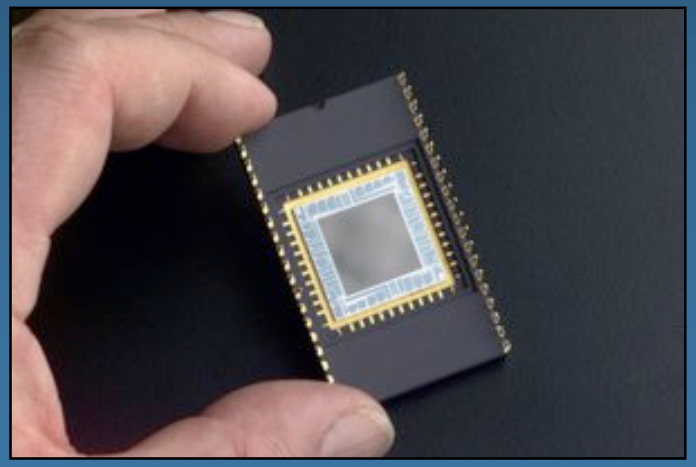
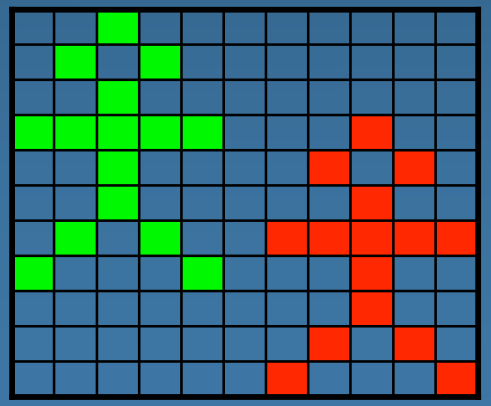
Light Microscopy Course 2008

“Digital” imaging
“Quantitative” imaging



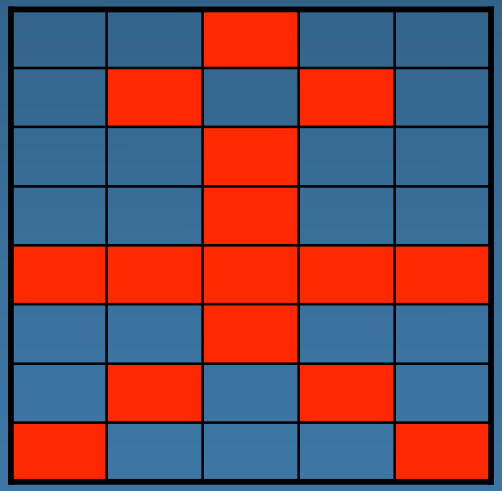
Quantitative Image Analysis?
…what does that mean?

- Pretty pictures are great for journal covers...
- Movies are great for visual presentation of images...
- Interactive 3D visualisation, data exploration...
- But for meaningful biological conclusions...
  - Scientists need numerical results from image data
  - Need to measure many objects
  - Need statistics from many images
  - Computers become useful!



What is an Image anyway..?

- An image is a representation of reality (not real)
  - Image of a point is not a point (Point Spread Function)
  - Pixelated by detector (CCD or point scanner)



A digital image of ???

Image Analysis
(Brain or Computer)

A stick man?
How do I know?
How can computer know?

What is an Image anyway..?

- Images contain information!
  - (not just pretty pictures)
    - Image data can be quantified / measured / analysed
    - Manipulate Image = Changed Info
      (Brightness / Contrast - Extreme Caution!!!)
    - Lost information - lost forever!
    - Meta data (What, Where, When, How)

A digital image:
How many objects? How “bright” is it?
How big is it? What is it? etc.

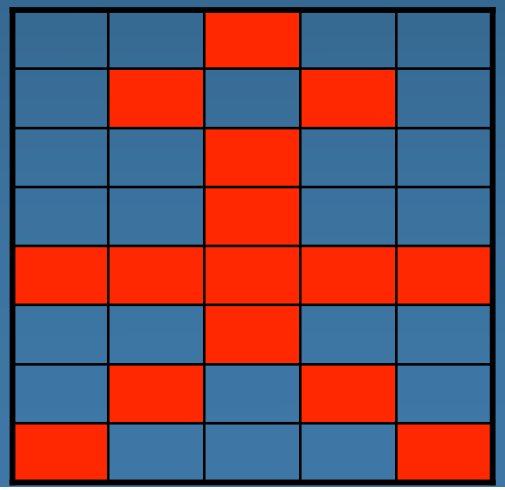
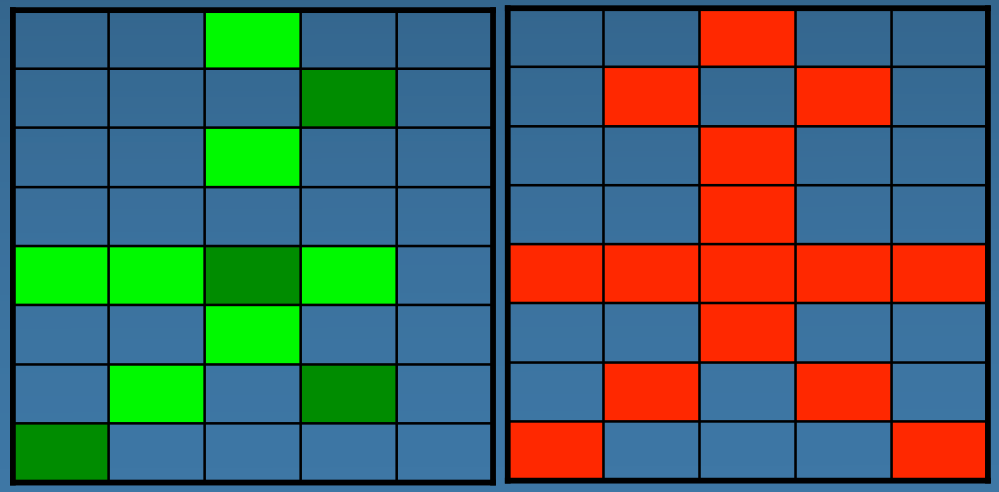


Image Data? What is it?

- Intensity is related to what? Something physical?
  - Dye concentration? Or is it? Why not?
  - Noisy Images? Averaging? Pixel Time?
- Comparison of 2 colours/dyes - Biology / BioChemistry / Interaction ?
- Shapes, Movement, Structure?

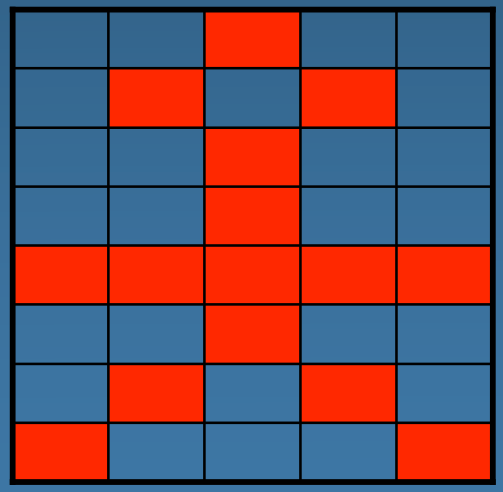
A digital image
With 2 channels / colours

What can you say here?



Photographer or Spectroscopist?

- We can show you how to
  - take pretty pictures (Art)
  - get useful information (Science)
  - make measurements (Quantitative Science!)
- You have to choose which you want to be!



This

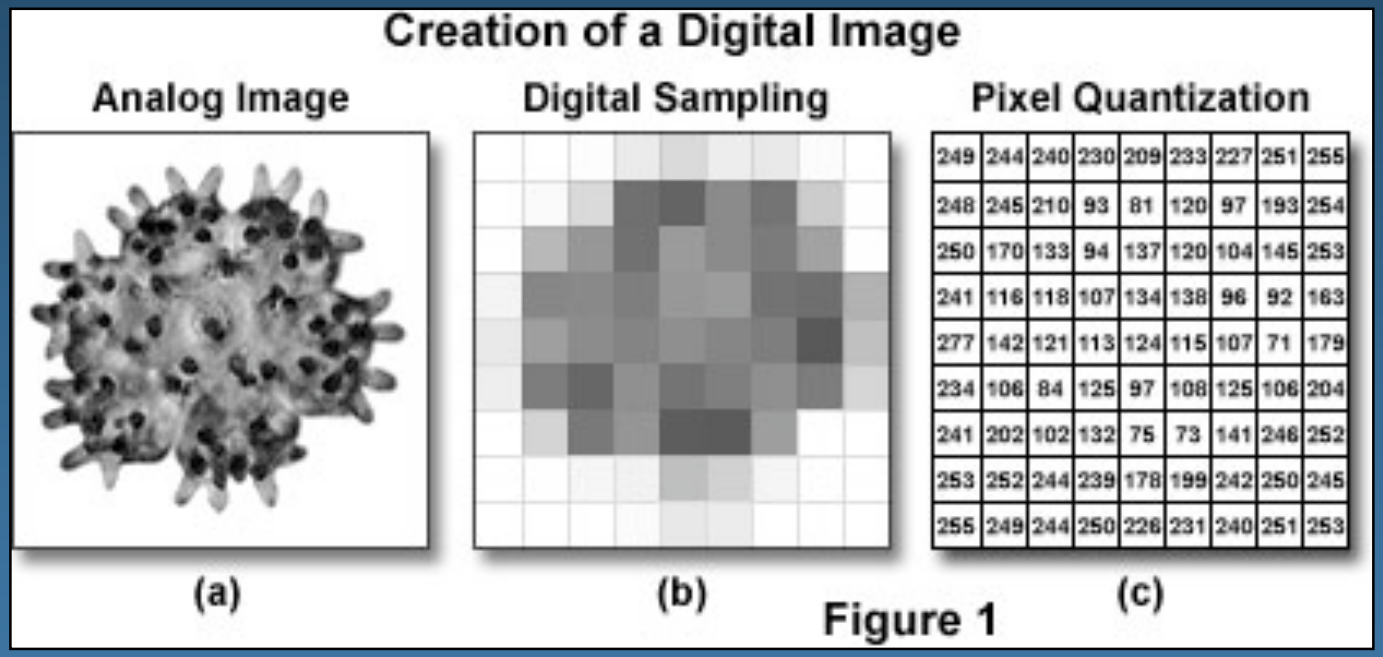
Is simply a way to "Visualise"

This

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

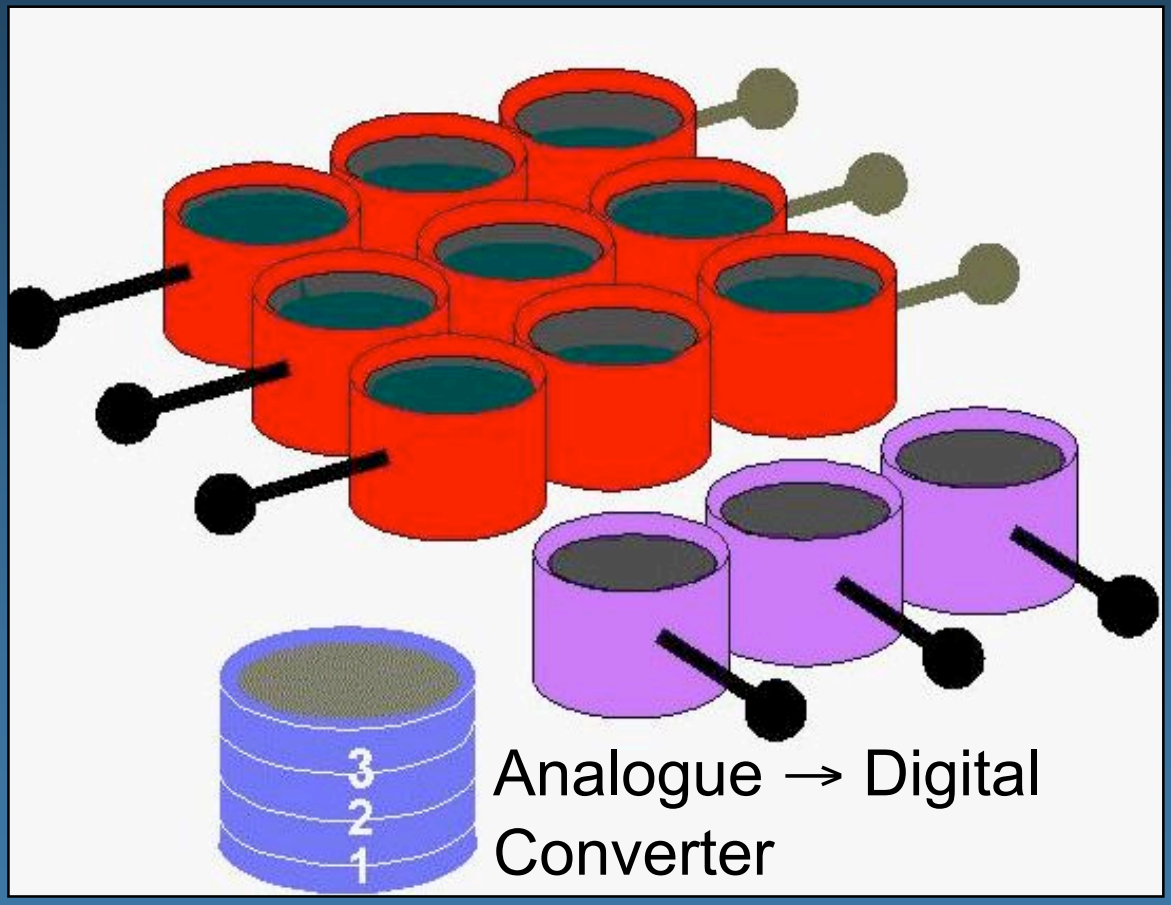
Digitisation

- Representation of an object, image or signal
  - by a discrete set of points or samples



Analog - continuous / Digital - discrete

Remember: the bucket brigade



Remember: the CCD bucket brigade

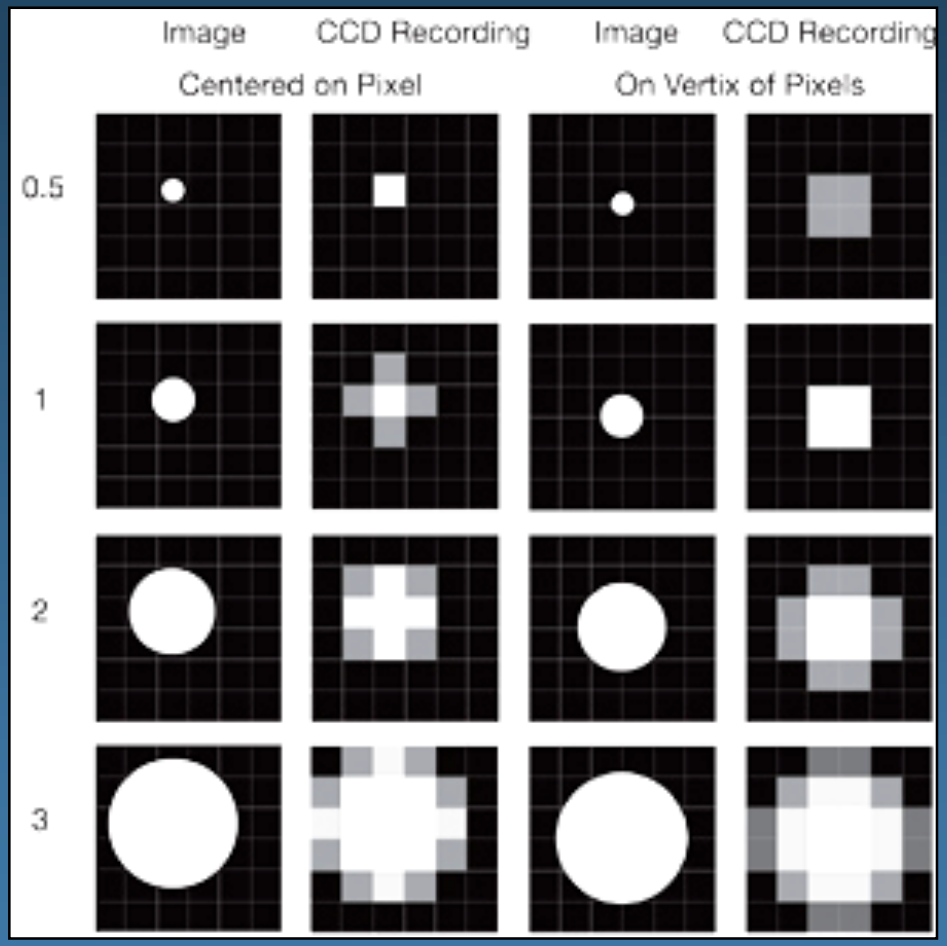
What do you digitize here?

SPACE

TIME

INTENSITY

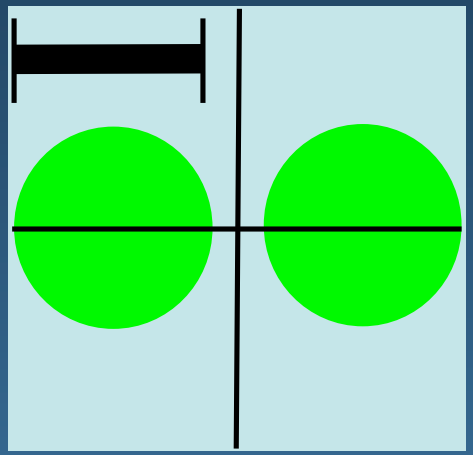
Digital spatial resolution

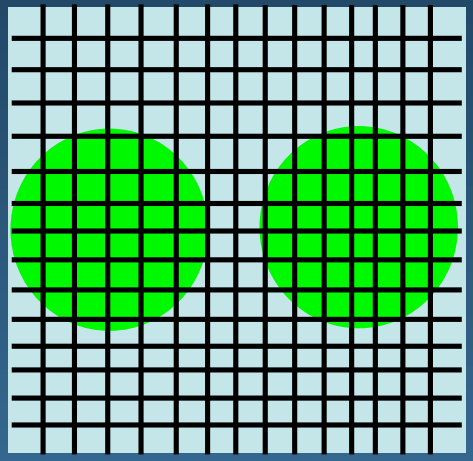


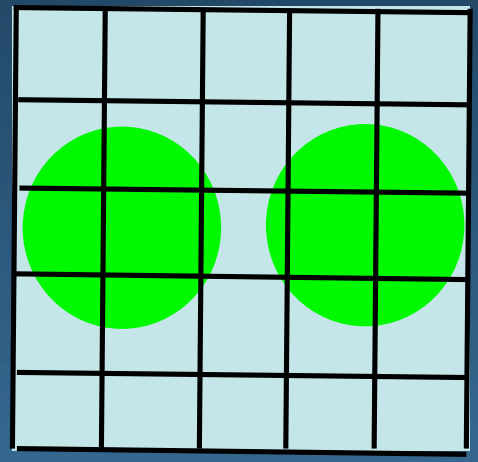
- Pixel size relative to projected image
- Image of object depends where it falls on detector

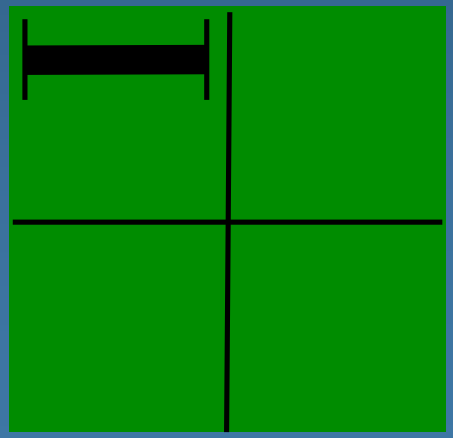
Digital spatial resolution

- Pixel size relative to projected image

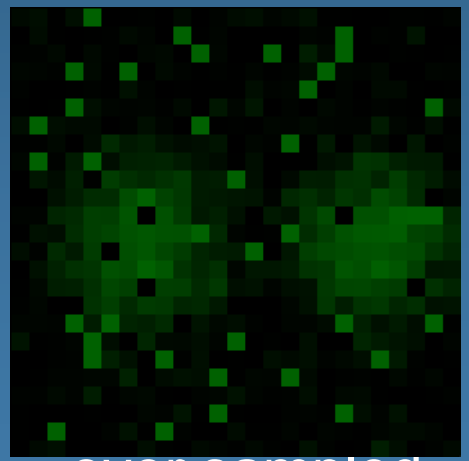




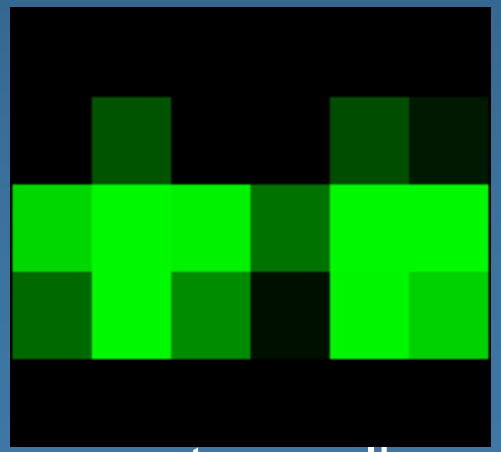




under sampled



over sampled

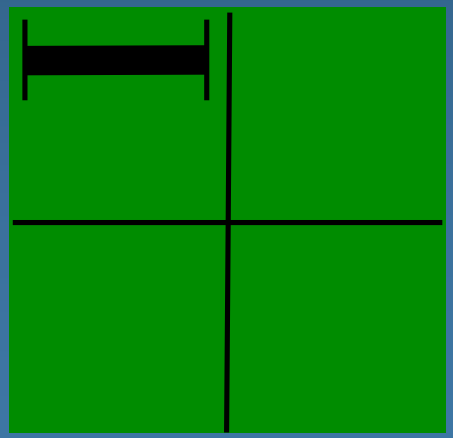


correct sampling

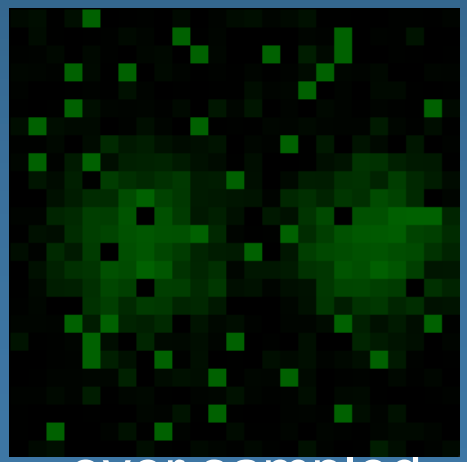
Pixel Size / Resolution

- "Correct" image size? (64x64, 512x512, 2048x2048)?
  - Get all information microscope can resolve, but files not too big
  - Proper spatial sampling (Nyquist sampling theory)
  - 2.3-3 pixels over optical resolution distance. (x, y and z)
  - Adjust "zoom", "binning" and image size (no of pixels).

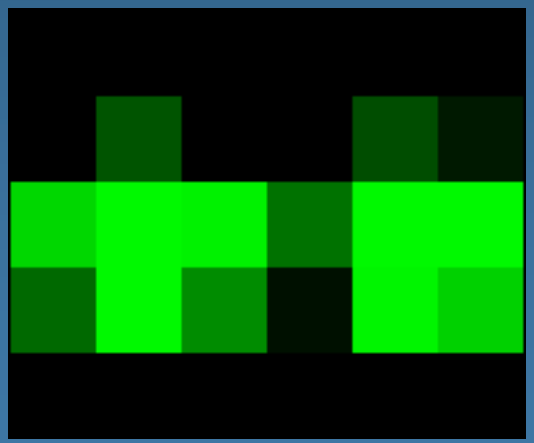
1 Airy unit



under sampled



over sampled



correct sampling

Harry Nyquist, 1889 - 1976

- Swedish - American
- engineer in telecommunications
- worked at Bell labs
- 138 US patents

Nyquist sampling criterion

- **General form:**

  Digital sampling frequency > analogue frequency x 2

- **Spatial representation:**

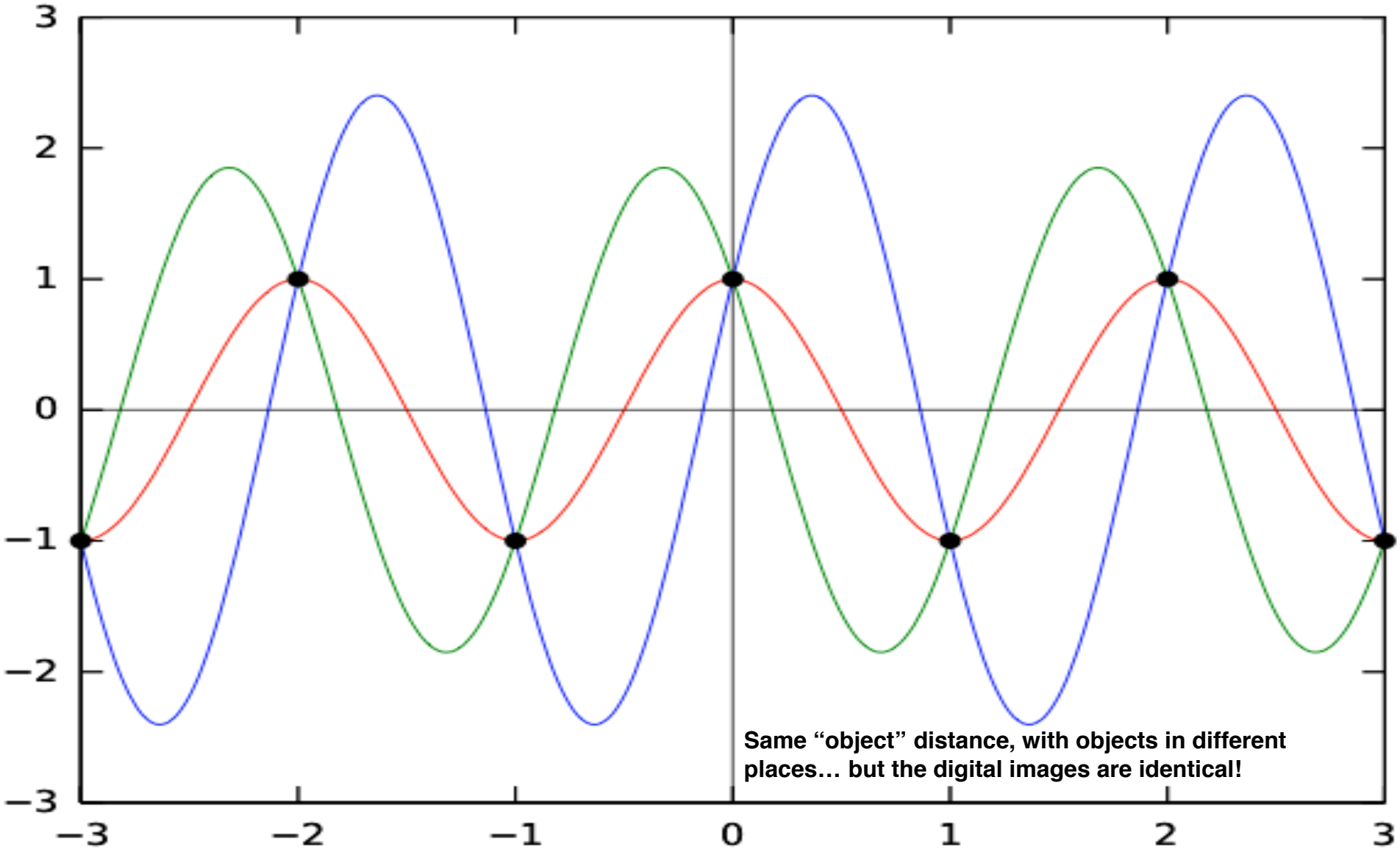
  Image pixel size x 2.3 <= smallest resolvable distance

- **Microscopy**

  Image pixel size x 2.3 <= optical resolution (d)

- **Aliasing - Moire patterns - info loss**

Nyquist sampling criterion



Nyquist sampling criterion

- example in imaging:
  - online demo:

Nyquist sampling criterion

- Resolution - pixel size calculations:

Objective (N.A.)	Optical Resolution limit (um)	Projected size on CCD (um)	Required pixel size (um)
4 x (0.20)			
10 x (0.45)			
40 x (0.85)			
60 x (1.40)			
100 x (1.40)			

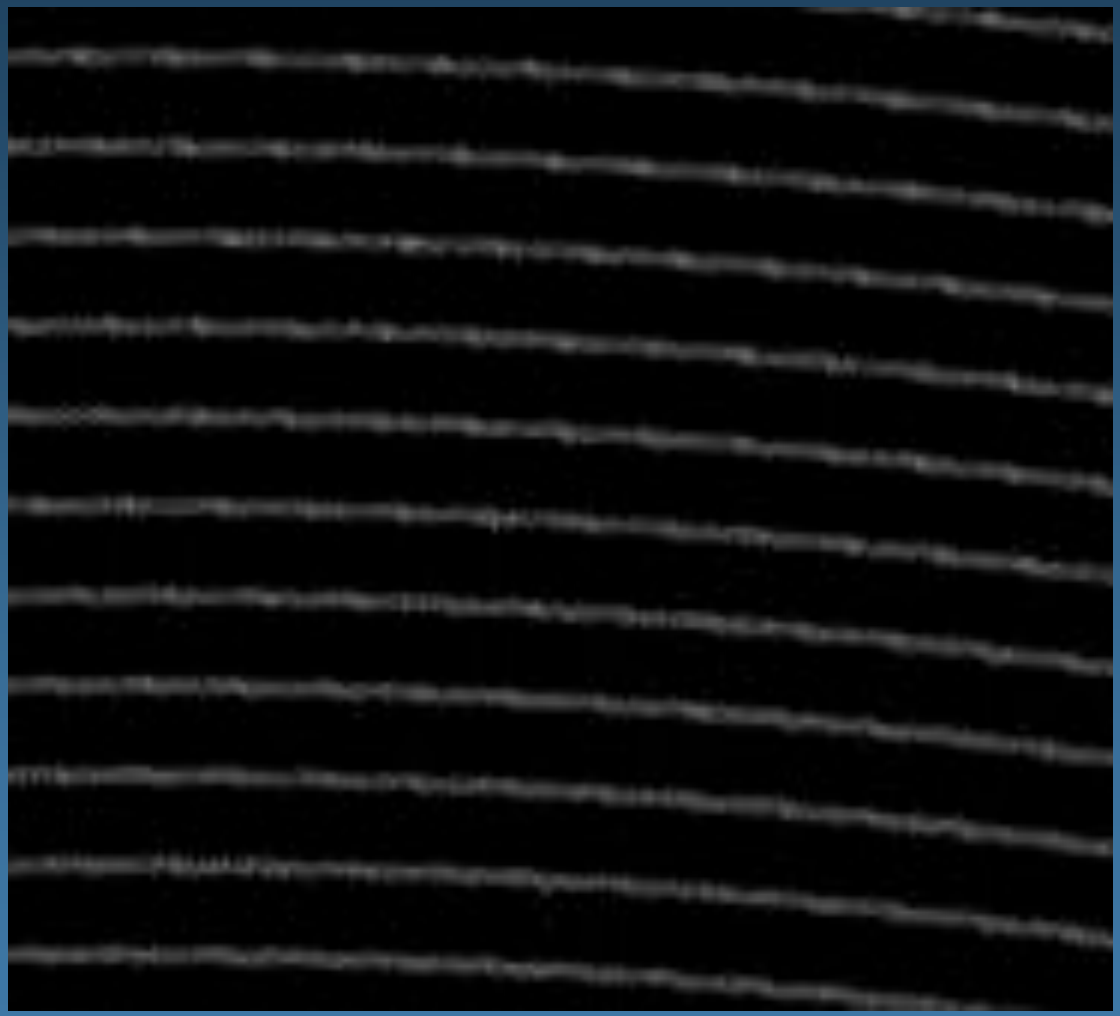
Nyquist sampling criterion

- Resolution - pixel size calculations:

Objective (N.A.)	Optical Resolution limit (um)	Projected size on CCD (um)	Required pixel size (um)
4 x (0.20)	1.30	5.2	2.26
10 x (0.45)	0.58	5.8	2.52
40 x (0.85)	0.30	12.24	5.32
60 x (1.40)	0.19	11.14	4.85
100 x (1.40)	0.19	18.57	8.07

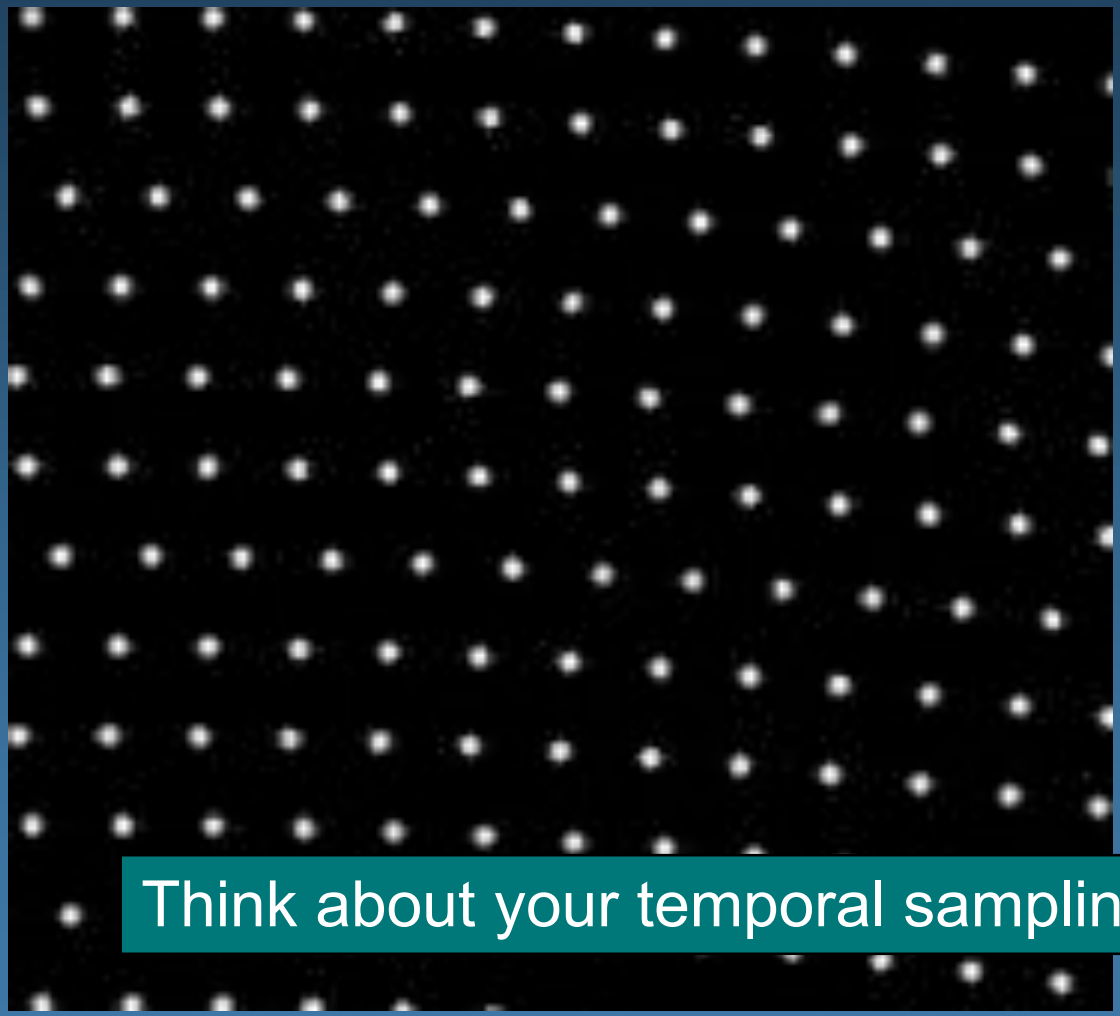
Think about your digital spatial resolution carefully!

Digital temporal resolution



- What could this image be?

Digital temporal resolution

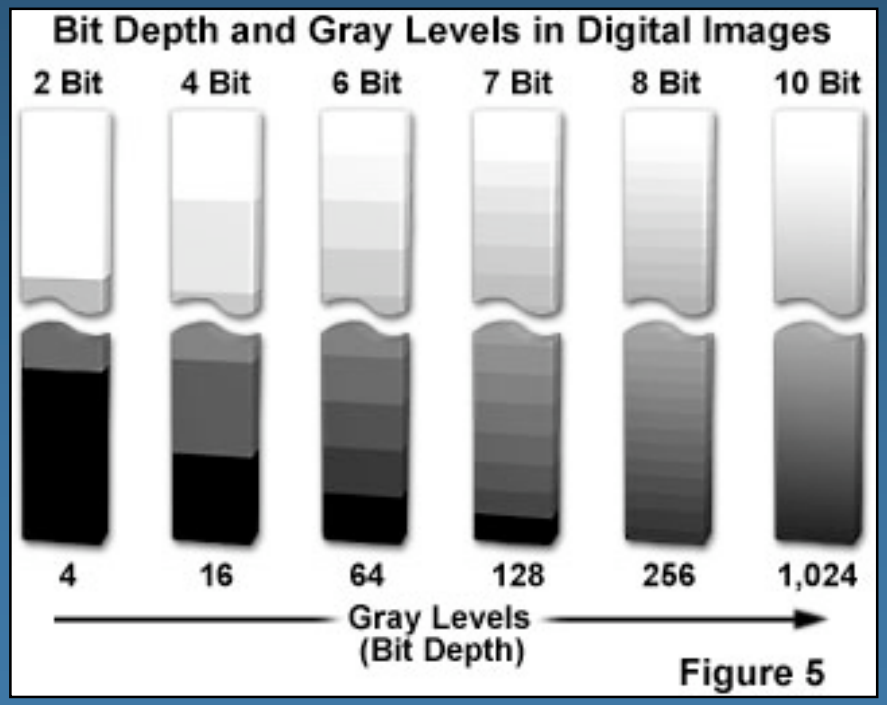


- What could this image be?
- A spinning disc full of holes!
- Consider speed of your trafficking vesicles...!

Think about your temporal sampling rate!

Digital intensity resolution

- Remember: bit depth, dynamic range and noise
  - Photon “shot noise” = square root of signal
  - Higher dynamic range = larger bit depth to resolve intensities



For a typical CCD:

$$\text{Best Possible Dynamic Range} = \frac{\text{Full Well Capacity}}{\text{Noise}}$$

- Signals within the range of the detector?
  - Your eyes lie! You can't see low intensities close to black!
    - Use Range Indicator / HiLo / OU and spectrum CLUTs
  - Adjust so brightest part is within detector range.
  - Remember to check z dir. also.
  - Don't over expose the image! Why not? Lost Info!

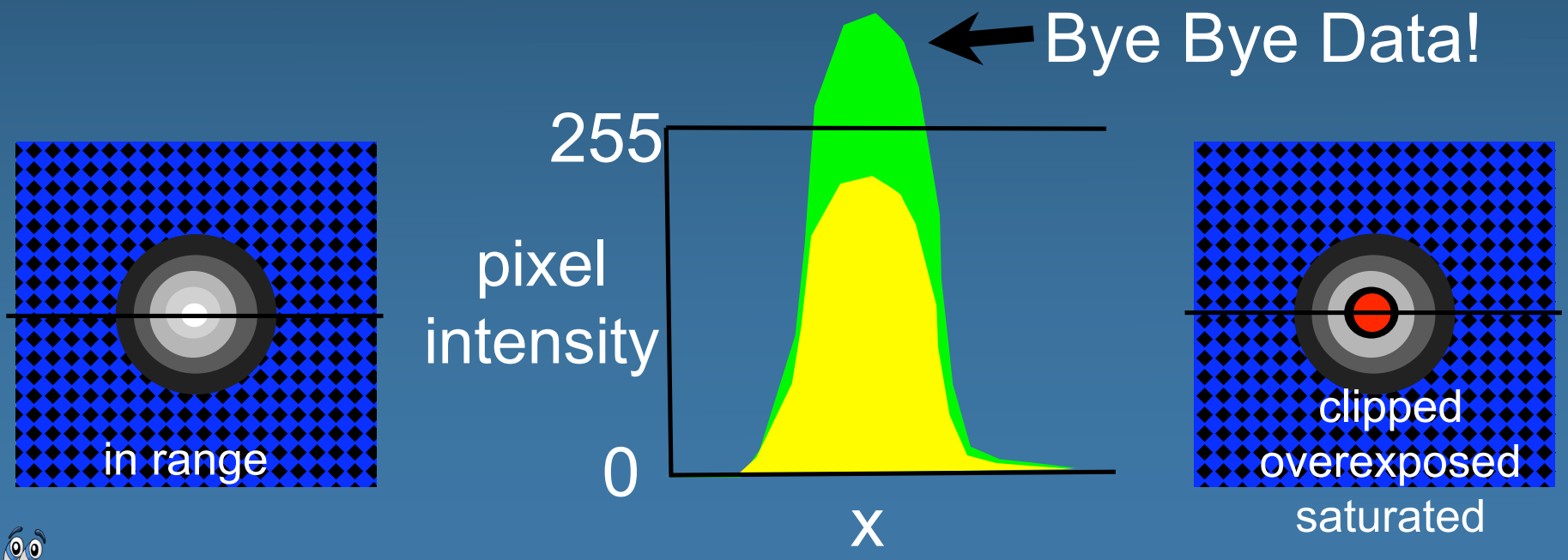
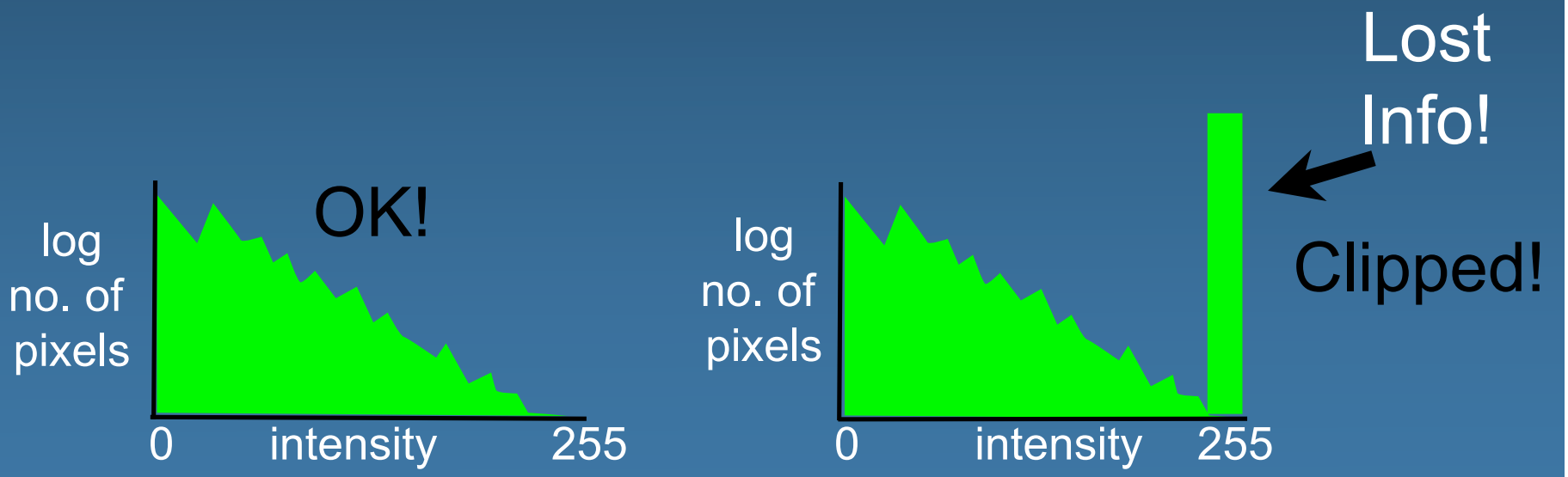


Image Intensity Histograms are your friends!

Use them!

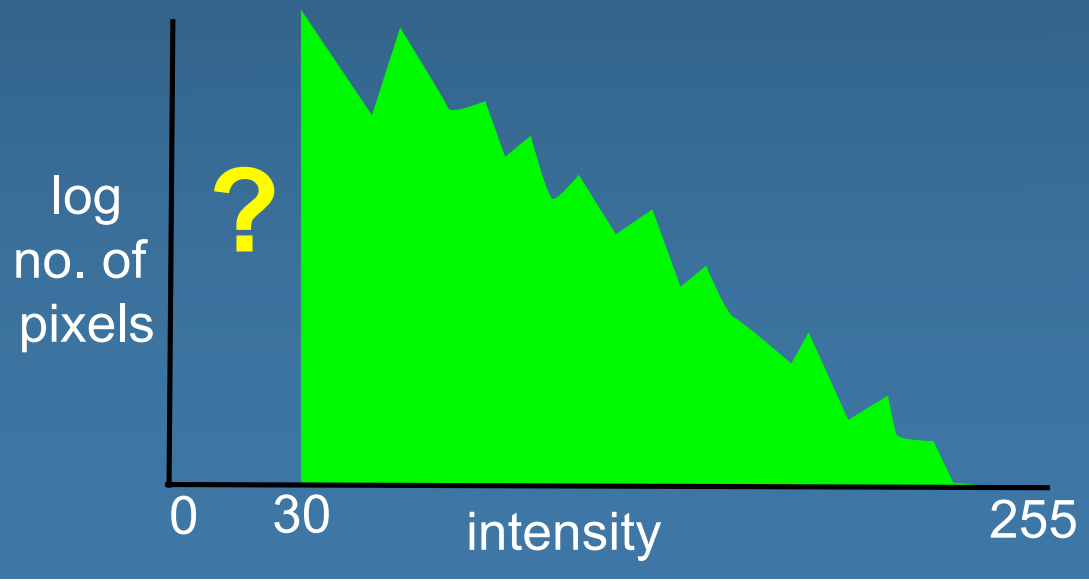


- What is “Background”?
  - Background Correction - Needed?.
  - Why? “background” close to zero, but keep low intensity info
    - What is “Background”? You decide!
    - Camera noise, unspecific staining, etc.

What is that Gap in the intensity histogram?

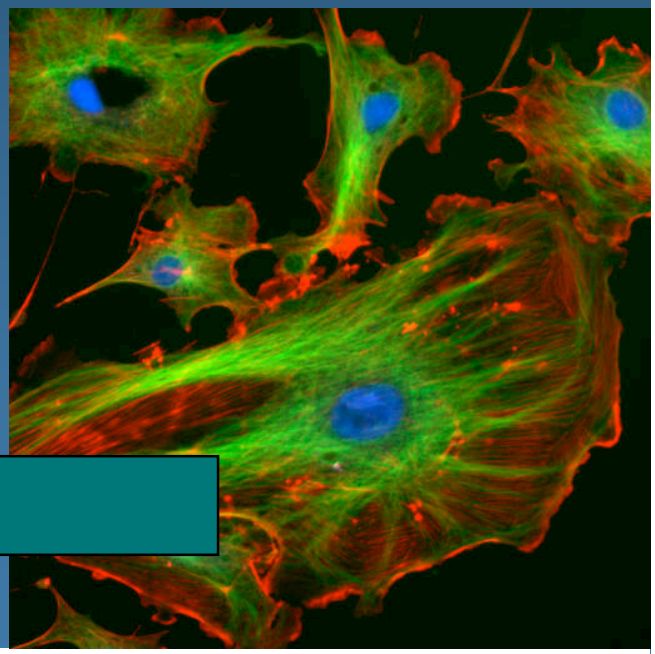
Background… Image intensity data starts at 30

So 30 = 0 !!!



Quantitative Microscopy - First Think...

- Choosing experimental and image processing methods:
  - What **BIOLOGY** am I trying to measure?
  - Do I need 3D, 4D, xD information? Resolution?
  - Choose / Optimise microscope system!
  - Statistics!
    - How many images / data points / etc?
  - **Controls!!!**



... and remember Nyquist!!