

S1

Hi and welcome to this lesson on Image Display, Histograms and Brightness Contrast Adjustment

S2

In this video we'll be approaching how digital images are displayed on a computer, and discuss ways in which image data can be represented. Finally we will see how the image display can be adjusted for improved visual inspection.

S3

In detail, we will see how digital images, which I will abbreviate as 'images' for simplicity's sake, can be represented. Typical forms are 2D intensity maps and histograms.

At the end of the video, we will go through the most simple way to adjust the display of the image through contrast enhancement and touch on the problem of image saturation.

After the video, you will have a few exercises to familiarize yourself with the ImageJ commands and we will address what qualifies as acceptable contrast enhancement and how to report it.

S4

Now let's refresh how we go from a real object to a digital image.

S5 What we have when looking at our monitor is the image of our object.

S6

To get there, we had to go through an imaging system, and a detection system that rasterizes (that is, discretises) the image.

S7

This gives rise to an array of values at different coordinates, which can be displayed on our screen

S8

This means that if we take a look at the underlying data that represents the image, we should see nothing but numbers.

If we take the Blobs image from ImageJ's File > Open Samples menu, and focus on the upper left corner, we see the image is made up of little squares. Inside each square, or pixel, a number is stored. This is the intensity value.

S9

So really, an image in this case is nothing more than a 2D array of numbers, where each cell is a pixel

S10

So we have a 2D array of pixels and we know how to display it. Now the interesting thing would be to be able to get some statistical information out of it, because images aren't just about show and tell. They contain an enormous amount of information.

A typical approach is to plot the distribution of the pixel intensities, or histogram of the image. This simply consists in counting the number of pixels that have a given intensity values.

S11

This short animation shows you how a histogram is computed. As we move through the image, we count how many pixels have a certain value and plot this value versus count on the right. The counts are accumulated and plotted on the live histogram on the right.

S12

In Fiji, we can use the Analyze > Histogram command to obtain a histogram plot from an open image, like in this example.

The histogram does not provide spatial information on where the pixels are, but just how many pixels of a certain value are on the image.

You can see that you can get a lot of information at a glance from a histogram, such as the mean intensity, standard deviation, count, min and max.

It is interesting to note that if we focus on the first bin of the histogram, we seem to have a lot of pixels that are exactly 0. While not unusual in itself, it is odd that the distribution is not continuously growing from zero. There seems to be a gap in the histogram.

This is because this image presents an artifact in the bottom right corner, where there is a strip of pure black, which is most likely not part of the image, but rather linked to a problem during the digitization of the image.

S13

Which leads us to an interesting question. What kind of artifacts can we discover with a Histogram or a visual inspection of an image?

Here you see three images of a western blot gel. Looking at them, intuitively you might say that the left one is under exposed, the right one looks 'off' or manipulated and the center one 'appropriate'. But can we get infer that information from the histogram in a more quantitative way?

Well it turns out we can. Note how in the leftmost image most of the pixels are in the 0 bin of the histogram. Similarly for the one on the right, most of the pixels are in the last bin of the histogram.

You can get this information if you read the 'Mode' of the histogram. That is, the value bin that has the most counts. Histograms where the mode is exactly 0 or 255 (in the case of 8-bit images) tend to raise a red flag. Why are most of the pixels pure black or white?

Usually this means we have saturation. That usually means something went wrong somewhere. Either the chemical revelation process was too long, the detector (a camera in this case) was not able to capture the intensities adequately or the image was manipulated somehow. You should see saturation as an irreversible loss of information which cannot be fixed after the acquisition. The only solution is to rerun the experiment, or reacquire the data. But if you look at these three images, you notice how each one enhances a different aspect of the data. The darkest image shows weak bands rather well, whereas the brightest image helps our eyes exclude unwanted background and focus on the strongest bands.

S14

If I show you the same 3 images again and tell you that all three are exactly the same, I believe I would have some sceptics among you. Didn't I just say that we should be weary of artifacts? Let's look at the histograms. All three images have the same underlying histogram! What has changed is the way the pixel intensities are mapped to the screen. This famous function we've been talking about. The left image considers everything below 100 values as black, the middle one uses the default mapping of 0 is black and 255 is white, and finally the right one considers everything above 150 as white. We've adjusted the brightness and contrast.

S15

In Fiji the brightness and contrast tool looks like this.

On the top you have a histogram as well as a line that shows how the pixel values are mapped to intensities on the screen.

The min slider defines which values will be black

The max slider defines which values will be white

The last two sliders modify what we call Brightness and contrast, but they are less easy to manipulate accurately for scientific data viewing.

S16

If I show you what that looks like in the software, you can see what happens when I move the min slider, the max slider, the brightness. And finally the contrast slider. Again, notice how the histogram does not change. Only the curve changes.

S17 (wrong bullet)

In this video we touched base with how images are displayed based on their pixel intensities, and on how we can already obtain relevant statistical data using histograms.

We've also seen how to use Fiji to adjust an image's brightness and contrast for visual inspection.

Finally I hope that you understand the problem with saturated images, which is an irreversible loss of information on your image, making quantification impossible.

We will follow up this video with a few exercises to familiarize yourself with the Fiji commands so that you can experiment for yourself.

Thank you for the attention