



- Bit depth
- Multi-channel images
 - RGB & Composite
- Special Images
 - File formats
 - Bio-formats

Hi! And welcome to this new lesson about Greyscale, color and special images. Today we'll see different types of images. And why we need these different types. First, we'll pay attention to the bit depth of Greyscale images. Then we'll introduce and compare to different formats to store and display colored images. Towards the end of this lecture video, we'll discuss images with special formats.

Notes

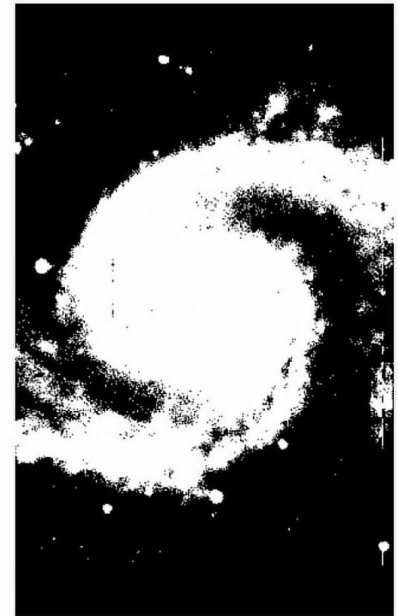
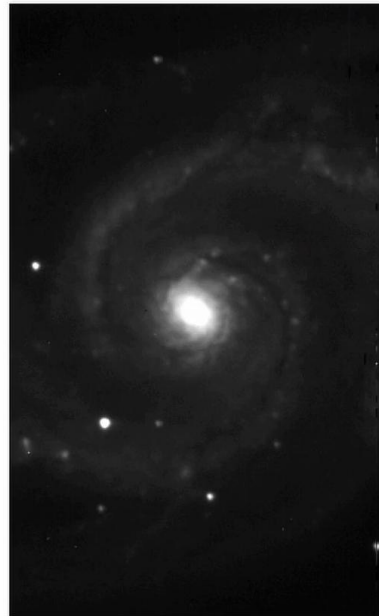
Summary



0m 05s

Grey Scale Images

Bits/Pixel	Range
1	0 or 1
8	0, 1, ...255



Until now we have used 8 bits images. Their pixels have 256 intensity value ranging from 0 to 255. That are typically displayed as monochrome black and white images. A mixture of black and white lead to a palette of grey colors. This is why we are often speaking of Greyscale images. You may have already encountered binary images. They have only 2 intensity values. 0 and 1 for example. Here we have pixels which are either black or white. In ImageJ there are no particular format for binary images. They use 8 bit format only using pixel value 0 or 255. Binary images are useful in order to detect objects. We'll see later in a different video how to do this. And how these objects are used in scientific image analysis.

Notes

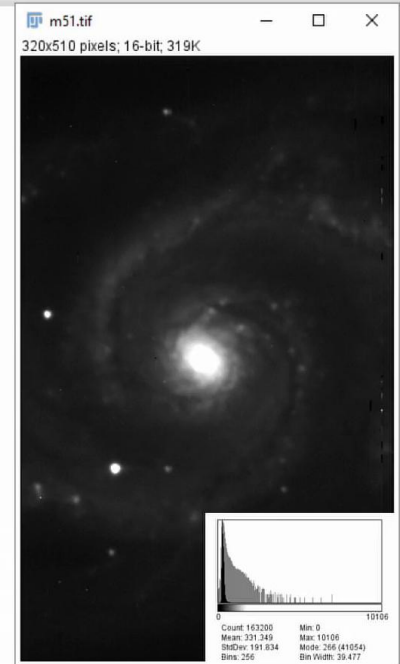
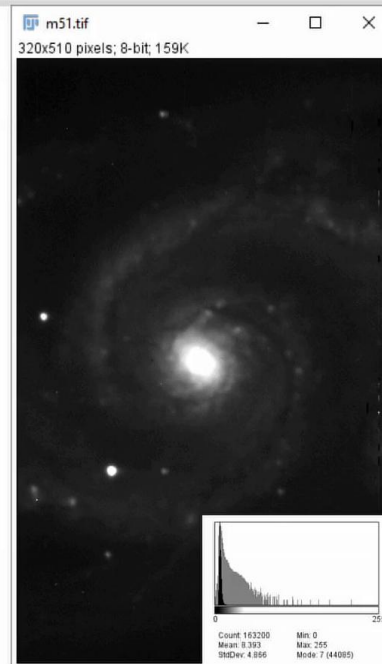
Summary



0m 32s

Grey Scale Images

Bits/Pixel	Range
1	0 or 1
8	0, 1, ...255
12	0, 1, ...4096
14	0, 1, ...16383
16	0, 1, ...65535



Now look at these 2 images. Do you see any difference? What if I told you that one of them is 8 bit, while the other is a 16 bit image? If you look at the histogram of the 2 images, you will see that you have more values available to store information. What does it mean? The bit depth is the number of bins you have at your disposal to store the information. Intensities in this case. With a 8 bit image we have 2 to the power of 8 = 256 values. While with the 16 bits image we have 2 to the power of 16 = 65536.

Notes

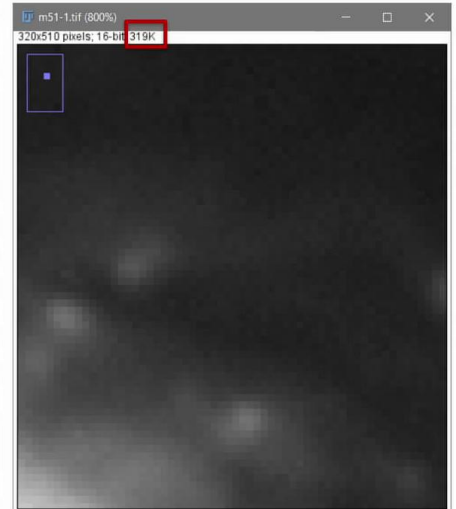
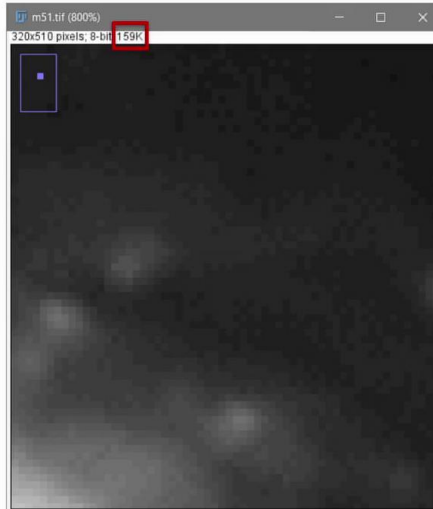
Summary



1m 24s

Grey Scale Images

Bits/Pixel	Range
1	0 or 1
8	0, 1, ...255
12	0, 1, ...4096
14	0, 1, ...16383
16	0, 1, ...65535



Now if we zoom in you may appreciate the difference a bit more. Depending of the settings of the screen. If you compare the pixels in the red square, you may see a bit more variety of grey in the 16 bits image. But if we get more visible, if we draw a line and retrieve the intensity profile along that line, this is what is shown in the boxes below the images. The profile of the 8 bit image has many plateau. While the 16 bit image shows more variability. I guess you realize now that these images are a lot more different than they first looked. Using ImageJ, you don't really need to open the histogram to know the bit depth. Indeed the image window that is written just below the image title followed by the size of the image. By the way, you can also find the file size and realize that it doubles When the bit depth goes from 8 to 16. Even if it is OK for a single image, you can see the implication. When you will require hundreds or thousands of images.

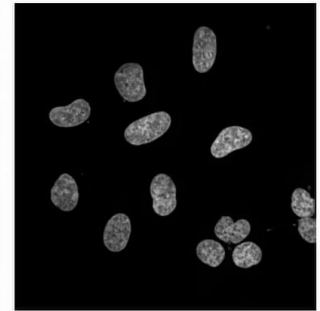
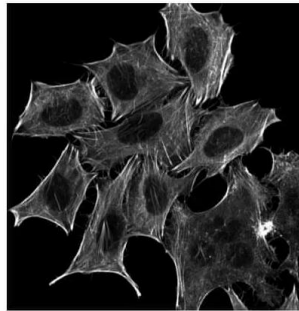
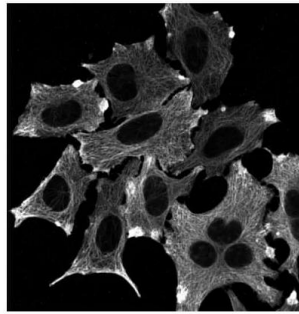
Notes

Summary



2m 14s

From Grey to Color Images



In life science, we usually have to deal with more than 1 staining. Because you are not only interested by the staining, but also interested in the context. The shape of the cell, some other compartments of the cell... In immunochemistry we can stain multiple targets of the same cell with different fluorophores, and image them. In this case we are looking at microtubules, actin and nuclei.

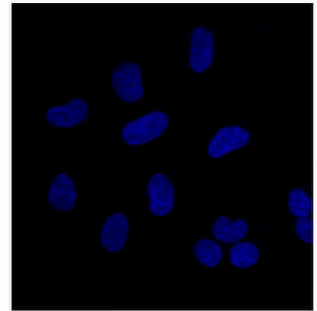
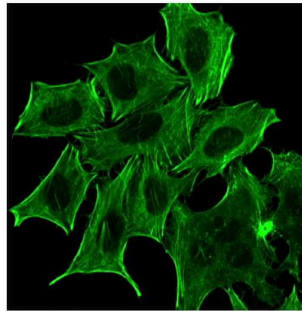
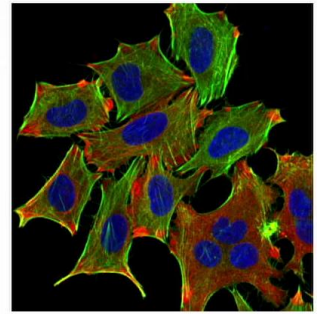
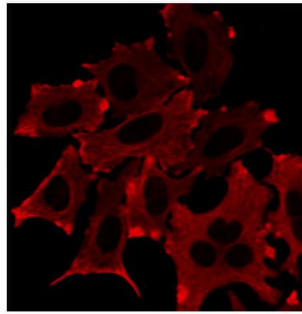
Notes

Summary



3m 26s

From Grey to Color Images



For each individual cell. As you knew previously, we can use different look up tables to get a colored scale instead of a grey. I guess you realize that you see the nucleus in blue a bit less than when they were grey. Right? Finally, we can merge these channels into a single image.

Notes

Summary



3m 53s

RGB VS Composite

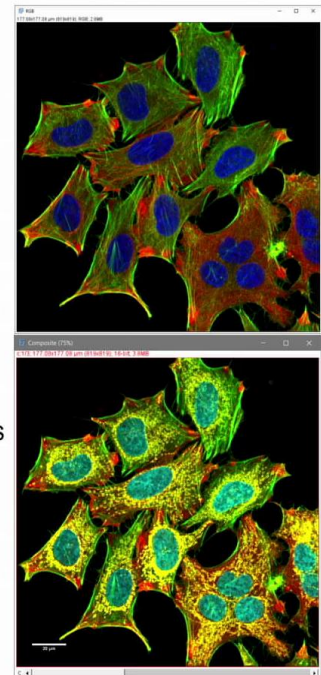


- RGB = 24 bits

- 8 bits Red
- 8 bits Green
- 8 bits Blue

- Composite

- More than 3 channels
- 8, 16, 32 bits



This is what we call an RGB image. With a bit further red channels and also a bit further green and blue channels. By the way, you will see that the window image mentioned is an RGB. Again, right below the title. Another way to display colored images is the composite mode. You can navigate between the different channels with the slider at the bottom of the image. And more importantly, this image can have more than 3 channels. So now we can add an extra one: an image of the mitochondria. It is important to know that if you have more than 3 channels RGB images are insufficient, and you will lose data. Even though they look the same, we have lost the ability to separate the channels. And we'll see that in the exercises. Another feature is that the composite image is no longer limited to 8 bits. The channels can be 8, 16, even 32 bits. Indeed, images can have more than 16 bits. In ImageJ, using 32 bits to each pixel means you can store floating points numbers (while 8 and 16 bits are only integer). You can also use negative numbers.

Notes

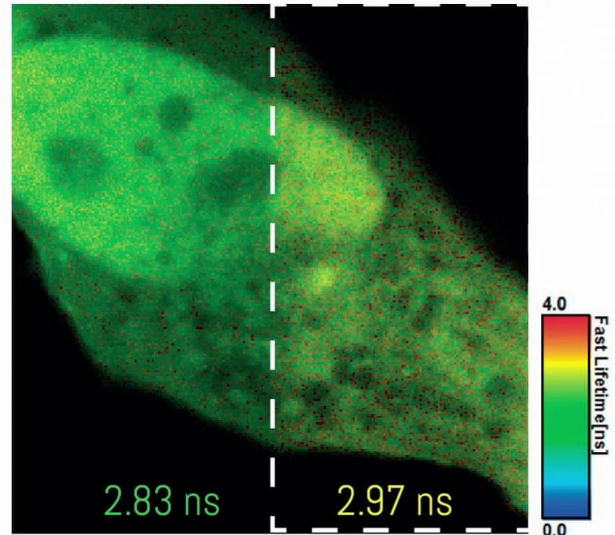
Summary



4m 15s



- 32-bit images
- 48-bit, ...



This will have a particular interest if we need to do some math on the image. Like a division or applying a square root. Or also to store floating point values. Like here for fluorescence lifetime microscopy.

Notes

Summary



5m 32s

File formats

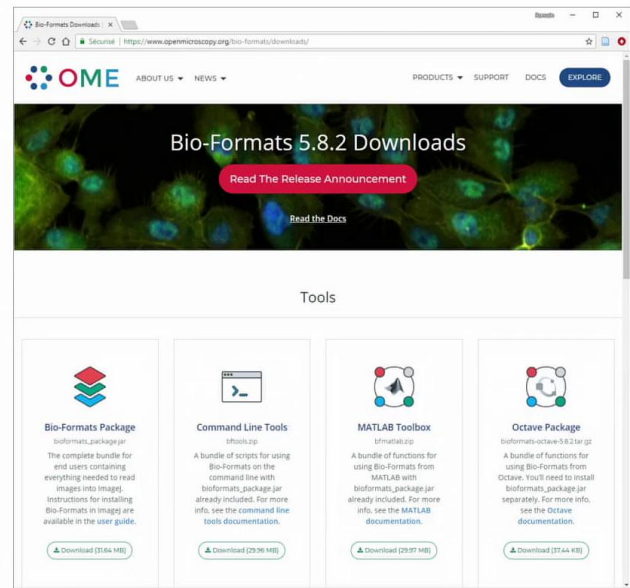


We saw that there are different types of images. Now let's look at how they are stored! Images can come from different hardware and software Built by different companies. As a consequence there are a variety of file formats.

- Notes

Summary





Fortunately, we benefit from the work of OME: The Open Microscopy Environment. Who made a plugin able to read more than 150 different formats.

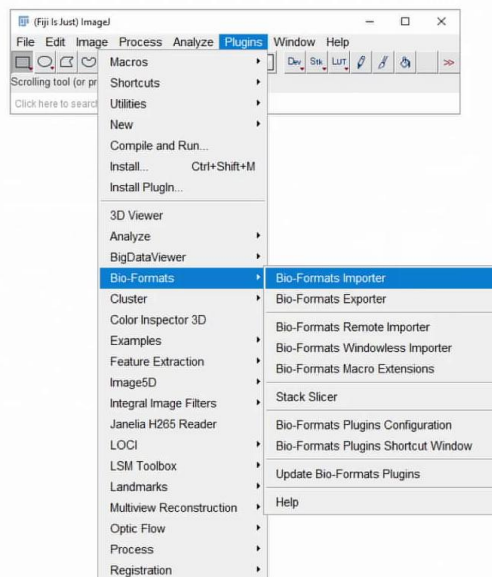
Notes

Summary



6m 02s

Bio-Formats in Fiji



So if you struggle with the opening of a non classical image, meaning when you drag and drop an image on Fiji and it's not opening, then you can simply go to plugin Bio formats: Bio format importer. And a new window will guide you through The opening of your special image.

Notes

Summary



6m 13s

Conclusion



- Bit-depths
 - Get more = Store more
- Distinguish Color Images
 - RGB VS Composite
- Use of Bio-formats

This is the end of the lesson greyscale color and special images. We saw together that you can use different greyscale image. Either 8, 16 or 32 bits. But the more information you get the more data you'll have to store. Just keep this in mind! Then we had a look to the difference between the RGB from the composite images. Finally we saw that many file formats exist. And that Fiji, thanks to bio formats, is capable to open most of them. Thank you! And see you next time!

Notes

Summary



6m 30s