ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Proper Feature Size

Image Processing & Analysis for Life Scientists

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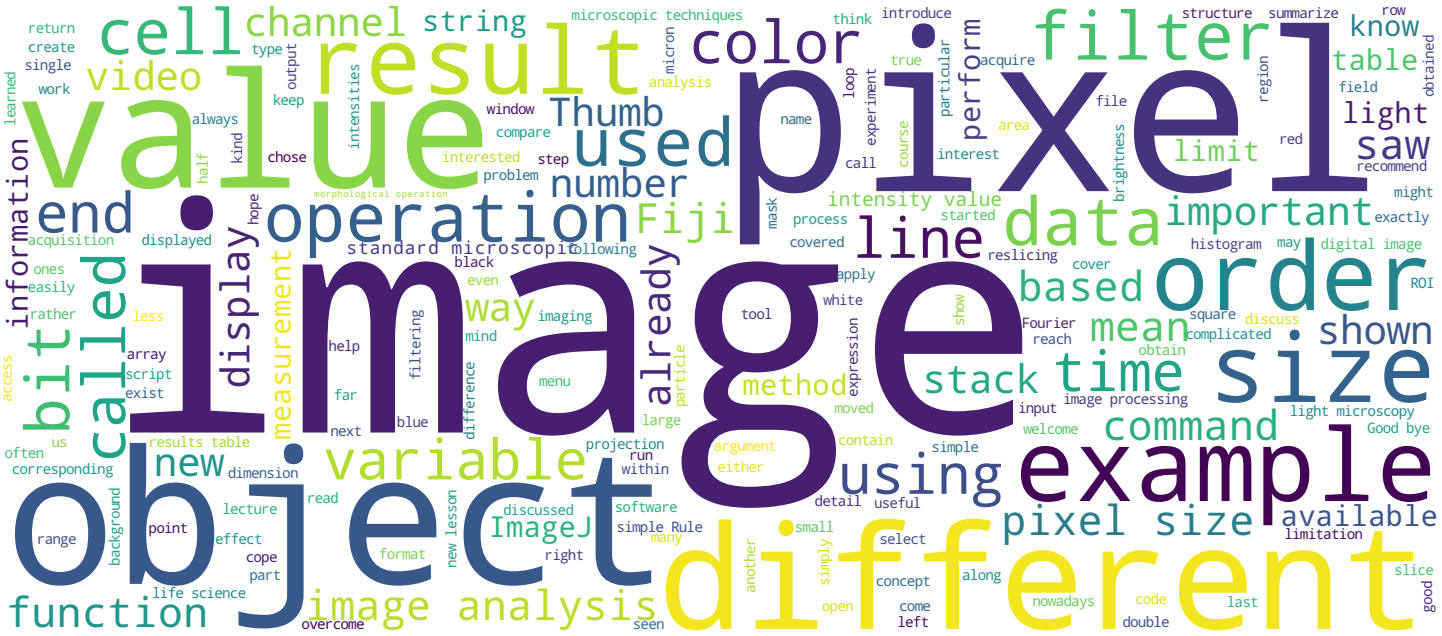

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Video

EPFL

Proper Feature Size

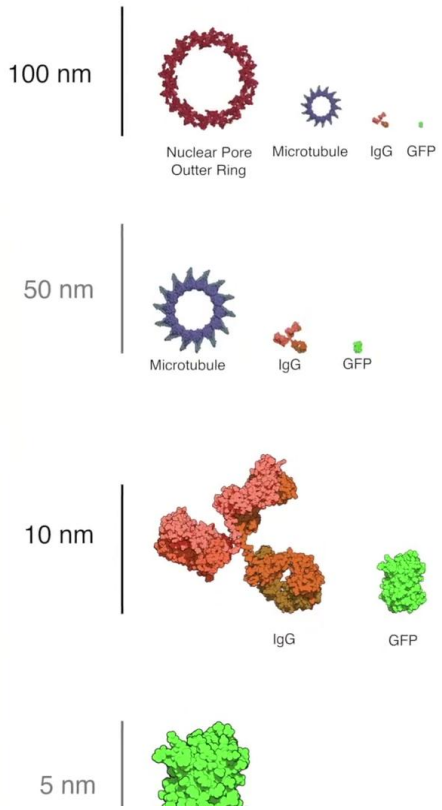
- Scales in Life Science
- Choosing Pixel Size
  - Rule of Thumb
- Limitation

Hi! And welcome to this new lesson during which we will see how to chose a proper feature size for image analysis. Today we'll have a quick look into the sizes covering several orders of magnitude we have to cope with in life sciences. We'll discuss how to adapt the pixel size based on its object size, and based on the image processing question. Finally we'll see what are the limits of object detection in light microscopy.

Notes

Summary

0m 07s

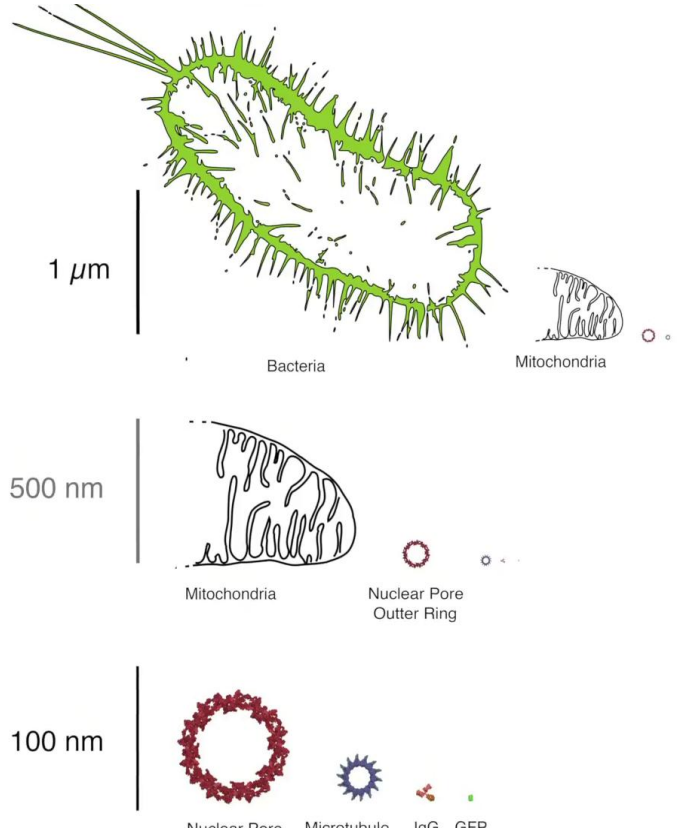


Let's take a virtual video trip to microscopic world. Atoms have a size a tenth of nanometer. They are form molecules like: adenosine tri-phosphate, the famous ATP. Amino acids. Which are the basic building blocks of proteins. Proteins like immunoglobulin, or fluorescent proteins belong to the nanometric scale.

Notes

Summary

0m 35s

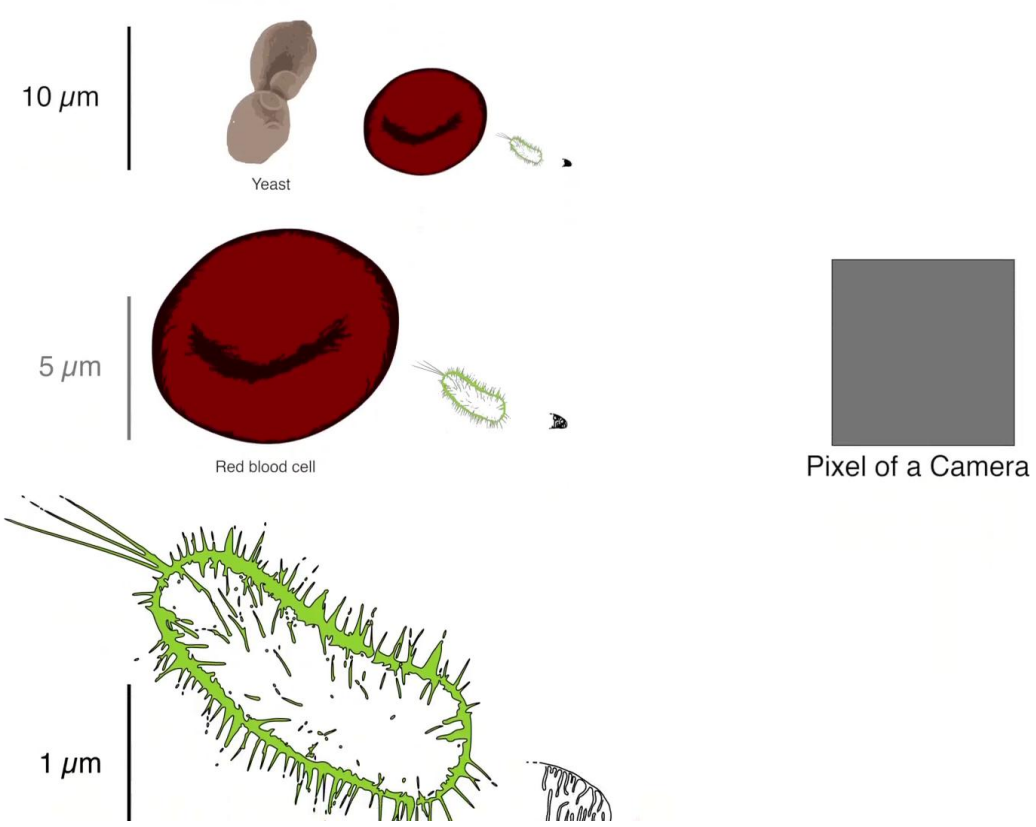


These proteins may also form larger structures like microtubules or Nuclear Pore complex. These structures can be associated to some organelles of cells from various size.

Notes

Summary

0m 58s

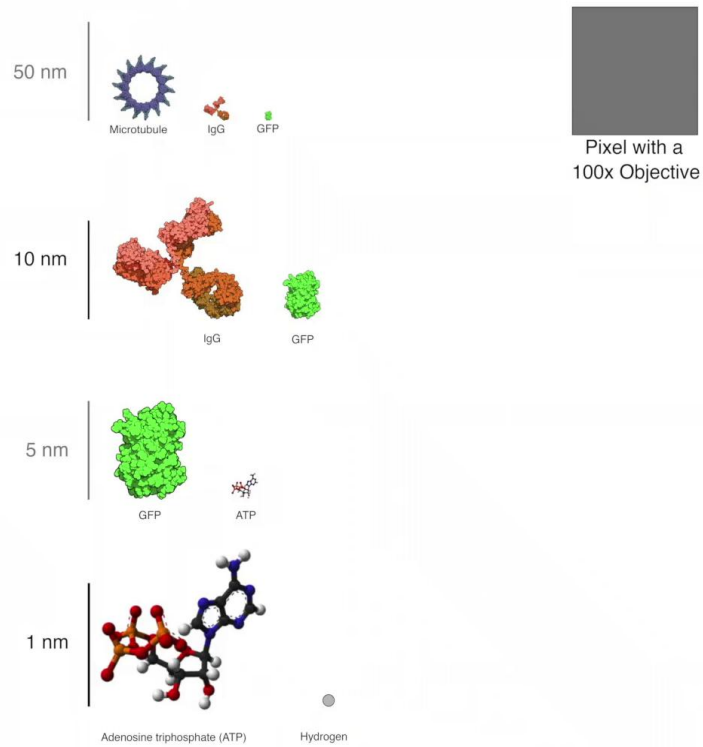


Now we can compare this to the physical size of a pixel on a common camera chip; and look at the effects on some magnification tools.

Notes

Summary

1m 13s



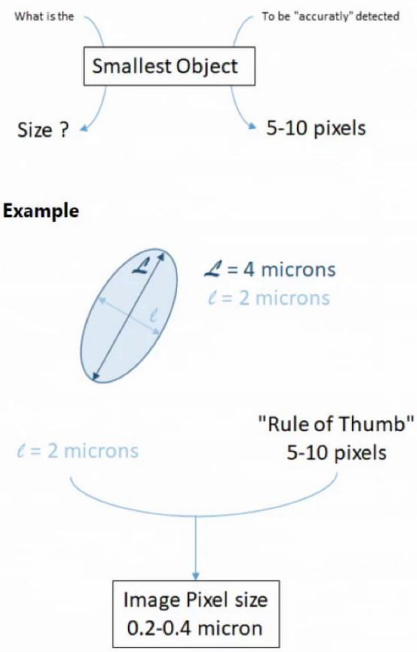
The ojectives of the microspcope allow us to get smaller pixels size.

Notes

Summary

1m 24s

Rule of Thumb



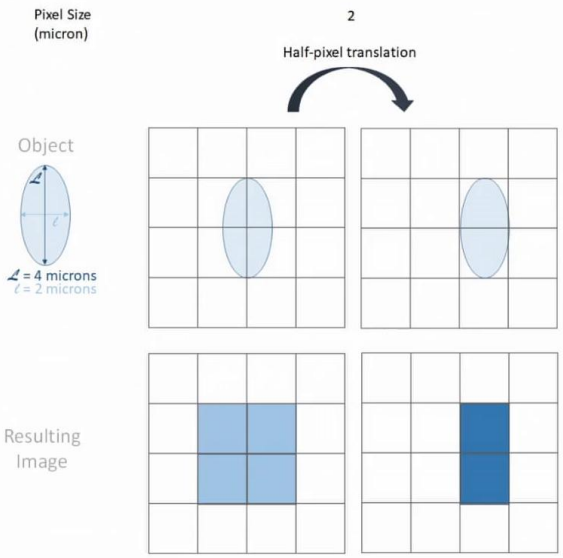
So, to be able to efficiently detect and analyse objects in your image it is useful to think about what is the size of your smaller object of interest. Then a good Rule Of Thumb is to define this object using between 5 to 10 pixels. Let use an example. Suppose we are interested by an object 4 microns long and 2 microns wide. The smallest lens is 2 microns. So we want to make these 2 microns fit inside 5 to 10 pixels. This means our pixel size should be somewhere between 0.2 and 0.5 microns per pixel.

Notes

Summary

1m 35s

Object Size & Intensity Measurement



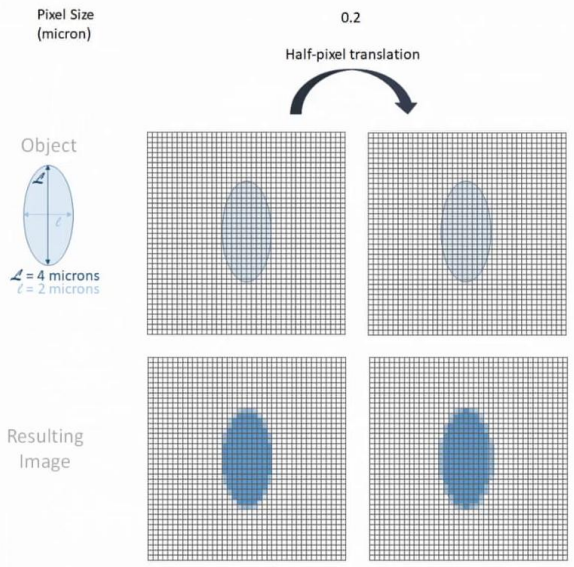
Why this arbitrary Rule Of Thumb? Here is a simple demonstration. Let's take pixels that are way larger, like 2 microns per pixel. If we try to acquire an image of this object we see on this squetch that if the object were moved by only half a pixel its size seems to double. Its average intensity drops because the object get spread over all the pixels.

Notes

Summary

2m 18s

Object Size & Intensity Measurement



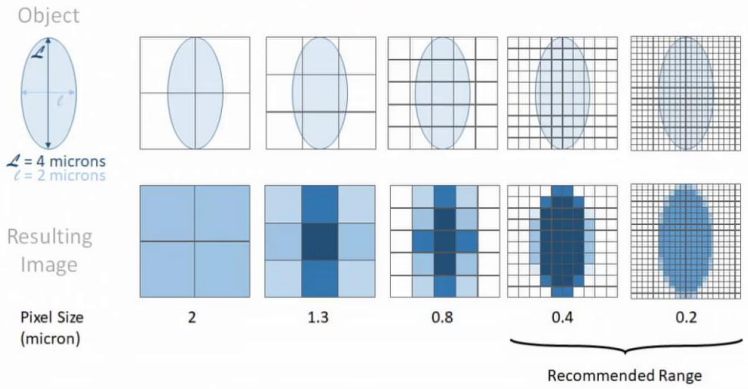
Now if we have pixels within the recommended range, you see that half a pixel translation affects the resulting image much less than before.

Notes

Summary

2m 47s

Recommended Sampling



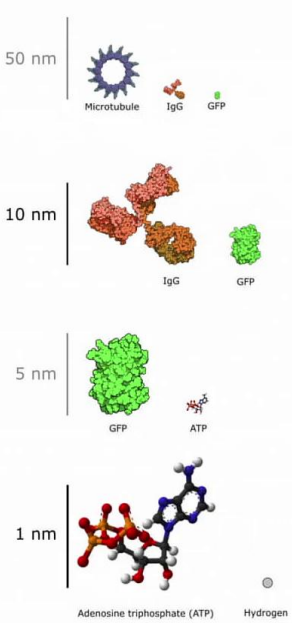
Of course this is a simple Rule Of Thumb which is a simplification of the Nyquist-Shannon Sampling Theorem.

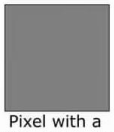
Notes

Summary

2m 57s

Limits





Unfortunately, it's a bit more complicated. As we saw a couple of minutes ago with a 100x objective on a standard microscope we can reach a pixel size around 50nm. Meaning we can accurately define objects up to 250 nm.

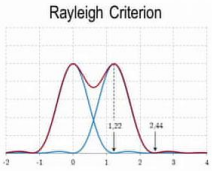
Notes

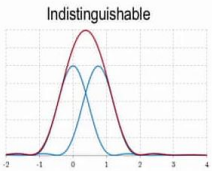
Summary

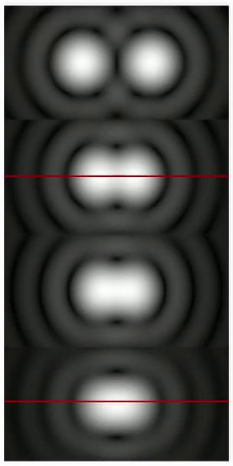
3m 06s

Limits

- Physical Law
  - The Abbe Diffraction Limit
  - 250 nm => 5 pixels = 50 nm / pixel







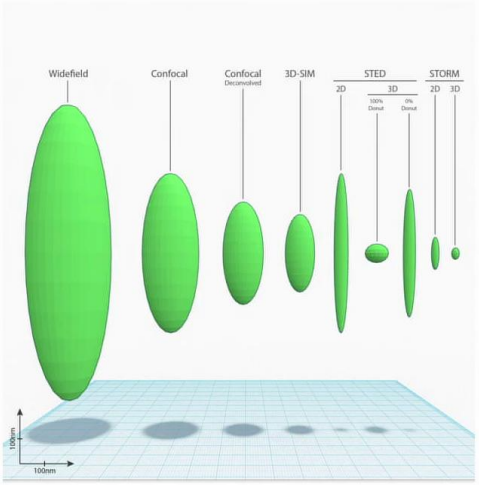
Which isn't in the range of Abbe diffraction limit: the maximum resolution standard microscopic techniques can achieve.

Notes

Summary

3m 24s

Super-Resolution



Fluorescence nanoscopy in cell biology.
Sahl SJ, Hell SW, Jakobs S.
Nat Rev Mol Cell Biol. 2017
PMID: 28875992

Why do I say standard microscopic techniques? Because nowadays we have access to some cutting edge techniques that combine knowledge from chemistry, mathematics, physics to overcome the physical laws of light and get access to super resolution imaging. I will not get into too much details. But for those interested by the field, we recommend you to read this publication.

Notes

Summary

3m 34s

Conclusion

- Scales From Proteins to Cells
- Recommended Sampling Rate
- Limits
  - Staying Rational
  - Super-Resolution Microscopy

This is already the end of this video. We saw the wide range of sizes in life science and the limits of standard microscopic techniques. We learned a simple Rule of Thumb to know which pixel size to use based on the size of an object. Finally, we quickly saw the origins of the limitations we have to cope with in light microscopy. And that interesting techniques exist and push beyond these limits. Thank you. Good bye!

Notes

Summary

4m 01s