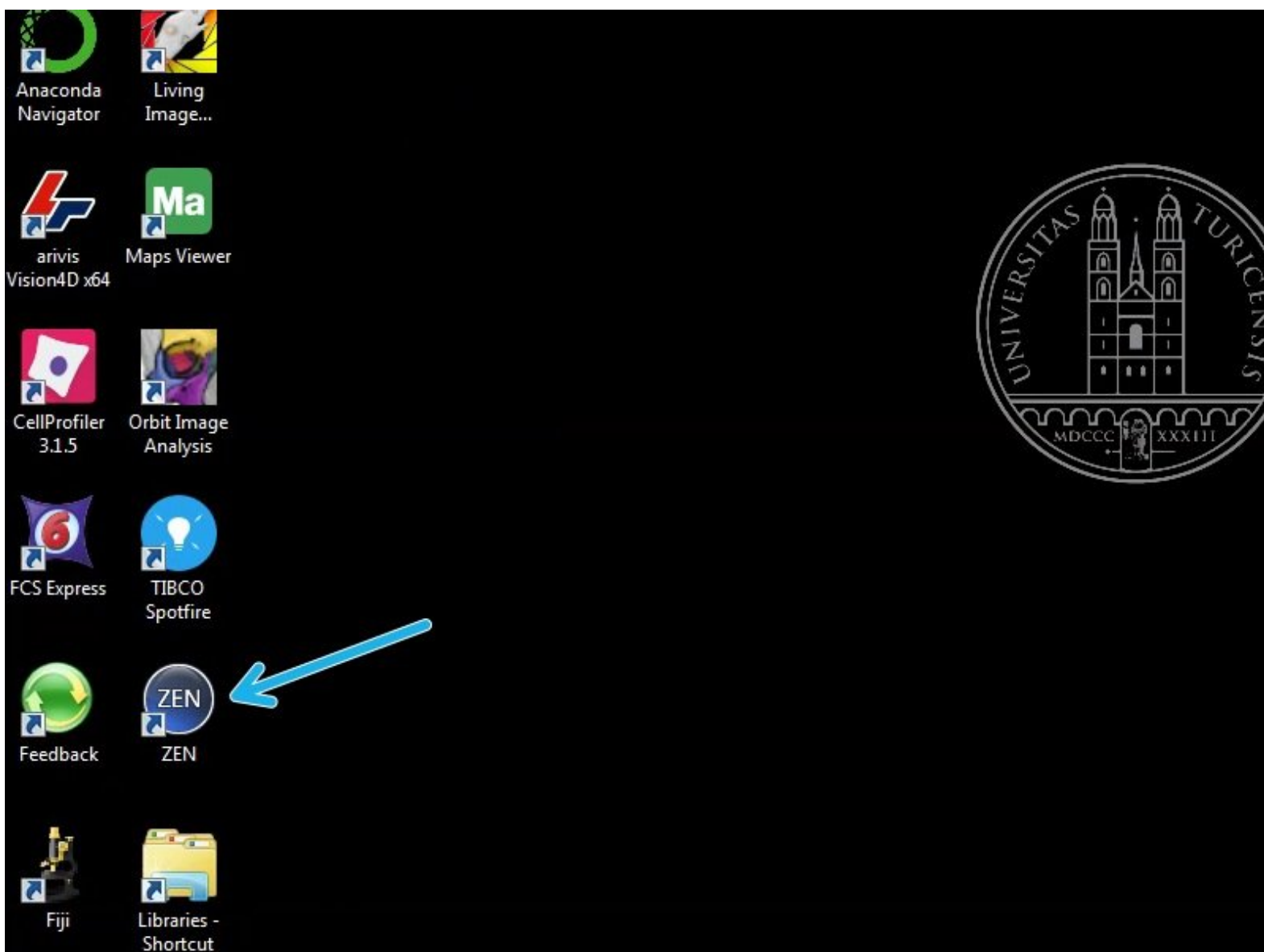


# Slidescanner - Zeiss Z1 - PartOptionalA: Export .czi Files to .tif

How to export .czi files to .tif using Zen blue.

Written By: Joana Raquel Delgado Martins

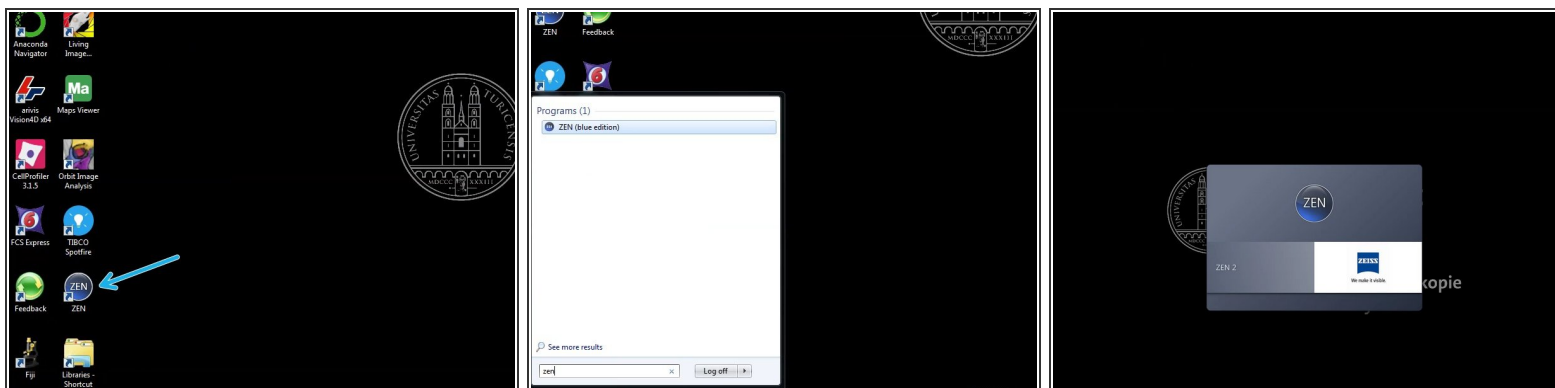


## INTRODUCTION

This guide describes the conversion of Zeiss AxioScan .czi files to .tif using Zen blue.

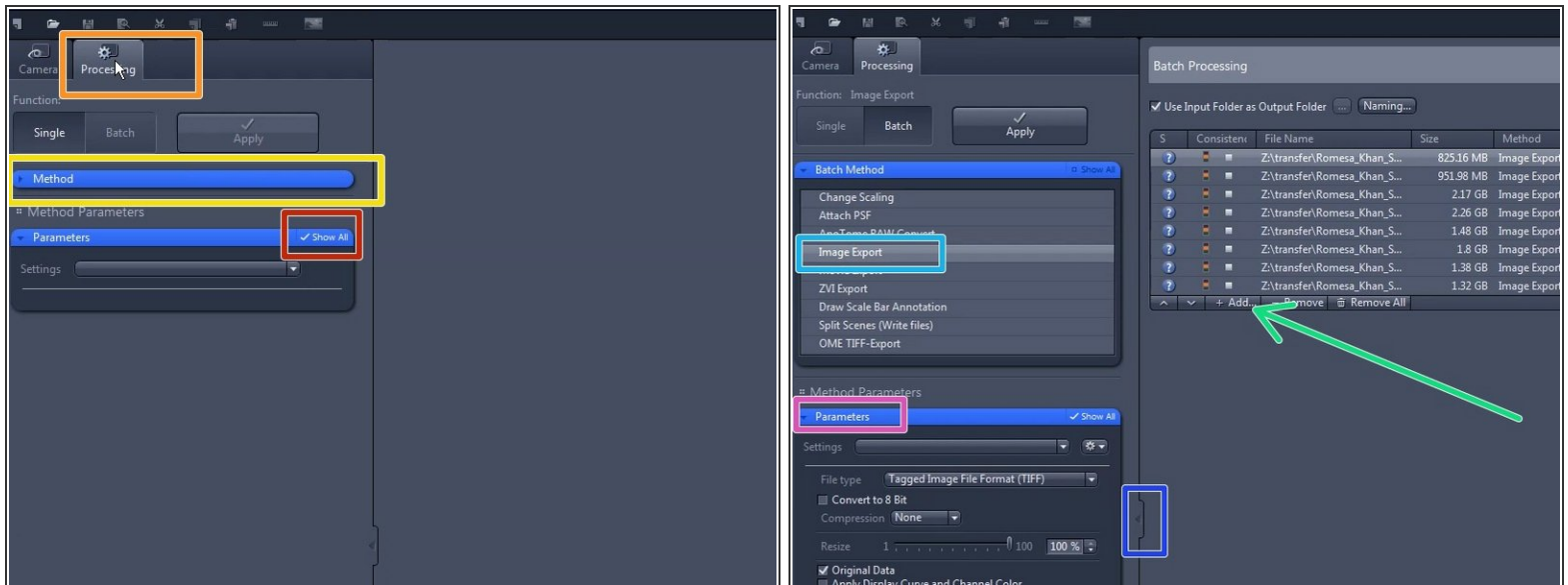
Please note that to directly visualize files generated by the Zeiss Axio Scan.Z1 slide scanner either Zeiss Zen, the open source tool QuPath or Bitplane Imaris are available on the ZMB virtual machines and can be used without prior file conversion to .tif.

## Step 1 — Open Zen Blue



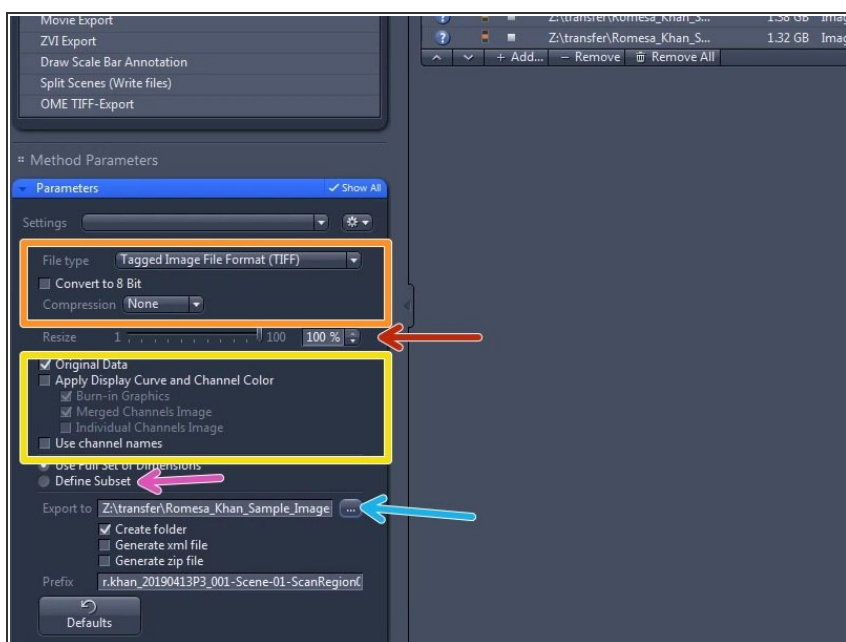
- Reserve and open a ZMB image processing virtual machine.
- Open Zen Blue.

## Step 2 — Batch parameters and add images



- Choose the processing tab. Choose if you want to export a single image ("Single") or use "Batch" to export multiple images.
- Expand the drop down menu "Method".
- Make sure the box "Show all" is checked for the Parameters menu.
- Choose "Image Export" on the "Methods" menu.
- Load your images for batch processing using "+Add".
- Select one file.
- Open the Parameters tool and select the desired settings (see next step).
- If you cannot see this panel look for this arrow to maximize it.

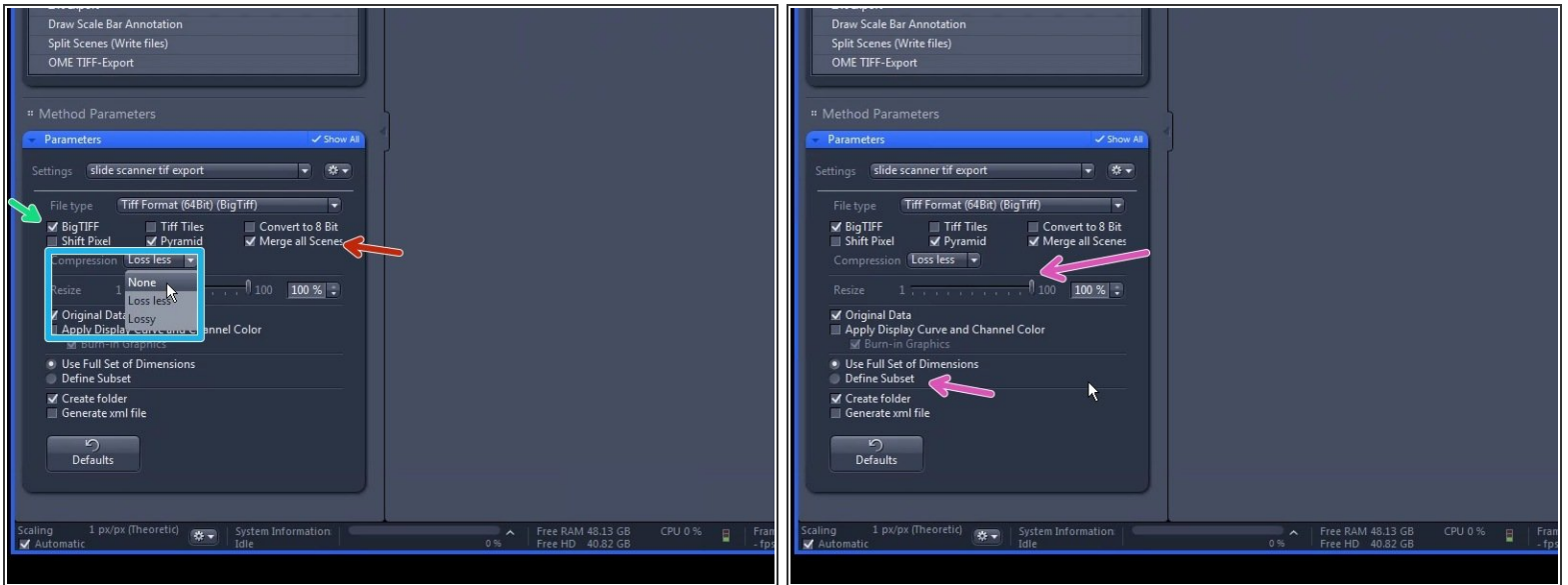
## Step 3 — Option 1 - Adjust export parameters (Tiff)



- For most file export you should choose Tagged Image File Format (TIFF) from the drop down menu.
- "Convert to 8 bit" - leave unchecked if you wish to further process/analyse your data. (recommended). Check if you wish to create images ready for visualization.
- Use lossless compression or no compression (for further analysis).
- Here you can resize the individual files.
- If you plan to further process your data in other software (e.g. using Ilastik) please uncheck "Apply Display Curve" and "Channel Color". It is recommended that you export the Original data (raw).
- If you want to export the images using the current visualization settings defined in the histogram view of Zen please check "Apply Display Curve" and "Channel Color".
- Here you can also select if you'd like to only export a Subset to reduce the size of individual files (eg. Divide into existing Tiles).

⚠ If an individual file is bigger than 4GB, you can either split the various scenes or choose to export it as "BigTiff". For more info see next steps.

## Step 4 — Option 2 - Adjust export parameters (BigTiff, for files larger than 4GB)

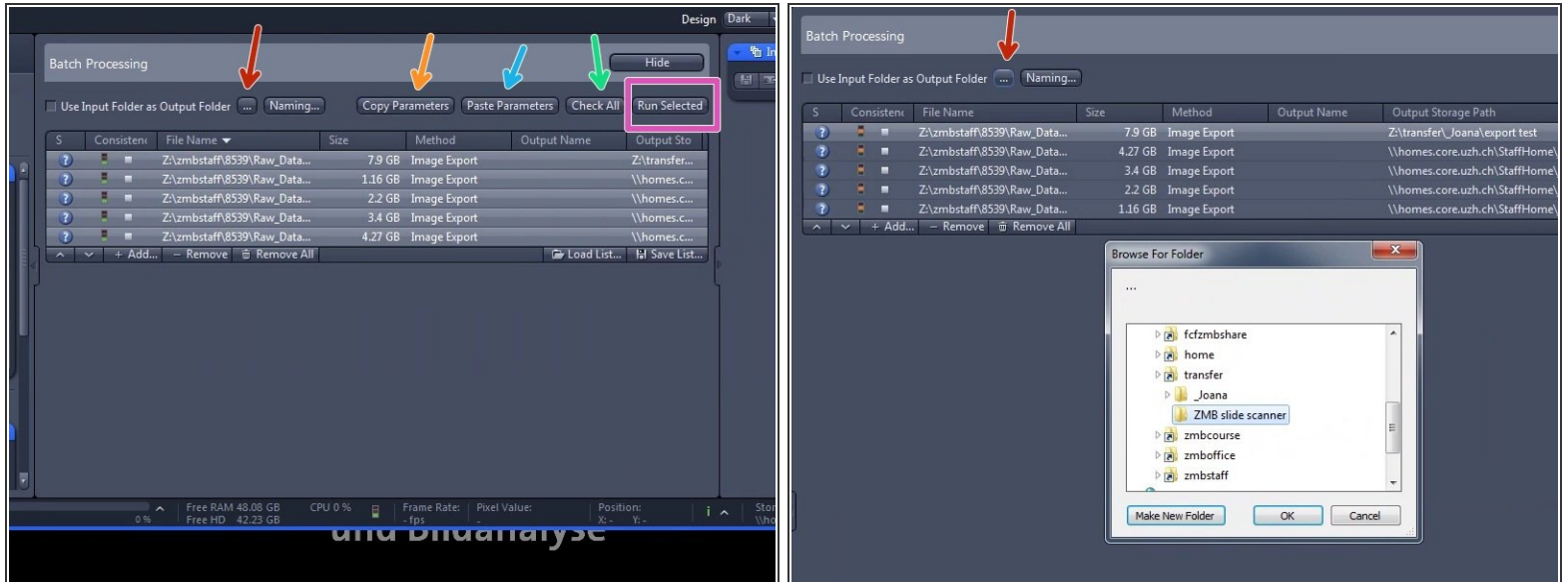


ⓘ If your files are bigger than 4GB you can choose to export them as BigTiff.

⚠ Be aware that although Fiji can deal with BigTiff data, it will be very slow. Also not all image processing software can open it (e.g. Ilastik). Consider exporting as subsets or using Imaris or QuPath for visualization and further processing.

- When you select BigTiff you'll have the following options to export:
  - BigTIFF checkbox - Activated: Generates a BigTIFF image if image size is larger than 4GByte. Deactivated: Generates a TIFF image with maximum size of 4GByte.
  - Compression - Choose either "None" or "Lossless" (compression in accordance with the Lempel-Ziv-Welch algorithm (LZW)).
  - To reduce file size you can also choose to export only subsets or resize the files if you do not need full resolution for an initial inspection.
  - Unchecking "Merge scenes" will create one individual file for each channel.

## Step 5 — Batch processing



- Once you've defined your settings choose the output folder where to save the exported images. You can also choose "Use the Input folder as Output Folder".
- Click on the "Copy Parameters" button.
- Select the images you would like to export with the same settings and click the "Paste Parameters" button.
- Click on the "Check All" button. All images in the list will be tagged with a green marker, if they have the correct settings.
  - You can also right click to "Check" only some files.
- Click on the "Run selected" button to start the export.

## Step 6 — Process / Visualize your data



- You can now visualize/process your data using different software:
  - Fiji
  - Imaris - convert the \*.tif using the Imaris File converter. Do not convert directly from \*.czi as it will create individual tiles.
  - QuPath can read \*.czi files directly (no need to convert initially to tif).