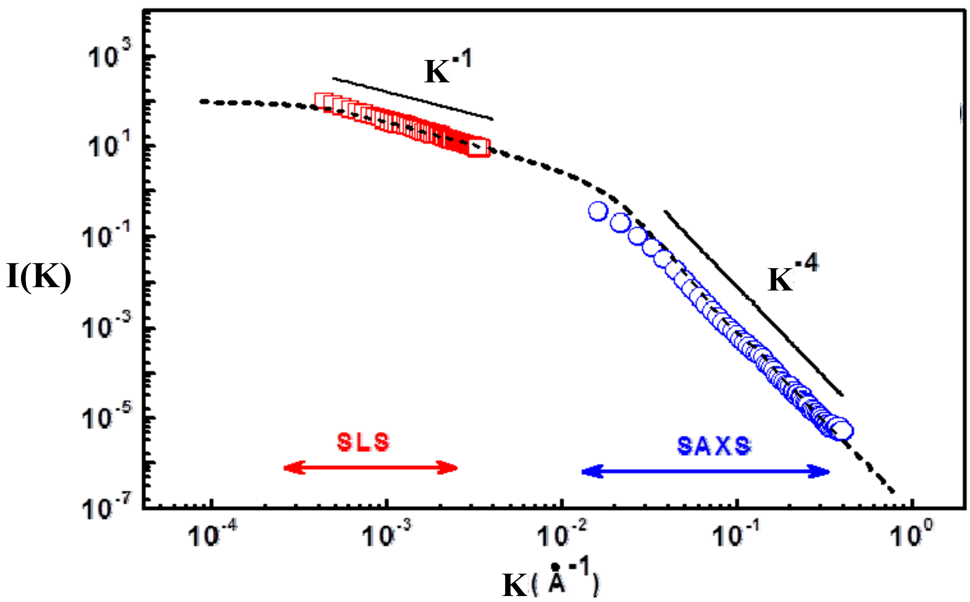
Licht, x-ray en neutronenverstrooiing

January 2015

Please use separate pieces of paper for OM, EM, and Scattering.
Don't forget to write down your name on every piece of paper!
You can write your answers in Dutch or English.

1. What are advantages and disadvantages of small-angle neutron scattering (SANS) in comparison to
    a. small-angle x-ray scattering (SAXS)
    b. static light scattering (SLS)

2. The graph below presents results of static light scattering (SLS) and small-angle x-ray scattering (SAXS) measurements in a suspension of clay particles.



a) The SAXS data is measured for $0.015 < K < 0.3$ Å^{-1}. What was the minimum θ_{min} and the maximum θ_{max} scattering angle in these measurements if the x-ray wavelength was $\lambda = 1$ Å?

b) The largest K value in the SLS data is about K=0.003 Å^{-1} and it is measured using a HeNe laser (wavelength $\lambda = 6328$ Å). Argue that the average refractive index of the sample is larger than 1.5.

c) On the basis of the presented results, what can you say about the shape of the clay particles? Motivate your answer!

d) Can one determine the radius of gyration of the particles using these experimental data? If yes, how? If not, why?

ASSM 2014-2015 - Exam Question EM

a. Using a formula, explain that the resolution of electron microscopes improves with higher acceleration voltages.

b. Why are HAADF-STEM images better suited for quantitative analysis than BF-TEM images?

c. Describe how the electron diffraction pattern looks like of (i) an amorphous material, (ii) a single crystal, and (iii) a polycrystalline material consisting of many crystallites.

d. Give the full name of the acronyms EDS and EELS. Mention one disadvantage of EDS, and one disadvantage of EELS.

e. A high-energy electron from the electron beam interacts with the sample material. As a result of this event, the electron from the beam loses energy and an X-ray is emitted. Explain that the energy loss of the beam electron is larger than the energy of the X-ray. In your explanation, consider the electronic energy levels of the atom in the sample.

Exam Questions ASSM Optical Microscopy

1) Wide-field Fluorescence Microscopy

(a) In class, I have stressed that (almost) any microscope has three major components; what are these major components in a wide-field fluorescence microscope? (do not be too general, focus on what is most important / needed specially for this type of microscope!)

(b) We have encountered the concept of "resolution limit" and found two – a theoretical and a technical – such limits. Define the two limits and relate them to the three major components mentioned above. Give a formula for the limits.

(c) You want to resolve structures with a typical size of 250 nm in your sample, using a wide-field microscope with a 40×/0.9 objective and a camera with a pixel size of 3.45 µm. Is this microscope capable of resolving these structures? If not, which resolution limit has to be improved, and how could you do this?

2) Fluorescence and FRET

(a) Explain FRET (Förster/Fluorescence Resonant Energy Transfer) using a Jabłoński diagram of the fluorophore(s) (you may ignore the triplet states). Explain why and how the fluorescence lifetime of the donor changes as a function of FRET efficiency. Make sure to define all terms used in your explanation and define rates in the Jabłoński diagram.

The fluorophore "Alexa 488" has an extinction coefficient of 65'000 M^{-1} cm^{-1}, a fluorescent lifetime of 4.1 ns and a quantum yield of 0.92.

(b) You can describe who strongly a molecule absorbs light using either of two quantities: The molar extinction coefficient ε [M^{-1} cm^{-1}] or the absorption cross section σ [cm^{-2}] = 1000 ln(10) ε / N_A.
Calculate the absorption cross-section of Alexa 488 and compare it to the physical size of the molecule without the coupling group; the bond length of benzene (and thus the radius of the ring) is 0.14 nm. Explain why the absorption cross-section is so much smaller/larger than the physical cross-section of the molecule.

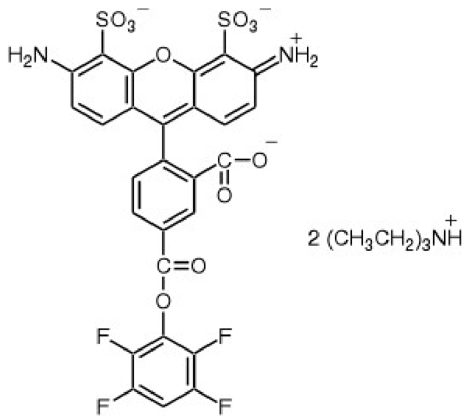


Figure 1: Alexa 488 fluorophore - the lower part, a carboxylic acid TFP ester, is a coupling group and can be ignored.

3) Super-resolution Microscopy

We have discussed several ways in which one can break the resolution barrier of a microscope.

(a) What is the starting point or "gold standard" of resolution that one has to beat in order to reach super-resolution?

(b) Using the example of Localization Microscopy (PALM or STORM), explain how super-resolution is achieved.

Equations

Lens equation:

$$\frac{1}{f} = \frac{1}{v} + \frac{1}{b}$$

v object distance, b image distance, f focal distance

Magnification:

$$M = \frac{b}{v}$$

Lensmaker equation:

$$1/f = (n-1).(1/R_1 + 1/R_2)$$

Numerical aperture NA = n sin(α) , with n the index of refraction and α the half angle of the illumination cone.

Airy-disk diameter:

$$d_{airy} = 1{,}22\,\frac{\lambda}{NA}$$

Resolution according to Rayleigh:

$$d_{\min} = 0{,}61\,\frac{\lambda}{NA}$$

Lateral resolution confocal microscope:

$$d_{\min,lat} = 0{,}45\,\frac{\lambda}{NA}$$

Axial resolution confocal microscope:

$$d_{\min,ax} = 1{,}4\,\frac{\lambda}{n.\sin^2\alpha}$$

Contrast: $\gamma = \dfrac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}}$

Energy of a photon: E = h.f , Planck's constant h = 6.63 x 10^{-34} J.s
Frequency of light: f = c/λ c speed of light (3 x 10^8 m/s) and λ the wavelength
Avogadro's number = $6{,}022\times10^{23}$
Charge of an electron e^- = 1.6x10^{-19} C