1 Point Spread Function (PSF)

- Consider the set-up of Fig.1. Replace the single dipole emitter by a pair of incoherently radiating dipole emitters separated by a distance $\Delta x = \lambda/2$ along the x-axis. The two dipoles radiate at $\lambda = 500$ nm and they have the same dipole strength. One of the dipoles is oriented transverse to the optical axis, whereas the other dipole is parallel to the optical axis. The two dipoles are scanned in the object plane and for each position of their center coordinate a signal is recorded in the image plane using a NA = 1.4 (n = 1.518), M=100X objective lens.

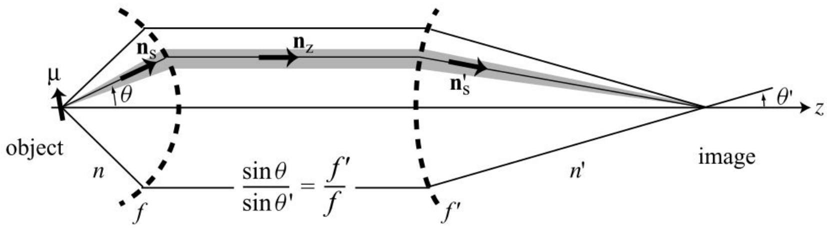
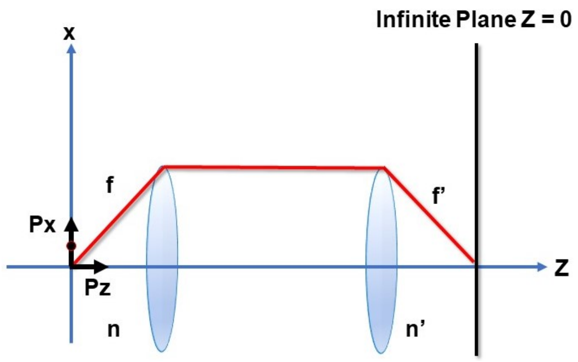


Figure 1: Configuration used for the calculation of the point-spread function. The source is an arbitrarily oriented electric dipole with moment μ. The dipole radiation is collected with a high-NA aplanatic objective lens and focused by a second lens on the image plane at $z = 0$.

(a) Determine the total integrated field intensity (s_1) in the image plane.

Calculating the power without pinhole.



The electric field is given by

$$E(\rho, \phi, z) = \frac{\omega^2}{\epsilon_0 c^2} \overleftrightarrow{G} . \vec{\mu},$$

where

$$\overleftrightarrow{G} \propto \begin{pmatrix} \tilde{I}_{00} + \tilde{I}_{02}\cos 2\phi & \tilde{I}_{02}\sin 2\phi & 2i\tilde{I}_{01}\cos\phi \\ \tilde{I}_{02}\sin 2\phi & \tilde{I}_{00} - \tilde{I}_{02}\cos 2\phi & -2i\tilde{I}_{01}\sin\phi \\ 0 & 0 & 0 \end{pmatrix}.$$

The power can be written as (the two dipoles are incoherent)

$$P \propto \int_0^\infty \rho d\rho \int_0^{2\pi} d\phi |E|^2 = \int_0^\infty \rho d\rho \int_0^{2\pi} d\phi\ (E_x^2 + E_y^2),$$

$$P \propto \int_0^\infty \rho d\rho \int_0^{2\pi} d\phi\ [(I_{00} + I_{02}\cos 2\phi)^2 + (I_{02}\sin 2\phi)^2] + \int_0^\infty \rho d\rho \int_0^{2\pi} d\phi 4 I_{01}^2,$$

where after integration over ϕ, $(\tilde{I}_{00}^2 + \tilde{I}_{02}^2)2\pi$ corresponds to μ_x and $4\tilde{I}_{01}^2 2\pi$ corresponds to μ_z respectively.

We can write $I_1 = \int_0^\infty \rho d\rho \tilde{I}_{00}^2$ as

$$I_1 = \int_0^\infty \rho d\rho \int_0^{\theta_{max}} \sqrt{\cos\theta}\sin\theta(1+\cos\theta) J_0\left[k'\rho\sin\theta(\frac{f}{f'})\right] d\theta$$
$$\times \int_0^{\theta_{max}} \sqrt{\cos\theta'}\sin\theta'(1+\cos\theta') J_0\left[k'\rho\sin\theta'\frac{f}{f'}\right] d\theta'.$$

Consider the closure theorem for Bessel functions,

$$\int x dx\ J_n(abx)\ J_m(a'bx) = \frac{\delta_{nm}\delta(a-a')}{ab^2}.$$

By using the closure relation, we can write I_1 as follows

$$I_1 = \int_0^{\theta_{max}} d\theta \cos\theta \sin^2\theta (1+\cos\theta)^2 \frac{1}{(k')^2(\frac{f}{f'})^2 \sin\theta}.$$

Now we can write $I_2 = \int_0^\infty \rho d\rho \tilde{I}_{02}^2$ as

$$I_2 = \int_0^\infty \rho d\rho \int_0^{\theta_{max}} \sqrt{\cos\theta}\sin\theta(1-\cos\theta) J_2\left[k'\rho\sin\theta(\frac{f}{f'})\right] d\theta$$
$$\times \int_0^{\theta_{max}} \sqrt{\cos\theta'}\sin\theta'(1-\cos\theta') J_2\left[k'\rho\sin\theta'\frac{f}{f'}\right] d\theta'.$$

By using the closure relation we can write I_2 as follows

$$I_2 = \int_0^{\theta_{max}} d\theta \cos\theta \sin^2\theta (1-\cos\theta)^2 \frac{1}{(k')^2(\frac{f}{f'})^2 \sin\theta}.$$

Now, for the μ_x dipole we get

$$\begin{aligned} I_1 + I_2 &\propto \int_0^{\theta_{max}} d\theta \cos\theta \sin\theta[(1-\cos\theta)^2 + (1+\cos\theta)^2], \\ &= 2\int_0^{\theta_{max}} d\theta \cos\theta \sin\theta(1+\cos\theta^2). \end{aligned}$$

Using $x = \cos\theta$, $dx = -\sin\theta d\theta$, where $\theta = 0, x = 1$, we get

$$\begin{aligned} I_1 + I_2 &\propto 2\int_{\cos\theta_{max}}^1 dx x(1+x^2) = [x^2]^1_{\cos\theta_{max}} + [\frac{2}{4}x^4]^1_{\cos\theta_{max}}, \\ &= [1-\cos^2\theta_{max}] + \frac{1}{2}[1-\cos^4\theta_{max}], \\ &= [\sin^2\theta_{max}] + \frac{1}{2}[1+\cos^2\theta_{max}][1-\cos^2\theta_{max}]. \end{aligned}$$

Considering $NA = \sin\theta_{max}$, we get

$$I_1 + I_2 \propto [NA^2] + \frac{1}{2}[2 - NA^2][NA^2].$$

Therefore, for the μ_x dipole we get the simplified equation

$$I_1 + I_2 = \frac{[NA^2]}{(k')^2(\frac{f}{f'})^2}\left[1 - \frac{NA^2}{2}\right].$$

For the μ_z dipole we get $I_3 = 4\int_0^\infty \rho d\rho \tilde{I}_{01}^2$,

$$\begin{aligned} I_3 = &\int_0^\infty \rho d\rho \int_0^{\theta_{max}} d\theta \sqrt{\cos\theta}\sin\theta(1+\cos\theta)J_1\left[k'\rho\sin\theta(\frac{f}{f'})\right] \\ &\times \int_0^{\theta_{max}} \sqrt{\cos\theta'}\sin\theta'(1+\cos\theta')J_1\left[k'\rho\sin\theta'\frac{f}{f'}\right]d\theta', \end{aligned}$$

By using the closure relation we can write I_3 as follows

$$I_3 = \int_0^{\theta_{max}} d\theta \ \cos\theta \ \sin^4\theta \frac{4}{(k')^2(\frac{f}{f'})^2\sin\theta} = \int_0^{\theta_{max}} d\theta \ \cos\theta \ \sin\theta(1-\cos^2\theta)\frac{4}{(k')^2(\frac{f}{f'})^2}.$$

Considering $x = \cos\theta$, $dx = -\sin\theta d\theta$, where $\theta = 0, x = 1$, we can write

$$\begin{aligned} I_3 &\propto \int_{\cos\theta_{max}}^1 dx \ x(1-x^2) = \left[\frac{x^2}{2}\right]^1_{\cos\theta} - \left[\frac{x^4}{4}\right]^1_{\cos\theta}, \\ &= \frac{1}{2}(1-\cos^2\theta) - \frac{1}{4}(1-\cos^4\theta). \end{aligned}$$

Considering $NA = \sin\theta_{max}$, we get

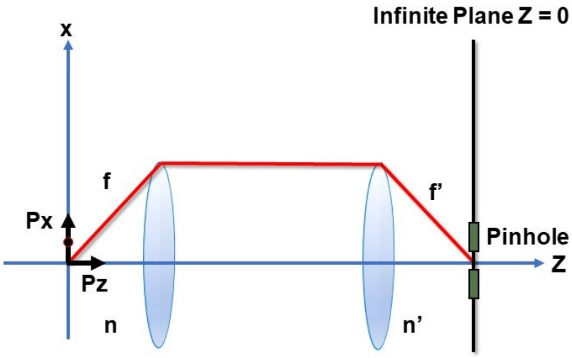
$$I_3 \propto \frac{NA^2}{2} - \frac{1}{4}\left[NA^2(2-NA^2)\right],$$

and finally

$$I_3 = NA^4 \times \frac{1}{(k')^2(\frac{f}{f'})^2}.$$

Using the magnification $M = \frac{f'}{f}$, the total power can be written as $P = \left[NA^2 + \frac{NA^4}{2}\right] M^2$, but since $\overleftrightarrow{G} \propto \frac{f}{f'}$, the power is $P = \left[NA^2 + \frac{NA^4}{2}\right]$ (independent of the magnification).

(b)Calculate and plot the recorded image (s_2) if a confocal detector is used. Use the paraxial approximation.



Calculating the power with pinhole.

In this situation we cannot use the closure relation because of the pinhole. Assume that the pinhole area is very small. Hence the formula for the power can be written as

$$P = \int_0^{\Delta\rho} \rho \, d\rho[\tilde{I}_{00}^2 + \tilde{I}_{02}^2]2\pi + 4\int_0^{\Delta\rho} \rho \, d\rho \tilde{I}_{01}^2 2\pi.$$

Consider the asymptotic behavior of Bessel functions

$$J_0\left[k'\rho\sin\theta'\frac{f}{f'}\right] \approx 1,$$

$$J_1\left[k'\rho\sin\theta'\frac{f}{f'}\right] \approx \frac{k'\rho\sin\theta'\frac{f}{f'}}{2},$$

$$J_2\left[k'\rho\sin\theta'\frac{f}{f'}\right] \approx \frac{k'\rho\sin\theta'(\frac{f}{f'})^2}{8}.$$

Then we have,

$$I_{00} = \int_0^{\theta_{max}} \sqrt{\cos\theta}\sin\theta(1+\cos\theta)d\theta.$$

Considering $x = \cos\theta$, $dx = -\sin\theta d\theta$, where $\theta = 0, x = 1$, we can write

$$I_{00} = \int_{\cos\theta_{max}}^{1} dx\ (1+x)\sqrt{x}.$$

Similarly for I_{01} we obtain

$$I_{01} = \int_{0}^{\theta_{max}} \sqrt{\cos\theta}\sin^2\theta \frac{k'\rho\sin\theta}{2} d\theta,$$

$$I_{01} = \int_{\cos\theta_{max}}^{1} dx\ (1-x^2)\sqrt{x}\ \frac{k'\rho f}{2f'}.$$

Similarly for I_{02} we obtain

$$I_{02} = \int_{0}^{\theta_{max}} \sqrt{\cos\theta}\sin\theta(1-\cos\theta)\frac{(k')^2(\rho)^2\sin^2\theta\ (\frac{f}{f'})^2}{8} d\theta,$$

$$I_{02} = \int_{\cos\theta}^{1} dx\ (1-x)\sqrt{x}(1-x^2)\ \frac{(k')^2(\rho)^2 f^2}{8(f')^2}$$

If you consider the diffraction integrals as a function of numerical aperture, NA, then you can write I_{01} and I_{02} as follows:

$$I_{00} = f_{00}(NA),$$

$$I_{01} = f_{01}(NA)k'\rho\frac{1}{M},$$

$$I_{02} = f_{02}(NA)(k')^2\ (\rho)^2\frac{1}{8M^2}.$$

Thus the power can be written in terms of I_{00}, I_{01} and I_{02} as

$$P = 2\pi\int_0^{\Delta\rho} \rho d\rho\{f_{00}^2 + \frac{f_{02}^2(k')^4\rho^4}{64M^4}\} + 8\pi\int_0^{\Delta\rho} \rho d\rho f_{01}^2\frac{(k')^2\rho^2}{M^2}$$

For μ_x we can write:

$$2\pi\int_0^{\Delta\rho} \rho d\rho = \pi(\Delta\rho)^2 = \delta A,$$

where δA is the pinhole area. Similarly for μ_z

$$8\pi\int_0^{\Delta\rho} \rho d\rho\frac{(k')^2(\rho)^2}{M^2} = 2\pi\frac{(k')^2\ (\Delta\rho)^4}{M^2} = \frac{2(k')^2}{\pi M^2}[\delta A]^2.$$

Hence the power can be simply expressed as follows:

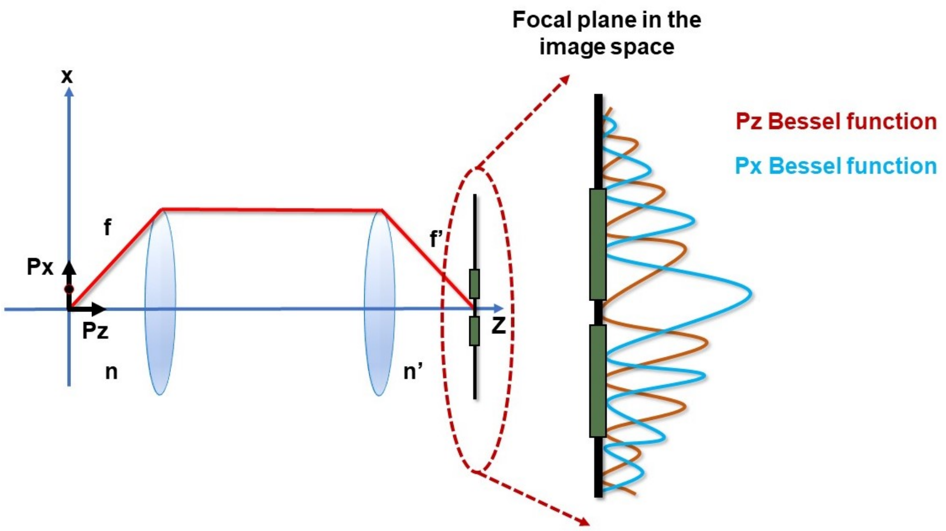
$$P = \frac{1}{M^2}\left[f_{00}^2 NA\,(\delta A) + f_{01}^2\; NA\,\frac{2(k')^2}{\pi M^2}(\delta A)^2\right],$$

where one can notice that now the power is also a function of the magnification, as intuitively expected.

Also, in the case of with and without the pinhole we have assumed that the there is no displacement between the two dipoles.

(c) Discuss what happens in (a) and (b) if the dipoles are scanned at a constant height $\Delta x = \lambda/4$ above the image plane.

In the case of (a), without pinhole the power was independent of the position, but, in the case of (b), with pinhole, the power is dependent on the position.



If we compare the μ_x Bessel function and μ_z Bessel function on the focal plane, we can see that μ_z forms a doughnut shape where as μ_x has an Airy pattern. This is the case of two dipoles aligned in same plane. If the two dipoles are shifted, the corresponding PSF will also shift. The distance between the maxima of μ_x and secondary maxima of μ_z corresponds to Δ.

In the object space Δ can be expressed as $\Delta = \frac{\lambda}{2}$, whereas in the Image space Δ can be expressed as $\Delta = M\frac{\lambda}{2}$

Thus if we move one dipole, the transmission will change accordingly due to the presence of the pinhole. For example, in the confocal detection, since we are not imaging the PSF it is possible to happen that if we have a second emitter, which is shifted, then the intensity of the shifted emitter becomes comparable to the first one even if they are having different orientations. So, this can make difficulty in interpreting the data. This problem can be improved by confocal microscopy. In confocal microscopy, it will be possible to suppress the excitation from the shifted dipole.

- A continuously fluorescing molecule is located at the focus of a high NA objective lens. The fluorescence is imaged onto the image plane as described in section related to the PSF. Although the molecule's position is fixed (no translational diffusion) it is rotating in all three dimensions (rotational diffusion) with high speed. Calculate and plot the averaged field distribution in the image plane using the paraxial approximation.

The paraxial point spread function (PSF) for a molecule oriented along x or y is

$$\lim_{\theta_{max} \ll \pi/2} |\mathbf{E}(x, y, z = 0)|^2 = \frac{\pi^4}{\epsilon_0^2 n n'} \frac{\mu_x^2 + \mu_y^2}{\lambda^6} \frac{\mathrm{NA}^4}{M^2} \left[2 \frac{J_1(2\pi\tilde{\rho})}{2\pi\tilde{\rho}} \right].$$

Here, $\tilde{\rho} = \frac{\mathrm{NA}\rho}{M\lambda}$ and $|E_x|^2 = |E_y|^2$.

For a molecule oriented along z

$$\mathbf{E}(\rho, \phi, z) = \frac{w^2}{\epsilon_0 c^2} \overleftrightarrow{G}_{PSF}(\rho, \phi, z) \cdot \mu_z,$$

where

$$\overleftrightarrow{G}_{PSF}(\rho, \phi, z) = \frac{k'}{2\pi i} \frac{f}{f'} e^{i(kf - k'f')} \times \begin{pmatrix} \tilde{I}_{00} + \tilde{I}_{02} \cos 2\phi & \tilde{I}_{02} \sin 2\phi & -2i\tilde{I}_{01} \cos\phi \\ \tilde{I}_{02} \sin 2\phi & \tilde{I}_{00} - \tilde{I}_{02} \cos 2\phi & -2i\tilde{I}_{01} \sin\phi \\ 0 & 0 & 0 \end{pmatrix} \sqrt{\frac{n}{n'}}.$$

Hence,

$$\mathbf{E}^z(\rho, \phi, z) = \frac{w^2}{\epsilon_0 c^2} \frac{k'}{8\pi i} \frac{f}{f'} e^{i(kf - k'f')} \sqrt{\frac{n}{n'}} \begin{pmatrix} \cos\phi \\ \sin\phi \\ 0 \end{pmatrix} (-2i\tilde{I}_{01}),$$

where

$$\tilde{I}_{01} \int_0^{\theta_{max}} \sqrt{\cos\theta} \sin^2\theta J_1(k'\rho \sin\theta \frac{f}{f'}) e^{ik'z(1 - \frac{1}{2}(\frac{f}{f'})^2 \sin^2\theta)},$$

with $M = \frac{n}{n'} \frac{f'}{f}$.

In the paraxial approximation, $\cos\theta \approx 1$ and $\sin\theta \approx \theta$, so that

$$\tilde{I}_{01} \simeq \int_0^{\theta_{max}} \theta^2 J_1(k'\rho\theta \frac{f}{f'}) \mathrm{d}\theta$$

because $z = 0$. Next, we use the fact that

$$\left(\frac{1}{z} \frac{d}{dz} \right)^m \left(z^{n+1} J_n(z) \right) = z^{n-m+1} J_{n+m}(z).$$

In our case $n = m = 1$, hence

$$\left(\frac{1}{z}\frac{d}{dz}\right)\left(z^2 J_1(z)\right) = zJ_2(z),$$
$$\int z^2 J_1(z) = z^2 J_2(z).$$

If $x = k'\rho\ (f/f')\theta$ and $dx = k'\rho\ (f/f')d\theta$

$$\begin{aligned}\tilde{I}_{01} &= \left(\frac{f'}{f}\frac{1}{k'\rho}\right)^3 \int_0^{x_{max}} x^2 J_1(x)\mathrm{d}x,\\ &= \left(\frac{f'}{f}\frac{1}{k'\rho}\right)^3 x_{max}^2 J_2(x_{max}),\\ &= \frac{f'}{f}\frac{1}{k'\rho}\theta_{max}^2 J_2(k'\rho\frac{f}{f'}\theta_{max}).\end{aligned}$$

Writing $k'\rho\frac{f}{f'}\theta_{max} = 2\pi\tilde{\rho}$, we get

$$\tilde{I}_{01} = \theta_{max}^3\left(\frac{J_2(2\pi\tilde{\rho})}{2\pi\tilde{\rho}}\right),$$

and

$$\mathbf{E}^z(\rho,\phi,z) = \frac{w^2}{\epsilon_0 c^2}\frac{k'}{4\pi i}\frac{f}{f'}e^{i(kf-k'f')}\sqrt{\frac{n}{n'}}\theta_{max}^3\frac{J_2(2\pi\tilde{\rho})}{2\pi\tilde{\rho}}\begin{pmatrix}\cos\phi\\ \sin\phi\\ 0\end{pmatrix}\cdot\mu_z.$$

. Using $(w/c)^2 = (k/n)^2 = (2\pi/\lambda)^2$, $k' = 2\pi n'/\lambda$ and $\theta_{max}^3 = \mathrm{NA}^3/n^3$, we obtain

$$\mathbf{E}^z(\rho,\phi,0) = -\frac{1}{\epsilon_0}\frac{\pi}{\lambda^2}\frac{2\pi n'}{\lambda}\frac{f}{f'}e^{i(kf-k'f')}\sqrt{\frac{n}{n'}}\frac{\mathrm{NA}^3}{n^3}\frac{J_2(2\pi\tilde{\rho})}{2\pi\tilde{\rho}}\begin{pmatrix}\cos\phi\\ \sin\phi\\ 0\end{pmatrix}\cdot\mu_z,$$

$$|\mathbf{E}^z|^2 = \frac{1}{\epsilon_0^2}\frac{4\pi^4}{\lambda^6}\frac{\mathrm{NA}^6}{M^2}\frac{1}{n'n^3}\left[\frac{J_2(2\pi\tilde{\rho})}{2\pi\tilde{\rho}}\right]^2\mu_z^2.$$

The PSF is the sum of the PSF for all 3 components, i.e.,

$$\mathrm{PSF} = |\mathbf{E}^x|^2 + |\mathbf{E}^y|^2 + |\mathbf{E}^z|^2.$$

We remark that the PSF associated with the z orientation exhibits a doughnut shape and it is proportional to NA^6 instead of NA^4.

2 References

1. Principles of Nano-Optics (Second edition) by Lukas Novotny