Exercise 05

1 Fluorescence microscopy

1.1 Wide-field Fluorescence Microscopy

Solve questions (a-f) for the wide-field microscope sketched in Figure 1 using the following assumptions:

- Geometrical optics principles are valid (i.e. no diffraction)
- Solve the problem in 3 dimensions (x, y, z) by assuming circular symmetry
- For simplicity: filters are not shown, but assume the camera only detects fluorescence
- The two dots are assumed to be two idealized fluorophores where we assume $I_{\text{em}} = I_{\text{ex}}$
- For simplicity assume the fluorophores to be disks of radius r_p instead of spheres.
- $f = 20\,\text{mm}$, $a = 30\,\text{mm}$, $P_{\text{ill}} = 1\,\text{W}$, $d = 1\,\mu\text{m}$, $r_p = 20\,\text{nm}$

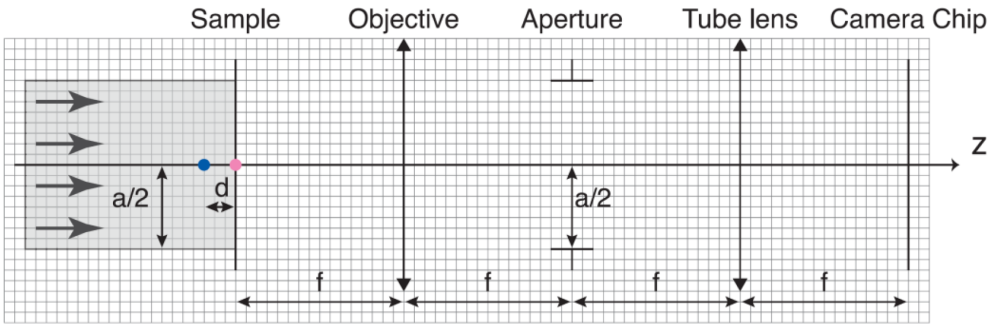

Figure 1: Schematic of a wide-field microscope with two fluorescent molecules (one in focus and one out-of focus).

(a) Please trace the marginal rays for the two point sources (one in focus, and one at a distance d from the focus plane).

(b) What is the NA of this system?

(c) Assume that the total laser illumination is I_{ill} and that your fluorophores have a radius of r_p. How much light power is exciting the two fluorophores? (Neglect "shadowing effects" by the particle in front.)

(d) For the fluorophore in focus, assume it to be a point light source emitting in all directions. What is the percentage of light that is collected by this microscope? Express the percentage as a function of the aperture opening, a.
Hint: Area of a spherical cap of a sphere is $A_{\text{cap}} = 2\pi r^2\,(1 - cos\,(\alpha))$.

(e) What is the image size on the camera of the point source that is out-of-focus by a distance d? Express the image size as a function of d and a.

(f) What is the percentage of the light from the point source that is out-of focus by a distance d that is collected on the camera? (Assume the camera chip size to be of infinite size.)

1.2 Confocal Fluorescence Microscopy

Solve questions (a-d) for the confocal microscope sketched in Figure 2 using the following assumptions:

- Geometrical optics principles are valid (i.e. no diffraction)
- Solve the problem in 3 dimensions (x, y, z) by assuming circular symmetry
- For simplicity: filters are not shown, but assume the camera only detects fluorescence
- The two dots are assumed to be two idealized fluorophores where we assume $I_{\text{em}} = I_{\text{ex}}$

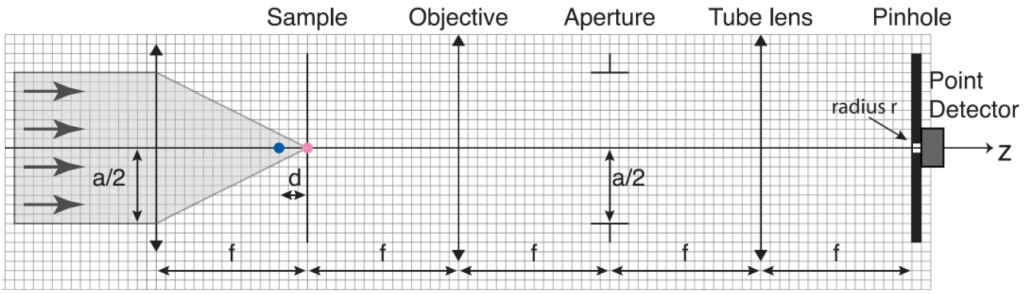

Figure 2: Schematic of a confocal microscope with two fluorescent molecules (one in focus and one out-of focus).

- For simplicity assume the fluorophores to be disks of radius r_p instead of spheres.
- $f = 20\,\text{mm}$, $a = 30\,\text{mm}$, $P_{\text{ill}} = 1\,\text{W}$, $d = 1\,\mu\text{m}$, $r_p = 20\,\text{nm}$, $r = 250$ nm.

(a) Please trace the marginal rays for the two point sources (one in focus, and one at a distance d from the focus plane).

(b) How much light power is exciting the two point sources in the confocal case? Assume again a total laser power of I_{ill} and a radius of r_p for the sources (and neglect shading effects). Give your results as a function of the out-of focus distance d and the diameter a of the laser beam.

(c) Behind the pinhole (of radius r) there is a detector. Please calculate how much light power is detected from the fluorophore in focus and out-of focus. Express the power as a function of P_{ill}, a, r_p, d and r. Determine the detection power for a given pinhole radius.
Hint: We assume a spherical emission from the fluorophore. The microscope can only get a part of this emission depending on its numerical aperture.

(d) Compare the results obtained in (c) to those in exercise 1(d) and 1(f). Comment on the optical sectioning property of the confocal microscope.