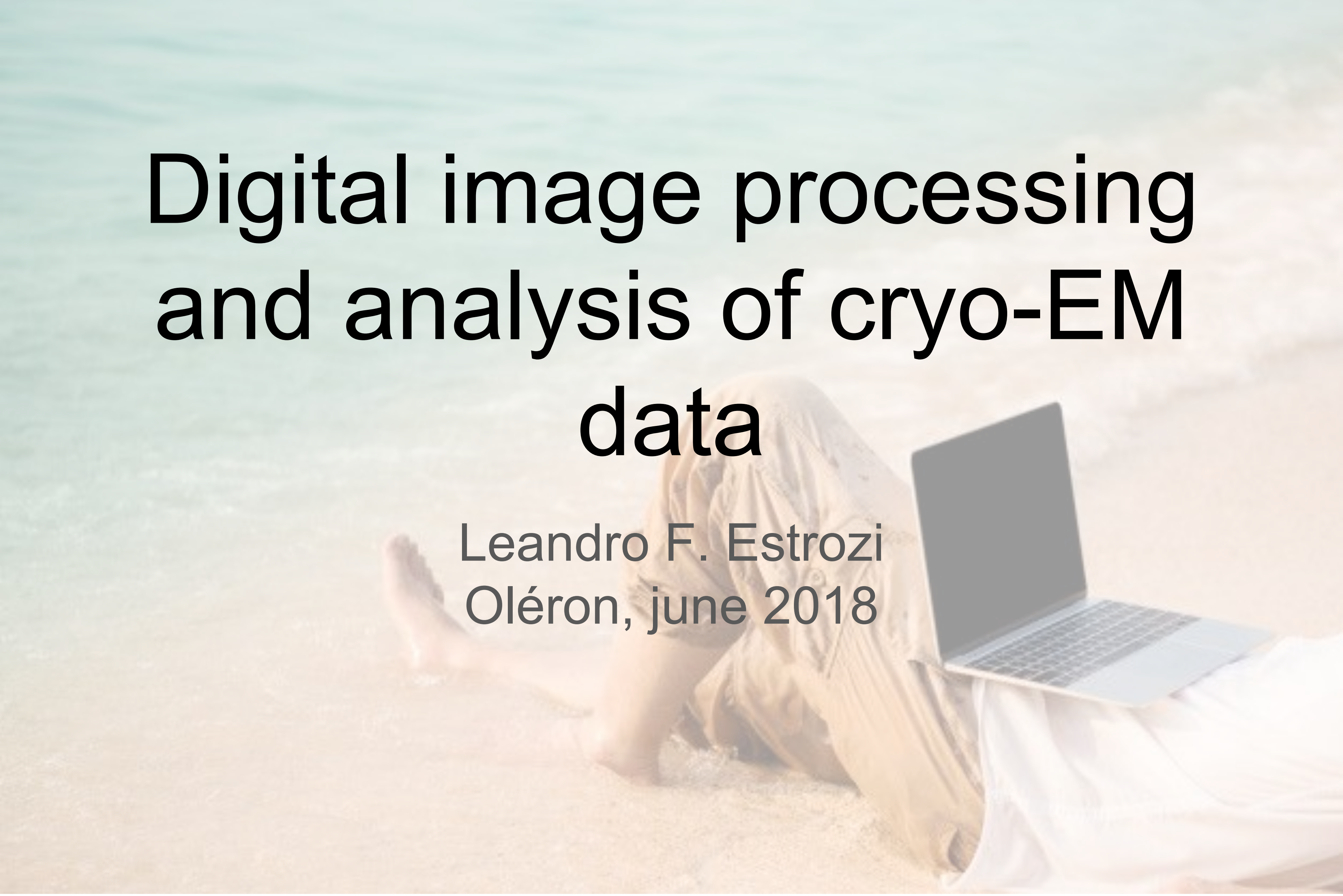


Digital image processing and analysis of cryo-EM data

Leandro F. Estrozi

Oléron, june 2018

Key concepts

Fourier transform

Point spread function (réponse impulsionnelle du système)

Convolution and deconvolution

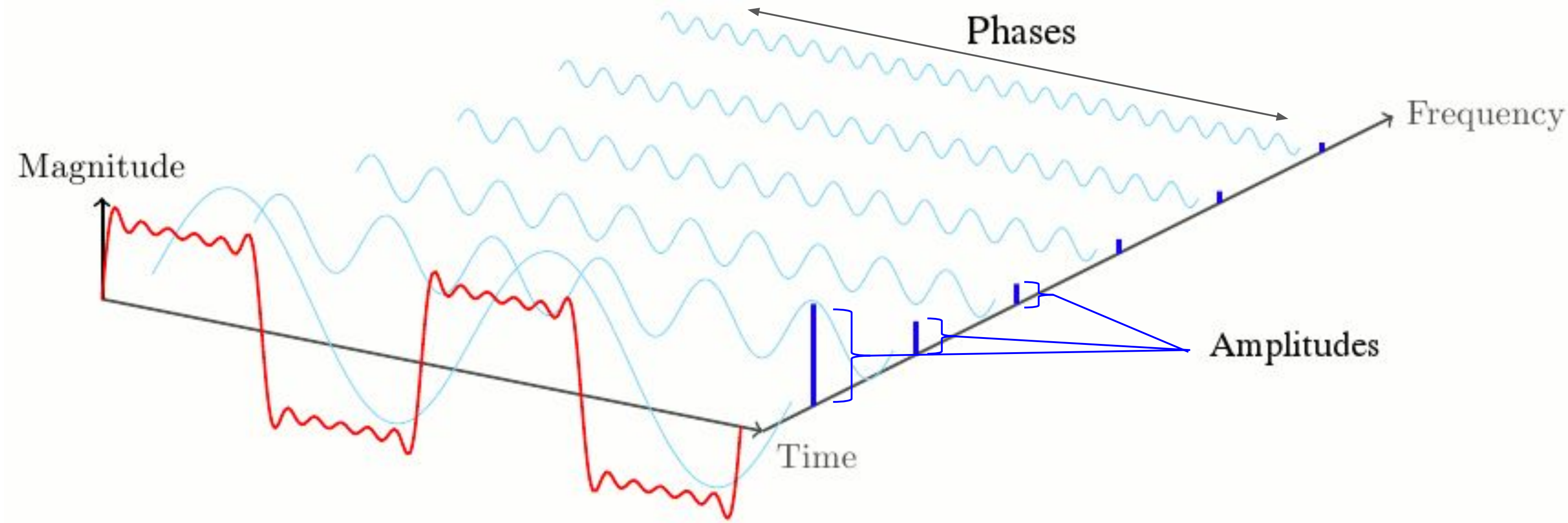
Low, High and Band pass filtering

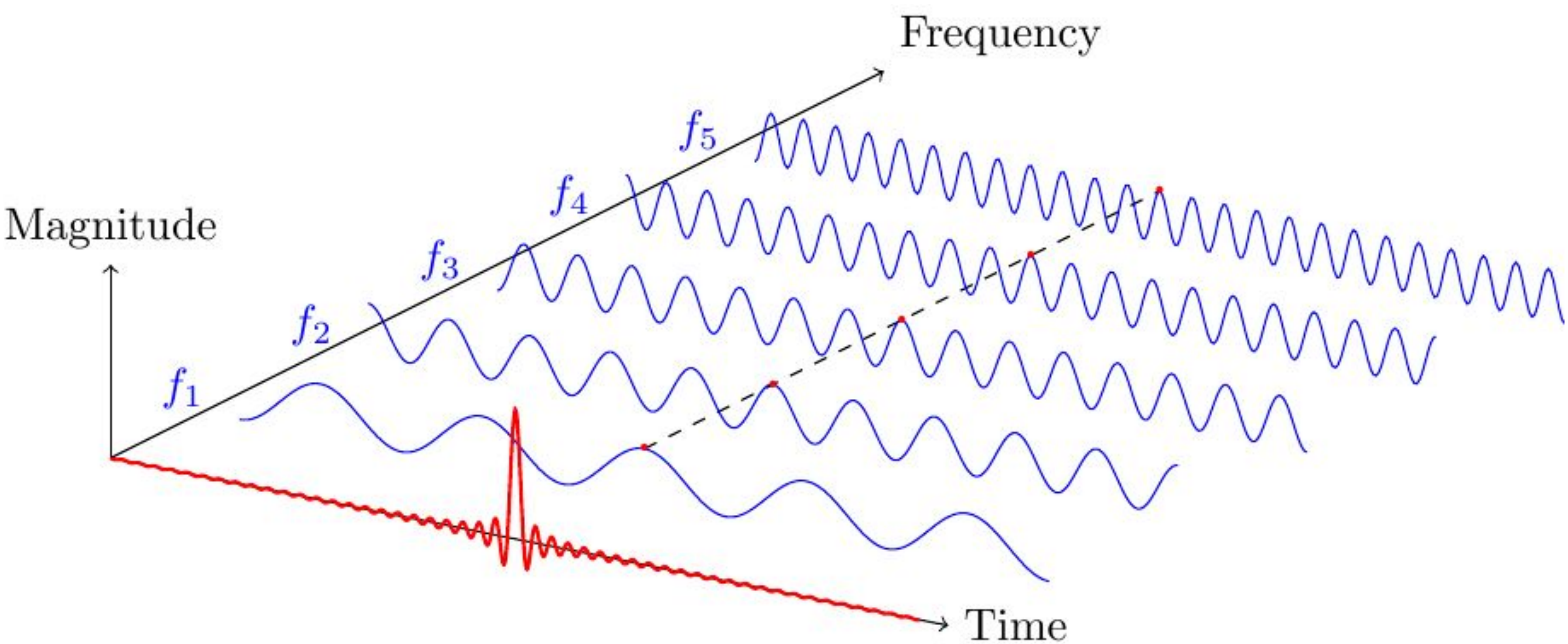
Signal to noise ratio

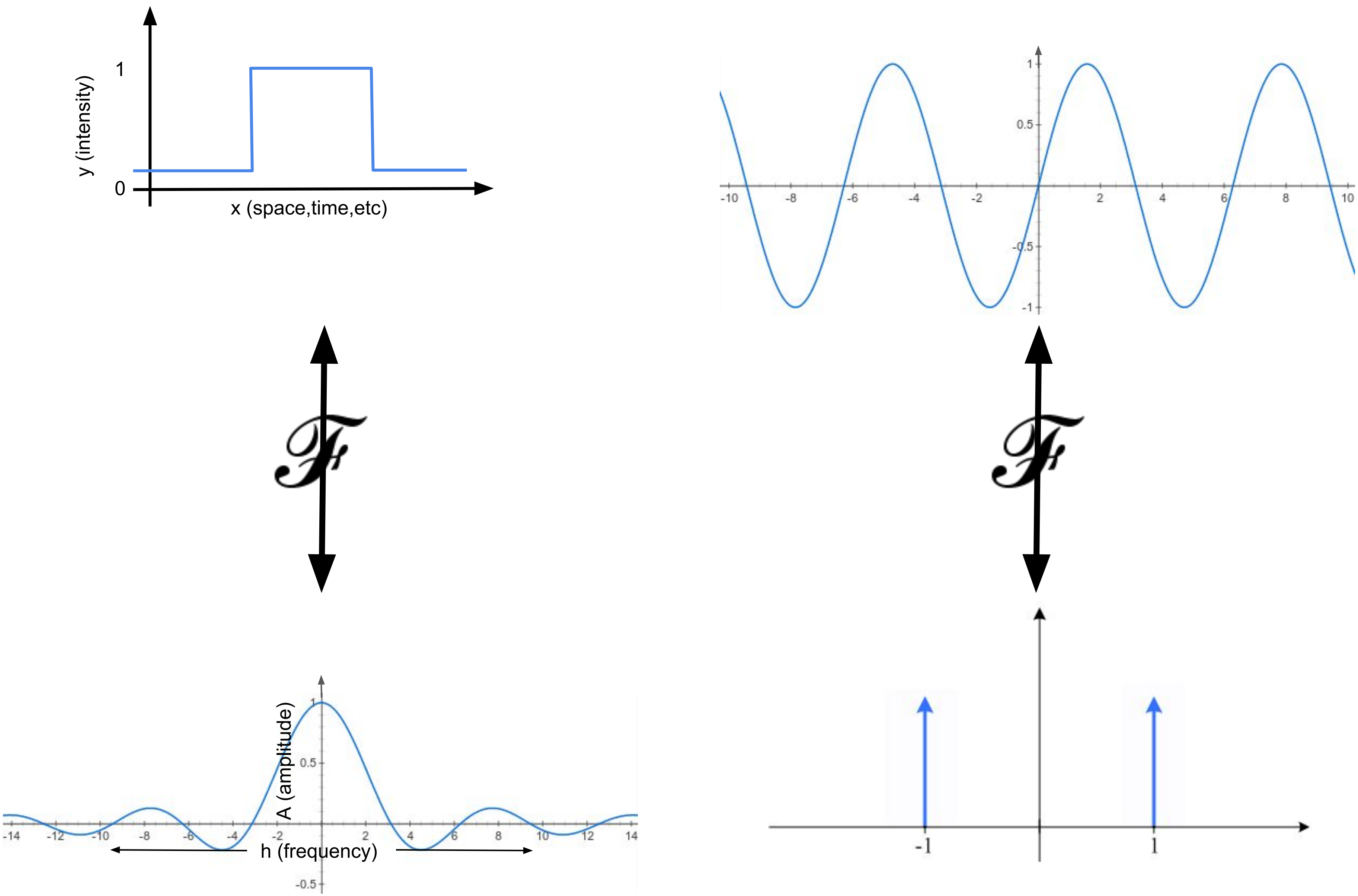
Central slice theorem and 3D reconstruction

Digital image representation

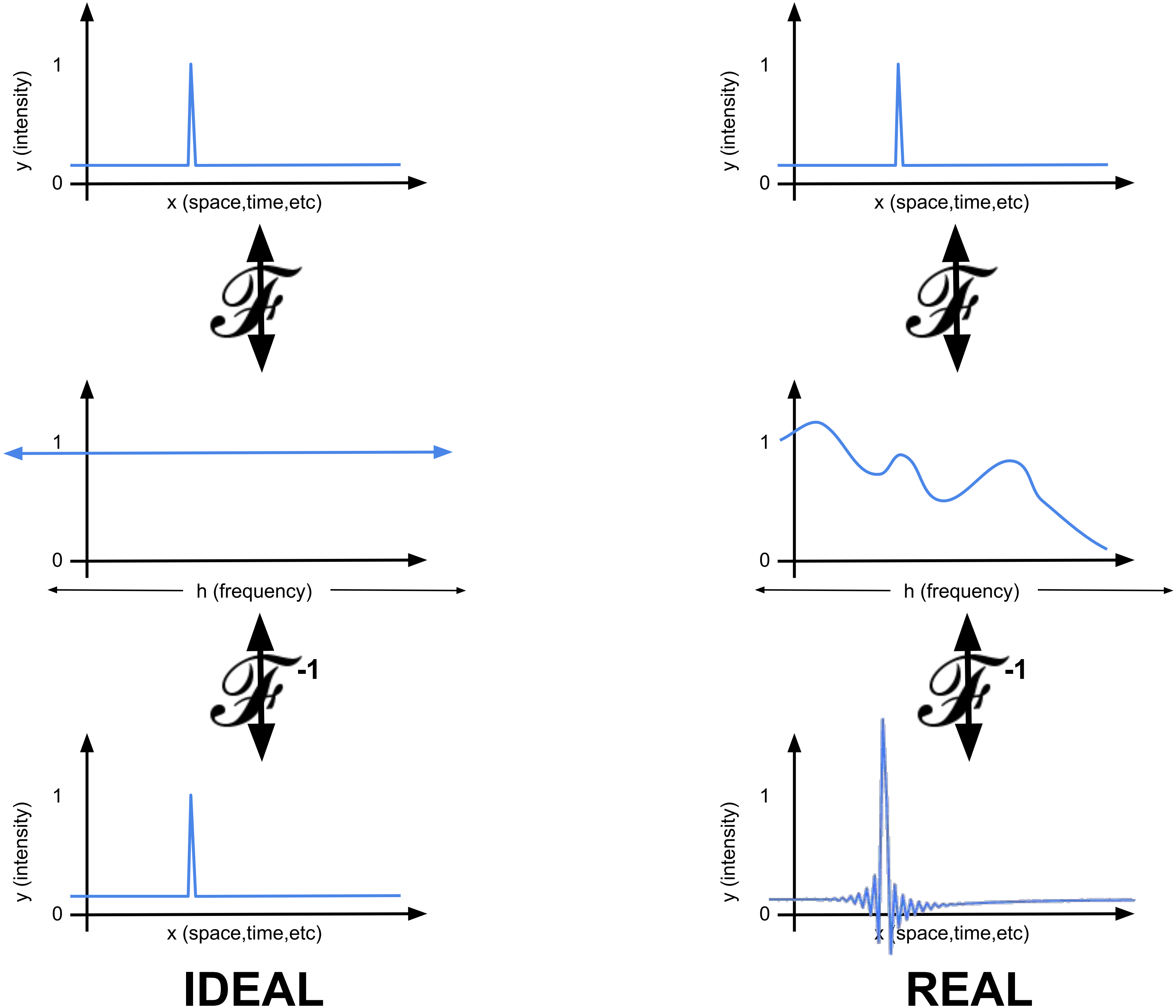

mp3
Marcia
"Oregon, My Oregon"
Land of the
Land of the
Empire Builders, Land of the Gold-en West; Con-quered and held by free men,
rose and sunshine, Land of the sum-mer's breeze; Lad-en with health and vig-or,
Fair-est and the best. On-ward and up-ward ev-er, Forward and on, and
Fresh from the Western seas. Blest by the blood of mar-tyrs, Land of the set-ting
on; Hail to thee, Land of He - roes, My O - re - gon.
sun; Hail to thee, Land of Prom - ise, My O - re - gon.
SPLOID GIF
wiseGEEK


Phases
Frequency
Magnitude
Amplitudes
Time


Frequency
Magnitude
f_1
f_2
f_3
f_4
f_5
Time

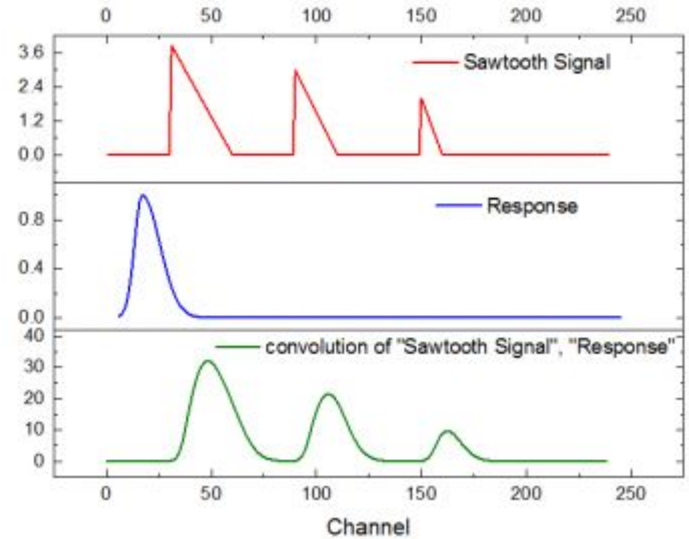


y (intensity)
1
0
x (space,time,etc)
A (amplitude)
h (frequency)
-1
1



y (intensity)
1
0
x (space,time,etc)
-1
h (frequency)
IDEAL
REAL

$$
\begin{aligned}
(f * g)(t) &\stackrel{\text{def}}{=} \int_{-\infty}^{\infty} f(\tau) g(t - \tau) \, d\tau \\
&= \int_{-\infty}^{\infty} f(t - \tau) g(\tau) \, d\tau.
\end{aligned}
$$



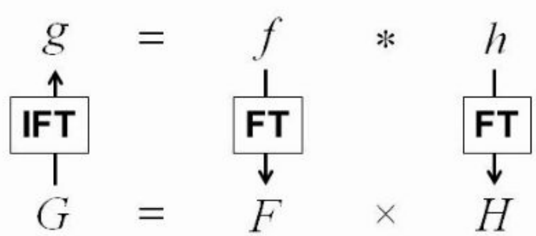
From: https://www.originlab.com/doc/Origin-Help/Convolution

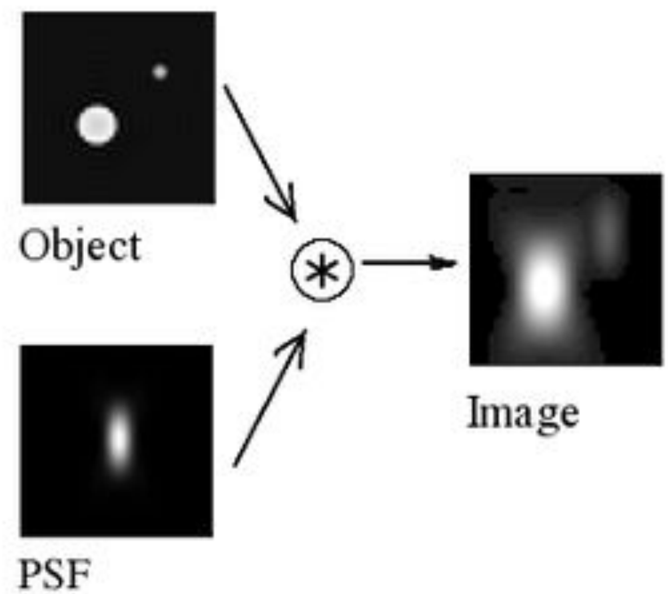
Spatial Domain *(x)* | **Frequency Domain *(u)***

$g = f * h \quad \longleftrightarrow \quad G = FH$

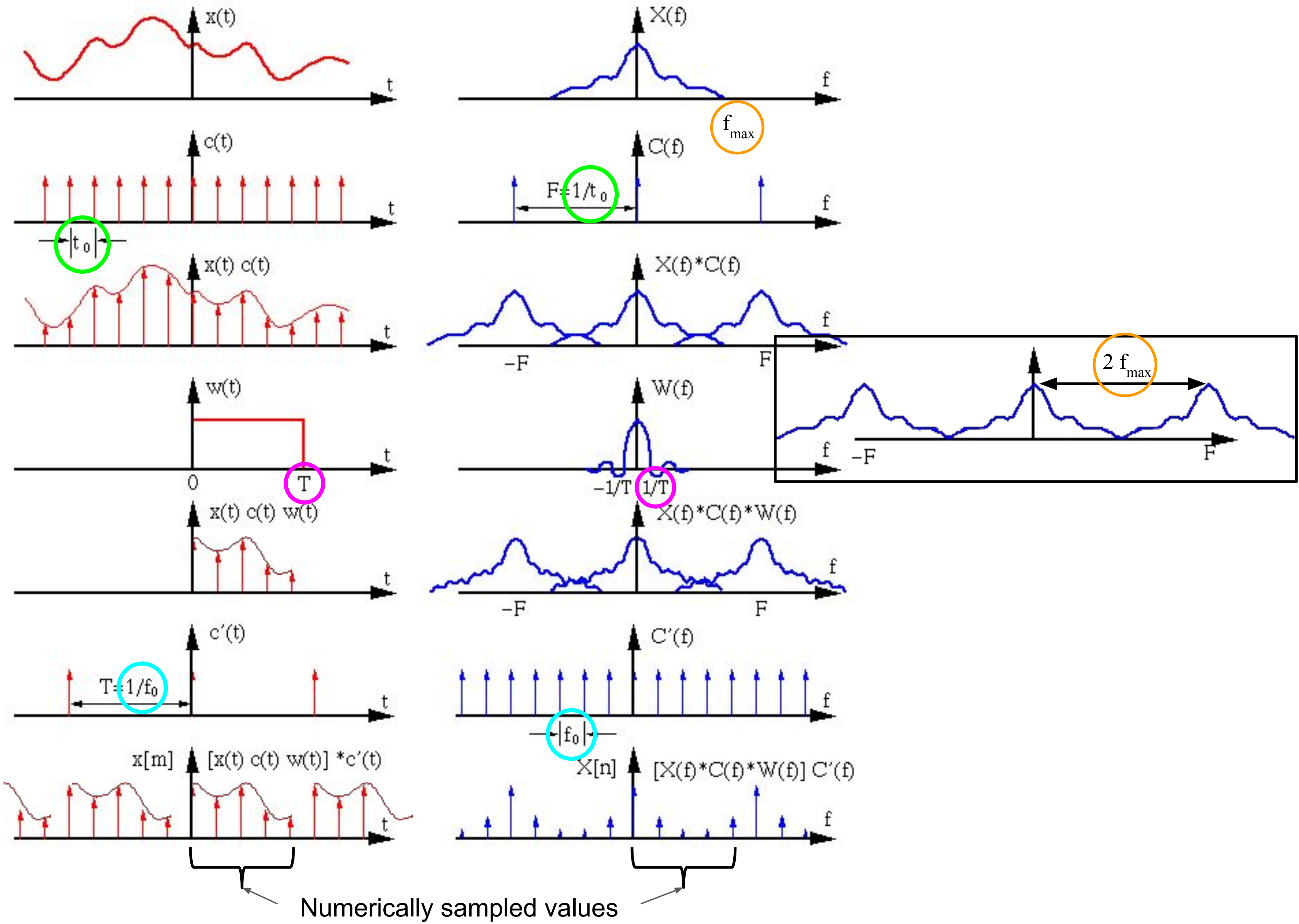
$g = fh \quad \longleftrightarrow \quad G = F * H$

So, we can find *g(x)* by Fourier transform



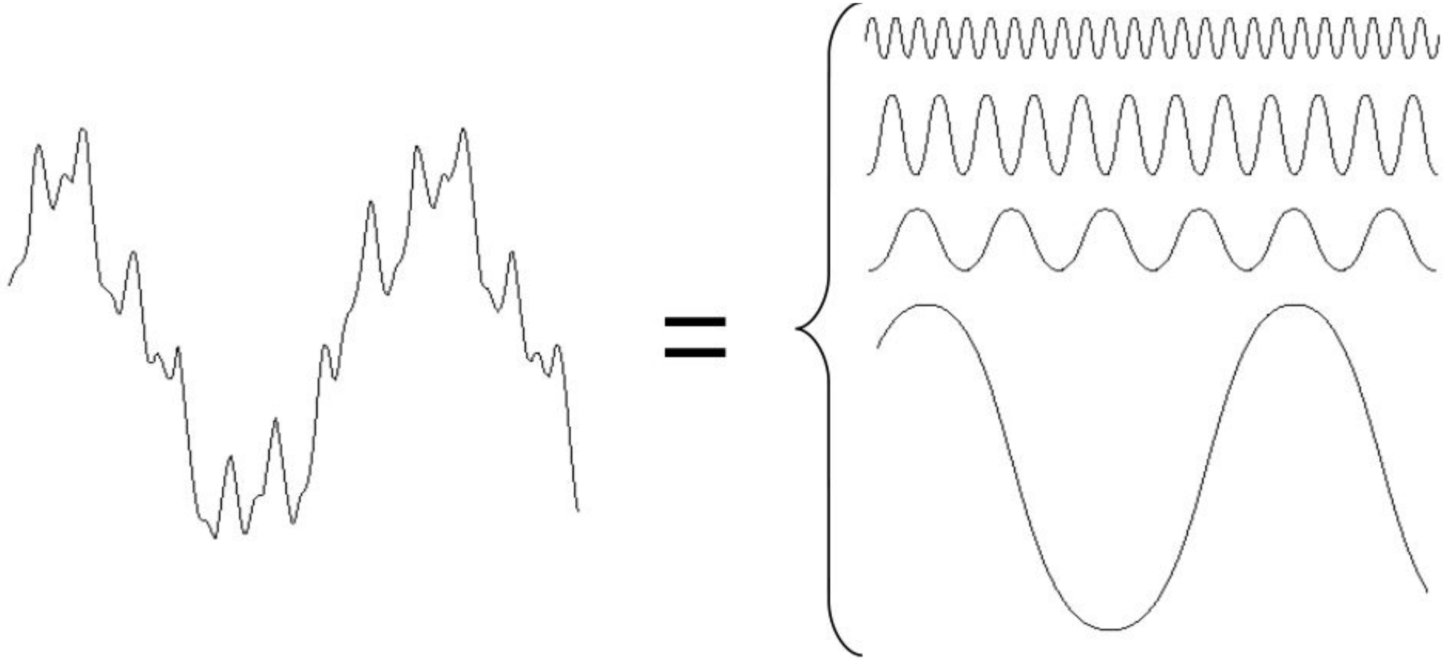


from: https://en.wikipedia.org/wiki/Point_spread_function



from: http://fourier.eng.hmc.edu/e101/lectures/Image_Processing/node2.html

$$f(t) = a_0 + \sum_{n=1}^{\infty} a_n cos(nf_0 t) + \sum_{n=1}^{\infty} b_n sin(nf_0 t)$$



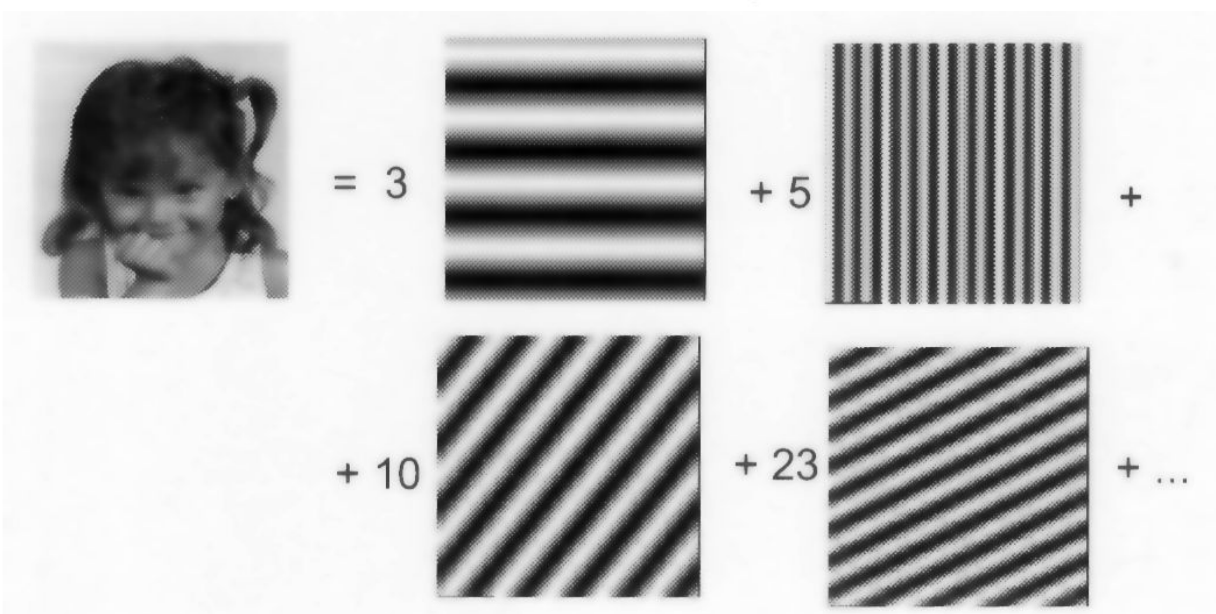
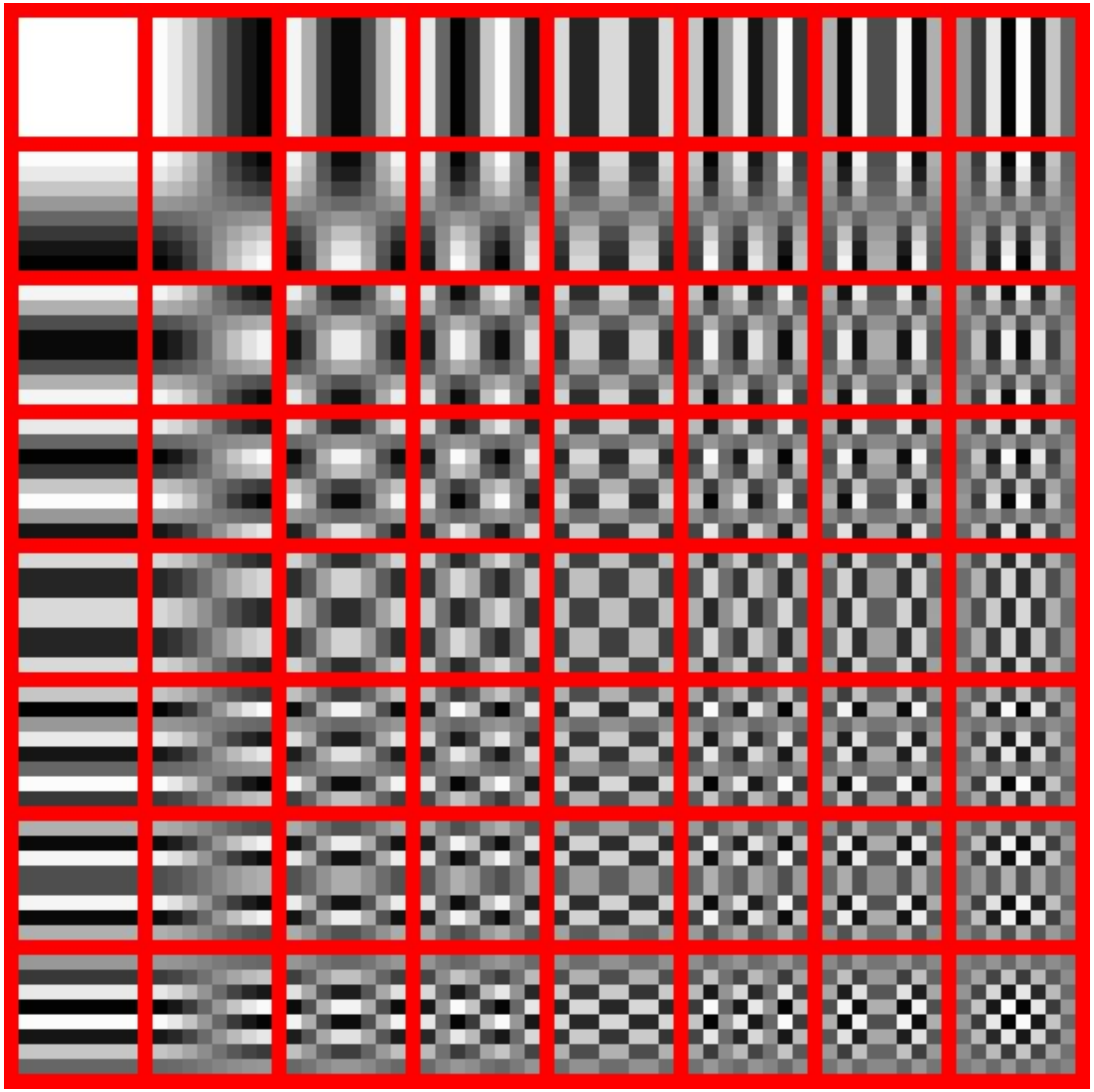
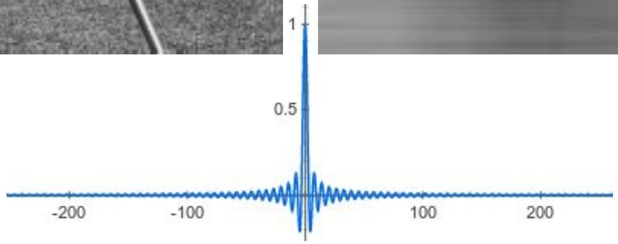


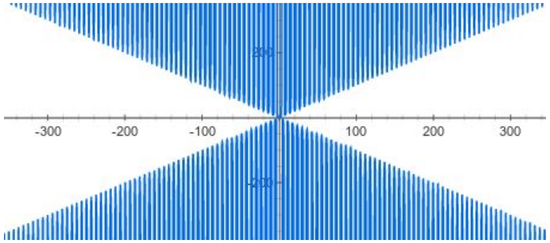
Image frequency decompostion

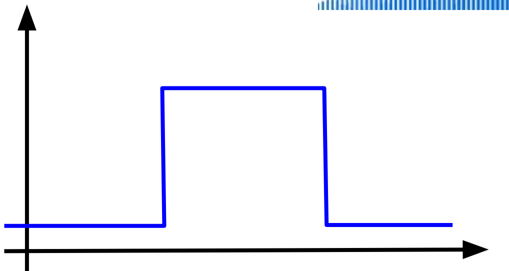


Resulting image | **Weighted component** | **Fourier component**

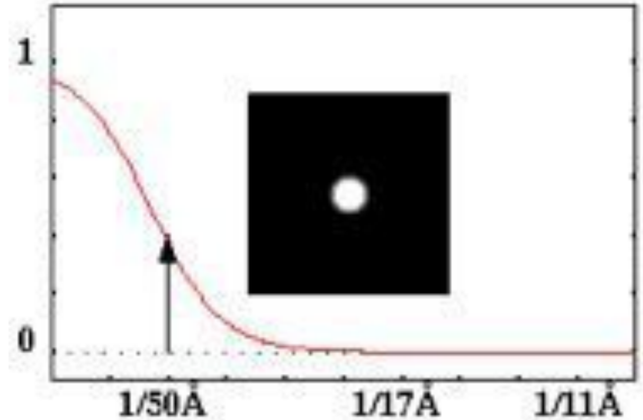
Motion blur (le flou cinétique)





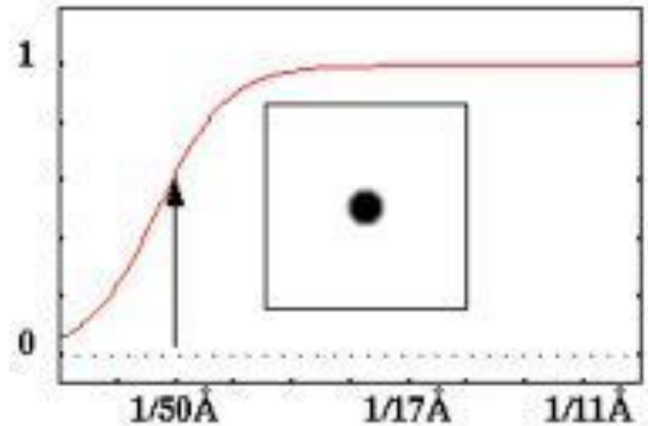


Fourier space filtering



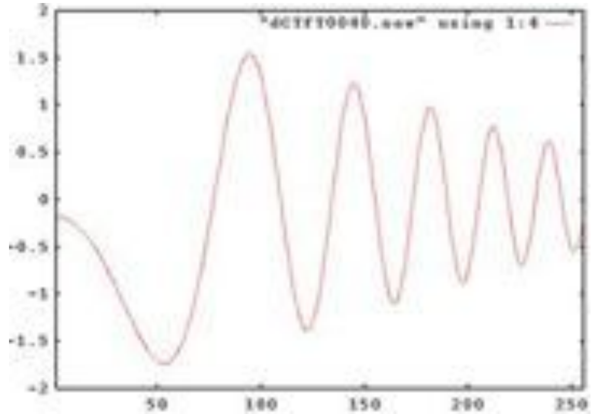
low-pass

Fourier space filtering

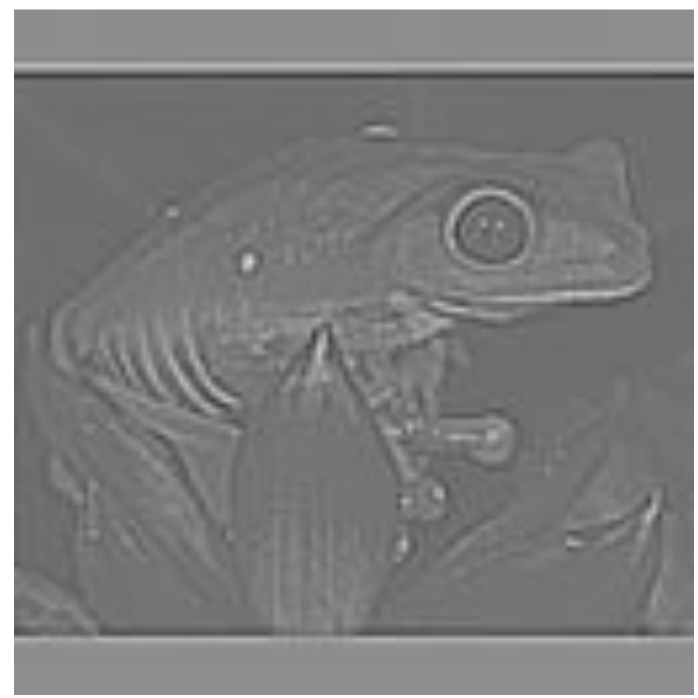


high-pass

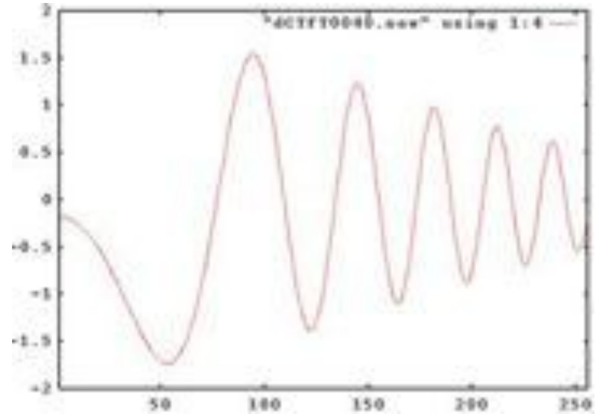
Fourier space filtering



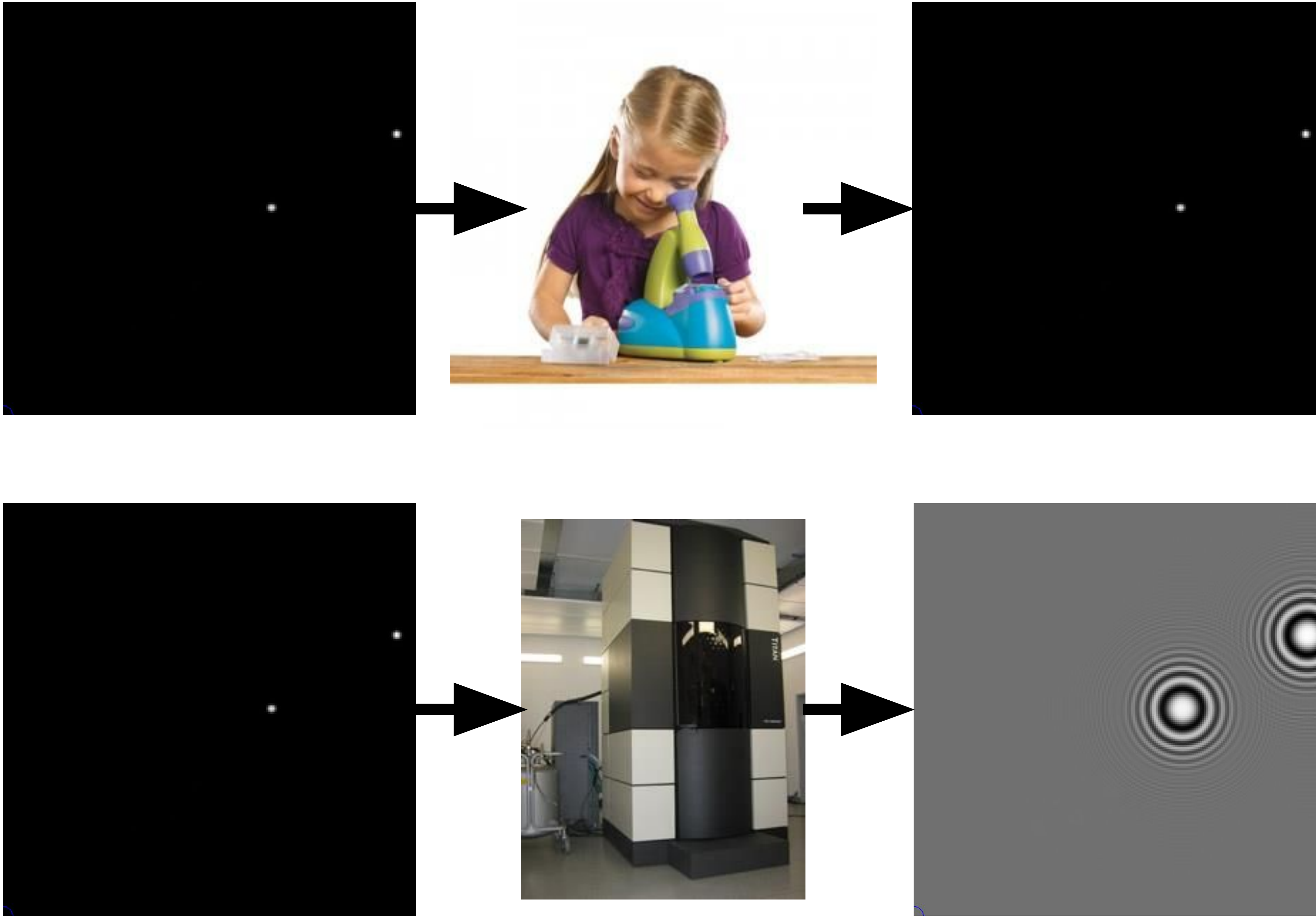
CTF

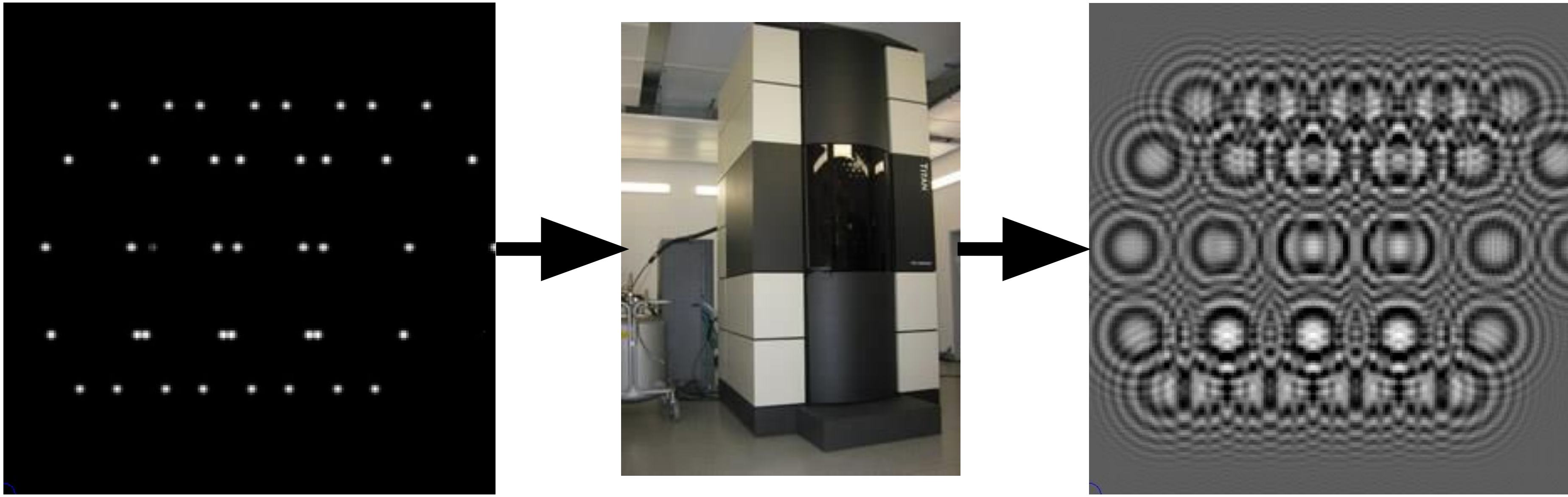


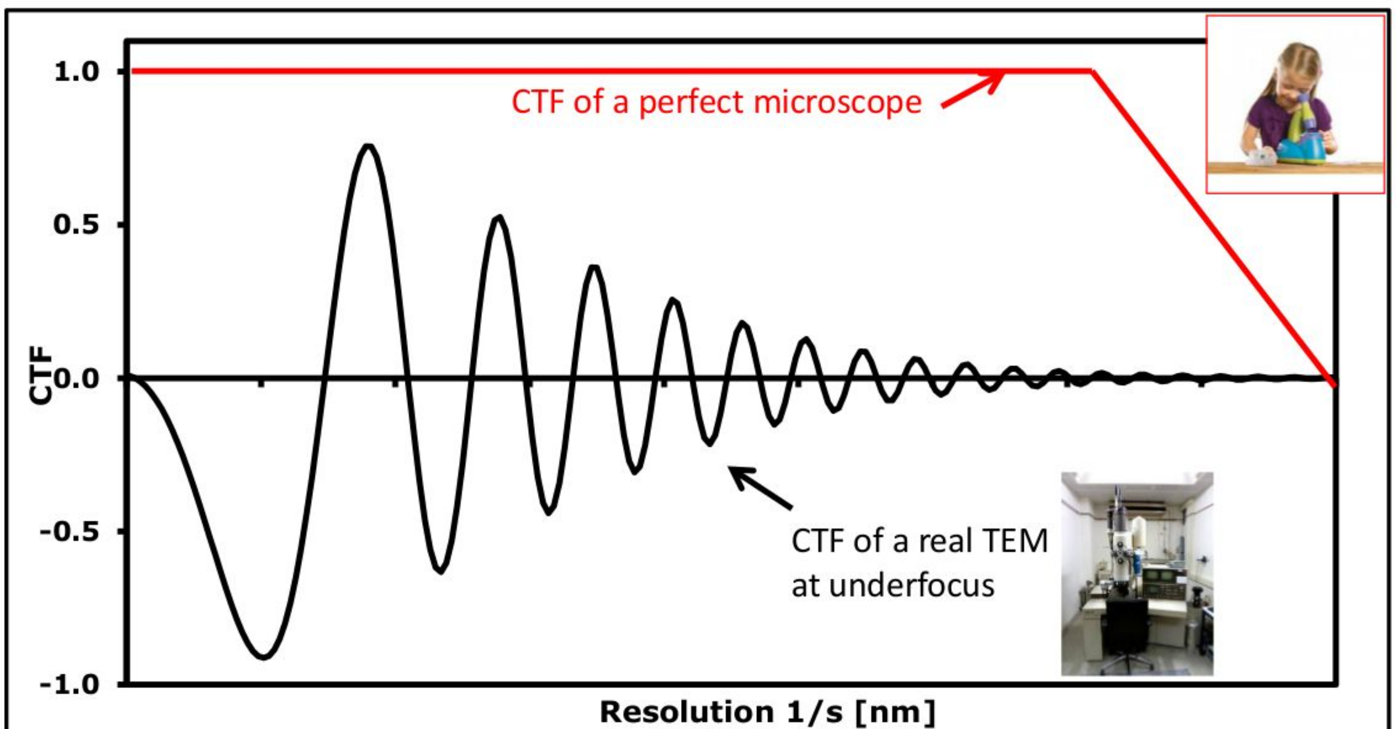
Fourier space filtering

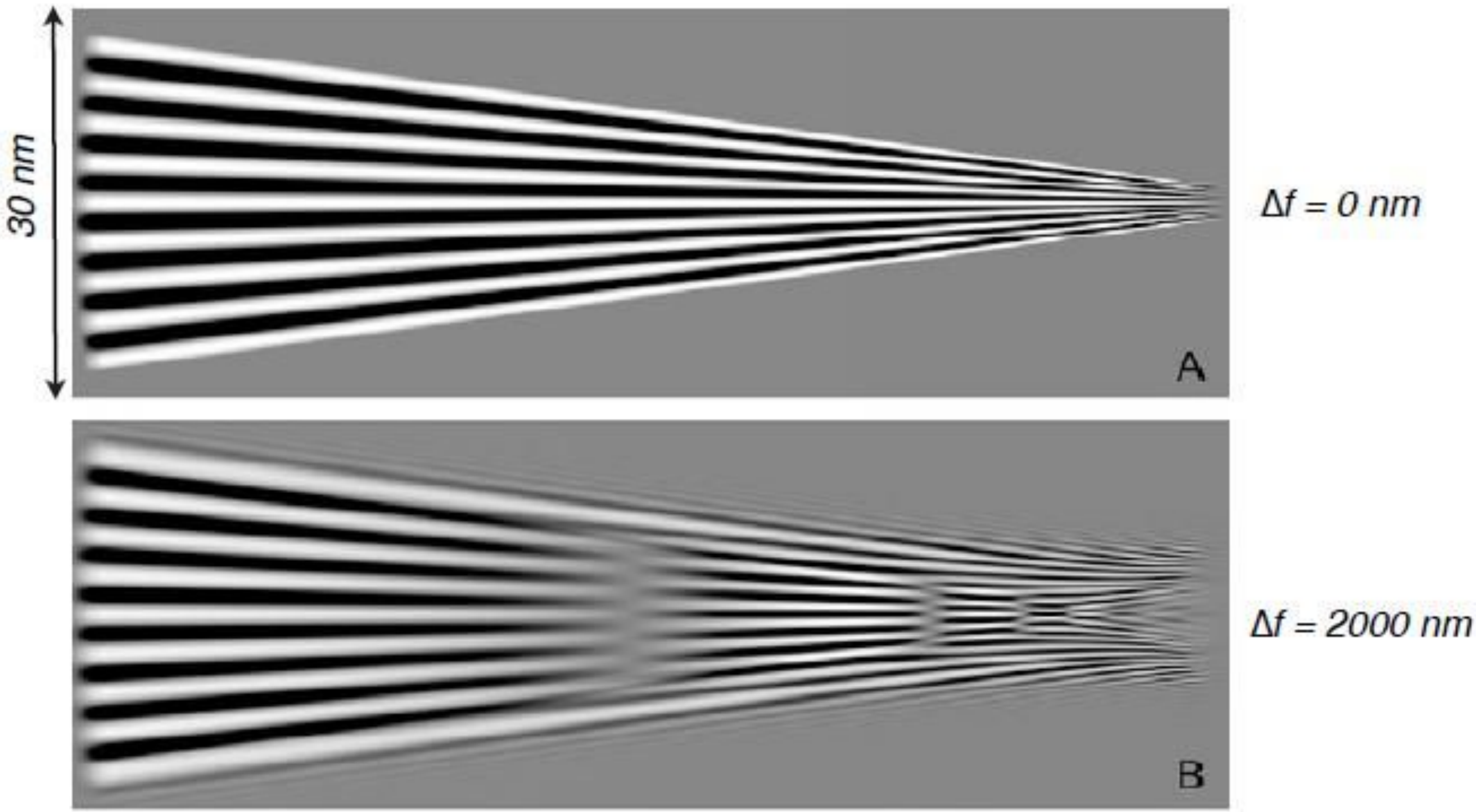


CTF-corrected


TITAN

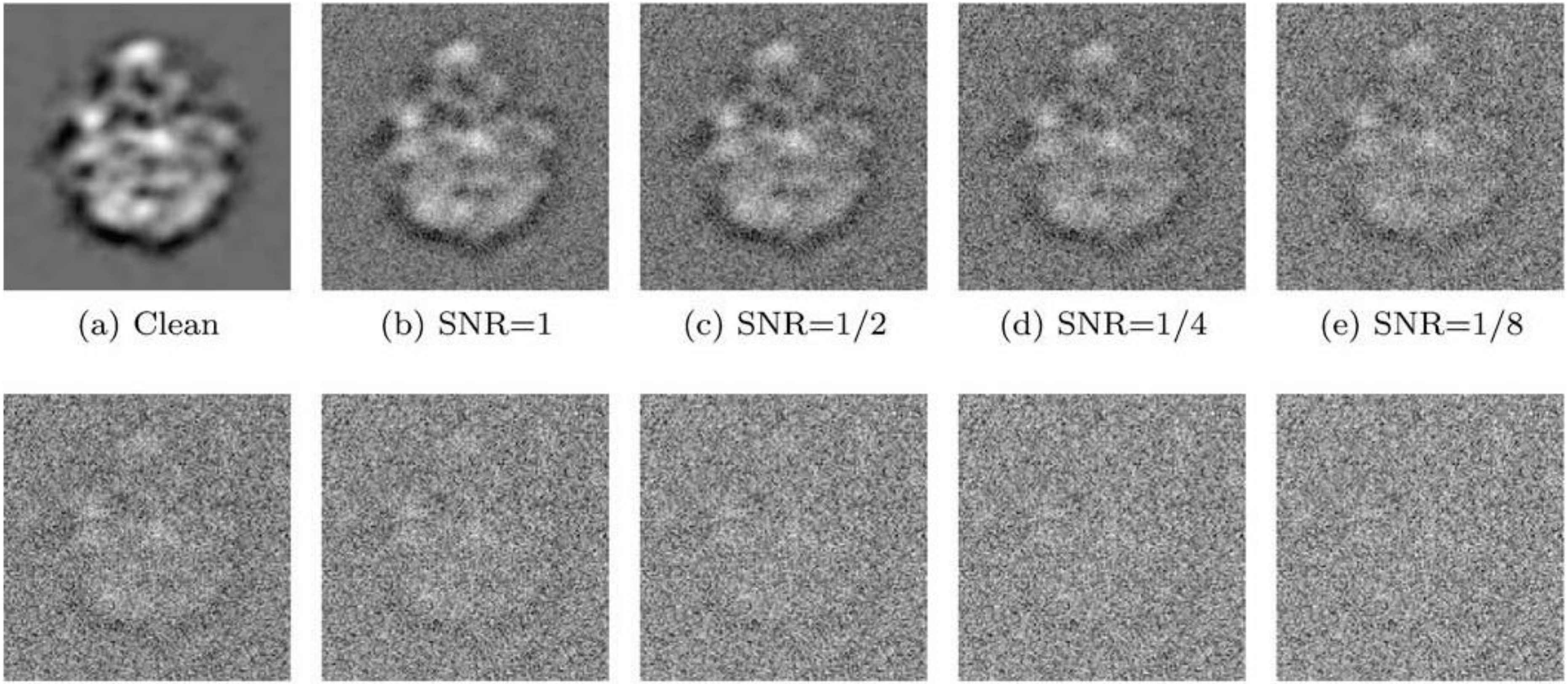



1.0
0.5
0.0
-0.5
-1.0
CTF
CTF of a perfect microscope
CTF of a real TEM
at underfocus
Resolution 1/s [nm]



Downing & Glaeser, Ultramicroscopy 2008

Signal to noise ratio



(a) Clean (b) SNR=1 (c) SNR=1/2 (d) SNR=1/4 (e) SNR=1/8

(f) SNR=1/16 (g) SNR=1/32 (h) SNR=1/64 (i) SNR=1/128 (j) SNR=1/256

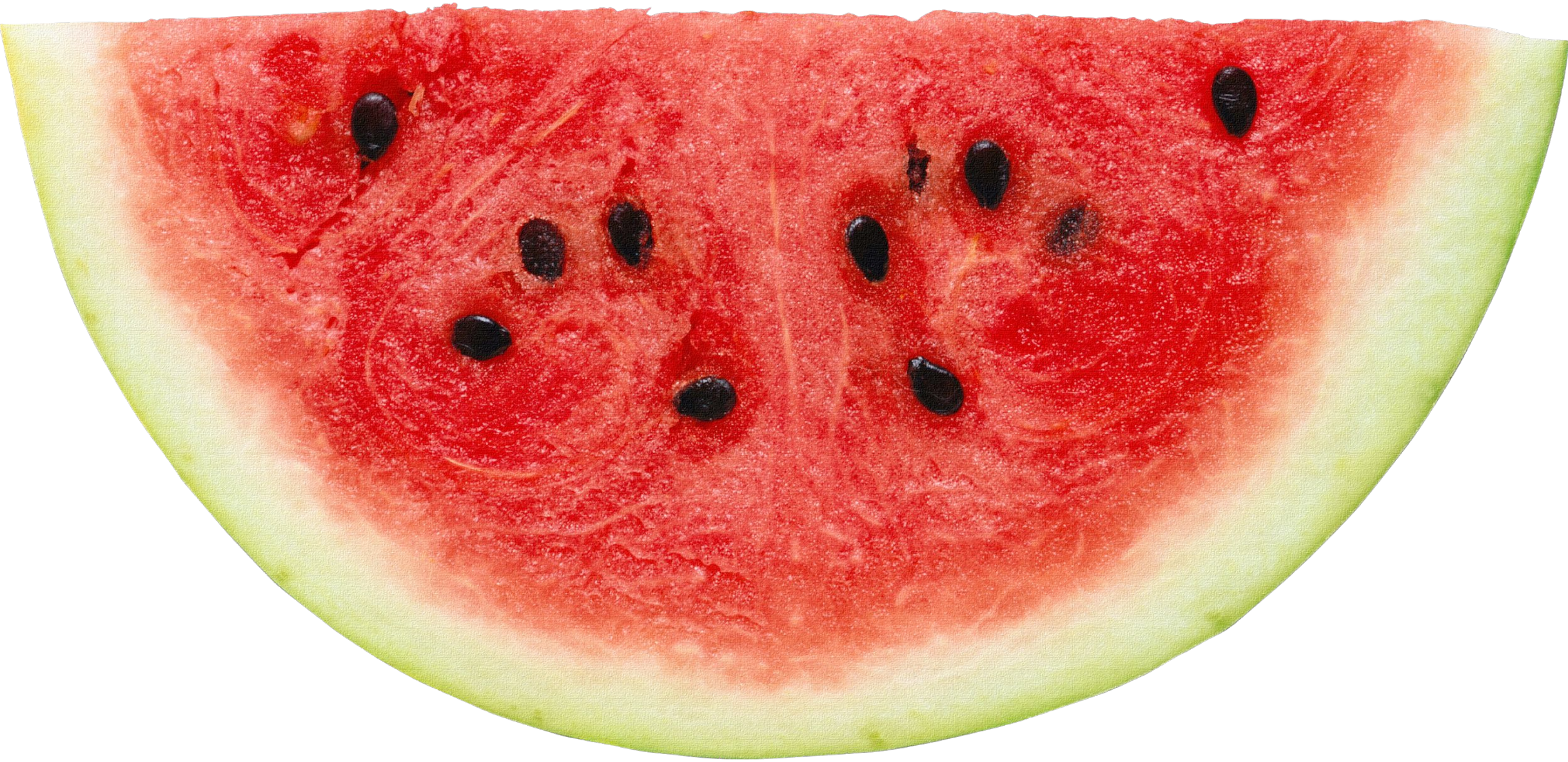
$$\mathrm{SNR} = \frac{\sigma^2_{\mathrm{signal}}}{\sigma^2_{\mathrm{noise}}}.$$

$$SSNR(r) = \frac{\sum_{r_i \in R} \left| \sum_{k_i} F_{r_i,k} \right|^2}{\frac{K}{K-1} \sum_{r_i \in R} \sum_{k_i} \left| F_{r_i,k} - \bar{F}_{r_i} \right|^2} - 1$$

From: https://www.semanticscholar.org/paper/Detecting-consistent-common-lines-in-cryo-EM-by-Singer-Coifman/639029f2c4af8d9f384d623695e3dcb43590fc02

Projection theorem (or Fourier slice)

$$F(h,k,l) = \iiint_{-\infty}^{\infty} \rho(x,y,z) e^{2\pi i(xh+yk+zl)} dxdydz$$



Projection theorem (or Fourier slice)

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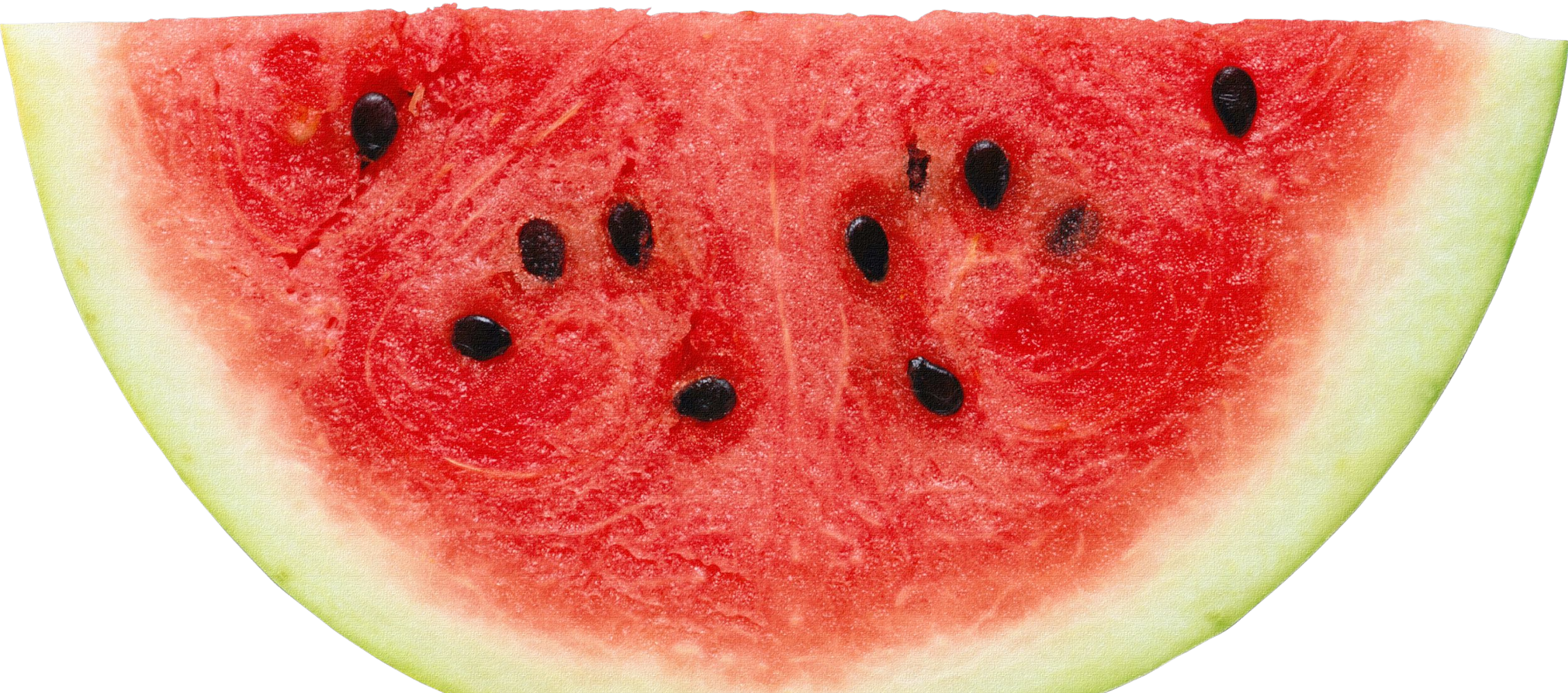
$$F(h,k,l) = \iiint_{-\infty}^{\infty} \overbrace{\int_{-\infty}^{\infty} \rho(x,y,z) dz}\, e^{2\pi i(xh+yk+zl)} dxdydz$$



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$$F(h,k,l) = \iiint_{-\infty}^{\infty} \overbrace{\underbrace{\int_{-\infty}^{\infty} \rho(x,y,z)dz}}\, e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iiint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk+zl)} dxdydz$$

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$$F(h,k,l) = \iiint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy \int_{-\infty}^{\infty} e^{2\pi i(zl)} dz$$

Projection theorem (or Fourier slice)

$$F(h,k,l) = \iiint_{-\infty}^{\infty} \rho(x,y,z) e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iiint_{-\infty}^{\infty} \overbrace{\underbrace{\int_{-\infty}^{\infty} \rho(x,y,z)dz}}\ e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iiint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy \underbrace{\int_{-\infty}^{\infty} e^{2\pi i(zl)} dz}$$

$$F(h,k,l) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy\ \delta(l)$$

Projection theorem (or Fourier slice)

$$F(h,k,l) = \iiint_{-\infty}^{\infty} \rho(x,y,z) e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iiint_{-\infty}^{\infty} \overbrace{\underbrace{\int_{-\infty}^{\infty} \rho(x,y,z)dz}} \; e^{2\pi i(xh+yk+zl)} dxdydz$$

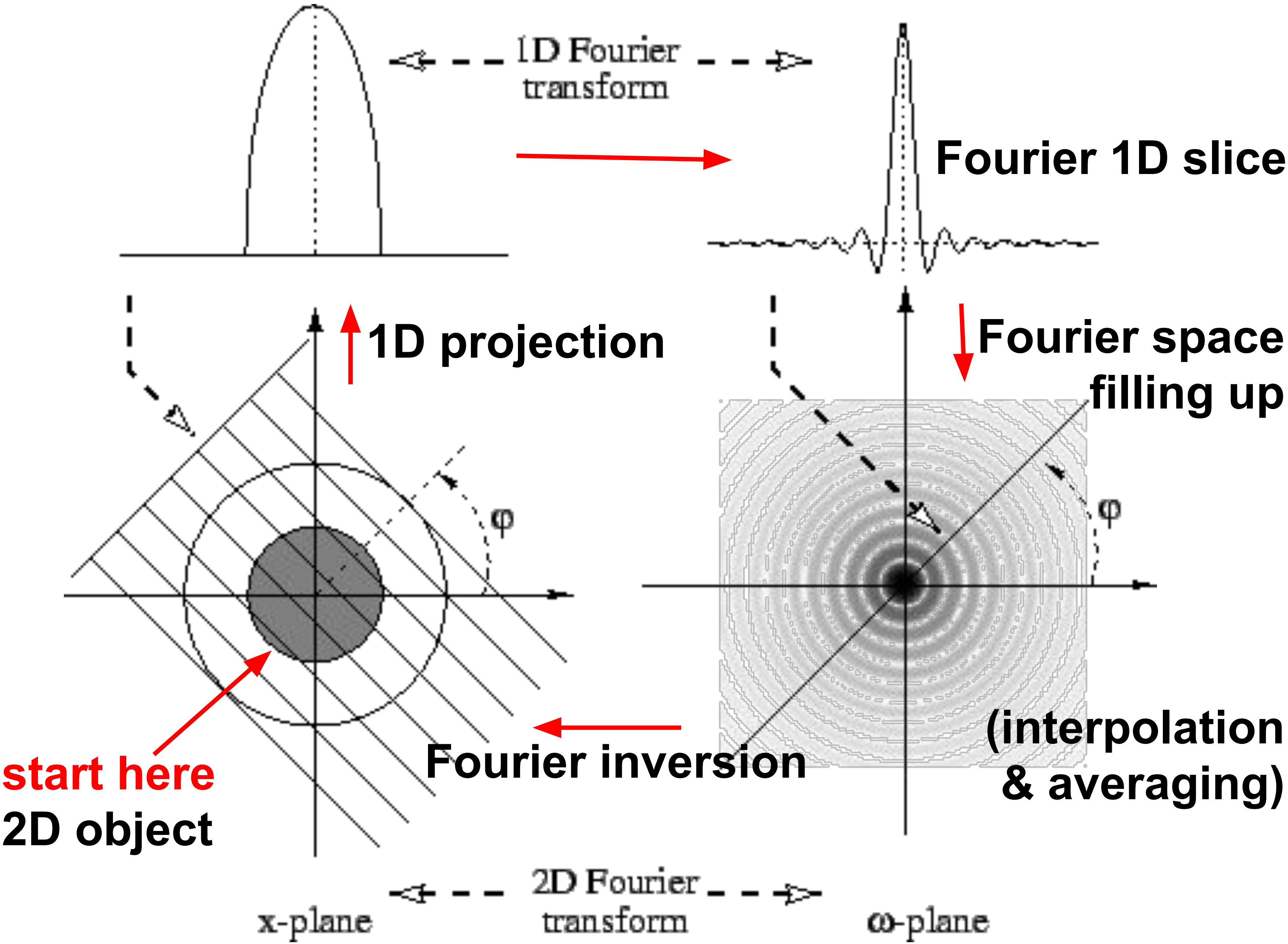
$$F(h,k,l) = \iiint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk+zl)} dxdydz$$

$$F(h,k,l) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy \; \underbrace{\int_{-\infty}^{\infty} e^{2\pi i(zl)} dz}$$

$$F(h,k,l) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy \; \delta(l)$$

$$F(h,k,0) = \iint_{-\infty}^{\infty} Img(x,y) e^{2\pi i(xh+yk)} dxdy$$

Projection (or Fourier slice) theorem 2D



Projection (or Fourier slice) theorem 3D

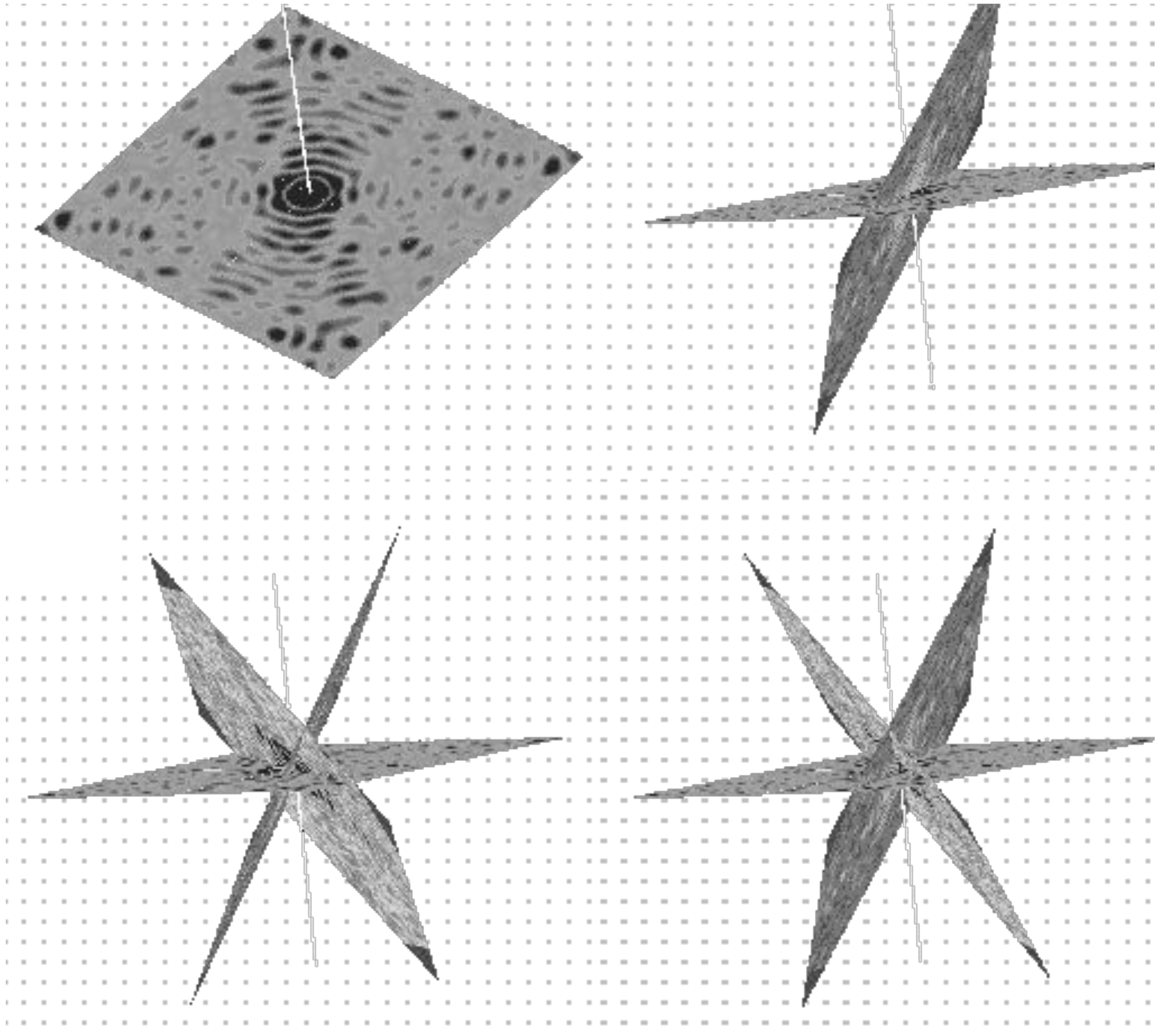
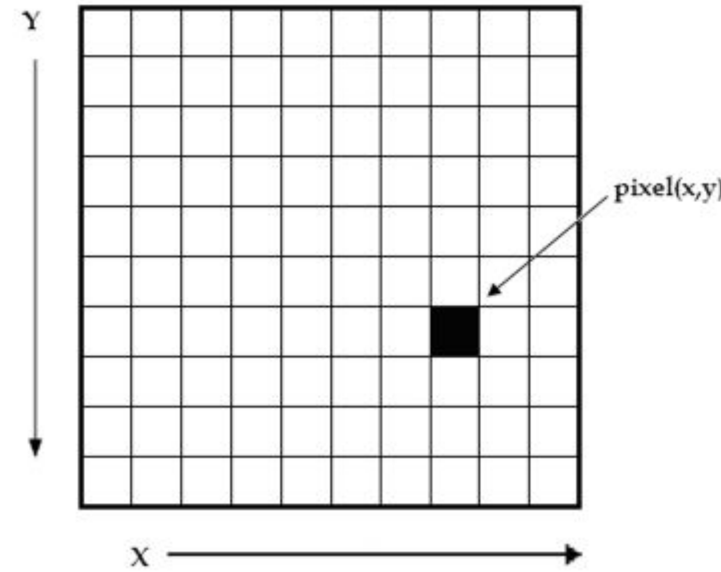
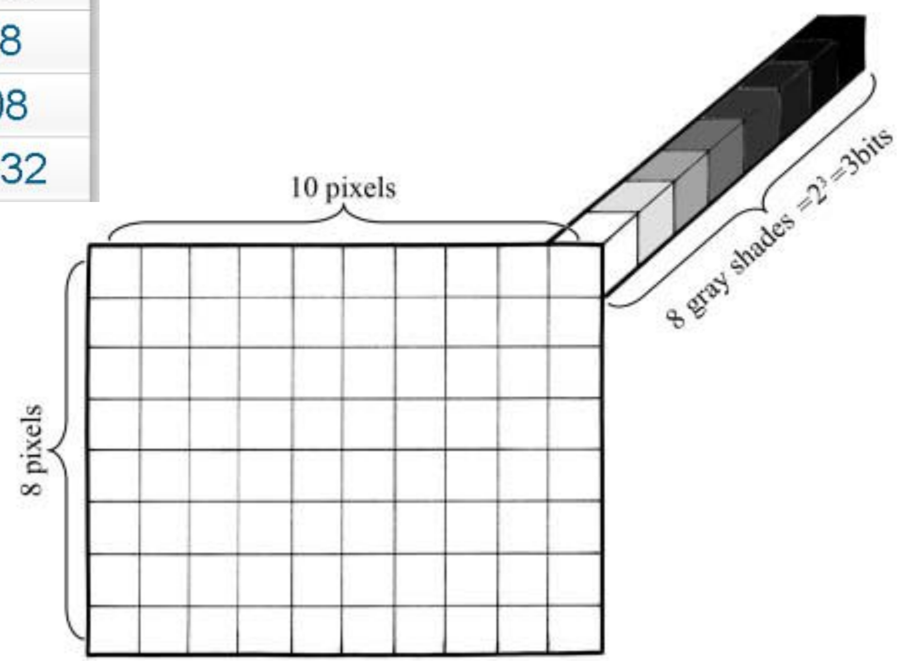


Image sampling and quantization

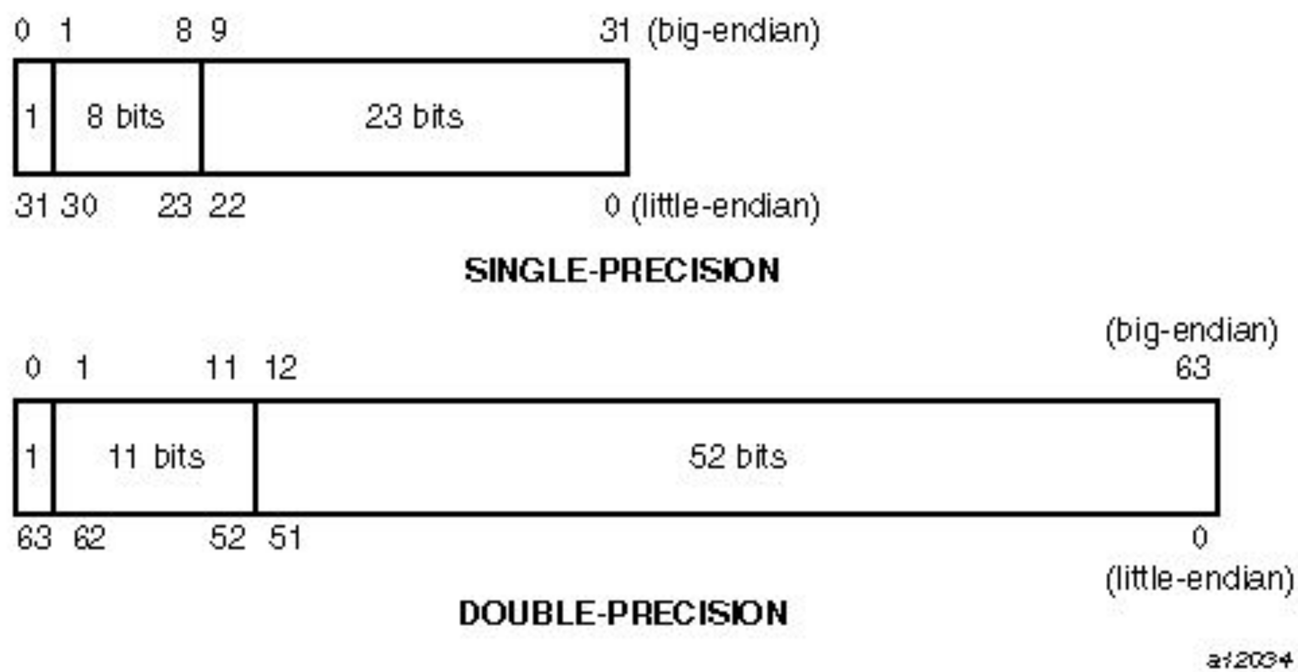
DATA TYPE	MIN_VALUE	MAX_VALUE
unsigned char	0	255
signed char	-128	127
unsigned short int	0	65535
signed short int	-32768	32767
unsigned int	0	65535
signed int	-32768	32767
unsigned long int	0	4294967295
signed long int	-2147483648	2147483647
float	-3.4 *10^-38	3.4 *10^38
double	-1.7*10^-308	1.7*10^308
long double	-3.4*10^-4932	1.1*10^+4932





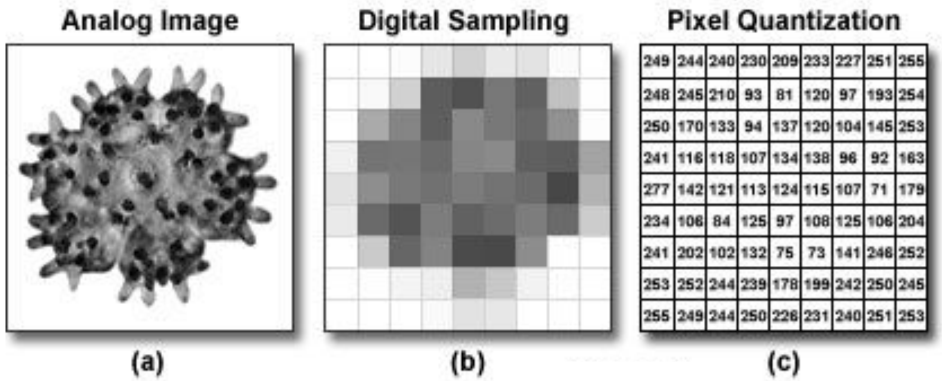
Floating-point number representation

$$\underbrace{6.63}_{Mantissa} \times \underbrace{10^{-34}}_{Exponent}$$

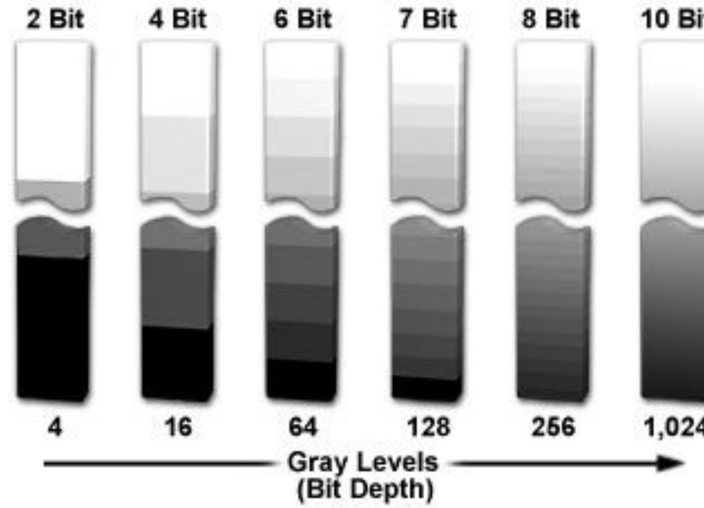


Digital image representation

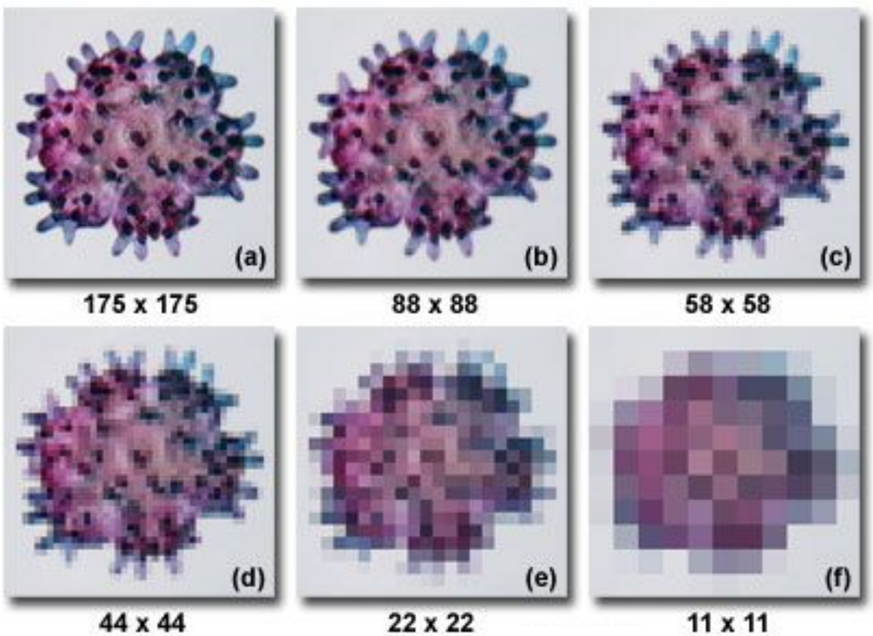
Creation of a Digital Image



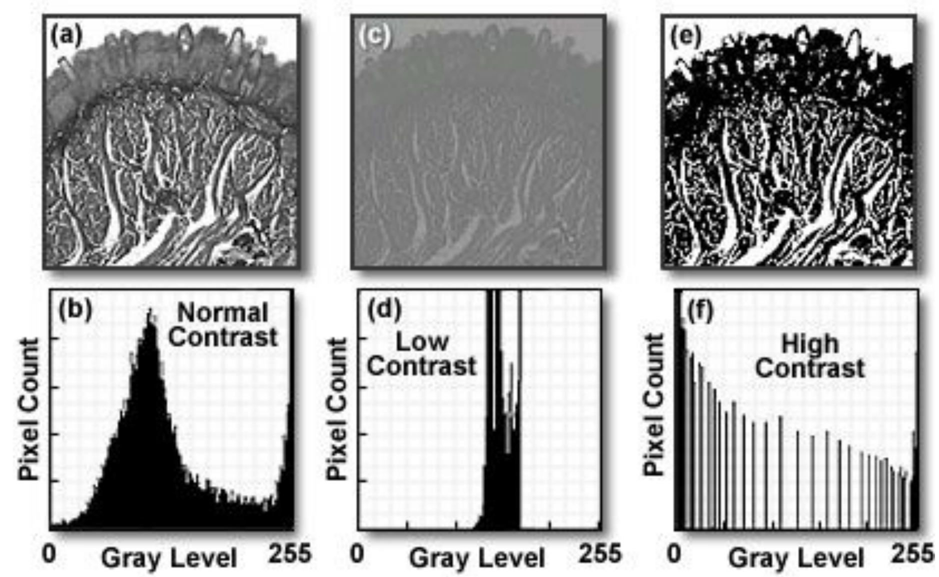
Bit Depth and Gray Levels in Digital Images



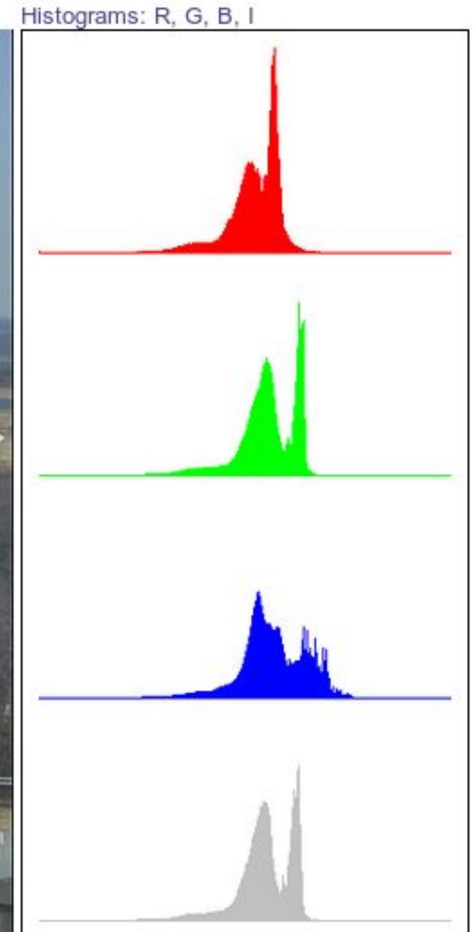
Spatial Resolution Effect on Pixelation in Digital Images



Grayscale Histograms and Contrast Levels in Digital Images



from: http://hamamatsu.magnet.fsu.edu/articles/digitalimagebasics.html

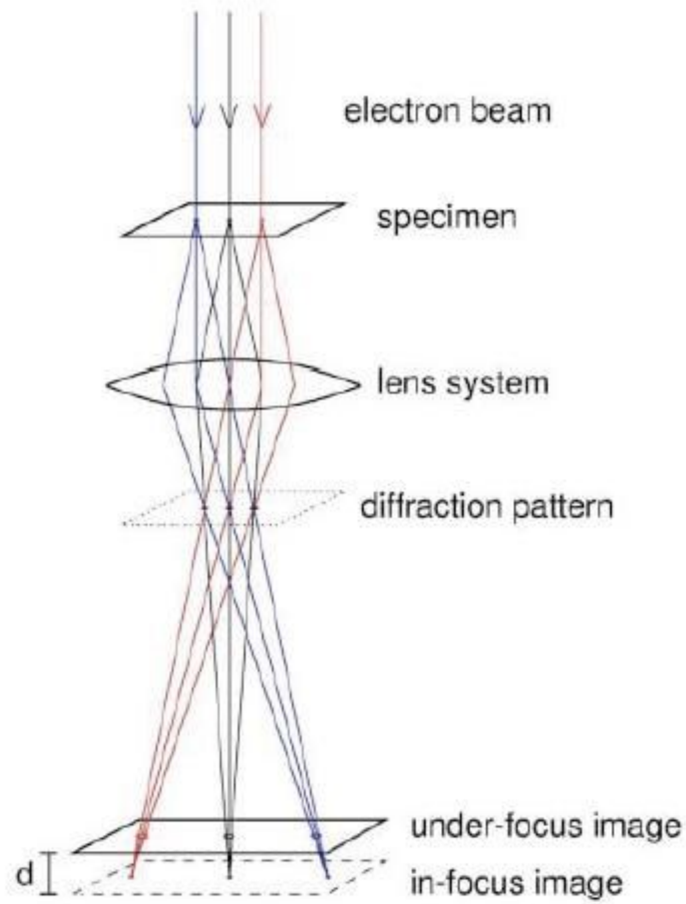


from: http://demo.ipol.im/demo/27/

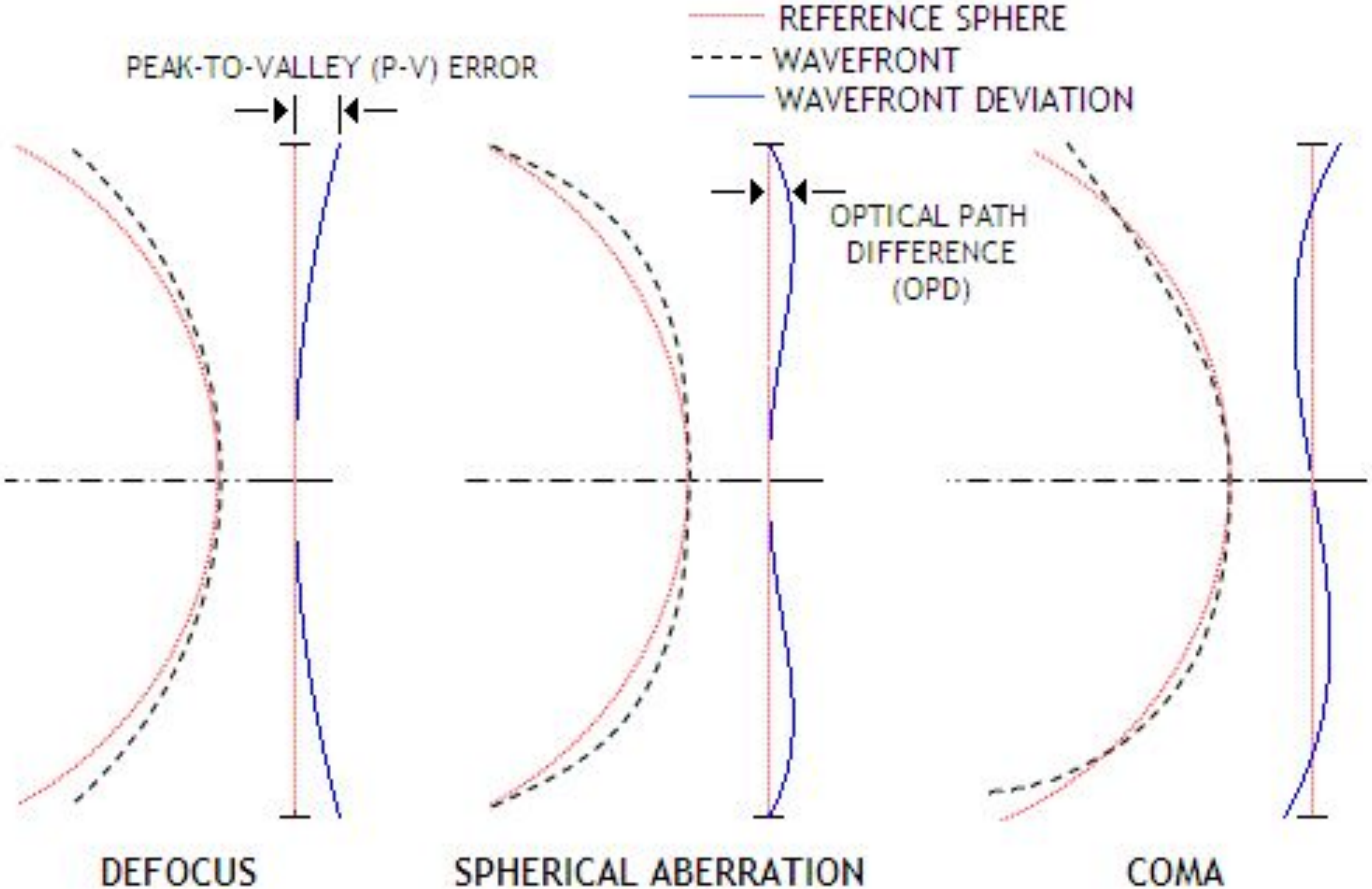
from: http://demo.ipol.im/demo/27/

Definitions

1. *Information:* any entity or form that resolves uncertainty or provides some answer to some kind of question.
2. *Data:* something from which information can be extracted (or not).
3. *Image:* a visible impression containing information obtained by a camera, telescope, microscope, or other device.
4. *Digital image processing:* to perform operations on images using digital equipment (e.g. computers).
5. *Digital:* data represented as a finite sequence of finite discrete values.
6. *Operation:* any of various mathematical or logical processes (such as addition, multiplication, etc) of deriving one entity from others according to a rule.
7. *Digital image analysis:* process by which information is extracted from images.



Volkmann & Hanein, 2002



from: http://www.telescope-optics.net/aberrations.htm