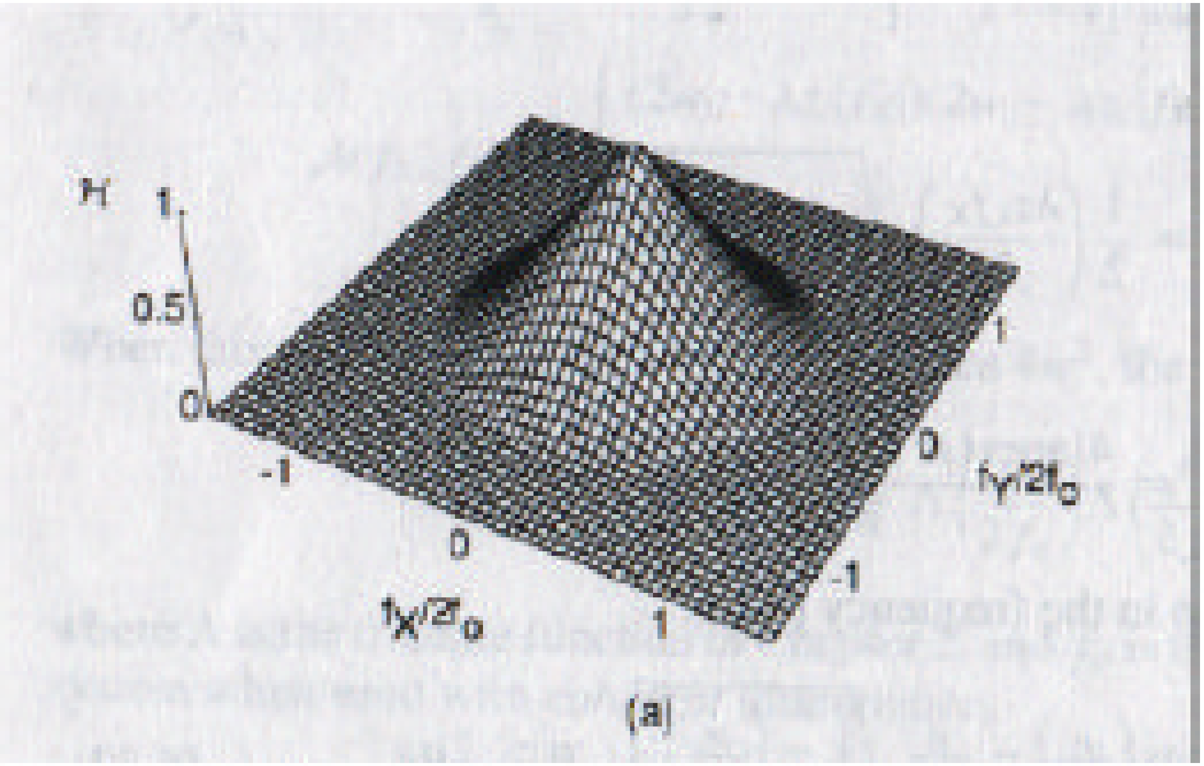
Optics and Microscopy II



Microscopic contrast and resolution

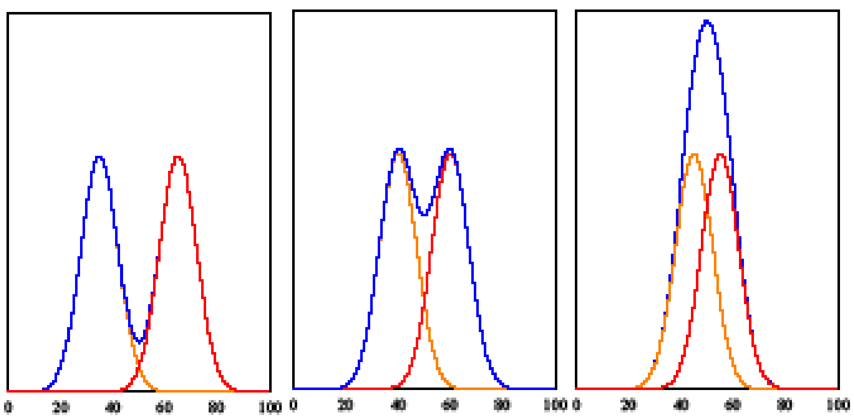
Two of the most important and difficult to quantify aspects of an optical microscope are its ability to generate contrast and it ability to resolve fine structures

What is contrast? Contrast refers to an “intensity” difference between a specimen of interest and its background.

Optically contrast is defined as the visibility:

$$V = \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}}$$

What is resolution? Resolution defines how fine we can see … how far apart two objects have to be for them to be distinguishable.



Rayleigh’s Criterion:

Two objects are distinguishable if their centers are separation by further than their full width at half maximum

Huygens-Fresnel Principle

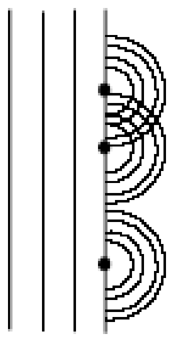
Diffraction

Diffraction can be considered as a more advanced treatment of interference effect. The basic physics of the two phenomena are identical. While we have treated interference as light originating from point sources (like the double slit experiment), diffraction considers interference of light from finite size objects such as an aperture.

Diffraction effect can be easily seen when light is restricted into dimensions that are comparable to its wavelength. For coherent light source, like a laser, diffraction effects can be readily observed. An example is sending laser light through a narrow sli

The treatment of diffraction effects started in the 1700s-1800s wi the introduction of the Huygens-Fresnel Principle.

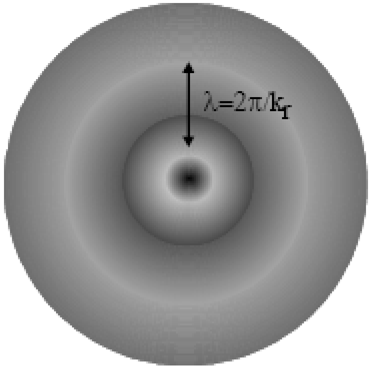
Huygens-Fresnel Principle: Every unobstructed points of a wave front are a source of secondary spherical wave. The optical field far away can be determined by the interference of the secondary waves.



The Huygen's principle can be derived directly from the wave equation assuming the electric field can be treated as scalar quantities. It is quite a bit of work and I will not go through it here.

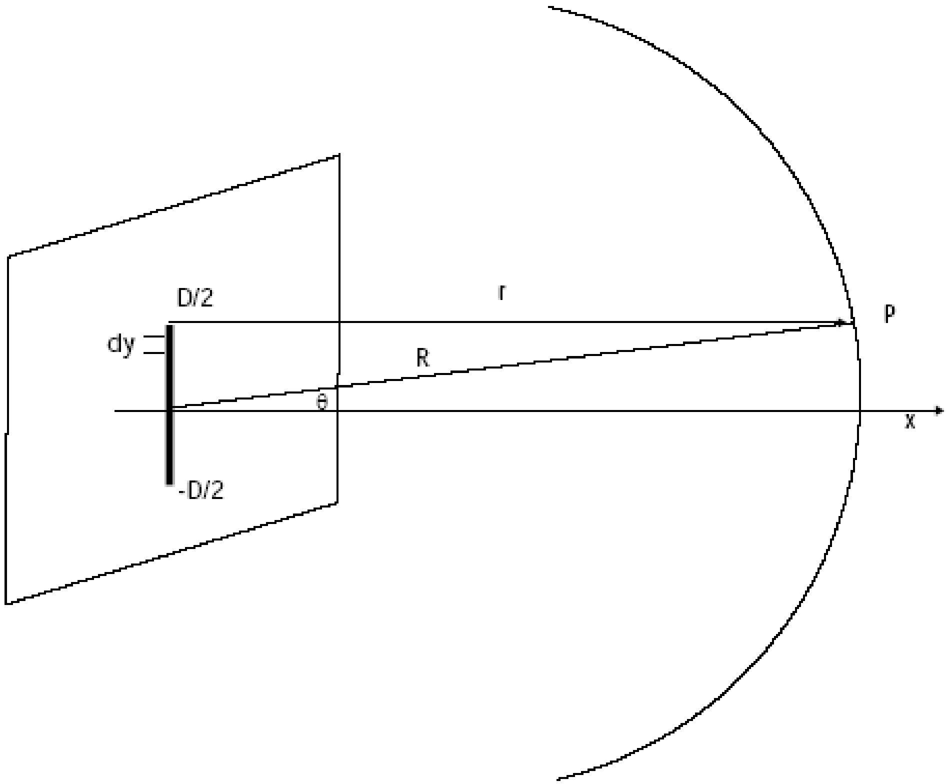
Spherical Wave Solution

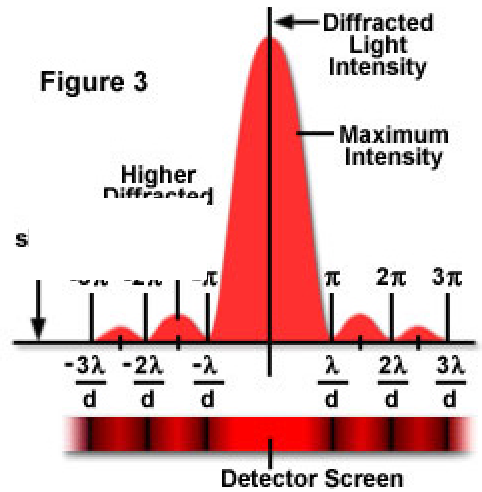
$$\vec{E}(r,t) = \vec{E}_o \frac{\sin(k_r.r - \omega t)}{r} \quad \text{and } ck_r = \omega$$



Diffraction I

Single slit diffraction is a result of the interference of light due to its wave nature

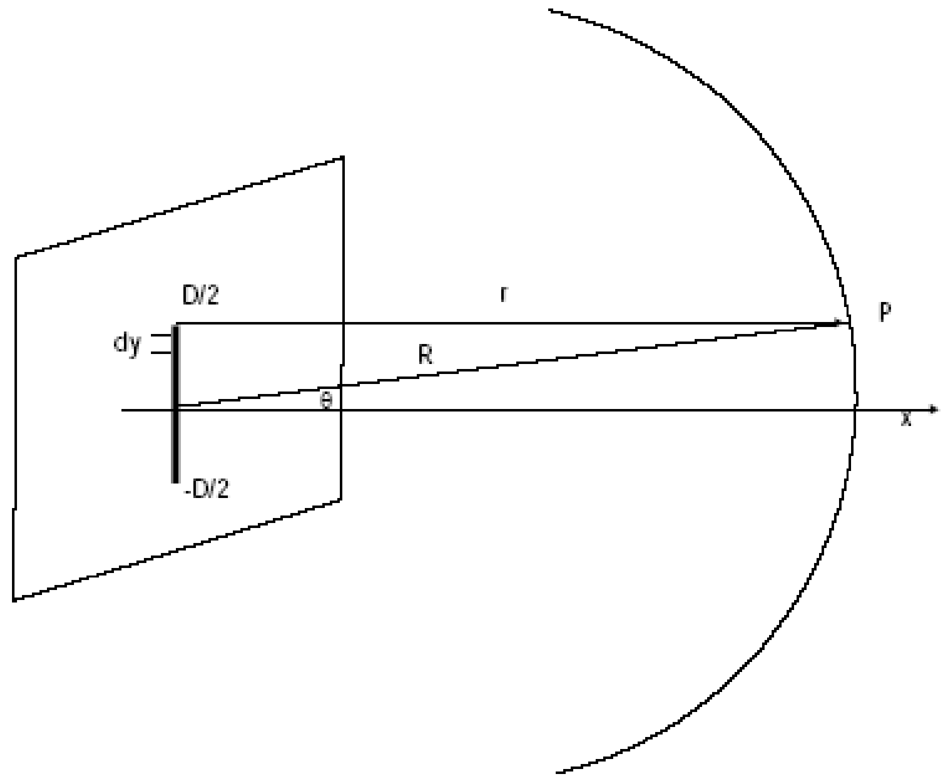




Diffraction II

Single Slit Experiment

Let's consider the simplest diffraction situation that of a finite size slit:



The contribution of element dy at position P can be calculated from Huygen's principle:

$$dE = \frac{\overline{E}}{r}\sin(\omega t - kr)dy$$

Diffraction III

In the far field (Fraunhoffer) limit, R>> D

We can approximate r by R and the distance r can be approximated as a function of R, y and θ (using Law of cosine):

$$r = R - y\sin\theta + (\frac{y^2}{2R})\cos^2\theta + \ldots$$

Keeping terms to first order in y, we have

$$dE = \frac{\overline{E}}{R}\sin(\omega t - kR + ky\sin\theta)$$

The total field at P is:

$$E = \frac{\overline{E}}{R}\int_{-D/2}^{D/2}\sin(\omega t - kR + ky\sin\theta)dy$$

Therefore, we have

$$E = \frac{\overline{E}D}{R}\frac{\sin[(kD/2)\sin\theta]}{(kD/2)\sin\theta}\sin(\omega t - kR)$$

Diffraction IV

Defining $\beta = (kD/2)\sin\theta$ and calculating the intensity at P we have:

$$I(\theta) = \frac{1}{2}(\frac{\overline{E}D}{R})^2(\frac{\sin\beta}{\beta})^2 = I(0)\sin c(\beta)$$

Note that sinc function corresponds to a number of fringes reflecting the fundamental interference effect of diffraction.

The maxima and minima location of the intensity can be identify by the first and 2nd derivatives of I(θ):

$$\frac{dI}{d\beta} = I(0)\frac{2\sin\beta(\beta\cos\beta - \sin\beta)}{\beta^3} = 0$$

From the 2nd derviative, we get the minima is corresponding to the solution of

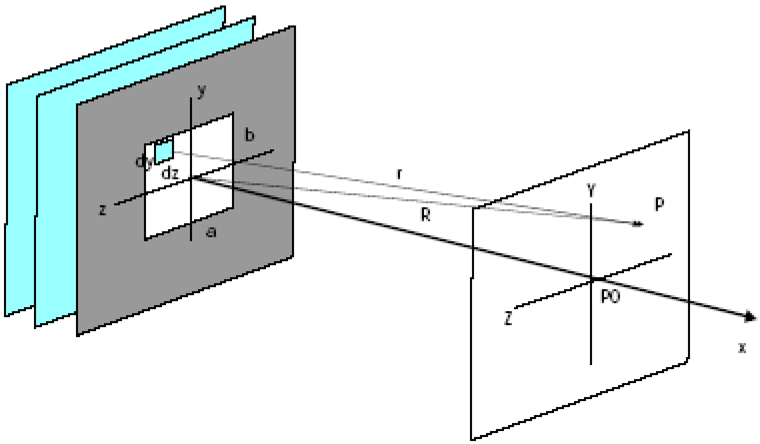
$$\sin\beta = 0$$

The maxima corresponds to the solutions of:

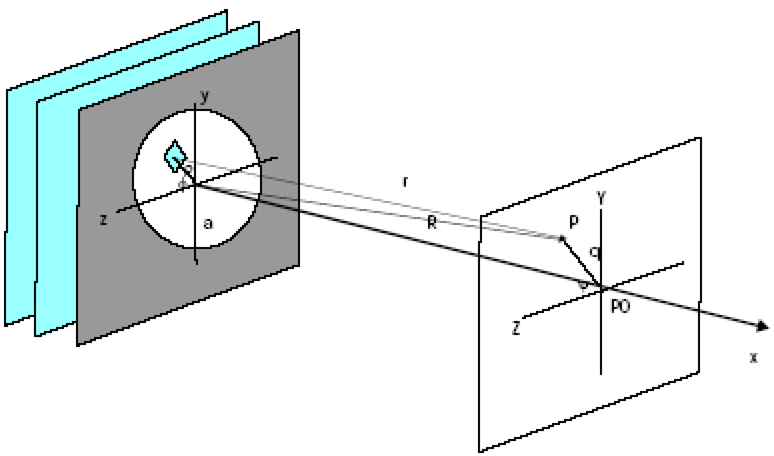
$$\tan\beta = \beta$$

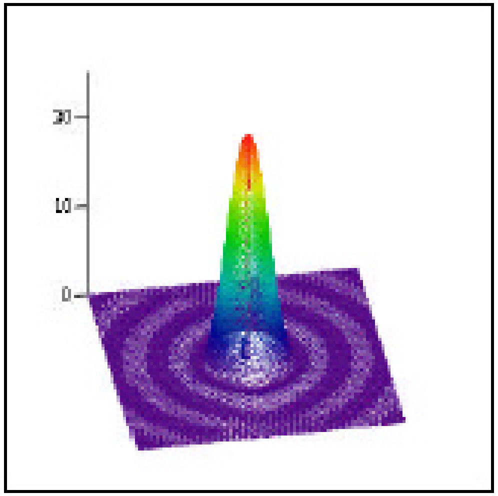
Note that in this derivation, we have ignored near field effects (Fresnel diffraction) as well as the vector nature of the electric field.

Diffraction V



$$I(\theta) = I(0)\left[\frac{2J_1(ka\sin\theta)}{ka\sin\theta}\right]^2$$





Microscopy imaging can be consider as the diffraction from a circular aperture with a lens for focusing – diffraction results in “broadening” of the focal point.

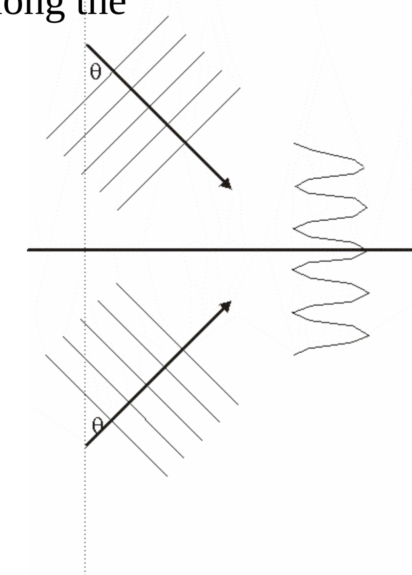
Fourier Optics I

Recall the interference of two plane waves

$$\vec{E}_1(\vec{r},t) = \vec{E}\cos(\vec{k}_1.\vec{r} + wt)$$
$$\vec{E}_2(\vec{r},t) = \vec{E}\cos(\vec{k}_2.\vec{r} + wt)$$

$$I(\vec{r},t) = 2I + 2I\cos(\vec{k}_1.\vec{r} - \vec{k}_2.\vec{r})$$

In the case where the waves incident symmetrically and looking at the intensity along the y axis



$$\vec{k}_1 = k\sin q\hat{x} + k\cos q\hat{y}$$
$$\vec{k}_2 = k\sin q\hat{x} - k\cos q\hat{y}$$
$$\vec{r} = y\hat{y}$$

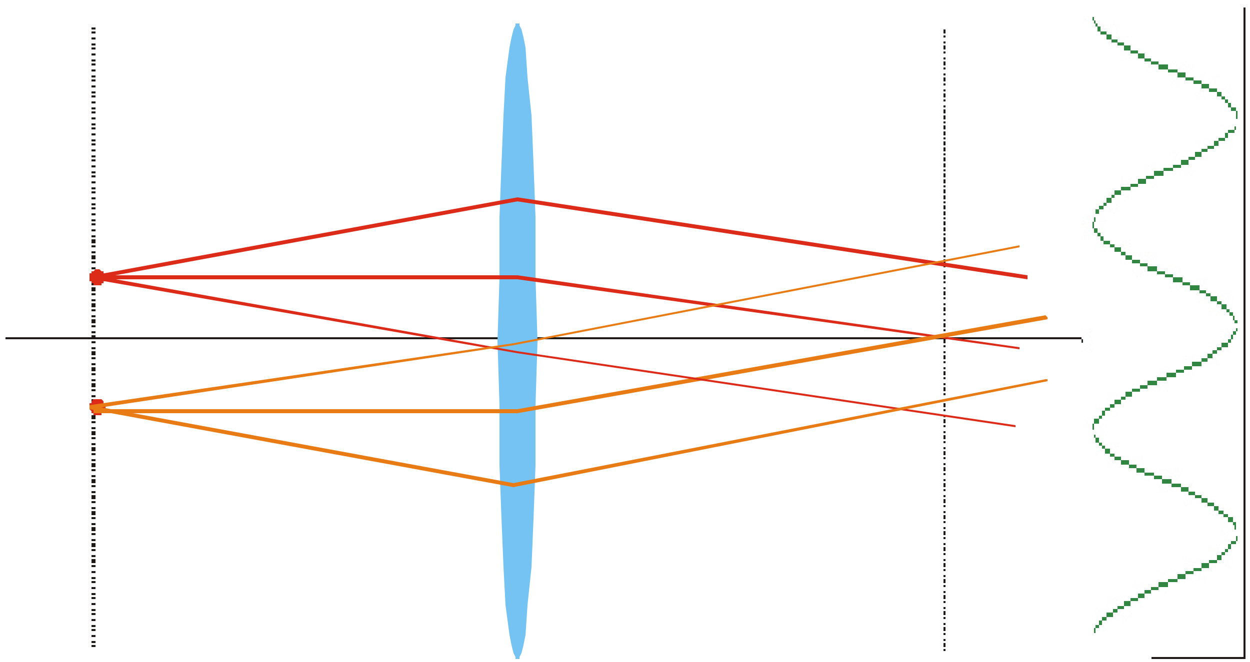
The intensity has a simple distribution depend on angle θ:

$$I(\vec{r},t) = 2I(1 + \cos(2k\cos qy))$$

Note that when angle is zero degree (light wave counter propagating), the highest frequency oscillation is observed at spatial frequency: $2k = 2p(\frac{2}{l})$. When the waves are parallel, angle is 90 degree, the spatial frequency is zero (constant intensity light).

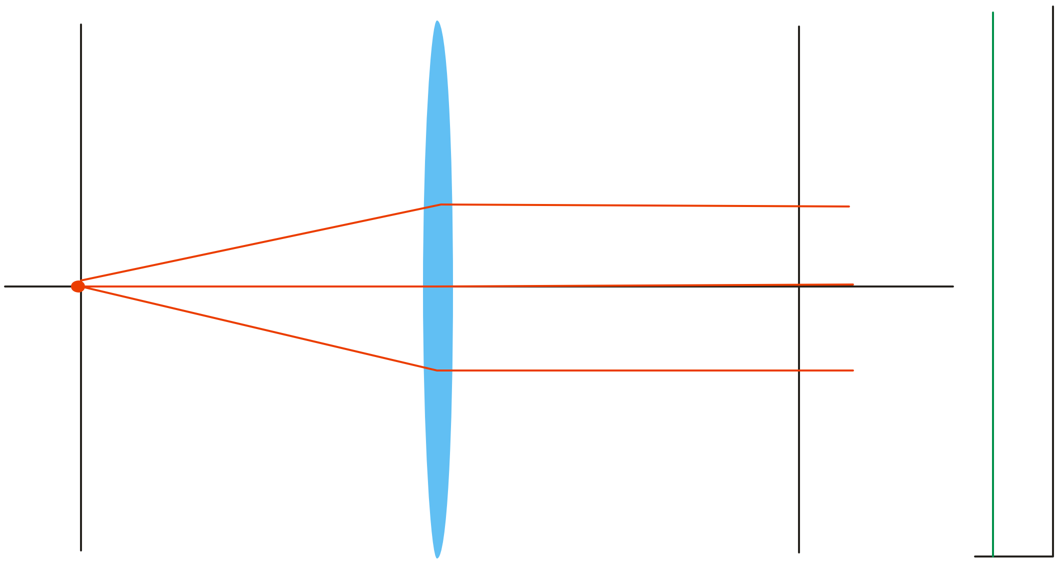
Fourier Optics II

Consider two point source at the focal plane of a lens, the light rays become collimated plane waves after the lens and interference is observed.



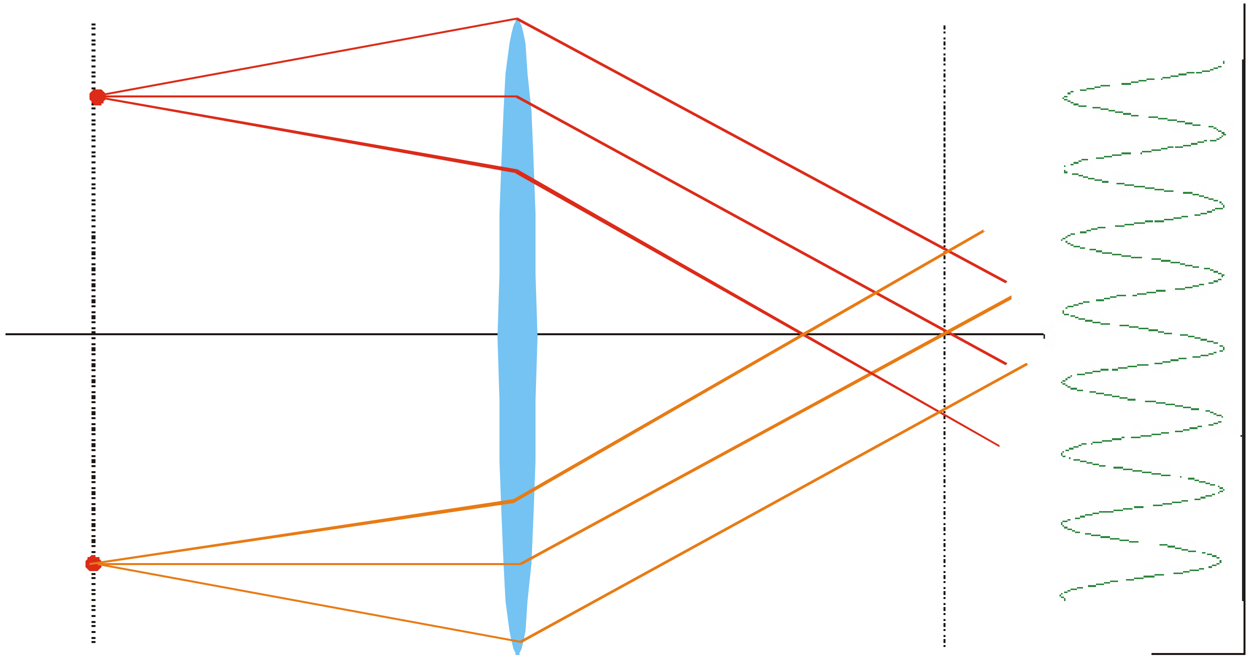
Fourier Optics III

What happen when the two sources coincide? Only parallel plane waves are generated.



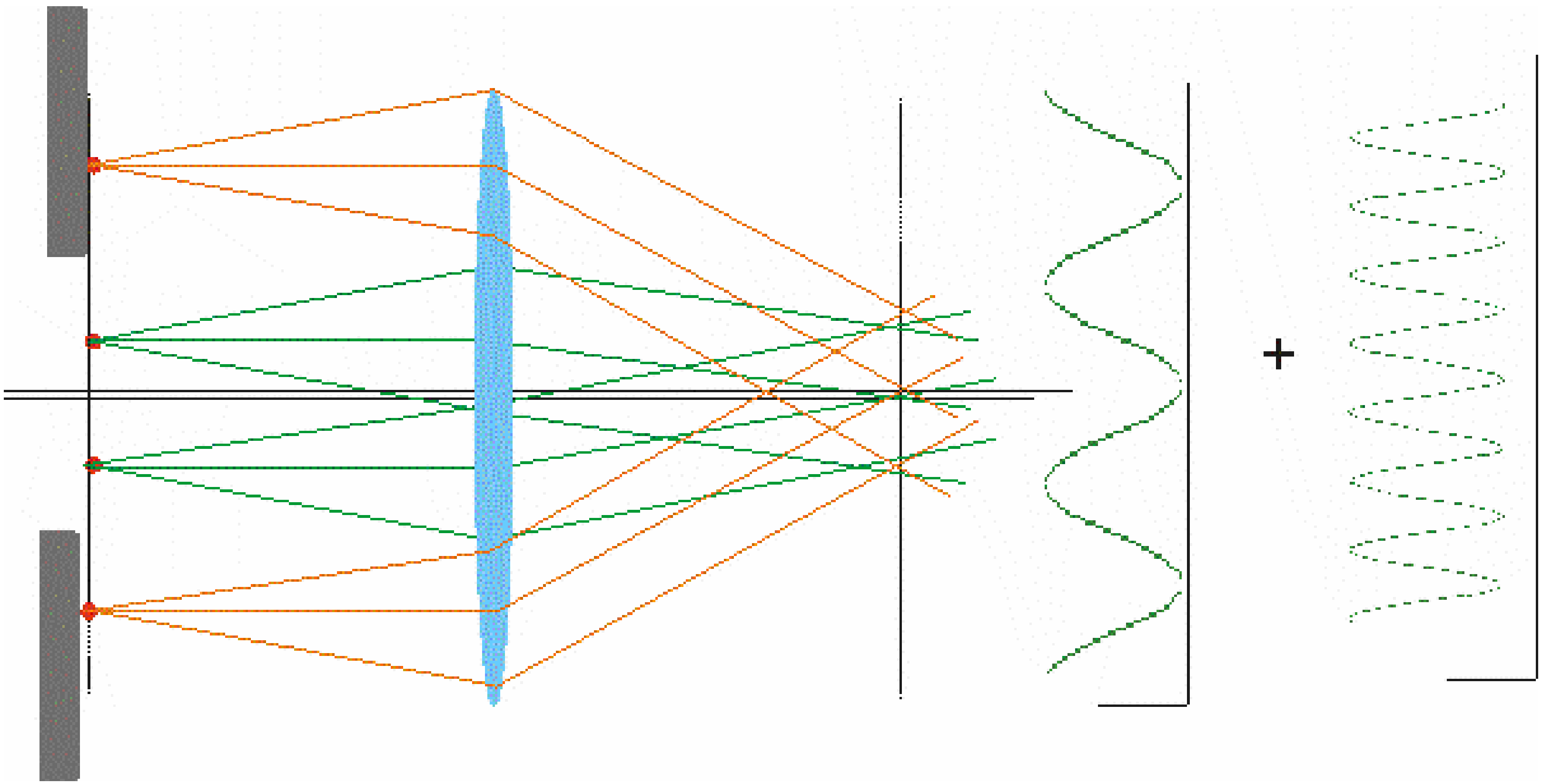
Fourier Optics IV

What happen if the point sources are made further apart?

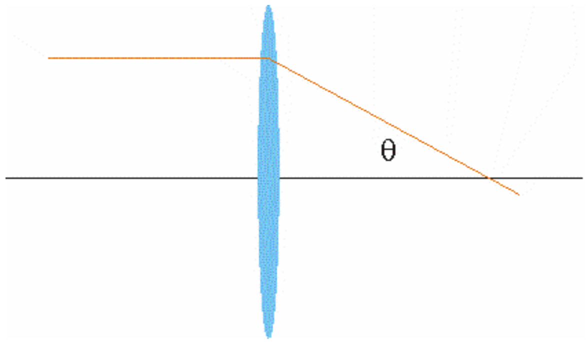


Resolution viewed from Fourier Optics

Light emission from any object in the specimen plane can be Decomposed into its Fourier components. Which Fourier component will pass the finite aperture of the objective lens? Low frequencies!



Resolution viewed from Fourier Optics II



$$NA = n\sin(q)$$

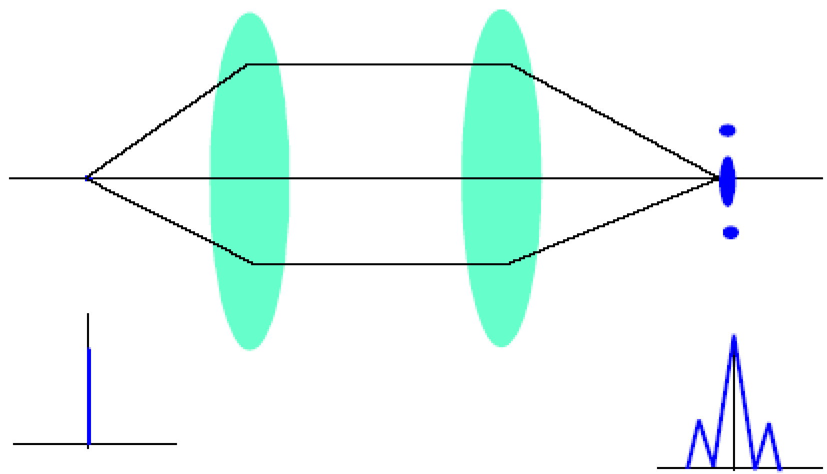
What is the maximum frequency that can be pass? Consider the case of a very large lens (numerical aperture, NA approach one). The waves will approach counter propagating and the maximum frequency is:

$$k_{\max} = 2p\left(\frac{2}{l}\right)$$

Note that maximum spatial frequency is a function of wavelength. Shorter wavelength implies higher frequency (resolution) imaging.

At a given wavelength, we should expect a resolution of about $\frac{l}{2}$

Resolution viewed from Fourier Optics III



Point Spread function

More quantitative analysis shows that:

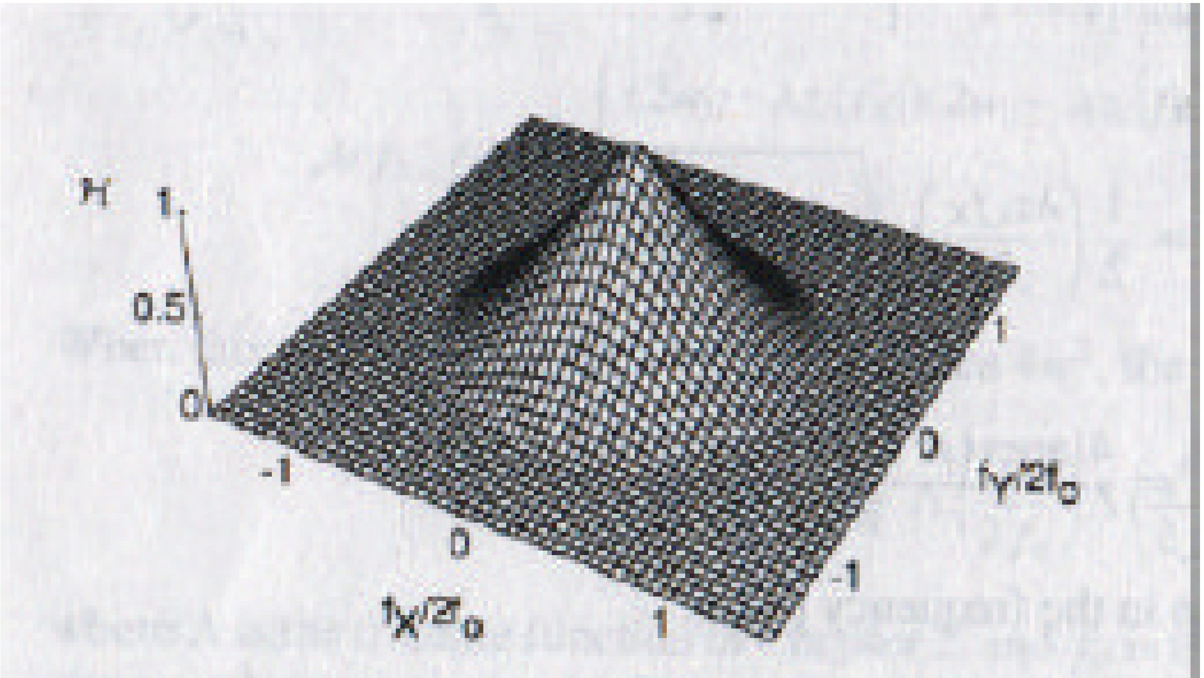
$$I(\theta) = I(0)\left[\frac{2J_1(ka\sin\theta)}{ka\sin\theta}\right]^2$$

$$J_1(ka\sin q) = J_1\left(\frac{2p}{l}a\frac{r}{f}\right) = J_1\left(\frac{2p}{l}\frac{a}{f}r\right) = J_1\left(\frac{2p}{l}NAr\right)$$

$$J_1(x) = 0 \quad at \quad x = 3.83 \quad \Rightarrow \quad r_{\min} = \frac{0.6\,l}{NA}$$

Resolution viewed from Fourier Optics VI

$$OTF(k) = \mathcal{F}(PSF(r))$$



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