2.3 Microscopy: Exercises

Lecture 9+10: More Image formation & Super Resolution

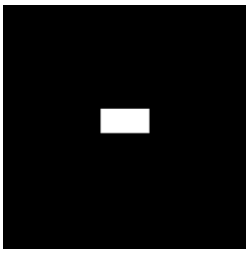
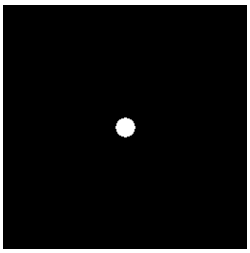
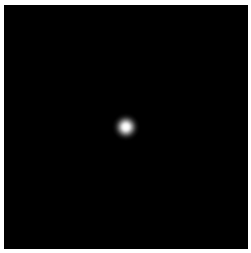
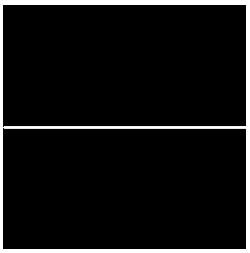
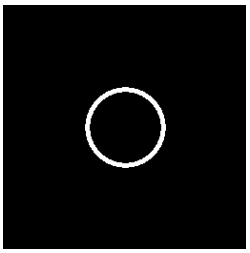
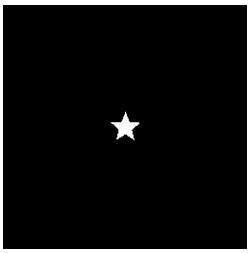
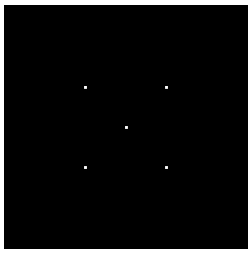
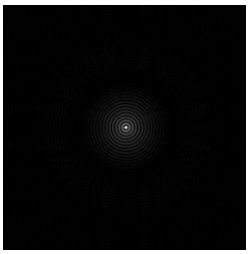
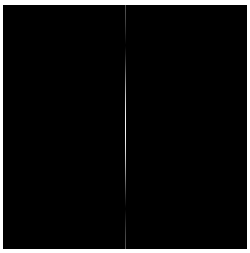
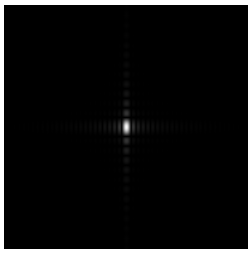
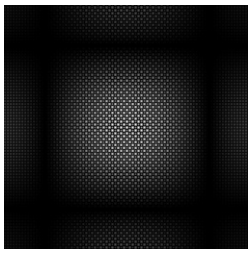
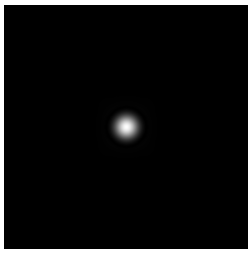
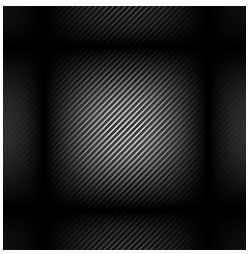
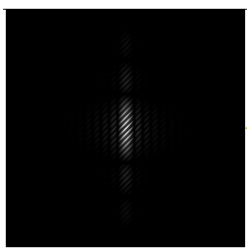
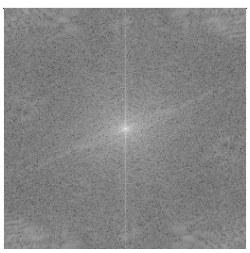
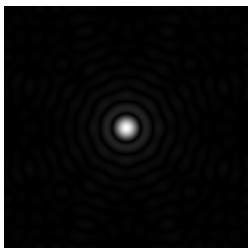
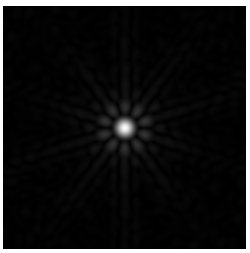
Christian Eggeling, Kai Wicker and Patrick Then

12.06.2018

1. Fourier Memory

Find the corresponding Fourier transform for each picture!

(All Fourier transforms below are showing the square of the absolute of the FT, scaled for better visibility, with picture h additionally using a logarithmic scale instead of a linear one)

 1.	 2.	 3.	 4.	 5.
 6.	 7.	 8.	 9.	 10.
 a	 b	 c	 d	 e
 f	 g	 h	 i	 k

2. Super resolution with dSTORM

A biological sample was stained with an organic dye (quantum yield=50%, absorption cross section $\sigma=5*10^{-16}$ cm²) and is observed with an epifluorescence microscope.
The objective (63x) has a numerical aperture of NA=1.4 (oil immersion). A laser (λ=650nm, P=100mW) is used to illuminate the sample. The total transmission of the system from laser to sample is 70%.
Using an illumination tube lens focused onto the back focal plane of the objective, the sample is illuminated with a collimated beam in a 50 µm wide area (for sake of simplicity, assume an equal intensity distribution).
Accounting for transmission losses (from lenses and filters), photon collection efficiency (over the solid angle of the objective lens) and quantum efficiency of the camera, about 15% of the emitted photons can be detected.
The microscope can be used to image the sample in widefield and (d)STORM mode.

Calculate the maximum achievable resolution in

a) Normal wide field mode

b) dSTORM mode (assuming 20 ms exposure per frame)

What do you expect in the two modes when imaging microtubuli or mitochondria?

Some hints and useful formulas:

For (d)STORM, the acchievable resolution (or localization precision) d can be approximated by

$$d=\frac{\Delta x}{\sqrt{N}}$$

where Δx is the resolution in normal epifluorescence mode and N the number of detected photons per localization event (i.e. the number of photons detected from one molecule in one image).
Calculate the number of photons emitted by the dye using the given values:
Start by calculating the power density on the sample and remember the calculations from homework assignement 3 (How much power is required to excite a fluorophore at a given rate k).
Don’t forget to take quantum yield and total detection efficiency of the setup into consideration.