Microscopy
Problem Set 5

July 3, 2019

Please hand in the solutions of this problem set next seminar on July 9 of 2019.

12 Resolution

a) What is resolution? Explain with your own words using your knowledge of diffraction.

b) Derive the Abbe formula. Explain each variable you are using.

13 Stimulated emission depletion (STED) microscopy

a) Draw a basic STED setup labeling each component.

b) Explain how the point spread function is created in STED to obtain sub diffraction imaging.

c) Write down how the Abbe formula is modified. Explain with a graph the dependence of the resolution on the intracavity intensity and the saturation intensity.

d) What is limiting the resolution in this technique?

14 Single molecule localization microscopy

a) Which is the difference between PALM and STORM?

b) Which is the difference between STORM and dSTORM?

15 Achieving super resolution with dSTORM

A biological sample was stained with an organic dye (the quantum yield is = 50% and the absorption cross section, $\sigma = 5 \times 10^{-16}\,\mathrm{cm}^2$) and is observed with an epifluorescence microscope. The objective ($63X$) has a numerical aperture of $NA = 1.4$ (oil immersion). A laser ($\lambda = 650\,\mathrm{nm}$, $P = 100\,\mathrm{mW}$ 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 $50\,\mu\mathrm{m}$ 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 widefield mode

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

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

Some hints and useful formulas:

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

$$d = \frac{\Delta x}{\sqrt{N}} \tag{1}$$

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 Exercise 8 (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.