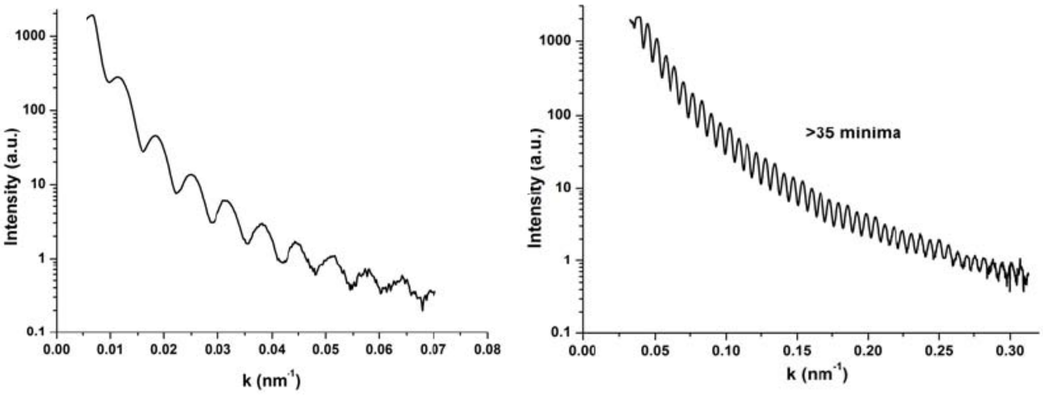
SCATTERING [100 points total]

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 either in English or in Dutch.

1. Explain the idea of contrast variation using the H/D substitution. Is it used in light, x-ray or neutron scattering? Why is it useful? [15 points]

2. What is the difference between the Guinier range and the Porod range? What type of information can be obtained from the measurements in these two cases? [15 points]

3. The graphs below present results of small-angle x-ray scattering measurements in a suspension of silica spheres. The left and right graphs present results for two different ranges of the scattering wavevectors: $0.006 < K < 0.07\ nm^{-1}$ (left) and $0.03 < K < 0.3\ nm^{-1}$ (right). The total K-range covered by these two measurements is therefore between $K_{min} = 0.006\ nm^{-1}$ and $K_{max} = 0.3\ nm^{-1}$.



a). What was the minimum θ_{min} and the maximum θ_{max} scattering angle in these measurements if the x-ray wavelength was $\lambda = 0.1$ nm? [15 points]

b) Argue that the colloidal particles were very monodisperse. [10 points] (The authors have estimated from these data that the variation of the diameter was less than 0.5%).

c) Despite the very low polydispersity, the minima in the scattering profile are not very deep. What can be the reason? [15 points]

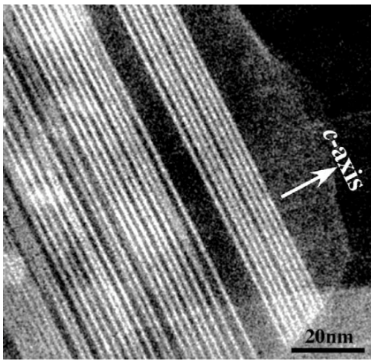
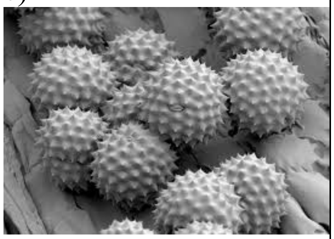
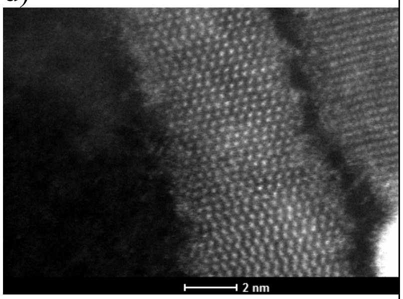
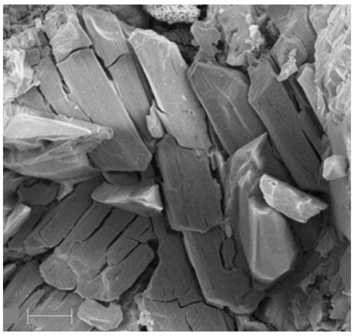
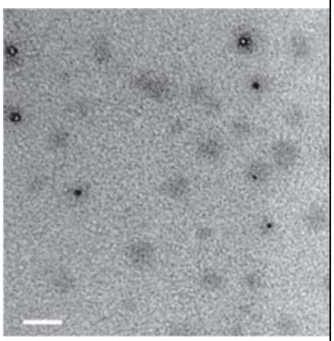
4. Imagine that one studies the same system (as above) using light scattering.

a) What range of K can be reached with light having the wavelength of $\lambda = 628$ nm? Assume that the average refractive index in the sample is $n = 1.5$ and that the scattering angle can be varied between 5 and 180 degrees. [15 points]

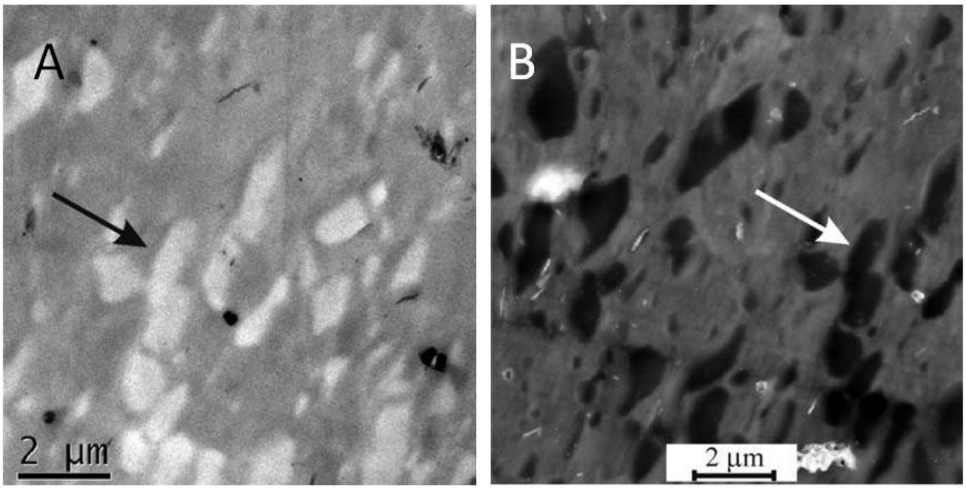
b) Can light scattering supplement the x-ray data shown above? Are there advantages/disadvantages in using light? Describe at least 3 advantages and/or disadvantages of using light in this case. [15 points]

Electron microscopy [14 points total]

Answers to each question should not exceed 2-3 sentences!

a) 	b) 	c) 
d) 	e) 	f) 

1. By which of those three methods have the images been acquired: Bright-field transmission electron microscopy (BF-TEM), high-angle annular dark field scanning TEM (HAADF-STEM), or scanning electron microscopy (SEM)? (3 P)



A thermoplastic vulcanized (TPV) unstained specimen imaged in different modes: (A) bright field conventional TEM image (approximately in focus); (B) high-angle annular dark field (HAADF) scanning TEM image of the same area as imaged in A (arrows indicate same specimen features).

2. Why is the contrast between A (bight field TEM in focus) and B (HAADF STEM) inverted? (3 P)

3. The contrast in A could be increased by defocusing. (*i*) how is this defocus-based contrast called and (ii) how is this additional contrast generated? (3 P)

4. Why is almost exclusively bright-field defocused-based imaging used for studying biological molecules in the frozen-hydrated state ('cryo-EM')? (2 P)

5. To precisely identify different types of atoms STEM imaging is often combined with Energy-dispersive spectrometry (EDS/EDX). Briefly describe how inelastic interaction of the electron beam with an imaged atom can generate characteristic X-rays. (3 P)

Optical Microscopy

Question weights: a), b), c), d), f) 1 point, e) 2 points; **TOTAL 7 points**.

In a FRET experiment GFP is used as a donor and Alexa500 as an acceptor. The fluorescence lifetime of GFP in the absence of Alexa500 amounts to 2.8 ns. Furthermore, the quantum efficiency of GFP is 0.3 and the Förster distance of the GFP-Alexa500 pair is 4.8 nm.
One Alexa500 dye molecule is positioned at a distance of 5.0 nm from the GFP.
a) Calculate the energy transfer efficiency in this situation.
b) Calculate the fluorescence lifetime of GFP.

Multiple Alexa-500 molecules are now positioned around the GFP, all at a distance of 5.0 nm w.r.t. the GFP. This results in an increase of energy transfer and further reduction of the donor lifetime .
c) Calculate the number of Alexa500 molecules that is required to reduce the fluorescence lifetime to less than 0.7ns. Assume that there are no interactions between the Alexa500 molecules.

A confocal microscope is equipped with a 60x oil immersion objective with an NA of 1.4. The microscope has a solid-state laser operating at a wavelength of 532 nm. The index of refraction of the immersion oil =1.57.
d) Calculate the detection volume of the confocal microscope in litres.

e) Explain the following concepts in 5 lines per concept max.:
- STED Microscope
- Numerical Aperture
- Spherical aberration
- Point spread function

A two photon excitation microscope is equipped with a laser with a repetition rate of 50 MHz, wavelength of 800 nm and a pulse width of 120 fs. The specimen is imaged using 2 mW of laser light. The microscope is equipped with a 60x oil immersion objective with an NA of 1.4.
f) Calculate the peak intensity of the laser light (in W/cm^2) in the focus of the microscope objective.

Equations for OM

Lens formula: $\frac{1}{f} = \frac{1}{v} + \frac{1}{b}$, $\frac{1}{f} = (n-1)(\frac{1}{R_1} + \frac{1}{R_2})$

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

NA = n sin(α)

Airy-disc diameter: $d_{airy} = 1{,}22 \frac{\lambda}{NA}$

Resolution Rayleigh, conventional microscope: $d_{\min} = 0{,}61 \frac{\lambda}{NA}$

Lateral resolution Rayleigh, confocal: $d_{\min,lat} = 0{,}45 \frac{\lambda}{NA}$

Axial resolution (FWHM) confocal: $d_{\min,ax} = 1{,}4 \frac{\lambda}{n.\sin^2 \alpha}$

Contrast: $\gamma = (I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$

Signal-to-noise ratio: $S / N = N_{sig} / (\sqrt{N_{sig}} + D + B)$

Nyquist criterion: $N_{samples} \geq\sim 2 \cdot d_{\min}$

Absorption: $I / I_0 = \exp(-\mu C d)$

Quantum efficiency $Q = N_{em}/N_{abs}$

Energy transfer efficiency:

$E = 1 - I_{D+A}/I_D$, $E = 1 - \tau_{D+A}/\tau_D$, $E = R^6_o / (R^6_o + r^6)$, $\tau = 1/\Sigma k$ en $Q = k_{rad}/\Sigma k$

Collision quenching: $I_0 / I = \tau_0 / \tau = 1 - K_D \cdot [Q] = 1 + k_q \cdot \tau_0 \cdot [Q]$

Index of refraction oil = 1.55, index of refraction water = 1.33

Speed of light $3.00 \cdot 10^8$ m/s