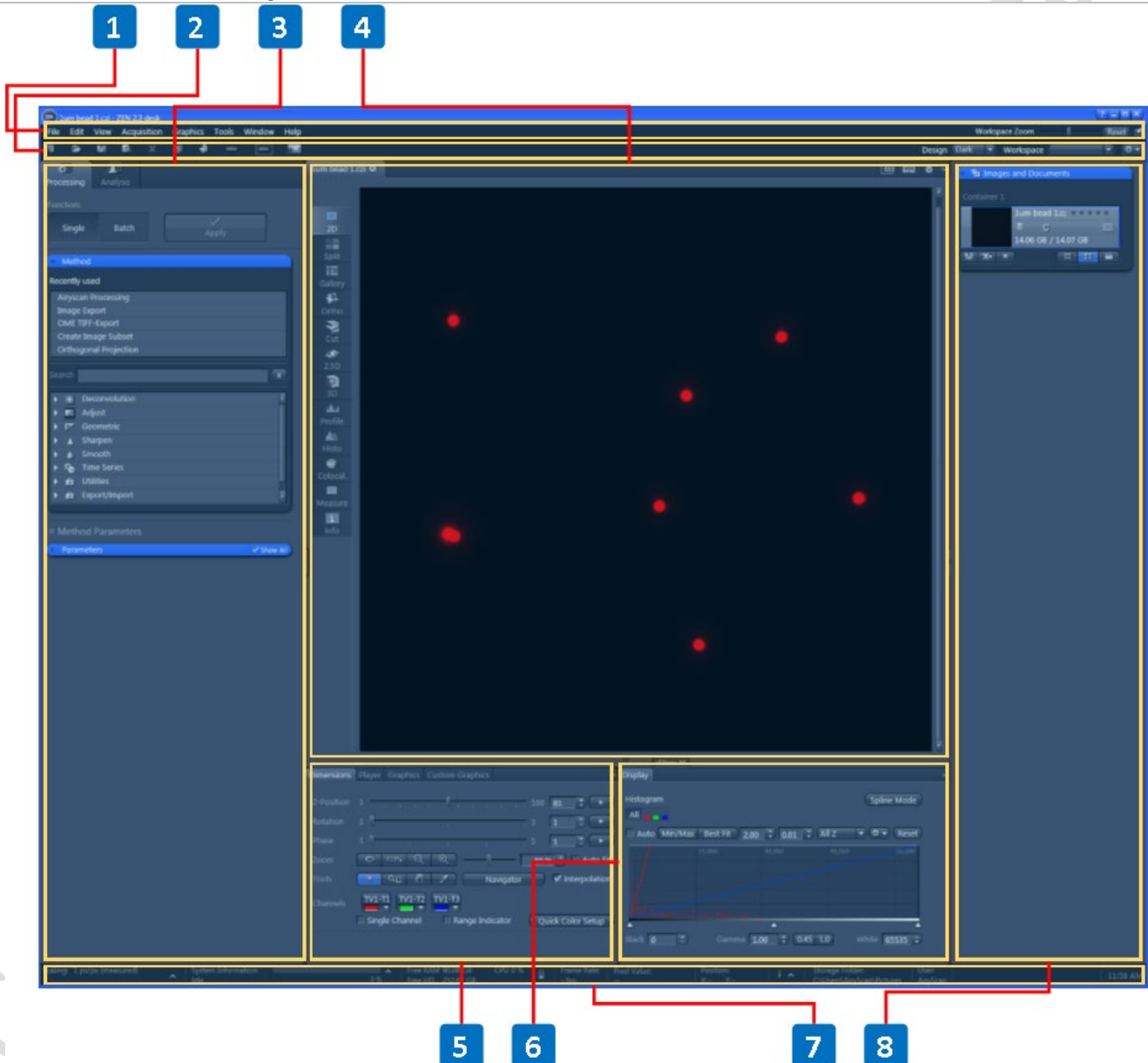
click on Start | All Programs | Carl Zeiss Microscopy | ZEN | ZEN (blue edition) entry (blue icon).

Click on “ZEN desk” button.



Graphical user interface description:




The terms will be used throughout this document to locate different buttons.

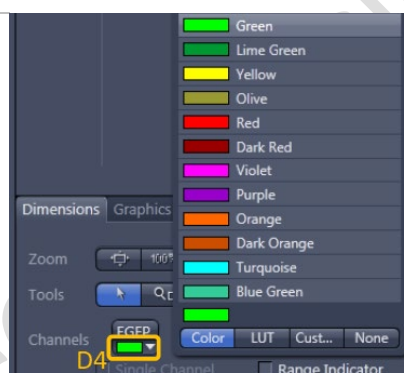
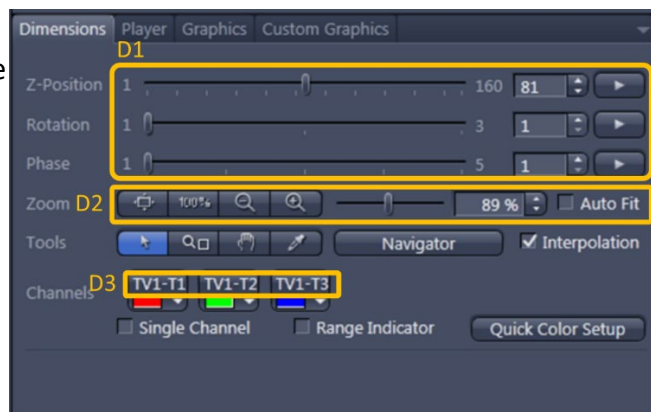




1. Menu bar
2. Tool bar
3. Left tool area
4. Centre screen area
5. Dimension tab
6. Display tab
7. Status bar
8. Image library

D. Display settings

Once an image has been imported it will be shown in the centre screen area. There are display settings below the window for users to view the acquired image.

1. Dimensions tab can be used to toggle images acquired in time series, z-stack or detector phase with sliders.
2. Zoom can be toggled between “fit”  or 100% zoom mode . Alternatively, you can scroll with mouse in the centre screen area while pointing to the area of interest with cursor.
3. Channels can be shown individually with “single channel” option or toggled ON or OFF per channels.
4. Click on the colour swatch  to change pseudo-colour for each channel if necessary.









5. In display tab, upper threshold (“white”) and lower threshold (“black”) can be set for each of the channels to adjust contrast.
6. Click “All” to adjust for all channels at the same time. Or click each of the representing pseudo-colour to adjust contrast for that channel.
7. Alternatively, click “Min/Max”  or “Best Fit” .

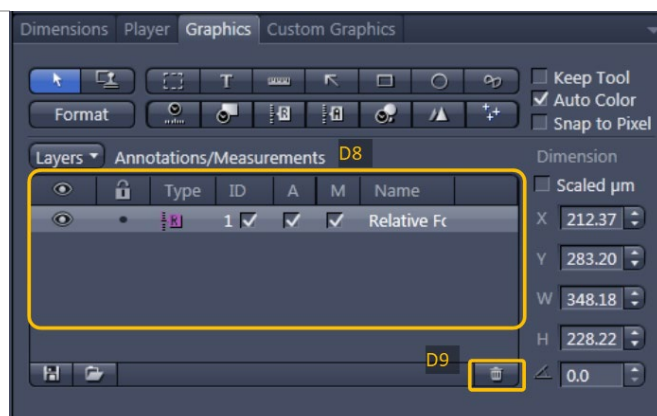
** Note: adjusting contrast will not change your data’s brightness.




Graphics tab

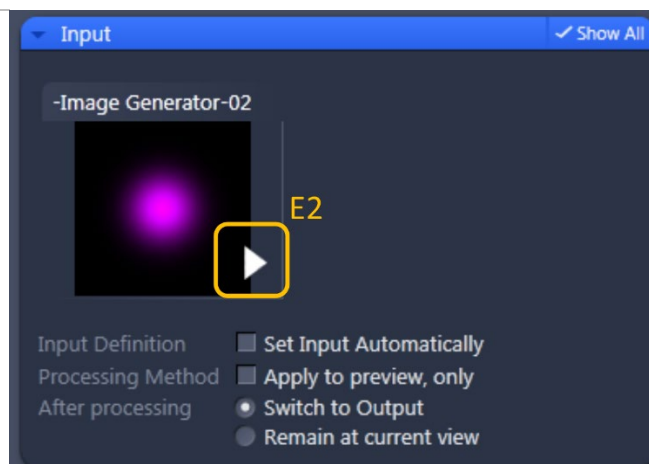
Annotations can be added onto the acquired image.

8. Region of Interest tool 
 - Insert text 
 - Add Scale bar 
 - Line profile tool 
 - Rectangle, circle, freeform shapes 
9. All annotations assigned to your image will be listed in the table.
10. Click delete  to eliminate any unwanted annotations.

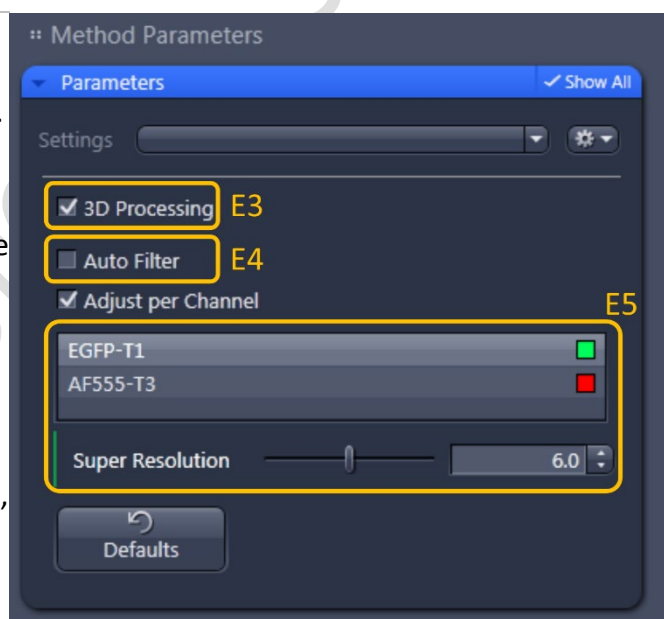


E. Airyscan processing

1. Find Airyscan processing function under “Utilities”/”Airyscan processing”
2. Load an image file acquired with Airyscan to Input by clicking on the white triangle button 



3. If your image contains z-dimension with optimized interval (50% of optical section thickness) 3D Processing option will be available.
4. Airyscan is pre-set to use “Auto Filter”. You can disable the check box it to manually adjust filtering. You can first process your data set once using the Auto Filter and then check the value that was actually applied by the Airyscan processing function. This value is stored in the metadata of the processed image and can be accessed using the Info view.
5. If your Airyscan image has two or more channels, you can check on the “adjust per channel” function to enable manually adjusting filtering for each channel.



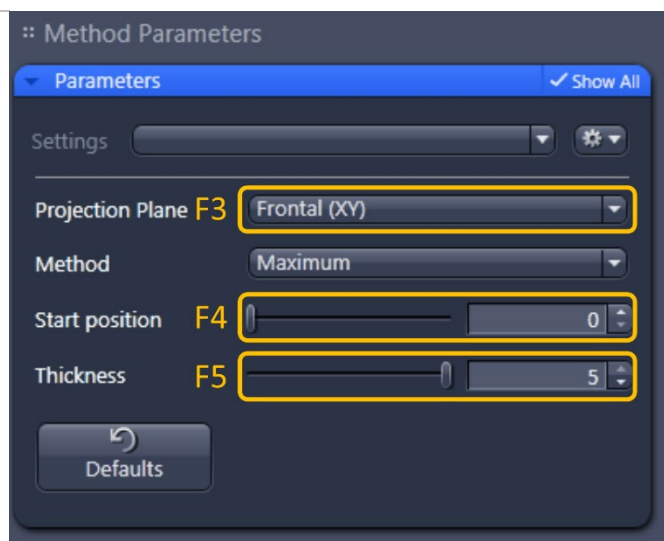
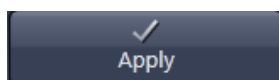
6. Click Apply



F. Orthogonal Projection (AKA maximal projection in ZEN Black Edition)

1. Find orthogonal projection under “Geometrical”/ “Orthogonal projection”
2. Load a z-stack image to input window following step E2.
3. Select “Projection Plane”: Frontal (XY); Transverse (XZ); Sagittal (YZ)
4. Adjust start position.
5. Adjust how many slice of the stack you want to project.

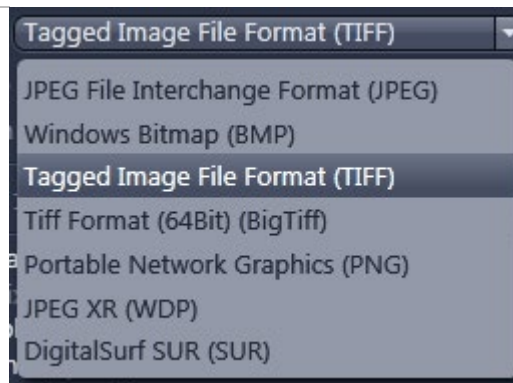
6. Click “Apply”



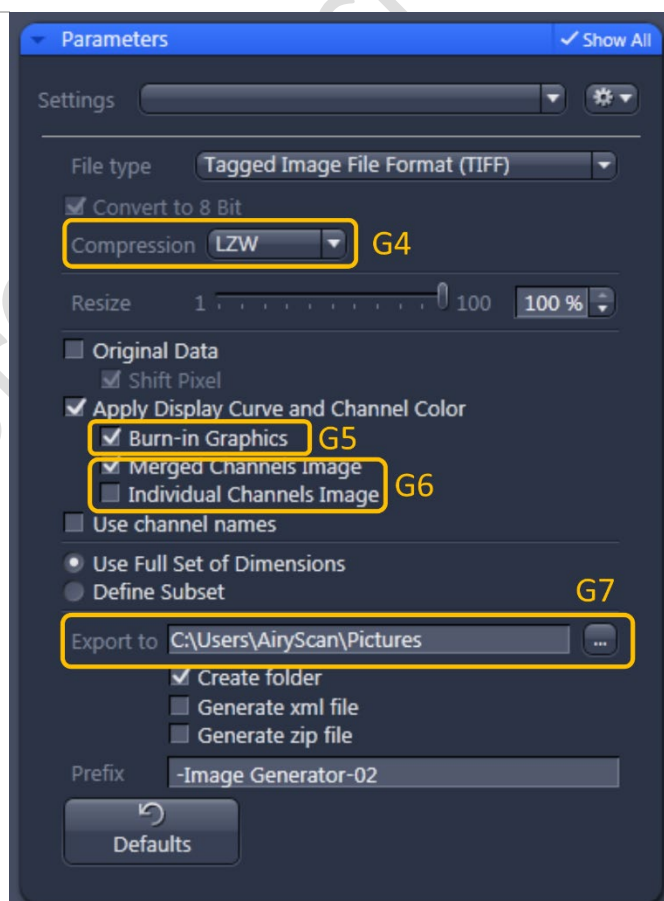
7. A new file will be created in the image library console (right side).

G. Image Export

1. Find Image Export function under “Export / Import” / “Image Export”
2. Load an image file following Step E2.
3. Select which file type to be exported

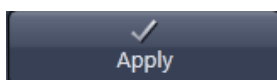


4. If you select tagged Image File Format (TIFF), you can select LZW or ZIP lost-less compression. If you want to retain 16 Bit images for downstream analysis, select “None” as compression.
5. Select “Burn-in Graphics” to show scale bar / time-series on the exported image.
6. There is an option for exporting merged channels and / or individual channels
7. Set a file directory for the exported image to be saved.



8. If “Define Subset” is selected. Specific channels, Z-position range, time range or region can be extracted and exported.

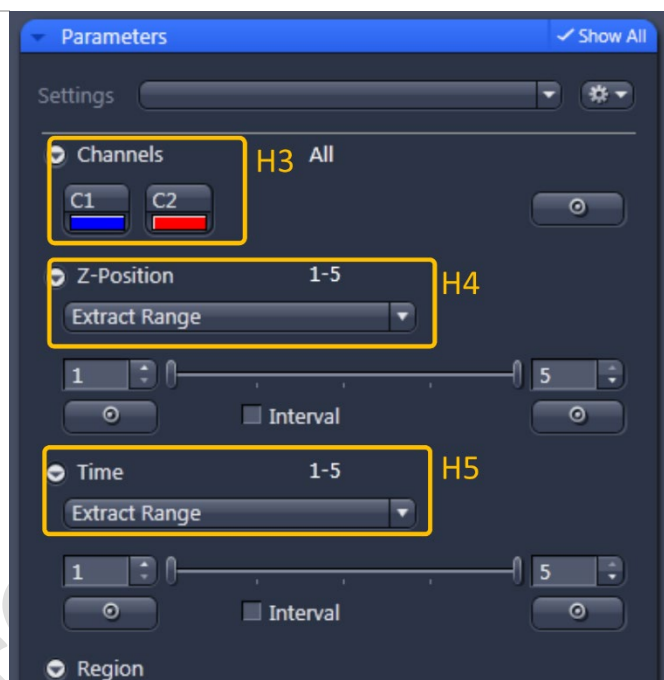
9. Press Apply



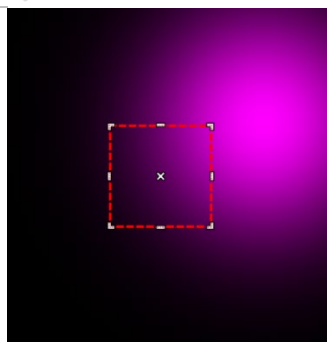
H. Image subset

A function similar to the “Define Subset” function in Image export can be used to create *.czi files with a portion of the dimensions or a multi-dimensional image file.

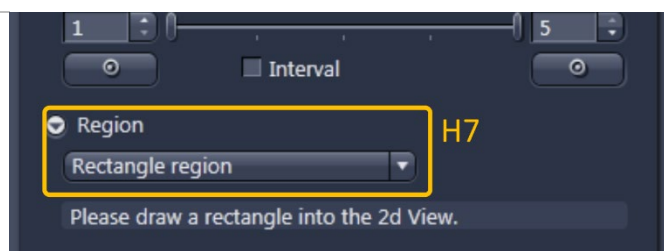
1. Find the function under “Utilities” / “Create image subset”
2. Load an image file following Step E2
3. Select channel
4. Select z-position (single or range)
5. Select time frame (single time point or range)



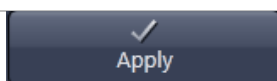
6. To subset a certain region of an image, create an ROI using tools in the graphics tab (Step D8).



7. At the region option. Select “rectangle region”




8. Press Apply



I. 3D view and Movie Export

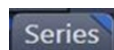
After acquiring a z-stack image set. Beside from combining the z-stack images into one plane as in 'orthogonal projection' (described in Chapter F in earlier content). One might wish to have the 3D structure spun around for viewing.

1. Click on a z-stack acquired image. An icon in image library will indicate if the image involves z-stack module: .

2. GoTo '3D' tab on left hand side of the preview area.



3. GoTo 'Series' tab under preview area.



4. Under 'Series' tab
5. Render series: Turn around X / Y / Z.
6. 360° or Partial Panorama
<N.B.> Select 'Start', 'Stop' angle and spin direction if partial panorama option is selected.
7. Play preview to confirm this is the series you wish to capture.
8. Click 'Apply'. A new file should be generated.
9. GoTo 'Process'. Find a function under Export / Import" / "Movie Export".



10. Load an image file following Step E2.
11. Adjust saving parameters.
12. File type selection
AVI (M-JPEG compression)(uncompressed)(DV)
WMF (WindowsMedia)
MOV (H.264) (MPEG4)
13. Image data selection
Burn In Graphic When activated: Burns the graphic elements into the image. The pixels under the graphic element (e.g. scale bars) are overwritten. The burnt-in graphic elements cannot be subsequently modified.
Merged / Individual channels image can be activated separately or together.
14. Export destination
'Export to'
The path of the export folder is displayed automatically in the display field. To change the file path, click on the button to the right of the display field.

15. Click 'Apply'

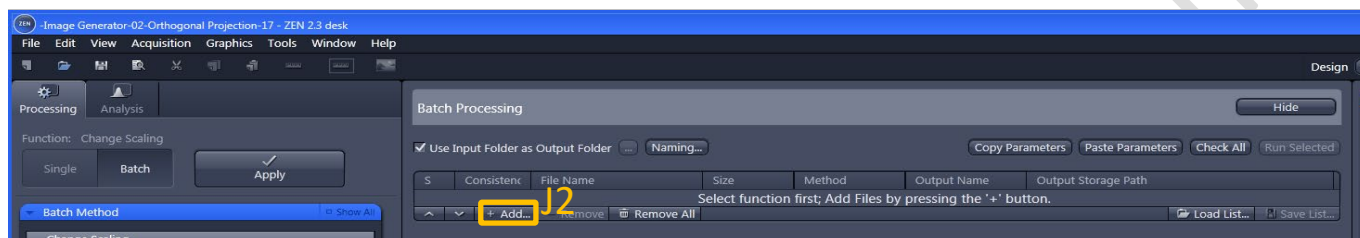
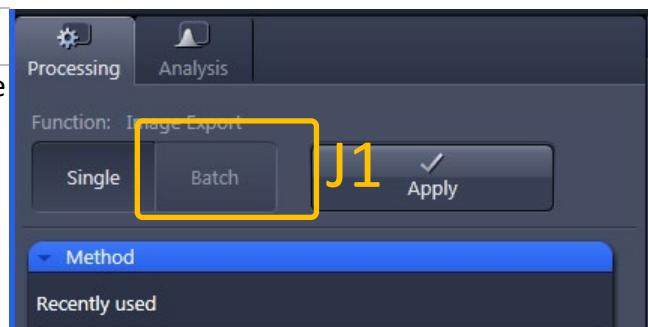


J. Batch Processing

One of the unique feature of Zen Blue Edition is Batch processing function. Essentially, multiple processing functions can be applied to multiple files.

16. Enter Batch mode.

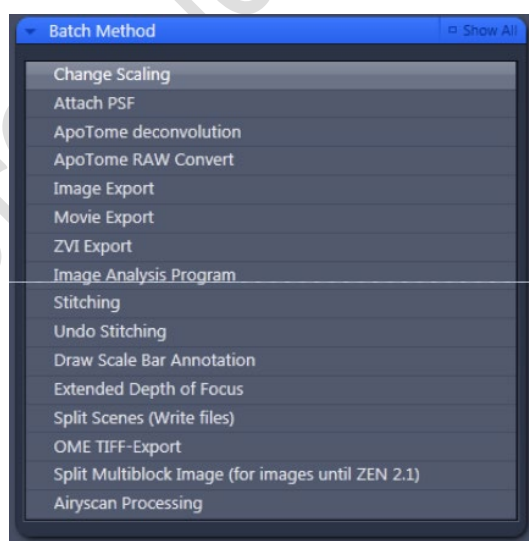
17. Click “Add” to select a folder containing all of the files to be processed.



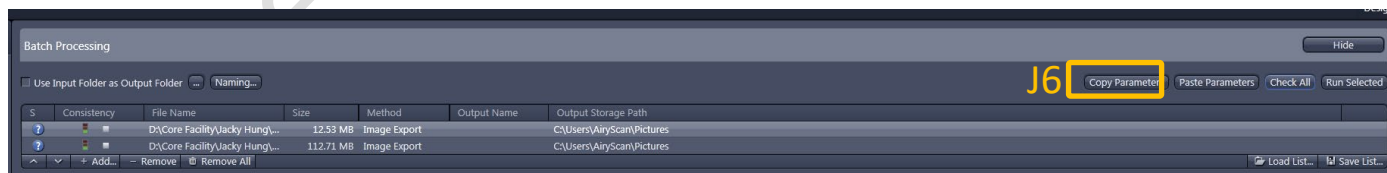
18. Select the first image file.

19. Select a processing method from list.

20. Adjust parameter of processing method.

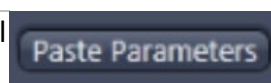


21. Copy parameter from the first file.

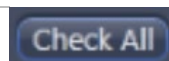


22. Select the rest of the file from list.

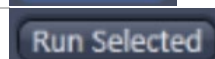
23. Click “Paste parameters” to apply setting onto all of the files



24. Select all of the files. Click “Check All”



25. Click “Run Selected”



26. Batch processed file will be saved in the original folder of source files.