2.3 Microscopy: Exercises

Lecture 6&7: (In-)Coherent Imaging and Confocal Microscopy

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15.05.2018

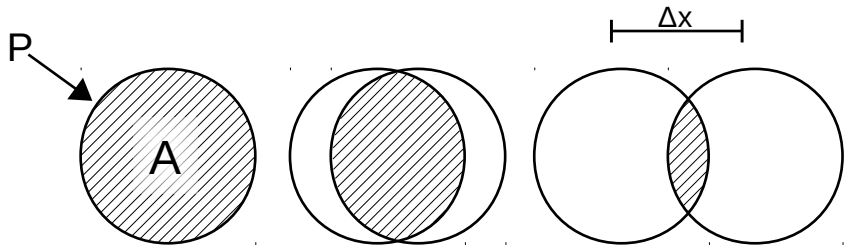
1. (In-Coherent) Imaging

The normalized transfer function $H(f_x,f_y)$ of an optical system links the frequency spectra of incoming and outgoing intensity distributions as

$$G_{out}(f_x,f_y)=H(f_x,f_y)G_{in}(f_x,f_y)$$

In an incoherent imaging system, $H(f_x,f_y)$ is known as the optical transfer function (OTF). Assuming that the system is aberration-free and diffraction limited, the OTF can be obtained by calculating the normalized overlap of the pupils for different offset, i.e.:

$$H(f_x,f_y)= \textit{area of overlap / total area}$$



Calculate the OTF for such a system with circular pupils!

2. Confocal Microscopy

A confocal laser scanning microscope is used to image a sample stained with a fluorescent dye (Alexa Fluor 488, excitation maximum 490 nm, emission maximum 525 nm). The microscope is equipped with an oil immersion objective (M=100x, NA=1.4, n_{oil}=1.518). A laser @488 nm is used for excitation of the sample.

a) Assuming the theoretical case of an infinitely small pinhole, what is the maximum achievable resolution as compared to a widefield microscope?

b) What is a more realistic assumption for the pinhole and what is the „real“ advantage of the confocal microscope?