Module 2: Superresolution

HW Problem Set

1. The Rayleigh criteria is given as: $\theta_{min} = 1.22\frac{\lambda}{D}$, where θ_{min} is the smallest resolvable angle, λ is the wavelength of light used and D is the diameter of the aperture or lens used. On the other hand, the Abbe limit is given as: $d = \frac{\lambda}{2\,n\sin(\alpha)} = \frac{\lambda}{2\,\mathrm{NA}}$, where d is the smallest resolvable angle, n is the refractive index of the medium between the object and the optical system, α is the biggest scattering angle (incident on the optical system) and NA is the numerical aperture. Since superresolution microscopy involves both, how do you relate them?

2. Calculate the magnification of an object placed 6.20 mm from a compound microscope that has a 6.00 mm focal length objective and a 50.0 mm focal length eyepiece. The objective and eyepiece are separated by 23.0 cm.

3. Consider the STED principle from one of your reading materials.

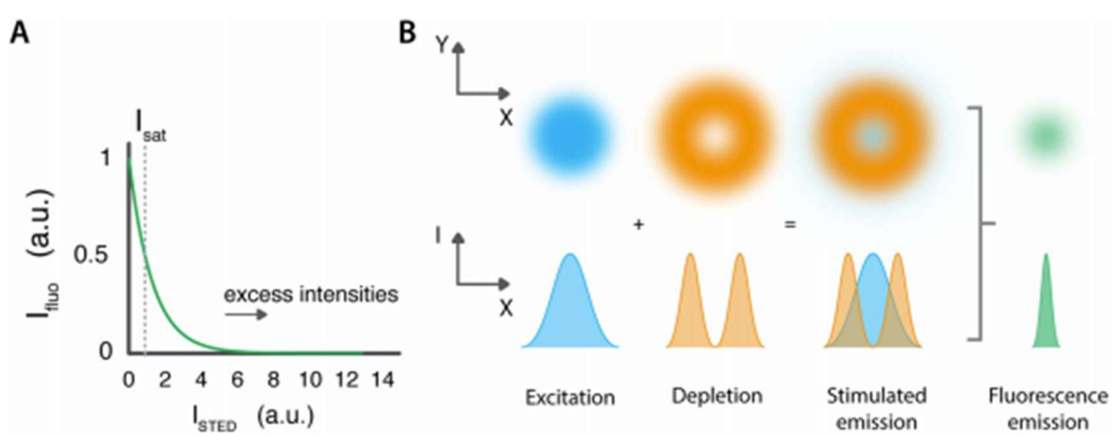

Figure 35. The STED principle. (A) The ability of a fluorophore to emit fluorescence decreases nearly exponentially with the intensity of the beam de-exciting the fluorophore by stimulated emission. I_{sat} is defined as the intensity at which the fluorescence signal is reduced by 50%. (B) The confocal excitation beam is approximately Gaussian in shape while the depletion beam is donut shaped, i.e. it features a central region of (near) zero intensity. After stimulated emission of fluorophores in the donut region, spontaneous emission from the center most molecules can be recorded. Modified from Göttfert *et al* [70].

In STED, the achievable resolution is strongly tied to the efficiency of the stimulated emission process, which in turn is directly proportional to the depletion beam intensity. Moreover, a high-intensity beam results in a steeper intensity gradient between the central zero and donut crest, reducing the diameter of the zero-intensity region from which fluorescence is ultimately collected. STED resolution and its relation to the depletion intensity can elegantly be expressed as a modified version of Abbe's formula:

$$d = \frac{\lambda}{2 \cdot NA \cdot \sqrt{1 + \frac{I}{I_{sat}}}}$$

where, I is the applied STED intensity and I_{sat} is the saturation intensity of the fluorophore, with *d* a measure for lateral resolution. Based on this expression, comment on the resolution of a STEAD microscope.