



- Reslicing
- Kymograph
- Interpolation

Welcome to this lecture covering the topic of Reslicing. This is a procedure which allows you to display 3D data and have a look at it from a different angle. Therefore, it is somehow related to projections which had been already covered in the last lecture. First, I will introduce the concept of reslicing. Then, I will talk about so called kymographs which are a very prominent example where the reslicing method is used in order to analyse moving objects. At the end, I will discuss pitfalls of reslicing which are stemming from the necessity of pixel interpolation. But now let's get started.

Notes

Summary



0m 05s

Reslicing

t=0

1	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

1	0	0	0	0	0	0
---	---	---	---	---	---	---

t=1

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

0	0	1	0	0	0	0
---	---	---	---	---	---	---

t=2

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	1	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

0	0	0	0	1	0	0
---	---	---	---	---	---	---

t=3

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	1

0	0	0	0	0	0	1
---	---	---	---	---	---	---

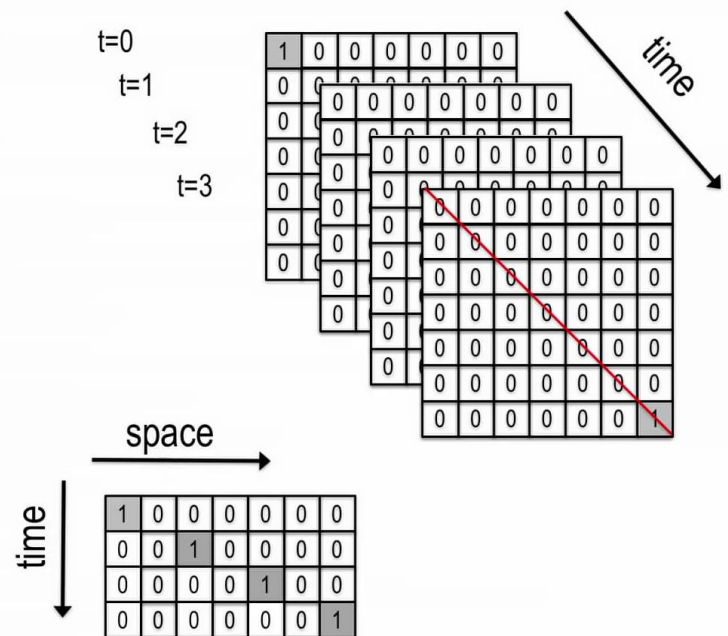
Whenever you want to use the reslicing procedure you need to have at least two images of identical size. The most typical examples are z or time stacks. Although nothing speaks against using these operations on other stacks, for example, spectral data, as well. Let's assume we have a stack of four images. It is not of importance whether they are from a time series or from a z-stack. In order to perform the reslicing method, we need to draw a line. This can be a straight line or even a free-hand one. For simplicity let's start with a straight line. The line we have selected is shown in red. Now, we just have to extract the intensity values along that line. So we are doing nothing else than a line measurement. You can find the intensity values of the line beneath the image. Now we switch to the next image of the stack and repeat what we have done with the first slice. We again obtain a row of numbers. You can surely imagine what will happen after this step. Exactly! We go to the next image and repeat the exact same procedure. And now we repeat that procedure until the last image of the stack. After this had been done we just have to assemble the obtained lines of data.

Notes

Summary



Reslicing



The obtained lines have the same length. So they can be easily arranged in a two dimensional array. And a 2D array is nothing else than an image. So we have converted a 3D dataset into a 2D image. So we performed a data reduction or a so called reslicing. In fact, if we think of our 3D data as a cube then we do a virtual cut along the z or t axis and we look at the surface which was created by that cut.

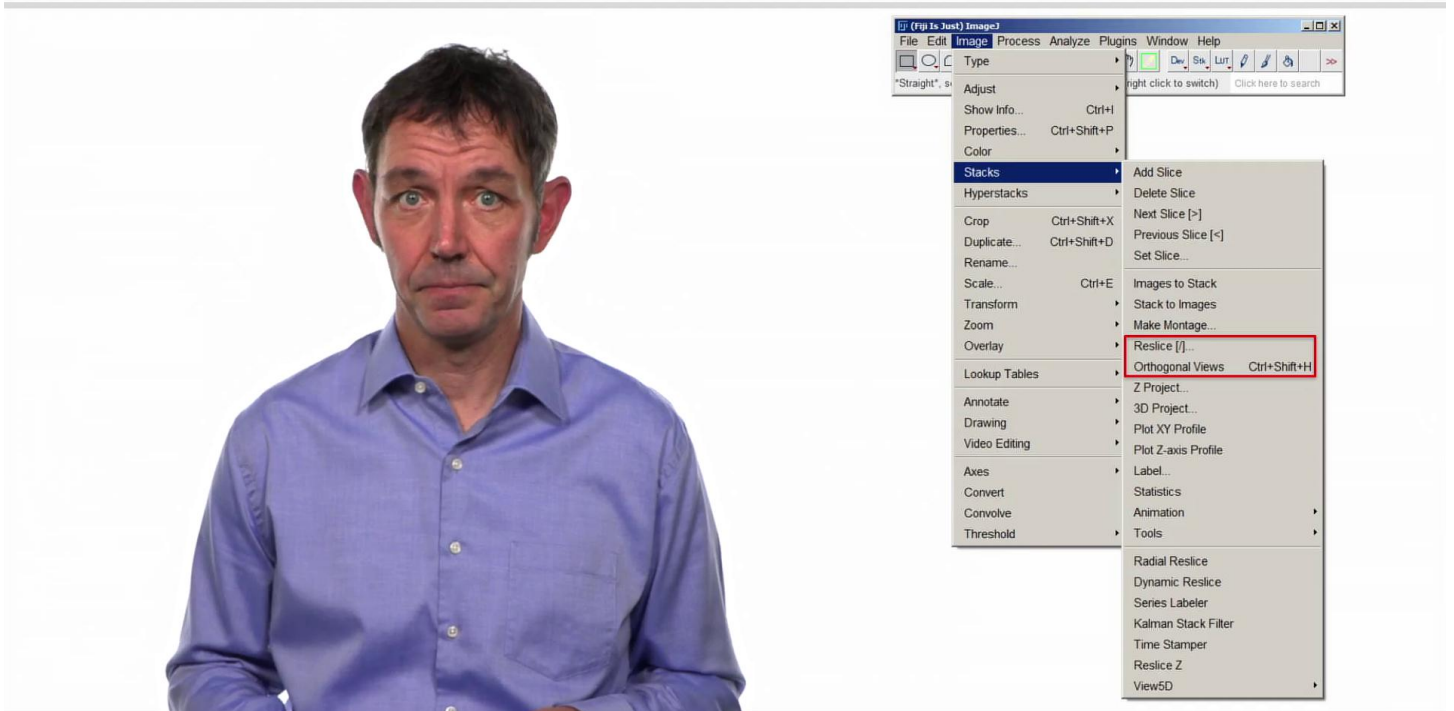
Notes

Summary



2m 11s

Reslicing



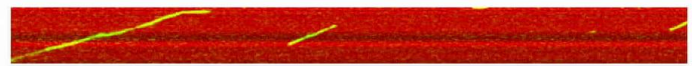
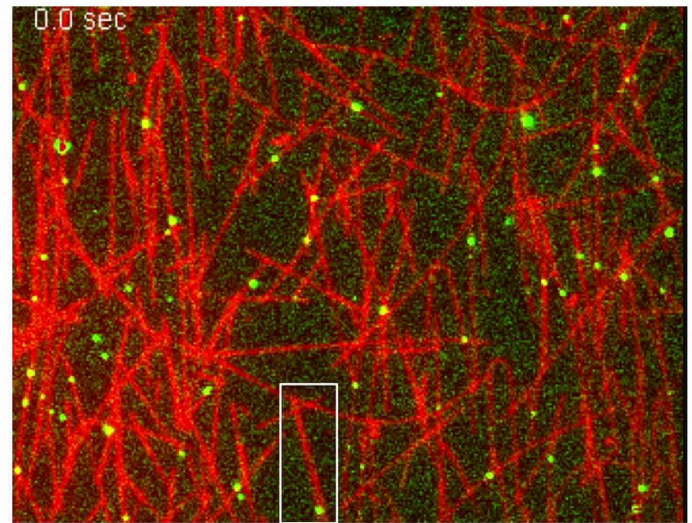
In Fiji/ImageJ the reslicing operation can be found in the submenu Stacks which can be found in the Image menu. Here you can also find the Orthogonal Views which we will briefly discuss later.

Notes

Summary



Kymograph



time

After that rather theoretical explanation I want to demonstrate how reslicing can be used in order to analyze moving particles. In the displayed movie you can see in vitro polymerized microtubules shown in red and the molecular motor kinesin displayed in green. As you can see this motor moved along the microtubules, using them as tracks. If we want to analyze the speed of these moving dots we have various options. One very efficient way is to perform a kymograph analysis. As we already know the tracks all we have to do is to draw a line along such a track. The white rectangle highlights the region where a line was drawn along the track. The reslicing command was then applied and resulted in the image shown beneath the movie. It is a kymograph also, termed time-space plot. Please keep in mind that we can easily swap the axes. This is important for any further analysis.

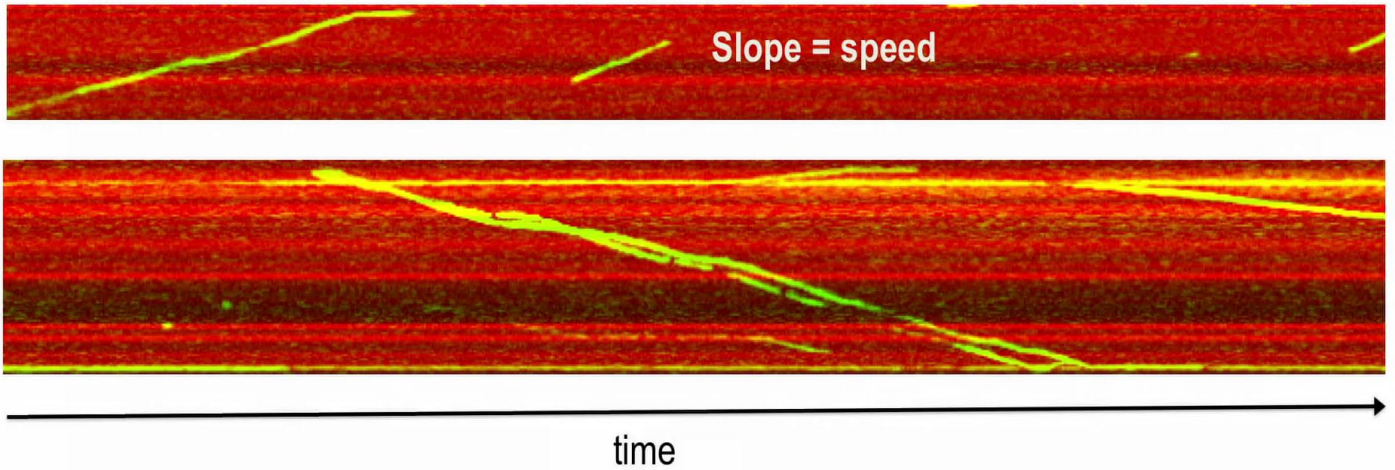
Notes

Summary



2m 59s

Kymograph



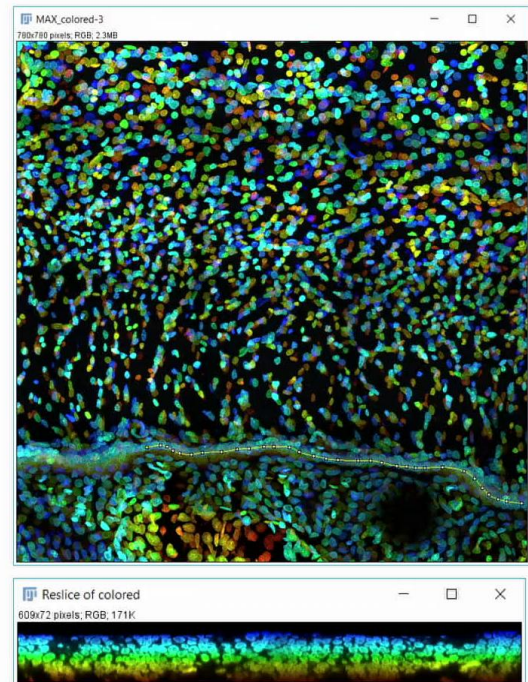
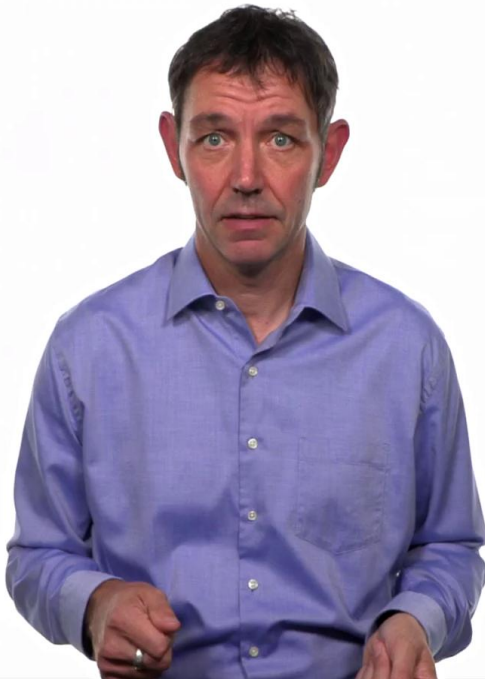
Let's have a closer look at the obtained images. Each line visible in the kymograph corresponds to a moving particle. The velocity can be deduced from the slope of that line. Horizontal lines are therefore particles which are not moving. Thus, with this rather simple operation we've read a lot of information. We rapidly get an idea about the number of moving particles, their speed. This is exactly the strength of this kymograph analysis.

Notes

Summary



4m 05s



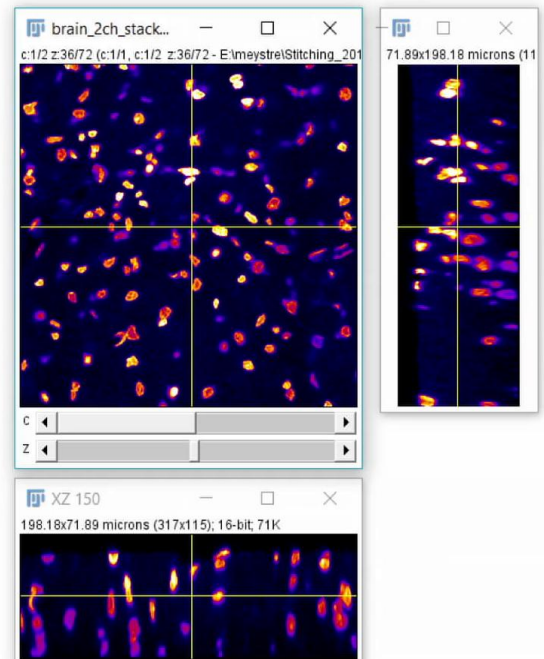
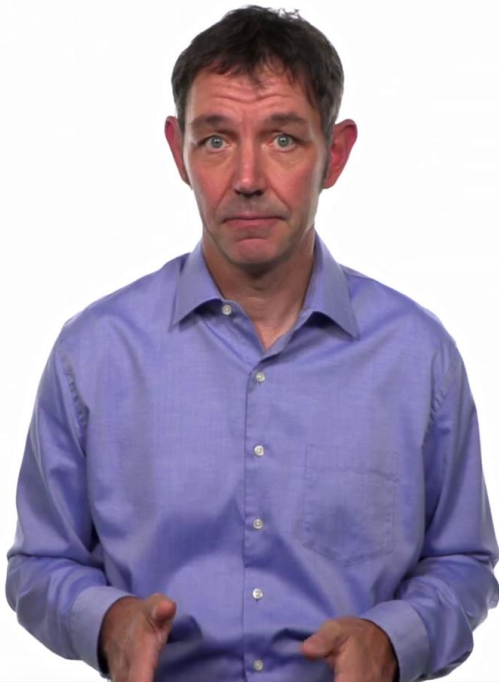
Until now, we have applied the reslicing operation on time stacks. But we can also use them for the analysis of z-stacks. This is demonstrated in the example shown here. The upper image is a maximum intensity projection of a z stack. The nuclei of a brain section were color coded based on their axial (Z) position. The projection clearly reveals a zone of high nuclei density. We can trace this zone by drawing a line, and then perform a reslice operation on the stack. It returns the small image shown beneath the projection. One clearly sees that the cells form kind of a wall. So they are close in xy as well as in z. The reslicing operation is a powerful tool in order to illustrate exactly this fact. The shown example combines various operations we have discussed so far. For example, color coding, projections and reslicing.

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Summary



4m 37s



Therefore it underlines that typically a combination of images processing and analysis steps are needed in order to extract meaningful data out of an image. The orthogonal viewer al shown here is also based on the reslicing operation. Here, two orthogonal lines can be freely moved over the image stack. For each of them the reslice operation is shown instantly in the images next to the stack. By moving the line, a new slice is automatically calculated. This orthogonal viewer is a very powerful tool in order to rapidly inspect 3D datasets.

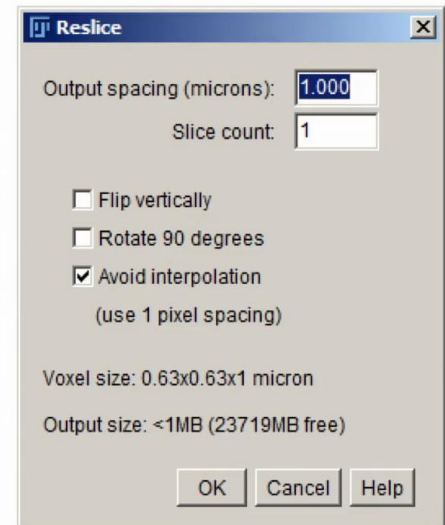
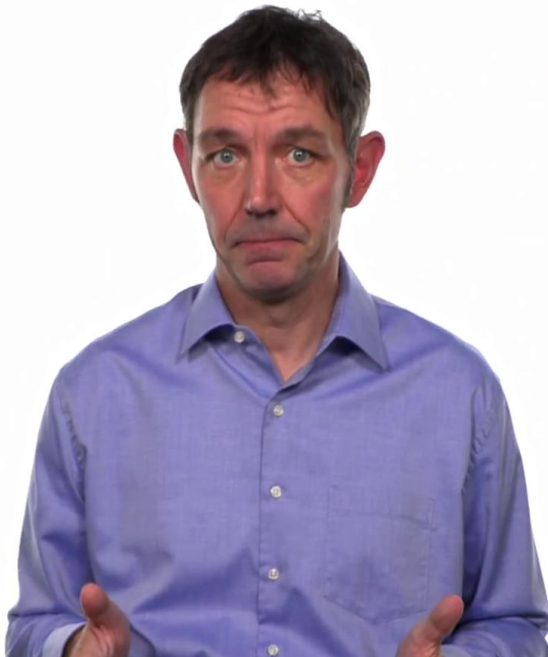
Notes

Summary



5m 40s

Interpolation



We have used the reslicing operation in different contexts. Towards the end of the lecture, I just want to briefly mention one of the pitfalls. When you execute the Reslice operation in Fiji/ImageJ, the following window pops up. You see that you can set various options. Most of them are easy to understand like flip or rotate. A bit more complicated is the term 'interpolation'. So what does it mean exactly?

Notes

Summary



6m 20s

Interpolation



0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

Only in a very few cases we will select lines which are always falling exactly onto the pixel raster of the image. Just look at the example shown here. In case we want to apply the reslice operation on that image, we have an issue. If we select all the pixels which are touched by the line, we end up with a line consisting of 11 pixels. This is longer than the diagonal of the square which is 7 pixels long. So this obviously makes no sense. A reasonable selection could look like the yellow/orange selection. However here we clearly ignore pixels which are covered by the line. In case we want to include them as well, we have to make an interpolation. One simple way is to include the pixel left and right from that line and calculate the mean value. By doing so, we would include all the pixels which are covered by the line. However, also pixels which are not covered by the line will be taken into account. And we can even go a step further and include all the surrounding pixels as shown here. If we take the average of all the five pixels this operation is, identically, to first filter the image and then extract the line as shown in the first example.

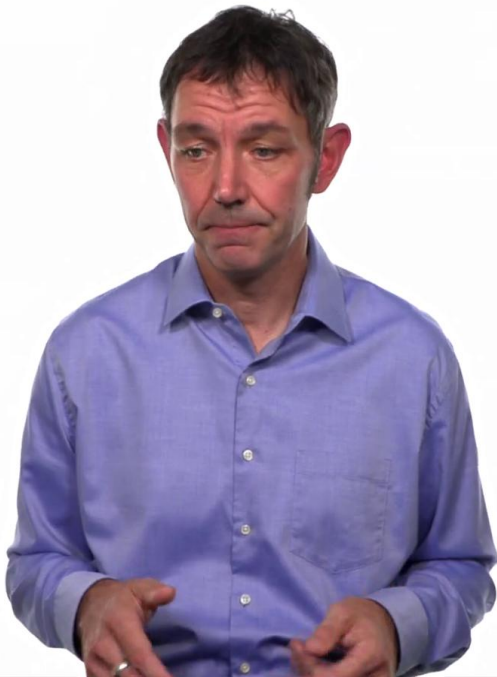
Notes

Summary



6m 48s

Interpolation



0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

For you, it is important to remember that different results can be obtained when performing a reslice operation. Therefore it is important to properly document which parameters had been used. Otherwise it will be tedious, or even impossible, to reproduce your results.

Notes

Summary



8m 13s

Summary



- Visualization of 3D data
- Reduces data size
- Applicable to time and space stacks
- Limitation: interpolation

So let's summarize. We have discussed the concept of reslicing 3D data sets. This is a way to inspect large datasets in a quick and intuitive way. One can either use orthogonal lines or even a freehand selection. The reslice operation is using the pixel values along that line in order to create a new image. This is done by going through each image of the stack. This operation can be used on z-stacks and on time stacks. In the latter case, this is extremely useful in order to analyse the movement of particles. I hope to see you next week. Take care and good bye.

Notes

Summary



8m 31s