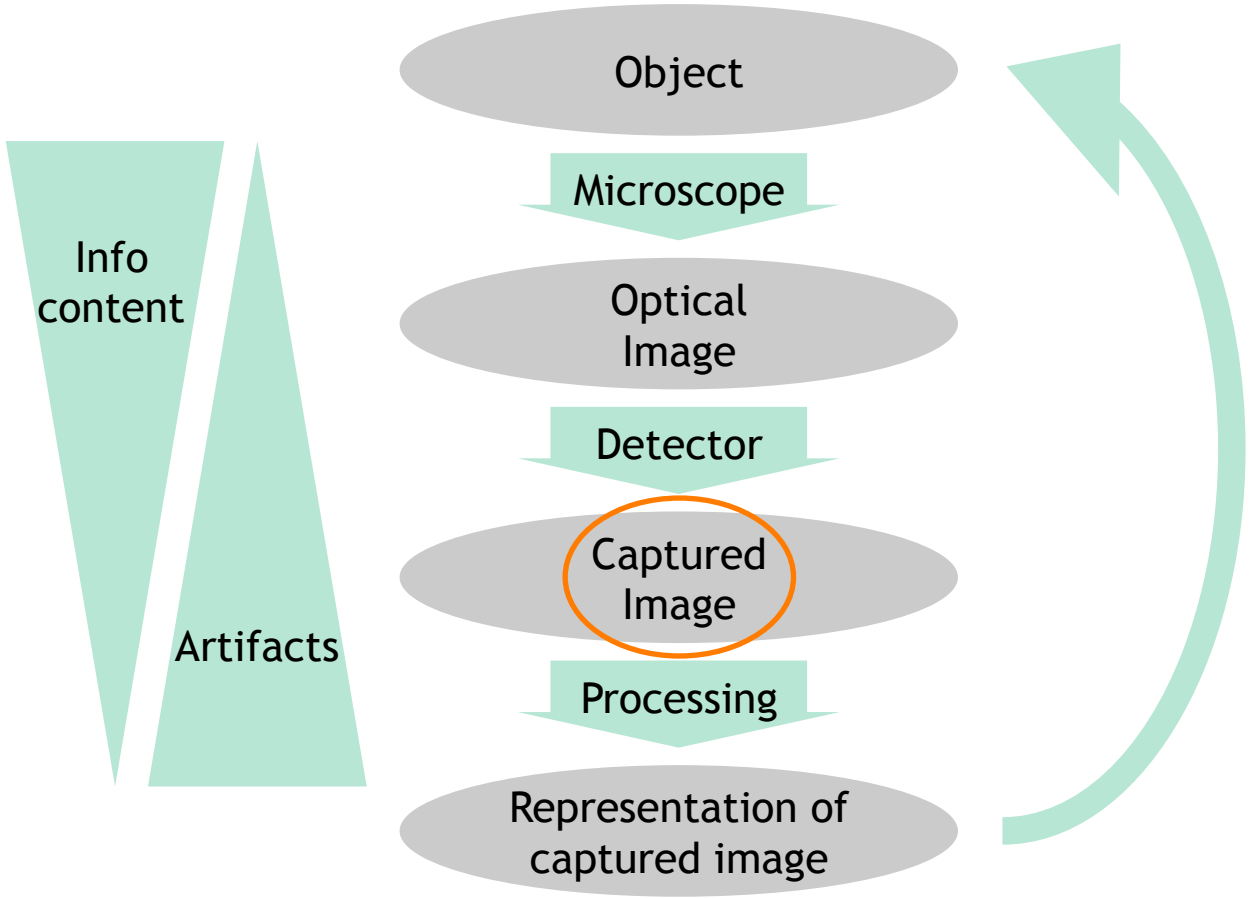

Recording digital images
considerations before even touching the microscope
http://doktorspinn.com/wp-content/uploads/2013/04/digital-tunnel-wallpaper1.jpg


ZEISS
C-APOCHROMAT
63x/1,2 W Korr UV-VIS-IR
421787-9970
0,15
0,17
0,19


ZEISS
C-APOCHROMAT
63x/1,2 W Korr UV-VIS-IR
421787-9970
0,15
0,17
0,19

Goal of the imaging workflow is to extract information about an object of interest



Extract information about the object of interest

=

Interpretation of representation of captured image knowing the formation process of this representation

We are looking for a highly sophisticated format to capture images

We offer: beautiful optical images formed in state of the art microscopes

The **ideal candidate** must:

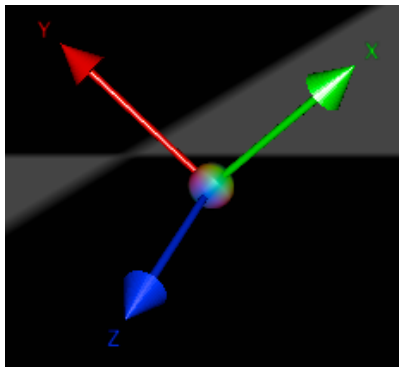
- **Preserve** as much **information** of the optical image as necessary
- Be **storable without changing information** content over time
- **Not** introduce **artifacts**

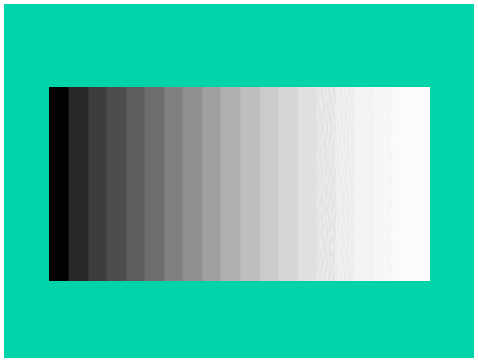
Use of an **efficient storage** medium (cost, space, material) and **convenient processability** are a strong plus

We are looking for a highly sophisticated format to capture images

What about digital images?

Digitization of an image means its conversion into an array of discrete values in different dimensions





Space

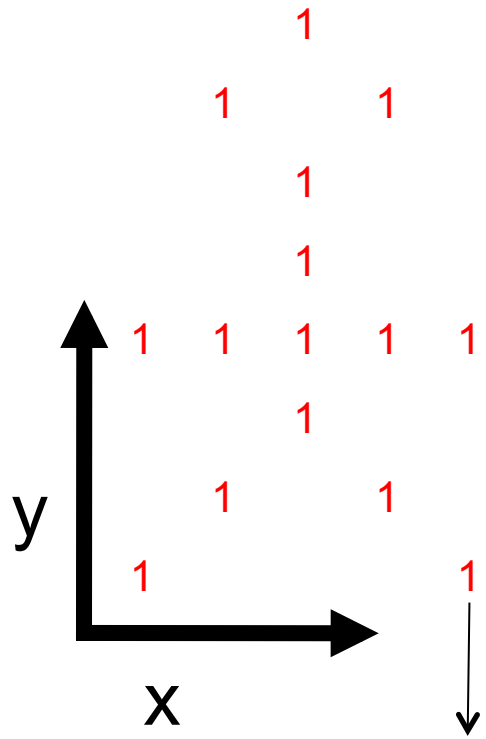
Intensity

Time

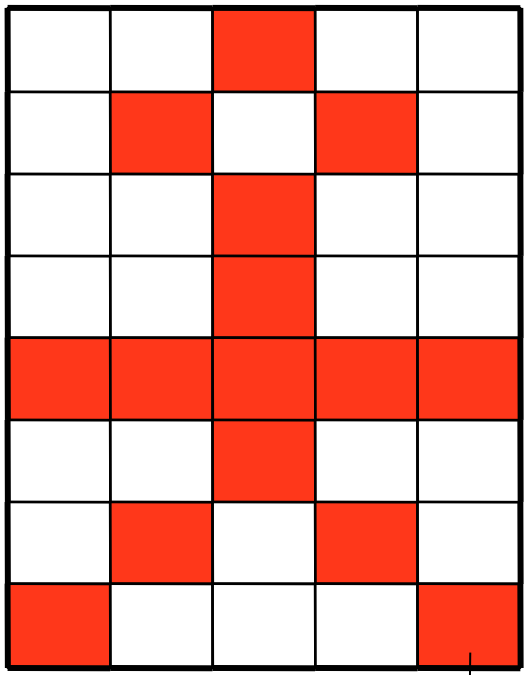
Digital Image

Digital Movie

Here you have an array of discrete values in 2-D space - a digital image

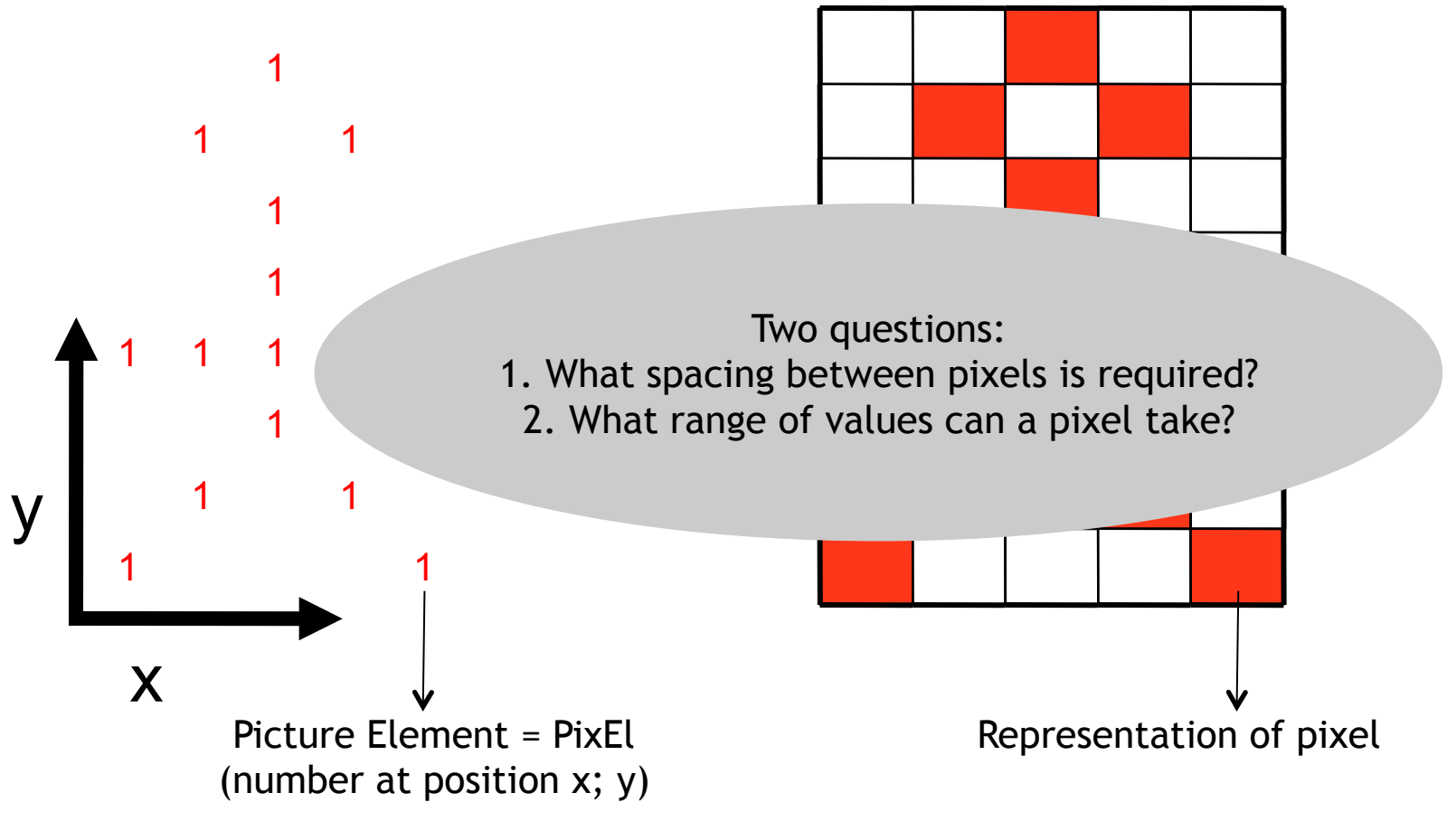


Picture Element = PixEl
(number at position x; y)

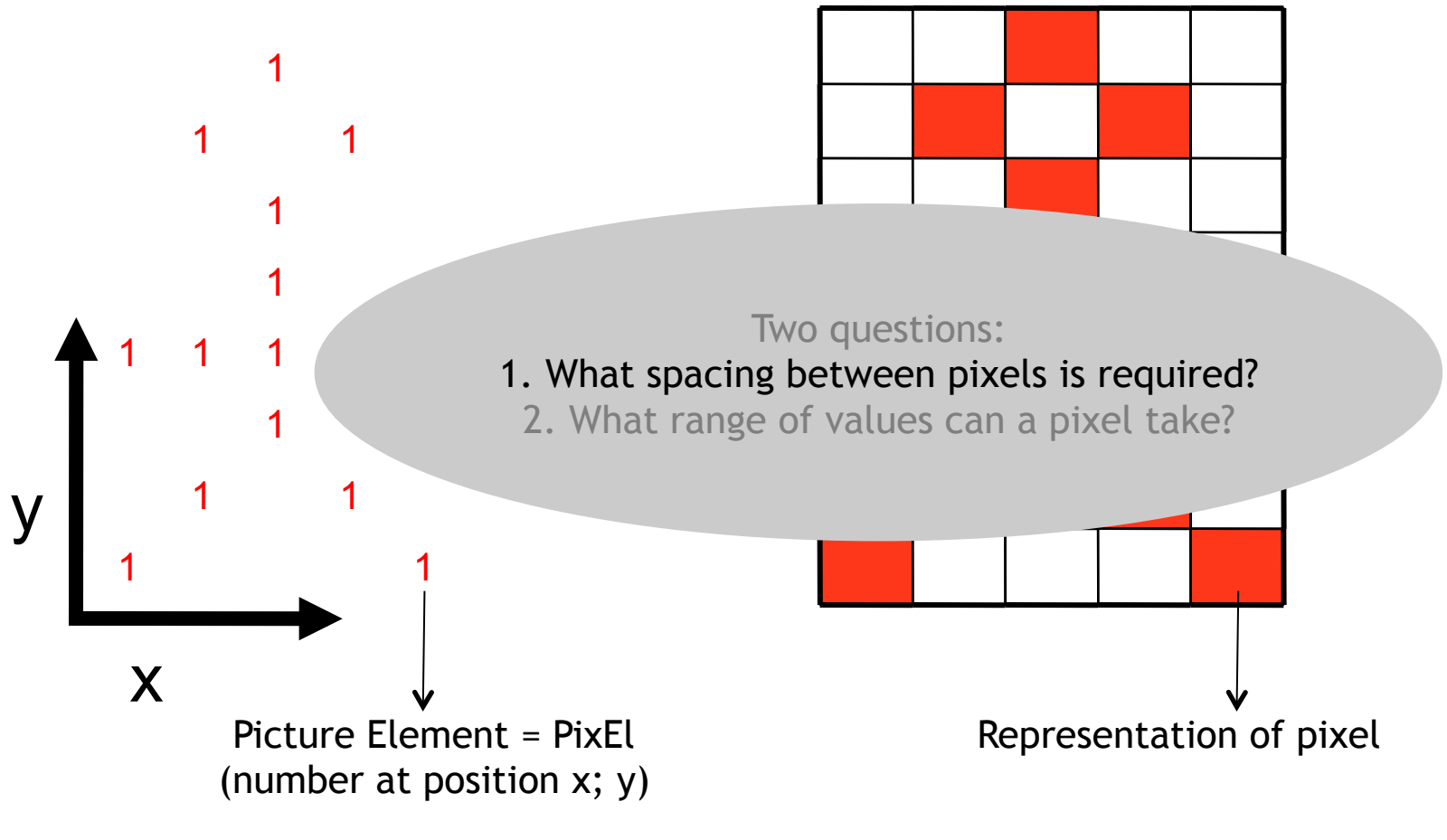


Representation of pixel

Here you have an array of discrete values in 2-D space - a digital image

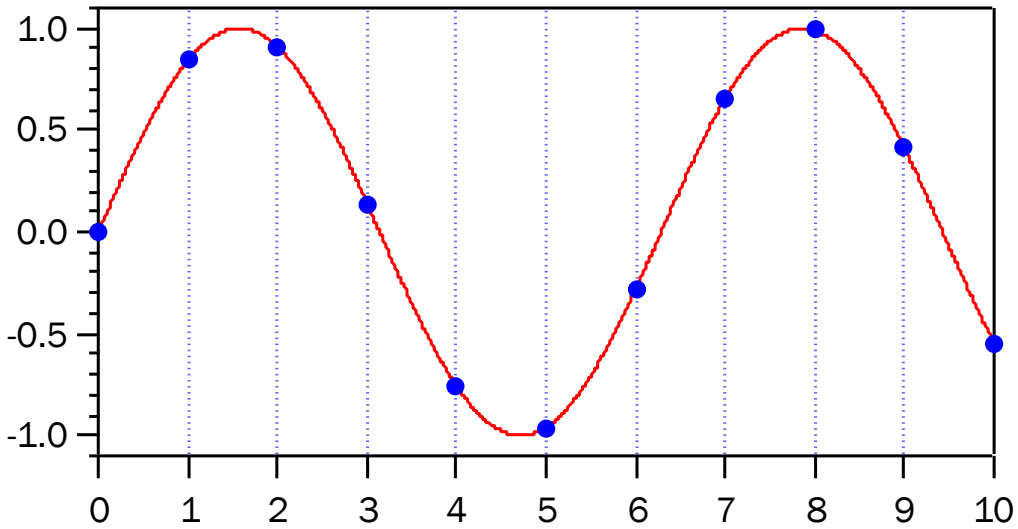


Here you have an array of discrete values in 2-D space - a digital image



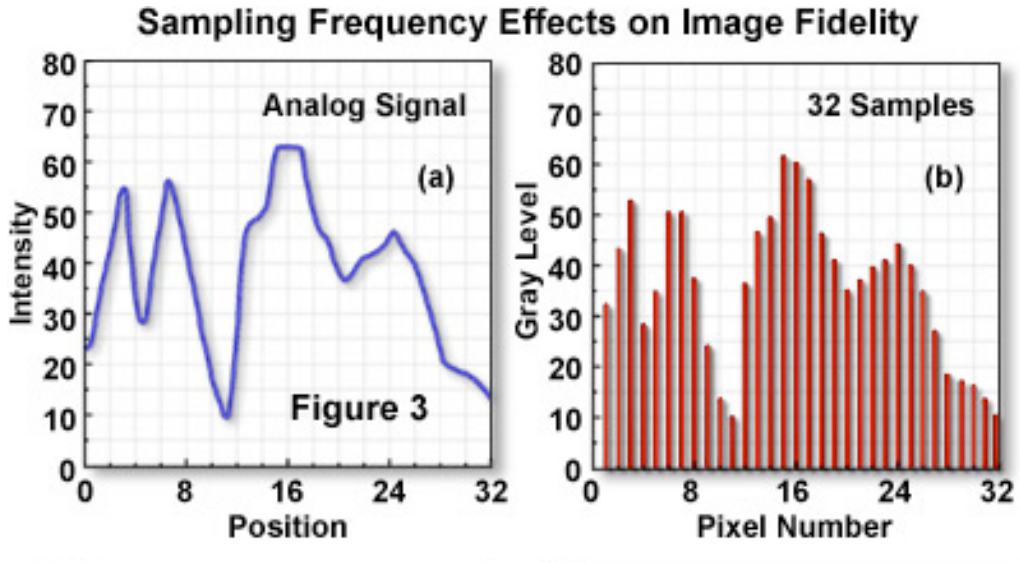
Creating a digital signal involves discretization of the continuous signal by sampling

Sampling of signal



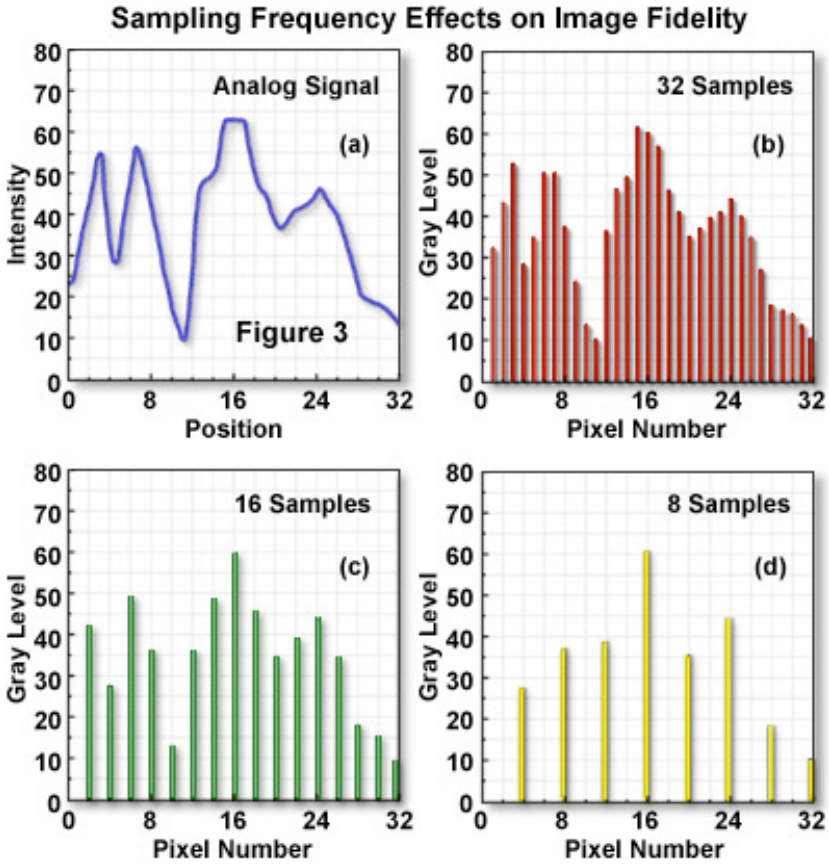
Features of continuous signal/image can be reconstructed from sampled digital image if certain criteria are fulfilled.

The rate of spatial sampling is critical to what detail (or spatial frequencies) can be recovered



http://www.olympusmicro.com/

The rate of spatial sampling is critical to what detail (or spatial frequencies) can be recovered



http://www.olympusmicro.com/

Nyquist-Shannon sampling theorem (perfect world):

If smallest feature to be resolved is sampled more than 2x, then this feature can be reconstructed.

Oversampling: Sampling rate > 2x

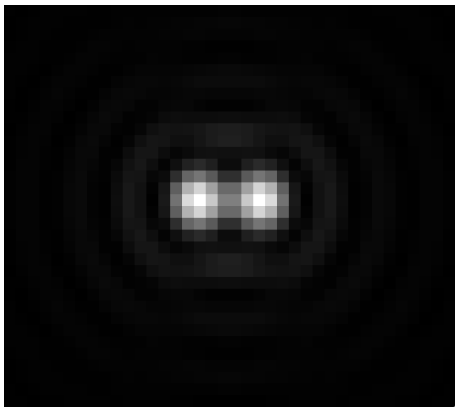
- Ok in terms of detail
- More data

Undersampling: Sampling rate < 2x

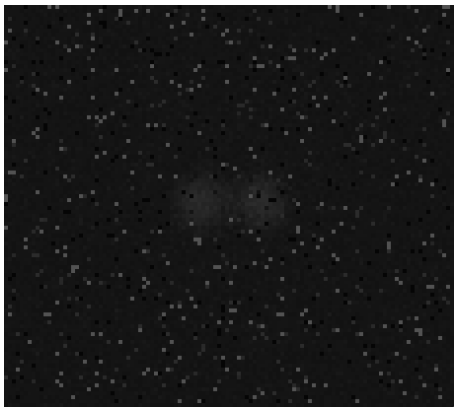
- Missed detail
- False detail (aliasing)!!!

In practice appropriate sampling should be 2.5 to 3x per smallest resolvable feature

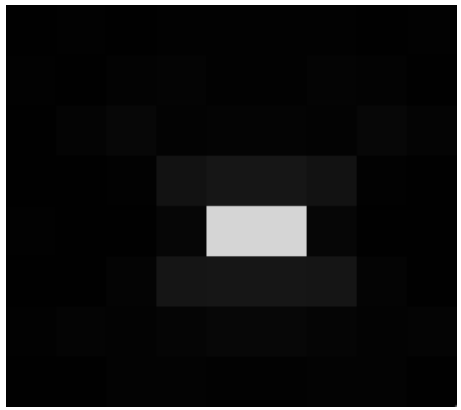
http://www.olympusmicro.com/



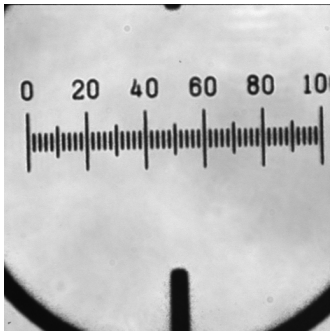
Good sampling



Oversampling
(more data, noise)



Undersampling
(lost detail, aliasing)



microscopic ruler

Calibrate your pixelsize!

Let’s consider the following example:

We have a 60x/1.4 oil objective available on our microscope and want to go for maximum spatial resolution in our digital image.

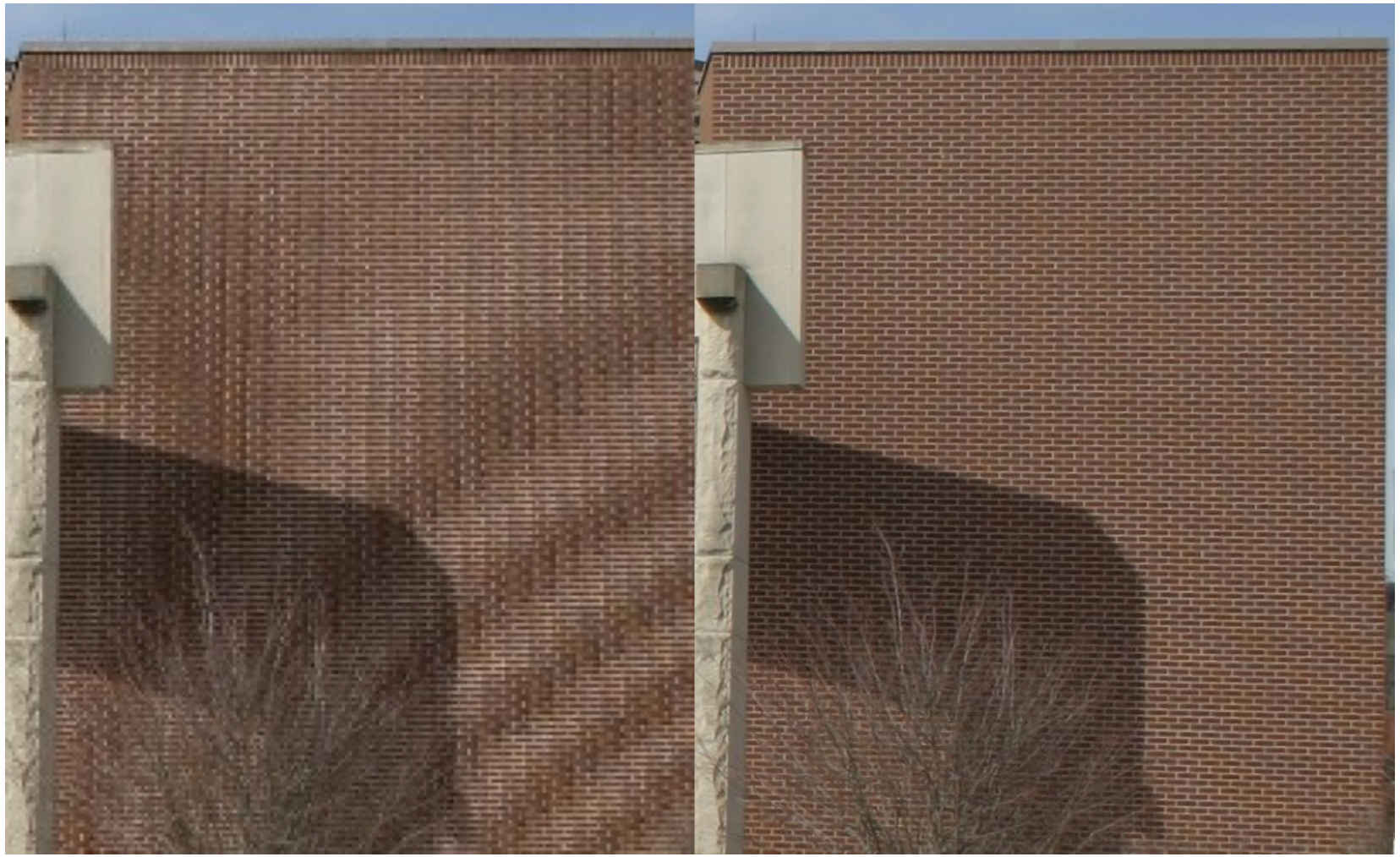
What camera dexel size do we need?

Matching or at least knowing a mismatch of the optimum sampling rate is crucial

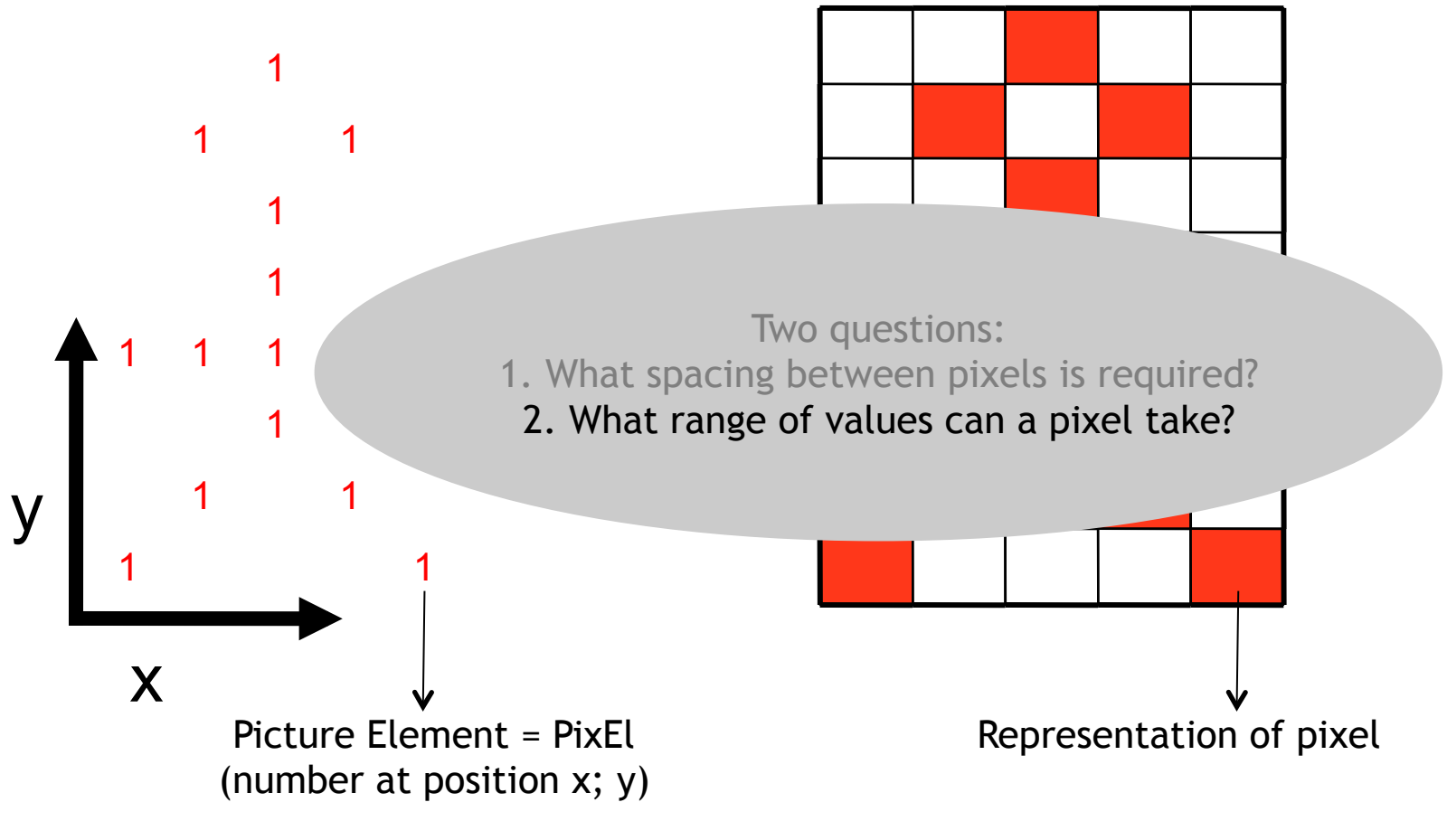
Assumptions: 550nm; d=lambda/2NA; pix=d/3

Objective (N.A.)	Optical Resolution limit (nm)	Projected size on CCD (um)	Required CCD dexel size (um)
4x (0.2)	1375	5.5	1.8
10x (0.4)	687	6.87	2.3
40x (0.75)	367	14.67	4.9
40x (1.3)	212	8.46	2.8
60x (1.4)	196	11.79	3.9
100x (1.4)	196	19.64	6.55

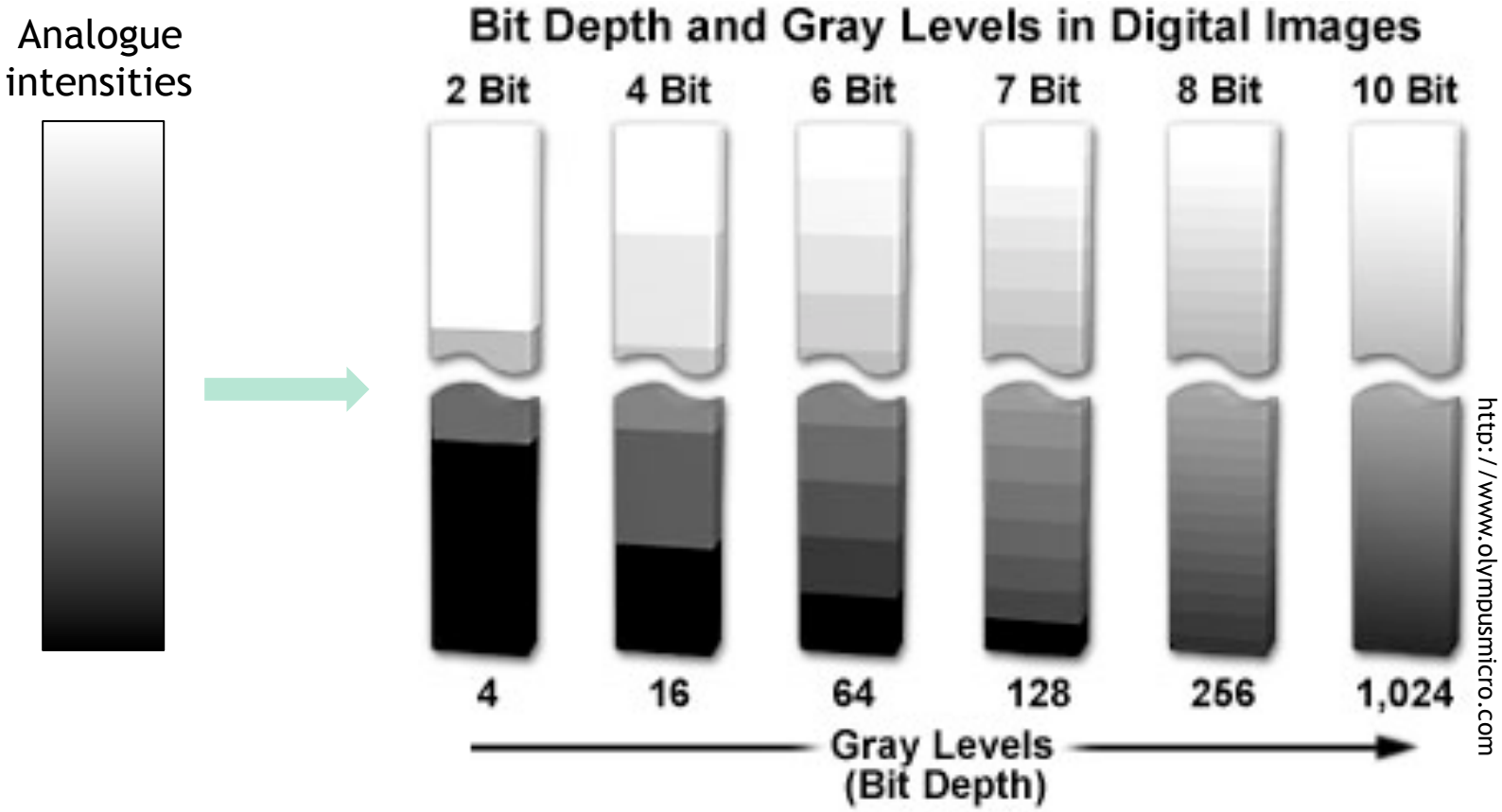
Undersampling does not only kill detail but can result in aliasing which may occur as Moiré pattern



Here you have an array of discrete values in 2-D space - a digital image



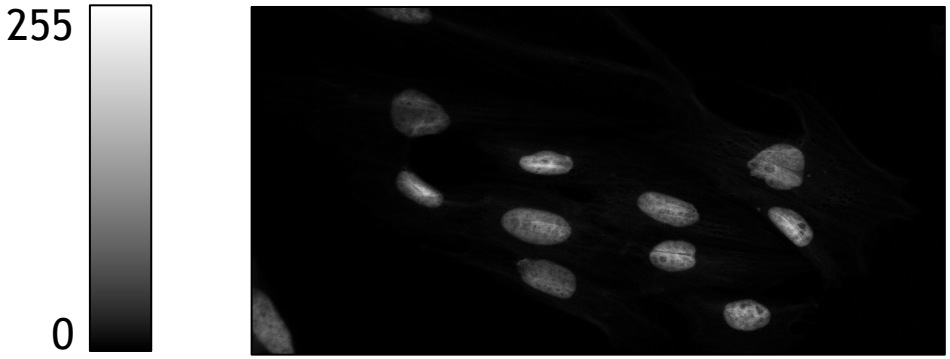
Analogue intensity is quantized into discrete grey levels during digitization



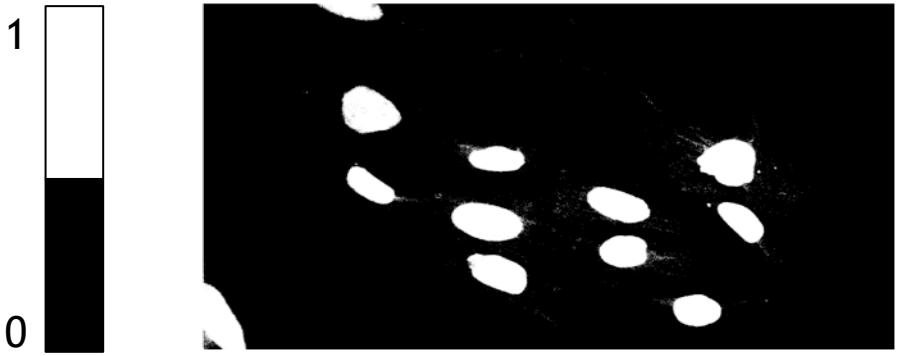
The bit depth required depends on your sample and application

Dynamic range: ratio of maximum and minimum intensities

8 bit greyscale image

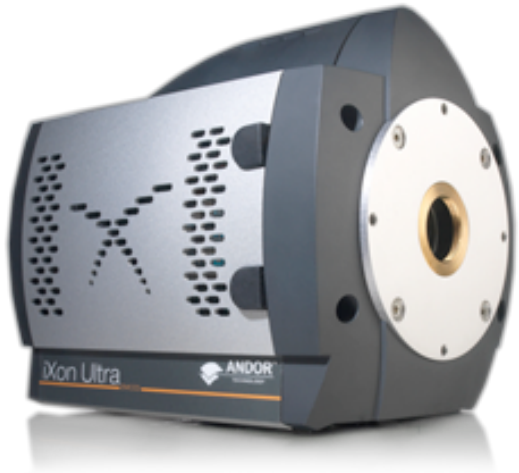


1 bit binary image

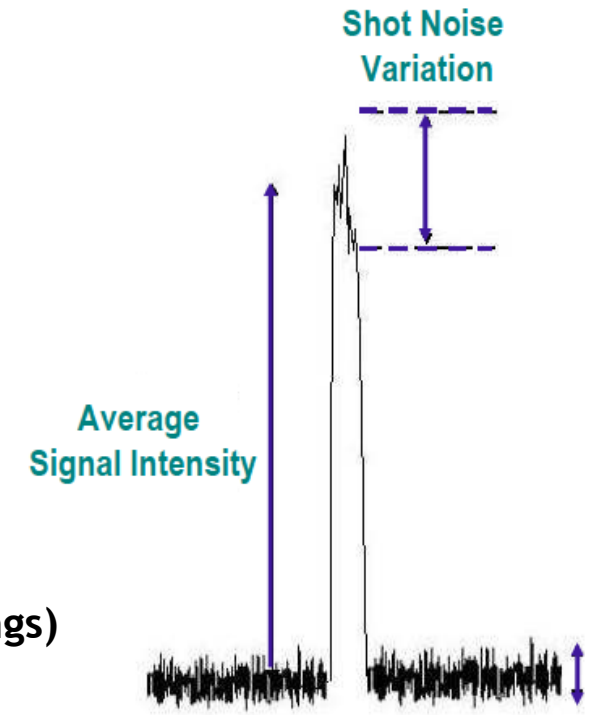


Dynamic range of interest must match the capabilities of the detector

Dynamic range: ratio of maximum and minimum intensities

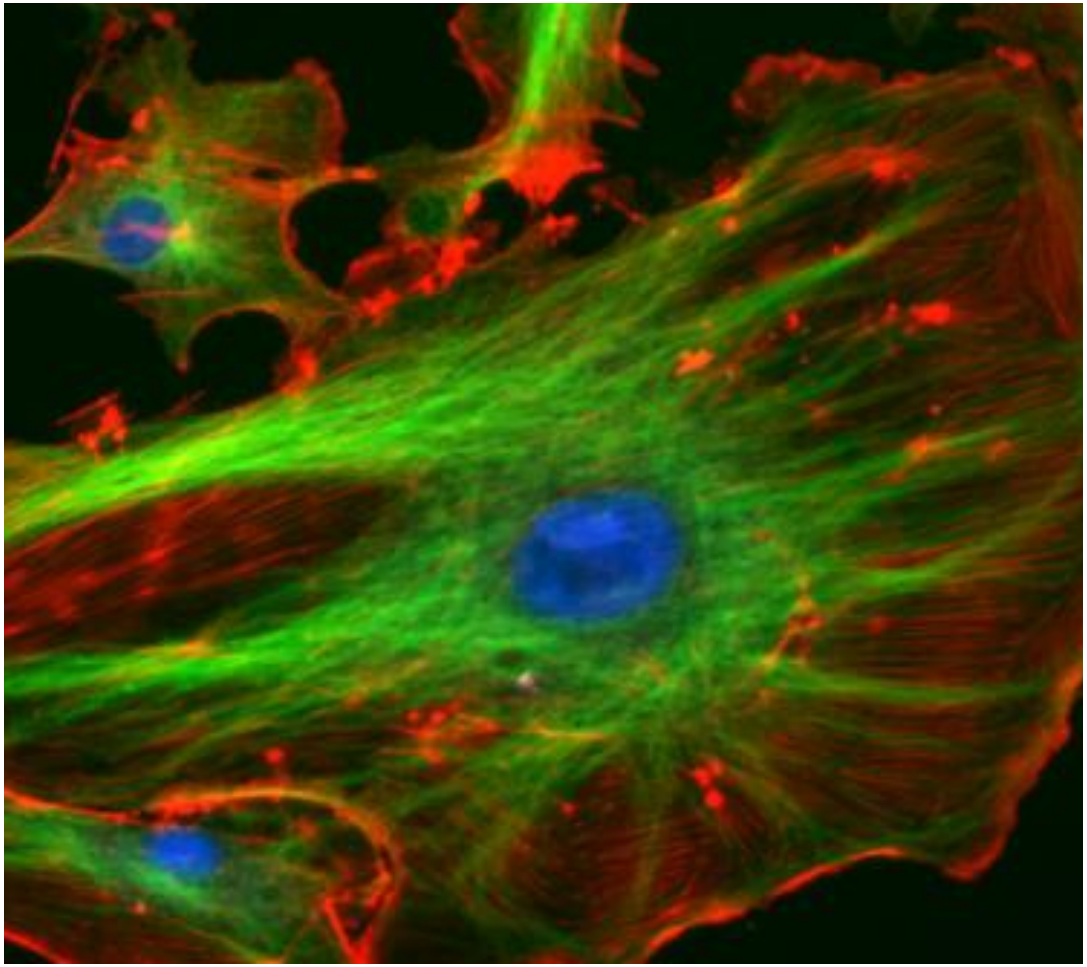


DR ≈ 20000 (depends on settings)



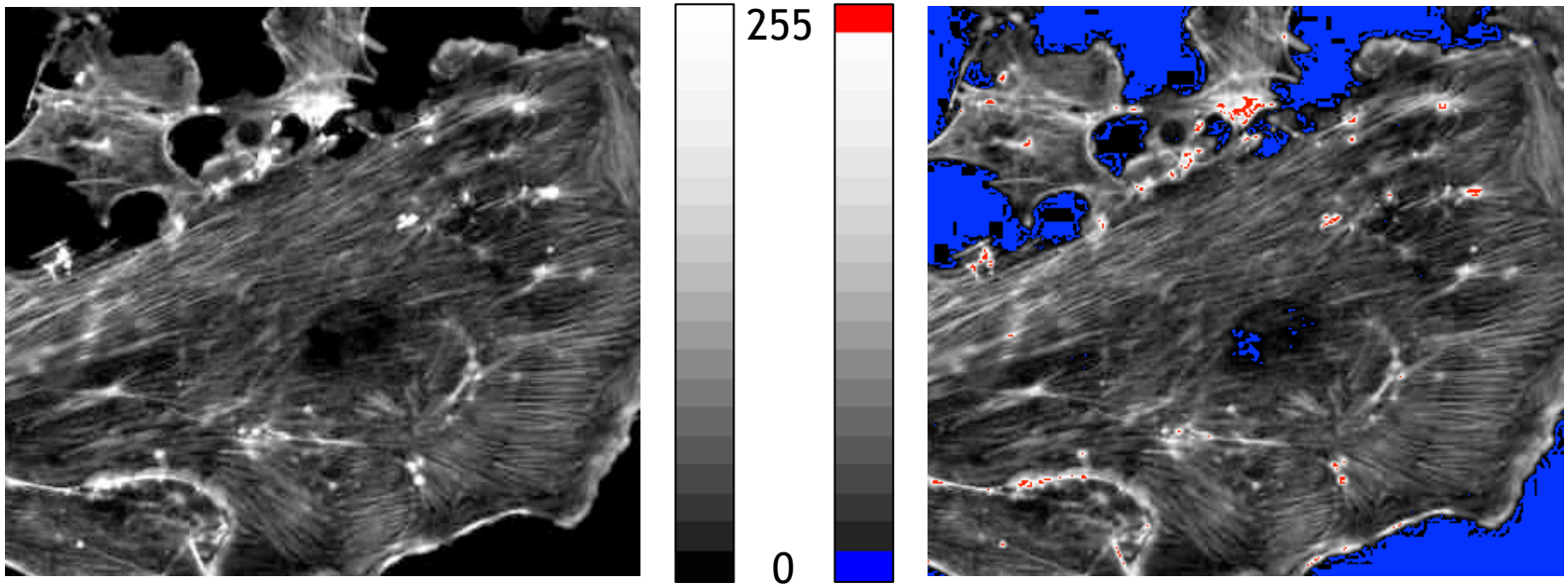
If the dynamic range in the optical image exceeds that of your detector you are in trouble. (workaround e.g. HDR mode)

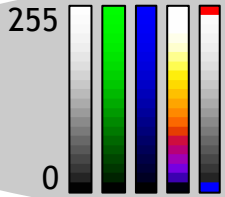
Everything allright with this image?



Lookup tables are your friends - use them according to your needs

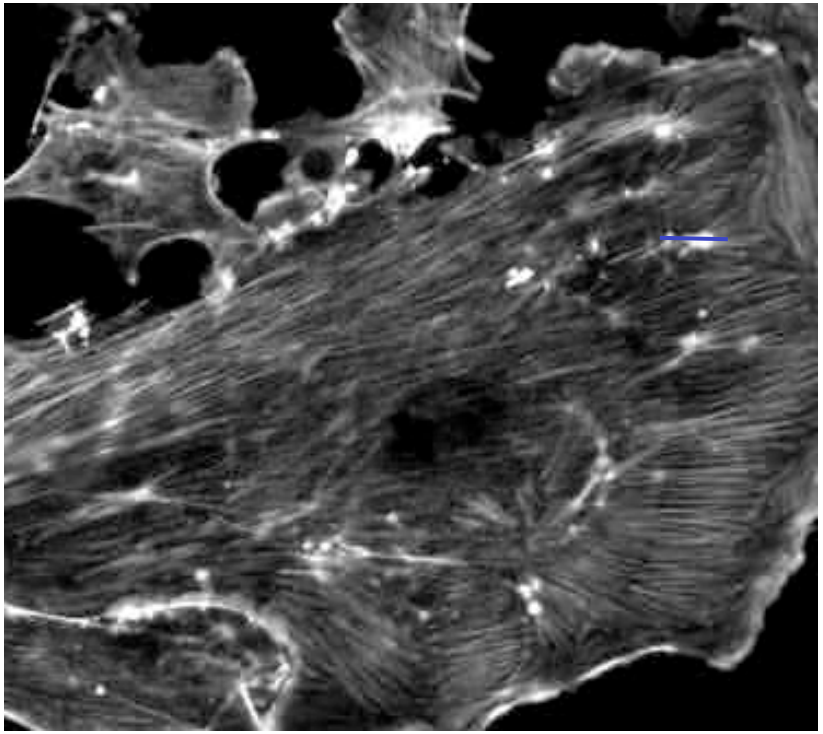
HiLo lookup table to directly visualize saturation levels

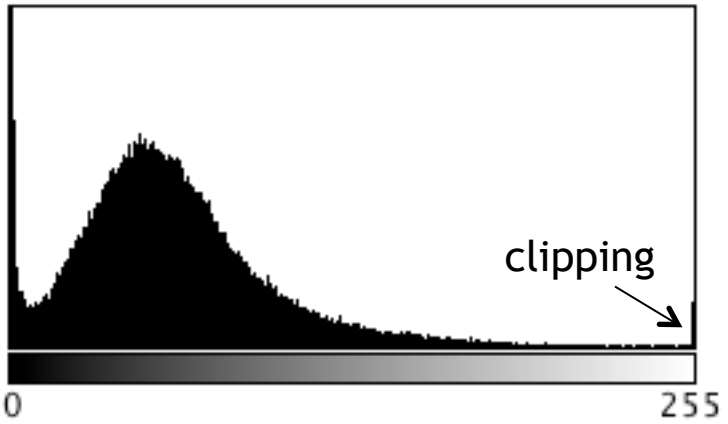




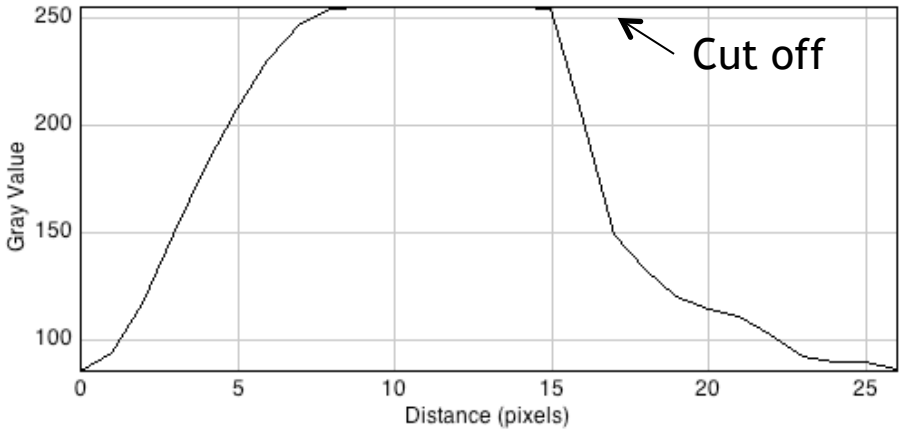
Whatever lookup table you use - if you want to quantify your image you have to measure!

Intensity histograms are our friends as well and allow to check for saturation of your image





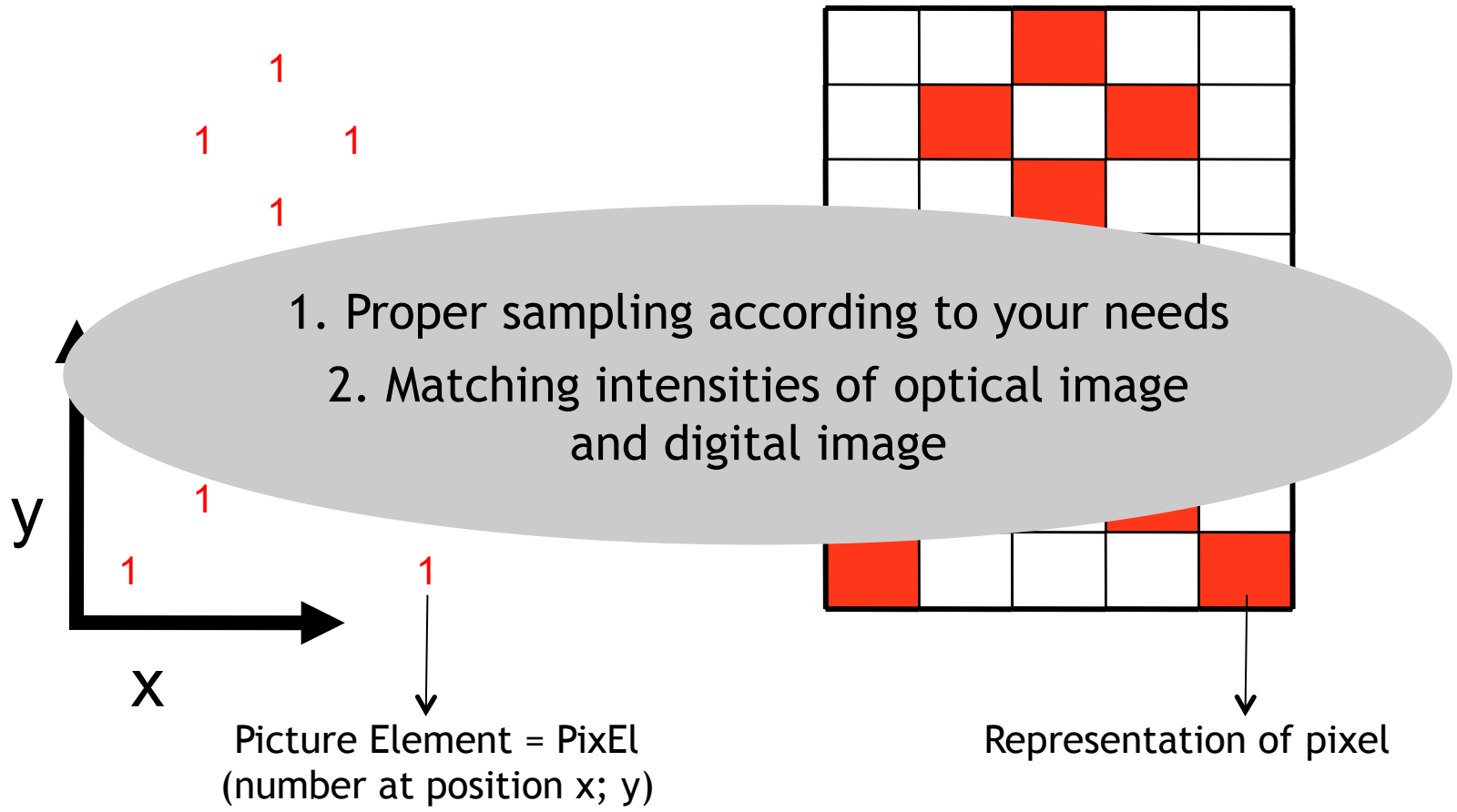
Count: 123876 Min: 0
Mean: 56.968 Max: 255
StdDev: 44.162 Mode: 0 (14363)



How do you measure the height of Frauenkirche?

You use an image which contains the height information

The essence…


1
1
1
1
1
1
1
y
x
1. Proper sampling according to your needs
2. Matching intensities of optical image and digital image
Picture Element = PixEl
(number at position x; y)
Representation of pixel

There is no rule without exception

Typical conflicts between imaging parameters

Goal	Larger Field of view	Detect dim features in ‘HDR sample’	Fast recording of field of view
Solution	Larger pixelsize	Increase detection sensitivity	Lower pixel number => larger pixels
Consequence	Undersampling	Clipping due to limited dynamic range of sensor	Undersampling

Information lost and artifacts introduced at the level of the detected digital image cannot be undone!!!