Digital imaging

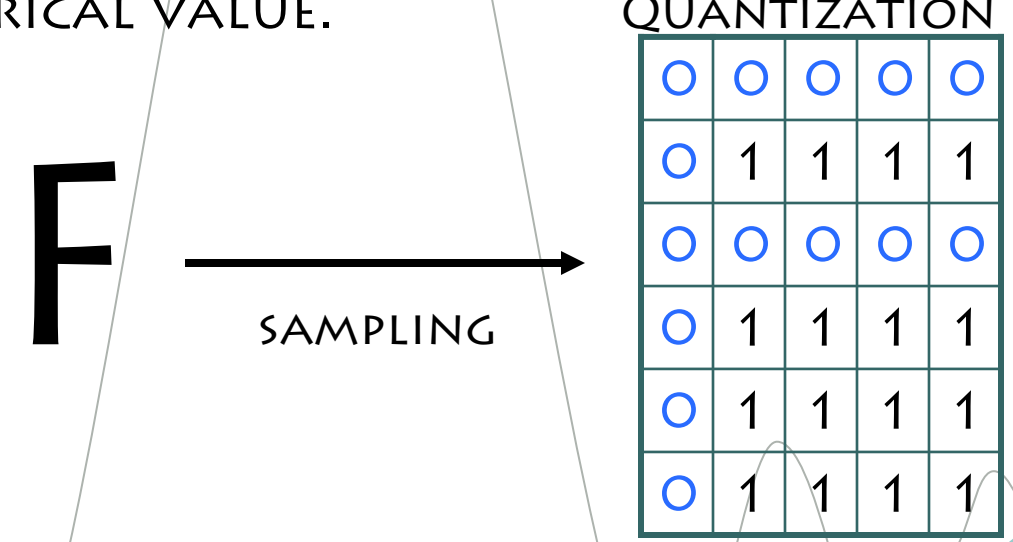
Analog image – continuous tone

- For the human
- Is Any natural image captured with an optical instrument, i.e. microscope, camera.
- Represented on film as a continuous display of shades ranging from light to dark.
- Represented on an electrical device as a series of signal fluctuations.
- represented mathematically as a continuous range of values representing position and intensity.

Digital imaging

Digital Image

- For computers
- Consists of an array of picture elements, pixels, where each pixel represents a numerical value.

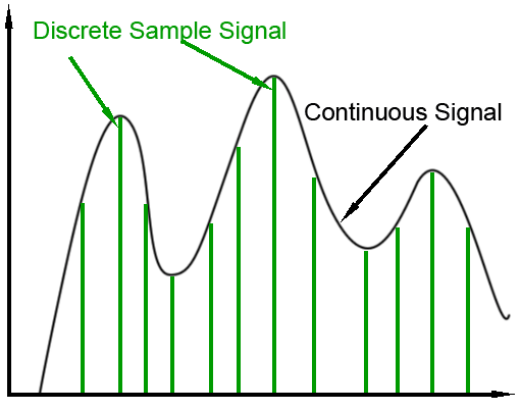


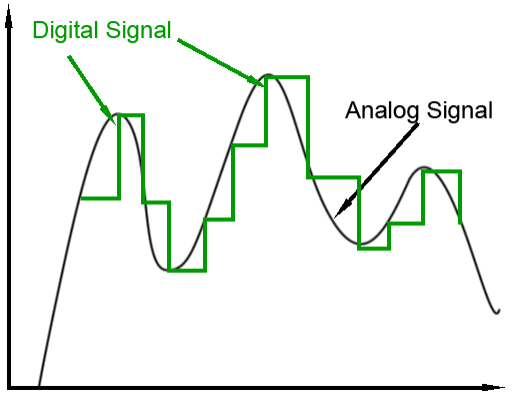
Digital imaging

How do we Get from analog Images to digital Images?

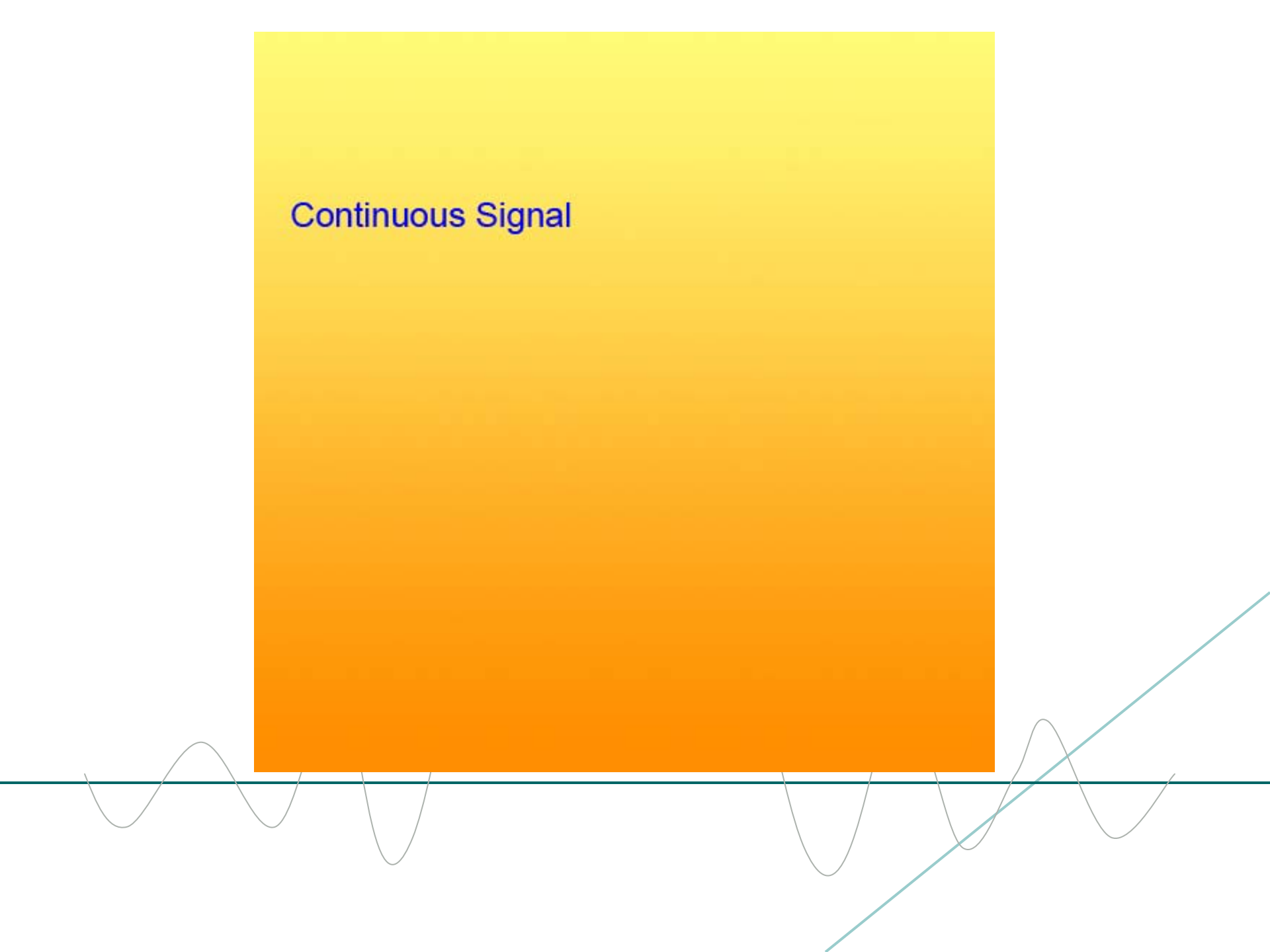
consists of a 2 part procedure involving

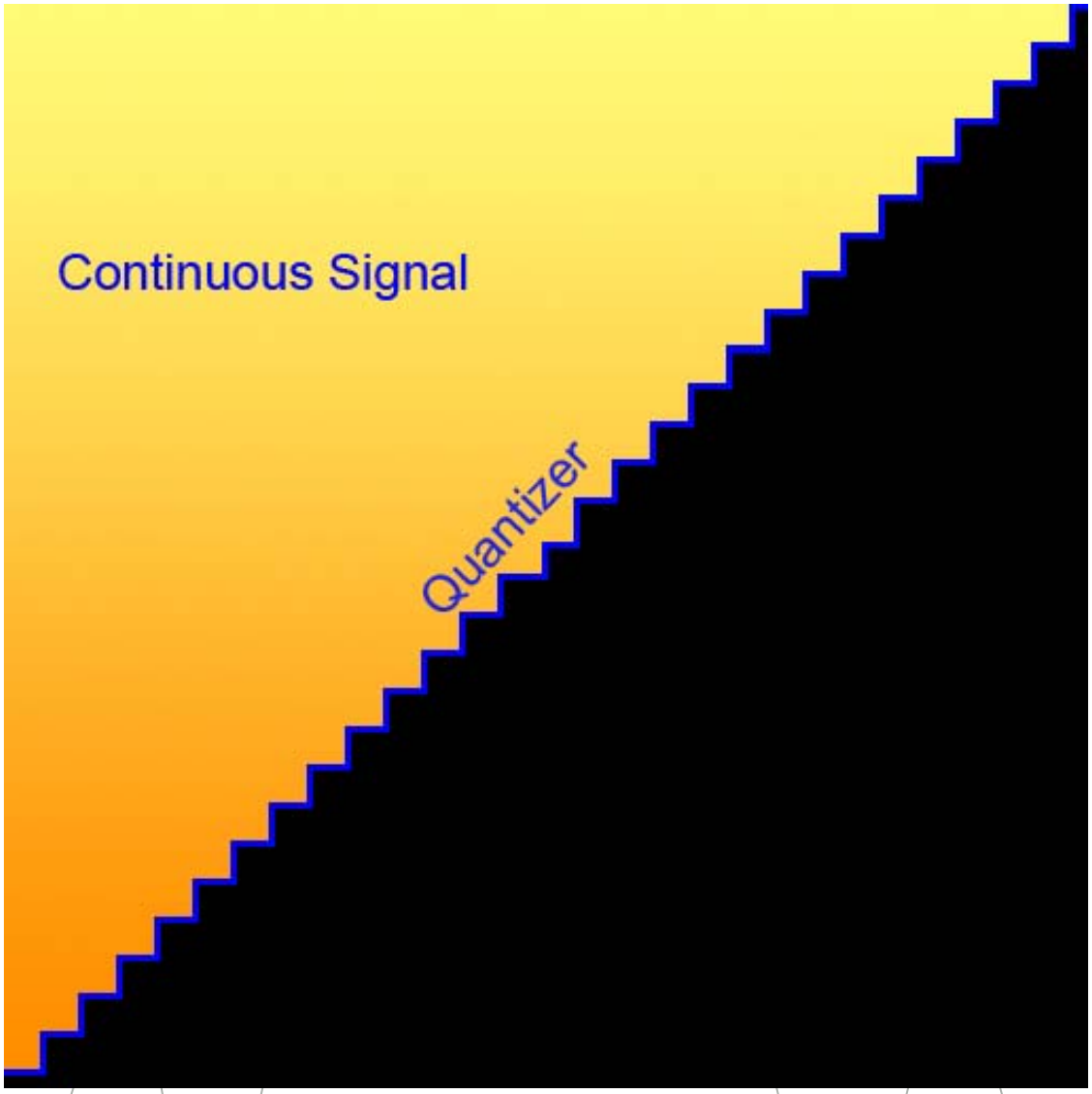
sampling and quantization or digitizing

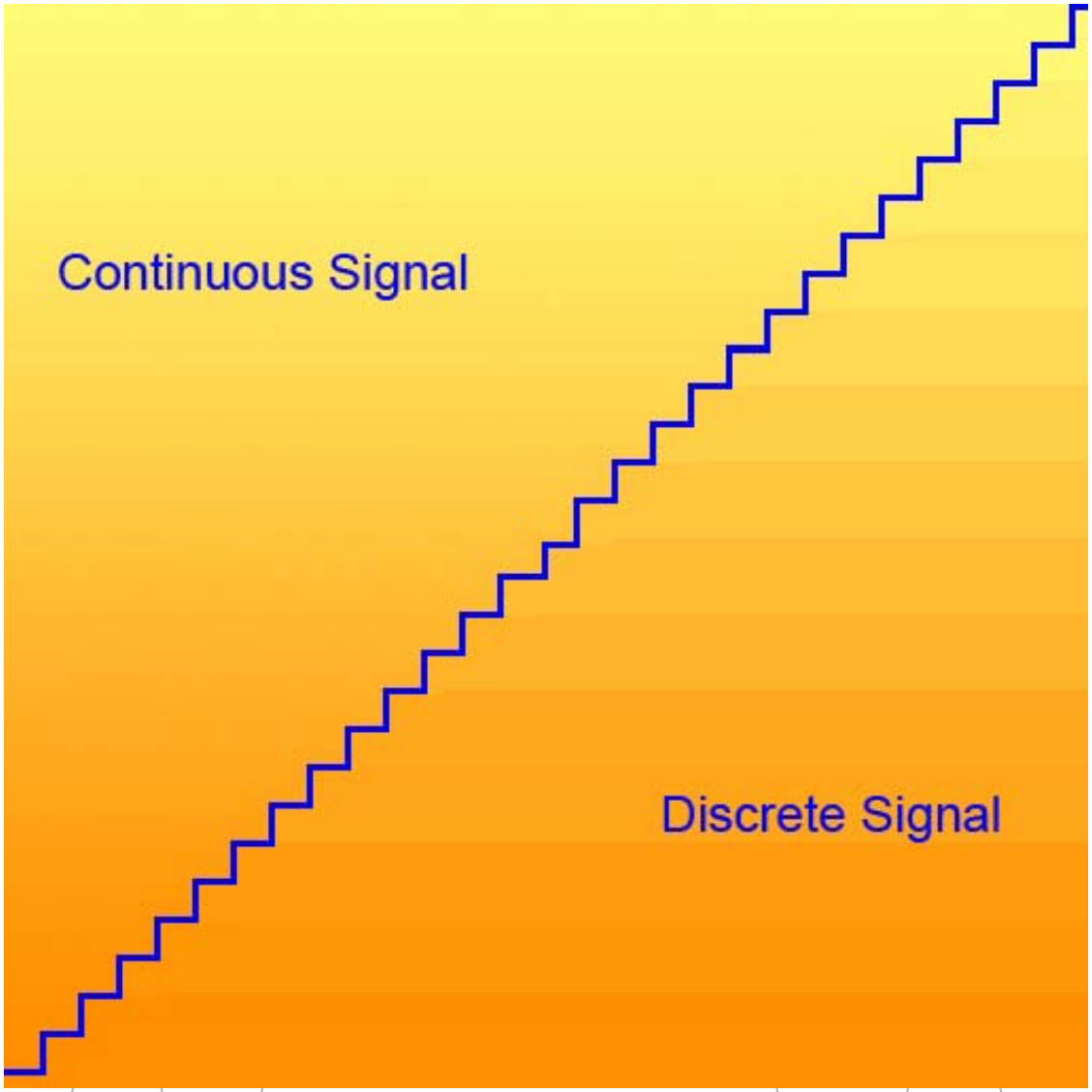




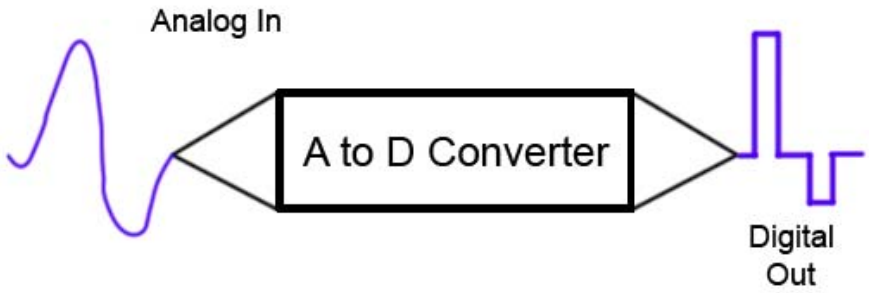
Continuous Signal




Continuous Signal
Quantizer


Continuous Signal
Discrete Signal

Digital imaging



Sampling

The intensity (continuous signal) in the analog image is measured at successive locations or at a particular rate (discrete signal), and forms an array consisting of discrete points, F(x,y) where F represents the intensity of the pixel, while x and y are the positional coordinates.

- An electronic device called An Analog to digital converter (A/D), performs this conversion.
- The digital number generated is proportional to the magnitude of the voltage or current.

Digital imaging

Sampling

- Interval – samples are taken every T seconds.
- Frequency – the number of samples taken within a second.
- Density - Defines the number of pixels recorded per distance or time.
  - Larger pixel = smaller sampling density
  - Smaller pixel = Larger sampling density
    - i.e. candy in a jar, the smaller the pieces, the more candy you can fit in the jar, the density of the candy in the jar increases.
  - This is set when you acquire your microscope image , you cannot change it post-acquisition
  - It is Determined by the zoom factor in confocals (magnification and detector size in wide field scopes)

Digital imaging

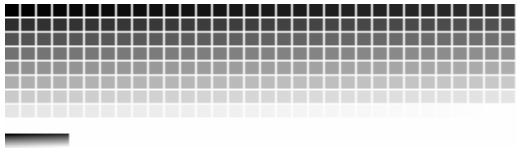
Quantization or digitizing

digital brightness values are assigned to the intensity array, resulting in the picture element or pixel.

- pixel bit depth - Each pixel consists of a series of bits, or binary digits, that the computer recognizes.
- The greater the bit depth, the more gray levels or increased dynamic range, is stored in each image. This results in more information for quantitative analysis.

Digital imaging

An Accurate image representation is dependent on the number of bits available to quantize the analog signal.

Bit depth	Gray levels	
2^1	2	
2^2	4	
2^4	16	
2^8	256	
2^{12}	4096	
2^{16}	65,536	

Digital imaging

Microscope Resolution

- the smallest distance between two points that still allows the points to be viewed as individual points.
- Determined by Objective's numerical aperture and excitation wavelength

Digital Image resolution

- the degree to which the image most accurately represents the analog image acquired by the microscope.
- Determined by the total number of pixels and the range of intensities for each pixel.

Digital imaging

How do we obtain the most accurate digital image?

The Nyquist Theorem

Specifies the sampling interval required to faithfully reconstruct a pure sine wave as a function of its frequency.

It requires a sampling interval equal to two to three times the highest spatial frequency of the specimen to accurately preserve the spatial resolution in the digital image.

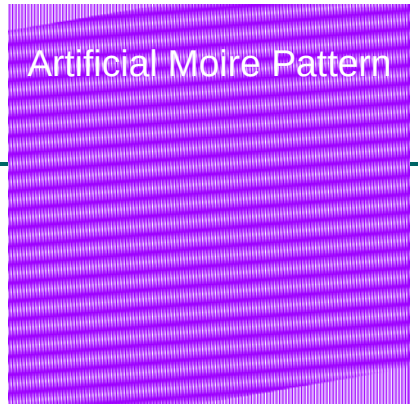
Digital imaging

Sampling issues

- Undersampling - Details in the analog image are sampled at a rate less than twice their spatial frequency; too few samples are acquired.
  - Spatial aliasing occurs; high frequency information will show as Low spatial frequency features that are not actually present, this is Often seen in regularly spaced, repetitive patterns.
  - Pixels are too far apart compared to the high frequency detail in the image.

Oversampling – details In the Analog image are sampled at a rate higher than twice their spatial frequency.

- increased sampling per scan area, which is obtained by increasing the optical zoom too high.
- Fails to improve resolution



Digital imaging

Example

Optical resolution of 63x 1.4 NA objective

R_{XY} = 0.4*λ/N.A. (Rayleigh criteria for Confocal)

R_{XY} = 0.4*543/1.4

R_{XY} = 155 nm

Scan format

number of pixels per line x lines per frame.

Scan field

determined by the objective magnification and the optical zoom.

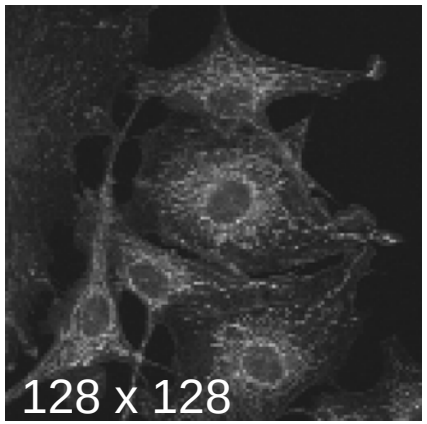
Digital imaging

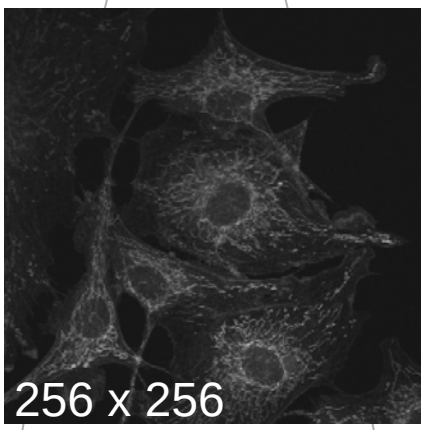
Images acquired with the 63 x objective at zoom 1.

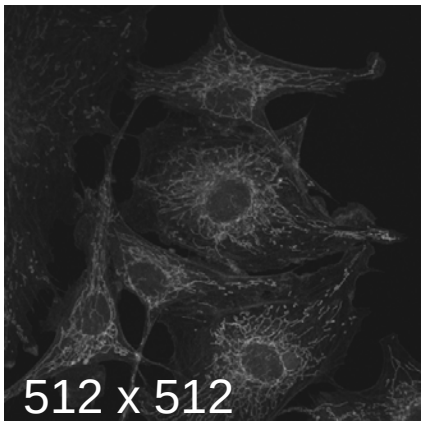
Scan field (um)	scan format (pixels)	Pixel size (um)
142.86 x 142.86	128 x 128	1.12
142.86 x 142.86	256 x 256	0.56
142.86 x 142.86	512 x 512	0.28
142.86 x 142.86	1024 x1024	0.14
142.86 x 142.86	2048 x 2048	0.07

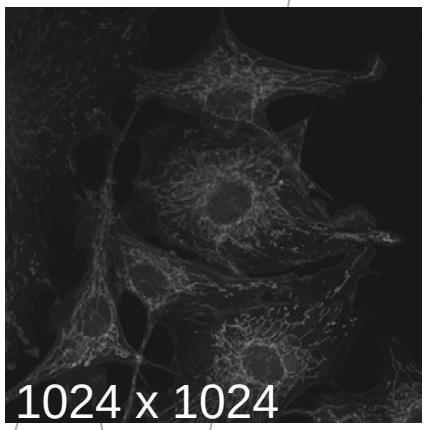
Note pixel size is reduced as the scan format is increased. Acquisition time greatly increases as scan format increases.

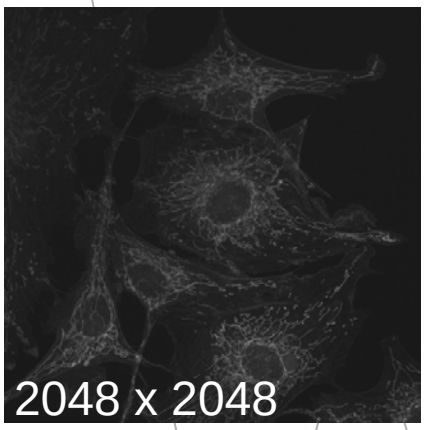
Digital imaging







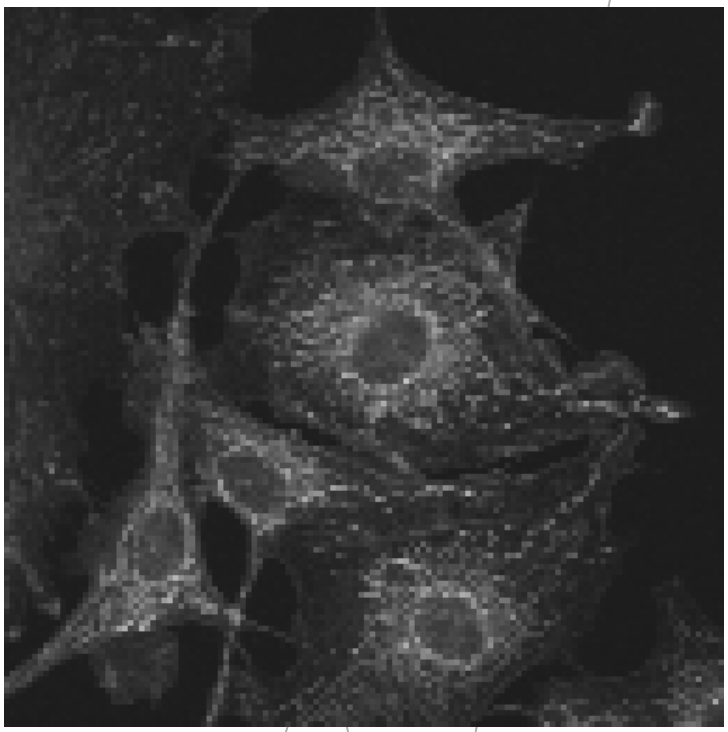




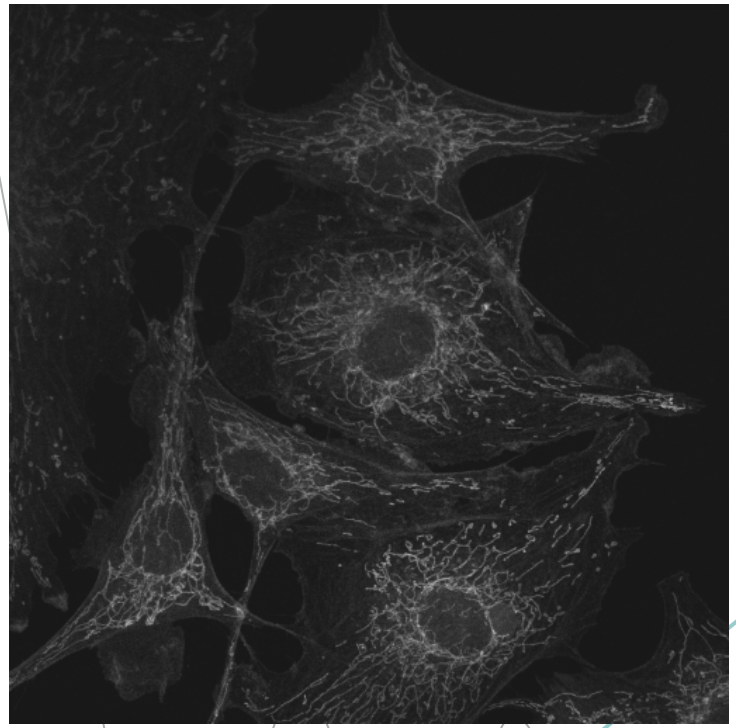
Images acquired at zoom 1

Digital imaging

128 x 128 pixels Vs. 512 x 512 pixels



Each pixel = 1.12 um



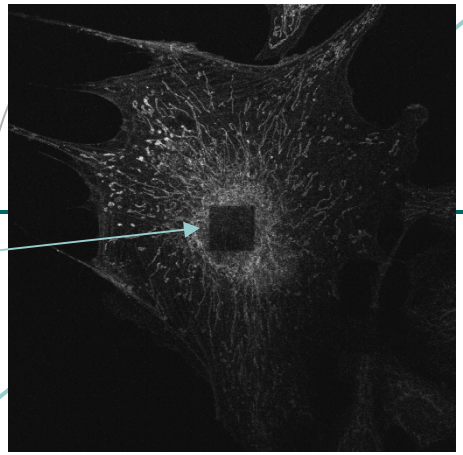
Each pixel = 0.28 um

Digital imaging

Scan format (um) and pixel size (um)
63x/1.4 N.A. objective

Scan format	Pixel size @ zoom 1	Pixel size @ zoom 10
128 x 128	1.12	0.11
256 x 256	.056	0.06
512 x 512	0.28	0.03
1024 x 1024	0.14	0.01
2048 x 2048	0.07	0.01

Note: bleaching increases dramatically as zoom and scan format increase.



Digital imaging

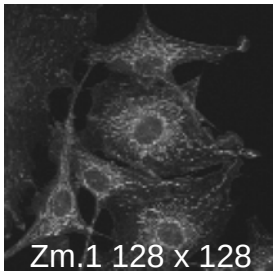
Optical Zoom

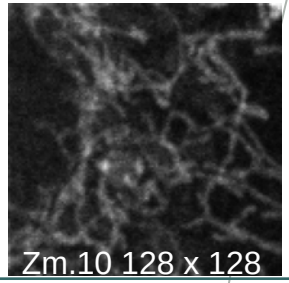
- Zooming in will reduce the scanned area with the same number of pixels, therefore the pixel size is reduced.
- The image is magnified and the resolution is improved; however this is limited by the resolution of the objective.

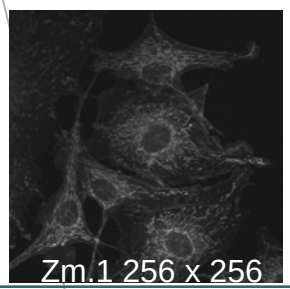
Digital zoom

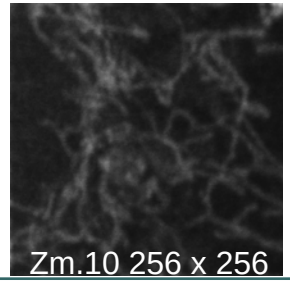
- Mathematically enlarges the image by interpolation of the pixels
- Uses the original number of pixels in the image, often resulting in a blurry image.

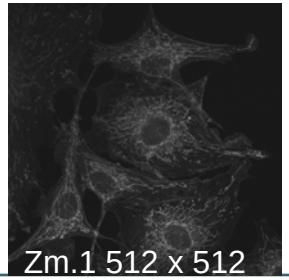
Digital imaging

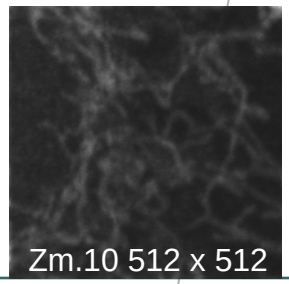

Zm.1 128 x 128

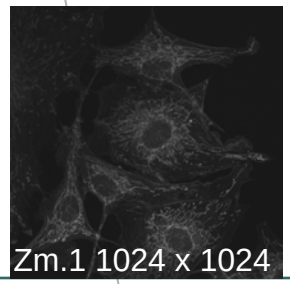

Zm.10 128 x 128

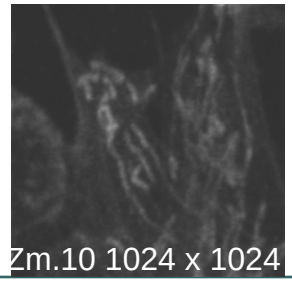

Zm.1 256 x 256

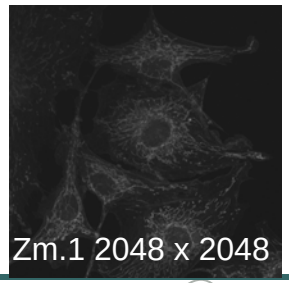

Zm.10 256 x 256

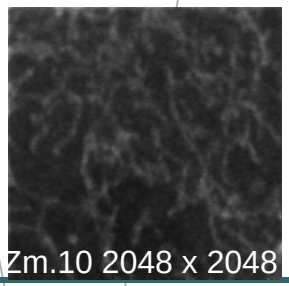

Zm.1 512 x 512


Zm.10 512 x 512


Zm.1 1024 x 1024


Zm.10 1024 x 1024


Zm.1 2048 x 2048

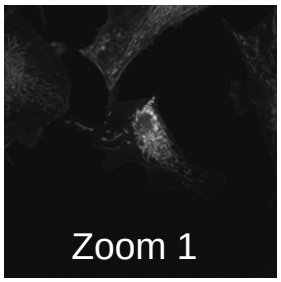

Zm.10 2048 x 2048

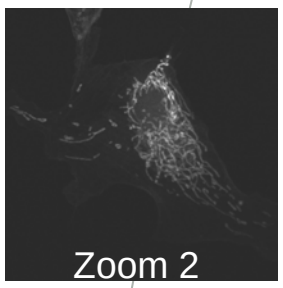
Digital imaging

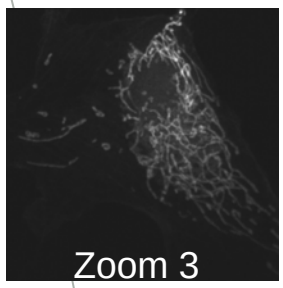
Zoom factor versus pixel and image size.
Scan format, 512 x 512, 63x/1.4 N.A.

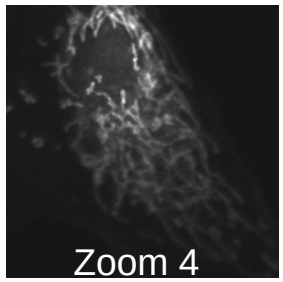
Zoom Factor	Pixel size (um)	Image size (um)
1	0.28	142.86
2	0.14	71.43
3	0.09	47.62
4	0.07	35.71
5	0.06	28.57
6	0.05	23.81
7	0.04	20.41
8	0.03	17.86
9	0.03	15.87
10	0.02	15.87

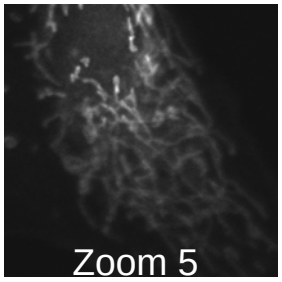
Digital imaging

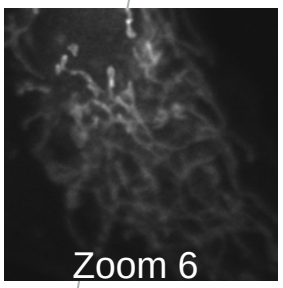


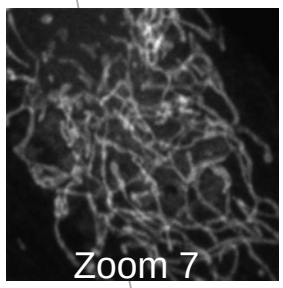


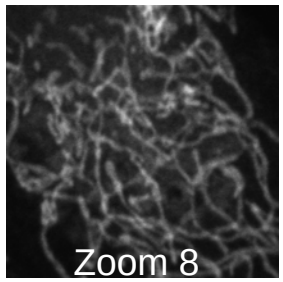


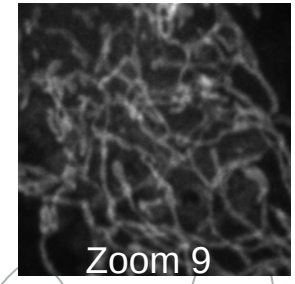


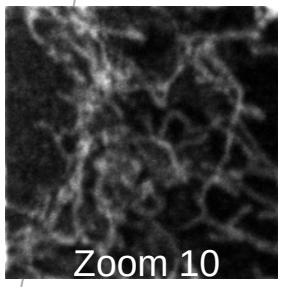












Digital imaging

Remember, for 63x/1.4N.A. the R_{XY} = 155 nm

Therefore, according to the nyquist criteria, the pixel size should be about ½ the minimum spacing.

Pixel size = R_{XY}/2.0 = 155/2.0 = 77.5 nm = 0.078 um

One should theoretically use a zoom factor of 3 or 4 to fulfill the nyquist criteria

Zoom Factor	Pixel size (um)	Image size (um)
3	0.09	47.62
4	0.07	35.71