Study of the diffraction in the microscope: Annular condenser

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Abstract. In this work we study the diffraction in the microscope when an annular condenser is used to illuminate the object. We calculate the point spread function (PSF) for a pinhole in an opaque screen illuminated with an annular condenser, consisting in an 1D array of incoherent point sources. We compare it with the PSF for a self-luminous point object, finding that the central disk of the diffraction pattern is narrower and the first intensity minimum is deeper for illuminated objects. We also analyze the resolution of the system by means of the intensity profile produced by two points objects, finding that two self luminous point objects are better resolved than two illuminated objects at the same distance. This suggests that the correlation introduced in the object diminishes the resolution in the former case.

1. Introduction
The effects caused in the diffraction pattern by the use of annular apertures have been extensively studied, because of the higher resolution achieved in the system given the sharpening in the central peak of the diffraction pattern. Annular apertures also causes a higher intensity in the secondary lobes of the diffraction pattern, although it may be reduced using different techniques (e.g. Sheppard & Choudhury 2004). The resolution of the system may also be increased by using an annular condenser. Although this method to increase the resolution is well known, there may be found in the literature very few studies on the subject (e.g. McKechnie 1972 and Nayyar & Verma 1976). In this work we analyze numerically the characteristics of the diffraction pattern of two pinholes illuminated by an annular condenser.

In a previous work (Ciocci, Echarri & Simon 2010), we developed a numerical model to calculate the diffraction pattern produced by a high numerical aperture microscope, considering a self-luminous, unpolarized point object, and taking into account the vectorial nature of light into the Huygens-Fresnel principle. We calculate the diffraction pattern considering the vectorial nature of light, solving the Kirchhoff integral with a method that combines ray tracing with a Monte Carlo routine. The aim of the present work is to study the image formation in the microscope in the case that an annular condenser is used to illuminate the object. To do this, we adapted the previous model to calculate the diffraction pattern for illuminated objects. In section 2 we present the theoretical basis for the simulation of the system, in section 3 we describe our simulations, and in section 4 we analyze our results and present the conclusions.
Figure 1. Action of the optical system on each component of the total electric field projected on a reference sphere in two directions: one normal to the plane defined by the two ray vectors, $\vec{r}$ and $\vec{r}'$, and the other within this plane. The normal component $\vec{E}_\perp$ does not change modulus nor direction when going through the system. The parallel component of the electric vector $\vec{E}_\parallel$ does not change modulus when going through the system.

2. Theoretical background
We developed a method to compute the Kirchhoff diffraction integral taking into account the vectorial nature of light. In this method we use as a reference the plane defined by the object ray $\vec{r}$ and the corresponding image ray $\vec{r}'$, and two reference spheres centered one in the object and the other one in its image (see figure 1). We project the electric field in two directions, one contained in the reference plane ($\vec{E}_\parallel$) and the other in a direction perpendicular to that plane ($\vec{E}_\perp$). The reference plane is characterized by the unit vector normal to its surface, given by

$$\hat{v}_\perp = \frac{1}{|\vec{r} \times \vec{r}'|} \vec{r} \times \vec{r}'$$

and therefore we can obtain the two electric field components, $\vec{E}_\perp$ and $\vec{E}_\parallel$, as

$$\vec{E}_\perp = \vec{E} \cdot \hat{v}_\perp$$

$$\vec{E}_\parallel = \vec{E} - \vec{E}_\perp$$

The effect of the optical system on the electric field depends on the component considered: $\vec{E}_\perp$ does not change its modulus nor its direction (assuming ideal antireflex treatments in every optical surface), while $\vec{E}_\parallel$ does not change its modulus, although its direction changes. In this way compute the electric field for one ray at the position of the geometric image of the object. We calculate the electric field in the entire image plane considering the propagation of a spherical wave in the direction of the image ray.

The illuminating system is an annular condenser, which is modeled as a 1D array of incoherent point sources. We assume that the electric field produced by the condenser has unit modulus and that it has two orthogonal components: one tangent to the annulus of the condenser, and the other one normal to the ray that leaves the condenser and arrives to the object. The object under study is an opaque screen with a pinhole. To compute the diffraction pattern of the object, we first calculate the diffraction integral of the electric field in the object using the method described above. Then we calculate the diffraction integral in the microscope. For this purpose, we assume that a high resolution microscope objective is aberration free, that it does not show birrefringence nor polarization, that it satisfies Abbe sine condition, and that the system has a symmetry axis.
Figure 2. The left image corresponds to the simulated image of a self-luminous point object, calculated using a vectorial diffraction theory. The right image corresponds to a simulation of a single pinhole in an opaque screen illuminated with an annular condenser. In both cases the magnification and the numerical aperture of the optical system were the same (m=100; NA=0.866), and the intensity scale is inverted. Notice that the central disk of the diffraction pattern is narrower and the first intensity minimum is deeper for the illuminated object.

3. Numerical simulations

3.1. Point spread function

The point spread function (hereafter PSF) is the system response to an unitary stimulus. This response completely characterizes the system, and allows to compute the image of any object by means of the superposition integral (e.g. Goodman). Using the method described in the previous section, we compute numerically the PSF of the system by means of the simulation of the image of a single point. This simulation uses a Monte Carlo technique to combine the result of the ray tracing method described above for a large number of randomly generated rays. We compare the results obtained for both self-luminous and illuminated objects.

The PSF of a self-luminous object is presented in Figure 2. To calculate the PSF in the illuminated case, we develop a second Monte Carlo algorithm that chooses points in the annular condenser at random, and computes the rays from these point to the pinhole. The electric field is then diffracted in the pinhole using the method described in section 2. This field constitutes the input of our Monte Carlo ray tracing method described above, which computes the final image through the microscope. We show the image obtained for the illuminated object in Figure 2. We also calculate the intensity profile for both images, which we present in Figure 3.

3.2. Resolution

We studied the resolution of the optical system according to Rayleigh criterion, comparing the intensity profile produced by two pinholes in an opaque screen when they are close to each other.

We obtained the simulated image of two self-luminous point objects as the sum of the intensities for each point, given that they are not correlated (Figure 4). The general procedure to compute the image of two pinholes illuminated with an annular condenser, is similar to that described in section 3.1 for a single pinhole. In this case there is a correlation between the electric field on both pinholes, and therefore for each random point chosen in the condenser, we compute the electric field in the image plane as the sum of the electric field produced by each pinhole. We calculate the intensity distribution for that particular point in the condenser, and then iterate over all the random points in the condenser, adding the corresponding intensities in the image plane. We show the image obtained in this case in Figure 4. We also compute the intensity profile for both images (Figure 5).
Figure 3. Intensity profiles for both self-luminous and illuminated objects. We note that the central disk of the diffraction pattern is narrower and the first intensity minimum is deeper for the illuminated object.

Figure 4. Simulated images of two just resolved point objects for both studied cases, two self-luminous point objects (left image), and two pinholes in an opaque screen illuminated with an annular condenser (right image). In both cases the magnification and the numerical aperture of the optical system were the same (m=100; NA=0.866), and the intensity scale is inverted. Note that the two objects are more separated in the second case, indicating that the resolution is poorer than in the first case.

4. Analysis and conclusions
We studied the effects on the diffraction pattern produced by a microscope when an annular condenser is used to illuminate a point object. In the first place, we computed the PSF for an illuminated single pinhole, and compare it with the PSF for a self-luminous point object, finding that the central disk of the diffraction pattern was narrower and the first intensity minimum was deeper for illuminated objects. These results suggest that the resolution of the system for illuminated objects should be greater than that achieved for self-luminous objects, and that the contrast in the image should be also greater for illuminated objects.

We analyzed the resolution of the optical system according to Raileygh criterion, comparing the intensity profile produced by two points placed close to each other. We considered that two point objects under coherent illumination are resolved if the joint intensity profile shows a central hollow. We found that, contrary to what we expected from the PSFs, for illuminated objects the resolution is worsen (by \(~33\%\) in the case studied) rather than increased. We argue that the decrease in resolution is due to the correlation introduced in the object when they are
Figure 5. Intensity profiles for two just resolved point objects, for both studied cases: self-luminous and illuminated objects. Note that the two objects are more separated in the second case, indicating that the resolution is poorer than in the first case.

illuminated, as no phase shift between the pinholes was considered. Clearly, further studies are needed to understand the origin and nature of this effect.

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