



Topic 07 – Background correction and unsharp masking

Background intensity can have different causes like detector offset, scattered light, background light, etc. The background can change over time.

Exercise 7.1 – Subtract mean background value

- a) Open the image *nuclei-rhod.tif*. Apply the *hilo lut*. Use the B&C tool to clearly distinguish a background region. Draw a line selection on the background and create a profile plot using *Analyze>Plot*. Make an area selection in the background and measure the mean intensity. Subtract it using *Process>Math>Subtract*. Create the profile plot again.
- b) Open the image *actine-stack.tif*. Play the movie by pressing on the play-button of the stack. Make a small rectangular selection on the image and plot the z-axis-profile from the *stk*-button. What can you tell about the background? Write a macro that subtracts the mean of a rectangular selection for each slice of the stack. Hints:
 - To iterate over the slices use:

```
for (i=1; i<=nSlices; i++) {  
    ...  
}
```
 - You can set the current slice with `setSlice(i)`.
 - To get the mean value of the current selection you can use the command `getStatistics` (see Image's macro-docu for details).
 - You can use the macro-recorder to find out how to subtract a value from the image.
 - You need to remove the roi before calculating the subtraction, otherwise it will only be applied to the roi. You need to restore the roi before the next iteration.

Exercise 7.2 - Inhomogeneous background

- a) Open the image *root2.tif*. Draw a vertical line on the background again and create the profile plot. As you see this time the background baseline is not constant. The image is darker in the top area and lighter in the bottom area. Measure the mean and subtract it from the image. Create the profile plot again. Is the gradient still there?
- b) One way to get rid of the gradient is to use a pseudo-flatfield correction. Duplicate the image and create a background estimation by applying a gaussian-blur filter to the copy. Choose the radius just big enough so that the signal is not visible in the image any more. Measure the mean on the background image. Use the Image Calculator to divide the input by the background, creating a 32-bit result. Multiply the result with the mean value you measured on the background image. Create a profile plot on a line selection on the background. Is the gradient still there?



- c) Apply the operation *Process>Subtract Background* to the image and create the profile plot. What radius do you have to use to get a good result?

Exercise 7.3 – Unsharp Masking

By subtracting a slightly blurred version of the image we can increase the apparent sharpness.

- a) Open the image *hst2.tif*. Zoom in 2 or 3 times. The image appears somewhat blurred or unsharp. Duplicate the image and apply a *Gaussian blur* with sigma 0.8. Multiply the result with the factor 0.6 and subtract the result from the original image.
- b) Revert the image and try the command *Process>Filters>Unsharp Mask*