NCMI Single Particle Workshop
Baylor, March 14-17th 2011

Image processing theory
Richard Henderson

• Fourier synthesis and analysis
• Central section theory and 3D reconstructions
• Contrast transfer theory
• B-factors, resolution, radiation damage
Baron Jean-Baptiste-Joseph Fourier introduced the idea that an arbitrary function could be represented by a single analytic expression. Fourier came upon his idea in connection with the problem of the flow of heat in solid bodies.

The formula
\[ x/2 = \sin x - \frac{(\sin 2x)}{2} + \frac{(\sin 3x)}{3} - \cdots \]

was published by Leonhard Euler (1707-1783) before Fourier.

Fourier used a polished iron ring of diameter \(~30\text{cm}\) held in place by wooden supports and heated by an adjustable Argand burner. Six holes were drilled halfway into the ring, four of which held thermometers on the Réamur scale (the space between the ring and the thermometer being filled with mercury - as were the other two holes). To achieve the steady state one point in the ring was heated while rest of the ring was allowed to radiate heat freely and on the whole results agreed very well with Fourier's theory.
Some 1D examples of Fourier series

The first four Fourier series approximations for a square wave.

http://www.ies.co.jp/math/java/trig/graphFourier/graphFourier.html
Some different ways to express 1D Fourier summations, where \( D = \) repeat distance (unit cell)

\[
f(x) = \frac{A_0}{2} + A_1 \cos \left( \frac{2\pi x}{D} - a_1 \right) + A_2 \cos \left( \frac{2\pi x}{D/2} - a_2 \right) + \ldots
\]

\[
= \frac{A_0}{2} + \sum_{n=1}^{\infty} A_n \cos \left( \frac{2\pi n x}{D} - a_n \right)
\]

\[
f(x) = \frac{A_0}{2} + \sum_{n=1}^{\infty} \left[ A_n \cos a_n \cdot \cos \frac{2\pi n x}{D} + A_n \sin a_n \cdot \sin \frac{2\pi n x}{D} \right]
\]

which in turn can be written as

\[
f(x) = \frac{A_0}{2} + \sum_{n=1}^{\infty} a_n \cos \frac{2\pi n x}{D} + \sum_{n=1}^{\infty} b_n \sin \frac{2\pi n x}{D}
\]

\[
f(x) = \sum_{n=-\infty}^{+\infty} C_n \ e^{i \frac{2\pi n x}{D}}
\]

How to find the Fourier coefficients in 1D

The coefficients are derived in an analogous way and we have the pair of equations

\[
f(x) = \sum_{n=-\infty}^{+\infty} C_n \ e^{\frac{i 2\pi n x}{D}}
\]

\[
C_n = \frac{1}{D} \int_{D} f(x) \ e^{-\frac{i 2\pi n x}{D}} \ dx
\]
Example: a simple Fourier series

We now use the formulae above to give a Fourier series expansion of a very simple function. Consider a sawtooth function (as depicted in the figure):

\[ f(x) = x, \quad \text{for} \quad -\pi < x < \pi, \]
\[ f(x + 2\pi) = f(x), \quad \text{for} \quad -\infty < x < \infty. \]

In this case, the Fourier coefficients are given by

\[ a_n = \frac{1}{\pi} \int_{-\pi}^{\pi} x \cos(nx) \, dx = 0. \]
\[ b_n = \frac{1}{\pi} \int_{-\pi}^{\pi} x \sin(nx) \, dx = 2 \frac{(-1)^{n+1}}{n}. \]

And therefore:

\[ f(x) = \frac{a_0}{2} + \sum_{n=1}^{\infty} \left[ a_n \cos(nx) + b_n \sin(nx) \right] \]

\[ = 2 \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n} \sin(nx), \quad \text{for} \quad -\infty < x < \infty. \quad (*) \]
Reproduced, by permission, from
W. de Beauclair, "Verfahren und Geräte zur mehrdimensionalen Fouriersynthese",
Akademie-Verlag, Berlin 1949.

from Max Perutz, Nobel Lecture 1962
from Taylor & Lipson (1964) “Optical Transforms”
Fig. 32. (a) Optical transform of a continuous helix. (b) Optical transform of a helix with 10 points per turn. (c) Optical transform of a helix with 5 points per turn.
from Misell, 1978: effect of lattice disorder on the diffraction pattern
reproduced from Harburn et al, Atlas of Optical Transforms, 1975
from Misell, 1978: Optical transforms: spatial filtering of ‘Mickey Mouse’
reproduced from Harburn et al, Atlas of Optical Transforms, 1975
Optics
Diffraction from a grating \( D \sin \theta = n \lambda \)

Crystallography in 3D
Bragg’s law \( 2d \sin \theta = n \lambda \)

Note different definition of \( \theta \) crystal tilt angle is half the diffraction angle
Maximum dose = 5e12 Å²
for organic or biological specimens

Strongest spots
protein ~ 10^-5 I₀
paraffin 10^-2 I₀

diffraction pattern

under focused image
in-focus image
Fig. 3.8. Focus series of a thin carbon film, plus gold particles to assist in focusing. The optical diffractograms are alongside each image: (a) optimum defocus, (b) 150 nm underfocus, (c) 210 nm underfocus, (d) 250 nm underfocus. $C_{s}$ and $\Delta f'$ are determined by measuring the radii (or diameters) of the dark circles corresponding to minima in the optical transform. $E_0 = 125$ keV, image bar = 10 nm, diffraction bar = 3.0 nm $^{-1}$. (P. Sieber, unpublished.)
Dubochet et al. 1987
CTF for 200keV electrons
Cs = Cc = 2.0mm

**Thick line:** $\beta = 0.3$ mrad
$\Delta E = 1.6$eV, simulating a tungsten electron gun

**Thin line:** $\beta = 0.015$ mrad
$\Delta E = 0.5$eV, simulating a field emission gun (FEG)

Typical CTFs: from Baker & Henderson
“A brief look at imaging and contrast transfer

\[ \chi = -2\pi/\lambda(\Delta F \theta^2/2 - C_s \theta^4/4) \]
\[ \text{CTF} = -\sin(\chi) \]

Spatial incoherence
0.03, 0.07, 1.4 mrad

Temporal incoherence
\[ \Delta E = 0.6, 1.2, 2.4 \text{ eV} \]
Cs = 1.4, kV = 100

Spatial frequency = \( f \)
defocus = \( z \)
3D specimen

Different 2D projected images

2D Fourier transforms

2D transforms are sections of 3D Fourier transform

Fourier Inversion

3D map
Bacteriorhodopsin 3.5 Å
Number of particles in projection/\(\mu m^2\) in 800 Å thick ice film (separation)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10mg/ml</th>
<th>2mg/ml</th>
<th>0.5mg/ml</th>
<th>0.1mg/ml</th>
<th>20(\mu g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 kD</td>
<td>48000 (45Å)</td>
<td>10000 (100Å)</td>
<td>2500 (200Å)</td>
<td>500 (450 Å)</td>
<td>100 (1000 Å)</td>
</tr>
<tr>
<td>50 kD</td>
<td>10000 (100Å)</td>
<td>2000 (220Å)</td>
<td>500 (400Å)</td>
<td>100 (1000Å)</td>
<td>20 (0.2(\mu m))</td>
</tr>
<tr>
<td>250kD</td>
<td>2000 (220Å)</td>
<td>400 (500 Å)</td>
<td>100 (1000 Å)</td>
<td>20 (0.2(\mu m))</td>
<td>4 (0.5(\mu m))</td>
</tr>
<tr>
<td>1 MD</td>
<td>500 (400Å)</td>
<td>100 (1000Å)</td>
<td>25 (0.2(\mu m))</td>
<td>5 (0.4(\mu m))</td>
<td>1 (1(\mu m))</td>
</tr>
<tr>
<td>5 MD</td>
<td>100 (1000Å)</td>
<td>20 (0.2(\mu m))</td>
<td>5 (0.4(\mu m))</td>
<td>1 (1(\mu m))</td>
<td>0.2 (2.2(\mu m))</td>
</tr>
<tr>
<td>25 MD</td>
<td>20 (0.2(\mu m))</td>
<td>4 (0.5(\mu m))</td>
<td>1 (1(\mu m))</td>
<td>0.2 (2.2(\mu m))</td>
<td>0.04 (5(\mu m))</td>
</tr>
</tbody>
</table>

Table of expected number of particles in cryoEM.

"Given the concentration of the molecules of interest, how many particles per square micron should you see in the image if the frozen specimen has the same concentration of molecules that you expect from the sample concentration?". The number/\(\mu m^2\) as well as the expected particle separation is given. If you make a grid and find either many more or many less particles than you expect, then something fishy is going on. For example all the particles might be sticking to the carbon (if too few are seen in the holes) or the blotting operation might be concentrating the particles (if there are too many), but you can make up hundreds of explanations.
Rotational power spectral analysis: Crowther & Amos (1971), RFILTIM
# Key individuals and key concepts

<table>
<thead>
<tr>
<th>Individual</th>
<th>Concept/Invention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph Fourier</td>
<td>Fourier analysis &amp; synthesis</td>
</tr>
<tr>
<td>Ernst Abbe</td>
<td>Abbe theory of the microscope</td>
</tr>
<tr>
<td>Lawrence Bragg*</td>
<td>Bragg’s law for diffraction geometry</td>
</tr>
<tr>
<td>Max von Laue*</td>
<td>observation of X-ray diffraction from crystals</td>
</tr>
<tr>
<td>Fritz Zernike*</td>
<td>Zernike phase plate &amp; phase contrast microscopy</td>
</tr>
<tr>
<td>F. Thon</td>
<td>Thon rings</td>
</tr>
<tr>
<td>Otto Schertzer</td>
<td>Schertzer focus</td>
</tr>
<tr>
<td>Ernst Ruska*</td>
<td>inventor of electron microscope</td>
</tr>
</tbody>
</table>

- Fourier analysis & synthesis
- Cross correlation, real and complex
- Convolution
- Fourier shell correlation (FSC)
- Contrast Transfer Function (CTF)
- Modulation Transfer Function (MTF)
- Detective Quantum Efficiency (DQE)