ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Digital Images and Visual Perception

Image Processing & Analysis for Life Scientists

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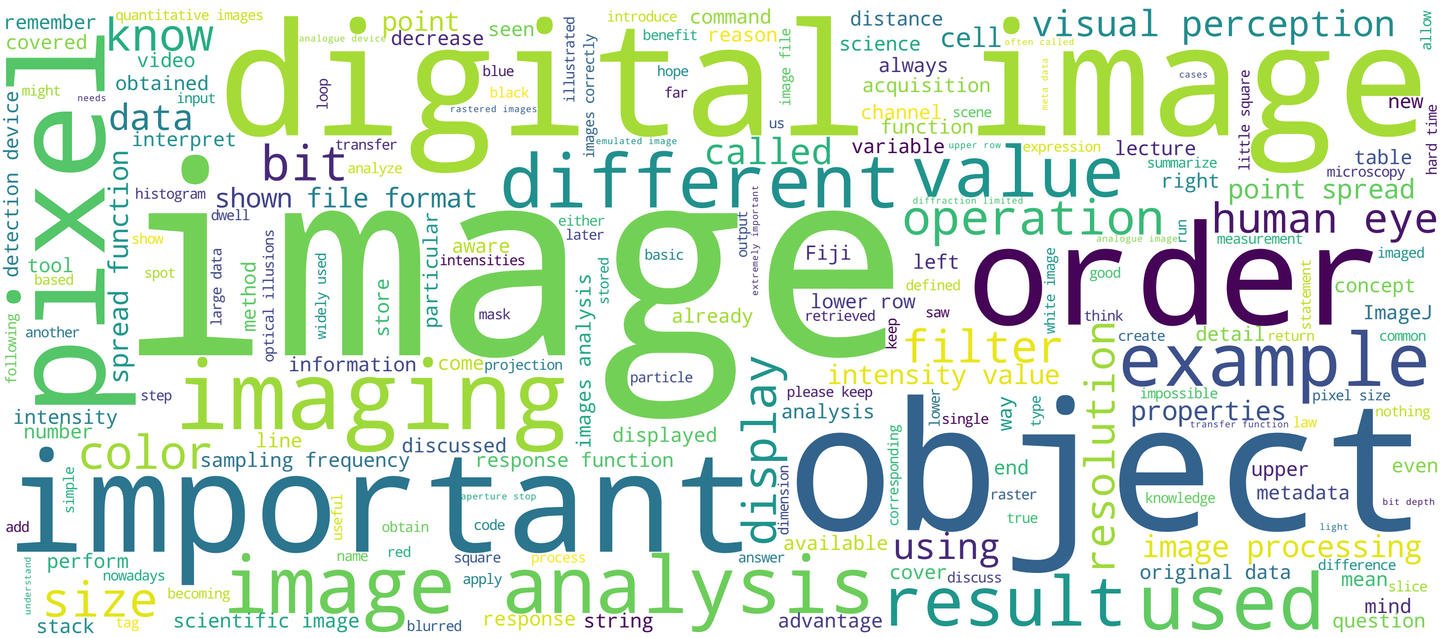

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Outlook

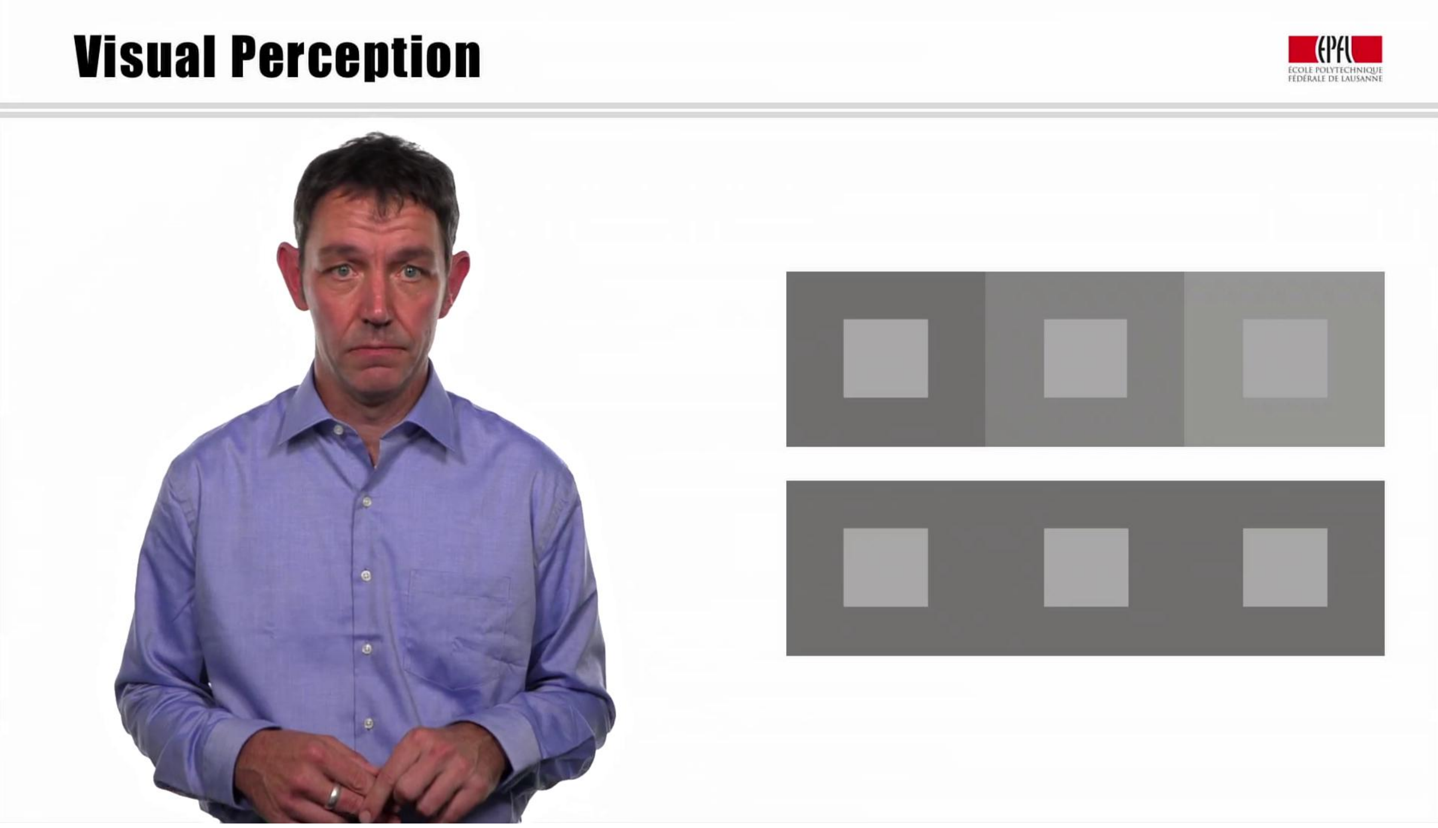
- Visual Perception
- Digital Images
- Resolution/Sampling Frequency
- Acquisition Workflow
- File Formats

You definitely have already been exposed to digital images. Nowadays, you will often encounter them in your everyday life, as as well through your scientific career. Nevertheless, be aware that it is has not always been like this. 20 years ago, almost exclusively analogue devices were used to acquire images. Today we'll have a hard time to come across such an analogue device. Due to the wide spread use of digital images, Image Analysis is becoming more and more important. Especially in sciences, as processing digital images is much easier than processing analogue ones. Today we'll cover the basics of digital image processing. We'll discuss visual perception. The properties of digital images. How digital images are acquired. And file formats to store these images.

Notes

Summary

0m 06s

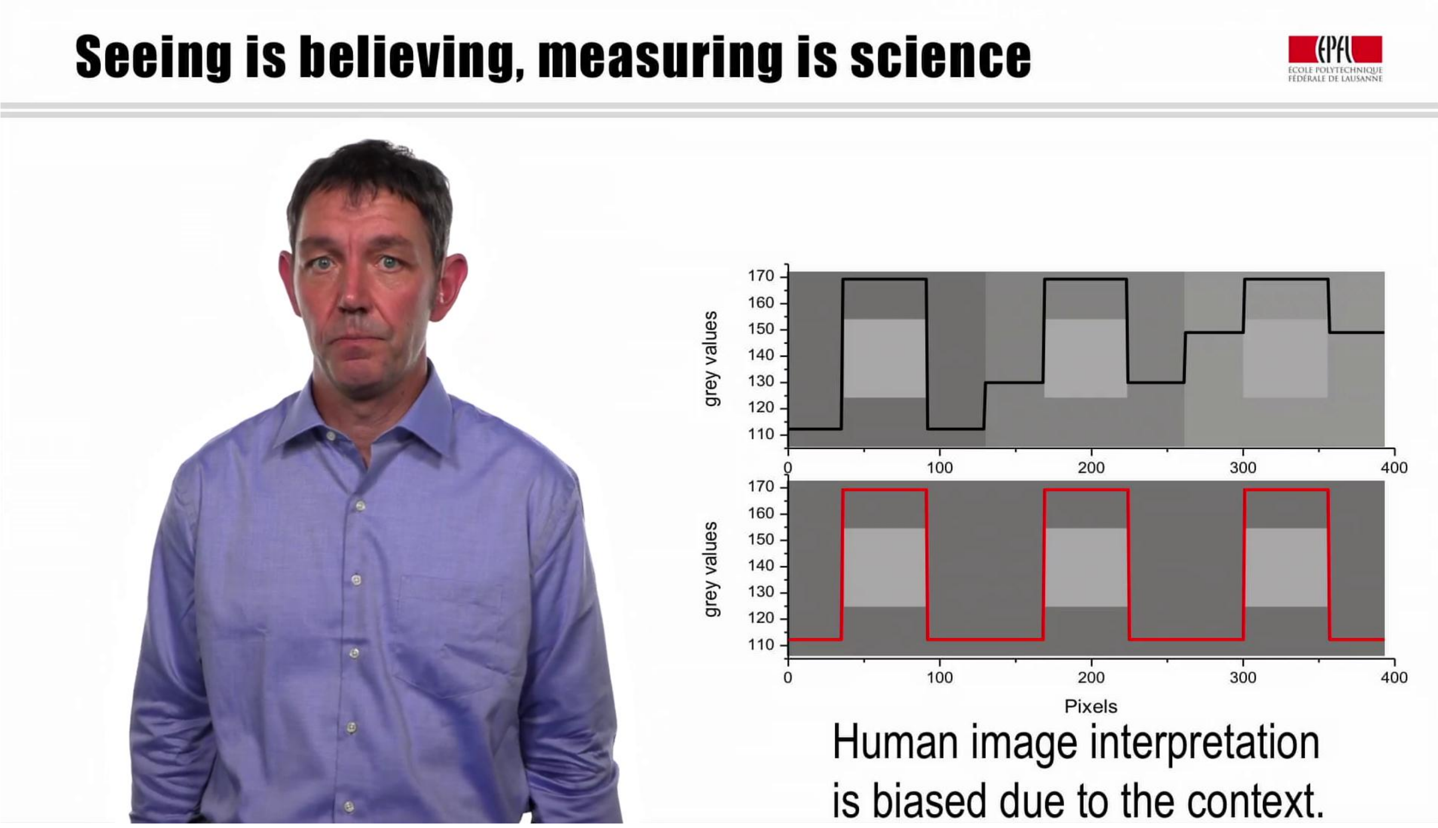


Moreover, I will outline what we do gain by digital image analysis. Before answering these questions, it is worth to dwell on a related one: can we trust the human eye in general? The human eye and the underlying visual perception has been optimized throughout evolution. Moreover, it is one the most powerful and versatile combination of detection device and analysis units attached to it. However, how really accurate is it, if it really comes down to quantitative images analysis? Are there any examples where we have to be careful how we perceive images? Just have a look of 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 of intensity in the lower row or either in the upper one. I'm pretty sure that some have already experienced similar or even the same optical illusions.

Notes

Summary

0m 58s

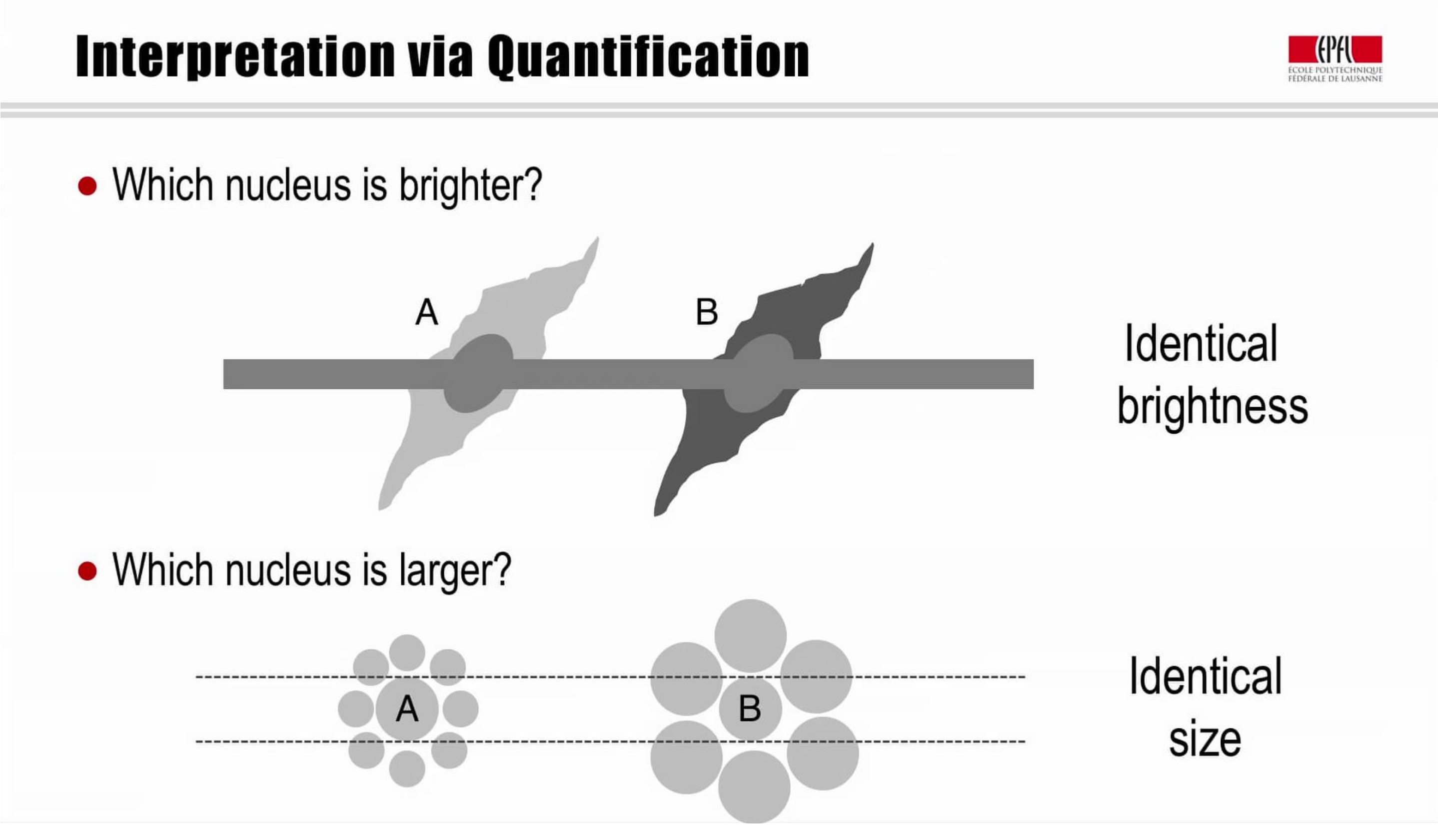


In fact, if we analyze the pixels intensity quantitatively, it turns that the intensity is the same in all of the 6 squares. However, we tend to see a decrease of intensity in the upper row. Obviously, the interpretation of the human eye is sensible to the context. As the surrounding square is getting less grey, we're having the same impression for the other one. The reason is that visual perception is optimized when seeing differences in the scene. This has been surely an advantage in former times in order to see enemies or identify a trait. But nowadays, in particular in science, it's a dangerous feature.

Notes

Summary

2m 01s



Therefore, if you want to make a statement on an 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 this lecture series, we will introduce the tools and concepts how to perform such an unbiased analysis. So you might ask: why are these optical illusions relevant for image analysis in life sciences? Look at these images here! What would you say if I asked you to compare the intensities again ? You already might know the answer. The nuclei in A and B are equally bright. And there are also no size difference between A and B in the lower row. So if it comes down to quantitative images analysis, we cannot fully trust our eye. Therefore, it is always better to use unbiased image processing routines in analysis tools.

Notes

Summary

2m 45s

Image Quantification

- Reproducible
- High Throughput
- Portable
- Unbiased

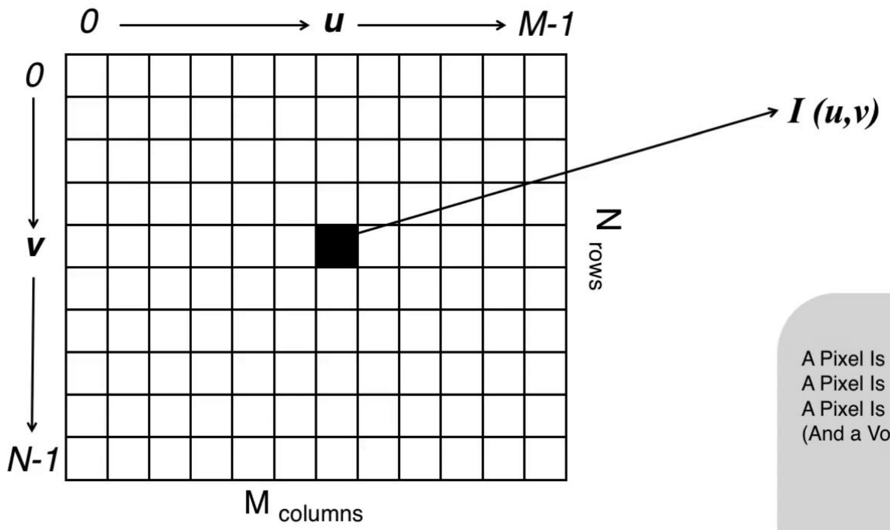
So let's briefly think about the benefits of quantitative scientific image analysis. The most important feature is the reproducibility. If properly documented, an image analysis based on digital image processing can be repeated everywhere at anytime. It can be scaled so that large amount of data can be treated the exact same way. And any bias coming for example from visual perception can be avoided. Therefore, digital images analysis is a very powerful tool, and provides reliable data which, in most of the cases, cannot be retrieved from just looking at the image.

Notes

Summary

3m 51s

Definition Digital Image



$I\ (u, v) \in \mathbb{P}$
$and\ \ u, v\ \in \mathbb{N}$

A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* A Little Cube)

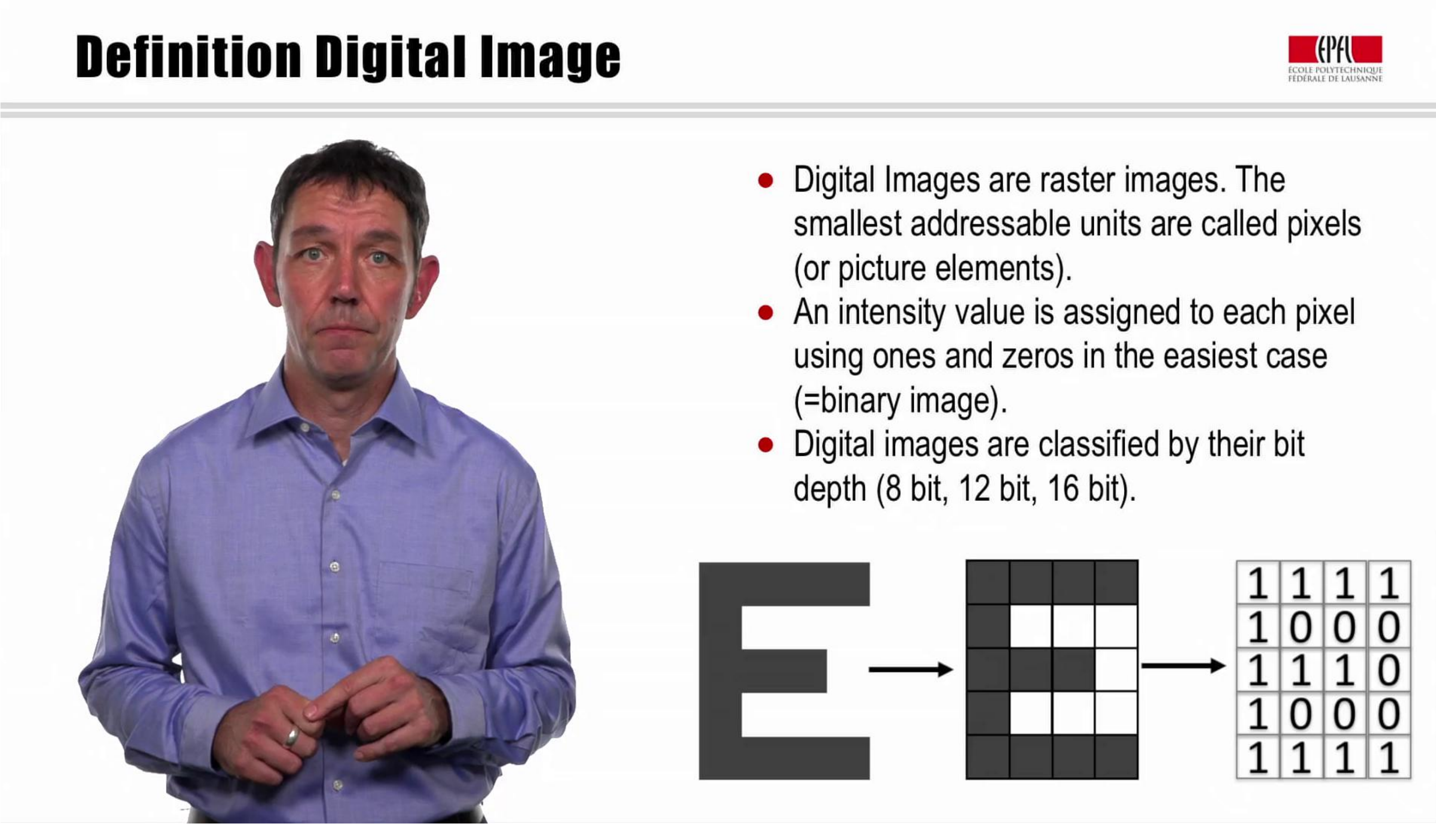
Technical Memo 6
~ Alvy Ray Smith
July 17, 1995

After that brief introduction of visual perception, how biased it can be, as well as the benefits of quantitative images analysis, we will dig a bit more into the properties of digital images. What is a digital image? And what is the main difference between a digital image and analogue 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 value, which is called: a pixel value or pixel intensity. This value is discrete and you'll later learn that the maximum reachable value scales with the so called bit depth. For now it is important to remerber that typically only whole numbers are allowed. For the display of the 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 0 black, and the highest 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 this not entirely correct. It is more appropriated to see them as infinitesimal small sample units without a shape or a size.

Notes

Summary

4m 34s



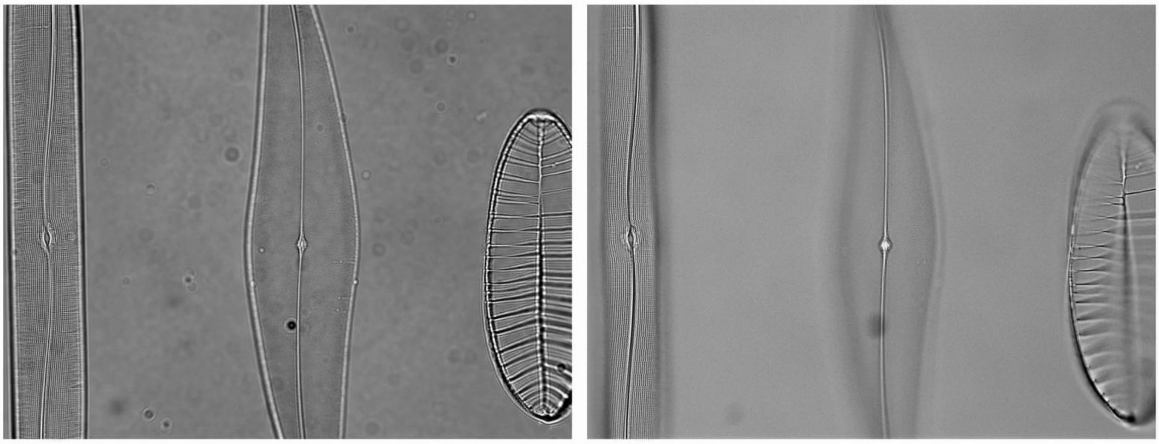
On the left hand side, you can see the transition of an analogue image to a digital one; and how this image is stored. An analogue image is consisting of continuous values which need to transfer into values with concrete steps in the case of a digital image. This is the case for the intensity. But it also is true for the distance and the size of the pixel. We will dwell a bit more on the details of that topic when talking about sampling frequency.

Notes

Summary

5m 59s

Images are Artefacts



Two images of same object (sample) imaged with the same microscope/objective!

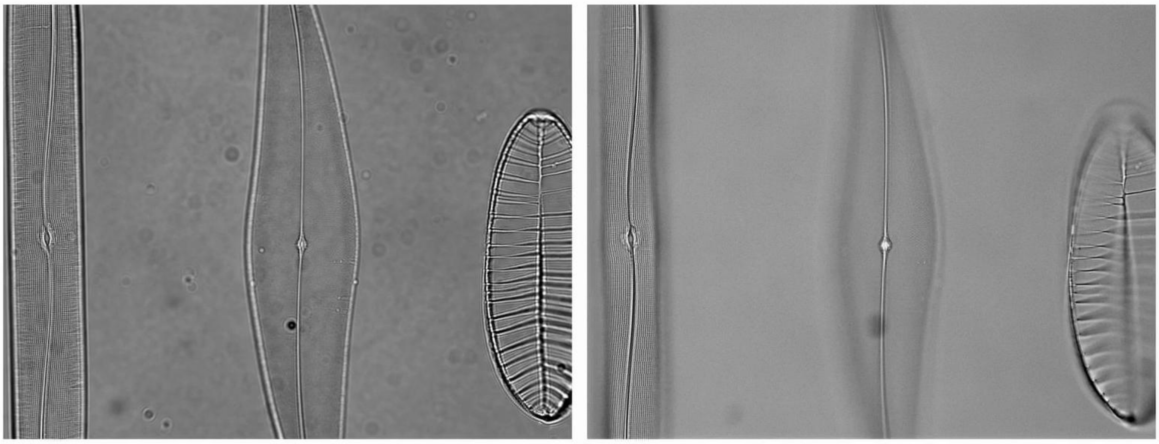
For now it is important to memorize that digital images are rastered images being nothing else than a matrix of integers. We have now formally defined a digital image. Now we will discuss how images are formed and recorded. At foremost, it is important to keep in mind that images and objects are distinct from each other. The aim of image analysis is to first analyse the image, and to use the information gathered to come to a conclusion about the object itself. 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 you formulate it a bit more scientifically, the transfer function of the imaging system. In every lens based system (we will exclusively use images from such system here) 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.

Notes

Summary

6m 33s

Images are Artefacts



Two images of same object (sample)
imaged with the same microscope/objective!

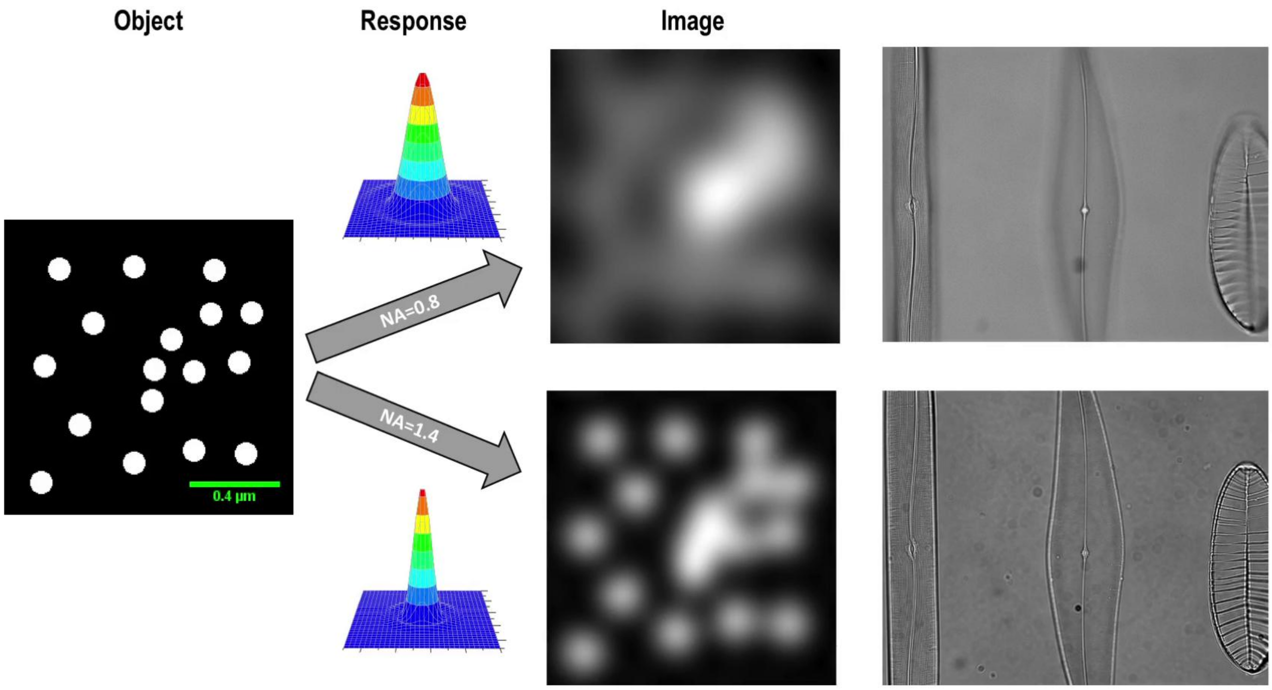
How important the transfer function is, is illustrated by the 2 images shown here. The same object so called diatom was imaged with the same microscope 2 times, by varying the aperture stop of the microscope. The result: 2 completely different images of the same object. This example serves to illustrate 2 facts. An image is always only one out of many representations of the object. And it is important to know the imaging system as well as its settings.

Notes

Summary

8m 02s

Images are Artefacts



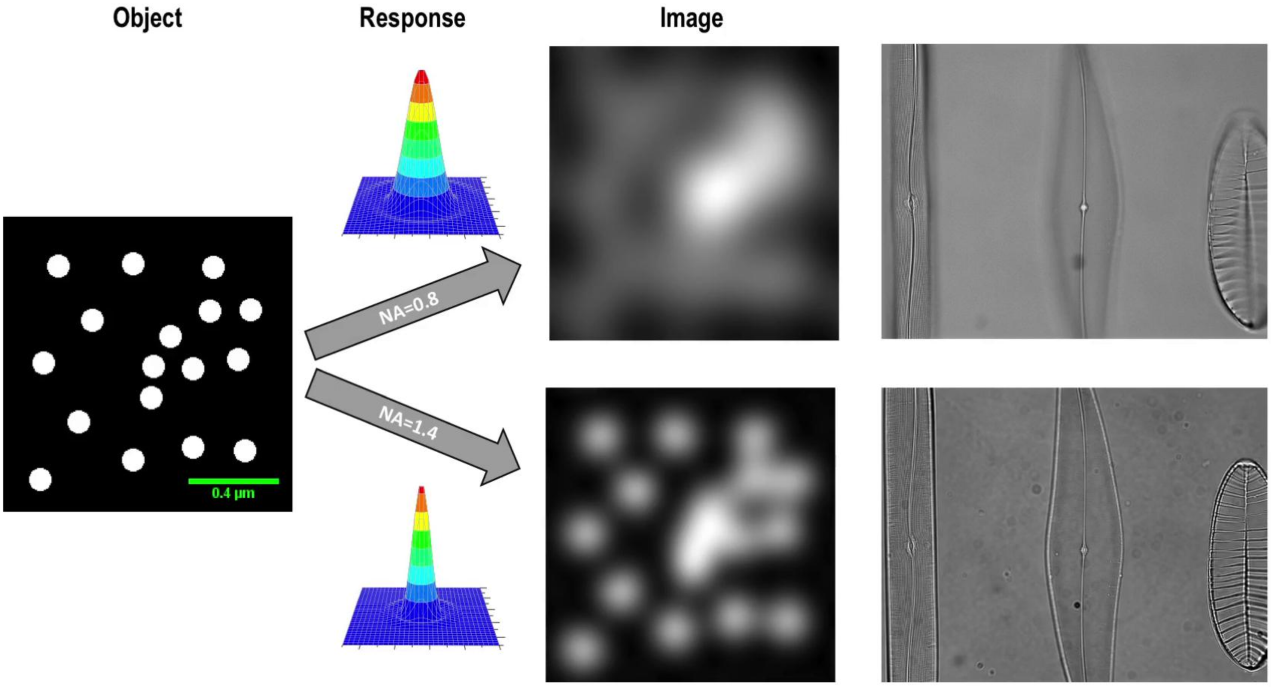
So let's have a closer look at the transfer or response function of the imaging system. Mathematically, the image of the object is obtained by convoluting the object with the response function of the imaging system. In light microscopy, the response function is often called the point spread function. A response or point spread function is responsible for the resolution power of the imaging system. This is shown in the 2 emulated images. 2D objects were convoluted with 2 point spread functions differing in size. The degree of blurriness is higher in the upper one. It is impossible to distinguish individual objects in that image. In the lower image, some discs are clearly visible. Others are not. Here you can see that the sharp edges of the objects are blurred in the image. The size of the point spread function is ruined 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 meaningfully. In ideal case, it can be retrieved from the meta data of the image. You will encounter the importance of meta data several times throughout this lecture.

Notes

Summary

8m 40s

Images are Artefacts

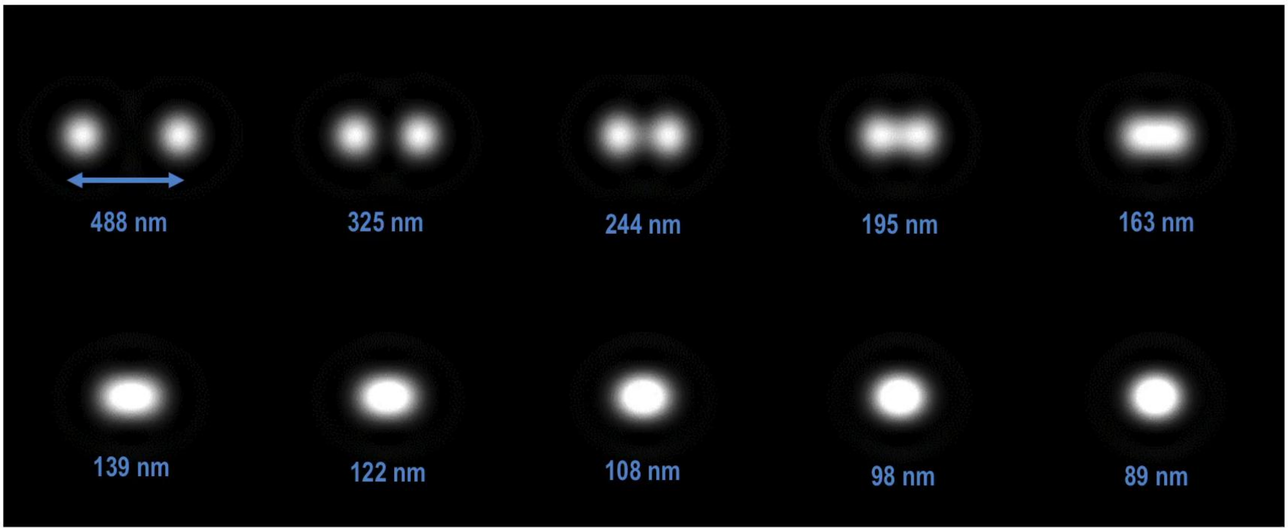


It means in essence that relevant information of the acquisition is stored along with the image. Coming back to the diatom images: Closing the aperture stop is decreasing the resolution of the imaging system. This is the reason why the upper image looks much more blurred than the lower one.

Notes

Summary

10m 03s



Wavelength: 488nm; NA=1.4 **Rayleigh criterion**: 212 nm

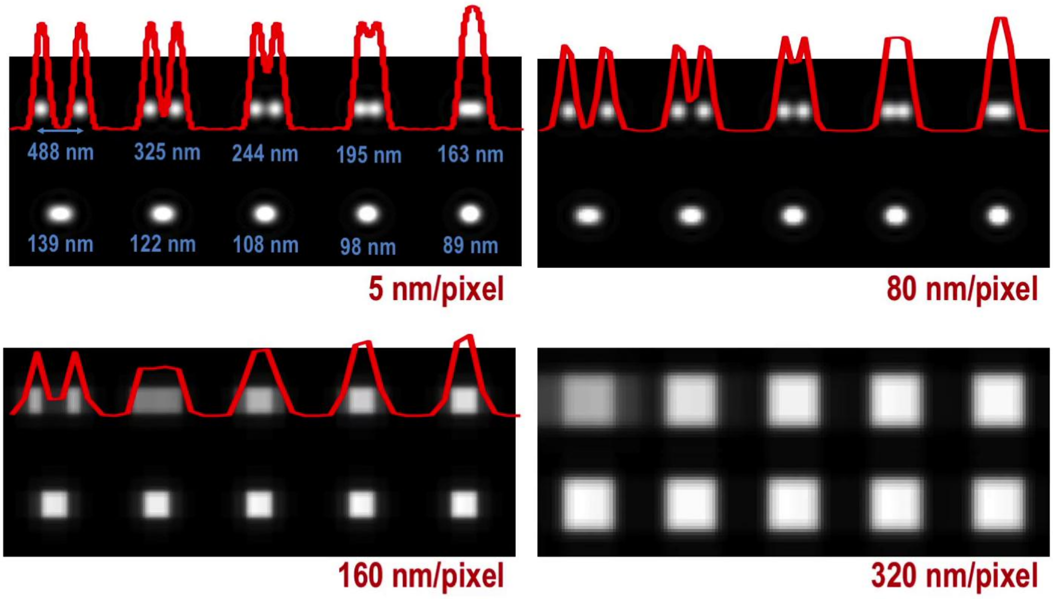
The point spread function is directly linked to the resolution. This term is important in order to understand the sampling frequency of the images later on. So let us briefly touch it without going into the details. It is illustrated in the following slide. Two diffraction limited spots are displayed, 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 2 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 minimum distance when the 2 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.

Notes

Summary

10m 25s

Digital Sampling



Wavelength: 488nm; NA=1.4 **Rayleigh criterion**: 212 nm

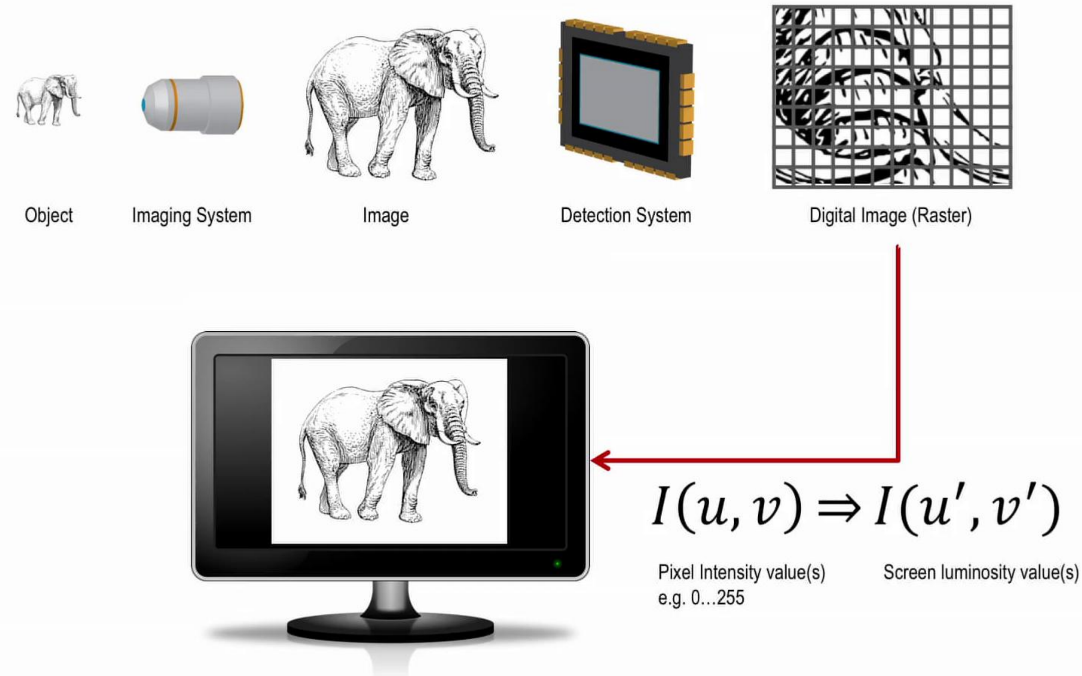
This is one of the reasons why it is important to be aware of the parameters when doing image analysis; and store them ideally along with the image. Digital sampling is equally important as resolution. What does that mean exactly? Remember what I said about pixels. There are not squares but rather infinitesimally small sampling points. How important the distance of these 2 point is can be seen in the following images displayed here. It is the emulated image which serves to explain resolution. Now we have add one layer of complexity and changed the sampling frequency, a.ka. pixel size. Not too surprisingly, we're losing resolution if we increase the pixel size. In particular, the image in the right corner is illustrating this finely. 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, they need to be matched in order to see both. There's almost no difference between the images in the upper row. However, the left image it's 250 times bigger than the one int the right. The optimal match between the resolution and sampling frequency is defined by the Shannon-Nyquist sampling theorem. Without goint to details just remember that this smallest resolvable structure within an image shall be sampled at least twice.

Notes

Summary

11m 32s

Image formation workflow



We have now discussed the individual steps of image acquisition workflow. So let's assemble it! An object is imaged. The produced image is detected. This results in a raster, or digitally representation of the image of the object. The response of the imaging system as well as the parameters creating the rasters are important. The functions involved to display the images are also extremely important. However they will be covered in an additional video. Each step can be considered a mathematical operation. In order to interpret the resulting images correctly, one needs to know the operation as good as possible. This is why it is to important to know the properties of the imaging system, including its detection devices; and ideally stored as metadata with the image.

Notes

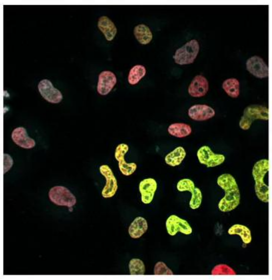
Summary

13m 14s

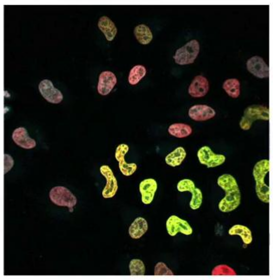
Image File Formats

- Lossless compression
  - TIFF (Tagged Image File Format)
  - GIF (Graphics Interchange Format)
  - BMP (Windows bitmap)
  - HDF5 (Hierachical Data Format)
- Lossy Compession
  - JPEG (Joint Photgraphic Experts Group)
  - JPEG 2000 (lossless and lossy storage)

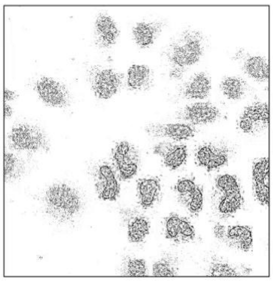
TIFF



JPEG



TIFF - JPEG



I know that we have covered quite a bit of theory. And you might be already a bit 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 be used? What are the advantages and pitfalls of the different imaging 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 and reports, the conversion can be useful. As the compression is not visible for the human eye. The most spread file format for images in science is TIFF, which stands for: Tagged Image File Format. It uses a lossless compression method. So the original data can always be restored. It also allows to link a tag to the image.

Notes

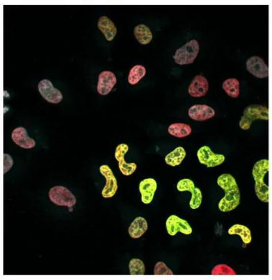
Summary

14m 13s

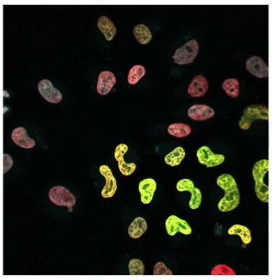
Image File Formats

- Lossless compression
  - TIFF (Tagged Image File Format)
  - GIF (Graphics Interchange Format)
  - BMP (Windows bitmap)
  - HDF5 (Hierachical Data Format)
- Lossy Compession
  - JPEG (Joint Photgraphic Experts Group)
  - JPEG 2000 (lossless and lossy storage)

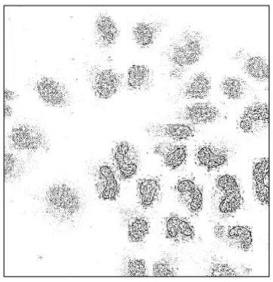
TIFF



JPEG



TIFF - JPEG



And 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 an BMP are also saving the original data. But they are not very widely used in image process. 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.

Notes

Summary

15m 51s

Summary

- **Image Analysis**
  - Quantification by avoiding human bias
  - High Throughput
  - Reproductible
  - Portable
- **Digital Images**
  - Carry the response of the acquisition system
  - Raster images with discrete intensity values (different bit depth)

Let's summarize what we have heard so far, and which are the underlying basics for scientific image 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 bigger. 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 us to store the so called metadata with the images. Therefore it is widely used in scientific image processing and analysis. And remember: the human eye is biased! Quantification can therefore be misleading. Digital image analysis, on the contrary is reproducible, can be easily shared with others, and can be used for the analysis of large data cells. With this one, we're are coming to the end. I hope to see you next time. Bye bye! And take care!

Notes

Summary

16m 27s