Optical Microscopy: Lecture 4

Interpretation of the 3-Dimensional Images and Fourier Optics

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Using scanning optical or acoustical microscopes it is possible to reconstruct structure of three-dimensional objects. How the image we obtain is related to the structure of the real object? Is it possible to simulate images of simple-shaped bodies obtained by optical or acoustical microscopes?
Acoustical images are difficult for direct interpretation: Ultrasound images of a fetus during seventeen of development (left) and an artist’s rendering of the image. (after Med. Encyclopedia, 2005)

“By striving to do the impossible, man has always achieved what is possible.”

Bakunin
The Fourier Transform

What is the Fourier Transform?

The continuous limit: the Fourier transform (and its inverse)

\[ F(\omega) = \int_{-\infty}^{\infty} f(t) \exp(-i\omega t) \, dt \]

\[ f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} F(\omega) \exp(i\omega t) \, d\omega \]

Fourier analysis is named after Joseph Fourier, who showed that representing a function by a trigonometric series greatly simplifies the study of heat propagation.

Jean Baptiste Joseph Fourier (1768 – 1830)
The Fourier Transform

Consider the Fourier coefficients. Let’s define a function $F(\omega)$ that incorporates both cosine and sine series coefficients, with the sine series distinguished by making it the imaginary component:

$$F(\omega) = \int f(t) \cos(\omega t) dt + i \int f(t) \sin(\omega t) dt$$

where $t$ is the time and $\omega$ is the angular frequency; $\omega = 2 \pi f$, where $f$ is the frequency

$$F(\omega) = \int_{-\infty}^{\infty} f(t) \exp(-i\omega t) dt$$

$F(\omega)$ is called the Fourier Transform of $f(t)$. It contains equivalent information to that in $f(t)$. We say that $f(t)$ lives in the “time domain,” and $F(\omega)$ lives in the “frequency domain.” $F(\omega)$ is just another way of looking at a function or wave.
The Fourier Transform and its Inverse

\[ F(\omega) = \int_{-\infty}^{\infty} f(t) \exp(-i\omega t) \, dt \]

\[ f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} F(\omega) \exp(i\omega t) \, d\omega \]

So we can transform to the frequency domain and back. Interestingly, these functions are very similar.

There are different definitions of these transforms. The \(2\pi\) can occur in several places, but the idea is generally the same.
What do we hope to achieve with the Fourier Transform?

We desire a measure of the frequencies present in a wave. This will lead to a definition of the term, the spectrum.

\[
\mathcal{F} \left\{ E(t) \cos(\omega_0 t) \right\} = \int_{-\infty}^{\infty} E(t) \cos(\omega_0 t) \exp(-i \omega t) \, dt
\]

\[
= \frac{1}{2} \int_{-\infty}^{\infty} E(t) \left[ \exp(i \omega_0 t) + \exp(-i \omega_0 t) \right] \exp(-i \omega t) \, dt
\]

\[
= \frac{1}{2} \int_{-\infty}^{\infty} E(t) \exp(-i [\omega - \omega_0] t) \, dt + \frac{1}{2} \int_{-\infty}^{\infty} E(t) \exp(-i [\omega + \omega_0] t) \, dt
\]

\[
\mathcal{F} \left\{ E(t) \cos(\omega_0 t) \right\} = \frac{1}{2} \tilde{E}(\omega - \omega_0) + \frac{1}{2} \tilde{E}(\omega + \omega_0)
\]

Example:
\( E(t) = \exp(-t^2) \)
**Fourier Transform of a rectangle function: \( \text{rect}(t) \)**

\[
F(\omega) = \int_{-\tau}^{\tau} \exp(-i\omega t) dt = \\
\frac{1}{-i\omega} \left[ \exp(-i\omega \tau) - \exp(i\omega \tau) \right] \\
= \frac{1}{-i\omega} \frac{2\tau}{(\omega \tau)} \left( \exp(i\omega \tau) - \exp(-i\omega \tau) \right) = \\
= 2\tau \frac{\sin(\omega \tau)}{(\omega \tau)}
\]

\[
F(\omega) = 2\tau \text{sinc}(\omega \tau) = \\
= A \times \text{sinc}(\omega \tau)
\]
\[ Sinc(\omega \tau) = 0 \iff \omega_0 \tau = \pi \]

\[ \omega_0 = \frac{\pi}{\tau}; \quad f_0 = \frac{\omega_0}{2\pi} = \frac{1}{2\tau} \]

- \textit{Sinc}(x) is the Fourier transform of a rectangle function.
- \textit{Sinc}^2(x) is the Fourier transform of a triangle function.
- \textit{Sinc}(x) describes the axial field distribution of a lens.
- \textit{Sinc}^2(ax) is the diffraction pattern from a slit.
- It just crops up everywhere...
Fourier Transform of a rectangle function: \( \text{rect}(t) \)

\[
\omega_o = \frac{\pi}{\tau}; \quad f_o = \frac{\omega_o}{2\pi} = \frac{1}{2\tau}
\]

\( \tau = 1 \)

\[
\tau = 5
\]

\[
\tau = 10
\]
Example: the Fourier Transform of a Gaussian function

\[ \mathcal{F}\{\exp(-at^2)\} = \int_{-\infty}^{\infty} \exp(-at^2) \exp(-i\omega t) \, dt \]

\[ \propto \exp(-\omega^2 / 4a) \]
The Fourier Transform Properties

- **Linearity** \( af(x, y) + bg(x, y) \Leftrightarrow aF(u, v) + bG(u, v) \)

- **Shifting** \( f(x - x_0, y - y_0) \Leftrightarrow e^{-j2\pi(ux_0 + vy_0)} F(u, v) \)

- **Modulation** \( e^{j2\pi(u_0x + v_0y)} f(x, y) \Leftrightarrow F(u - u_0, v - v_0) \)

- **Convolution** \( f(x, y) * g(x, y) \Leftrightarrow F(u, v)G(u, v) \)

- **Multiplication** \( f(x, y)g(x, y) \Leftrightarrow F(u, v) * G(u, v) \)

- **Separable functions** \( f(x, y) = f(x)f(y) \Leftrightarrow F(u, v) = F(u)F(v) \)
Scale Theorem

The Fourier transform of a scaled function, \( f(at) \):

\[
\mathcal{F}\{ f(at) \} = F(\omega/a) / |a|
\]

Proof:

\[
\mathcal{F}\{ f(at) \} = \int_{-\infty}^{\infty} f(at) \exp(-i\omega t) \, dt
\]

Assuming \( a > 0 \), change variables: \( u = at \)

\[
\mathcal{F}\{ f(at) \} = \int_{-\infty}^{\infty} f(u) \exp(-i\omega [u/a]) \, du / a
\]

\[
= \int_{-\infty}^{\infty} f(u) \exp(-i \, [\omega/a] \, u) \, du / a
\]

\[
= F(\omega/a) / a
\]

If \( a < 0 \), the limits flip when we change variables, introducing a minus sign, hence the absolute value.

(From. Prof. Rick Trebino, Georgia Tech.)
The Scale Theorem in action

\[ f(t) \quad \rightarrow \quad F(\omega) \]

- **Short pulse**: The shorter the pulse, the broader the spectrum!
- **Medium-length pulse**: This is the essence of the Uncertainty Principle!
- **Long pulse**: (From Prof. Rick Trebino, Georgia Tech.)
Nyquist theorem: to correctly identify a frequency you must sample twice a period.

So, if $\Delta x$ is the sampling, then $\pi / \Delta x$ is the maximum spatial frequency.
The Nyquist–Shannon sampling theorem is a fundamental result in the field of information theory, in particular telecommunications and signal processing. Sampling is the process of converting a signal (for example, a function of continuous time or space) into a numeric sequence (a function of discrete time or space). The theorem states:

**Theorem:** If a function $x(t)$ contains no frequencies higher than $B$ hertz, it is completely determined by giving its ordinates at a series of points spaced $1/(2B)$ seconds apart.

OR In order to correctly determine the frequency spectrum of a signal, the signal must be measured at least twice per period.

In order for a band-limited (i.e., one with a zero power spectrum for frequencies $f > B$) baseband ($f > 0$) signal to be reconstructed fully, it must be sampled at a rate $f \geq 2B$. A signal sampled at $f = 2B$ is said to be Nyquist sampled, and $f = 2B$ is called the Nyquist frequency. No information is lost if a signal is sampled at the Nyquist frequency, and no additional information is gained by sampling faster than this rate.

The *Nyquist Sampling Theorem* states that to avoid aliasing occurring in the sampling of a signal the sampling rate should be greater than or equal to twice the highest frequency present in the signal. This is referred to as the *Nyquist sampling rate*. 
The Fourier Transform of 2-D objects

Scanning of the intensity along a line

Intensity of the contrast of the image along a line

Fourier Transform of the intensity of the contrast of the image along a line

Intensity of the contrast of the image along a line
The Fourier Transform of 2-D objects

\[ I \quad \log\{|\mathcal{F}\{I\}|^2+1\} \quad \angle[\mathcal{F}\{I\}] \]

FT of an Image (Magnitude + Phase)

The Fourier Transform of 2-D objects

\[ I \quad \text{Re}[\mathcal{F}\{I\}] \quad \text{Im}[\mathcal{F}\{I\}] \]

FT of an Image (Real + Imaginary)

The Fourier Transform in Space

Gallanger et al., AJR. 190. 2008
Example of the Fourier Transform in Space

Bahadir Gunturk
Light Waves: Plane Wave

**Definition:** A plane wave is a wave in which the wavefront is a plane surface; a wave whose equiphase surfaces form a family of parallel surfaces (MCGrav-Hill Dict. Of Phys., 1985).

**Definition:** A plane wave in two or three dimensions is like a sine wave in one dimension except that crests and troughs aren't points, but form lines (2-D) or planes (3-D) perpendicular to the direction of wave propagation (Wikipedia, 2009).

The large arrow is a vector called the wave vector, which defines (a) the direction of wave propagation by its orientation perpendicular to the wave fronts, and (b) the wavenumber by its length. We can think of a wave front as a line along the crest of the wave.

\[ \phi = Ae^{i(k_x x + k_y y + k_z z - \omega t)} \]
The Fourier Transform in Space

Time Fourier Transform

\[ F(\omega) = \int_{-\infty}^{\infty} f(t) \exp(-i\omega t) \, dt \]

Fourier Transform in Space?

\[ \phi = \mathcal{A}e^{i(k_x x + k_y y + k_z z - \omega t)} \]

As \( \Phi \) must satisfy the Helmholtz equation, every solution can be decomposed into plane waves \( \exp(ik_x x + ik_y y + ik_z z) \) with wave vector \( \mathbf{k} = (k_x, k_y, k_z) \), \( |\mathbf{k}| = \omega / c \), and \( c \) being the velocity of sound in the coupling liquid. If \( k_x^2 + k_y^2 \leq k^2 \), then . Such waves are denoted as homogeneous waves.

\[
U(k_x, k_y, Z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \Phi(x, y, Z) \exp[-i(k_x x + k_y y)] \, dx \, dy
\]

\[
\Phi(x, y, Z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} U(k_x, k_y, Z) \exp[i(k_x x + k_y y)] \, dk_x \, dk_y
\]
The Fourier Transform in Space: Angular Spectrum

\[
U(k_x, k_y, Z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \Phi(x, y, Z) \exp[-i(k_x x + k_y y)] dx dy
\]

Conversely, the potential can then be written as the inverse Fourier transform of the angular spectrum.

\[
\Phi(x, y, Z) = \frac{1}{(2\pi)^2} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} U(k_x, k_y, Z) \exp[i(k_x x + k_y y)] dk_x dk_y
\]

The inverse Fourier transform represents the wavefield in the plane Z as a superposition of plane waves \(\exp(ik_x x + ik_y y)\).
The Fourier spectrum of the field at the plane $z = Z$, $U(k_x,k_y,Z)$ can be expressed through the Fourier spectrum of the field at the plane $z = 0$, $U(kx,ky,0)$.

$$U_i(k_x,k_y,Z) = U_i(k_x,k_y,0) \exp(ik_z Z)$$
Properties of the Fourier Transform

\[ U(k_x, k_y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \Phi(x, y) \exp\left[-i(k_xx + k_yy)\right] dxdy \]

The Fourier spectrum of the circular Function

\[ P(r) = \begin{cases} 1, & r < 1 \\ 0, & r > 1 \end{cases} \]

Is the jinc function:

\[ U(k_r) = A \frac{J_1(k_r)}{k_r} \]

\[ k_r = \sqrt{k_x^2 + k_y^2} \]

Axial Intensity Distribution

Debye integral can be simplified for $\rho = 0$.

$$\Phi(z) = u_0 f \exp(-ikf) \int_0^\alpha \exp(ikz \cos \theta) \sin \theta d\theta =$$

$$\frac{u_0 f \exp(-ikf)}{k_z} \left[ \exp(ikz) - \exp(ikz \cos \alpha) \right]$$

$$\Phi(z) = B \frac{\sin \left[ 0.5 k_z (1 - \cos \alpha) \right]}{0.5 k_z (1 - \cos \alpha)}$$

For small angles $\cos \alpha \sim 1 - 0.5 \sin^2 \alpha$, then

$$\Phi(z) = B \frac{\sin \left[ k_z \sin^2 \alpha / 4 \right]}{k_z \sin^2 \alpha / 4} = B \frac{\sin \left[ k_z \times NA^2 / 4 \right]}{k_z \times NA^2 / 4}$$

PSF: Point Spread Function
Field in the Focus and Fourier Spectrum Approach

\[ \Phi(\vec{r}) = A \int_{0}^{\alpha} \int_{0}^{2\pi} P(\theta, \phi) \exp(ikr(\cos \theta \cos \theta_p + \sin \theta \sin \theta_p \cos(\phi - \phi_p))) \sin \theta \, d\theta \, d\phi \]

In the limit \( f \to \infty \) (Debye approximation) this equation is valid throughout the whole space. It expresses the field in the focal region as a superposition of plane waves whose propagation vectors fall inside a geometrical cone formed by drawing straight lines from the edge of the aperture through the focal point, which, in contrast to the usual Debye integral, are weighted with \( P(\theta, \phi) \). Where \( P(\theta, \phi) \) is the Pupil Function.

We may say that each point on \( P(\theta, \phi) \) is responsible for the emission (and, by reciprocity, for the detection) of the plane wave component emitted along the line from the point on \( P(\theta, \phi) \) through the focal point.
Field in the Focus and Fourier Spectrum Approach

To find the angular spectrum of the emitted field in the focal plane, we set the third coordinate of $r$ equal to zero and substitute Cartesian coordinates for the angular integration variables. For the vector components of $k$ hold: $k_x = k \sin \theta \cos \phi$, $k_y = k \sin \theta \sin \phi$, $k_z = \cos \phi = \sqrt{k^2 - k_x^2 - k_y^2}$. The Jacobi determinant corresponding yields:

$$
\sin \theta d\theta d\phi = \frac{1}{k^2 \cos \theta} dk_x dk_y = \frac{1}{kk_z} dk_x dk_y
$$

Denoting the Cartesian coordinates of $r$ by $x, y, z$, Equation can be rewritten:

$$
\Phi_i (x, y, z = 0) = \frac{1}{(2\pi)^2} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(k_x, k_y) \exp \left[ i(k_x x + k_y y) \right] \frac{dk_x dk_y}{kk_z}
$$

Up to a constant $P(k_x, k_y)$ is defined as

$$
P(k_x, k_y) = \begin{cases} 
P(\theta, \phi), & k_x^2 + k_y^2 \leq k^2 \\
0, & \text{elsewhere} 
\end{cases}
$$
Field in the Focus and Fourier Spectrum Approach

\[ \Phi(x, y, 0) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} U(k_x, k_y, 0) \exp\left[i(k_x x + k_y y)\right] dk_x dk_y \]

The Pupil Function of an ideal lens can be described by a circle function:

\[ P(k_r) = \begin{cases} 
1, & k_r < k \sin \alpha \\
0, & \text{otherwise}
\end{cases} \]

Fourier transform of the circle function:

\[ \Phi(\rho) = A \frac{J_1(k \rho \sin \alpha)}{k \rho \sin \alpha} \]

Field in the Focus and Fourier Spectrum Approach

Equation (1) also tells us that the lens performs Fourier transform on the incident electromagnetic.

Plane wave passing the lens with a pupil function $P(x,y)$ transforms into jinc function in focal plane of the lens.
Lens as a Fourier transform

Fourier transform of letter “E”
Lens as a Fourier transform

Input laser beam
Gaussian

Output laser beam
Flat top

Focused laser beam
Airy pattern

Input laser beam
Gaussian

Output laser beam
super-Gaussian

Focused laser beam
Flat top

Focal πShaper

Radius, mm
20 μm

Courtesy to Vitali. Prakapenka, University of Chicago
Field in the Focus and Fourier Spectrum Approach

\[ U_i''(k'_x, k'_y) = U_i(k'_x, k'_y) \exp(ik'_z Z) \]

For the reflection microscope we have, since the backscattered wave propagates opposite to the \( z \)-direction. Combining all above we obtain the output signal of the reflection microscope as

\[
V(X,Y,Z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(-k_x, -k_y)P(k'_x, k'_y)g_s(k_x, k_y, k'_x, k'_y) \exp \left[ i(k'_x - k_x)X + (k'_y - k_y)Y + (k'_z - k_z)Z \right] \frac{dk'_x \, dk'_y \, dk_x \, dk_y}{kk'_z}
\]
X-Z scan through a steel sphere for a reflection microscope (OXSAM) at 105 MHz. The radius of the sphere was 560 μm. The semi-aperture angle of the microscope lens was 26.5°. Z=0 corresponds to focussing to the centre of the sphere. (b) X-Z scan through a steel sphere for reflection microscope calculated with the same parameter as in From Weise, Zinin et al., Optik 107, 45, 1997.
Acoustical images are difficult for direct interpretation: Ultrasound images of a fetus during seventeen of development (left) and an artist’s rendering of the image. (after Med. Encyclopedia, 2005)

“By striving to do the impossible, man has always achieved what is possible.”
Bakunin
Important conclusion from the theory we developed is that size of the spherical particle can be determined only from image taken by a *transmission microscope*. The size of the image of the spherical particle in reflection microscope is less than the real size of the particle and is equal to \( a \sin(\alpha) \), where \( a \) is the radius of the particle, and \( \alpha \) is semiaperture angle of the lens. This theory has found several direct application in practical microscopy: surface imaging and in developing Emulated Transmission Confocal Raman Microscopy.
Optical images of yeast cells on glass (100x objective) in transmission mode (a), in reflection mode (b). The red circles mark the position of the laser beam.
Optical images of yeast cells

Calculated vertical scans through a transparent sphere with refraction index of 1.05 and refraction index of surrounding liquid of 1.33: (a) reflection microscope with aperture angle 30°; (a) transmission microscope with aperture angle 30°.
Optical images of yeast cells

Sketch of the optical rays when cell is (a) attached to the glass substrate or (b) to mirror.
Optical image of the yeast bakery cells in the reflection confocal microscope. Rectangle shows the area of the Raman mapping. (a) Raman spectra of the cell $\alpha$ measured with green laser excitation (532 nm, WiTec system).

Map of the Raman peak intensity centered at 2933 cm$^{-1}$. The intensity of the 2933 cm$^{-1}$ peak is shown in a yellow color scale. (b) Map of the Raman peak intensity centered at 1590 cm$^{-1}$. The intensity of the 1590 cm$^{-1}$ peak is shown in a green color scale.
• Sinc function is a Fourier Transform of the rectangular Function.
• Distribution of the field in the focal plane is the spatial Fourier Transform of the Pupil Function of the Lens
• Fourier Spectrum Approach of Image in 3-D
• Contrast in Reflection and Transmission Microscopy
• Emulated Transmission Confocal Raman Microscopy
1. Present a definition of the Fourier transform (SO).

2. Describe difference in image formation of transmission and reflection optical microscope of 3-D objects. (KK).

3. Formulate and explain Nyquist Sampling Theorem (SO).

4. Derive a Fourier transform of the function $f(t) = \{1, \text{if } -1/2 < t < 1/2; \text{0 if } |t| > 1/2\}$ (KK).

5. Derive magnification of the compound microscope (KK and SO)