VisiView® Automated Shading and Background Correction

Shading ruins all our microscope images quality. As a consequence the intensity of objects can't be reliably compared across a single or multiple images. Also you won't publish images showing ugly holes in the signal. There are two main causes of shading in microscopy, number one is uneven illumination, number two is dirt.

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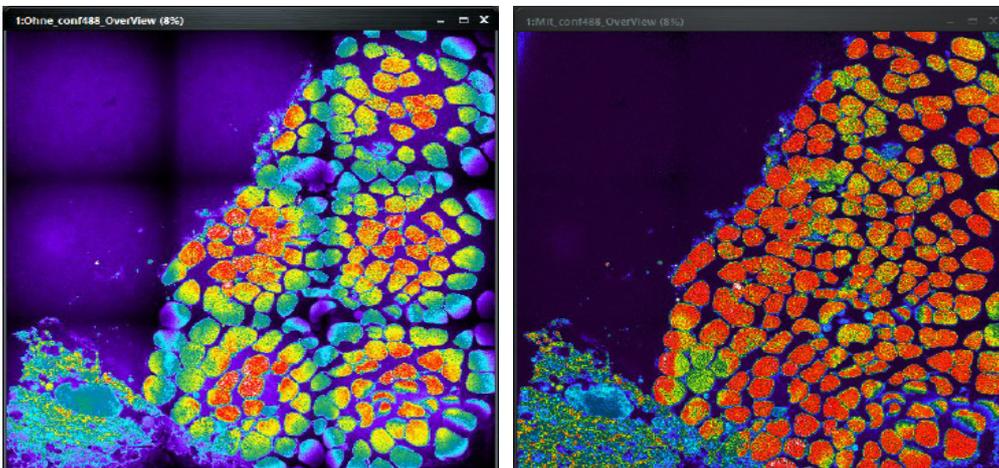
Figure 1: Scan slide acquisition of 3x3 images with 13x13mm field of a sCMOS camera and 63x/1.4 oil objective before shading correction and after

Get the Best Shading Correction

Of course the primary goal is to avoid shading optically. Therefore five hot tips how to avoid or get rid of shading:

1. Protect your microscope from dust (Filter dust out of the air, use dust covers, keep your microscope closed, avoid input of dust from outside)
2. Clean any exposed optical surface of your microscope regularily, especially the objective front lens.
3. Set up your trans or epi-illumination path according to „Köhler". If applicable do: 1.) Focus your sample 2.) close the field stop 3.) center field stop 4.) focus field stop with the condenser 5.) Open the field stop sufficiently 6.) Close the aperture stop as far as required to achieve best signal, resolution, contrast.
4. Visitron offers the VS-Homogenizer which achieve best flat laser illumination in field. Consider purchasing the Visitron VS-Homogenizer for your Spinning Disc Confocal which yields the best flat laser illumination in the field.
5. In TIRF or laser illumination in general laser interference is a strong source of shading. Visitron TIRF systems use spinning lasers to average laser interference.

But even all precautions can't avoid shading at 100 percent. Sometimes shading is intrinsic to the technique we use.

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Then software shading correction is a legal means of improving image data. Since version 5.0.0.8 VisiView offers a simple tool to acquire shading references and to apply them to acquired images automatically. The key to good shading correction and the most challenging part is to acquire proper reference images. The way you generate reference images differs fundamentally between fluorescence and transmitted light images. A good fluorescence reference requires a fully fluorescent slide with homogeneous dye distribution.
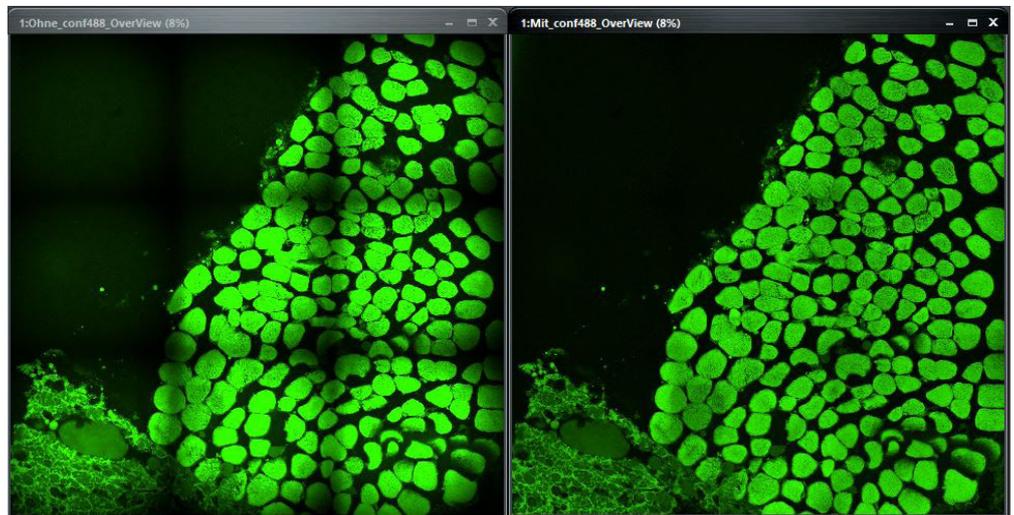
Figure 2: Scan slide acquisition of 3x3 GFP images with 13x13mm field of a sCMOS camera before shading correction and after correction
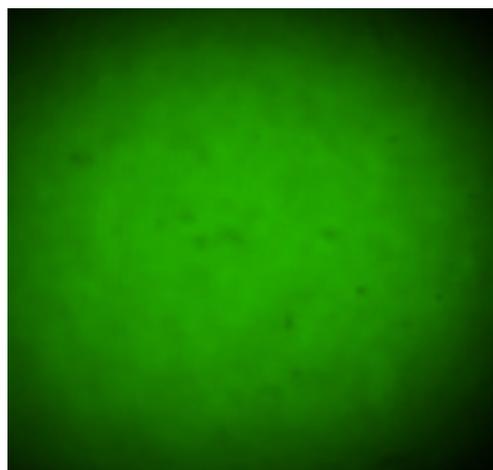

Figure 3: fluorescence shading reference example

Experience shows that highly concentrated dye solutions prove successful. Nevertheless sometimes it is helpful to average multiple suboptimal reference images to create a perfect one. VisiView allows for this by adding reference images straight out of live view.

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The transmitted light reference is in this regard easy to acquire as you just replace the sample slide by a clean and empty slide. The difficulty in acquiring a good transmitted light reference arises from the large degree of freedom in condenser settings and the accessibility of the condenser. Any change in the condenser focus or aperture requires a new reference. The best procedure to acquire transmitted light references is to set first the parameters for the experiment using your sample. In the second step you remove your sample and acquire the shading reference. Finally you start experiment with shading correction activated.

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Figure 4: transmitted light images with 13x13mm field of a sCMOS camera;
image with dust and stain before and after shading correction

Get the Best Reference Image

Of course reference images are specific for a certain light path and for certain camera parameters like the pixel size, binning, rotation, ROIs. In VisiView the light path is specified by illuminations and magnifications. Hence VisiView stores or modifies references for any combination of these settings.

Whether you will acquire pre-processed images you can select in the wavelength tab of the VisiView Acquire window. This decision will be wavelength/illumination specific.

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Optionally the VV Shading correction offers to subtract a background image or to use the feature also solely for background subtraction. For this you just do not define a shading image.
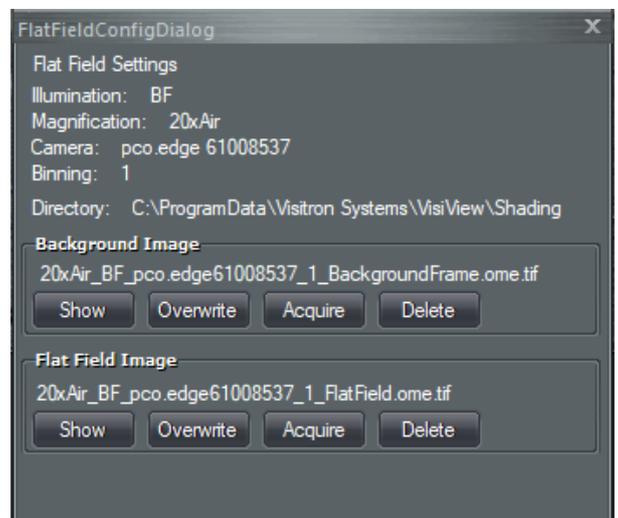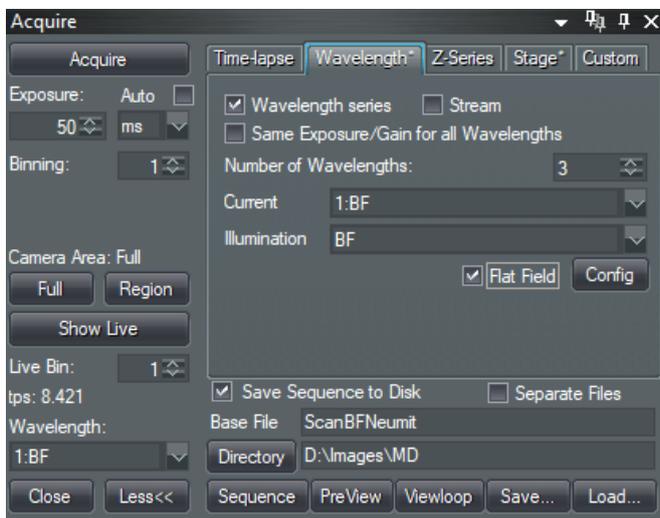
Of course the shading correction became also part of the Visiview Post-Processing and is available as image arithmetic method. Again VV retrieves the appropiate reference automatically.
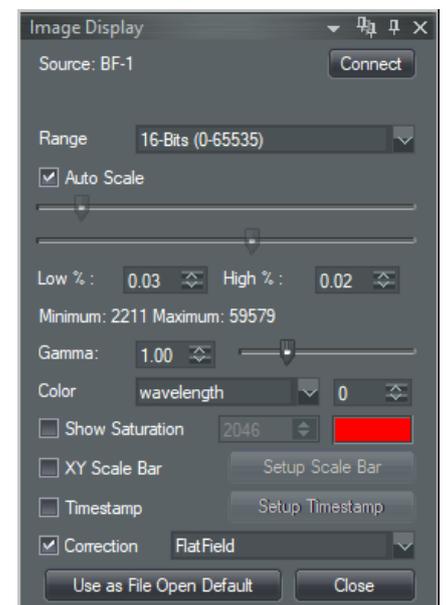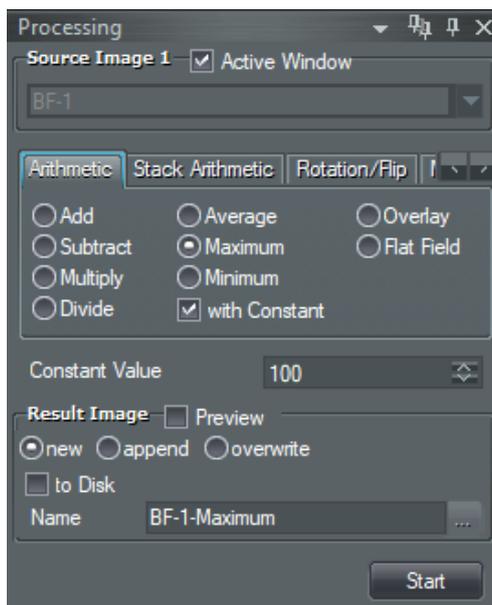
Last but not least the shading correction is also avalaible as realtime processing method which can be activated during acquisition and just affects image display but not the image data.