

Resolution of the microscope

Equipment

Laser on a 2-axis translation stage, 1-m optical rail, two lenses ($f = 10\text{-}15\text{ cm}$), lens holders, 2 adjustable slits (vertical and horizontal) on a 2-axis translation stage, screen, wire, tape measure.

Purpose

To understand and test Abbe's theory of microscope resolution. To understand geometric optics as the limiting case of physical optics.

Abbe's theory

Any imaging system has a finite limit of resolution, i.e. capability to generate distinguishable images of close objects. The principal reason limiting the resolution is the diffraction of light waves. Light rays are restricted by diaphragms and lens edges, leading to each infinitely small point being imaged as a diffraction spot of a finite size. Diffraction spots from nearby points may overlap with each other and become indistinguishable. The present experiment studies the diffraction resolution limit of a microscope objective.

The theory of microscope resolution was developed by German physicist *Ernst Karl Abbe* (1840 – 1905). Let us consider the simplest microscope, in which an object, located in the plane P_1 close to the focal point of the objective lens, generates a real, magnified, inverted image on a screen P_2 (Fig. 1). Suppose the object is a periodic grating, illuminated by coherent light. If the period of the grating is d , the period of its geometric image is

$$d' = ds' / s, \quad (1)$$

where s and s' are, respectively, the distances from the lens to the object and the image.

Abbe considered two stages of the rays' propagation from the object to the image. In the first stage, a pattern is formed in the back focal plane F of the objective. This pattern is considered as a source of secondary wavelets according to the Fresnel-Huygens principle. These wavelets form the image of the object.

The pattern in the back focal plane of the objective is the Fourier image of the object. To see this, let us Fourier decompose the light wave transmitted by the object

$$E(x) = \int_{-\infty-\infty}^{+\infty+\infty} \tilde{E}(k_x) e^{ik_x x} dk_x,$$

where

$$\tilde{E}(k_x) = \frac{1}{2\pi} \int_{-\infty-\infty}^{+\infty+\infty} E(x) e^{-ik_x x} dx$$

and x is the transverse coordinate in the object plane P_1 (we neglect the other transverse coordinate, y , for the time being). Each Fourier component of the object will generate a plane wave

$$e^{ik_x x - i\omega t} \rightarrow e^{ik_x x + ik_z z - i\omega t}$$

with $k_x^2 + k_z^2 = (2\pi / \lambda)^2 = \omega^2 / c^2$. This wave will propagate in the direction defined by angle θ with respect to the axis z of propagation, such that

$$\sin \theta = \frac{k_x}{k}$$

The objective lens will focus this wave into a single point $x' = f \sin \theta$, where f is the focal length of the lens.

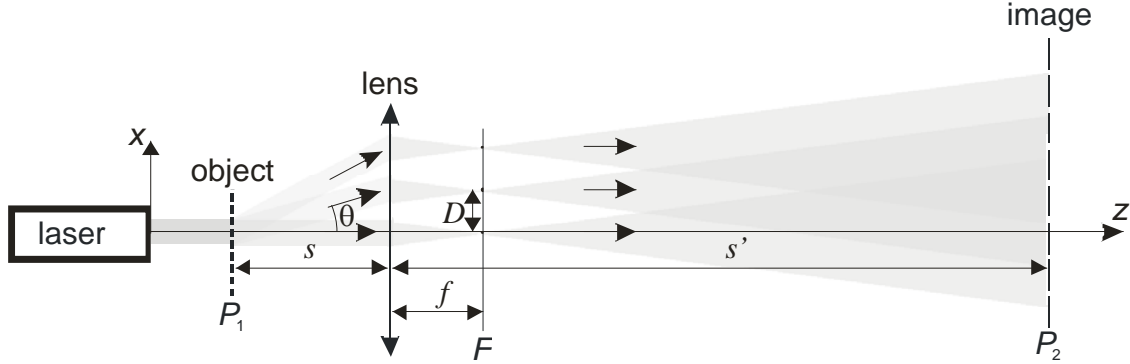


Fig. 1: Abbe's theory of microscope resolution

Now let us remember that the object is a grating. The diffraction maxima are produced at angles θ_m defined by the condition $d \sin \theta_m = m\lambda$. The picture in plane F will thus consist of equidistant bright dots of unequal intensities, positioned at locations

$$x'_m = fm\lambda / d ,$$

d being the grating period. Secondary wavelets, produced by these coherent sources, will interfere with each other, producing an image of the grating in plane P_2 .

Suppose now that we place a diaphragm into the F plane, so some of these diffraction maxima are blocked and do not participate in the formation of the image. Let us first consider an extreme case, when only the zeroth diffraction order is transmitted. The associated wavelet will not have anything to interfere with, so in plane P_2 you will only see a bright spot; there will be no image of the grating.

Now let us open the diaphragm slightly and position it asymmetrically, so that the zeroth and one of the first diffraction orders can pass. These diffraction orders, which in plane F have a form of two bright dots situated at distance $D = f\lambda / d$ from each other, will form a "two-slit" interference pattern in the P_2 plane. The period of this pattern is

$$\tilde{d}' = (s' - f) \frac{\lambda}{D}. \quad (2)$$

Expressing s' through s and f using the thin lens formula, we find that $\tilde{d}' = d'$, where d' is the period of the grating's geometric image given by Eq. (1).

We see that the transmission of the zeroth and first orders of diffraction in the F plane is sufficient to convey information about the most basic features of the image – in our case, the grating period. On the other hand, the double-slit interference pattern is sinusoidal, so it does not represent any finer details about the structure, such as e.g. sharp borders between bright and dark sections of the grating, are represented in the image. These details are conveyed by higher order diffraction maxima, which create narrower interference fringes in the P_2 plane.

Note also that if we block the first order diffraction maximum in plane F , but transmit the zeroth and second order maxima, we will again see a sinusoidal interference pattern in P_2 , but with the period $d'/2$.

Resolution of the microscope

We see from the above that in order for the microscope to resolve object's details of size d , its aperture size must be at least

$$A = f\lambda / d. \quad (3)$$

In an actual microscope, there is no artificial aperture in the F plane; its role is played by the finite diameter of the objective lens. However, if the object is close to the lens's front focal plane (as in the real microscope) the rays after the lens are almost parallel to the z axis, so a different location of the aperture does not compromise the above argument.

It is sometimes convenient to express the above result in terms of the *angular aperture* θ_{\max} (Fig. 1). In the paraxial approximation, and remembering that the object is close to the lens's focus, we can write $\theta_{\max} \approx A / f$. A microscope with a given angular aperture can resolve objects of size

$$d \geq \lambda / \theta_{\max}.$$

It is clear that the maximum possible value of the angular aperture is on the order of 1, so the resolution limit of any optical microscope is on the order of the wavelength.

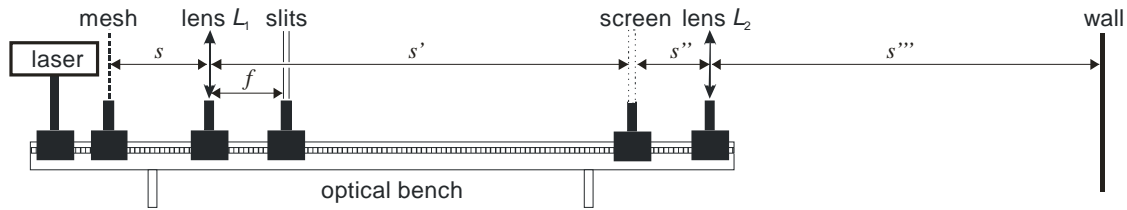


Fig. 2: experimental setup

Experimental setup

We use a periodic square wire mesh as our microscopic object. It can be viewed as two one-dimensional gratings placed perpendicular to each other. The diffraction picture in plane F will form a square pattern with the maxima located at $(x'_m, y'_n) = (fm\lambda / d, fn\lambda / d)$, where m and n denote, respectively, vertical and horizontal diffraction orders. We use a combination of a vertical and horizontal slits to independently block diffraction orders in each dimension. In this way we can, for example, transmit only the zeroth diffraction order with the vertical slit, and have the horizontal slit fully open. The image in the P_2 plane will then show a grating with only horizontal lines present.

The focal length of the objective lens L_1 has to be chosen relatively large, so the diffraction pattern in plane F is clearly visible. This means that the magnification of the microscope is limited and the image in plane P_2 is difficult to see. We address this issue by constructing a second magnification stage of the microscope, placing a second lens L_2 after P_2 and projecting a magnified image of this plane onto the wall (plane P_3). The setup is shown in Fig. 2.

Experimental procedure

1. Measure the focal lengths of both lenses with a precision of better than ± 0.5 cm. Feel free to choose any procedure for this measurement

2. Place the laser at the end of the optical rail. Write down its wavelength. Align the laser beam parallel to the rail. Set the mesh with the largest available period onto the optical rail immediately behind the laser. Place the screen onto the rail, 15-20 cm away from its other end. Place L_1 (it is advisable to use the lens with a larger focal length here) 15-20 cm away from the mesh.
3. Adjust the position of L_1 to generate a clear magnified image of the mesh on the screen. If you have difficulty identifying the image, try to minimize the size of the bright spot from the laser on the screen.
4. Write down the position of the screen. Remove it. Place lens L_2 at the far end of the rail. Adjust its position to produce a clear image of the mesh on the wall (1-2 m away from the rail). The image should consist of bright squares, arranged in a square pattern. You should see sharp borders between bright and dark regions of the image.
5. Measure s, s', s'', s''' . Verify them to be consistent with the focal lengths measured in step 1 (use the thin lens formula). Measure the mesh period in the image. Determine the overall magnification of your microscope by comparing with the period value printed on the mesh. Verify consistency with the values of s, s', s'', s''' .
6. Replace the mesh by the one with the smallest period. Observe the Fraunhofer diffraction pattern in the back focal plane F of L_1 .
7. Place the two slits in plane F . Fully open both of them. Center the slits on the diffraction pattern.
8. Close both slits so that only the $(0, 0)$ diffraction order is transmitted. Observe the image on the wall.
9. Fully open one of the slits so diffraction orders $(0, n)$ are transmitted. Observe the image on the wall. Repeat with the other slit.
10. Open and reposition one of the slits to transmit the zeroth and one of the first diffraction orders (the width of the other slit does not matter). Observe the periodic pattern on the wall. Measure the period and compare it with that measured in step 5. Gradually open the slit and observe the details reappear in the image.
11. Position the vertical slit to transmit the zeroth, one of the first, and one of the second diffraction orders. Block the first diffraction order with the wire supplied. Observe the periodic pattern on the wall. Measure the period and compare it with that measured in step 5.
12. Rotate the mesh by 45° . Open one of the slits, close the other to transmit only diffraction orders (n, n) . Measure the period of the pattern in the image plane.
13. For each mesh available, find the minimum width of one of the slits that (barely) allows observation of some periodic structure in the image. In order to get an accurate measurement, you should adjust not only the width, but also the position of the slit. Be sure to align each mesh parallel to the slit edges. Repeat each measurement several times to estimate the measurement uncertainty.

To be included with your lab write-up

1. Derivation of Eq. (2) and a proof that $\tilde{d}' = d'$.
2. Description of the procedure and the method of estimating the error in measuring the focal lengths of lenses (Procedure step 1).
3. Measurements and calculations for the step 5.
4. Measurements, observations (including image descriptions), and explanations for steps 6-12.
5. Plot of the aperture sizes obtained in step 13 versus the inverse mesh period (as printed on the mesh). Include the uncertainties and a theoretical line according to Eq. (3).