Question Set 2

Optics on the small scale

It is necessary to read and understand the papers. The lecture handouts are also helpful to understand what is important in the course. Note that the questions may require you to go beyond the papers specified here in order to find your answers.

For generalized optics see for example:

https://www.microscopyu.com/

http://micro.magnet.fsu.edu/

Questions for Review paper (Moerner)

Most of the questions can be answered by reading the paper[1], but it might be necessary to check the web as well.

1. How large is a typical fluorescent single molecule? E.g. fluorescein or GFP?
2. What are the benefits of single-molecule measurements over bulk measurements?
3. How are single-molecule measurements implemented? What are the most common single-molecule techniques used to make it possible to see single molecules? What do I need to consider in order to succeed in detecting single molecules?
4. How much light can I get from one single molecule? At what rate? What is the maximum total number of photons that I can get from one single fluorescent molecule?
5. (**) What is extinction coefficient and absorption cross-section? How can the absorption cross section be calculated from the extinction coefficient?
6. What is blinking? What is the physical basis for blinking?
7. What is bleaching? What is the physical basis for bleaching? What can you do to avoid bleaching?
8. In a typical experiment I would see spots in the field of view of my CCD camera. How do I know that I am looking at a single molecule?

9. What is FRET? What length scales can I probe using FRET? What are the limitations?

Questions for photoacoustic microscopy

Most of the questions can be answered by reading the paper[2], but it might be necessary to check the web as well.

1. Describe the key concept of the technique of photoacoustic microscopy! What property of the sample defines the contrast in the resulting image?

Questions on super resolution microscopy

Most of the questions can be answered by reading the review paper[4], but it might be necessary to check references that the papers does to the literature as well as the web.

1. What is the diffraction limit? What is the Rayleigh criterion? Make a drawing!
2. Can you localize the position of a point-source of light without violating the diffraction limit? Elaborate! What limits the level of uncertainty that you can reach? In practice how low uncertainty can you reach? What is FIONA?
3. Describe the basic idea of STORM (aka FPALM). How is it performed in practice? Are there any special requirements on the dyes used?
4. What resolution is it possible to reach using STORM? What the limits the attainable resolution?
5. How long time does it take to acquire data of a typical object?
6. How can 3D information be obtained in STORM?
7. How does STED work? What is the key idea? Does it need a pulsed laser?
8. What resolution is attainable in STED? What limits the resolution?
9. How well is the axial (along optical axis) resolution improved by STED? How can the situation be improved?
10. How are STED, GSD, RESOLFT related? What is the main fundamental difference between the techniques? What is the main technological difference between the techniques?
11. How long time does it take to acquire a typical image? In which ways has the frame rate been improved since the first STED experiments? Consider also other techniques that are related to STED.

References

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4. Huang, B., M. Bates, and X. Zhuang, *Super-Resolution Fluorescence Microscopy*. Annual Review of Biochemistry, 2009. **78**(1): p. 993-1016.
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