

Creating/converting a Slide for CASK Virtual Microscopy

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What is this manual about?

This manual describes how to convert digital slides to the Zoomify format that is used in the CASK viewer.

What do you need?

- a digital image of a slide.
- a computer with at least Windows 7 and *at least* 8GB RAM, to work efficiently you'll need 24-32GB RAM, see: Appendix 'Size limitations'
- software: specified per step in this manual

Steps: where to start?

This manual starts out with a Mirax file (.mrxs), as that is the format produced by the slide scanner in LUMC. If you have another image format, you can skip the Mirax conversion step and start with a later step:

- If you have a Mirax file (.mrxs): start with step 1
- If you have an image in any non-jpeg format (.tiff, .bmp, etc.): start with step 2
- If you have a .jpeg file: start with step 3

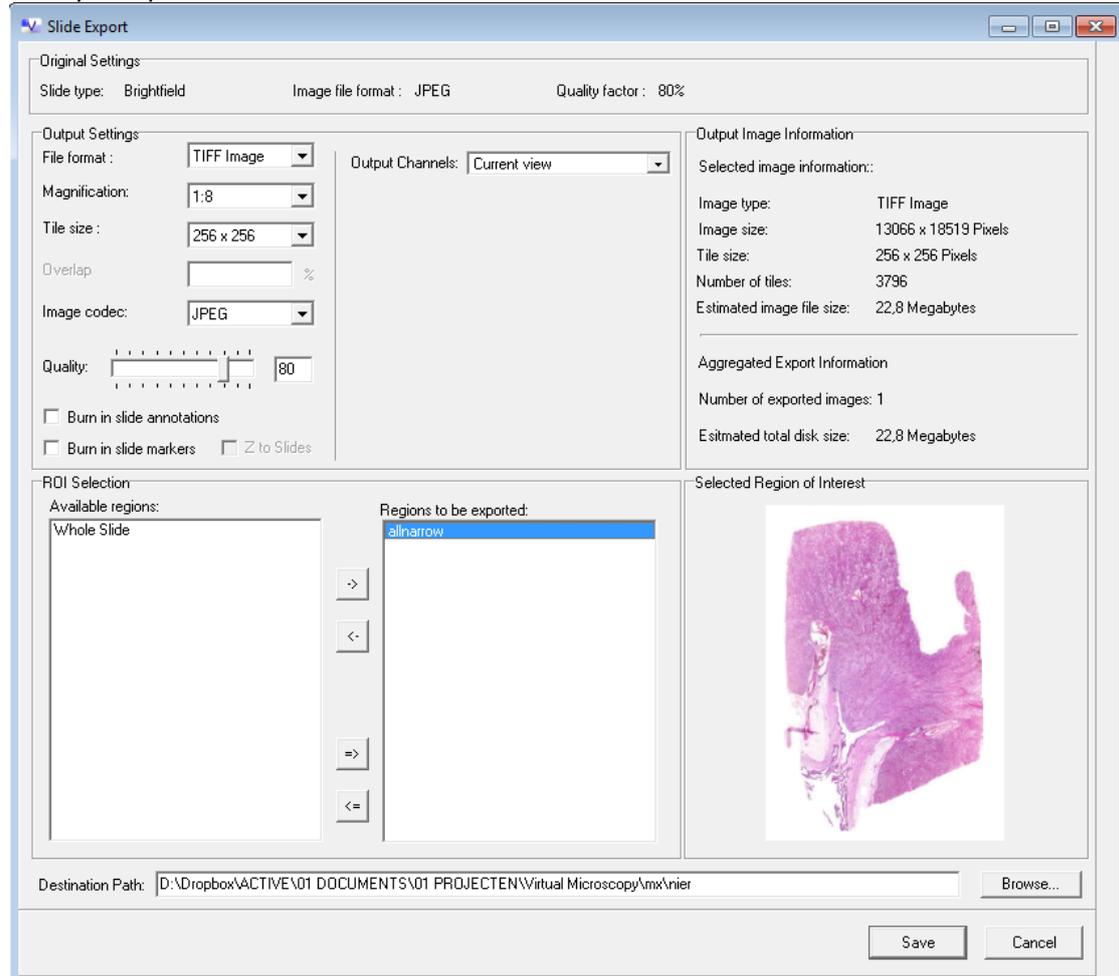
Step 1. Mirax export to tif/(jpeg) (~5-15 min).

- a. Install Panoramic Viewer if you use it the first time. Get it at: http://www.3dhistech.com/panoramic_viewer
- b. Load Mirax slide in Panoramic viewer.
- c. Create an annotation that contains the usable part of the slide and only a small white space around. This method allows to capture the whole slide and not export too much. You can freely choose a name for the annotation.



d. File> export. This results in a so-called 'tiled TIFF'.

Example export window:



Settings:

- **File Format:** TIFF Image
- **Magnification:** Use the maximum size 1:1 if possible. Due to size limitations you sometimes have to scale down large slides to 1:2, 1:4, etc.
IMPORTANT! Read appendix 'Size limitations' to determine which size you can handle.
IMPORTANT! Remember the chosen magnification, you will need it later on, when embedding the slide in the CASK Virtual Microscopy Viewer (see Manual 2: Manual 2. Setting up CASK Virtual Microscopy viewer and adding slides)!
- **Tile size:** 256 x256
- **Image codec:** JPEG
- **Quality:** 80
- **Regions to be exported:** choose the annotated area you made, it has the name you gave in the previous step. Choosing 'Whole Slide' may cut off part of the image and export unnecessary white space in another direction.
- **Destination Path:** choose a suitable location on your PC where you want the export to be placed (button 'Browse').
IMPORTANT! Prepare the file name for step 2. If you forget it and have to do this in step 2, the renaming takes a long time!
 - replace any spaces by underscores: 'bla bla bla' → 'bla_bla_bla'

- replace any diacritic letters by standard ASCII letters: 'éée' → 'eee'
- replace any upper case characters by lower case characters: 'Blabla' → 'blabla' (this is not absolutely necessary, but may prevent problems when you put your file on the server)

Step 2. Convert Tiled TIF/(jpeg) to Jpeg (2-90 min)

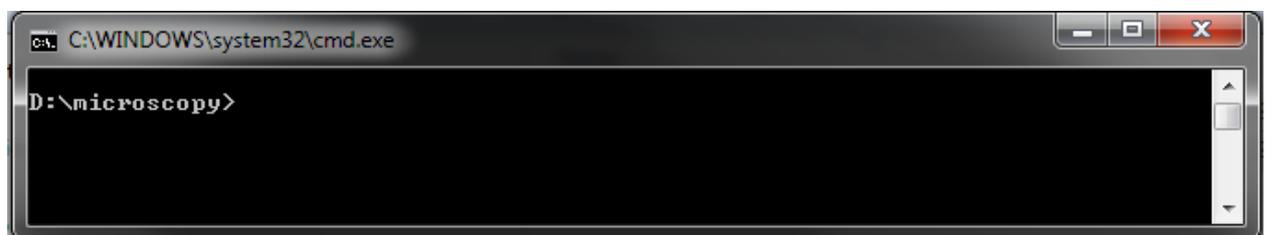
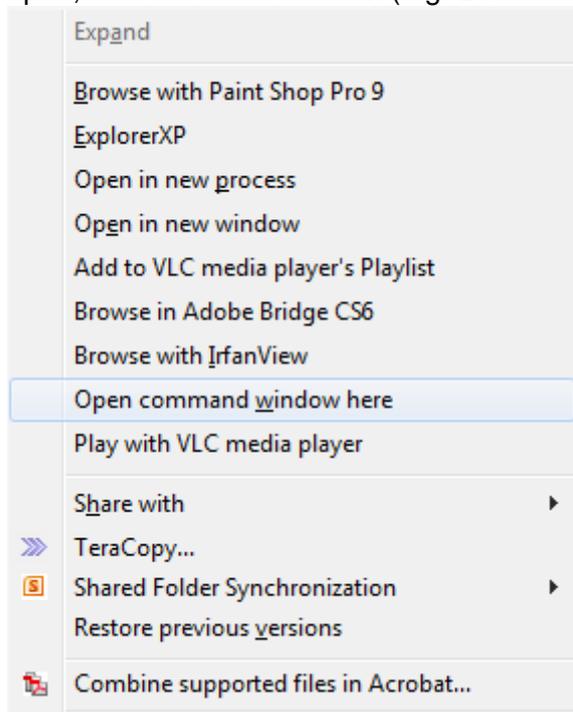
If you want the largest sizes (=best quality) and aren't afraid of a slightly more difficult interface (command-line) we advice ImageMagick. You will need preferaby at least 8GB or more RAM and the 64 bits version of Image Magick, see: Appendix 'Size limitations'

ImageMagick Commandline

- Install ImageMagick if you use it the first time. You can get it at: <http://www.imagemagick.org>
- Browse to the folder where your image is (if you did step 1, this is the .tif you exported). If you didn't do so in step 1, rename the file so it doesn't contain any spaces or diacritic characters. This renaming takes 10 min. or more !!

Windows 7:

- Press and hold 'Shift' button, Right-click the folder that contains the image and choose 'Open command window here'. A black background window (the command window) will open, with the folder location (e.g. D:\microscopy) pre-entered in it.

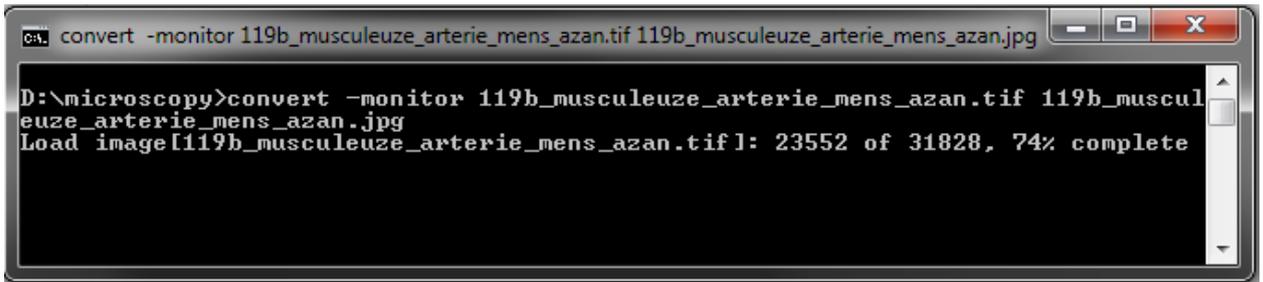


- In the command-window, behind the path, type:
 “convert –monitor” (without quotes)
- Browse to and select (click on) the *image file* to be converted.
- Press 'F2' to get to the rename-option
- Right-click the name, choose: Copy

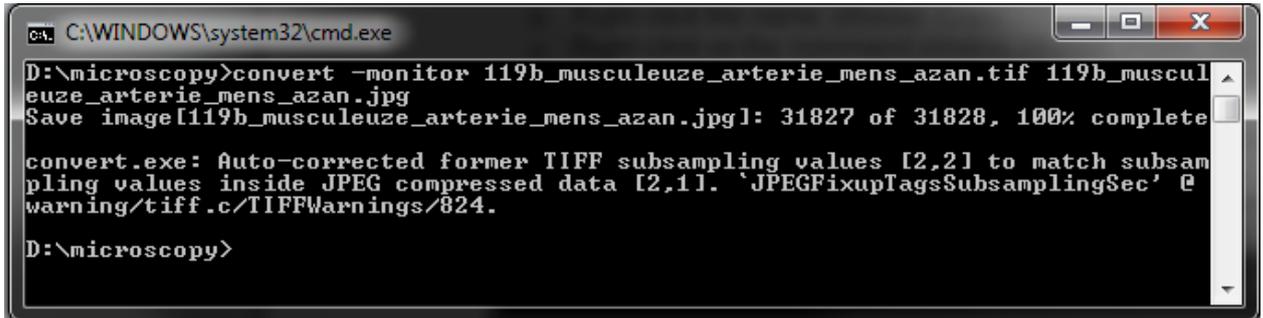
- h. Right-click on the *command-window*, choose: Paste. The name of your file will be included. If it didn't copy the extension (e.g. .tif), add it.
- i. Type a space
- j. Again right-click on the command-window, choose: Paste. The name of your file will again be included. Now give it the extension .jpg
- k. Now you should have something in the command window like:

```
D:\microscopy>convert -monitor carotis_human_he.tif carotis_human_he.jpg
```

- l. Press Enter button to run it.
- m. It will show the progress:



- n. After finishing, it might issue a warning message, but this doesn't seem to affect the result.



Windows XP (not tested, will need Image Magick 32bit):

- o Start> cmd brings up command line window
- o Navigate to the folder where the tif is. Use standard 'old-school DOS box commands to navigate, eg. 'cd' to go to a deeper directory or ../ to go a directory up.
- o type command: "convert -monitor image_name.tif image_name.jpg" (without quotes)
- o Press Enter button to run it.

Photoshop method: For images up to 32767 pixels max dimension you may use Photoshop

- Load the TIF in Photoshop or an other image editor capable of handling such large images.
- 'save for Web' as jpeg (Photoshop issues a warning that the exporter was not designed for such large formats, but it does work)

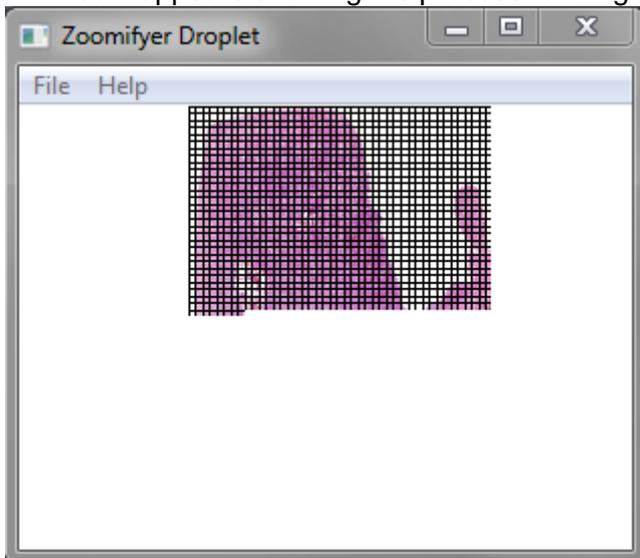
Not working:

It appears to be impossible to directly convert the tif/jpeg by Zoomify (step 3). Zoomify then issues a jpeg coding error. Renaming the tif to jpeg also doesn't work -> Zoomify closes directly.

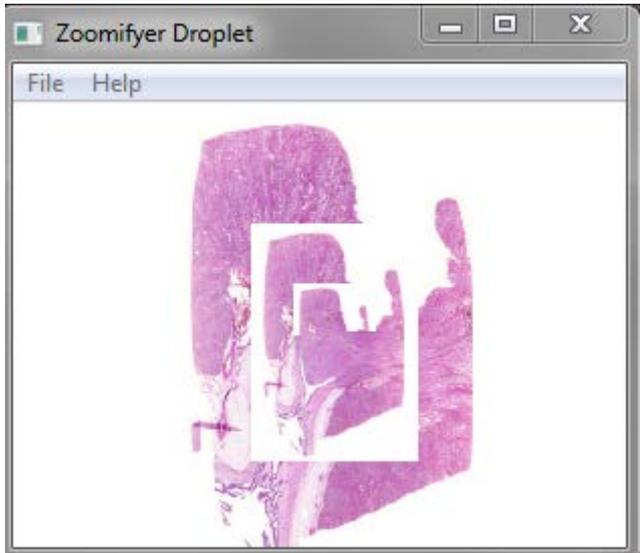
Step 3. Break the Jpeg into tiles (2-5 min)

- a. Get Zoomify-converter at:
info:
http://www.zoomify.com/support.htm#a_ZXv4 ,
Windows:
<http://www.zoomify.com/downloads/products/shipping/ZoomifyExpress4-Win.zip>
Mac:
<http://www.zoomify.com/downloads/products/shipping/ZoomifyExpress4-Mac.zip>
Zoomify-express version can handle files up to 256MB, enterprise version up to 768MB.
- b. Double click Zoomify converter.exe to run it.
- c. Open the jpeg in it.

A window appears showing the process running: one sees the tiles being formed.



Medium size: tiles indicated



Large size image

The process results in a newly created folder (named according to the jpeg input file) containing a series of subfolders: *TileGroup0*, *TileGroup 1*, *TileGroup 2*, etc and a file *ImageProperties.xml*.

	TileGroup0	4-2-2013 23:21	File folder
	TileGroup1	4-2-2013 23:21	File folder
	TileGroup2	4-2-2013 23:21	File folder
	TileGroup3	4-2-2013 23:21	File folder
	TileGroup4	4-2-2013 23:21	File folder
	TileGroup5	4-2-2013 23:21	File folder
	TileGroup6	4-2-2013 23:21	File folder
	TileGroup7	4-2-2013 23:21	File folder
	TileGroup8	4-2-2013 23:21	File folder
	TileGroup9	4-2-2013 23:21	File folder
	TileGroup10	4-2-2013 23:21	File folder
	TileGroup11	4-2-2013 23:21	File folder
	TileGroup12	4-2-2013 23:21	File folder
	TileGroup13	4-2-2013 23:21	File folder
	TileGroup14	4-2-2013 23:21	File folder
	TileGroup15	4-2-2013 23:21	File folder
	TileGroup16	4-2-2013 23:21	File folder
	ImageProperties.xml	4-2-2013 23:21	XML Document

Your slide is now ready to be embedded in the CASK viewer!

(see Manual 2. "Setting up CASK Virtual Microscopy viewer and adding slides")

Appendix Size limitations

There are two major limitations. The only 'hard' border is the JPEG format limitation. The second limitation is the amount of RAM in your PC. If you have 8GB or less you'll need a lot of patience. With 24GB RAM the whole process mostly can be done within half an hour per slide.

Step 1) Pannoramic export

This exports TIFF with codec JPEG or uncompressed TIFF.

- The JPEG format can handle images of maximally 65,535 px (in both directions). Attempting to export larger images will break the export process half way. Attempting to circumvent the limitation by using uncompressed TIFF format (max. 4GB) is useless as step 3 limits the file size, so you will need to compress to JPEG whatsoever in step 2.

Step 2) Conversion TIFF to JPEG*, **

You can use different image editors.

- Photoshop can handle a maximum number of pixels in any direction of 32,767px.
- If you aren't afraid of a slightly more difficult interface (command-line) we advice ImageMagick** that does not have this limitation. Little RAM is a major hold-up in this step, see below*.

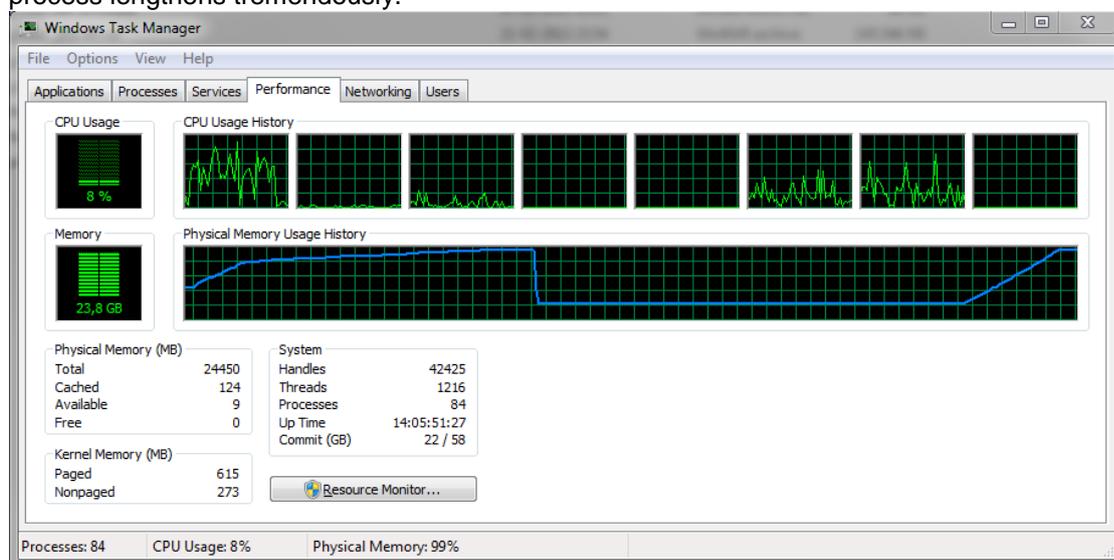
Step 3) Tiling of the jpeg with Zoomify

- The free Zoomify Converter handles images up to 256MB. The Enterprise version Zoomify Converter can handle images up to 768MB.

* The speed of the Image Magick conversion depends on the size of the image and the amount of RAM in your computer. To give an impression:

Image size ↓ / RAM →	8 GB	24 GB
100 MB	10 min	1.5 min
250 MB	25 min	1 hour (?)
400 MB		25 min

** The image below shows the Windows Task Manager during two consecutive slide conversions in Image Magick on a Windows 7 (64bit) 24 GB RAM PC. RAM usage increases steeply during the initial load phase and slightly in the consecutive conversion phase. As long as the system doesn't reach its limit, the process goes fast (1-2 min). When it does reach its limit (as in the 2nd slide below), the process lengthens tremendously.



** There is also an ImageMagick in graphical user interface (GUI) version, but this cannot handle larger sizes due to a Windows limitation:

