Provis shading correction

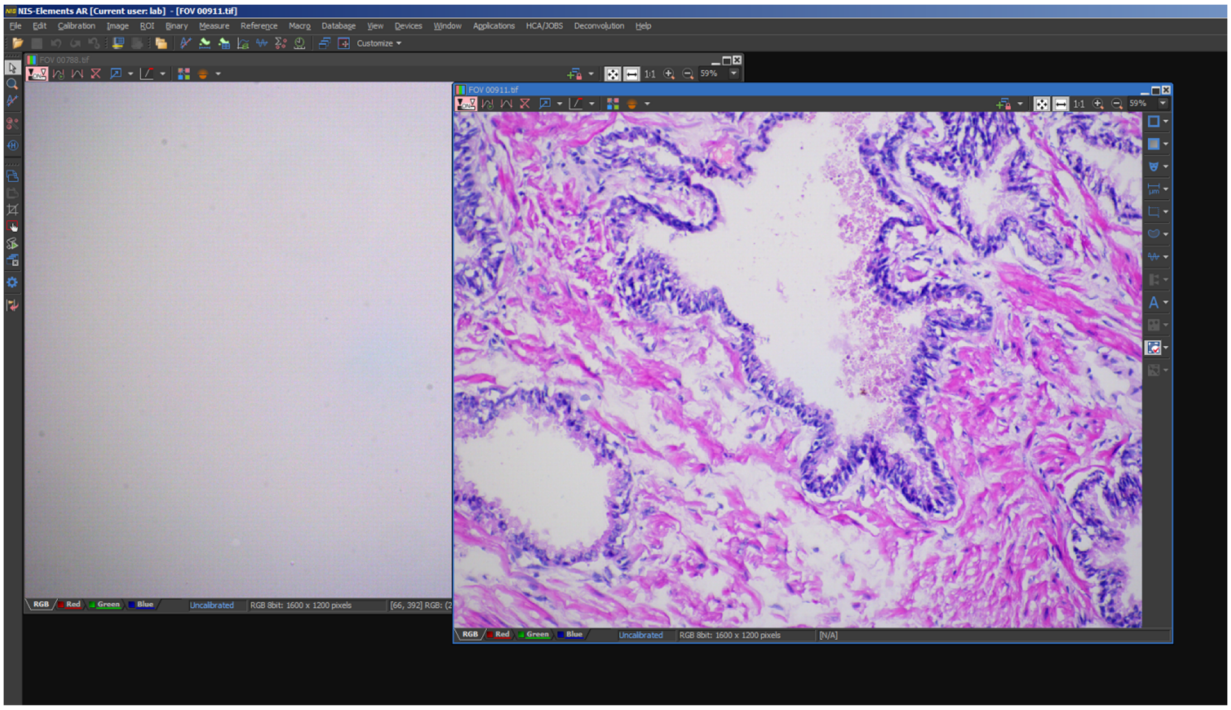
Basic shading Correction

When doing white light imaging on the Provis, always acquire a “correction image” every time you sit down to use the microscope.

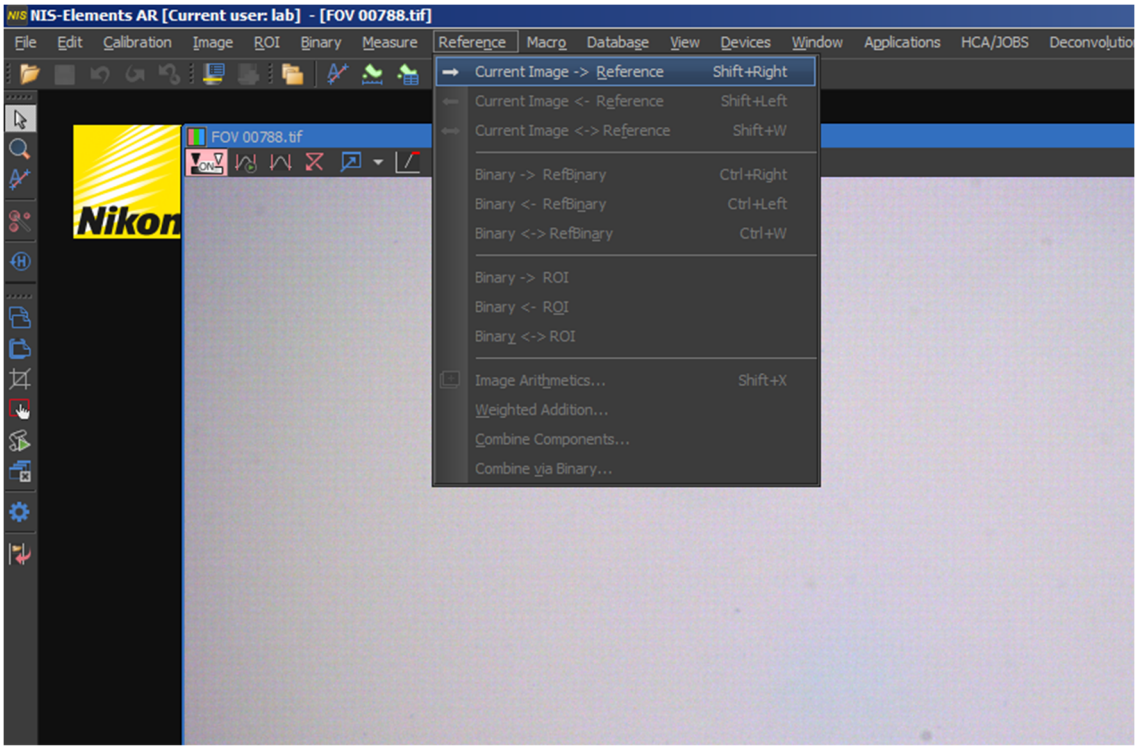
This image should be taken **after** you’ve set Köhler, chosen your exposure time, and white balanced. It should be an image **of nothing**, as clean of an image as you can get.

BACKUP YOUR DATA. We recommend doing this on a **COPY** of your data **ONLY**, in the event anything goes wrong.

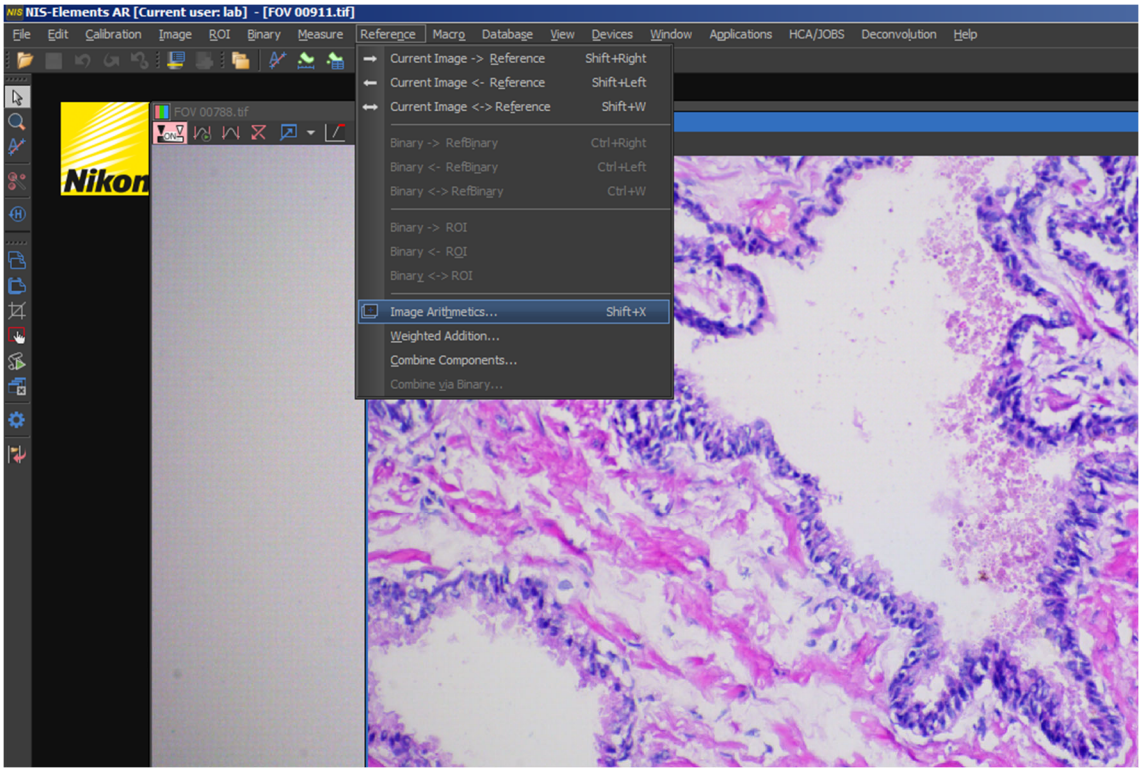
Open your correction and your normal image In NIS elements (the correction image may look strange. This is normal.)



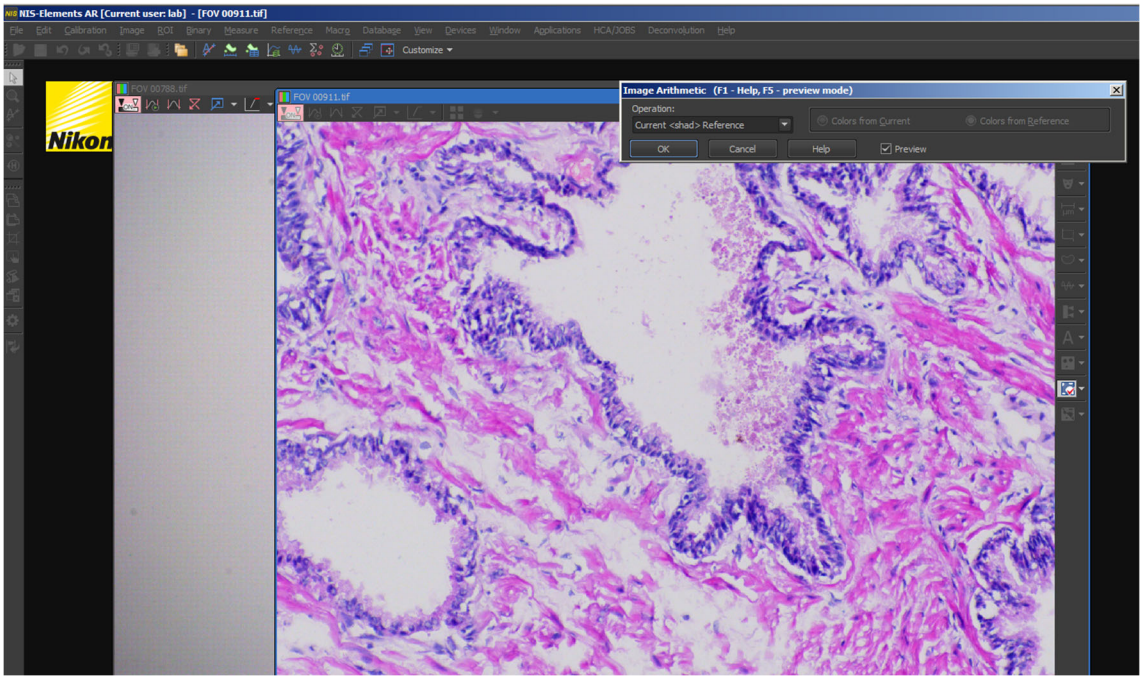
Select your correction image. Go to reference, and select “Current image -> reference”



Select your normal image. Go to “reference” and select “Image arithmetics”



Use “Current <SHAD> ref” and hit OK



Done! Your Shading correction has been used to remove any dirt or grime (inside the microscope) from your image, as well as uneven illumination of your field of view. Save your image.

You can now open any other images you have, and go to reference, Image arithmetics, and “current <SHAD> reference” for those images as well.

You do not need to re-define your correction image for each picture, but you do need to have a unique shading correction image for each imaging session.

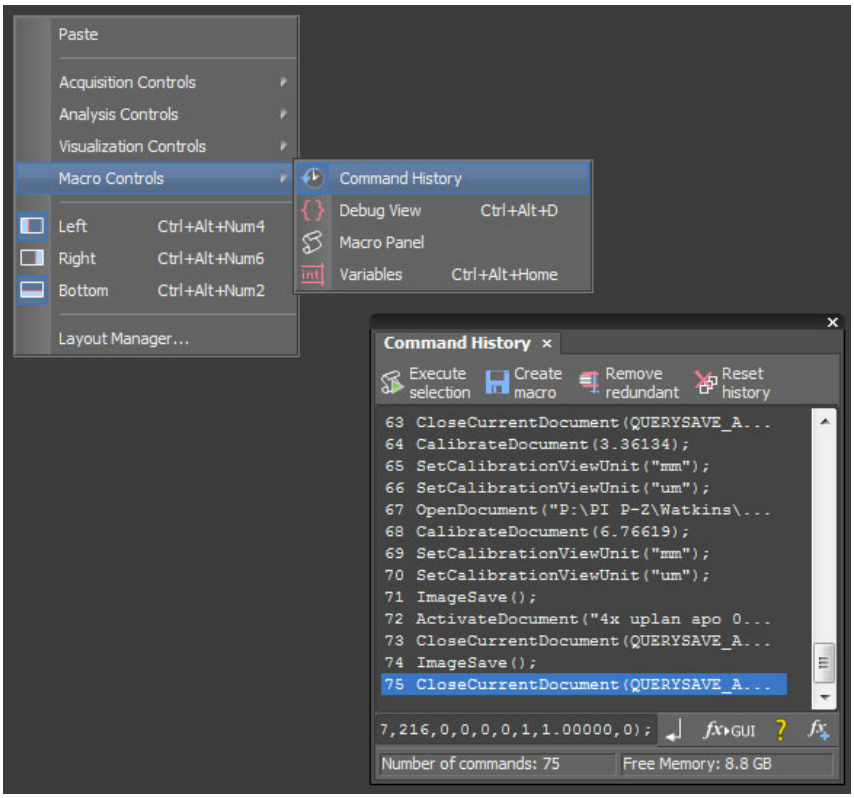
You can do this individually if you only have a few images, or you can run them as a batch.

Running your images as a batch

BACKUP YOUR DATA. We recommend doing this on a **COPY** of your data **ONLY**, in the event anything goes wrong.

Put all the images associated with the SAME shading correction (not including the shading correction itself) into a folder

Right click on the background of NIS elements, go to “macro controls” and open the “command history”



Hit “create macro” and paste the following:

ColorOperations(5,1);

CloseCurrentDocument(QUERYSAVE_YES);

To calibrate your document at the same time, add the line:

CalibrateDocument(1.00000);

Replace 1.00000 with your calibration number (pixel size), in um.

The calibration numbers (for the OLYMPUS PROVIS ONLY) are:

2x PlanApo 0.08 NA: **6.77** um/px

4x UplanApo 0.16 NA: **3.36** um/px

10x UplanSApo 0.40 NA: **1.35** um/px

20x UplanSApo 0.85 NA: **0.67** um/px

40x UplanApo 1.00 NA: **0.33** um/px

60x PlanApo 1.40 NA: **0.23** um/px

100x UplanSApo 1.40 NA: **0.14** um/px

To add an additional white balance your document at the same time, perform a white balance on an image (after applying the shading correction) in image -> adjust image -> white balance. The command history will give you a command similar to:

WhiteBalance(255,255,255,0,0,0,0,0,1.00000,0);

If you choose to also do a dynamics correction, it will look similar to:

WhiteBalance(216,217,216,0,0,0,0,1,1.00000,0);

These numbers are unique to your set, so use the numbers NIS elements generates for you, and not the example numbers used here. Add this line to your macro.

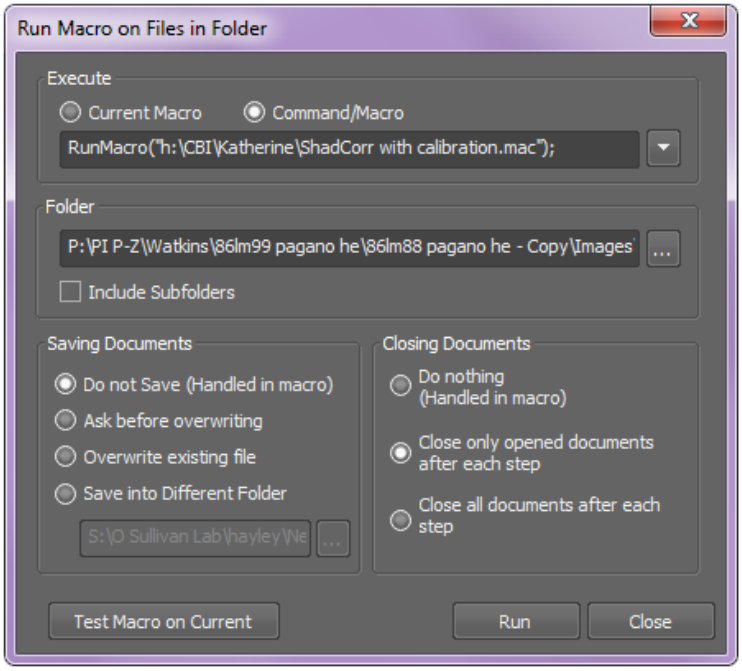
Be sure your macro is in an order that makes sense… so, it should “color operations” (shade correct) before “white balance”, and “close document” should be the last command in the sequence.

Save this macro. Note that the “color operations” and “close” commands can be used for any of your images, “calibrate” needs to contain a value that matches the objective you used, and the “White balance” command need to be unique to each sample set.

How to use this macro

Open your shading image. Go to “reference” and **set your current image as the reference**, like we did in step 1 on this document.

Go to “macro” and choose “run on files in folder.” You should see this window:



Under “execute,” choose “command/macro” and run macro -> choose the macro you saved earlier

Under “folder,” choose the location of the folder that contains your images

In this case, we told our macro to automatically save each picture when complete, so you can choose “Do not save (handled in macro)”

You can also ask Elements to close an image after it’s finished. If you have many images, it is a good idea to close them afterwards, so the program does not crash.

Hit “run,” and Elements will now pull up each image in your folder and perform the actions you instructed, save the resulting image, and then close the document (if desired).

You can add additional things to your macro, such as showing and burning a scale bar, scaling your LUTs, exporting as a TIFF or ND2, etc. although that is outside the scope of this guide. The “Command history” will be a useful tool for creating a macro, as it shows you the corresponding command of every action you perform, and many of these commands (not all) can be directly copied-and-pasted into a macro.