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You definitely have already been exposed to digital images. Nowadays you will have encountered them in your every-day life as well throughout your scientific career.

Nevertheless, be aware that it has not been always like this. Twenty years ago, almost exclusively analog devices were used to acquire images. Today you will have a hard time to come across such an analog device.

Due to the wide-spread use of digital images image analysis is becoming more and more important especially in science as processing of digital images is much easier than processing of analog ones.

Today we will cover the basics of digital images processing. We will discuss Visual perception, the properties of digital images, how digital images are acquired and file formats to store the images. Moreover I will outline what do we gain by digital image analysis.

Before answering this question, it is worth to dwell on a related one: Can we trust the human eye in general.

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The human eye and the underlying visual perception has been optimized throughout evolution. Moreover, even today it is one of the most powerful and versatile combination of a detection device and analysis unit attached to it.

Nevertheless, how accurate is it if it really comes down to quantitative image analysis. Are there even examples where we have to be careful how we perceive images?

Just have a look at the set of images on the left. If I ask you to comment on the intensity of the little square inside the big one. What would you answer?

Is there a difference in intensity in the lower row or rather in the upper one. I'm pretty sure that the one or the other has already experienced a similar or even the same optical illusion.

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In fact if we analysis the pixel intensity quantitatively it turns out that the intensity is the same in all of the six square. However, we tend to “see” a decrease of intensity in the upper row.

Obviously, the interpretation of the human eye is sensible to the context. As the surrounding square is getting less grey, we are having the same impression for the inner one. The reason is that the visual perception is optimized for seeing difference in a scene. This had been surely an advantage in former times in order to see enemies or identifying a trait. But nowadays in particular in science it is a dangerous feature.

Therefore, if you want to make a statement on the intensity within an image be aware of optical illusions. It is always recommended to measure intensities in an unbiased way and verify or falsify an assumption based on visual perception.

Throughout the lecture series, we will introduce the tools and concepts how to perform such an unbiased analysis.

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You might ask: why are these optical illusions relevant for image analysis in life sciences. You already might know the answer. The nuclei in A and B are equally bright. And there is also no size difference between A and B in the lower row.

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So, if it comes down to quantitative analysis, we cannot fully trust our eye. Therefore it is always better to use unbiased image processing and analysis tools.

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So let's briefly think about the benefits of quantitative scientific image analysis. The most important feature is its reproducibility. If properly documented an image analysis based on digital image processing can be repeated everywhere at any time.

And it can be scaled so that large amount of data can be treated the exact same way. And any bias, e.g. coming from visual perception can be avoided. Therefore digital image analysis is a very powerful tool and provides reliable data which in most of the cases cannot be retrieved from just looking at the images.

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After that brief introduction of visual perception, how biased it can be, as well as the benefits of quantitative image analysis we will dig a bit more into the properties of digital images.

What is an digital image and what is the main difference of an analog and a digital one?

Digital images are composed of small addressable units called pixels. Typically these units are assembled in a rectangular manner. Every pixel is defined by its coordinates and a value which is called the pixel value or pixel intensity. This value is discrete and you will later learn that the maximal reachable value scales with the so called bit depth. For now it is important to remember that typically only whole numbers are allowed. For the display of images we need to assign a color to an intensity value. For monochrome black and white images it is common to display the lowest value -typically zero- black and the brightest value white. But please keep in mind that this is just a convention.

It is very tempting to think of pixels as little squares but that is not entirely correct. It is more appropriate to see them as infinitesimal small sampling units without a shape or size.

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On the left hand side you can see the transition of an analog images to an digital one and how this image is store. An analog images is consisting of continuous values which need to transferred into values with concrete steps in the case of a digital images. This is the case for the intensity but it also holds true for the distance and the size of the pixel. We will dwell a bit more in detail on that topic when talking about the sampling frequency. For now it is important to memorized that digital images are raster images being nothing else than a matrix of integers.

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We have now formally defined a digital images. Now we will discuss how images are formed and recorded.

At foremost, It is important to keep in mind, that images and objects are distinct from each other. The aim of image analysis is to first analyze the image and use the information gathered to come to conclusions about the object itself. But we need to consider that the image of the object is only a representation of the object. It's appearance and properties are highly depending on the imaging system.

If you look at a black and white image of a colorful landscape you will have a hard time mapping the grey values in the image with its original color in the scene. This trivial example illustrates that it is extremely important to know the properties, or if we formulate it a bit more scientifically, the transfer function of the imaging system.

In every lens based system – and we will exclusively use images from such systems within this lecture- the image of an object is the convolution of the light emitted of the object and its surroundings with the response function of the imaging system.

How important the transfer function is, is illustrated by the two images shown here. The same object – a so called diatom- was imaged with the same microscope two times by varying the aperture stop of the microscope. The result: two completely different images of

the same object. This example serves to illustrate two facts: an image is always only one out of many representation of the object and it is important to know the imaging system as well as its settings.

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Let's have a closer look at the transfer or response function of the imaging system.

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Mathematically the image of the object is obtained by convoluting the object with the response function of the imaging system. In light microscopy the response function is often called the point spread function. The response or point spread function is responsible for the resolution power of the imaging system.

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This is shown in the two emulated images. 2D objects were convoluted with two point spread functions differing in size. The degree of "blurriness" is higher in the upper image. It is impossible to distinguish individual objects in that image. In the lower image some discs are clearly visible others are not. But even here you can see that the sharp edges of the objects are blurred in the image. The size of the point spread function is ruled by the law of diffraction. This is why microscopy is often called "diffraction limited". The knowledge of the response function is necessary in order to interpret the obtained images correctly and meaningful. In the ideal case it can be retrieved from the metadata of the image. You will encounter the importance of metadata several times throughout this lecture. It means in essence that relevant information of the acquisition is stored along with the image.

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Coming back to the diatom images: Closing the aperture stop is decreasing the resolution of the imaging system. This is the reason why the upper image looks much more blurred than the lower one.

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The point spread function is directly linked to the resolution. This term is important in order to understand the sampling frequency of images later on. So let us briefly touch it but without going into the details. It is illustrated in the following slide. Two diffraction limited spots are displayed and its distance is decreased from left to right in the upper as well as in the lower row. One may debate when the two points are not distinguishable but it is obvious that it is impossible to identify two objects in the lower row. Here the resolution of the system is not sufficient. Please keep in mind that the size of the spot is dictated by the law of diffraction and that the minimal distance when two spots are still visible will decrease when the spot is getting bigger. A bigger spot size or less resolution is expected when we decrease the NA of the imaging system or use longer wavelength. This is one of the reasons why it is important to be aware of these parameters when doing image analysis and store them ideally along with the image.

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Digital sampling is equally important as resolution. What does that mean exactly?

Remember what I said about pixels. They are not squares but rather infinitesimal small sampling points. How important the distances of these points is, can be seen in the four images displayed here.

It is the emulated image, which served to explain resolution. Now we have added one layer of complexity and changed the sampling frequency aka pixel size. Not too surprisingly we

are losing resolution is we are increasing the pixel size. In particular the image in the right corner is illustrating the finding.

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So what we can see is not only influenced by the resolution of the imaging system but also by the sampling frequency of the detection device. And obviously there is a good match between both. There is almost no difference between the images in the upper row. However, the left image is 256 times bigger than the one on the right.

The optimal match between resolution and sampling frequency is defined by the Shannon-Nyquist sampling theorem. Without going into the details: just remember that the smallest resolvable structure within an image shall be sampled at least twice.

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We have now discussed the individual steps of the image acquisition workflow. So let's assemble it. An object is imaged. The produced image is detected. This results in a rastered or digital representation of the image of the object. The response of the imaging system as well as the parameters creating the raster. The functions involved to display the image are also extremely important. However, they will be covered in an additional video.

Each step can be considered as a mathematical operation. In order to interpret the resulting image correctly one needs to know the operations as good as possible. This is why it is so important to know the properties of the imaging system, including its detection device and ideally store it as Metadata with the image.

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I know that we have covered quite a bit of theory and you might be already pretty tired.

Therefore I want to finish this introductory lecture with a rather practical aspect. At some

point we will have to save the data which we acquired digitally. And this poses the question which format shall we use. What are the advantages and pitfalls of the different formats?

The most common format “jpeg” is extremely versatile for non-scientific images. An extremely clever compression algorithm is used there. Information which is not visible to the human eye is discarded. This reduces the data size tremendously. However the data is lost. Therefore it is called a lossy compression. You can surely imagine that such data loss is unfortunate for scientific data processing. Therefore using this format is not recommended for original data. However for presentations and reports a conversion can be useful as the compression is not visible for the human eye.

The most widespread file format for images in science is tiff which stands for “tagged Image File format”. It uses – if at all- a lossless compression method, so the original data can be always restored. It also allows the link tag to the images. These tags are typically used to encode the metadata. How important the metadata is has been discussed in the lecture before. The data format gif and bmp are also saving the original data. But they are not very wide-spreadly used in image processing.

For large data sets the format HDF5 is becoming a real alternative.

The most important take home message for you is to remember that the file-format needs to be chosen carefully.

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So let's summarize what we have heard so far and which are the underlying basics for scientific images processing and analysis.

Digital images are rastered images with discrete intensity values. In order to resolve fine details or display a high dynamic range the intensity values need to become larger making also the image files size larger. All images carry the response of the acquisition system.

Knowledge of that response is key in order to interpret the images correctly. Ideally the

characteristics of the imaging system are stored with the images. The tiff file format allows to store the so called meta data with the images therefore it is widely used in scientific image processing and analysis.

As the human eye is biased quantification can be misleading. Digital images analysis on the contrary is reproducible, can be easily shared with others and can also be used for the analysis of large data sets.

I hope to see you next time again. Bye bye and take care.