

## **Process Description:**

"Shade correction" compensates for uneven illumination in the image. The illumination intensity on almost all microscopes higher in the center than near the edges of the movie, often by 15-20% or more. This illumination pattern also varies with wavelength, laser line, etc. and can therefore introduce error into the final ratio images. This illumination heterogeneity is corrected by taking "shade images" - images with the same illumination and acquisition settings, but taken of a blank area of a coverslip which contains no fluorescent objects. These images are then averaged together and filtered, and used to even-out the illumination in the actual fluorescence images by dividing each image by the illumination pattern.

## **Parameter Descriptions:**

### **Input Channels:**

This allows you to select which channels you want to perform shade correction on. This should be applied to all channels that are going to be used for ratioing or bleedthrough correction. Select the channels by clicking on them in the "Available Input Channels" box and then clicking "Select->" to move them to the "Selected Channels" box. You can un-select a channel by clicking the "Delete" button

### **Shade Image Channels:**

This box allows you to specify a directory containing the shade images corresponding to each channel to be corrected. You must specify a directory for each channel to be shade corrected, but the same directory may be specified multiple times (However, this is not recommended!) The directories specified should contain one or more "shade images". It is recommended to take 5 or more, as these will be averaged together to improve the correction. It is also recommended that separate shade-correction images be taken for each channel to be corrected.

### **3x3 Median Filter:**

If this box is checked, a median filter will be applied to the shade images prior to their use as a correction. This is useful because it minimizes the contribution of noise in the shade images, and removes "hot pixels" - pixels which have a much higher-than-normal background value (several hundred counts)

### **Gaussian Filter:**

If checked, the shade images will also be filtered (smoothed) using a gaussian filter whose sigma (in pixels) is specified by the value in the "Sigma" box. Larger sigmas will give smoother images, but too large of a sigma will cause loss of information in the correction images, and introduce artifacts near the image edge. A good starting value is 1. This can be used to further reduce noise in the shade images and is especially important if only 1 or a small number of shade image(s) are taken.

### **Normalize:**

If this box is checked, the shade correction images will be normalized prior to their application. This is

highly recommended. Normalization allows the pattern of illumination in the shade images to be used without the actual absolute intensity values affecting the correction.

Normalize to Mean 1:

If this option is selected, each shade correction image will be normalized so that its mean is equal to 1 prior to use in correction. This means the images will correct only the spatial pattern of illumination, not the overall illumination intensity.

Normalize to combined mean:

If this option is selected, the shade correction images will be normalized so that the combined mean of all shade images across all channels to be corrected is equal to 1 prior to their use in correction. This allows the relative illumination intensities in the different shade images to be taken into account when correcting. This option is only useful in certain circumstances, and is not generally recommended.