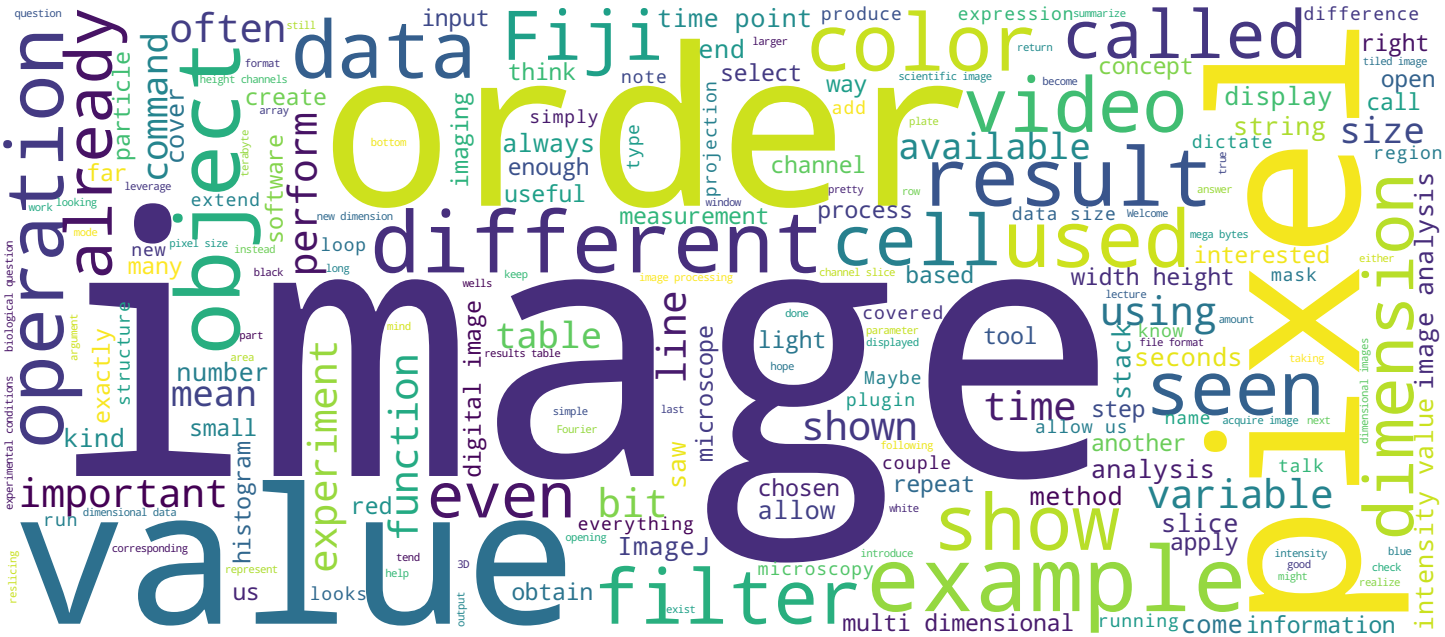
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Images With More Than Two Dimensions

Image Processing & Analysis for Life Scientists

Olivier Burri, Romain Guiet & Arne Seitz



Search MOOC

Video

EPFL

Summary

- Dimensions in Microscopy Images
- Handling Multidimensional Data
- Data Size
- Considerations for Image Acquisition

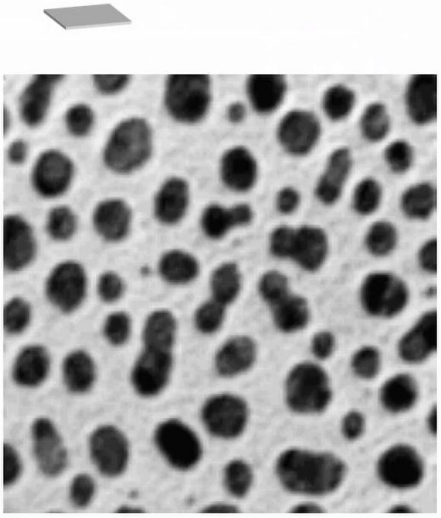
In this video, we'll be talking about multi dimensional images. We've seen that images can contain multi channels. Like an RGB image from a color camera. However, scientific data can have many more dimensions. First, we'll explore the more typical dimensions that make up scientific images. And then we'll see how multi dimensional data is visualized and manipulated in Fiji. We'll touch base on data size, and see the dangers of modern day acquisition systems. Which would lead us to talk about how you should approach image acquisition in microscopy.

Notes

Summary

0m 05s

N Dimensions



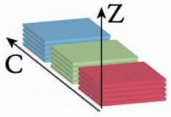
We've seen this image enough. Images have a width and a height. And each pixel encodes one value. You've recognized this image? We've used it a couple of times This is one of ImageJ's most famous images, called Blobs. It's so famous actually that it even has a dedicated shortcut under software: which is control B.

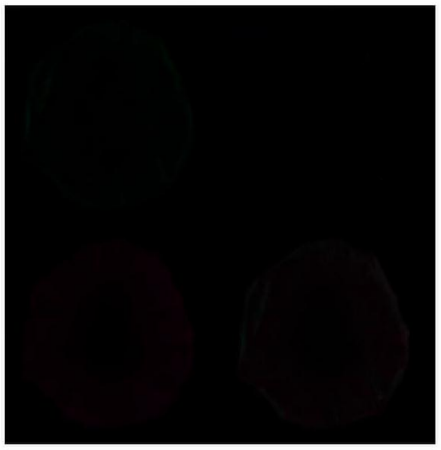
Notes

Summary

0m 34s

N Dimensions





Width, Height, Channels, Slices

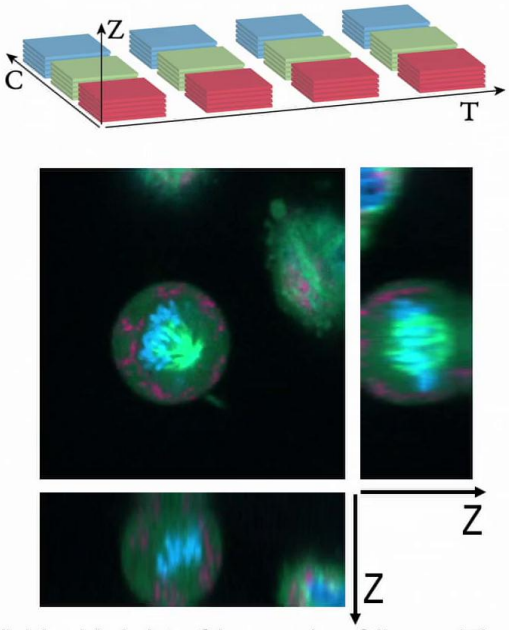
Now if we extend this image through a new dimension, we can for example code channel information. Here is an image acquired on 3 different channels. If we want to go one dimension higher, we can acquire images at different depths in order to produce a 3D image stack. Like the one shown here. Actually, this is already what we call a hyperstack. Because it has 4 dimensions: width, height, channels, and slices. The image here shows a montage of a cell in four channels. 4 cellular compartments are differently stained. In respect to the colors, you've seen already that they are totally arbitrary. They simply depend on the look up table that we've chosen.

Notes

Summary

0m 51s

N Dimensions



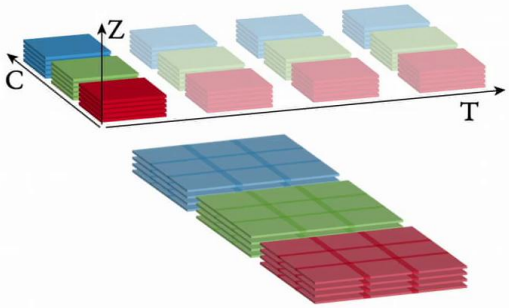
So where else can we go? Maybe we want to follow a cell overtime! So let's add that dimension! We can repeat an acquisition at a given time interval, in order to obtain information about the dynamic of a process. For example here, we have a cell getting ready for a division.

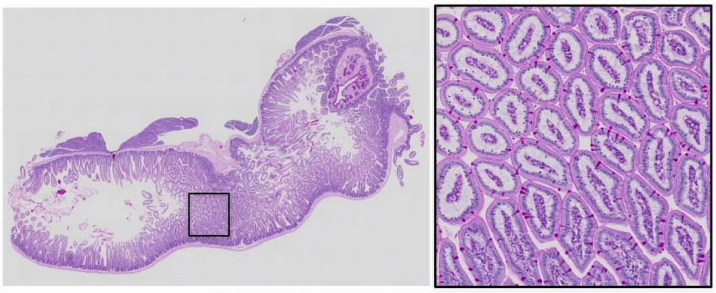
Notes

Summary

1m 30s

N Dimensions





Tiled Images

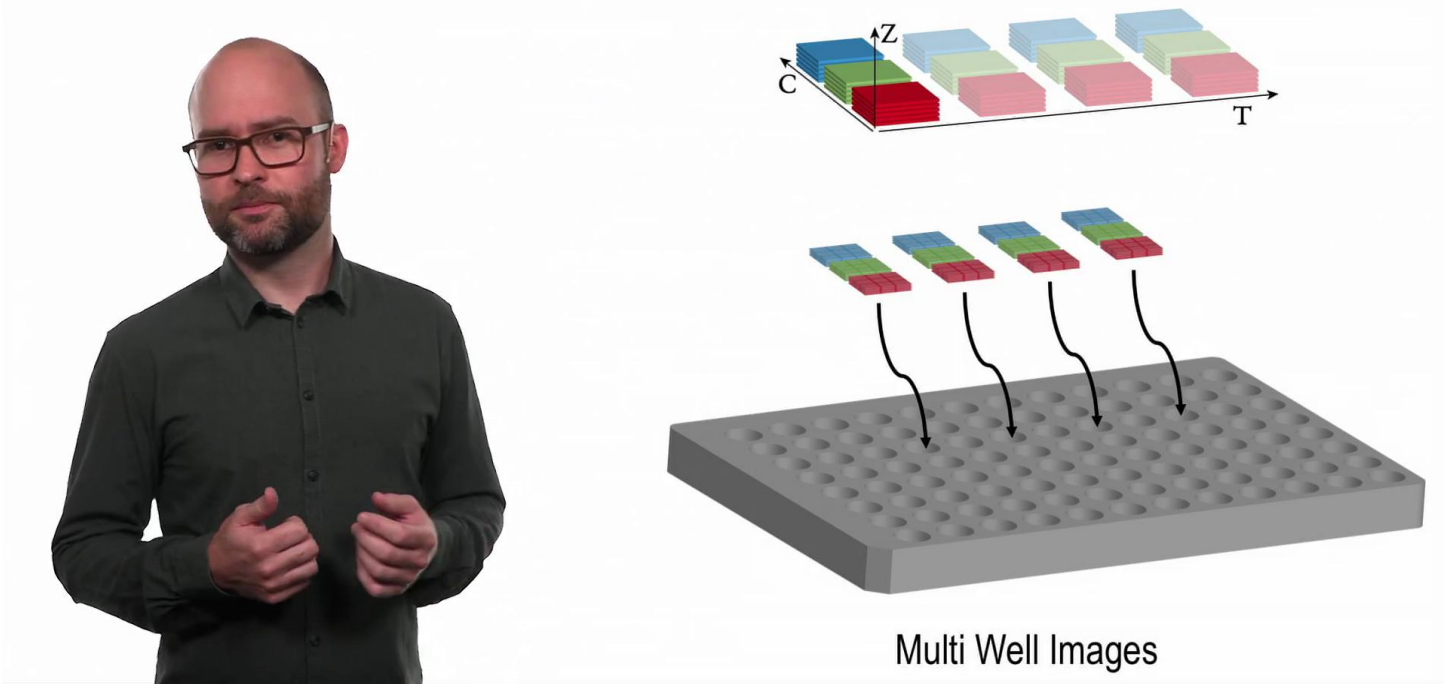
These are typically dimensions a scientific microscopy image can have. But what if we go even further? Well, not exactly a new dimension, we can think of not limiting ourselves to a single field of view. But instead, to acquire a tiled image. Here we show a 3 per 3 tiled images. So there are 9 images for each channel, slice, and, eventually, time point. Typical experiments that require tiling are full tissues section scan, using slice scanning microscope. This tends to result in images with over 10 thousands pixels, in either width or height; and allows a general view of the tissue that can extend down to cellular, or subcellular level.

Notes

Summary

1m 46s

N Dimensions

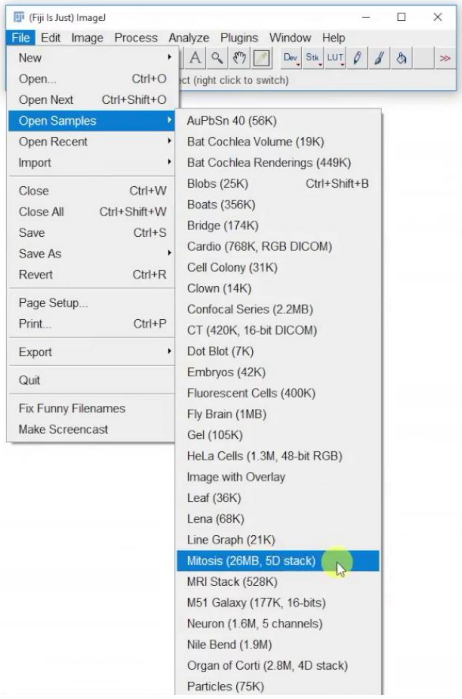


We can go even further by acquiring data in a multi well plate. With different experimental conditions per well. This means again that we repeat all the steps we've seen before. Once for each well. So we have 7 potential dimensions we can explore: Width, height, channels, slices, time points, tiles and wells. So let's quickly explore what that looks like in Fiji!

Notes

Summary

2m 23s

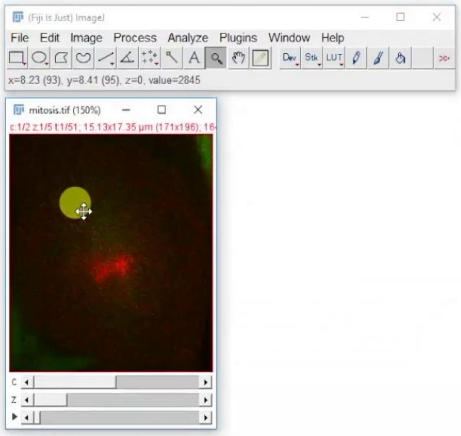


Here, we're opening a data set called mitosis.

Notes

Summary

2m 48s

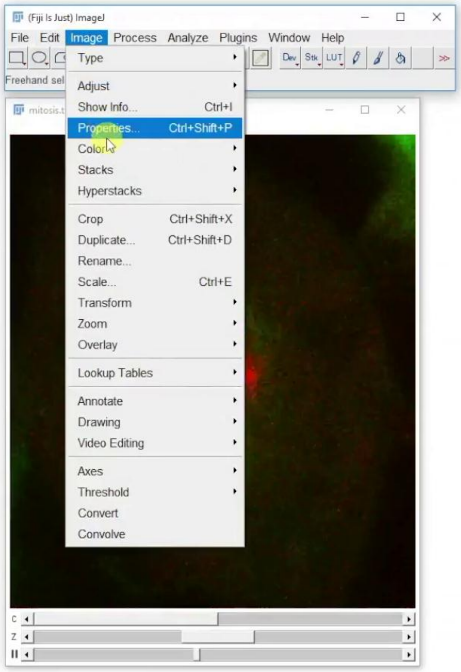


Which is a 5D stack available from the open samples menu. The images are rather small.

Notes

Summary

2m 53s

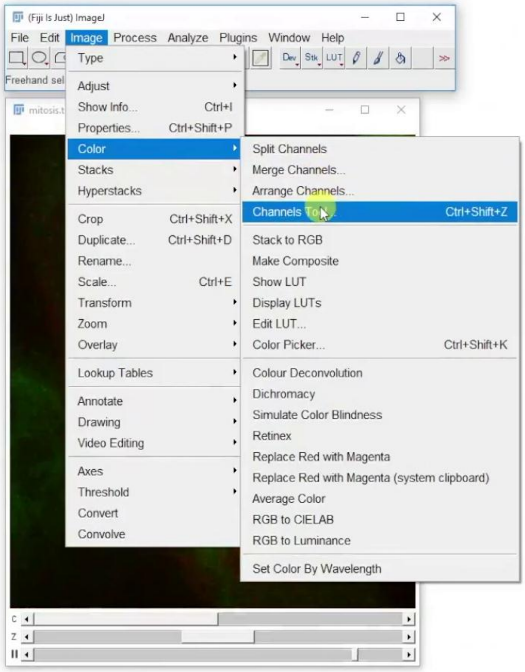


So we can use the zoom tool in order to simply increase the size of the image on our screen. Notice the multiple sliders at the bottom. One for the Z which is the depth, or the slices; and one for time. If we right click on the play button, we get a couple of animation options that allow us to select the play back speed for the video. And then, simply by pressing play, We get to observe a nice 2 channel cell mitosis.

Notes

Summary

2m 59s

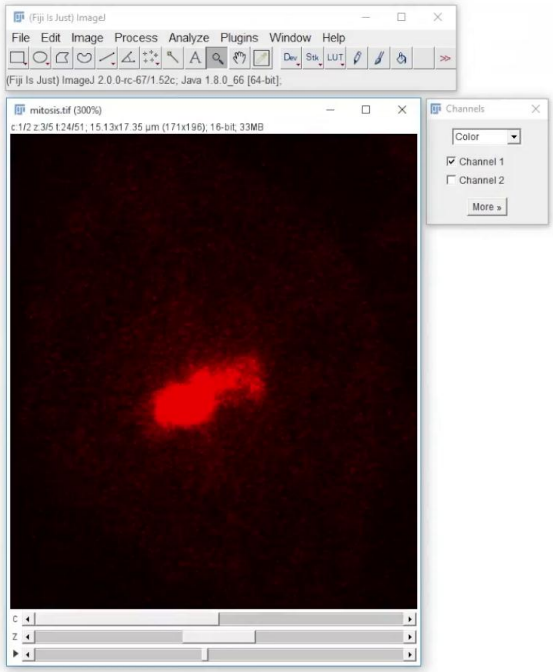


Now if we want to manipulate the channel data, we need to go to the channels tool.

Notes

Summary

3m 31s



Which is shortcut control shift Z. We see here that we have 2 modes: one composite, the other one color. The only difference there is that the first one allows you to show an arbitrary number of channels simultaneously, whereas the second one only shows one channel at a time.

Notes

Summary

3m 36s

Working With Fiji

- Max 5 Dimensions
  - XYCZT
- Large Data: BigDataViewer

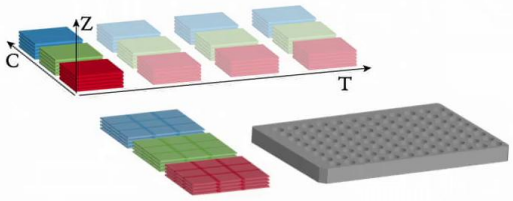
One thing to note is that the native Fiji image viewer can only show you up to 5 dimensions. Fields or Wells would have to be open as separate images, or combined using a plugin before viewing. Because of this rise in dimensionality over the years, New tools have emerged in order to better navigate this extra dimensional data. One of them is called BigDataViewer. Which is able to show image, even really big ones, with arbitrary dimensions very easily, provided you use the right file format.

Notes

Summary

3m 53s

Let's Talk Image Size



Reference: 8-bit Image, 1024 x 1024

Dimensions	# Images	Size
XY	1	1MB
XYC	3	3MB
XYCZ	3 x 10	30MB
XYCZT	3 x 10 x 20	600MB
Tiled XYCZT	3 x 10 x 20 x 9	5.4GB

- Biological Replicates x 3 : **16.2GB**
- In 96 Wells: **1.5TB**

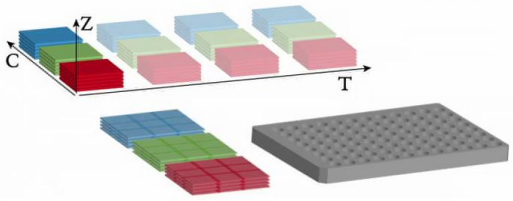
So why do we talk about large data? Let's walk through an experiment, as we increase the image dimensions, and see how this affects the size of the final data. As a reference, I'll be using a 2D single channel 8 bit image. That's taking at a 1024 by 1024 pixel. That represents about 1 mega bytes data. Typically, we're not just looking at 1 channel. Basic immunofluorescent images typically already have about 3 channels. So our data size starts ramping up. Maybe we want to include a few slices, in order to get a 3D representation of our data. And now we're already up to 30 mega bytes. Now if our experiment is on living cells, we probably need to capture a time series as well. Supposing 20 time points is enough, which is very small, We already up to 600 megabytes! To maximize the use of our sample, we decide to take multiple fields. Which takes us well over the giga byte limit! Now let's not forget we're also working with living organisms. We need to check the sample variation So we need to perform the experiment in triplicate. And now we're reaching up to 16 gigabytes. And all, wouldn't you know, lucky us, we have access to microscope that is able to perform all of this!

Notes

Summary

4m 22s

Let's Talk Image Size



Reference: 8-bit Image, 1024 x 1024

Dimensions	# Images	Size
XY	1	1MB
XYC	3	3MB
XYCZ	3 x 10	30MB
XYCZT	3 x 10 x 20	600MB
Tiled XYCZT	3 x 10 x 20 x 9	5.4GB

- Biological Replicates x 3 : **16.2GB**
- In 96 Wells: **1.5TB**
- Using a 16-bit Camera: **3.0TB**

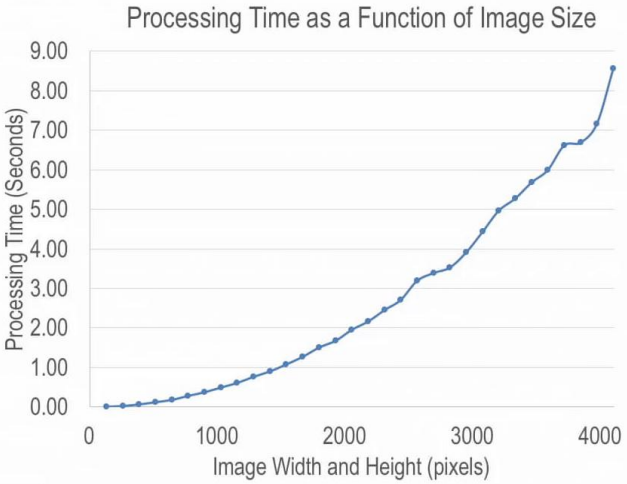
In a 96 well plate for all our experimental conditions at the same time. And we reach 1 terabyte for our single experiment. And then, finally, we realize that we have a very nice camera that is available. That can take images in 16 bits instead of 8. And now we're at 3 terabytes! So while this scenario might seem a bit simplistic, microscopes are now capable of generating this kind of data rather easily. High-throughput microscope can produce over a terabyte per day. And this raise considerable questions regarding data handling and storage for a laboratory.

Notes

Summary

5m 32s

Processing Time



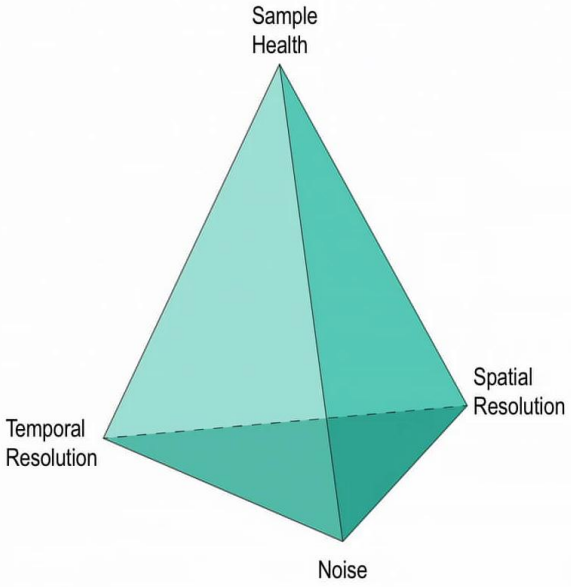
So the question we should really be asking is: Okay! So the microscope can do it. But does the experiment, or biological questions, really need all this data? Moreover, having the raw data is one thing. But the end goal is quantitative microscopy. So we want to consider the amount of image processing time we need for the analysis as well. As an example here, Running a simple operation in Fiji on images of larger and larger pixel size shows a quadratic dependence. Even though this operation is multi threaded, and running on an iron workstation. We see that it can take over 8 seconds to complete for a single 4000 pixel by 4000 pixel image.

Notes

Summary

6m 06s

There is Always a Compromise



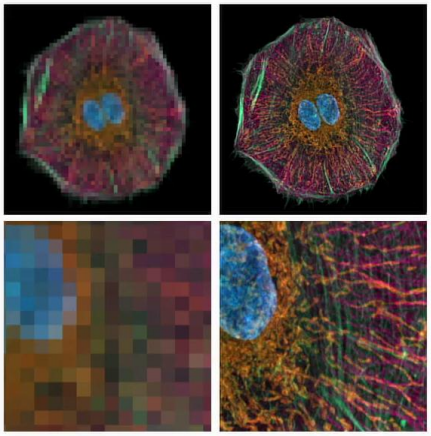
This considerations aside, there are actually constraints which very often do not allow us to go crazy on our data acquisition. There's always a compromise where we'll need to leverage how finely we want to sample our object, how quickly we want a image, and how pretty we want our image to be. And most importantly, keeping our sample healthy!

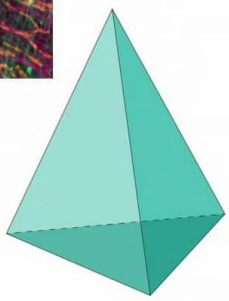
Notes

Summary

6m 44s

There is Always a Compromise





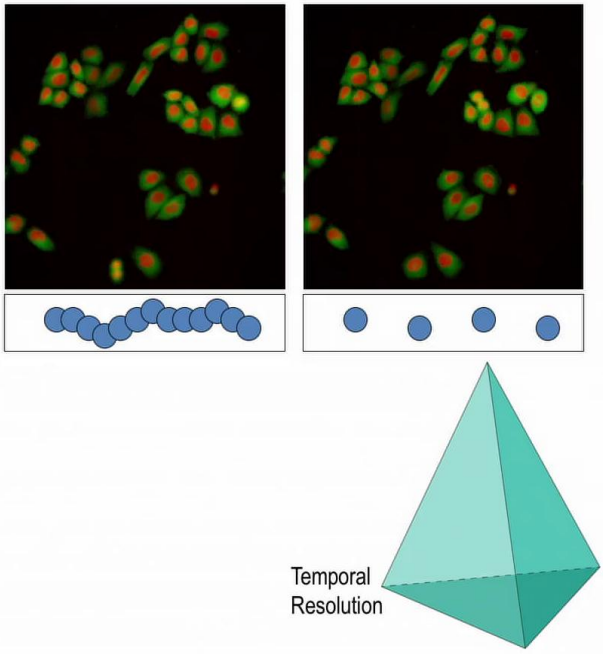
So comes the question: Are we interested in 10 micron nuclei in order to count cells? Or in 400 nm wide subcellular components? This will dictate our sampling rate. And more fine is not always better or nor useful.

Notes

Summary

7m 03s

There is Always a Compromise



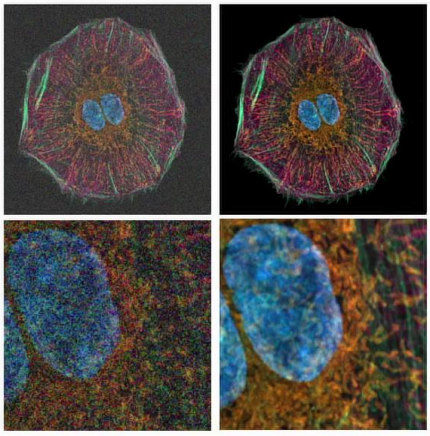
We need to leverage this with how often we want to image. Is the biological process fast or slow? More importantly, if we want to time point every five seconds, the entire acquisition process, (so changing channel, slices and positions) must all be done under 5 seconds.

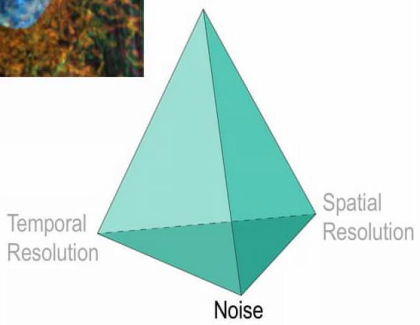
Notes

Summary

7m 18s

There is Always a Compromise





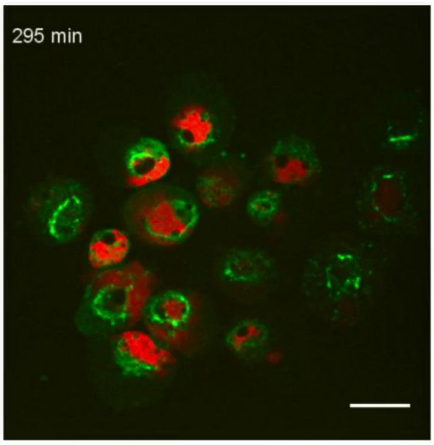
We could try to spend less time per slice. For example, lowering the exposure time. But it's usually at the expense of signal quality. Can we still see the structures we interested in if the images are noisier?

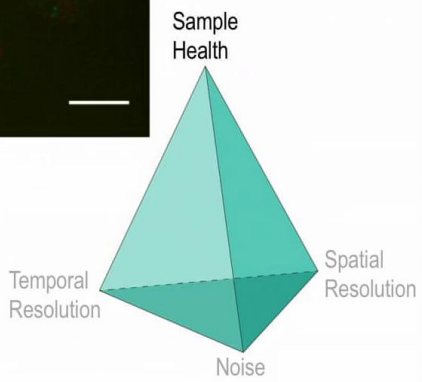
Notes

Summary

7m 34s

There is Always a Compromise





Finally, and most important of all, Whatever setting we have chosen, we have been useless if the sample cannot survive the imaging conditions. Sample health will always be the one parameter that will dictate how well and often we can image. The sample can bleach, die, or simple behave in a non natural way, simply because of the imaging condition. This is by far the most important point. You can image all you want as long as you can prove your sample remains healthy.

Notes

Summary

7m 44s

Conclusion

- Image are N-Dimensional
- Fiji provides Visualization Tools
  - Limited to 5 Channels
- Data Can Become Big Fast
- Everything is a Tradeoff
  - "Image what you *need*, not what you can"

Alright, that covers this video on multi dimensional images. We have seen how images have more dimensions Than just width, height and channels. And what kind of tools Fiji provides in order to visualize them. We've seen that we're limited to scrolling to 5 dimensions in the data set; but that there are tools to circumvent this when needed. We also went through a small example where we saw how data can become really big really fast. Finally we talked about how everything is a tradeoff. And that you will never get everything at once for an experiment. Think first of what you need to answer your biological question. Then acquire! Image what you need not what you can! Thank you! And goodbye!

Notes

Summary

8m 11s