Exporting large image data from the Leica *.LIF format using FIJI

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This document applies to users who are effectively using the motorized Leica DMI6000 as a microscope slide scanner and stitching (tile scanning) together multiple images to show large areas of a microscope slide. The stitched image files can be enormous, larger than the LAS-X software can export (usually any image over 1.5-2.0GB). There are a few options:

Use less resolution

- If you do not need multiple gigabytes of image information, you can use LAS-X to reduce the size of the file. It may not take a big reduction in file size to allow you to export.

  o Go to the PROCESS tab (top of the LAS-X software window) and choose the CROP tool. Draw a box around your sample to reduce the amount of unneeded space around the portions of the image that you want to keep. The box tool is located in the drawing tool icons right above the image itself. Cropping will create a new image file within the project and may reduce your image size enough to be able to export the new image from within LAS-X.

  o If cropping does not make your image size small enough, in the PROCESS tab look for the RESIZE tool. You can reduce the overall size of your image by a percentage with this tool. This will create a new file that has a lower image resolution, but this may be an appropriate choice for some types of images.

Use all the resolution that you captured

- FIJI is already installed on the Leica DMI6000 computer.

- For your lab or personal computer - download and install the FIJI version of the free image analysis software, ImageJ (Windows or MacOS). FIJI includes the BioFormats plugin for opening vendor specific file formats (like LIF files), as well as other useful plugin modules. For large image files, I would highly recommend that you use a 64bit operating system and the 64bit version of FIJI. See: https://fiji.sc/

FIJI (ImageJ)

- FIJI does have a version for Macintosh computers, so these steps should (in theory) be able to be performed on a MacOS computer.

- Install FIJI on a powerful computer in your lab. To work with enormous files, you need a high-end computer, with a speedy CPU, plenty of hard disk space, and lots of RAM. The average student laptop might not be up to the task. *(Note: I tested this on my work laptop, which is an older dual-core Intel i5 CPU running at 2.80GHz, with 16GB of RAM, and the laptop took several minutes for each step working with a 2.2GB LIF file.)*

- To simplify things:

  o Make sure the LIF file you are working with has only the single stitched image you want to export inside it. Create a new Project file if needed. This may require you to also have a copy of the free (Windows only) Leica LAS-X software installed on the computer you are using. To download LAS-X see: http://www.leica-microsystems.com/products/microscope-software/details/product/leica-las-x-ls/

  o Make sure you have the LIF file on your hard disk drive, not a USB flash or external hard disk drive plugged into your computer (even if it is USB 3.0, things will greatly slow down).

  o It would be a good idea to reboot your computer before following these instructions. Once you are logged in, make sure that this is the only task you are asking the computer to perform (don't run other software, it just uses up the RAM). Turn off as many background programs as you can.

- In FIJI use PLUGINS | BIOFORMATS | BIOFORMATS IMPORTER to open the LIF files.
- In the Bioformat Import options:
  - Choose "*View stack with: Image Browser*"
  - Uncheck "*Group files with similar names*", since you have (hopefully) only put one image in the LIF file.
- <u>VERY</u> IMPORTANT - It may take many minutes for images to open and then to be saved in different file formats. This waiting can be frustrating for those of us who are used to our computers responding to our commands quickly, but these oversized images are presenting a huge job to our desktop computer. FIJI is written in the JAVA programming language, which can be slow. <u>Even if it feels like nothing is happening, be patient.</u> This is especially true once you start exporting the file from FIJI, as this takes far longer than opening the file. Since FIJI is not particularly good about progress bars, one tip is that your exported image should be "done" when:
  - The file name finally appears in the folder you saved it to (it may take several minutes for this to happen).
  - When the file size stops changing. The final file size (in MB) should be very similar in size to that of the project file that you exported the image from.
- In FIJI use IMAGE | TYPE | RGB. This will create a new version of your image that says RGB at the top (no more channel slider at the bottom of the image). This step is required to mix all the color channels so that you can export.
- To save this file as a tiff image in FIJI, use FILE | SAVE AS | TIFF.
  (Please note: While it may be tempting to use the jpeg file format because the overall file size will be smaller, I do not recommend jpeg for science images as it tends to smear the crisp edges that we work so hard to see.)