

Image processing to optimise photomicrographs.

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Often the raw image taken in a photomicroscope has common technical flaws. Among them:

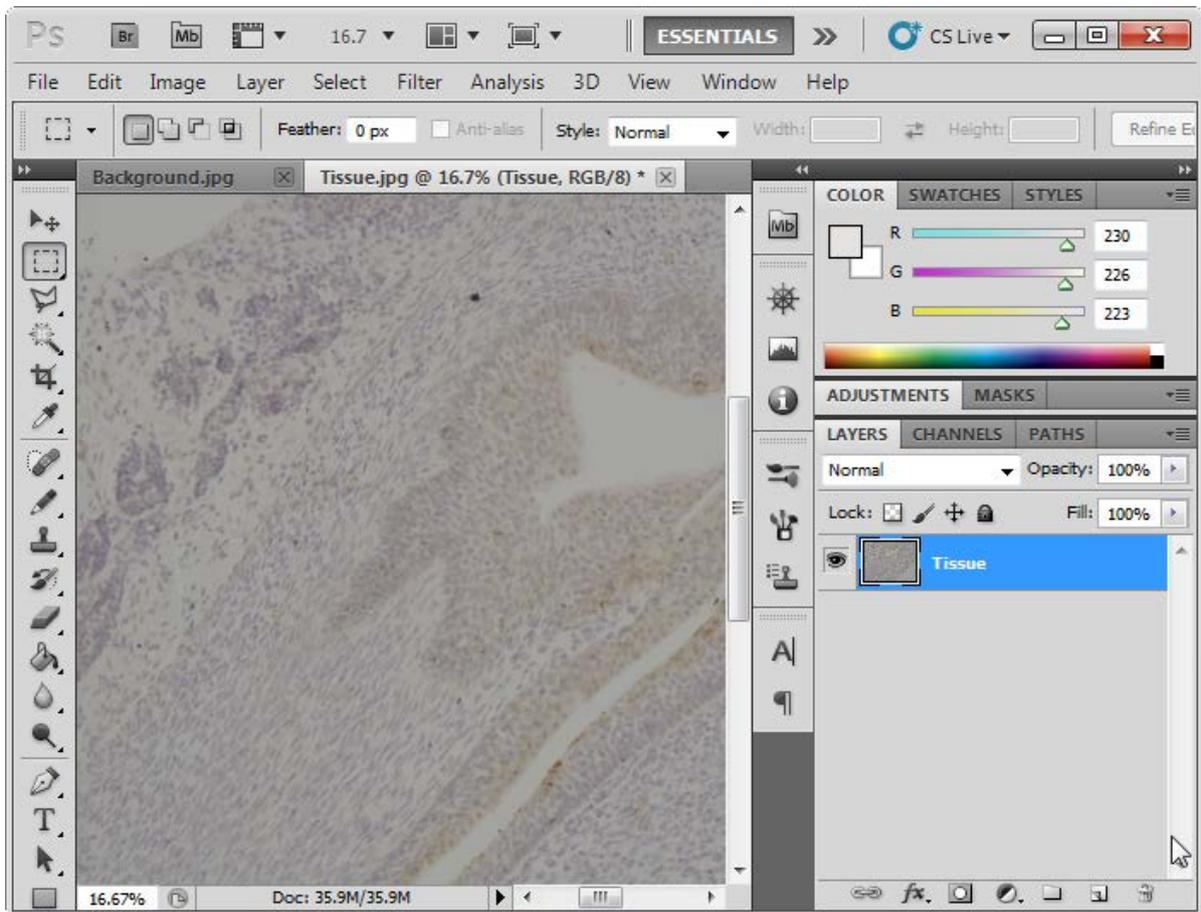
- Poor colour balance
- Uneven illumination across the field
- Poor contrast
- Unsharpness

Usually photomicrographs can be greatly improved by appropriate digital processing. Here are some steps to consider.

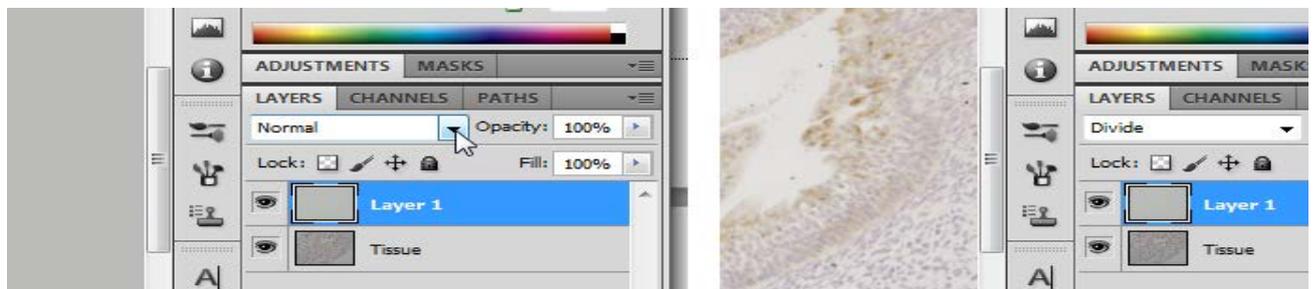
1. Take care at the onset to set up your microscope for optimal performance. For brightfield microscopy many people forget to set up the condenser correctly, which can seriously degrade the image you can take. Normally one establishes Köhler Illumination. If you are unsure how, read a guide (eg <http://www.olympusmicro.com/primer/anatomy/kohler.html> or <http://www.microscopyu.com/tutorials/java/kohler/>). Common issues include the condenser diaphragm closed too small (causes diffraction artefacts and low resolution) or not focused (poor resolution)
2. Make sure you focus your image accurately on the camera (most photomicroscopes allow you to adjust focus for the camera so that the focal plane for the camera and the eyepieces is about the same, but if in doubt check the image from the photomicroscope)
3. Set the camera to manual exposure, and adjust it to ensure the background (bits without any tissue) is nearly white, but not quite, and the darkest stained bits are not totally black. Do not adjust the condenser or light voltage after this step.
4. If the camera software allows, adjust white balance on your slide (click on/select a part of the section with no tissue to do this – you may need to move the tissue off the field of view)
5. When you take your image, take a matching image without the slide present, at exactly the same illumination and camera settings. You will later merge this background image with your tissue image – if the background illumination is uneven you can subtract the uneven background to get an evenly illuminated outcome.



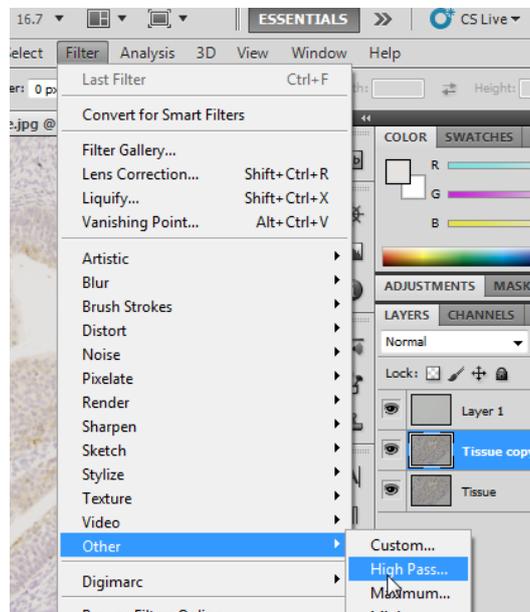
- Open both files in photoshop (or GIMP, the free, open source alternative, or any one of many alternative image editing programs). To avoid ambiguity I have selected the tissue section image and changed the name of the image layer to "tissue" (Photoshop defaults to "background")



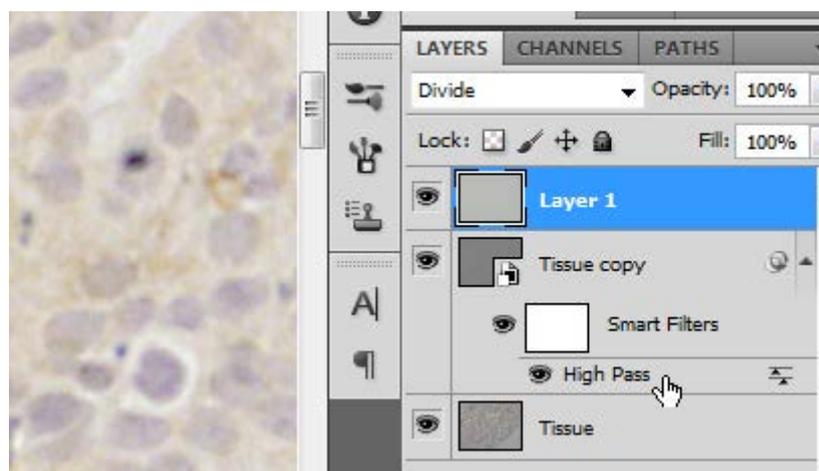
- Switch to the background image, select all and then copy (Ctrl-A , Ctrl-C). Switch to the Tissue section image and Paste (Ctrl-V) the background image. This will make a new layer over your tissue section (called "Layer 1"). Use the "Blending Options" drop down to change from "normal" to "divide" This will change the image you see from a copy of the background to the slide tissue with the background removed (this is what the "divide" blend does)



- Now for a bit of sharpening high-pass filter sharpening (I find this gives better results than the standard sharpen filter). Select the “Tissue” layer and duplicate it (Ctrl-J). This creates a new layer called Tissue Copy. Select Tissue Copy layer. Right Click on this and select “convert to smart layer”. Having a smart layer means you can later go back and change the settings for the filter you will apply, if you decide it needs tweaking. In the menu choose Filter :: Other :: high Pass.

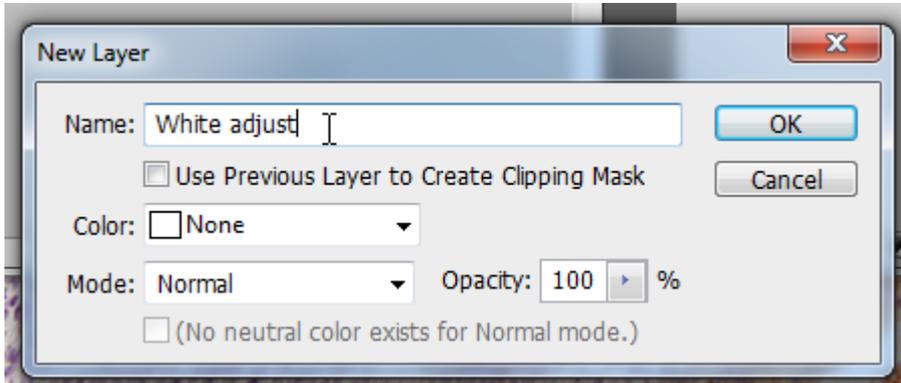


- In the High Pass filter dialog, adjust the radius setting so that you can just faintly see the edges in your original. Click OK and alter the layer blending options to “overlay”. You should now see a sharpened image. Check the sharpening effect by clicking the ‘eye’ icon beside the Tissue Copy layer to turn it off (no sharpening) and on (sharpened). For best assessment, set the image to 100% zoom (Ctrl-1) before you do this comparison. If the effect is too little or too much (you don’t want to introduce artefacts) adjust the filter (double click on the High-pass under the smart layer).

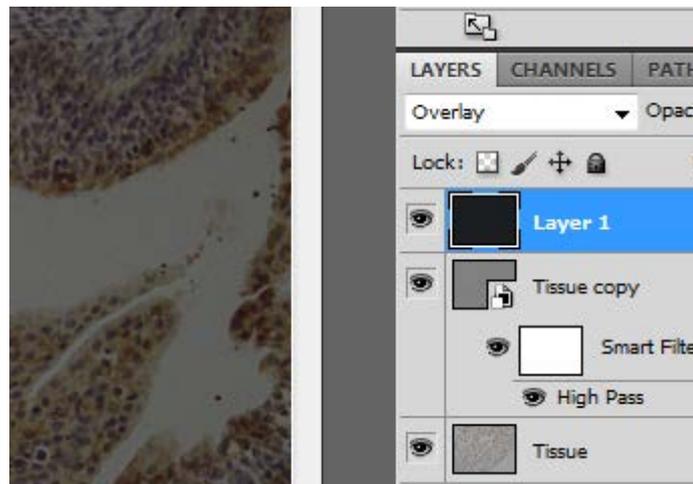


- If the overall density, white levels or contrast need further adjustment, there are a couple of further tweaks you can do.

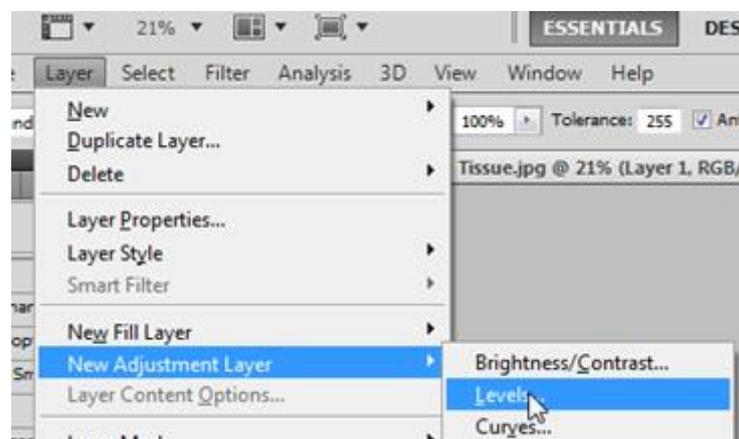
11. In this example the “white” areas are not really a neutral white. I tried the following: select the eye-dropper tool. Right click on the image in a “white” area (eg a gap between tissues). Select say a 5x5 average. This loads the sampled colour into the palette. Choose the rectangular marquee tool (deselect the dropper). Add a new layer (Shift-Ctrl-N) and call the new layer ‘White Adjust’



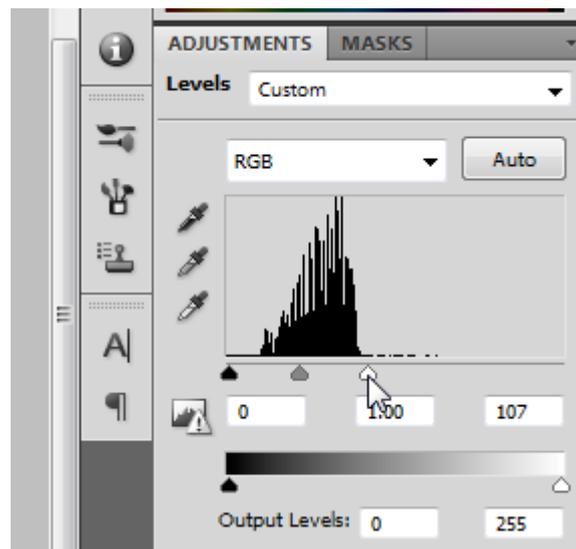
Drag this layer to the top. Ctrl A to select the whole layer. Choose the paint bucket tool (G), and click on the image. The image will appear to blank with the foreground colour.



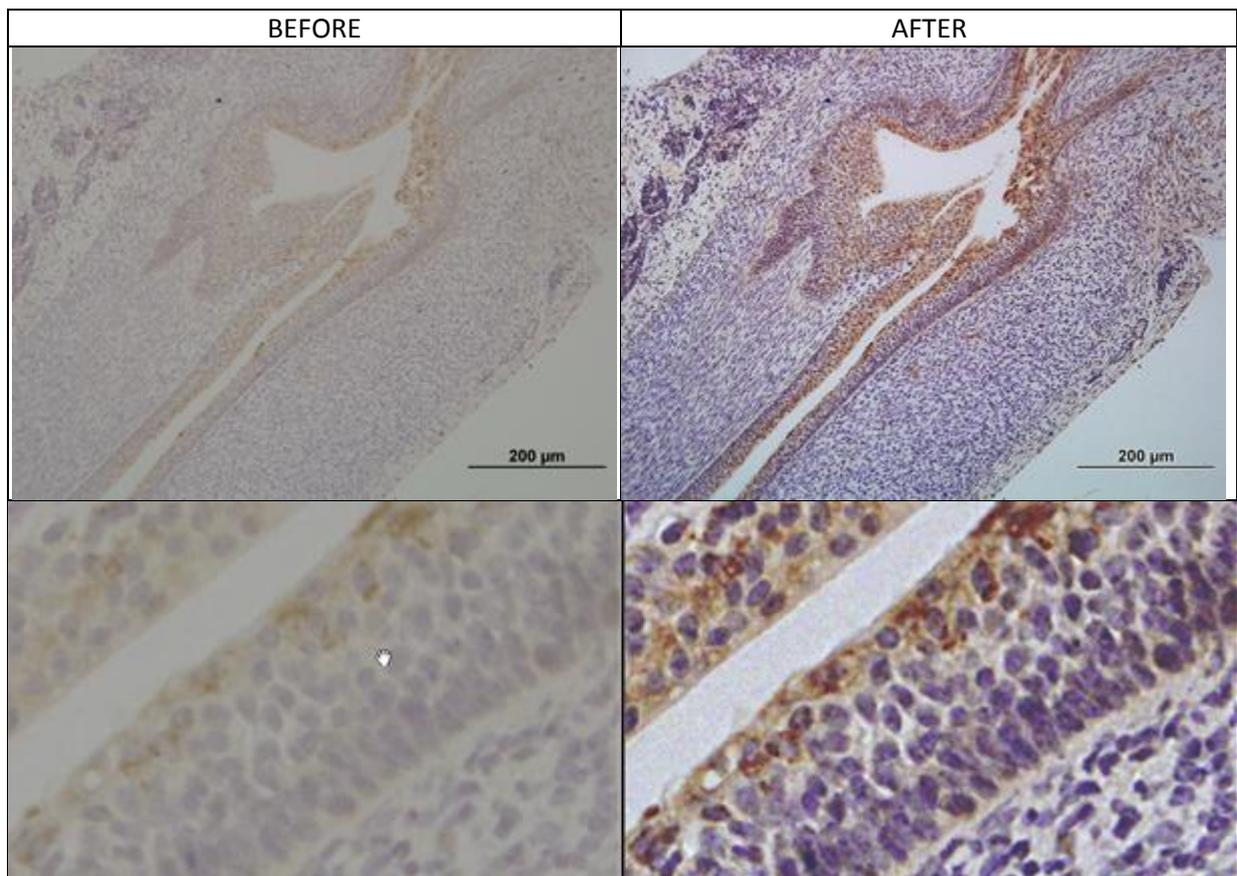
12. Add a levels adjustment layer,



13. Adjust the white level using the histogram as a guide



14. You now have (hopefully) a much improved image).



15. Save your file with all the layers (PSD or TIFF) so you can adjust things more later if you need to—warning probably a HUGE file) and save as jpeg so you have a smaller version as a working copy.

I am sure there are hundreds of other sequences of manipulation that would work fine too. Have a play and see what happens. If you keep a backup copy of your originals you can always start again. If you use layers in photoshop (or GIMP or ...) then you can keep the steps of your edits, and by flipping layers on or off you can check the effect of each adjustment to make sure you are happy before you progress.

Happy experimenting.