ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Image Projection Methods

Image Processing & Analysis for Life Scientists

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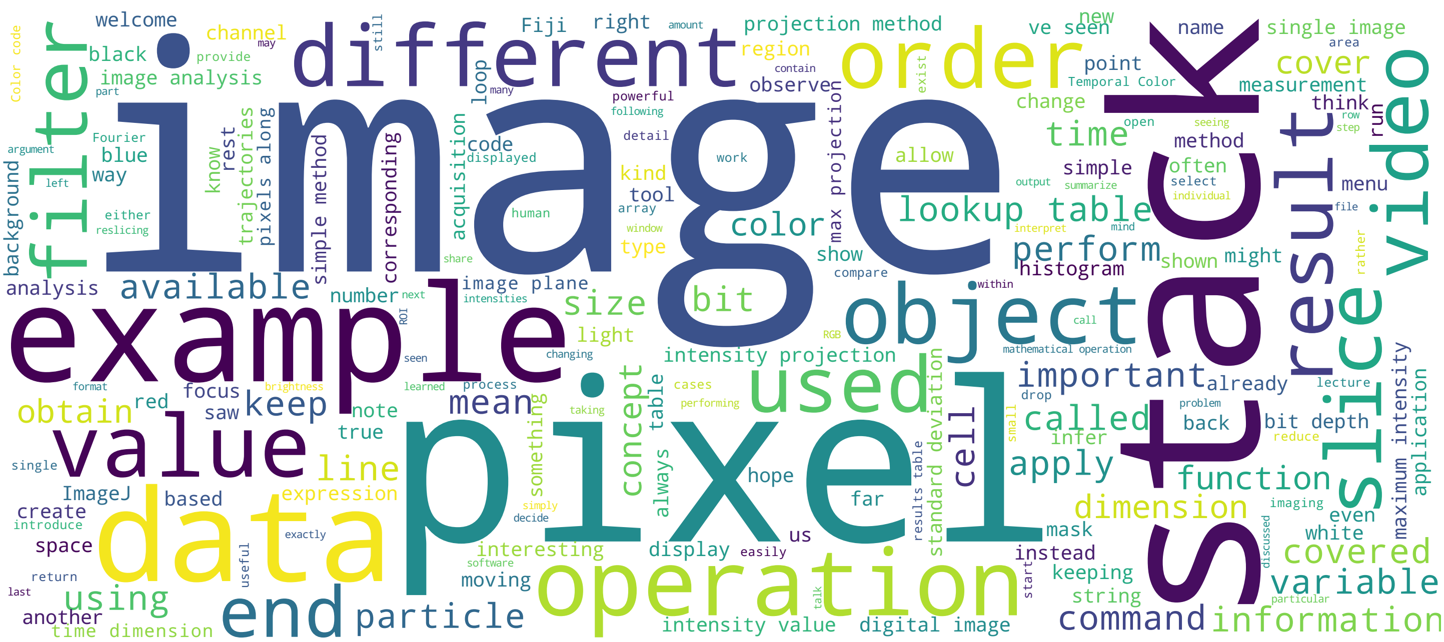

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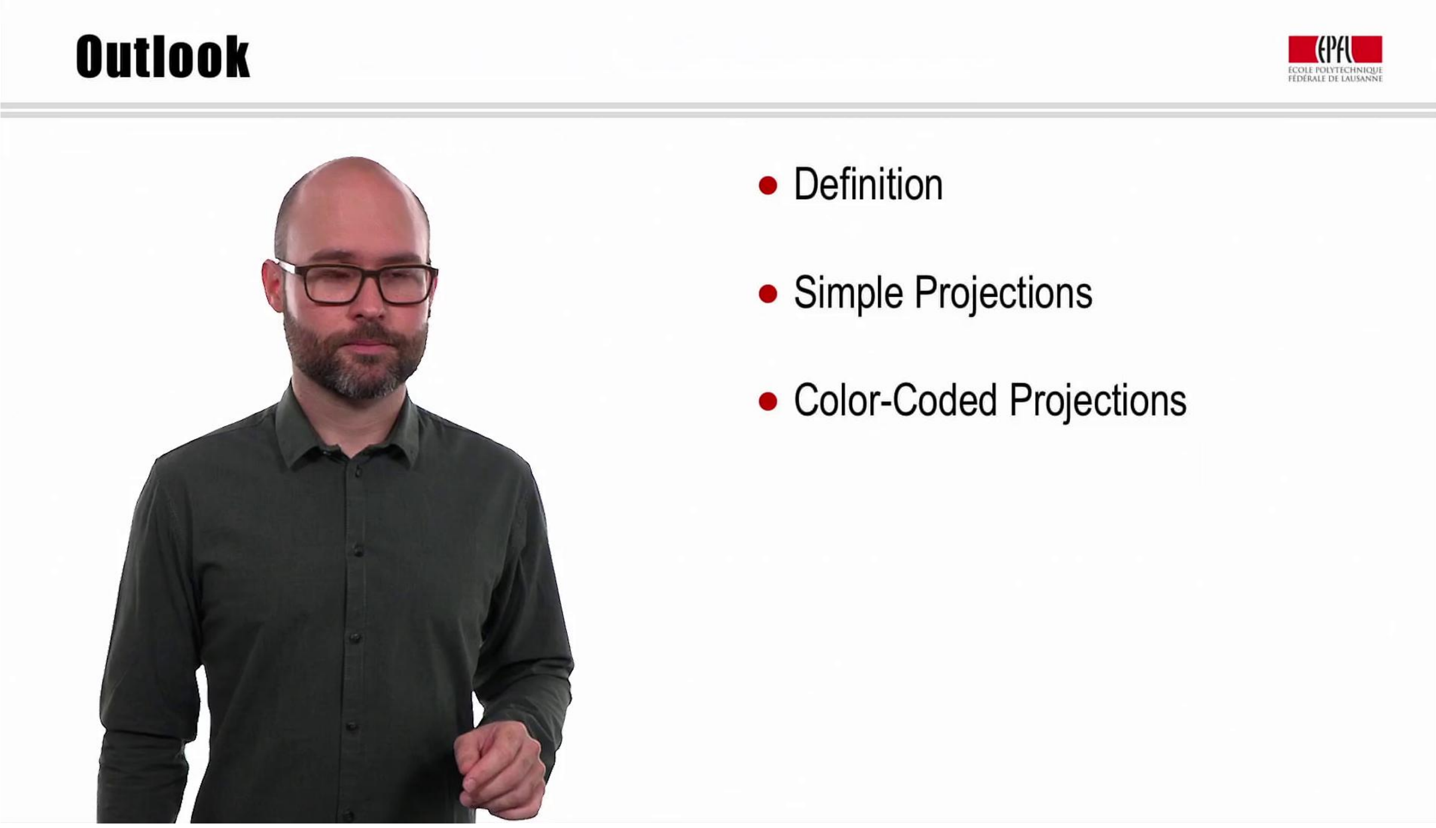


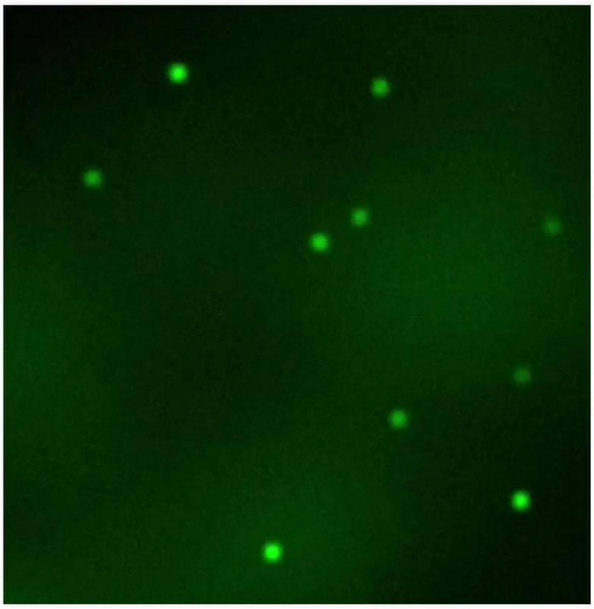
Hi! And welcome to this video where we will be discussing different ways in which we can project multidimensional image data. We will first see what we mean when we talk about image projection methods, and cover the most simple projections available in Fiji. Finally we will cover the concept of color-coded projections.

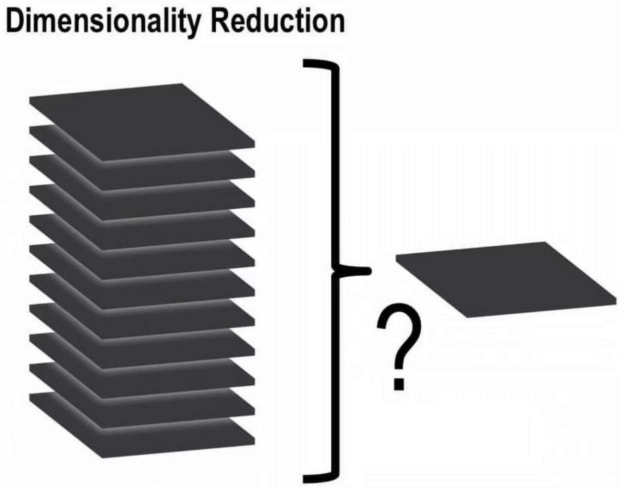
Notes

Summary

0m 05s

Projections: Approach



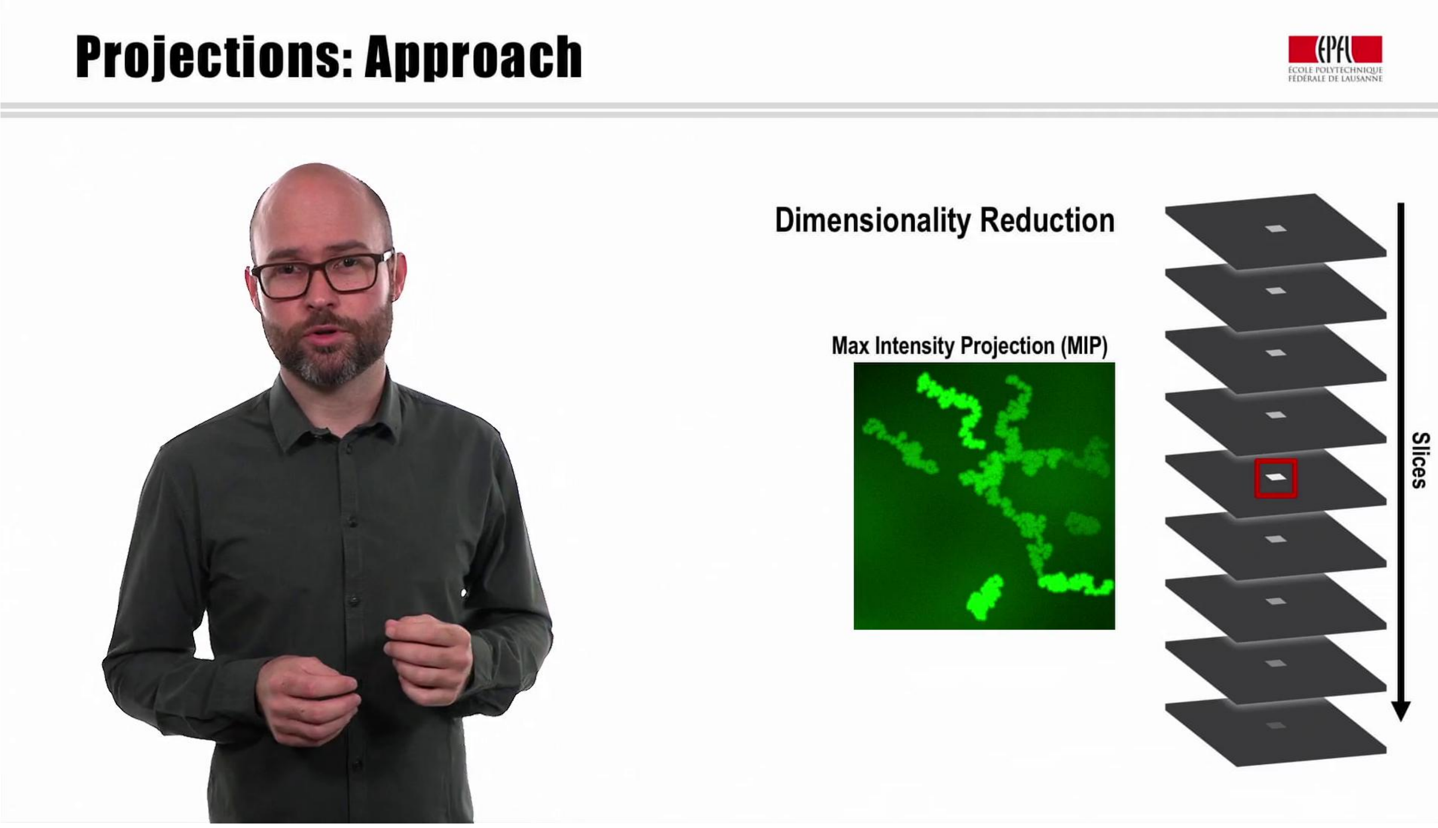


Projections are a very simple method of dimensionality reduction. For instance, this video, which represents particles undergoing directed motion, is quite simple to observe and interpret. But, as humans, we lack the capacity to observe the entire dataset at once. What would be interesting is to somehow collect all the information of the time dimension into a single image. So how do we go from a stack to a single image plane ? And what kind of operations can we perform?

Notes

Summary

0m 27s

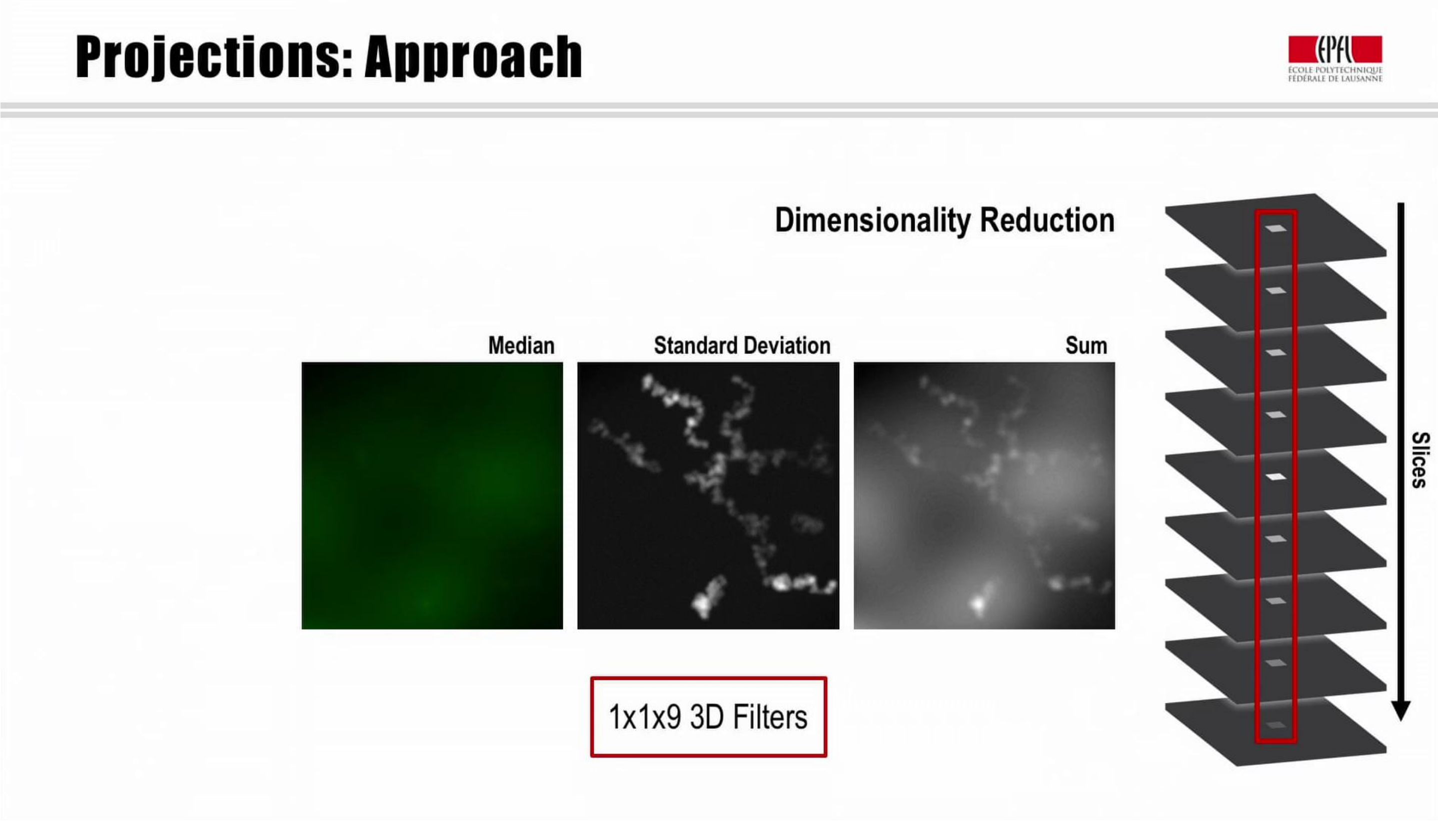


Let’s focus at a single image plane, where you see pixels represented as little squares. If focus on one of these pixels and isolate it, and then we look at the pixels that share the same xy coordinate throughout the stack, This third dimension could be coding depth, time, channels or any other dimension we’ve previously seen. These pixels now, usually, will have unique intensity values along each slice. So one thing we could do to reduce the stack to a single slice is to pick out the pixel with the brightest intensity, and discard the rest. Then, if we do the same for all the pixels along the stack, we can obtain what is called the Maximum Intensity Projection (MIP).

Notes

Summary

1m 00s



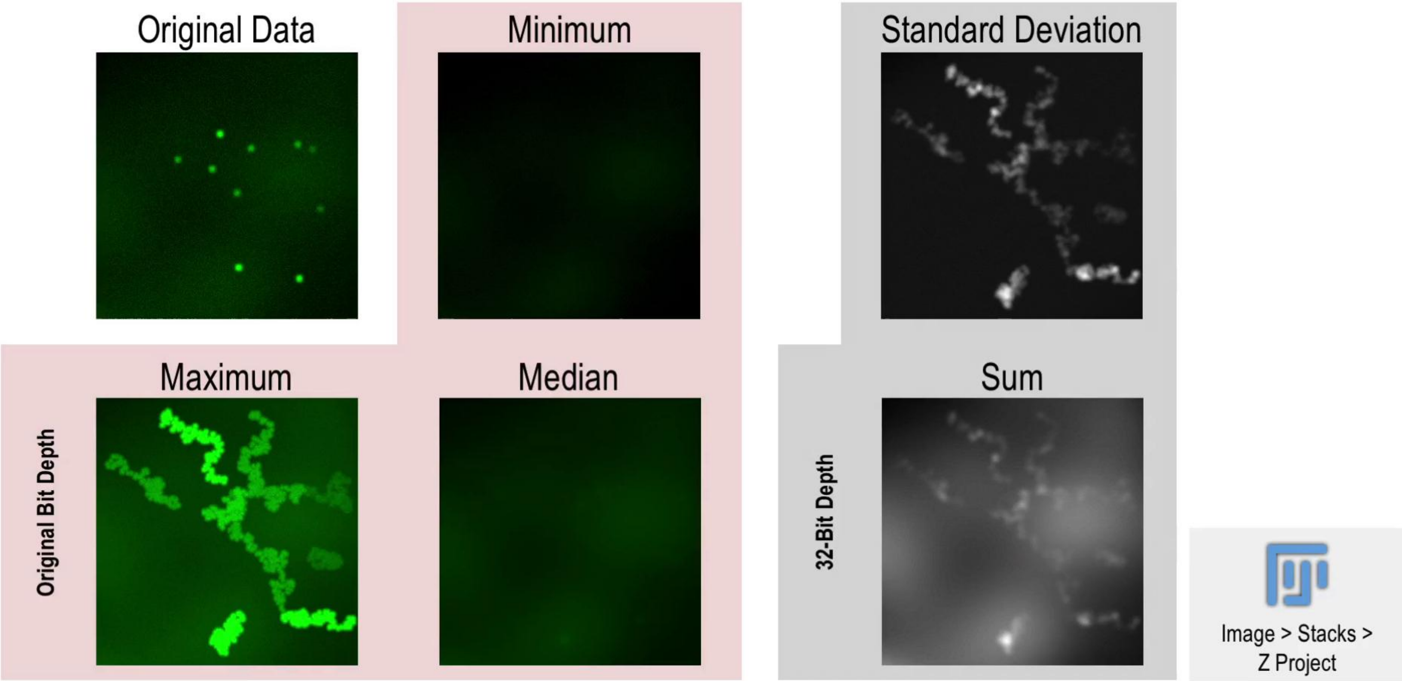
If we keep only the dimmest pixel along the stack, we get the minimum intensity projection. If instead of keeping a single pixel and discarding the rest, we decide to apply some sort of operation on all the pixels in the stack, we could, for example, compute the sum, or the standard deviation of all the pixels along the stack, or even their median value. If you think you've seen something like this before, it's because you can think of projections as a type of 3D filter. In this example, with the 9 slices we have, we are basically using a 1x1x9 3D nonlinear filter.

Notes

Summary

1m 47s

Projections



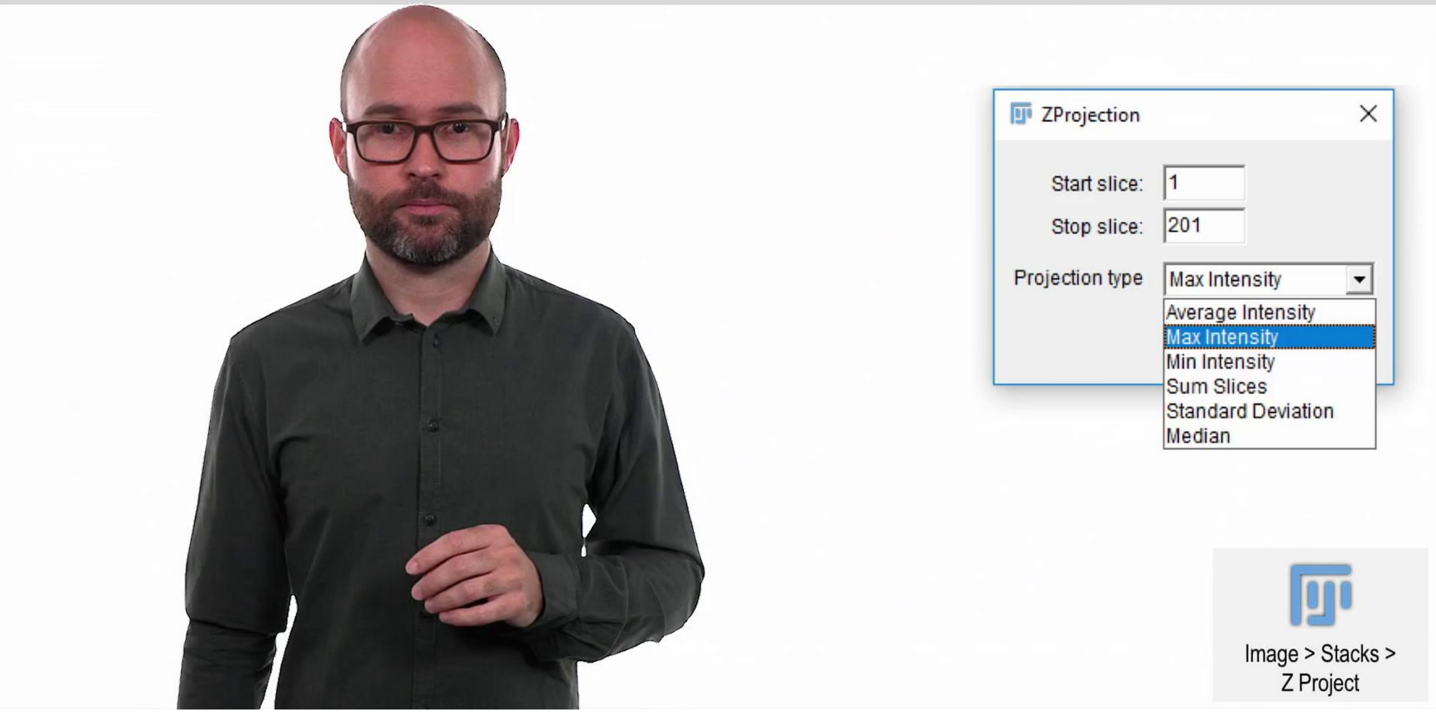
So going back to our data set, this is is a summary of what the different projections look like. One thing to note is that for the first 3 operations we've seen, the projections returned are in the original bit depth. However the projections that involve mathematical operations, like standard deviation, or mean, or sum, will return images that are in 32-bits. All this is made available through the Image> Stacks > Z Project command The command is very simple.

Notes

Summary

2m 24s

Projections

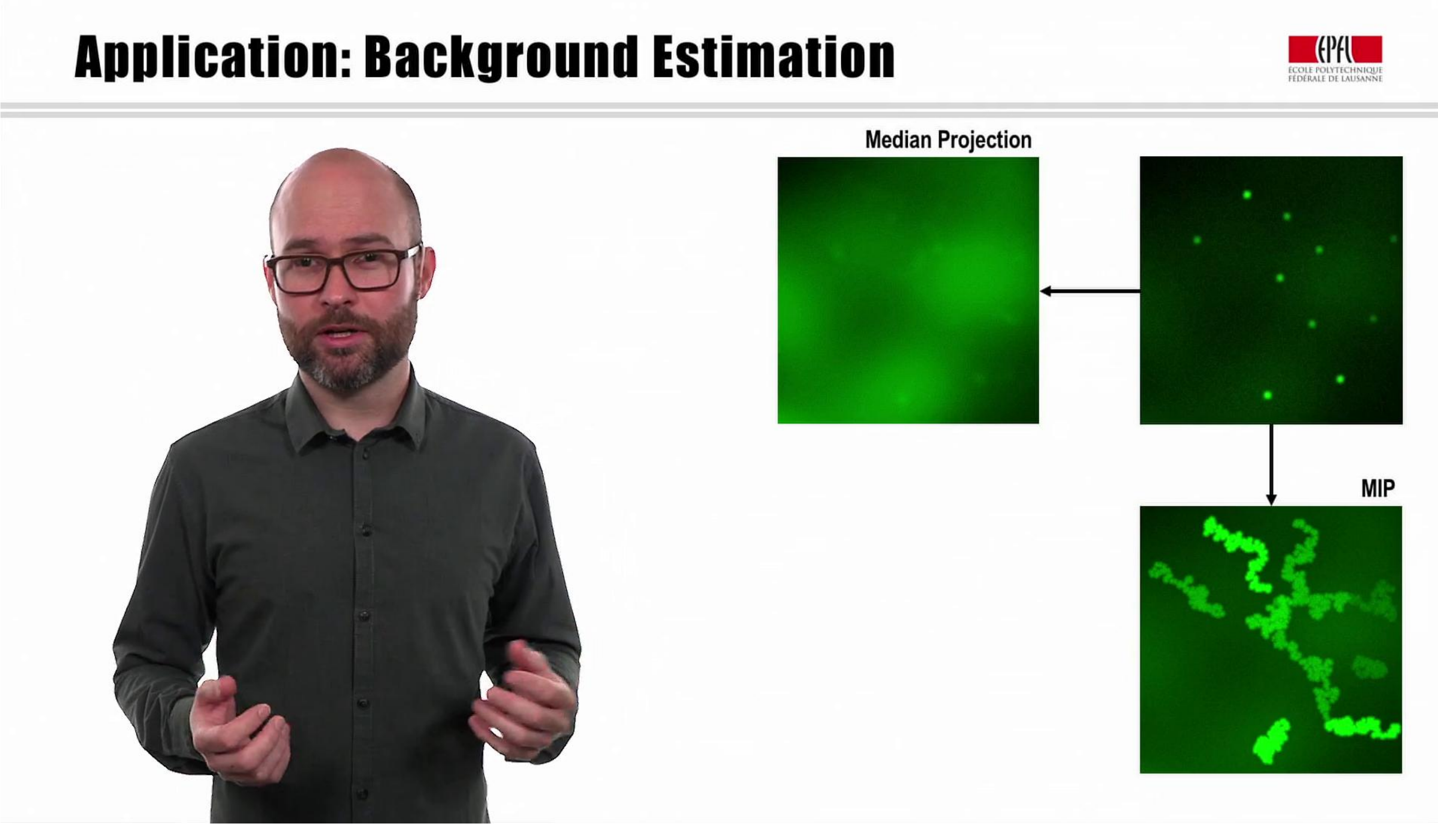


You provide it a stack, and it will perform the given operation on the given slice range.

Notes

Summary

2m 54s



Several operations are available from the scroll down menu Let’s look at an application for these methods. For example here, computing the max projection gives us a good idea of where the particles are. And we can observe their trajectories. But this is polluted by the uneven background of the acquisition. But if it is background, then, by definition, it is something that isn't moving or changing over time. The particles, however, they're moving through space, and during the movie, then each pixel is much more often a background pixel than a particle pixel. So a pretty good estimation of our background would be we the median projection, for example. We can then use this, and apply Image Math to subtract it to the original image; which will clean up our data.

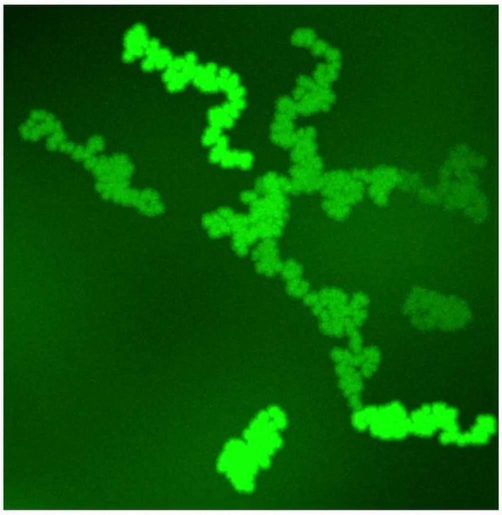
Notes

Summary

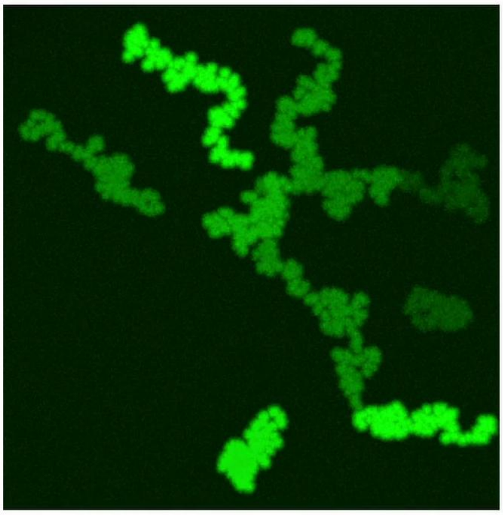
3m 00s

Application: Background Estimation

MIP



MIP
Corrected for Background

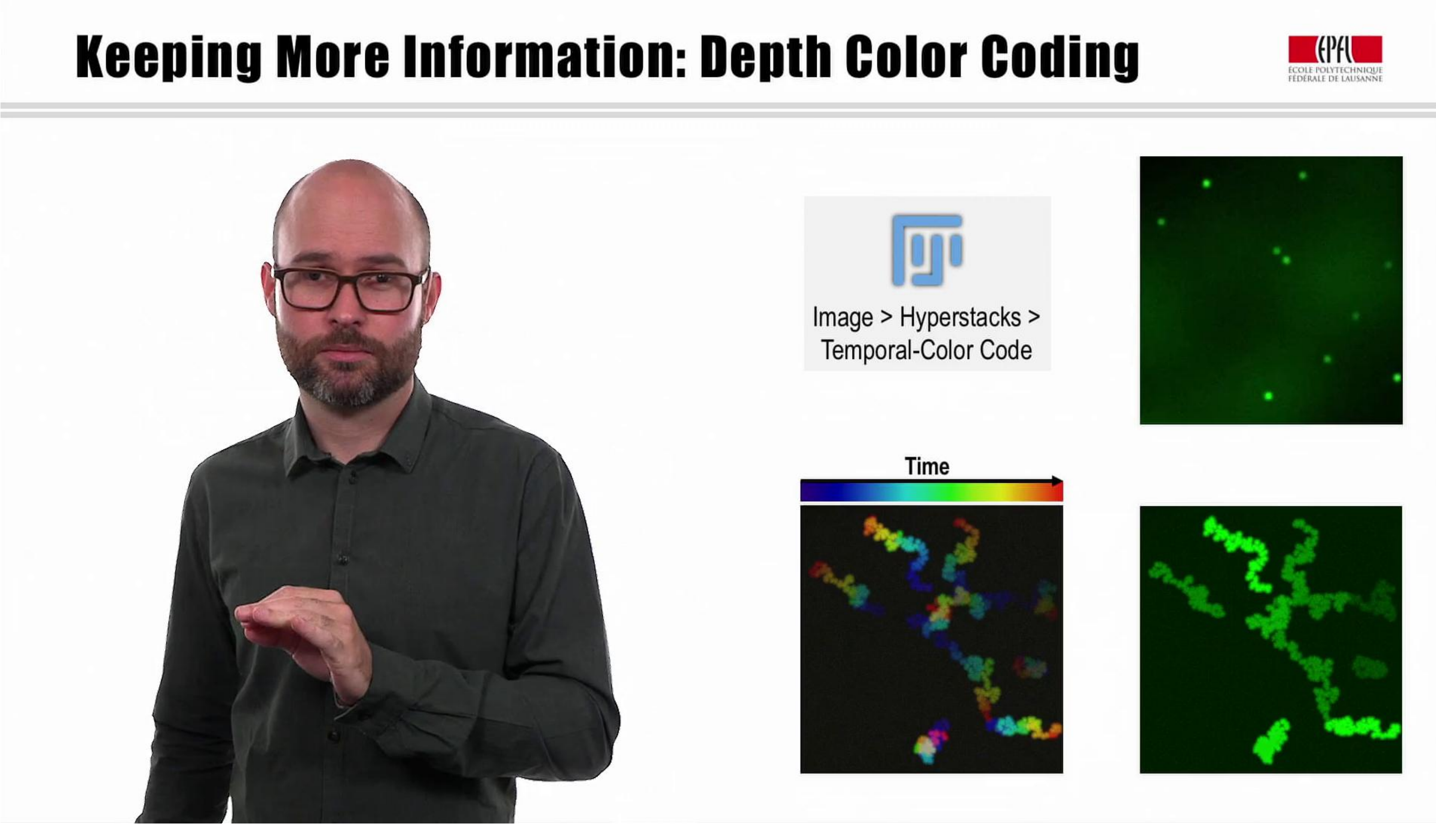


You can compare the change this does on our resulting maximum intensity projection.

Notes

Summary

3m 50s



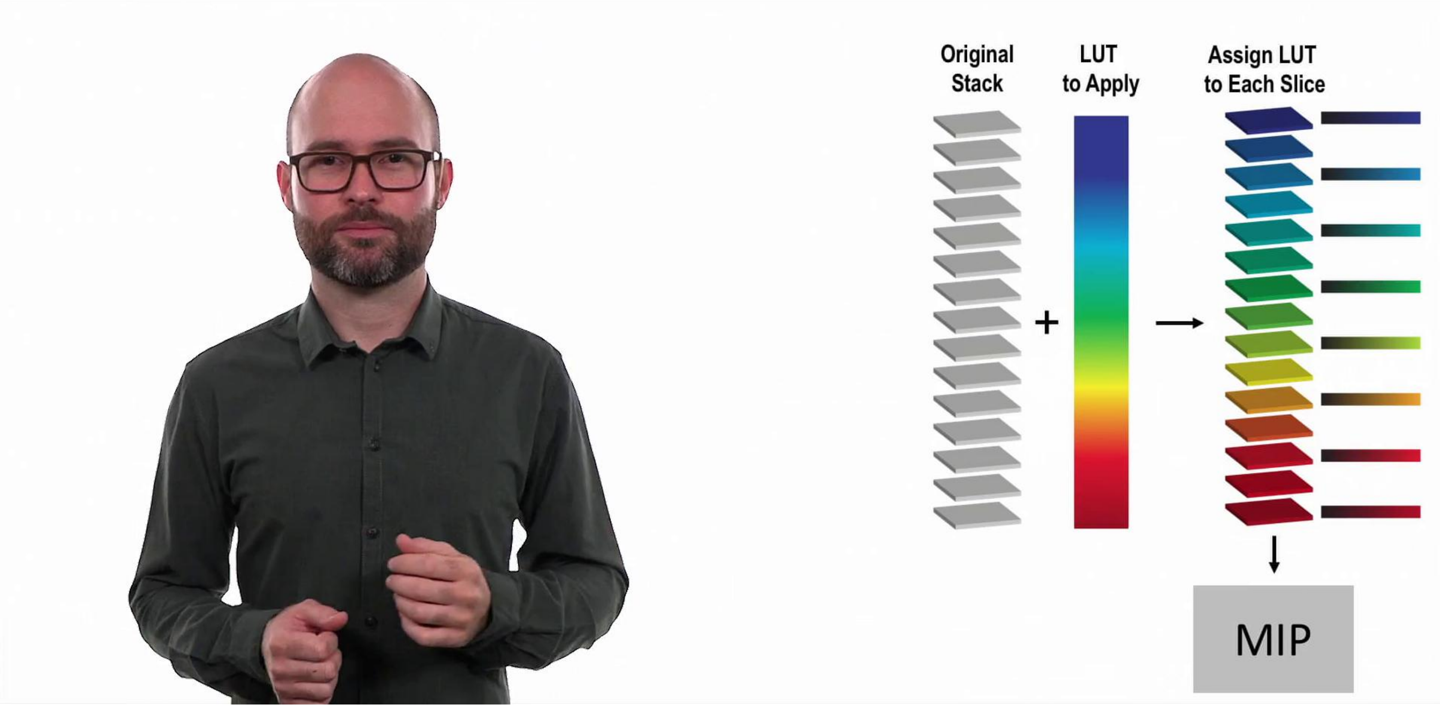
But if I were you, I would be a bit sad about what we have been doing so far. Because, basically, if our stack were composed of 100 slices, we have just thrown out 99 percent of our data by taking the max intensity projection! Indeed we are only keeping the value of one pixel amongst the 100 available to us for each x y position in the stack. So, ideally, we would like to try and maximize the amount of information we want to keep. In the examples here, there is one dimension that we have not made use of yet, that is still available to us. And that is Color. Fiji comes bundled with a command called Temporal Color code, which can apply a lookup table to each slice of your stack before performing a color max projection. So instead of getting something like what we've had before, where we've lost all time information, that is, where each particle starts and ends, we can get a much more detailed projection where we keep the directionality information of the particles. Here, using the Physics lookup table, we see where the particles begin their trajectories in blue and where they end in red. In cases like these it is important to include the lookup table that was used, in order to help people understand the image.

Notes

Summary

3m 55s

Keeping More Information: Depth Color Coding



Let's look at how this command works. It will take your original input stack, and the lookup table you would like to use. It will then assign a new lookup table to each slice of your stack. Which means each slice then has a lookup table that goes from black to the current color, based on the depth.

Notes

Summary

5m 11s

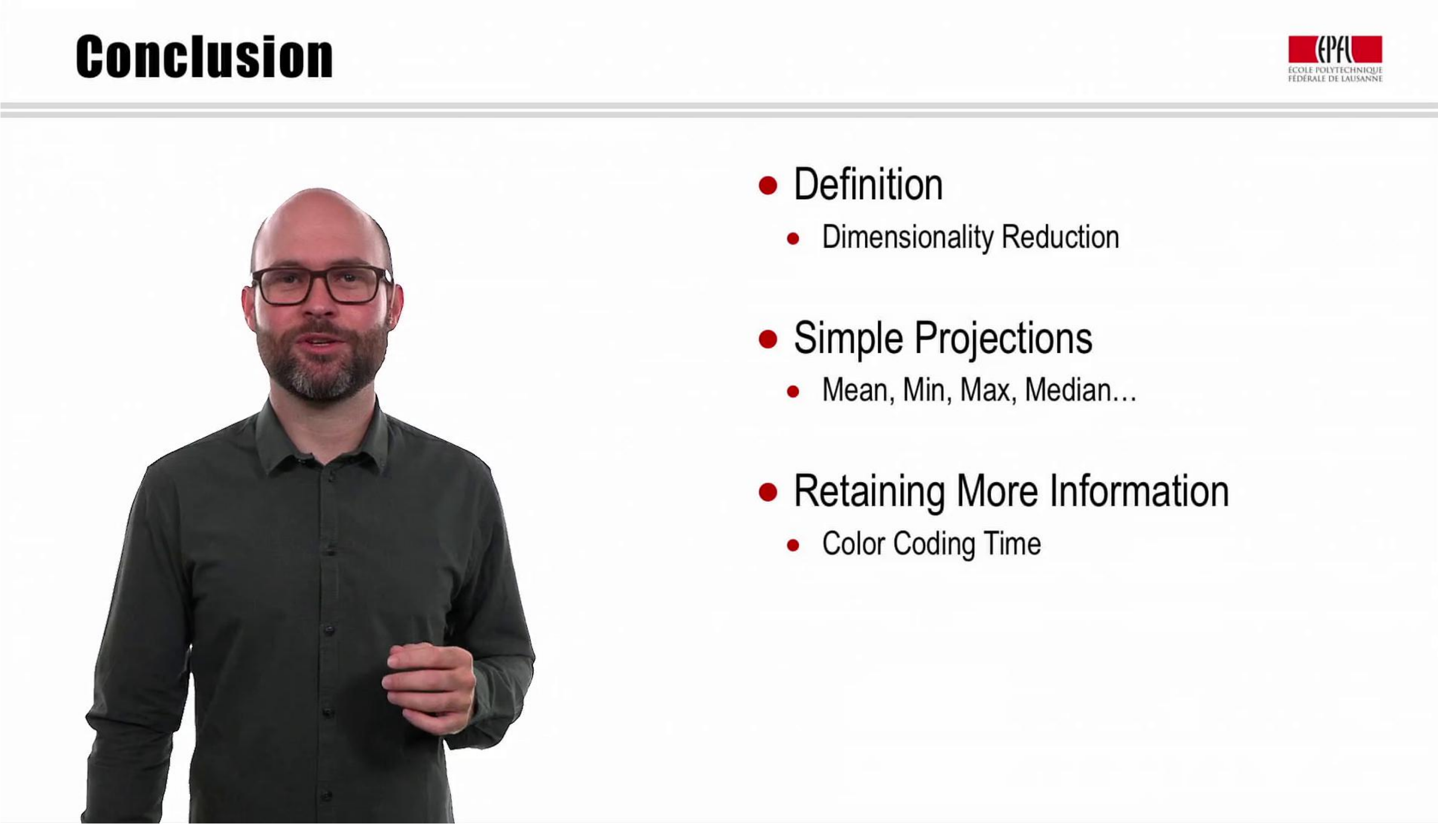
Good Resolution Leads to Useful Projections

The command then uses a maximum projection method to obtain the final image Projections are very powerful to help us interpret our data better by letting us see essential things at a glance. Like here, how we can infer the trajectories for each particle. But this only holds true for images where the sampling rate of your acquisition was high enough. Consider this case where only one time point out of four is kept before projecting. We can still see the trajectories and infer where the particles came from; But the moment we start having too dramatic a drop, in temporal resolution in this case, it becomes impossible to see which point belongs to which particle. Except for very isolated ones. This is actually the application of what you learned for spatial dimensions to the time dimension. What is true for space is true for time. as my colleague would say.

Notes

Summary

5m 33s



With that we are at the end of this video on projection methods. We have covered the simple methods available to us unsing Fiji, and covered the most interesting one, which is temporal color code. This concept, of trading one dimension for another (like here time to color) is a subject that will be covered more in detail when we will speak of reslicing methods. I hope you've enjoyed this video and look forward to seeing you in the exercises.

Notes

Summary

6m 27s