Basic Image Analysis (using ImageJ/FIJI)

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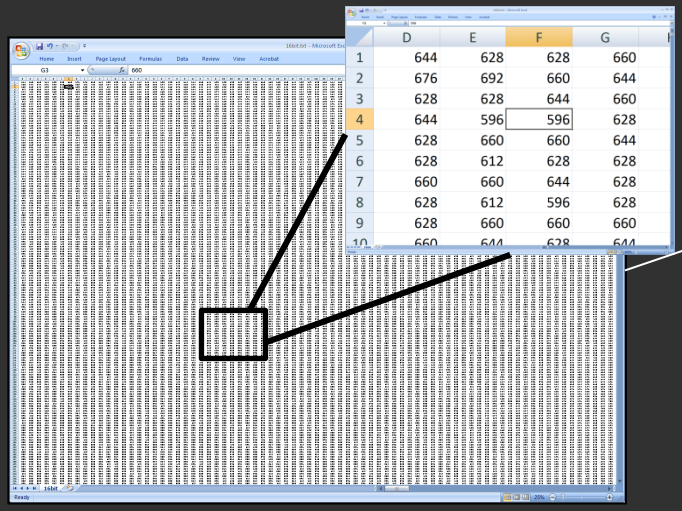
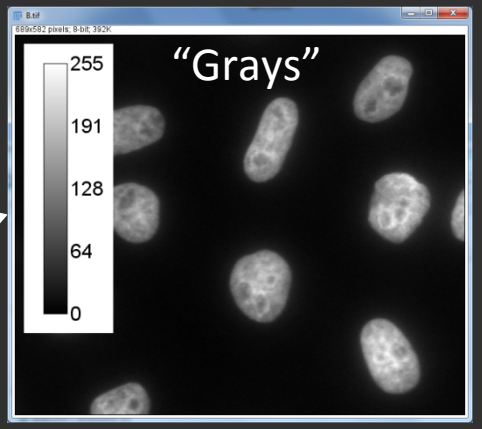
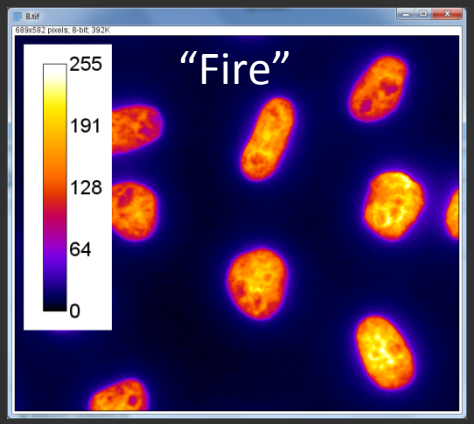
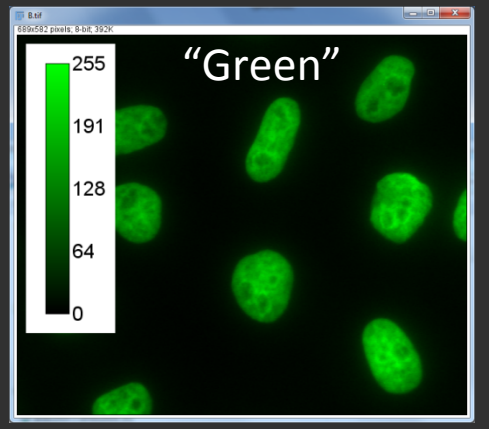
An image is a matrix of numbers



Lookup Table (LUT)







- 8-bit: 0,1,..,255 (2^8 - 1)
- 12-bit: 0,1,..,4095 (2^12 - 1)
- 14-bit: 0,1..,16383 (2^14 - 1)
- 16-bit: 0,1,..,65535 (2^16 - 1)

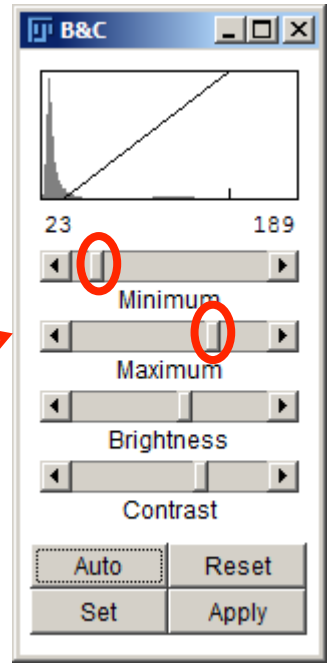
Image data inspection

- Image analysis starts on the microscope! The reason is that you can only later analyze images that have been acquired properly.
- Acquiring images on the microscope you have to make many decisions, such as choosing an exposure time and a detector gain; taking those decisions you need to know how a “good” image should actually look like.
- We will learn now what the criteria for a good image are and also learn about different ways and tools to check for those criteria.
- We will start with a white-board session on
  - Bit depth
  - Saturation
  - Offset
  - Dynamic range
- Also very important in this context is the concept of a signal to noise (S/N) ratio, which we will cover later.

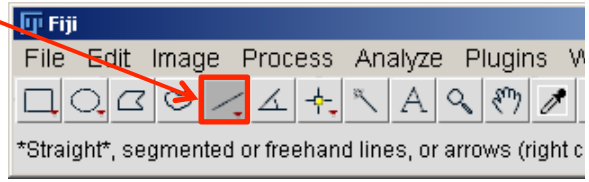
Image data inspection

- Open **"../image-inspection/A.tif"** ***(File..Open)***
- Also open **B.tif, C.tif, D.tif, E.tif**

- Use below methods (M1,M2,M3) to answer **Question 1**
- **M1**: Adjust the display ***(Image..Adjust..Brightness/Contrast)***
- **M2** :Examine gray values in whole image ***(Analyze..Histogram)***
- **M3**: Analyze gray values along a line ***(Analyze..Plot Profile)***



Question 1:
which of the images is best described by:
no_problem, **high_offset**, **low_offset**,
low_dynamic_range , **or saturation ?**



Optional Task 1: find (up to five) different ways of checking whether an image contains **saturated pixels**

Optional Task 2: modify the **good_dynamic_range** image such that it contains the *same values* as the **high_offset** image ***(Process..Math)***

Image data presentation

- The first step in any project involving image analysis is to visually inspect the images to see if they are actually worth analyzing.
- For example, often one wants to see whether a certain treatment caused an effect. For this it is very important to show the treated and non-treated images in the most quantitative and also in exactly the same way.
- The following practical will show you how to generate a good image data presentation.

Image data presentation

Open all files in "../image-presentation" and try to generate below images, using the indicated Fiji commands

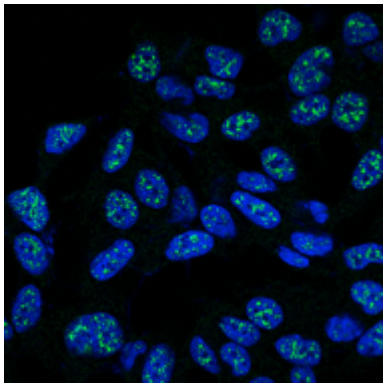
[Image > Color > Merge Channels]

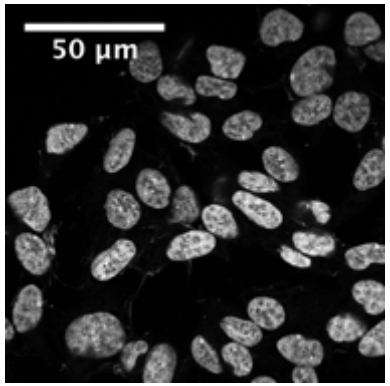
[Analyze > Tools > Scale Bar]

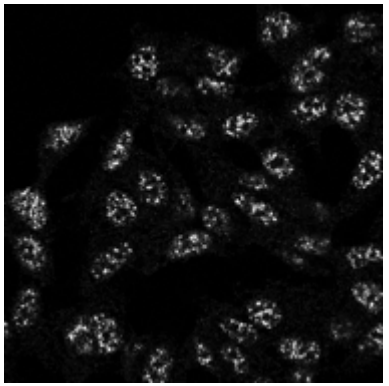
[Image > Adjust > Brightness&Contrast]
- [Set] "Propagate to all other open images"

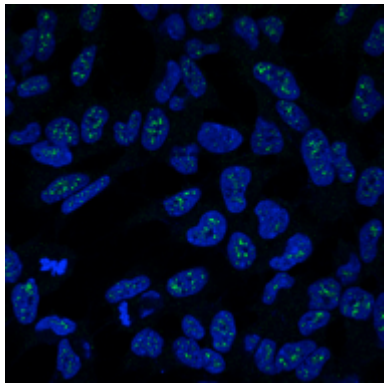
Positive control

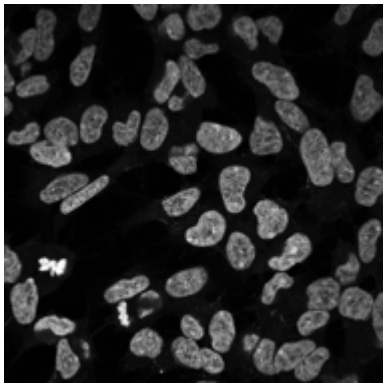
Negative control

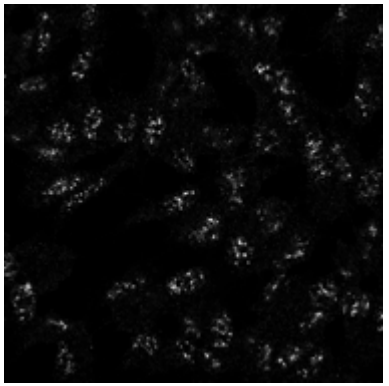


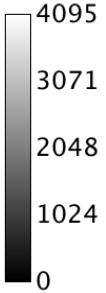












[Analyze > Tools > Calibration Bar]

To put the images into a PowerPoint presentation use:
[Edit > Copy to System] and then simple paste (CTRL+V) them into PowerPoint

Image format conversion

- Unfortunately, quite often, it is necessary to convert an image from one format into another, e.g. because certain applications can only read certain formats
- During such conversions many (bad) things can happen to the quantitative information in your image.
- It is thus of critical importance to always test the image content before and after you saved it in another format.

Image format conversion

- open **"../image-format-conversion/16bit.tif"** ***(File..Open)***

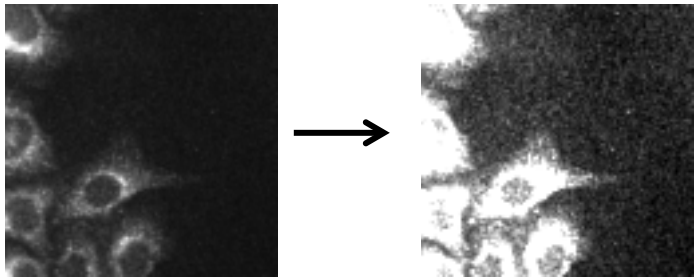
- ***Adjust the display that you actually see something***

- adjust Jpeg quality (0-100) to **10** ***(Edit..Option..Input/Output)***
- save as **Q10.jpg** ***(File..Save As..Jpeg)***
- repeat the last 3 steps for Jpeg qualities **75**, and **100**

→ **Q10.jpg**
Q75.jpg
Q100.jpg

- save as **im.png** ***(File..Save As..PNG)***

→ **im.png**



- adjust the display such that it appears saturated
  (Image..Adjust..Brightness/Contrast)
- save as **Q100_saturated.jpg** ***(File..Save As..Jpeg)***
- save as **im_saturated.png** ***(File..Save As..PNG)***

→ **Q100_saturated.jpg**
im_saturated.png

Task 1: compare the sizes of these files!
Task 2: compare the content of the images!
(you have to re-open the saved images!)

Image bit depth conversion

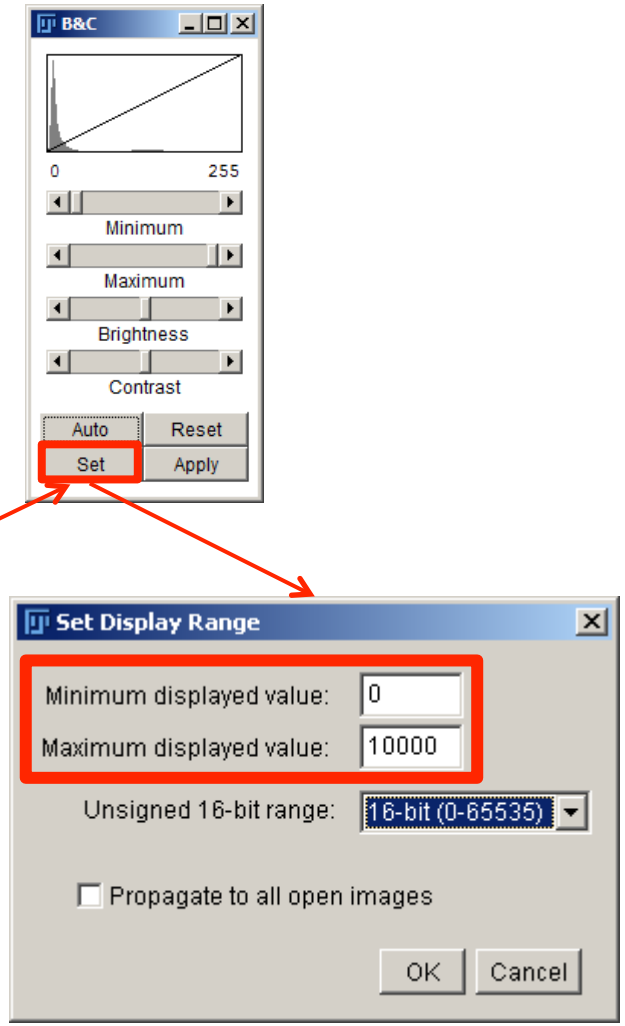
- You should avoid it at all cost, but sometimes to have to convert images from one bit depth to another, e.g.
  - Some old algorithms only work on 8-bit data
  - You need to save hard-disk space
  - You don't have enough RAM to process you images in 16bit
- During such conversions the gray values in your image are likely to be affected and you really have to make sure that you are in control about these changes, ensuring that you do not distort the scientific information in your data.
- In the following we will discuss 16bit to 8bit conversion in general and in particular how ImageJ handles this.

Image bit depth conversion discussion

- What are sensible ideas for converting images from 16 to 8 bit?
  - MinMax Bit-depth mapping
  - MinMax image content mapping
  - User adjusted mapping

Conversion to 8-bit format

1. Open **"../bit_conversion/16bit.tif"**
2. examine the gray values using for instance: ***Analyze..Histogram*** or ***Analyze..Plot Profile***
3. Duplicate the 16bit image ***(Image..Duplicate)*** **perform the next steps on the duplicated image!**
4. change display using*:* ***Image..Adjust..Brightness/Contrast..Set***
5. convert to 8-bit: ***Image..Type..8-bit***
6. Examine gray values of 8-bit converted image, using for instance: ***Analyze..Histogram*** or ***Analyze..Plot Profile***



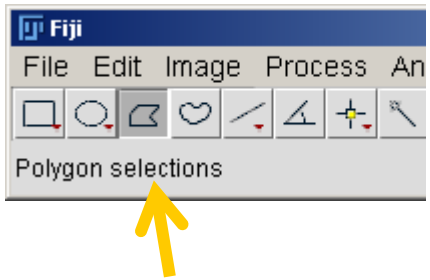
Take home message:
ImageJ uses the Minimum and Maximum display values and, when converting to 8bit, maps those to 0 and 255.

Measurements in images

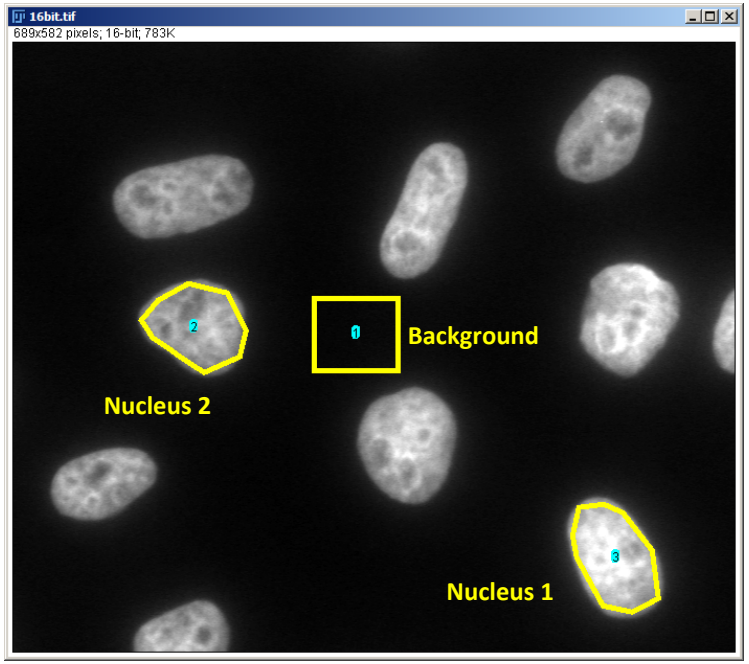
- These days, for scientific publications, it is typically not enough to just show "representative images" but you need to provide measurements and statistics!
- In the following we will practice a simple way to extract meaningful numbers from images.

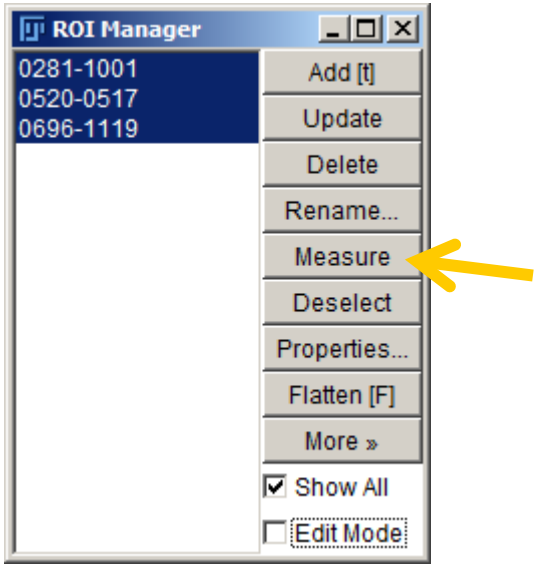
Manual measurements in multiple regions

Question: how much brighter is nucleus 1 than nucleus 2?
Optional Task: measure the area of all nuclei (which one is the largest)?



- open **"../bit-conversion/16bit.tif"**
- draw region for Nucleus 1 as shown below using the ***Polygon Selection***
- save the region ***(Analyze..Tools..ROI Manager..Add)***
- name the region "Nucleus 1" ***(Analyze..Tools..ROI Manager..Rename)***
- **Repeat above steps for Background and Nucleus 2**
- select "Mean Gray Value" measurement ***(Analyze..Set Measurements)***
- Select all regions and measure ***(ROI Manager…Measure)***

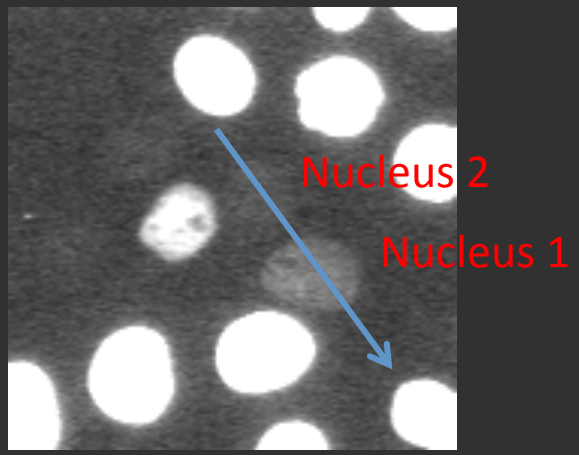


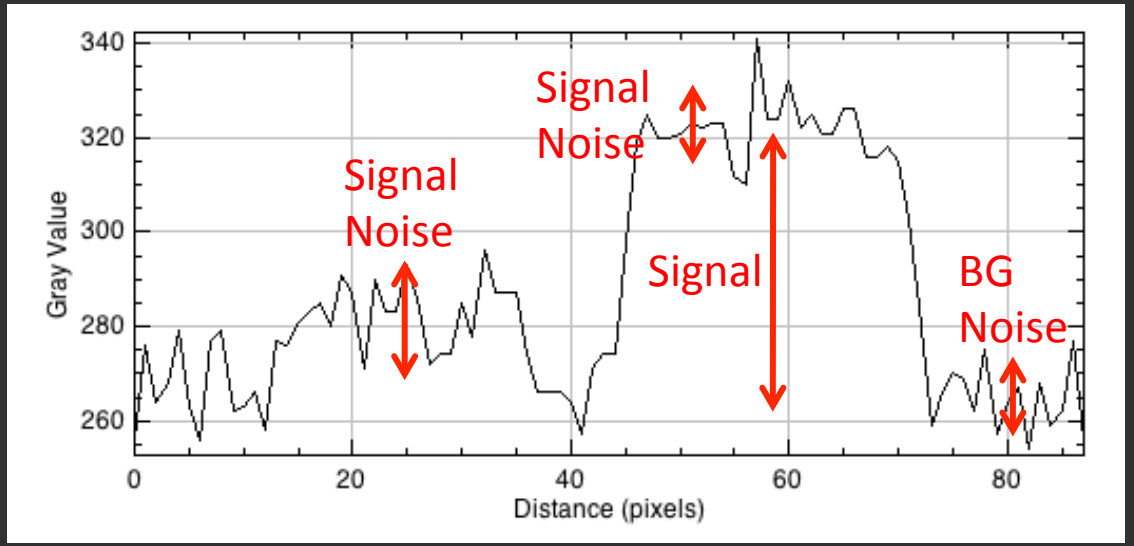


Measuring signal to noise in images

- Sitting on the microscope, you have to decide on many settings, including excitation intensity and exposure time.
- Typically you need to balance photo-toxicity and bleaching with image quality.
- In this context, image quality typically means “signal to noise ratio” (S/N). This is important, because if the S/N is too low, it will be difficult to detect your objects of interest and even more difficult to properly measure their intensity.
- Here, we will learn how to measure S/N in an image.

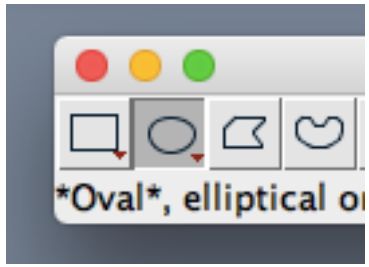
Measuring signal to noise



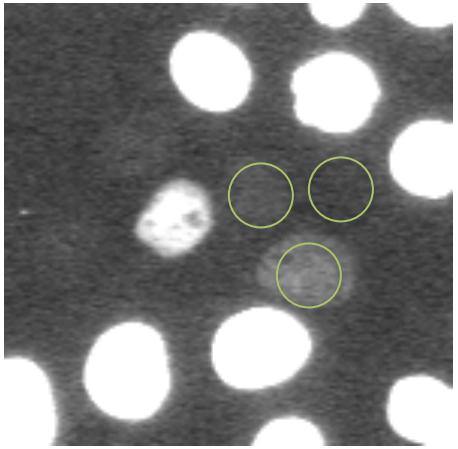


- Signal to Noise = (Mean(Object)- Mean(BG)) / Sqrt(Sdev(Object)^2+Sdev(BG)^2)
- Nucleus 1: 4.7
- Nucleus 2: 2.0

Measure signal to noise



- [File > Open] "../signal-to-noise/h2b-mcherry.tif"
- [Analyze > Set Measurements]
  "Mean gray value", "Standard deviation"
- Draw region as shown using the "Oval Selection"
- Save region [Analyze>Tools>ROI Manager>Add]
- Name region [Analyze>Tools>ROI Manager>Rename]
- Select all regions and measure [ROI Manager>Measure]
- Apply below formula to measure a S/N ratio:
  (Mean(Signal)-Mean(BG)) /
  Sqrt(Sdev(Signal)^2 + Sdev(BG)^2)



Label	Mean	StdDev
h2b-mcherry.tif:nucleus1	282.3675	7.8233
h2b-mcherry.tif:nucleus2	323.5301	13.2718
h2b-mcherry.tif:background	262.1205	8.3214