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Central European Institute of Technology
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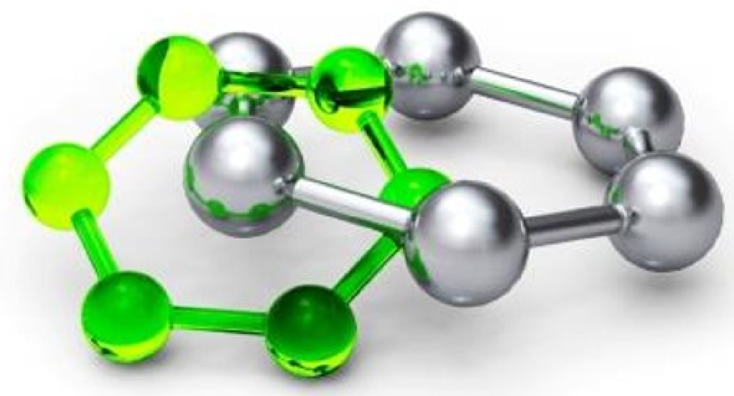
Image analysis II

C9940 3-Dimensional Transmission Electron Microscopy
S1007 Doing structural biology with the electron microscope

March 16, 2015



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Outline

Image analysis II

- Fourier transforms revisited
- Digitization
- Alignment
- Multivariate data analysis

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Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i k x} dx$$

f: function (1D) which we are transforming

x: real-space coordinate

i: √-1

k: spatial frequency

F(k): Fourier coefficient at frequency k

– complex, of the form *a* + *bi*

Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi ikx} dx$$

Euler's Formula: $e^{i\phi} = \cos\phi + i\sin\phi$

$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

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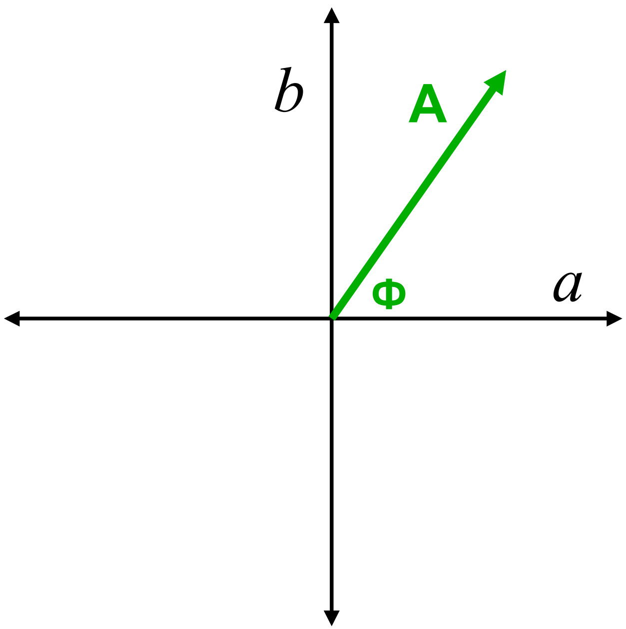
Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

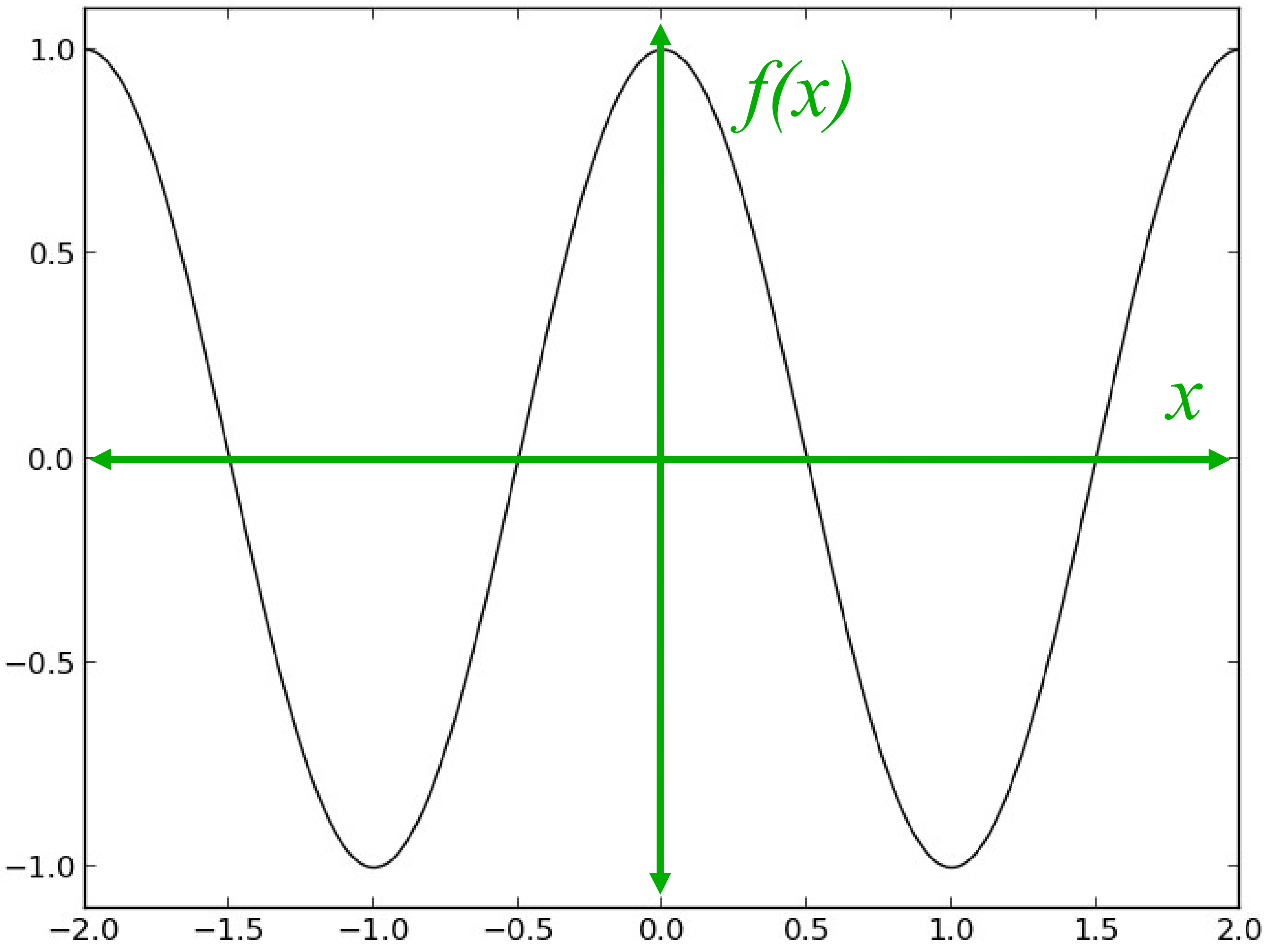
$$\boldsymbol{a} \quad +\boldsymbol{i} \quad \boldsymbol{b}$$



Amplitude, A: $\sqrt{a^2 + b^2}$

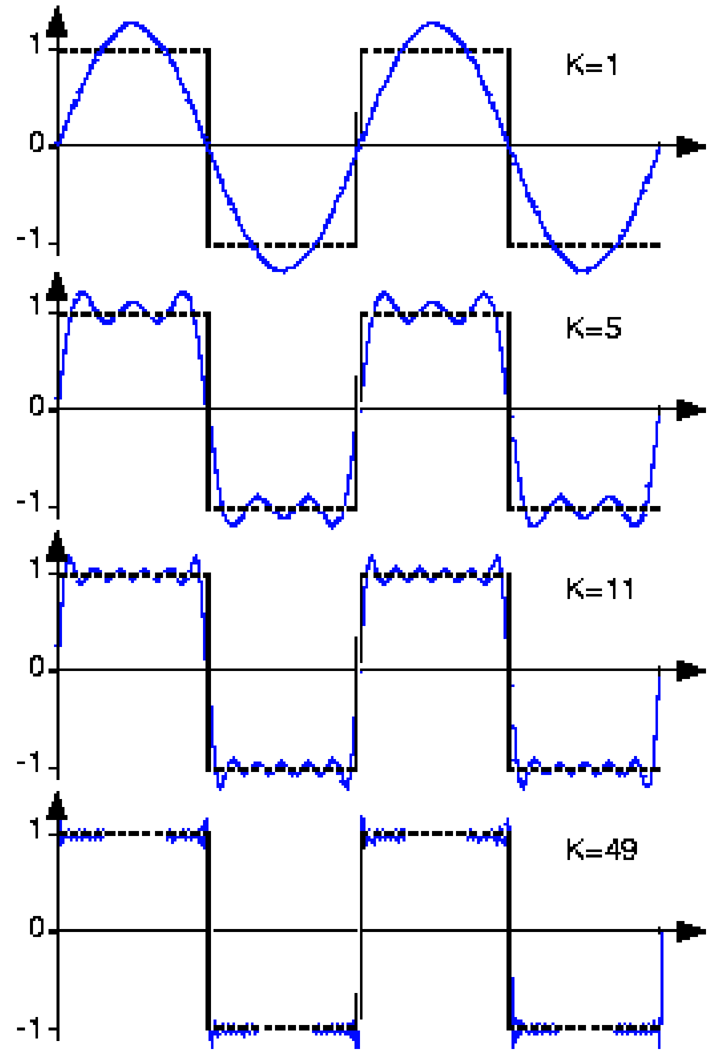
Phase, Φ: $\arctan \dfrac{b}{a}$

Fourier transforms: plot of cosine of x



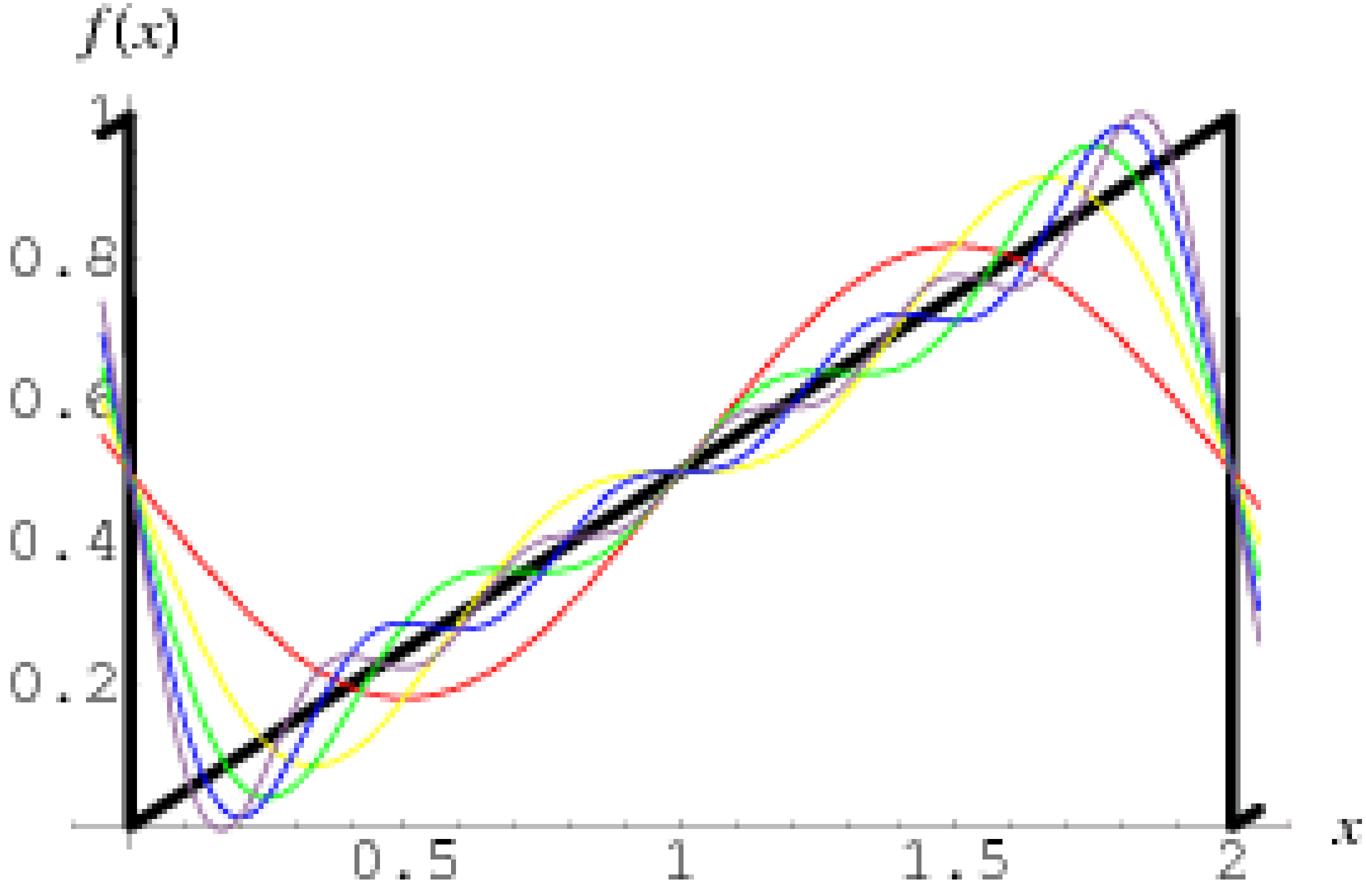
Fourier transforms: plot of step function

The higher the spatial frequencies (i.e., higher resolution) that are included, the more faithful the representation of the original function will be.



http://cnx.org

Fourier transforms: plot of sawtooth function



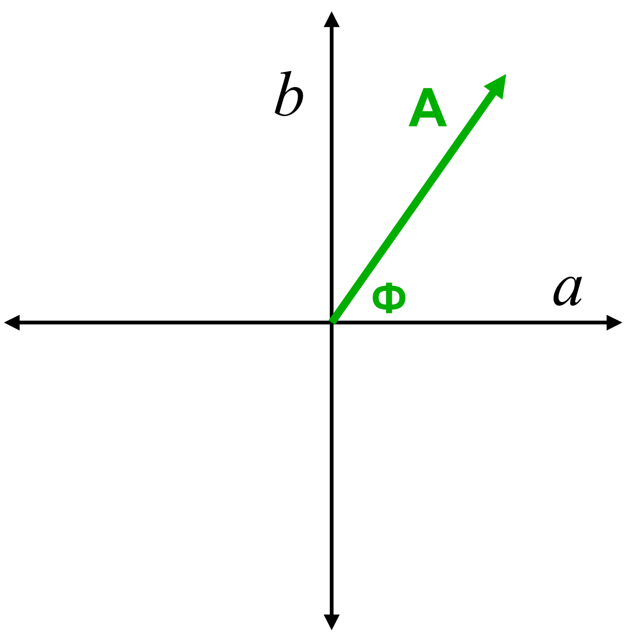
http://mathworld.wolfram.com

How do we calculate the Fourier coefficients?

Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

a $+i$ b

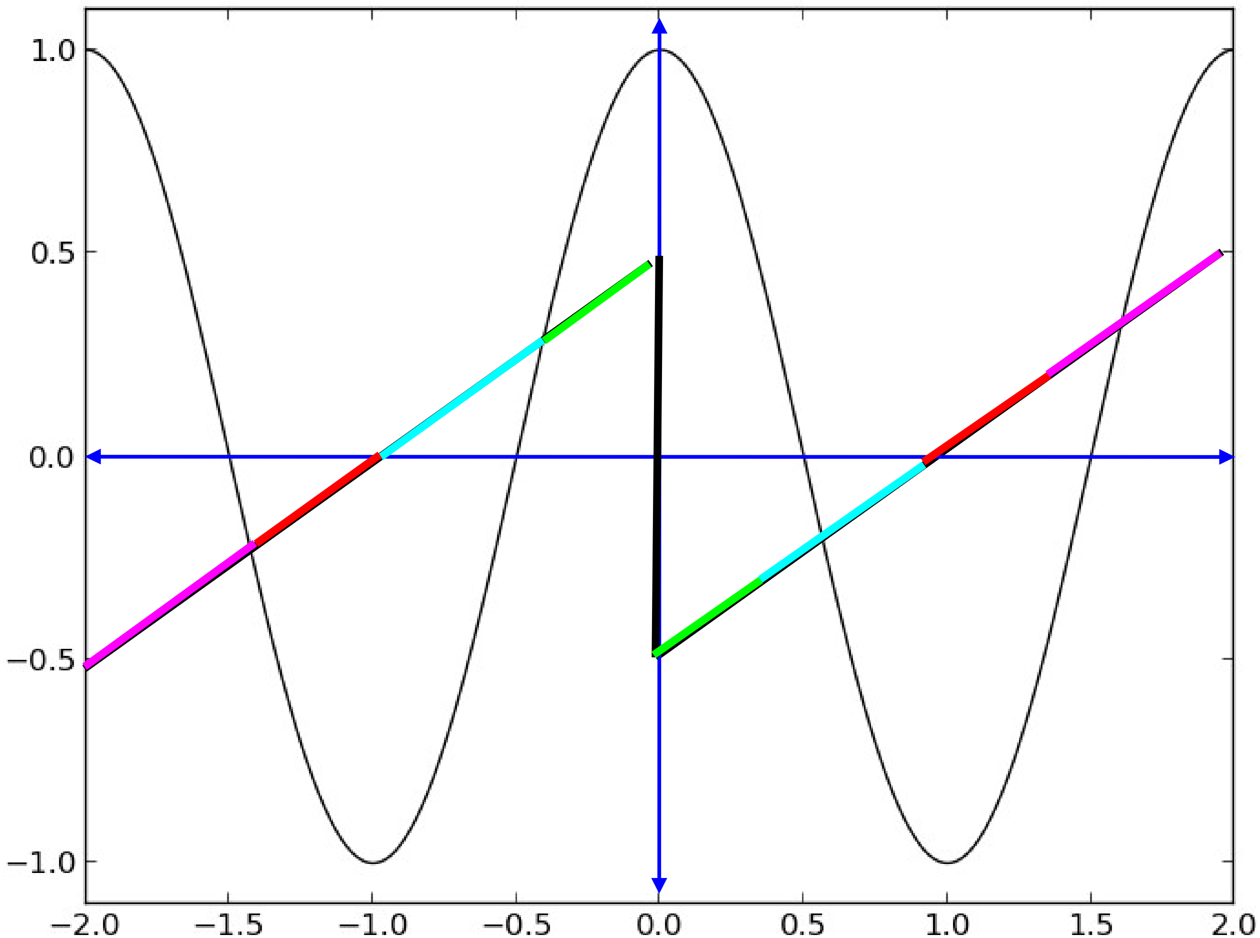


Amplitude, A: $\sqrt{a^2 + b^2}$

Phase, Φ: $\arctan \frac{b}{a}$

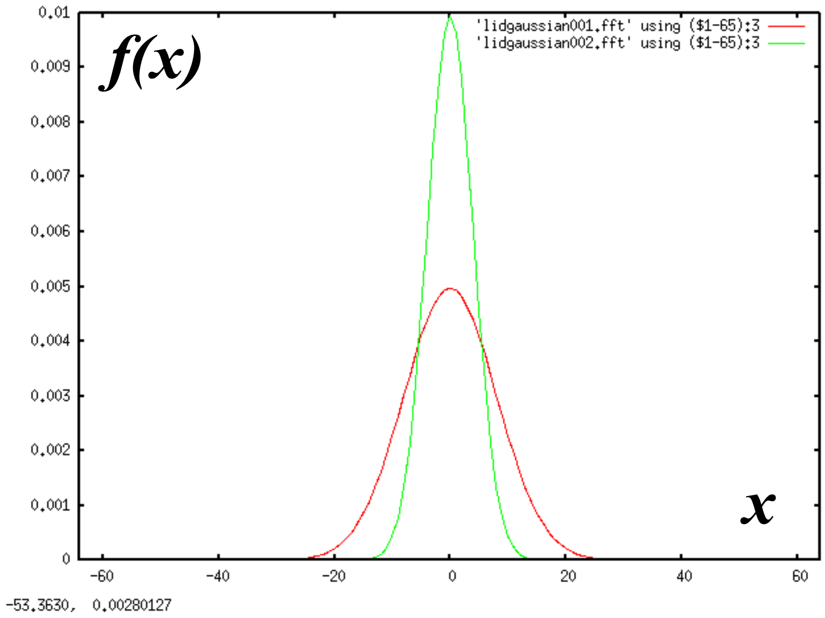
Why aren't we calculating the cosine terms?

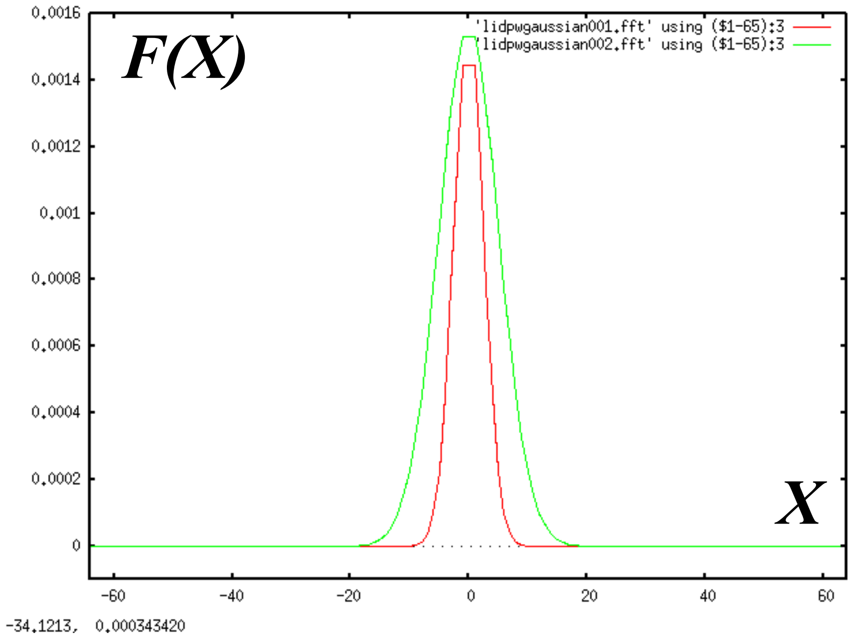
Fourier transforms: Sawtooth function



$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

Fourier transforms: plot of a Gaussian



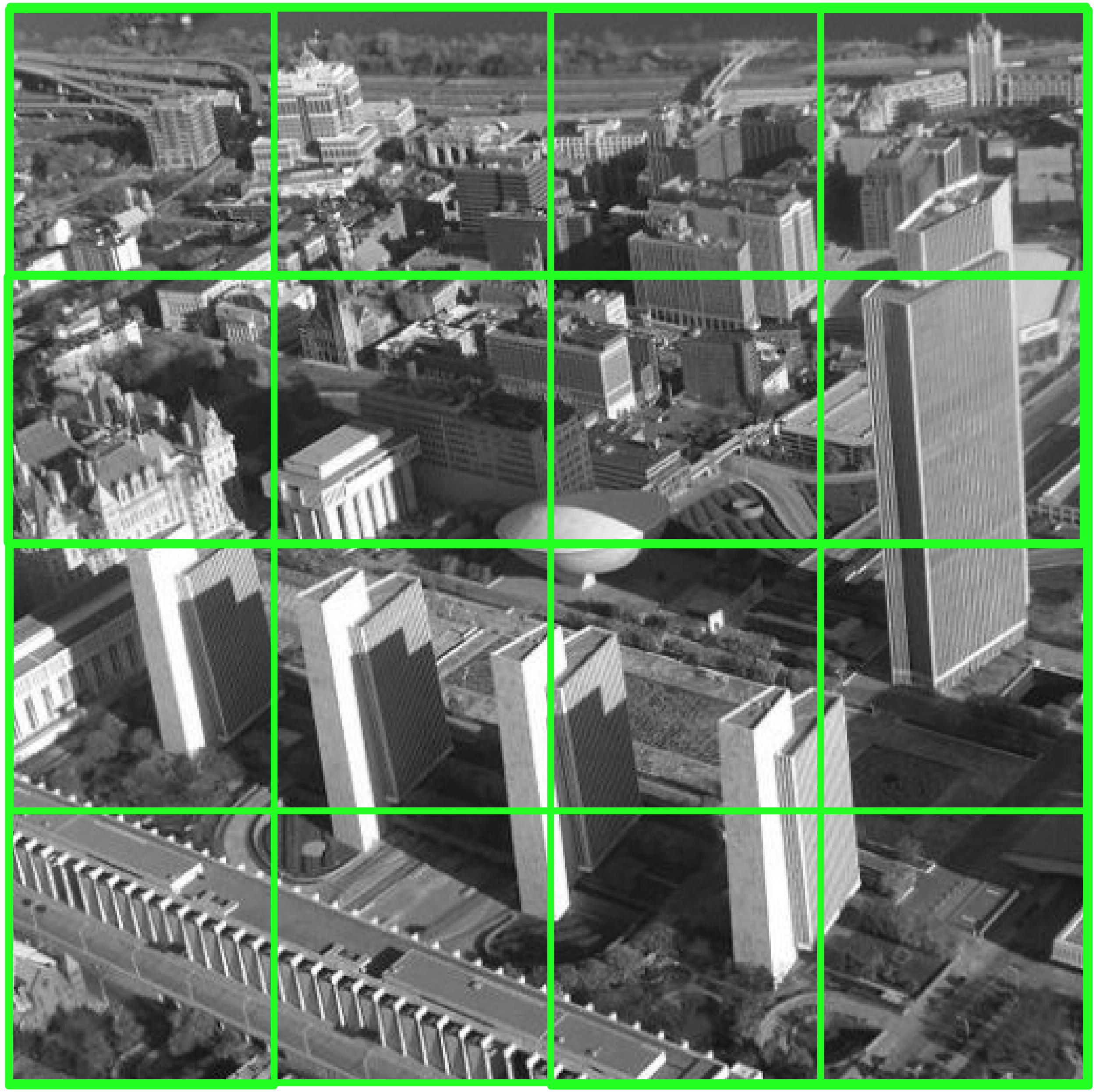


Outline

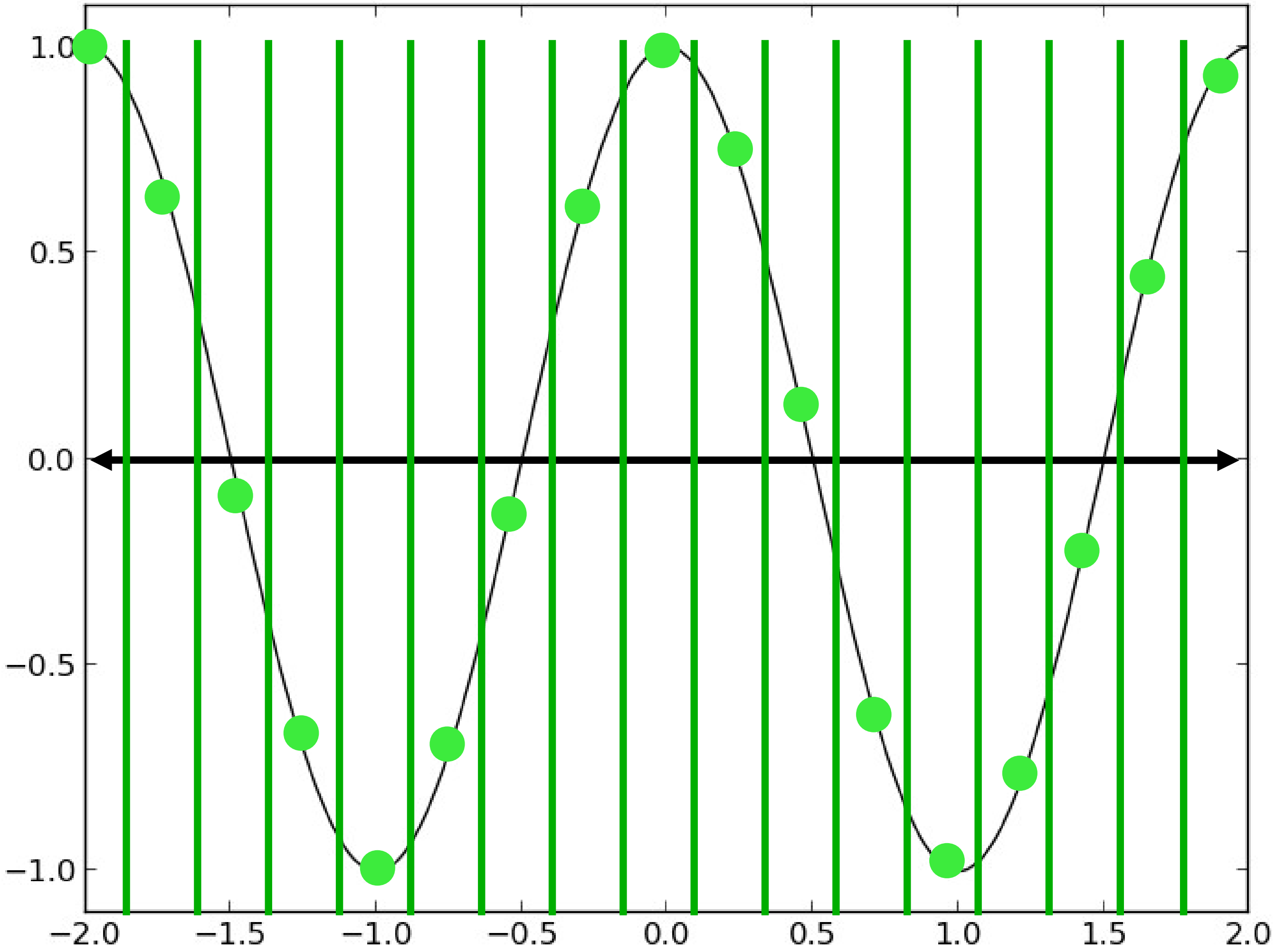
Image analysis II

- Fourier transforms revisited
- Digitization
- Alignment
- Multivariate data analysis

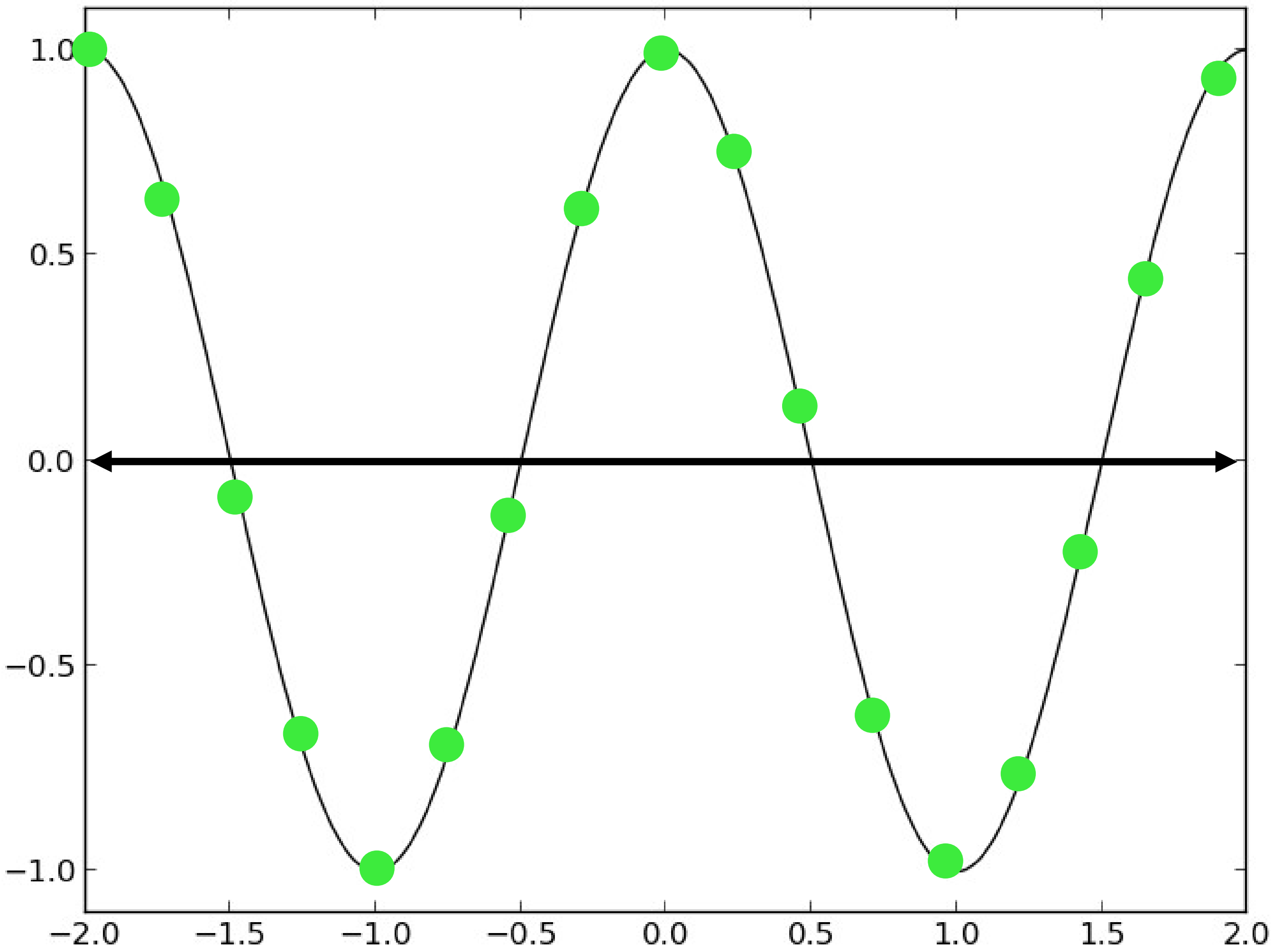
Digitization in 2D



Digitization in 1D: Sampling



Digitization: Is our sampling good enough?



Here, our sampling is good enough.

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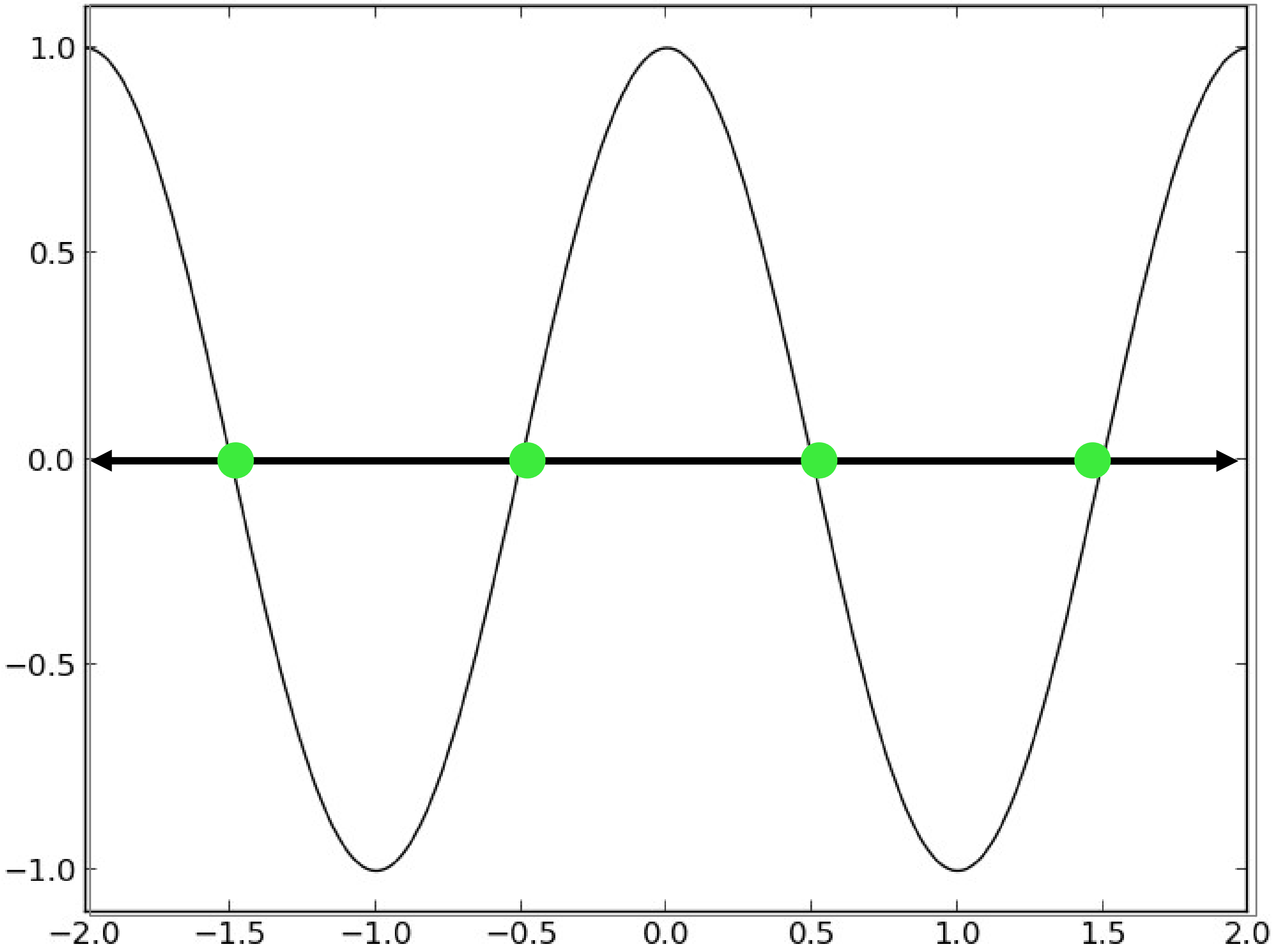
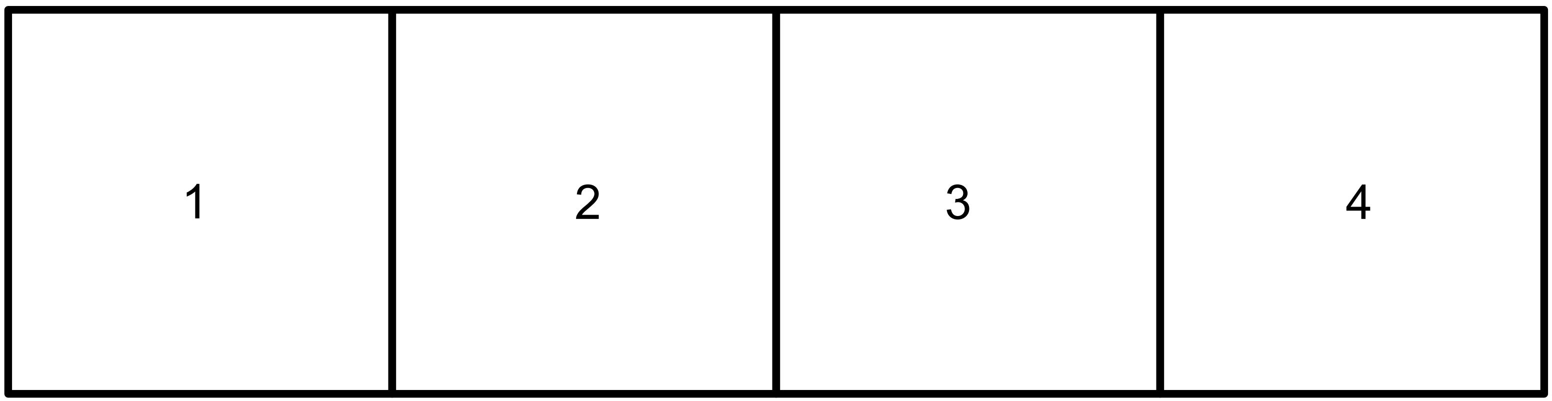
Digitization in 1D: Bad sampling



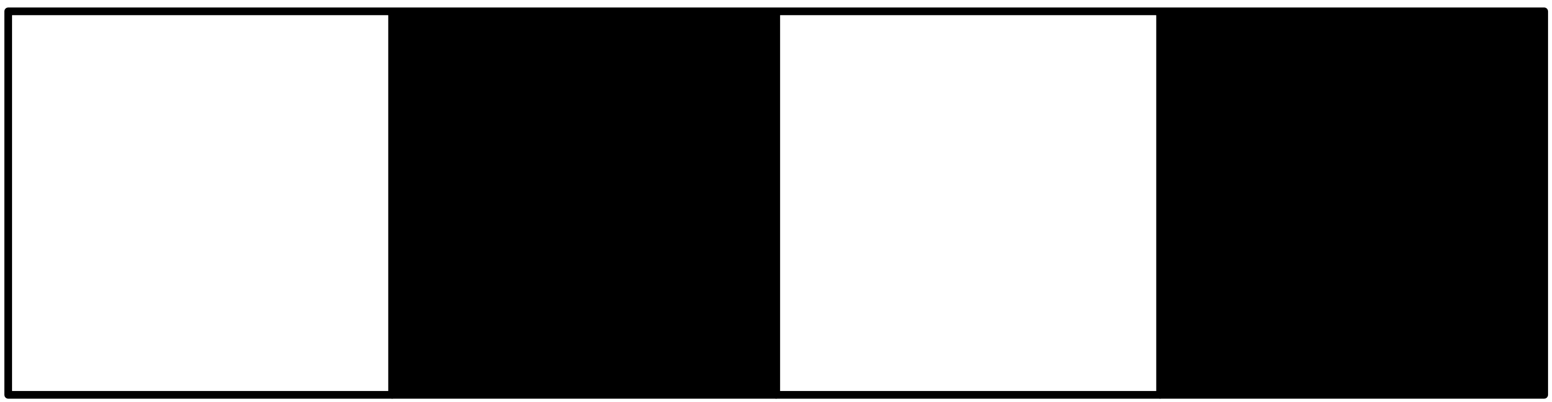
What's the best resolution we can get from a given sampling rate?



A 4-pixel “image”

In other words, what is the most rapid oscillation we can detect?

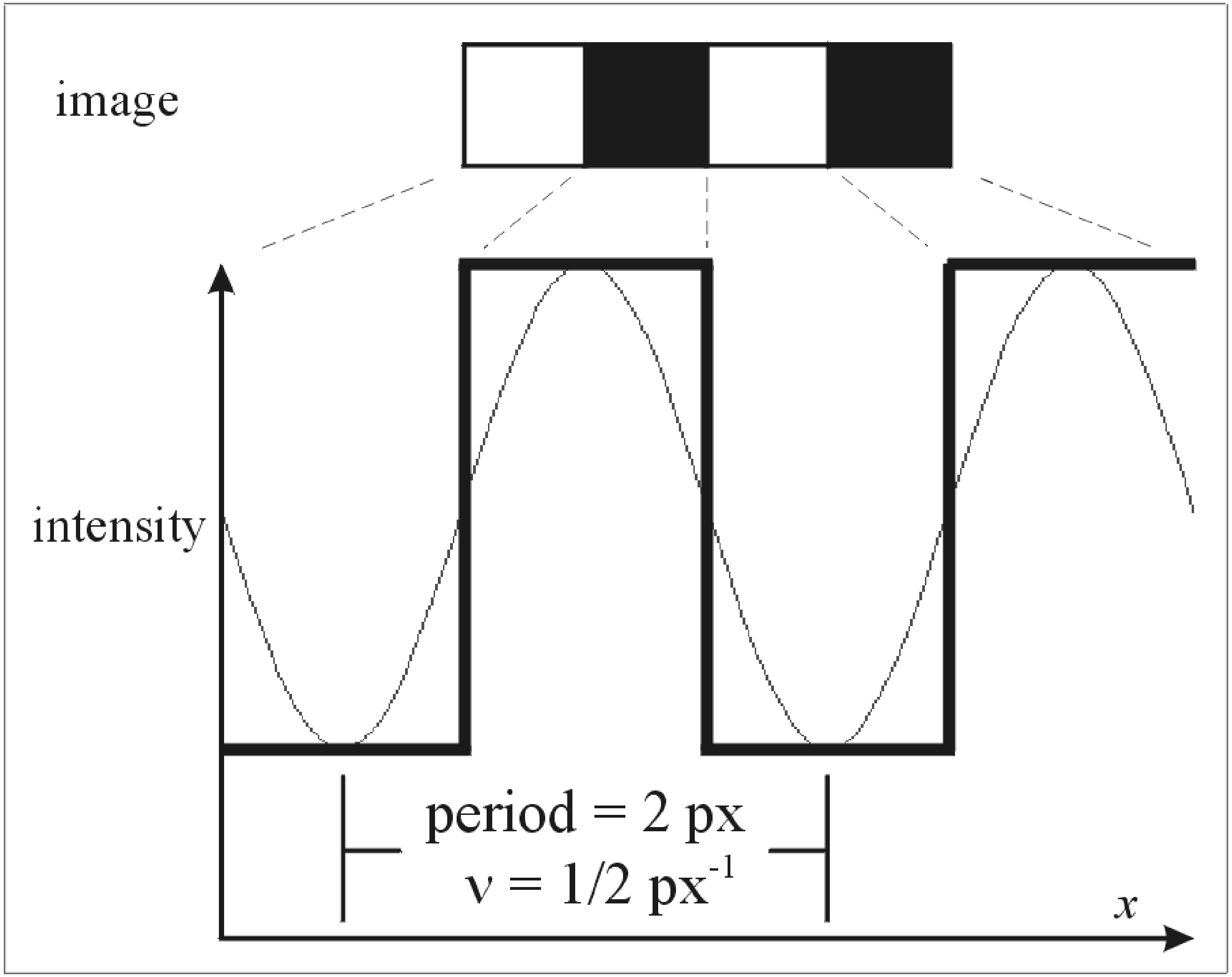
What's the best resolution we can get from a given sampling rate?



A 4-pixel “image”

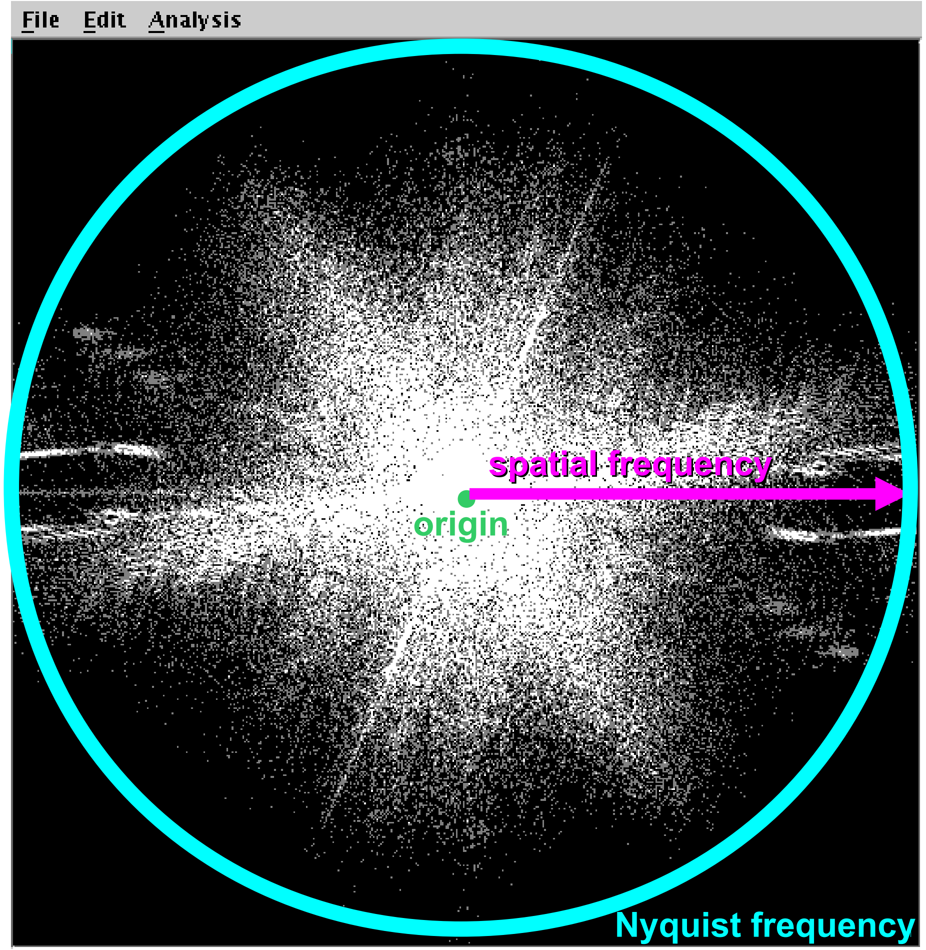
In other words, what is the most rapid oscillation we can detect?

ANSWER: Alternating light and dark pixels.



The period of this finest oscillation is 2 pixels.
The spatial frequency of this oscillation is 0.5 px^{-1}.
The finest detectable oscillation is what is known as “Nyquist frequency.”
The edge of the Fourier transform corresponds to Nyquist frequency.

Nyquist frequency



The period of this finest oscillation is 2 pixels.
The spatial frequency of this oscillation is 0.5 px^{-1}.
The finest detectable oscillation is what is known as "Nyquist frequency."
The edge of the Fourier transform corresponds to Nyquist frequency.

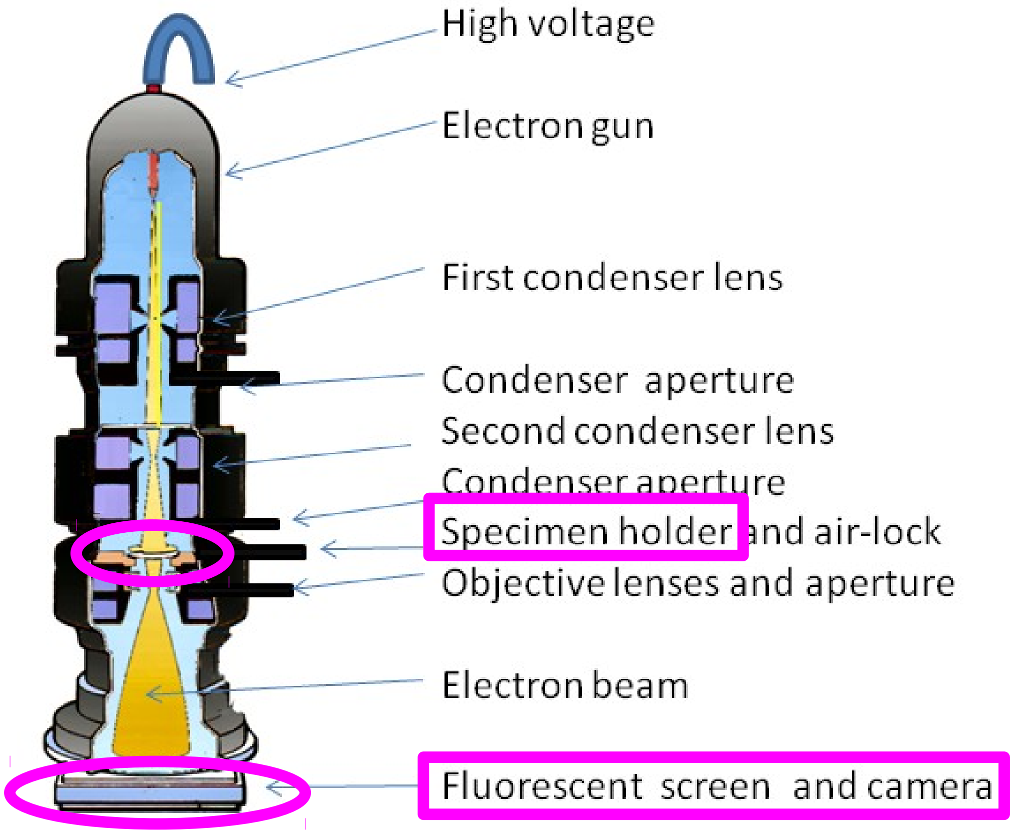
What do we mean by pixel size?

Typical magnification: 50,000X
Typical detector element: 15μm
(pixel size on the camera scale)

Pixel size on the specimen scale:
15×10^{-6} m/px / 50000 =
3.0×10^{-10} m/px = **3.0 Å/px**

In other words,
the best resolution we can achieve (or, the finest oscillation we can detect) at 3.0 Å/px is **6.0 Å**.

It will be worse due to interpolation, so to be safe, a pixel should be 3X smaller than your target resolution.

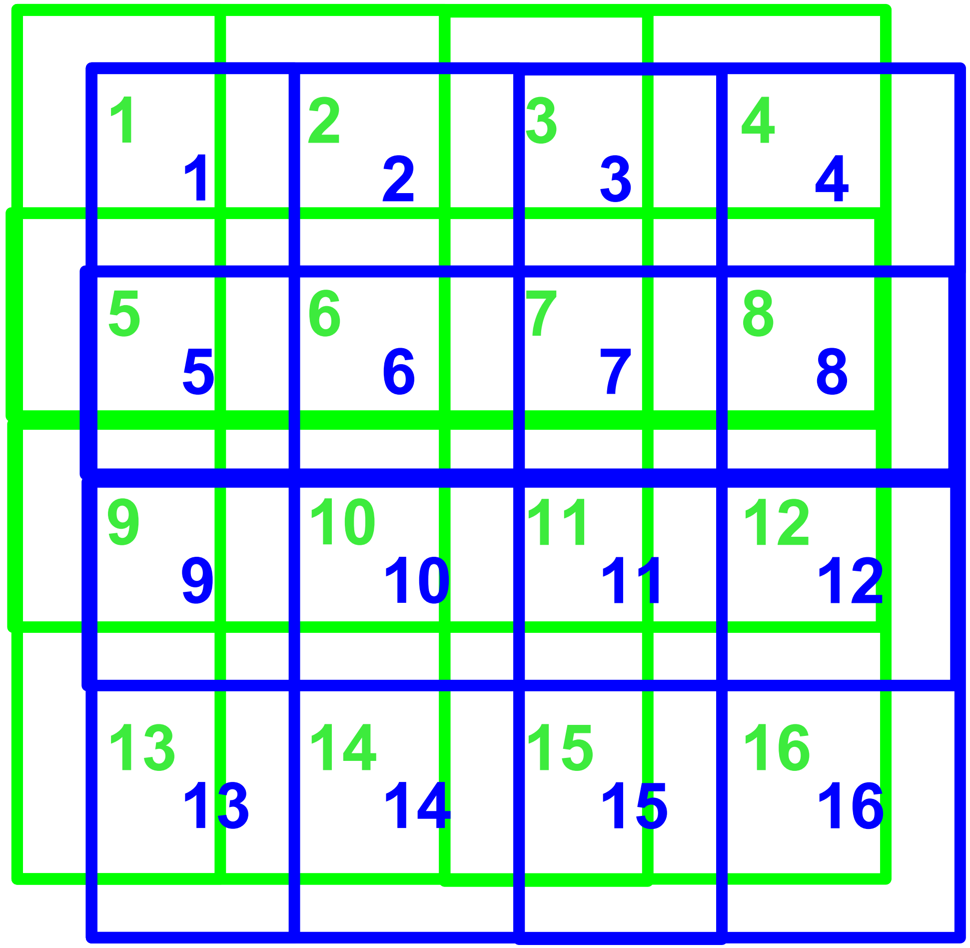


Transmission Electron Microscope

http://www.en.wikipedia.org

Interpolation

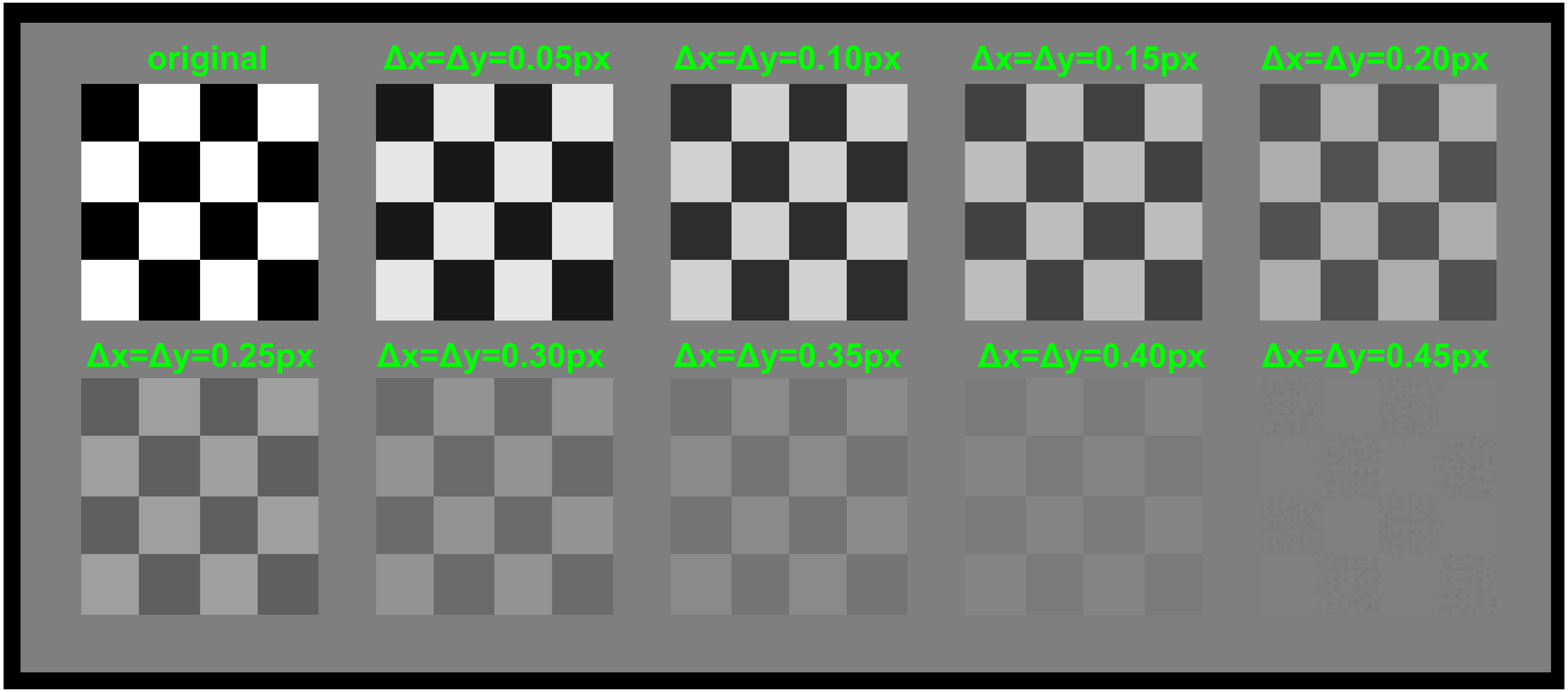
Shifts



Suppose we shift the image in *x* & *y*.

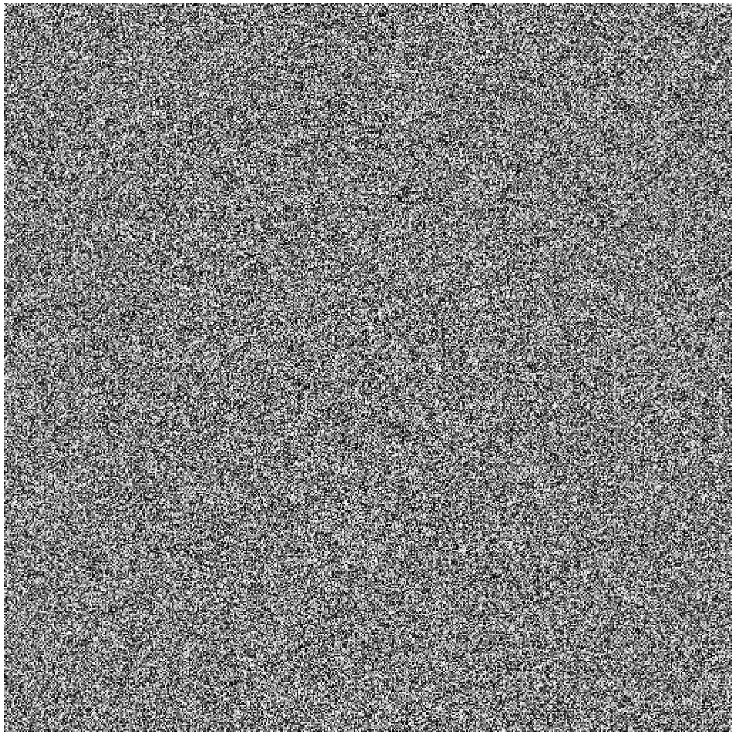
The new pixels will be weighted averages of the old pixels.

Effect of shifts

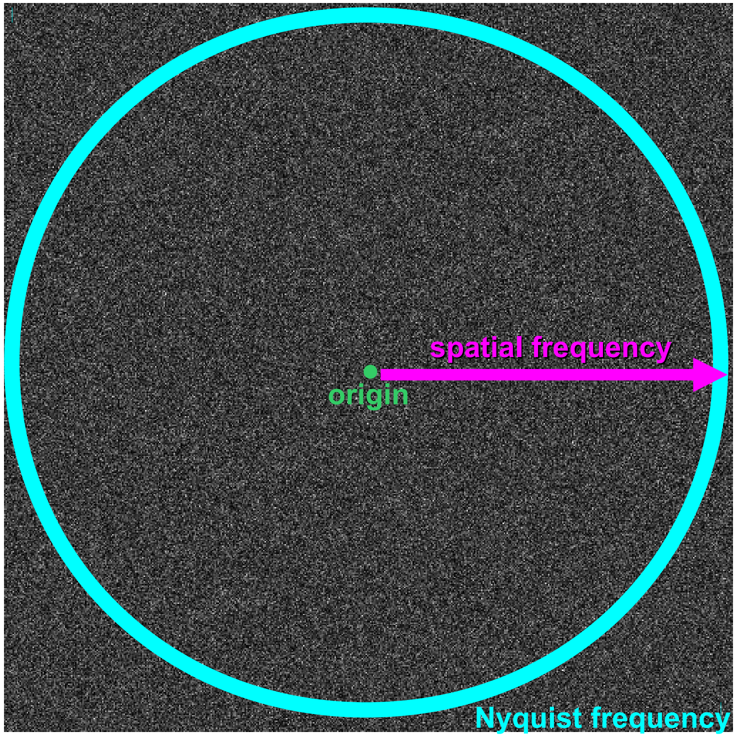


Two more properties of Fourier transforms: Noise

- The Fourier transform of noise is noise
- “White” noise is evenly distributed in Fourier space
  - “White” means that each pixel is independent



White noise



Power spectrum

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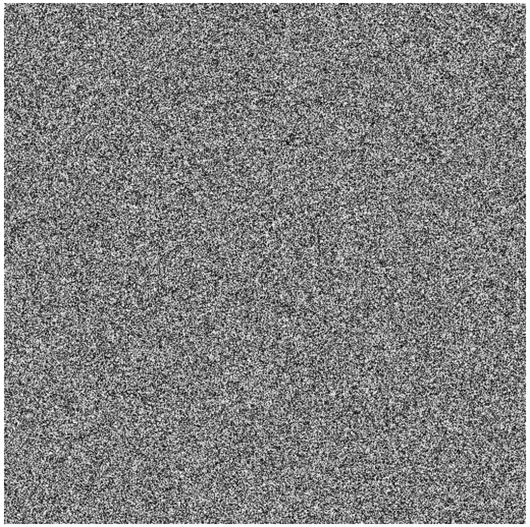
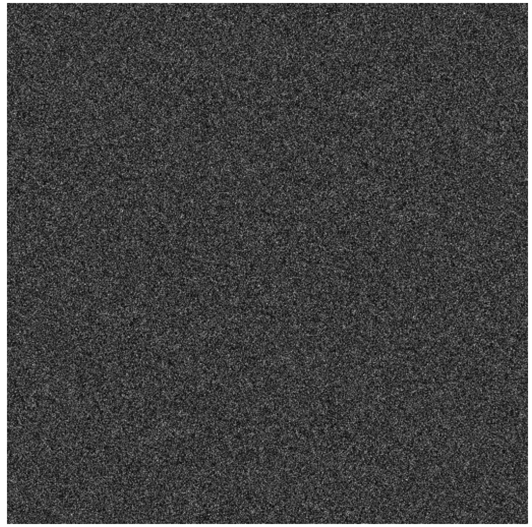
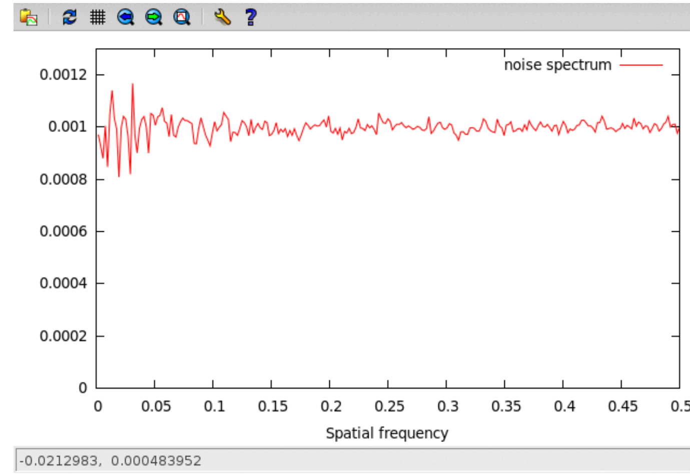
Effects of interpolation are resolution-dependent

Original





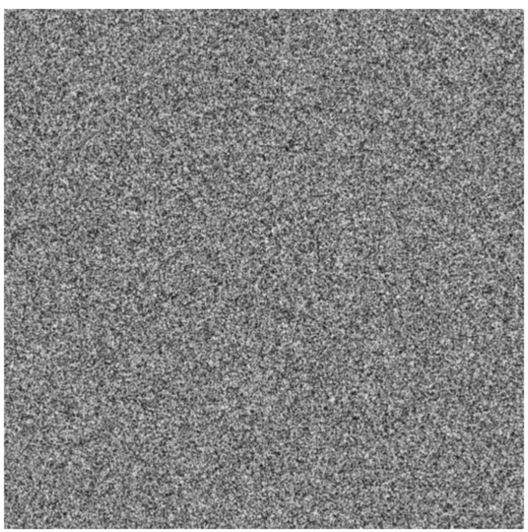


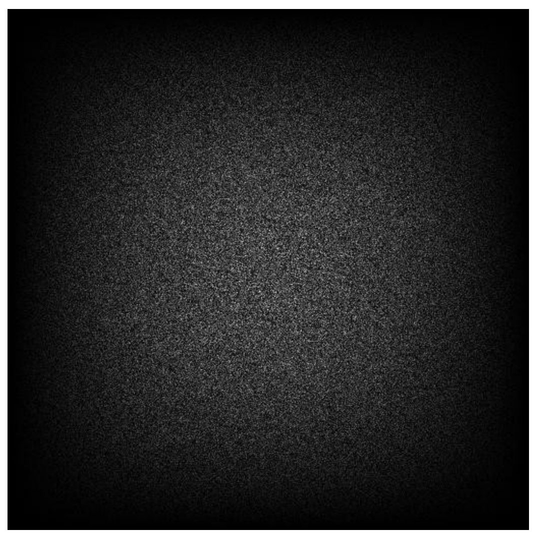
Image

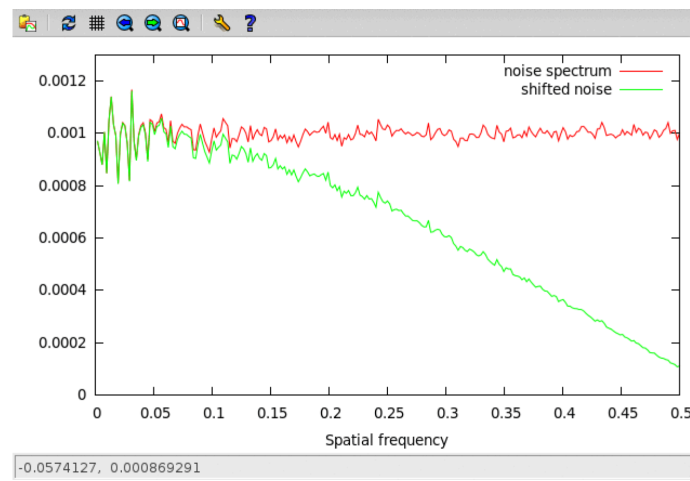
Power spectrum

Profile

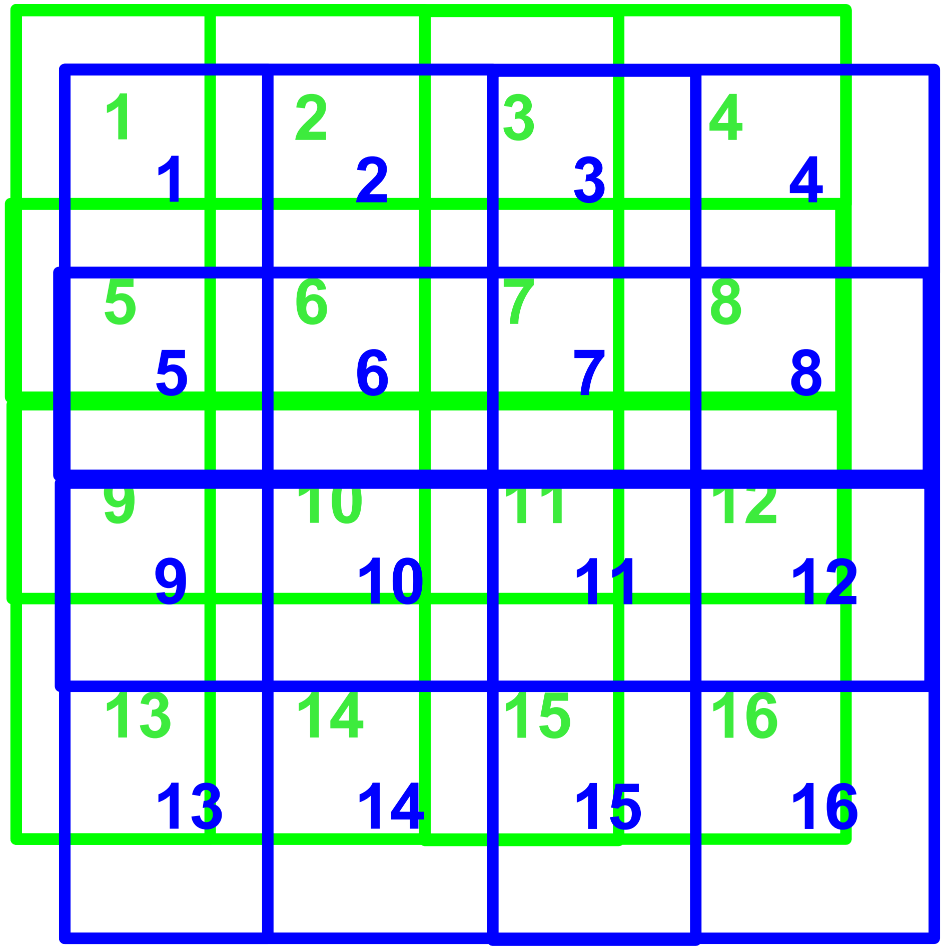
Shifted by (0.5,0.5) px







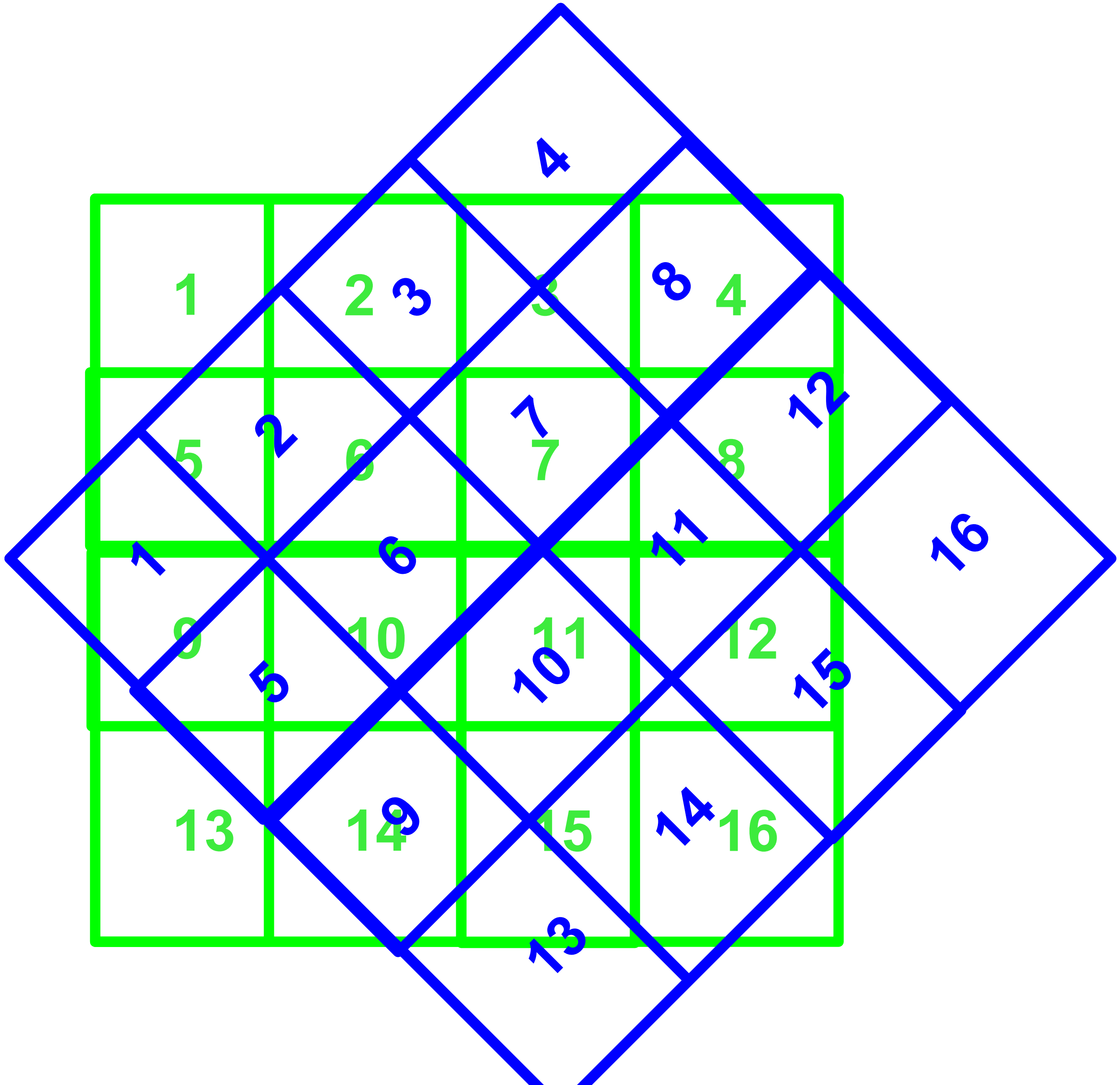
Rotation



Suppose we rotate the image.

The new pixels will be weighted averages of the old pixels.

Rotation



Suppose we rotate the image.

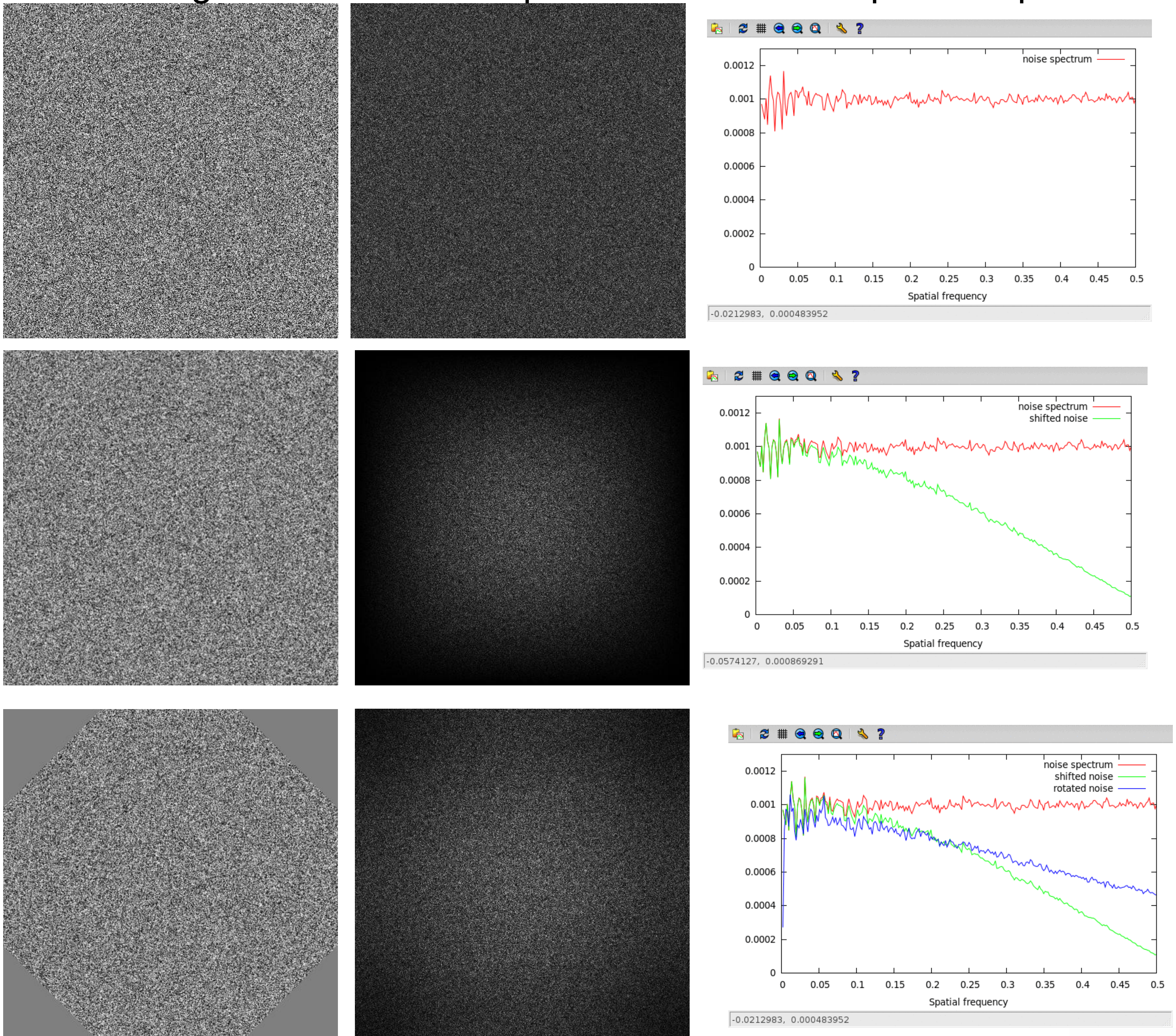
The new pixels will be weighted averages of the old pixels.

Image | Power spectrum | Power spectrum profile

Original

Shifted by (0.5,0.5) px

Rotated by 45°



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The degradation of the images means that we should minimize the number of interpolations.

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(P)review of 3D reconstruction: The parameters required

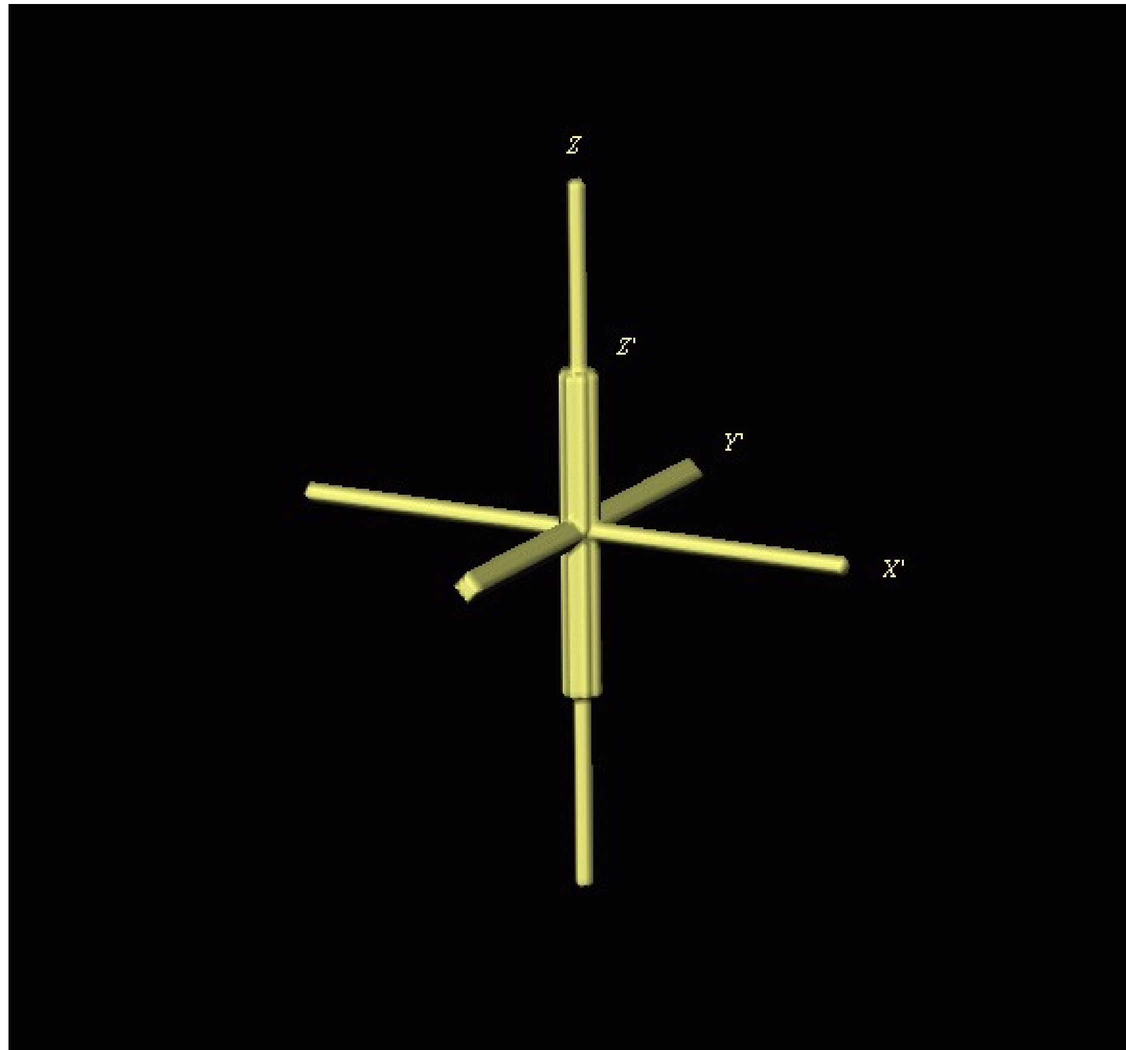
Two translational:

- Δx
- Δy

Three orientational (Euler angles):

- phi (about *z* axis)
- theta (about *y*)
- psi (about new *z*)

These are determined in 2D.



http://www.wadsworth.org

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How do find the relative translations between two images?

Translational alignment

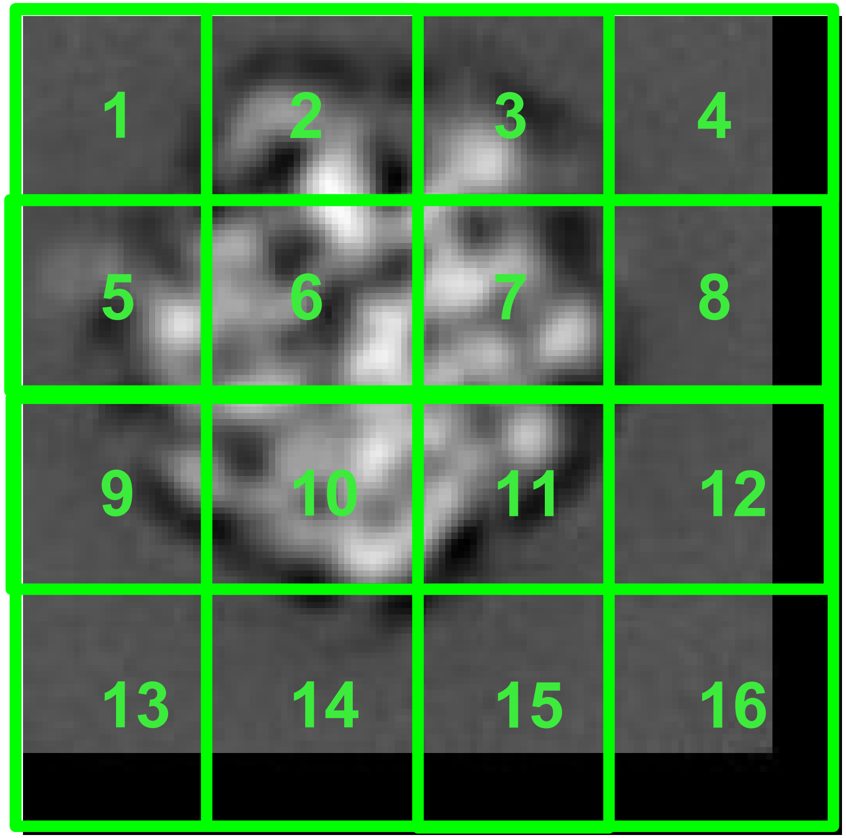

Image *f*

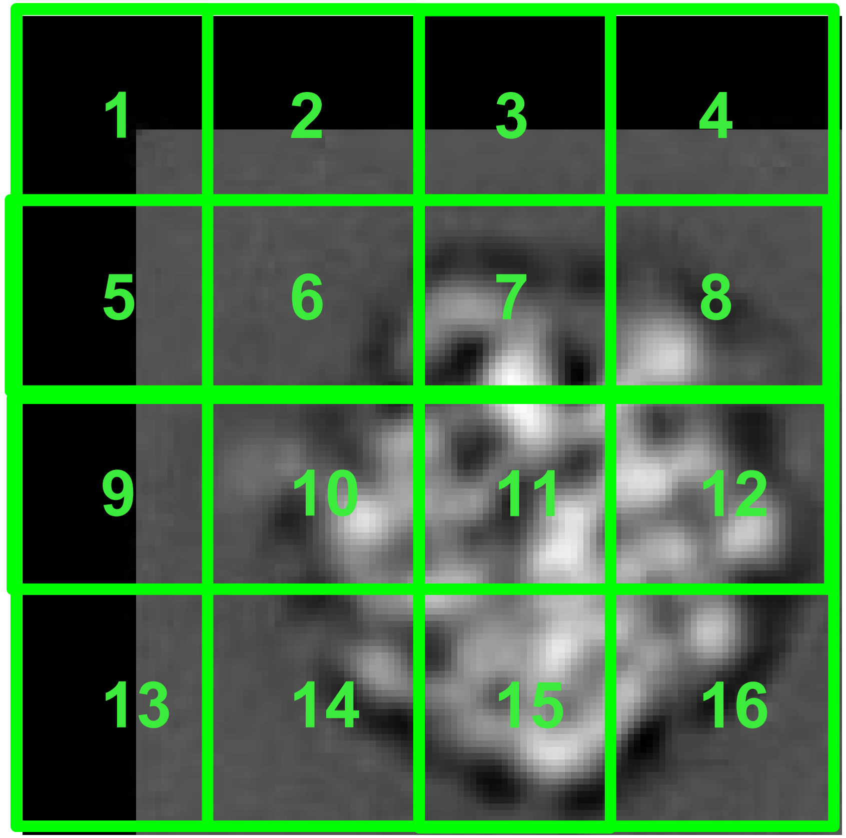

Image *g*

Cross-correlation coefficient:

$$\frac{\sum_{N=1}^{16} f(\vec{x})\, g(\vec{x})}{\sigma_f \sigma_g}$$

Cross-correlation coefficient

Cross-correlation coefficient: $$\frac{\sum_{N=1}^{16} f\left(\vec{x}\right) g\left(\vec{x}\right)}{\sigma_f \sigma_g}$$

If the alignment is perfect, the correlation value will be 1.

What if the correlation isn't perfect?

Translational alignment

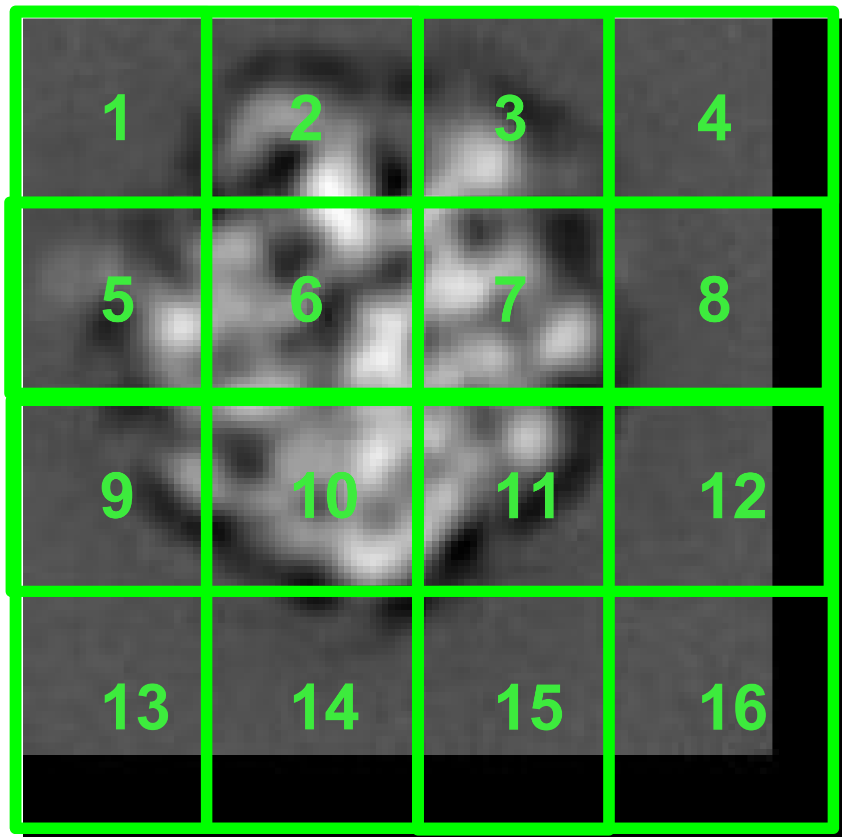

Image *f*

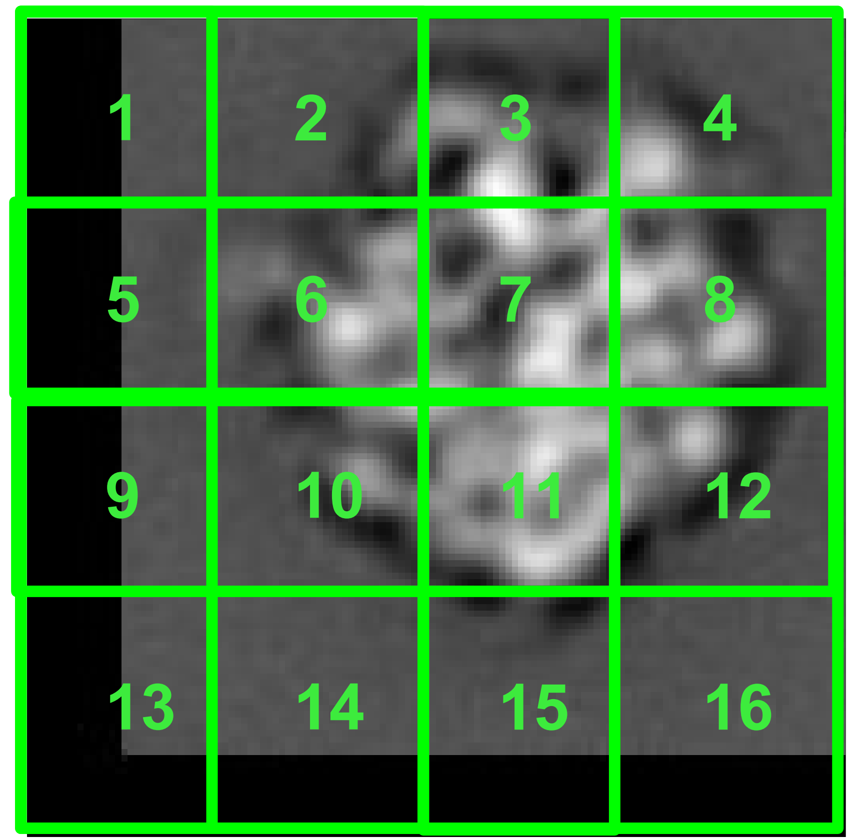

Image *g*

What if the correlation isn't perfect?

ANSWER: You try other shifts (perhaps all).

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Cross-correlation function (CCF)

Brute-force translational search is CPU-intensive

BUT

Fourier transforms can help us.

Some notation:			
	Real space	$f(x)$	$g(x)$
	Fourier space	$F(X)$	$G(X)$

Complex conjugate:
If a Fourier coefficient $F(X)$ has the form: a + bi
The complex conjugate $F^*(X)$ has the form: a - bi

$$F^*(X)\ G(X) = F.T.(CCF)$$

This gives us a map of all possible shifts.

Cross-correlation function (CCF)

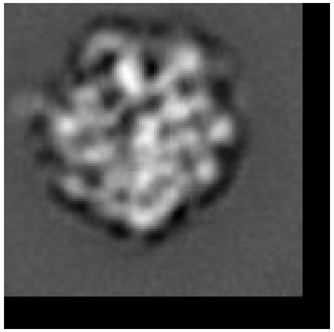


Image *f(x)*

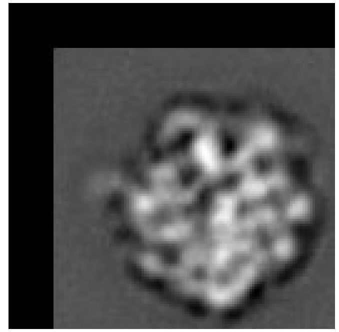
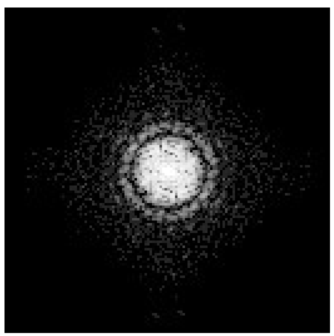
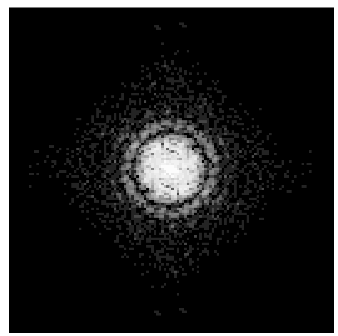


Image *g(x)*



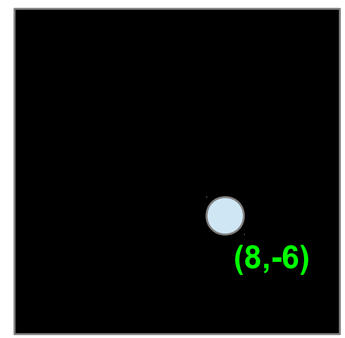
F.T. *F*(X)*
(complex conjugate)

x



F.T. *G(X)*

=



CCF

The position of the peak gives us the shifts that give the best match, *e.g.*, (8,-6).

That was an easy case.
We only needed to do translational alignment.
What about orientation alignment?

Orientation alignment

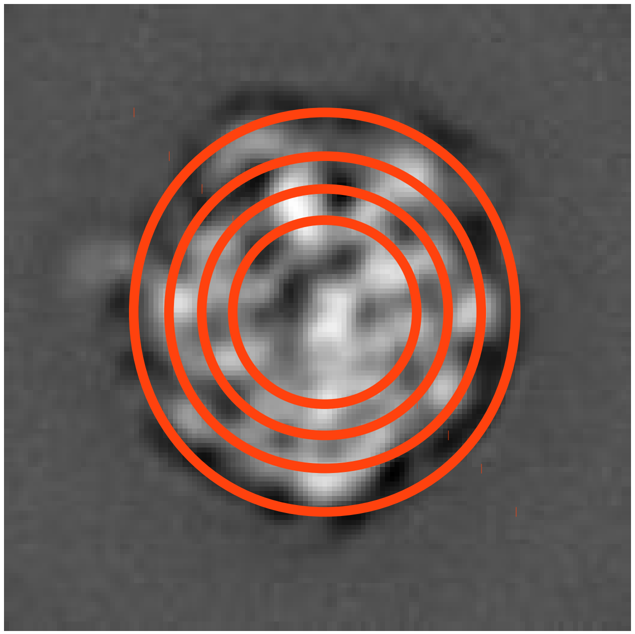


Image 1

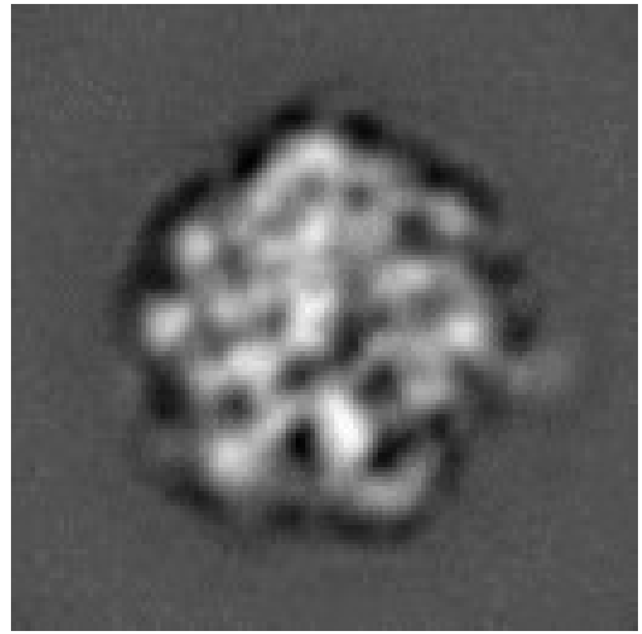


Image 2

We take a series of rings from each image, unravel them,
and compute a series of 1D cross-correlation functions.

Shifts along these unraveled CCFs is equivalent to a rotation in Cartesian space.

Orientation alignment

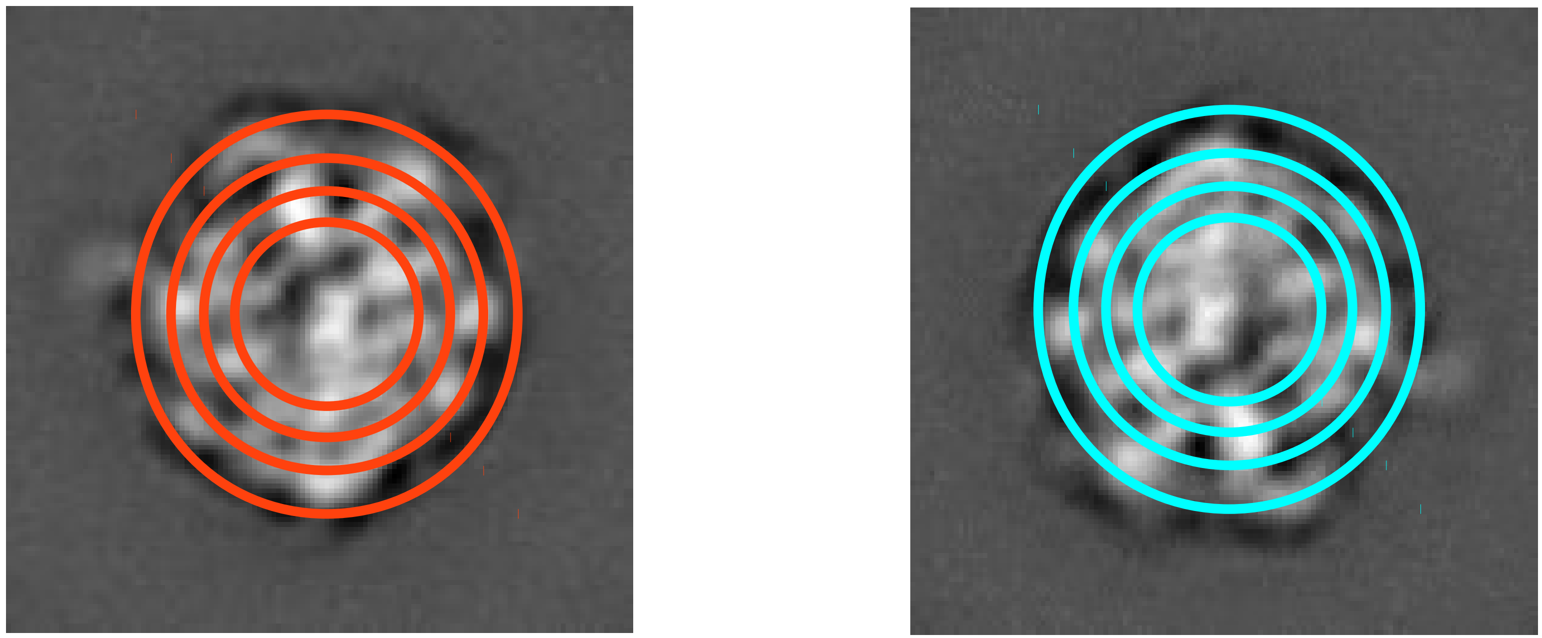


Image 1 Image 2

Which do you perform first?
Translational or orientation alignment?

Translational and orientation alignment are interdependent

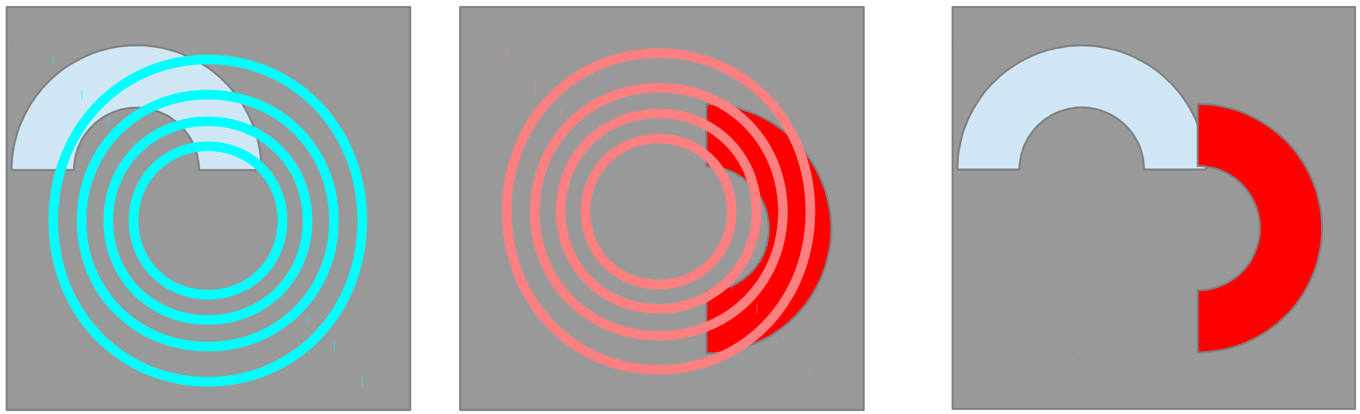
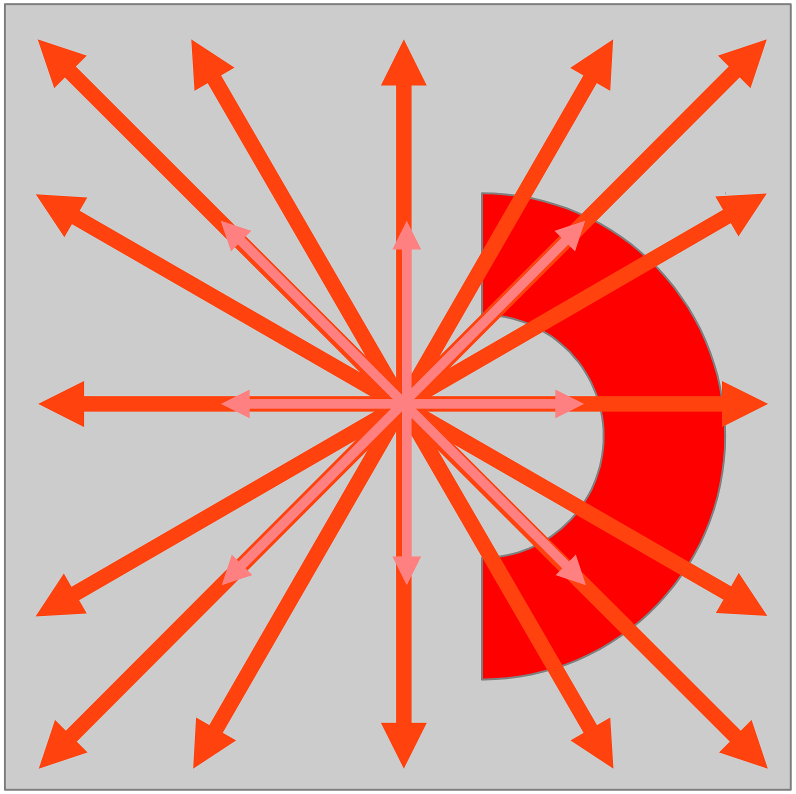


Image 1 Image 2 Superimposed

SOLUTION: You try a set of reasonable shifts, and perform separate orientation alignments for each.

Translational and orientation alignment are interdependent



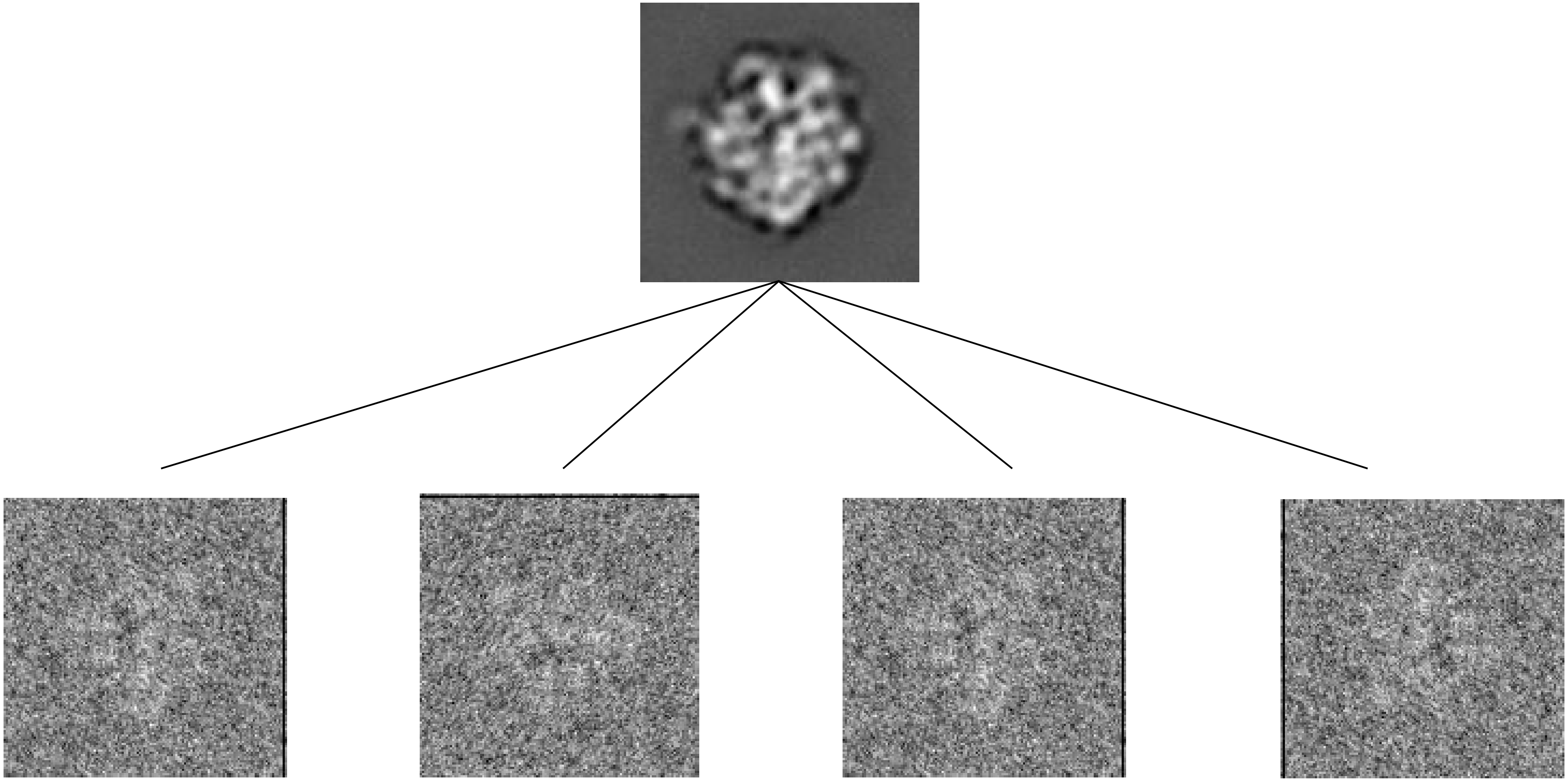
Set of all shifts of up to 1 pixel

Set of all new shifts of up to 2 pixels

Shifts of (0, +/-1, +/-2) pixels results in 25 orientation searches.

Different alignment schemes

Reference-based alignment

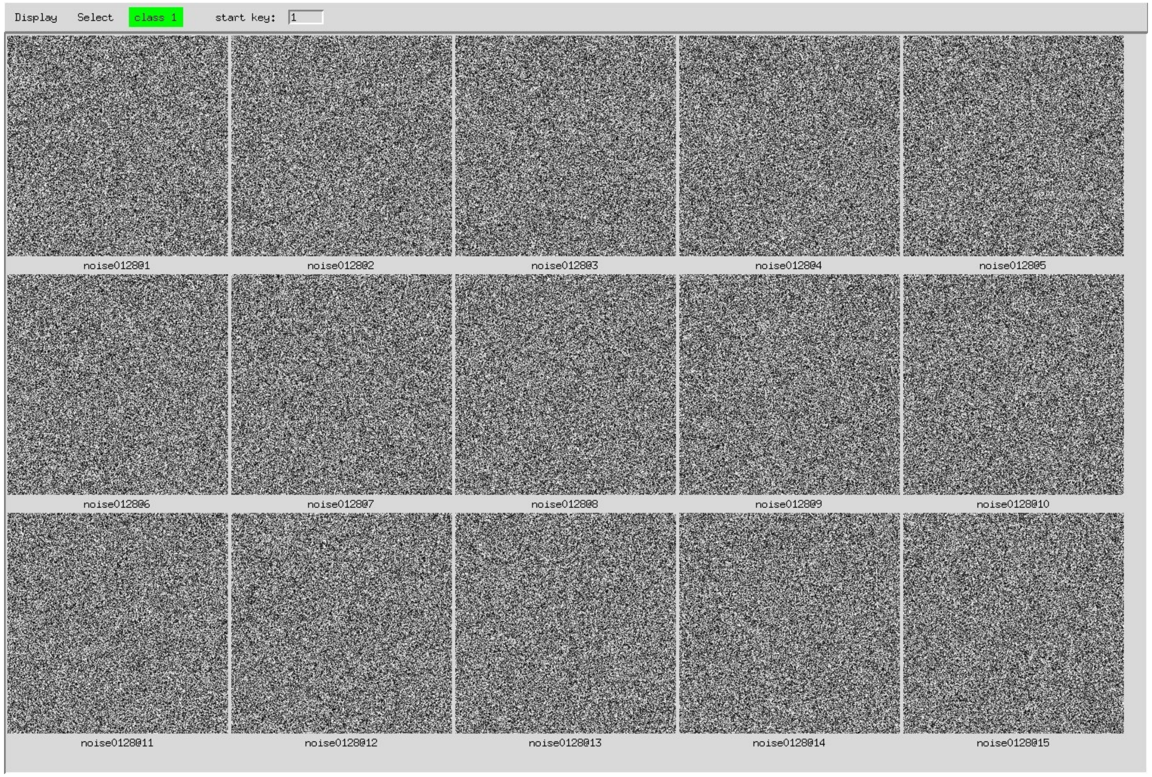


There's a problem with reference-based alignment:

Model bias

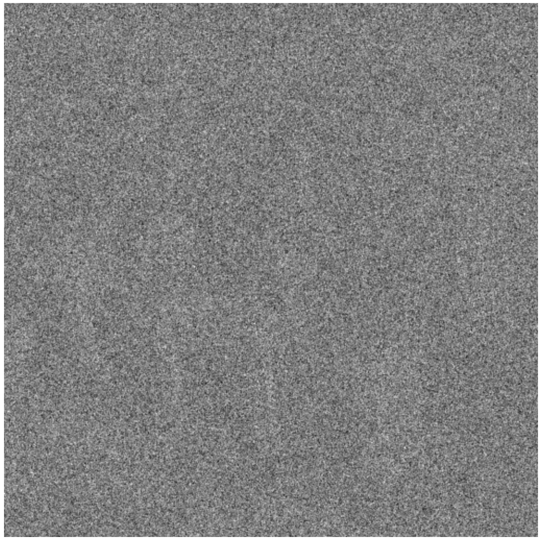
Model bias

Reference

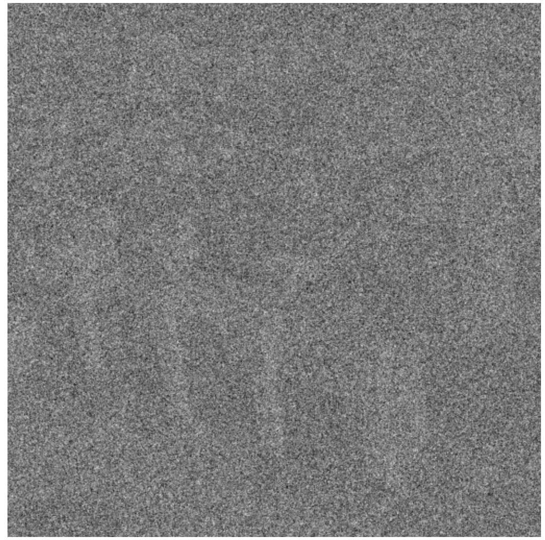


Images of pure noise

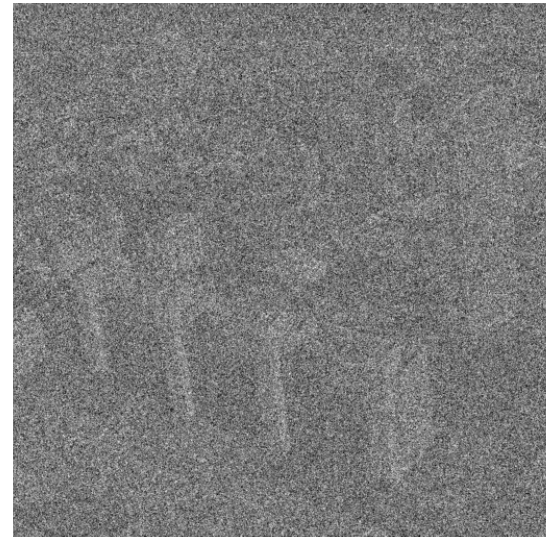
Averages of images of pure noise



N = 128

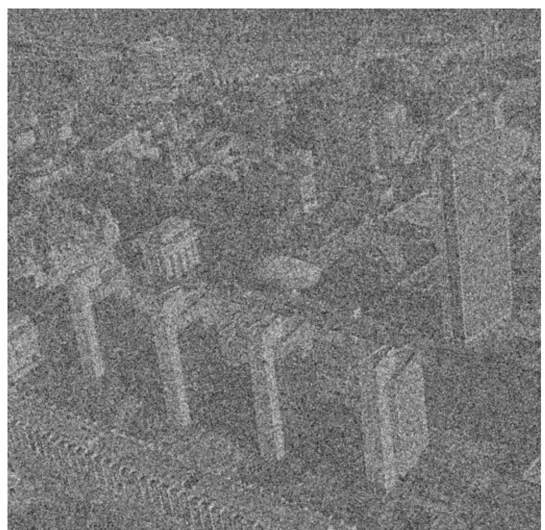


N = 256



N = 512

N = 1024

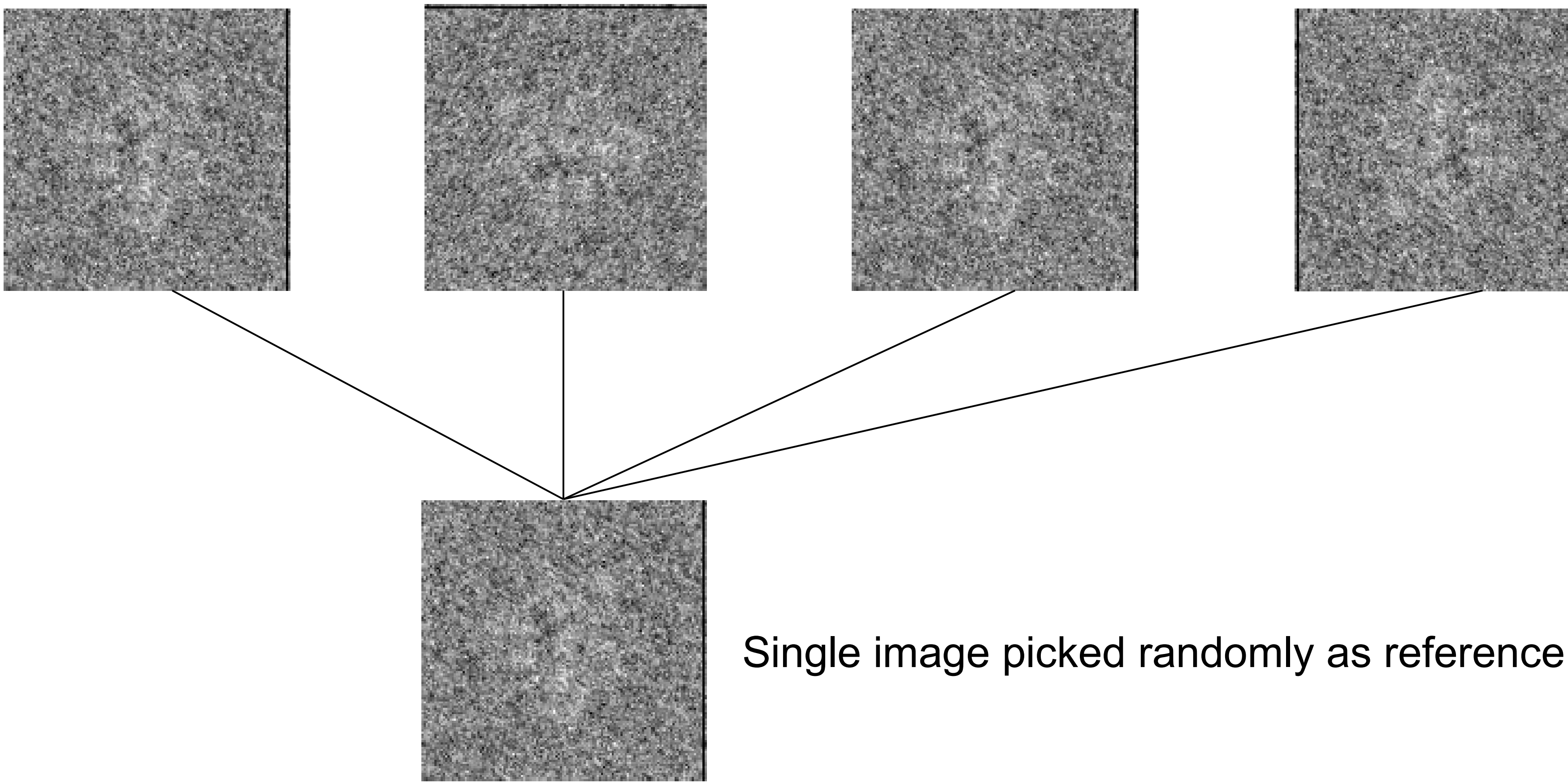


N = 2048

original

There are reference-free alignment schemes

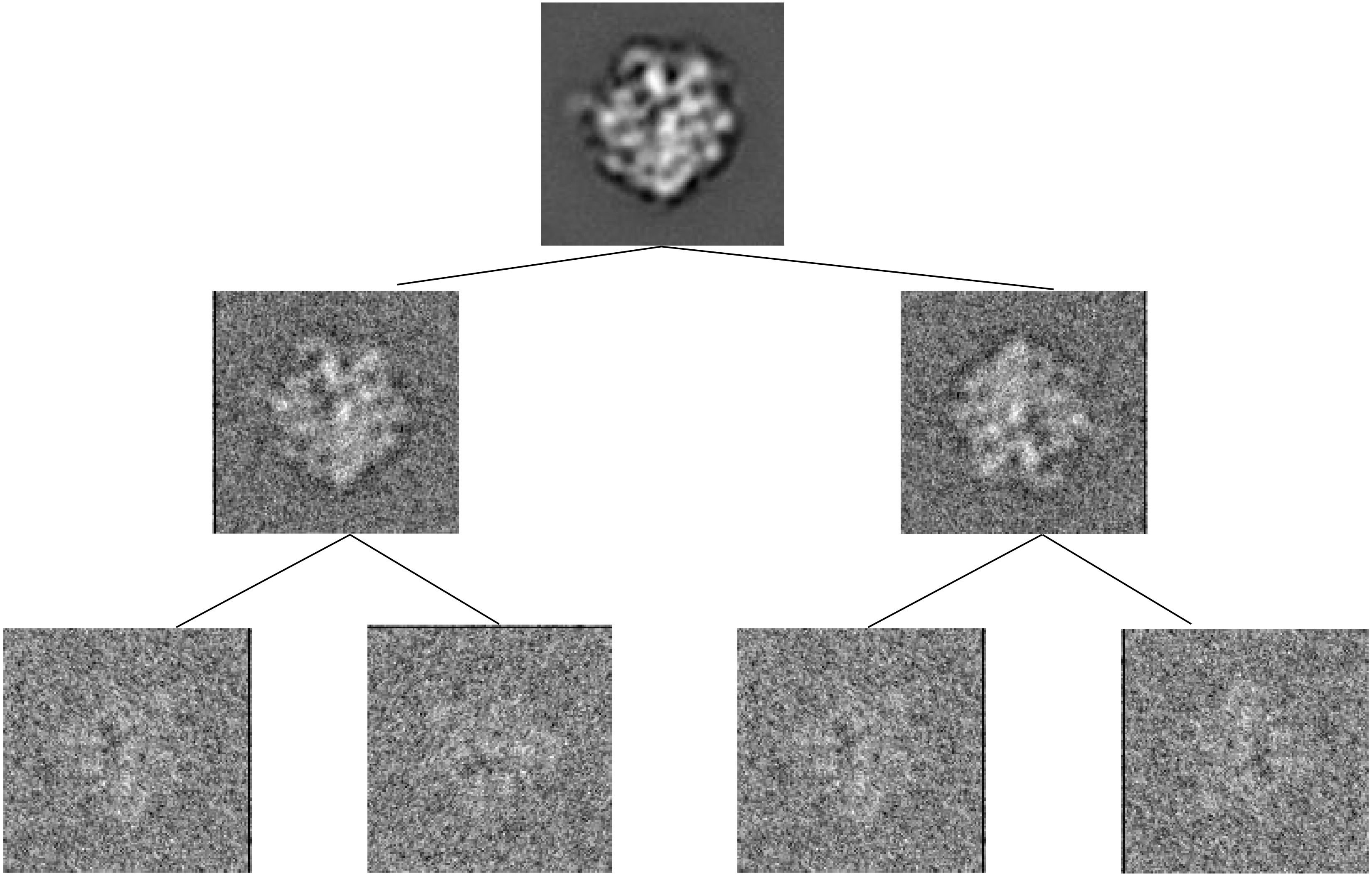
Reference-free alignment (SPIDER command AP SR)



Single image picked randomly as reference

Disadvantage: Alignment depends on the choice of random seed.

Pyramidal/pairwise alignment



Marco... Carrascosa (1996) Ultramicroscopy

You have aligned images,
but they don't all look the same.

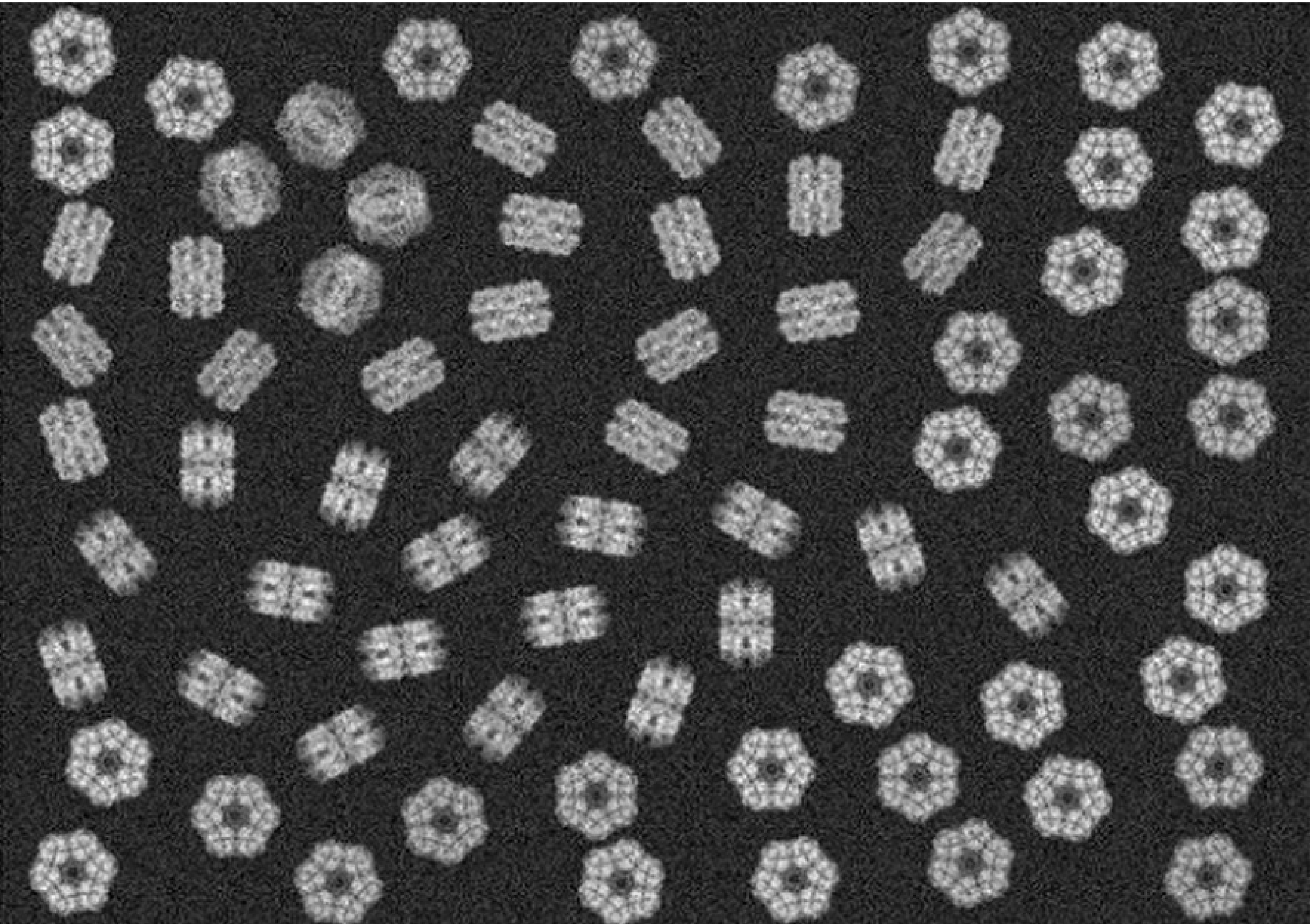


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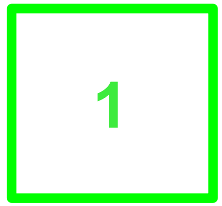
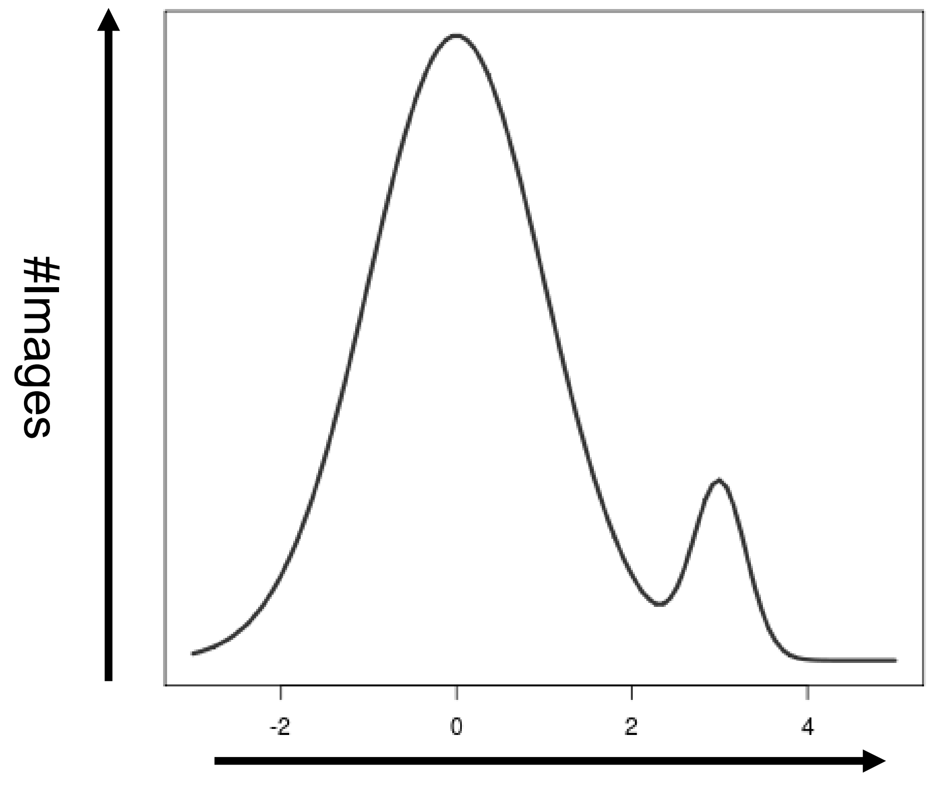
Image analysis II

- Fourier transforms revisited
- Digitization
- Alignment
- Multivariate data analysis

Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)



1-pixel image



http://isomorphism.es

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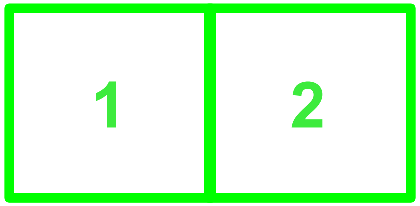
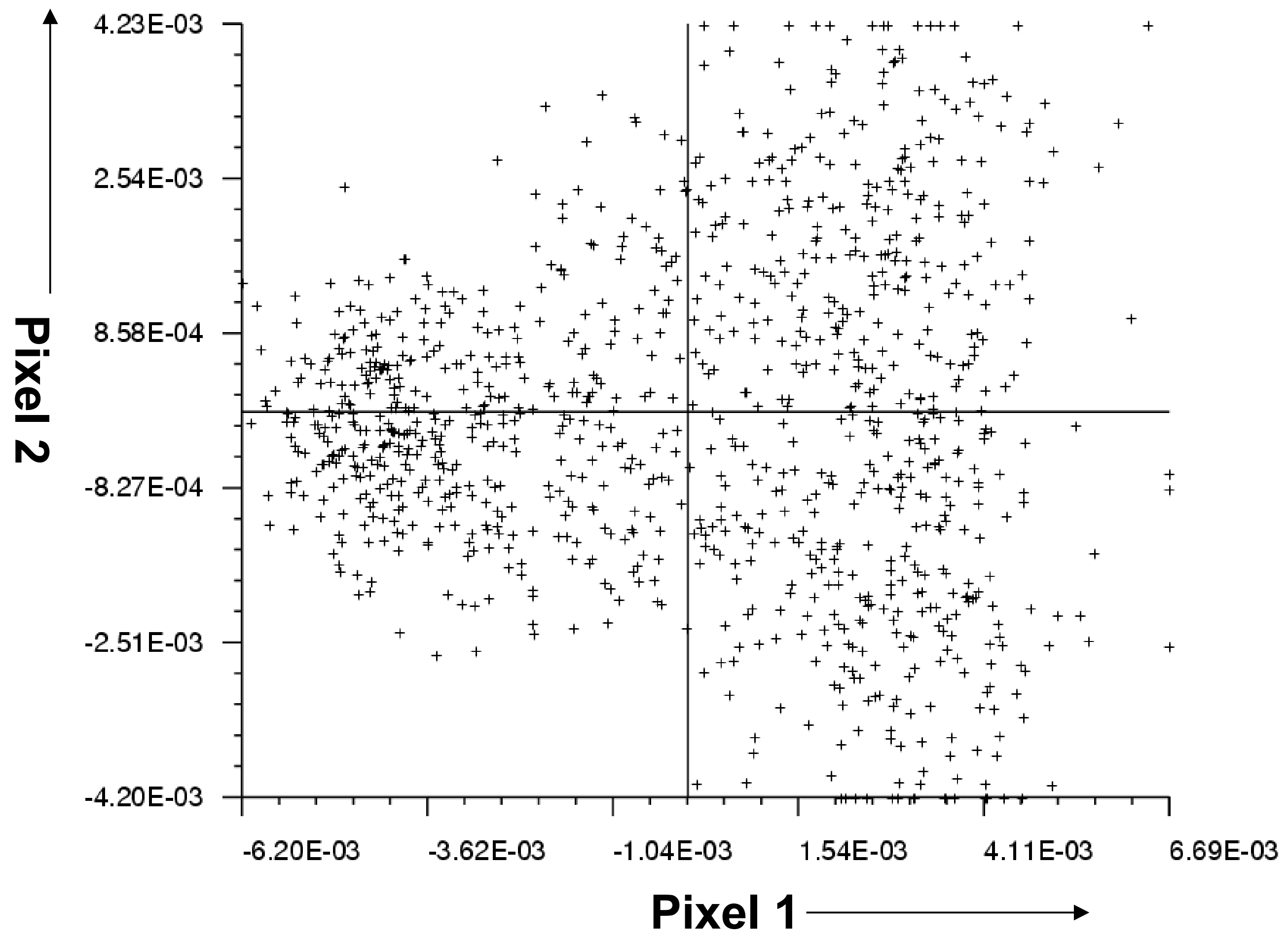
Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)



2-pixel image



Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

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9	10	11	12
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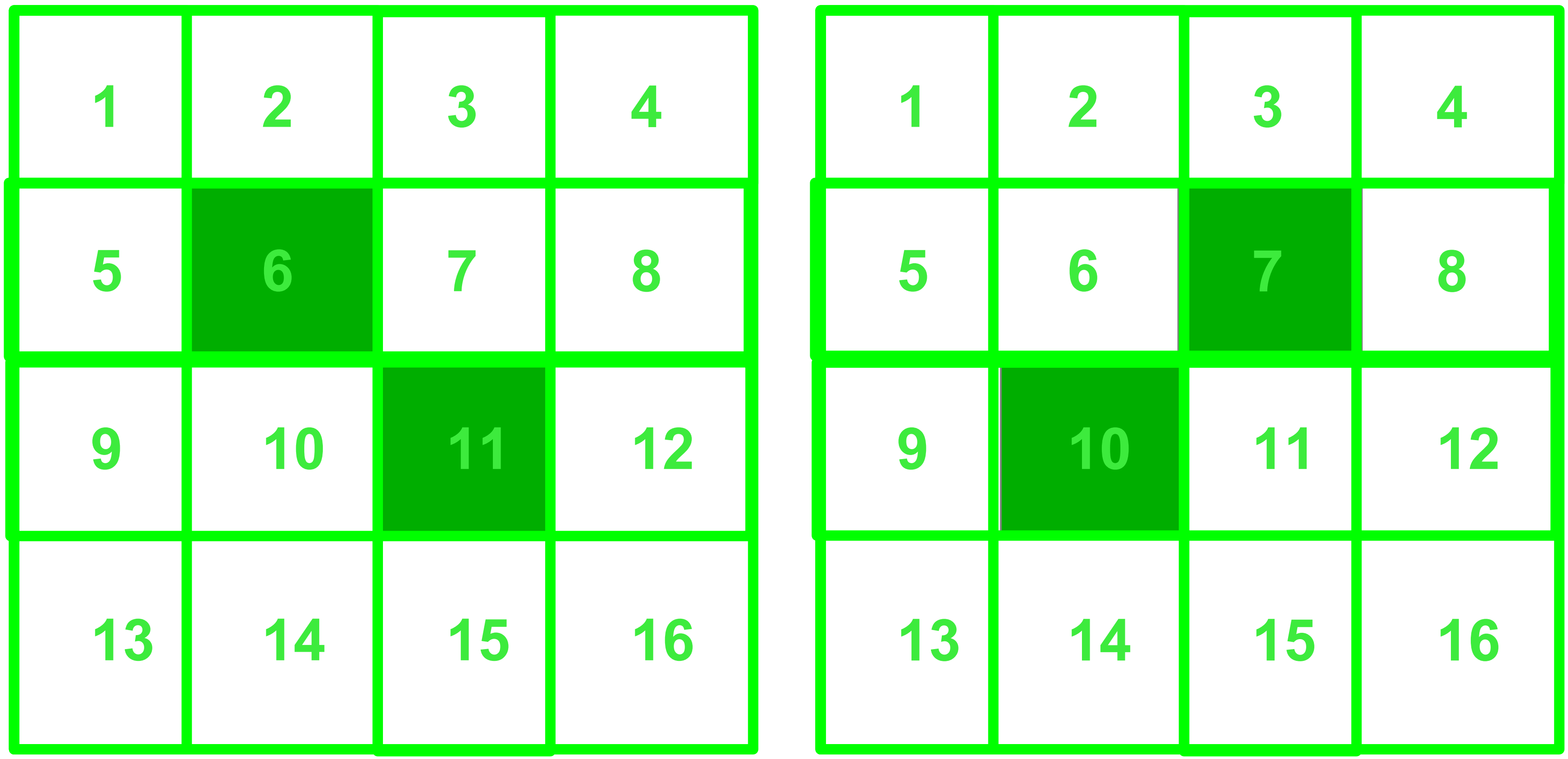
Now, we have a 16-dimensional problem.

Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)



Suppose pixel 6 coincided with pixel 11,
And pixel 7 coincided with pixel 10.
Then, we're back to two variables, and a 2D problem.

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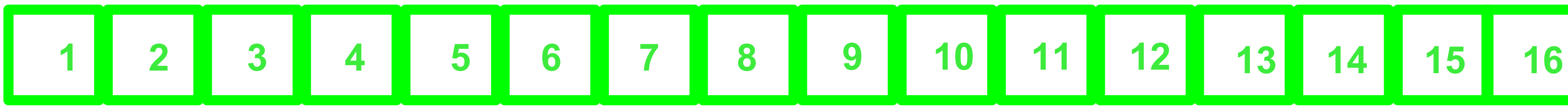
Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)



Our 16-pixel image can be reorganized into a 16-coordinate vector.

Covariance of measurements *x* and *y*:

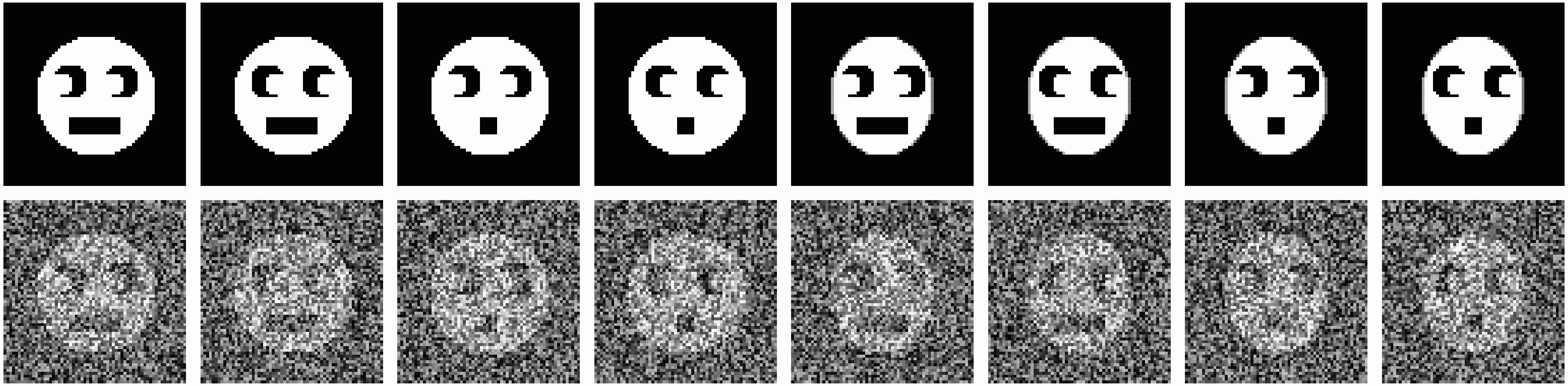
$\langle xy \rangle - \langle x \rangle \langle y \rangle$,

where $\langle x \rangle$ is the mean of *x*.

A high covariance is a measure of the correlation between two variables.

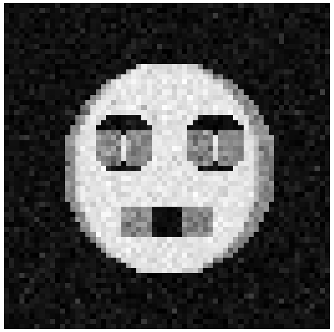
MDA: An example

8 classes of faces, 64x64 pixels



With noise added

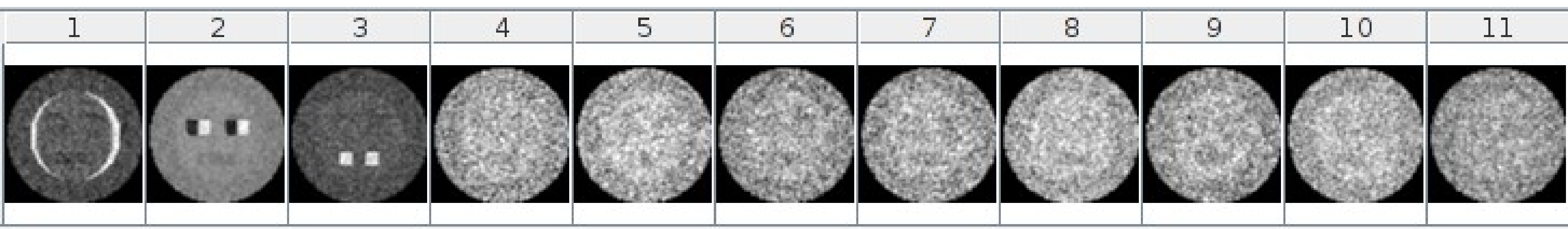
Average:



From http://spider.wadsworth.org/spider_doc/spider/docs/techs/classification/tutorial.html

Principal component analysis (PCA) or Correspondence analysis (CA)

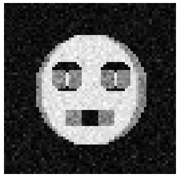
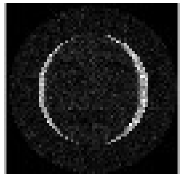
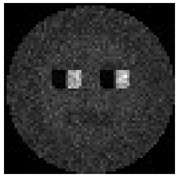
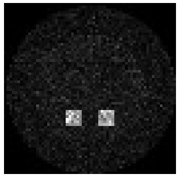
- For a 4096-pixel image, we will have a 4096x4096 covariance matrix.
- Row-reduction of the covariance matrix gives us "eigenvectors."
    - The eigenvectors describe correlated variations in the data.
    - The eigenvectors have 64 elements and can be converted back into images, called "eigenimages."
    - The first eigenvectors will account for the most variation. The later eigenvectors may only describe noise.
    - Linear combinations of these images will give us approximations of the classes that make up the data.



Eigenimages

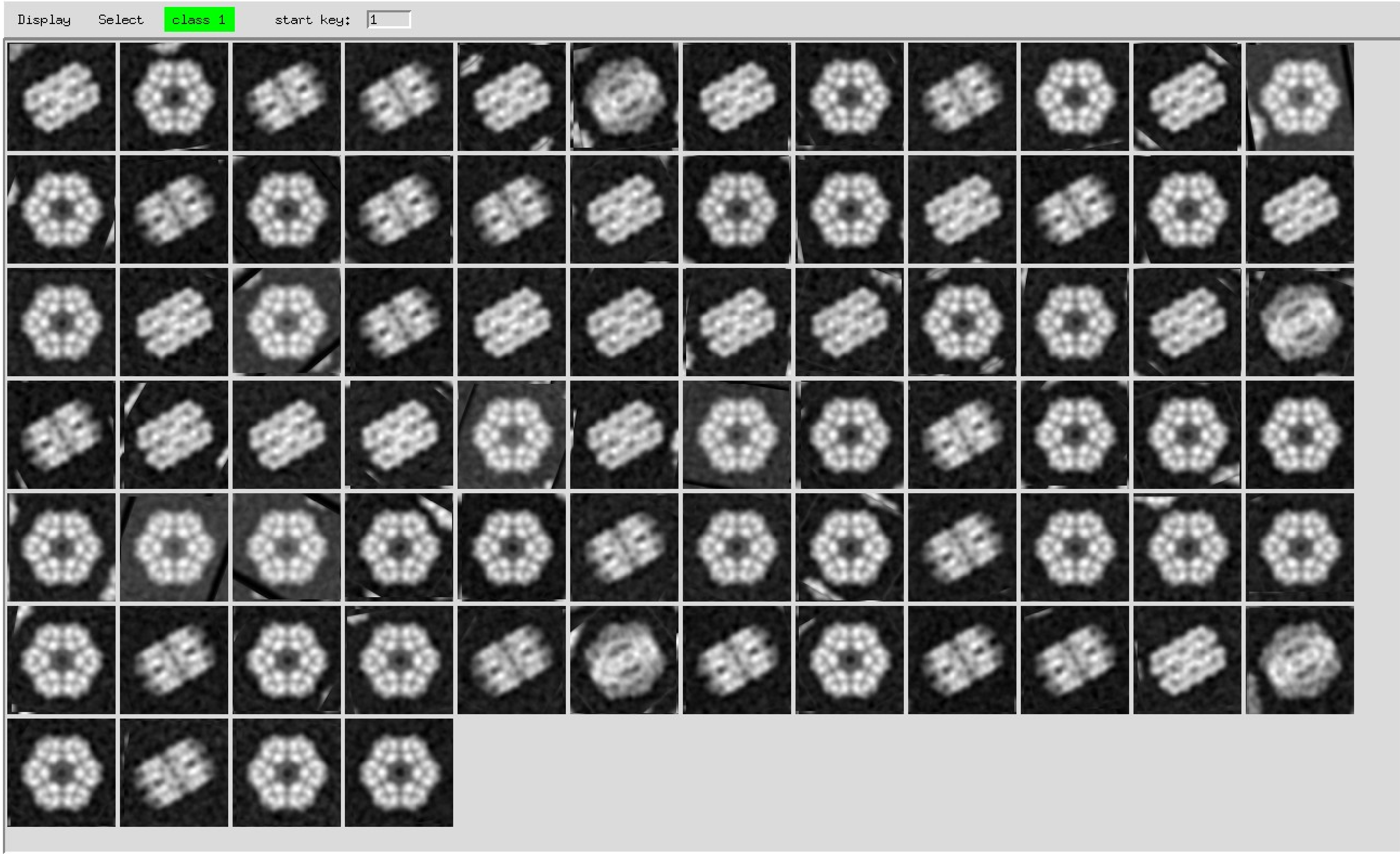
Reconstituted images

Linear combinations of these images will give us approximations of the classes that make up the data.

c_0  $+\ c_1$  $+\ c_2$  $+\ c_3$  $+\ \dots$

Average | Eigenimage #1 | Eigenimage #2 | Eigenimage #3

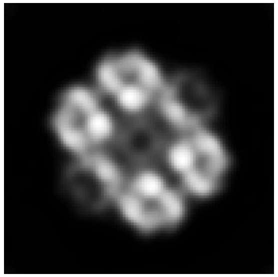
Another example: worm hemoglobin

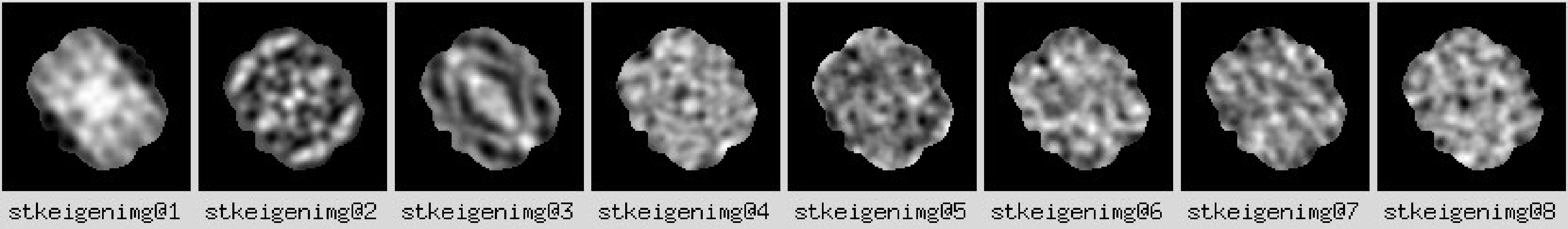


Phantom images of worm hemoglobin

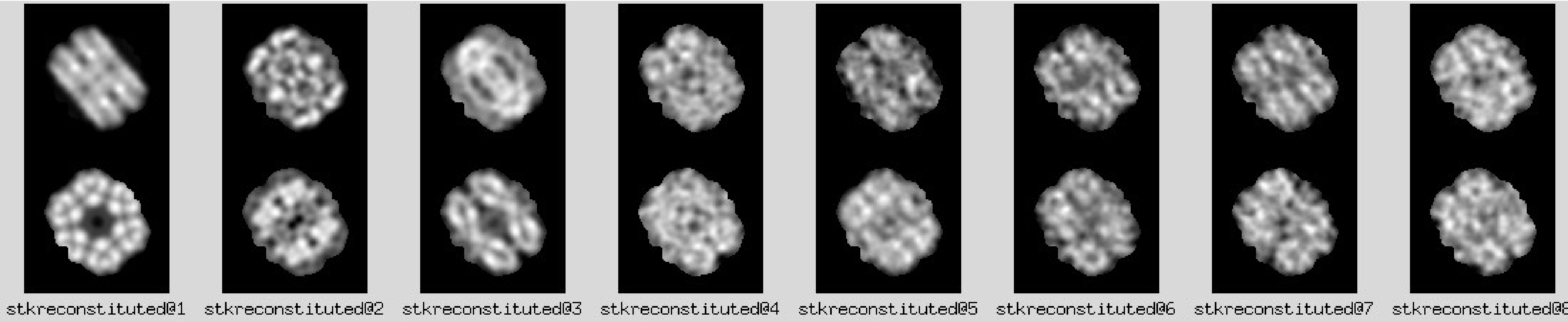
PCA of worm hemoglobin

Average:





$+c_0$ $+c_1$ $+c_2$ $+c_3$ $+c_4$ $+c_5$



$-c_0$ $-c_1$ $-c_2$ $-c_3$ $-c_4$ $-c_5$

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Next week:
Classification & 3D Reconstruction

Thank you for your attention

CEITEC

Central European Institute of Technology
Masaryk University
Kamenice 753/5
625 00 Brno, Czech Republic

www.ceitec.muni.cz | info@ceitec.muni.cz

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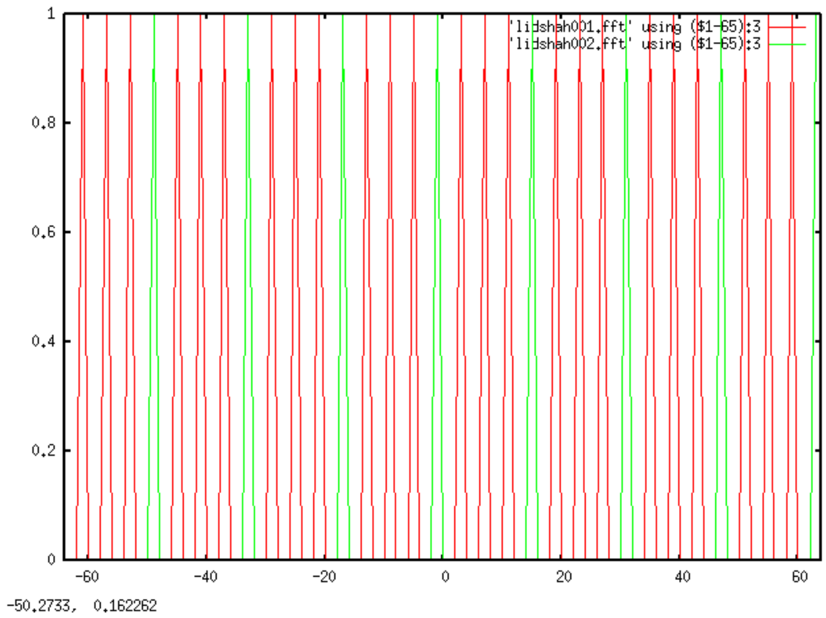
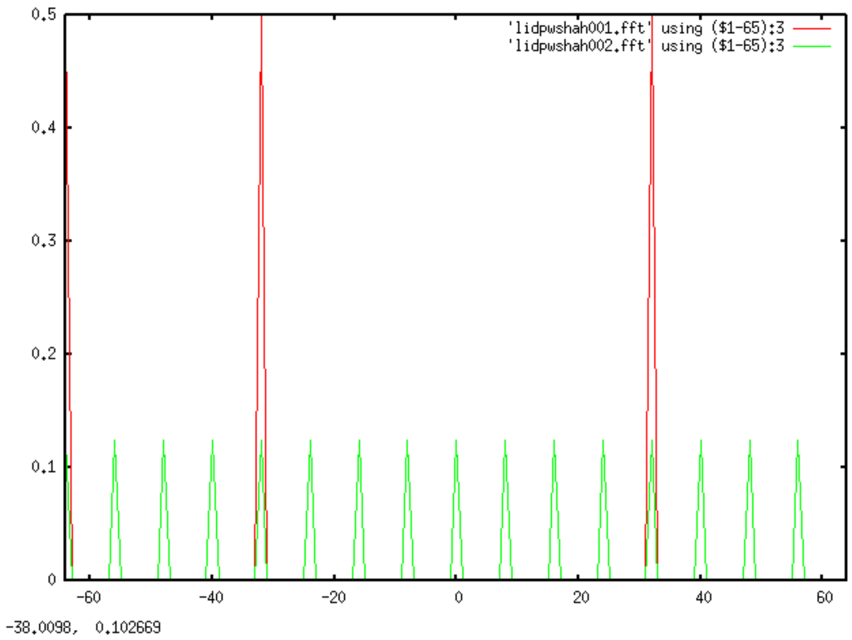
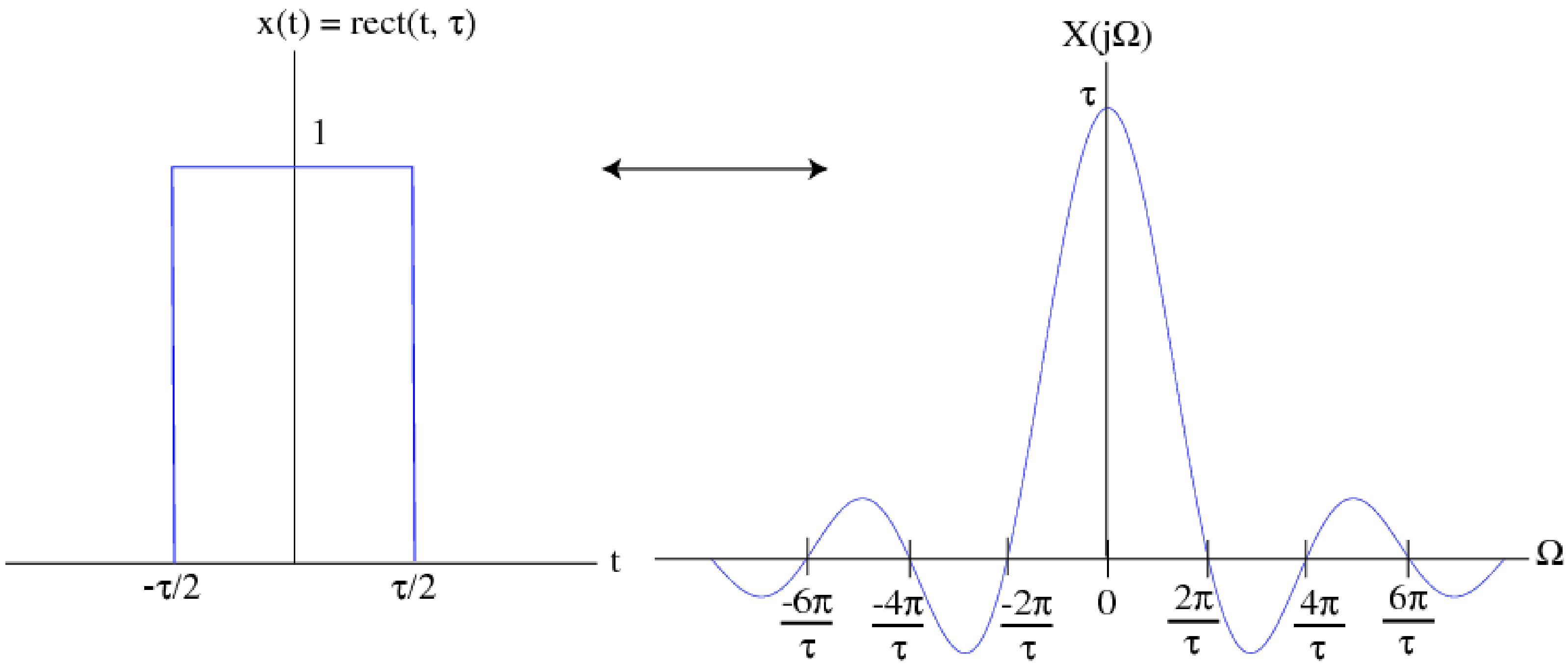
Some simple 1D transforms: a 1D lattice



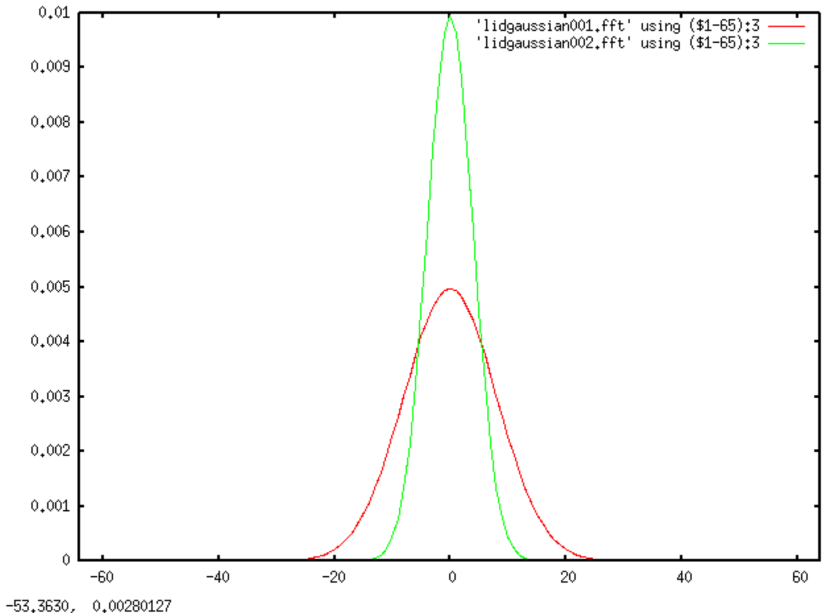


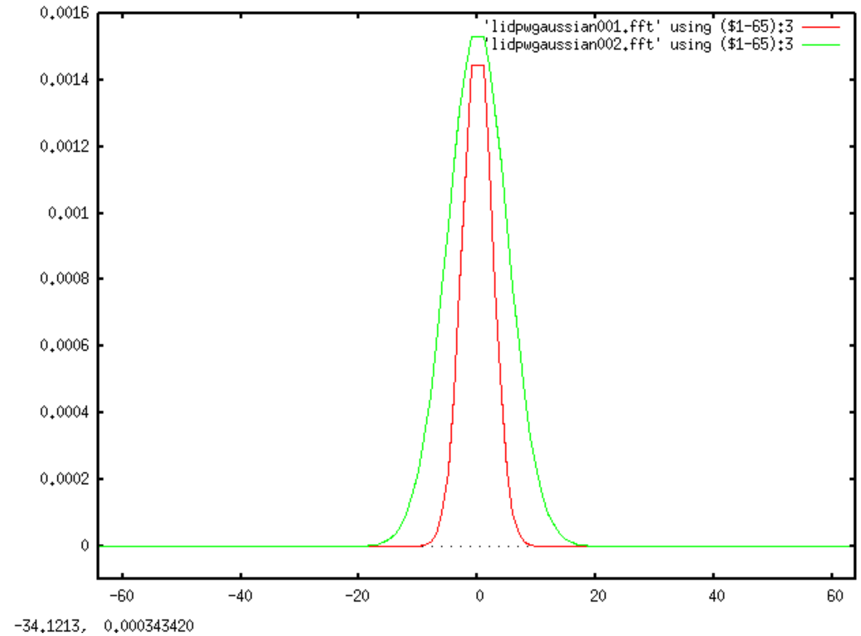
Some simple 1D transforms: a box



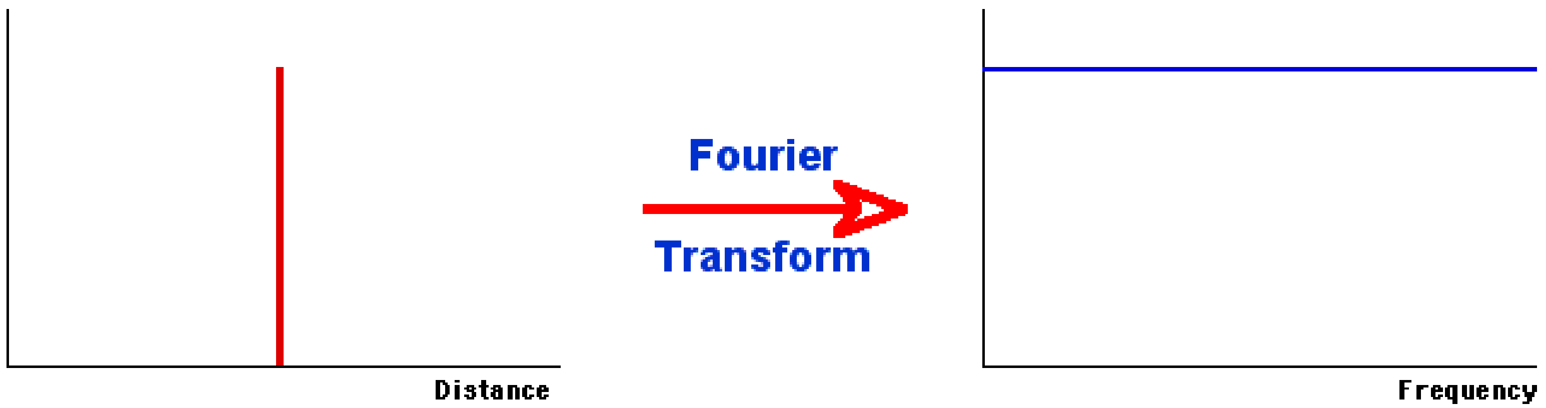
http://cnx.org

Some simple 1D transforms: a Gaussian





Some simple 1D transforms:
a sharp point (Dirac delta function)



http://en.labs.wikimedia.org/wiki/Basic_Physics_of_Nuclear_Medicine/Fourier_Methods

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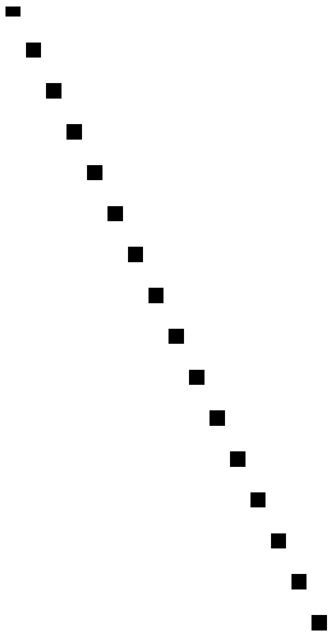
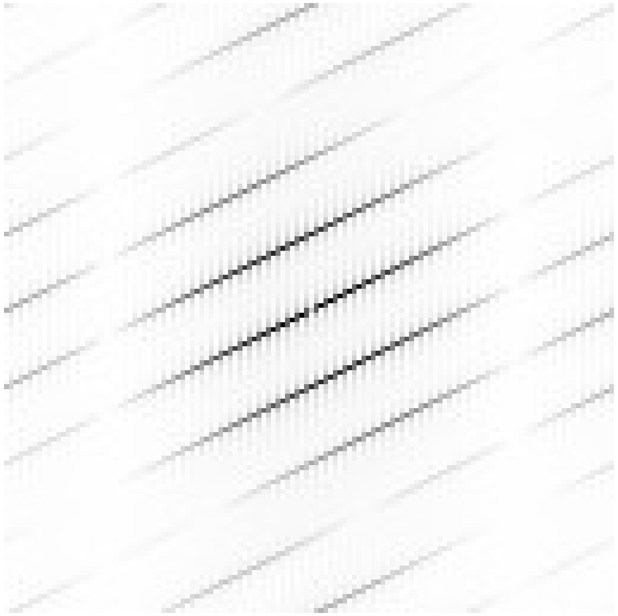
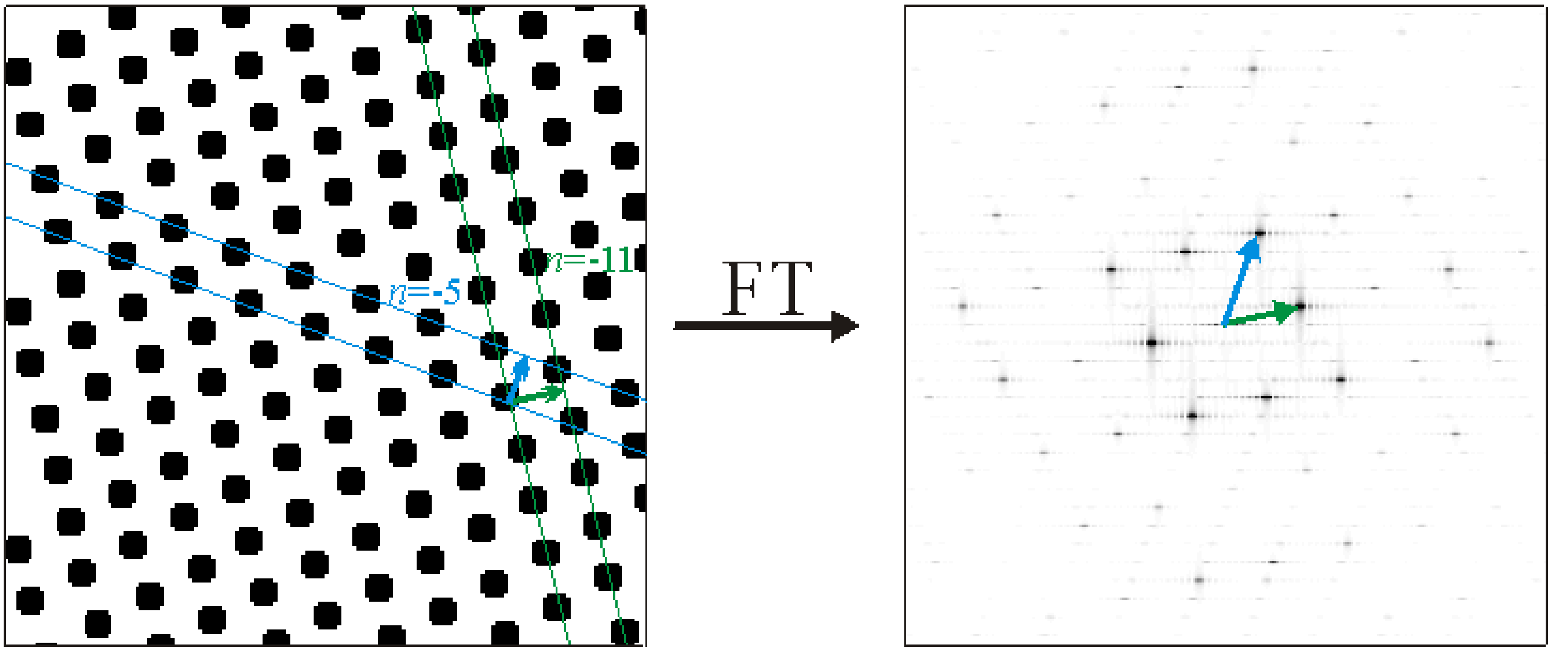
Some simple 2D Fourier transforms: a row of points

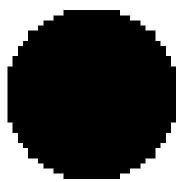


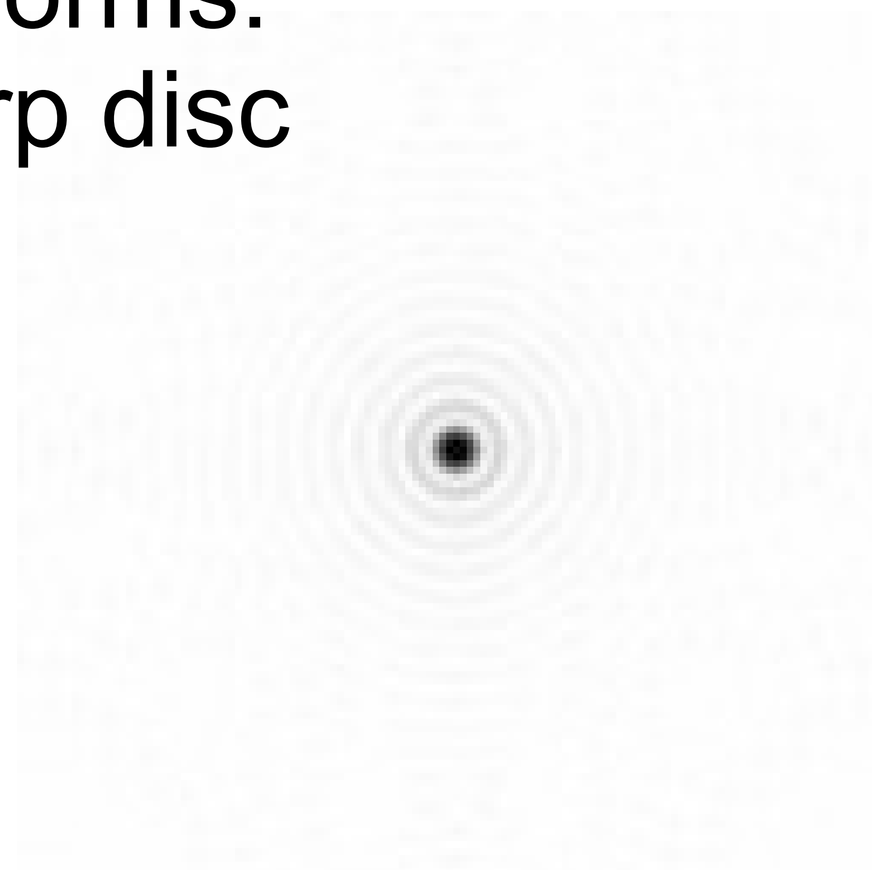


Some simple 2D Fourier transforms: a 2D lattice



Some simple 2D Fourier transforms:
a sharp disc





Some simple 2D Fourier transforms:
a series of lines