Topic 05 – Brightness and Contrast Adjustment

## Exercise 5.1 – Linear display-adjustments

When working with images you will often need to adjust the brightness and contrast of the displayed image in order to be able to see the interesting details. You can use the B&C-Adjustment tool. It allows to fix a minimum and a maximum value. Values below the minimum will be displayed black, values above the maximum with the maximum value possible for the image type. The display-intensity of values between the minimum and maximum are mapped by the line defined by the two points (for example (min, 0) and (max, 255) for 8-bit images).

a) Open the image *bc-adjust.tif* from the folder *05 - brightness and contrast adjustment.* Use the B&C-Adjustment tool (*Image>Adjust>Brightness/Contrast*) to optimise the display of the image.

b) Measure the total intensity (*IntDen*) in the image (make sure *Integrated density* is selected under *Analyse>Set Measurements*). Change the brightness and measure again. Does the measured intensity value change?

c) Measure the total intensity (*IntDen*) in the image. Change the brightness, press the *Apply-button* and measure again. Does the measured intensity value change?

d) On the image *cells.tif*, press the auto-button multiple times. Press the reset button. Make a selection on the background and press the auto button. Make a selection on the foreground and press the auto-button. What do you observe?

e) Can you describe what happens to the line that maps the intensity-values in the image to the displayed intensity values, when you move the *Brightness-slider* and when you move the *Contrast-slider*.
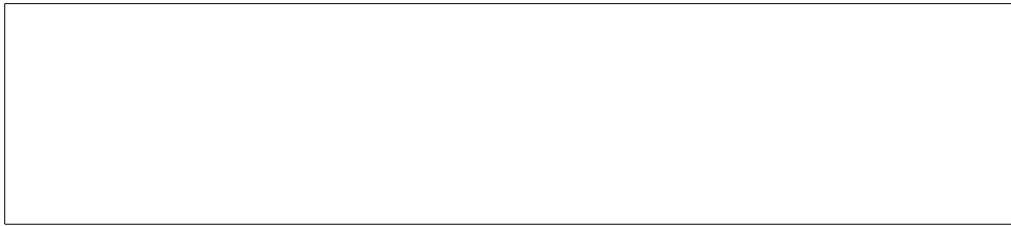
f) Another tool to change the contrast and brightness is the Windows/Level-Adjustment tool. Here you set the middle value and the size of the window around it. If you set the level to 15 and the window to 20, what are the corresponding min. and max. values?

Exercise 5.2 – Non linear display-adjustments

In cases where very high and very low intensities should become visible a non-linear display adjustment is needed, so that small values can become bigger without saturating already big values.

a) Open the image *cells.tif* from the folder *05 - brightness and contrast adjustment.* Try to use the B&C-Adjustment tool to make the intensities in the background visible. What happens to the bright values in the nuclei when you do this? Use *Process>Math>Gamma* to make a non-linear adjustment. Can you make the background-intensities visible, without saturating the bright spots on the nuclei?
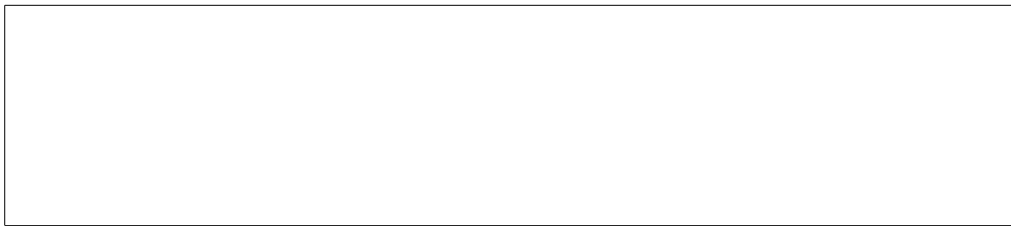
b) The command *Process>Math>Gamma* changes the pixel values in the image. You can use the macro-tool *GammaCorrectionTool* to modify the display using a gamma-function, without modifying the pixel values. Drag the link from the ImageJ macro-tools page onto the Image-launcher window. Press the gamma-button and click into the image. Drag to change the gamma value. The function is displayed on the image. Which gamma value gives a good display? Hint: Double-clicking on the gamma button displays the current gamma value in the option dialog. You can get rid of the function displayed on the image with *ctrl+shift+a*.

c) Add the GammaCorrectionTool-macro to your toolsets. To do this you need to save the file

into the *ImageJ/macros/toolsets* folder. The name of the file will be the name of the toolset in the toolset-menu (on the >> button).

Exercise 5.3 – Enhance Contrast

The enhance contrast tool, that can be found under *Process>Enhance Contrast*, has three different functions. When neither *Normalize* nor *Equalize* is selected, the display is adjusted in a way, that the given number of pixels becomes saturated. In this case the intensity values in the image are not changed. When Normalize is selected, a contrast stretching or normalization is done. The third function is the histogram equalization.

    a) Open the image *cells2.tif* from the folder *05 - brightness and contrast adjustment.* Use the histogram to find out the min. and max. intensities in the image. Set the min. and max. values for the display-adjustment to these values (you can use the set button on the B&C-adjuster). Is the display appropriate?

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    b) Run *Process>Enhance Contrast* and make sure that no option is selected. Enter 0.5% in the *Saturated Pixel*-field and press *ok*. What are the min. and max. values that will be set by the tool?

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    c) Display the histogram of the image. Run *Process>Enhance Contrast* again, but this time select the *normalize* option. Compare the histogram of the resulting image with the histogram of the original image.

    d) Run *Process>Enhance Contrast* again. This time select *Equalize Histogram*. Do it again, but hold down the *alt* key while pressing the *ok* button. Compare the histograms of the original image, the image after histogram-equalization and the image after histogram-equalization with the alt key pressed.

    e) OPTIONAL: Histogram-equalization calculates a normalized, cumulative histogram, i.e. each histogram value is replaced by the sum of the values up to this value and the values are normalized to the available range of greyvalues. Each greyvalue in the image is replaced by its value in the normalized, cumulative histogram

        1. Write a macro that displays the cumulative histogram of the image. Hints:
- Call *Plugins>New>Macro* to create a new macro.
- The histogram h of the image can be calculated using the command
  `getRawStatistics(area, mean, min, max, std, h);`
- You can use a for loop to loop over the histogram-values:
  ```
  for (i=1;i< h.length;i++)  {
  }
  ```

Within the loop you must replace the current value `h[i]` with the sum of `h[i]` and the preceding value.

- To create and show a plot, use the commands `Plot.create` and `Plot.show`. Look for the details on the Macro-Functions page of ImageJ (you can access it from the *Dev*-button on the startup-macros toolset).

2. Modify the macro to create and show the normalized cumulative histogram. The formula for the normalization is:

$$ncdf(g) = round\left(\frac{cdf(g) - cdf_{min}}{(M \cdot N) - cdf_{min}} \cdot 255\right)$$ where cdf is the cumulative histogram, cdf_{min} the minimum value in the cumulative histogram and M and N the width and height of the image.

3. In addition of plotting the normalized cumulative histogram, apply the histogram-equalization to the image by changing the value of each pixel with its value in the ncdf. You can use the macro functions `getWidth()`, `getHeight()`, `getPixel()` and `setPixel()`. You can use a nested loop to loop over all pixels of the image:
```
for (x=0; x<width;x++) {
 for(y=0; y<height; y++) {
 ...
 }
}
```

4. Modify the macro to use the sqrt of the histogram values when calculating the cumulative histogram. You need to adapt the normalization step accordingly.

Exercise 5.4 Lookup-tables

We will now change the display of our image by mapping the intensity values to colors. This is done by the use of lookup tables. A lookup table is a table that maps the 255 intensity values to 255 arbitrary colors. Colors are expressed by the proportion of the three basic colors red, green and blue.

a) Open the *Control Panel* from *Plugins>Utilities>Control Panel* (*ctrl+shift+u*). In the control panel go to *Image>Lookup Tables* and tear this menu off. Close the parent menu. Make sure that no image is opened. Click on the different lookup-tables. An image showing the colors for the 255 intensity values is displayed. Now open the image *cells2*.tif, do a histogram-equalization and apply the lookup-tables to it, by clicking on the lockup-tables again.

b) Open the *Rainbow RGB* lut. Can you tell in which color the intensity value 200 is displayed? What are the rgb-components of this color (use the list button on I*mage>Color>Show Lut*)?

c) The lookup table *HiLo* is useful to adjust the display of our image. 0 will be displayed in blue, 255 in red and values in between will be displayed in gray. Have a look at the HiLo

lookup-table. Use it in combination with the b&c-adjustment tool to optimize the display of the image *cells2.tif*.

d) Select the grey-lookup table. Use the lut-editor from I*mage>Color>Edit>Lut* to create a lookup-table that displays 0 in green, 255 in yellow and the values between 100 and 120 in different shades of red, becoming lighter with higher intensity. Save the lookup-table into the lut-folder, call Help>Refresh Menus and apply it to the image.

Exercise 5.5 Overlay of multiple channels

A common task in fluorescent microscopy is to create a combined image from the different channels. The task consists in creating an overlay of the different channels, in adjusting the display of each channel and eventually in transferring the settings from one overlay to another, to allow a visual comparison. In ImageJ this can be accomplished using so called hyperstacks. Hyperstacks allow to work with multidimensional images. The different dimensions are the x,y and z axis, the time and the channel (representing the color or wavelength).

a) Open the images *dapi 3.tif* and *rhod 3.tif*. Run the *Merge Channels...* command from the menu *Image>Color*. Try the Primary Colors button from the Lookup Tables toolset to change the colors of the two channels. Use the *Channels* tool from Image>Hyperstacks>Channel Tool... to switch channels on and off. Use the B&C-Adjuster to optimize the display of the two channels independently.

b) Save the hyperstack as tif-image. Close it and load it again. Create an rgb-snapshot, using the command *Convert to RGB* from the more-button of the *Channels* tool.

c) Create a second hyperstack from the images *dapi 5* and *rhod 5*. Transfer the display settings from the first hyperstack (dapi and rhod 3) to the second by using the propagate functionality of the set command on the b&c adjuster.

d) Create an overlay from the three images *b2RFP_gemDeltaC2_blue.tif*, *b2RFP_gemDeltaC2_green.tif* and *b2RFP_gemDeltaC2_red.tif* and adjust the display.

e) Create an overlay of the images *dapi 4.tif* and *Rhod 4.tif*. Correct the alignment by using select all (*ctrl+a*), cut (*ctrl+x*) and paste (*ctrl+v*). If the rectangular-selection tool is selected you can now move the pasted channel. After the correction, crop the image to get rid of the empty area.

f) Open the three images *Actine.stk*, *DAPI.stk* and *Gtub.stk*. Concatenate the three stacks with *Image>Stacks>Tools>Concatenate*. Use *Image>Hyperstacks>Stack to Hyperstack...* to create a composite image. Be careful to select the right order of dimensions (xyzct).

g) Make a z-projection of the result of step f. What do you get?