Modern Imaging, Spectroscopy and Diffraction Techniques
TIF 030 and FIM 150
October 30th, 2014

Aids: Formula sheets attached to the exam, “Physics Handbook”, calculator, and writing tools.

Total marks available from exam: 30
Marks required to pass: 12

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1. OM (4p)

a) You are observing a sample in transmission using an upright microscope equipped with infinity corrected optics. Sketch the image forming path from the lamp filament to the retina of the eye and mark all conjugate planes. (2p)
b) What is Köhler illumination and why it is important? (1p)
c) In wide-field fluorescence microscopy, one usually uses a so-called filter cube for obtaining good images. Where in the microscope is the filter cube placed and why is it placed there? (1p)

2. OM (3p)

a) Draw a Jablonski diagram of the low-energy quantum levels of a typical molecule and mark the following processes with arrows 1) electronic excitation; 2) vibrational relaxation; 3) intersystem crossing; 4) fluorescence; 5) phosphorescence; 6) Raman scattering; 7) infra-red absorption; and 8) FRET (assuming a second molecule around) (2p)
b) What is the difference between Raman and fluorescence processes? (1p)

3. OM (3p)+bonus 0.5p

In super-resolution techniques, such as STED and SIM, one uses fluorescence molecules.
a) explain the working principle of both STED and SIM. What property of fluorescent molecules is used to achieve super-resolution? Why STED and SIM can beat the diffraction limit? (2p)
b) estimate the maximum number of fluorescence photons that can be emitted per second by a fluorophore with quantum efficiency of QY=0.1 and fluorescence lifetime of 10 ns, assuming no photobleaching and negligible stimulated emission. (1p)
c) Name a Noble prize winner in 2014 who is the inventor of STED (bonus 0.5p)

4. OM (3p)

You are observing a sample consisting of transparent cells inside an aqueous buffer solution.
a) which technique would you use: dark-field or phase-contrast microscopy, and why? Describe the working principle of both methods? (2p)
b) according to Lorentz model sketch the wavelength dependence of refractive index of a typical dielectric. Which aberrations in optical microscopy arise due to this? (1p)

5. SEM (3p)

(a) Draw a schematic figure illustrating the depth of focus in the SEM. (1p)

(b) What type of electron source would you like to use for obtaining the best possible spatial resolution. Explain your answer (0.5p)

(c) Draw schematically the interaction volume for 20 kV electrons hitting a Ni sample. What signals are generated and from which depths are they coming? (1p)

(d) What happens to the interaction volume in (c) if the accelerating voltage is lowered to 5 kV? (0.5p)

6. TEM (3p)

a) Describe mass-thickness contrast in the TEM. Draw a ray diagram to show how this works. Include the specimen and the objective lens in the diagram. All other lenses can be omitted. (1.5 p)

b) How is amplitude contrast obtained by using the objective aperture? (0.5p)

c) The objective aperture is positioned according to Fig. 1. What will you see in the image? Make a drawing. (1p)

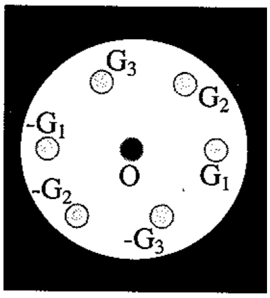

Fig. 1. Position of the objective aperture in a diffraction pattern

7. Electron diffraction (3p)

The diffraction pattern in Fig. 2 is obtained for an iron crystal with bcc crystal structure having the electron beam incident along a zone axis in a TEM operated at 200 kV.

a) Index the pattern. Explain how you check the validity of your indices. (1p)

b) Draw the Kikuchi lines corresponding to the 8 diffraction spots located closest to the 000. (0.5p)

c) The intensity of the diffraction spots in the schematic DP in Fig. 2 varies. What information is contained in the intensity distribution? Explain your answer. (0.5p)

d) What are the differences between a selected area electron diffraction (SAED) pattern and a convergent beam electron diffraction (CBED) pattern? Are there any advantages of using CBED instead of SAED (1p)

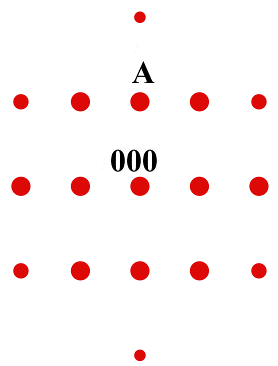

Fig. 2. Diffraction pattern from an iron bcc crystal.

8. STEM, EDS and EELS (4p)

(a) There are three main parts of EELS spectra. Describe them. (1p)

(b) You are to investigate the presence of light elements like oxygen and nitrogen in a TEM specimen. Would you prefer EDS or EELS? Why? (1p)

(c) Assume that you would like to investigate if there is segregation of an element to a grain boundary. Explain the effect of specimen thickness and acceleration voltage on the quality of the analysis. (1)

(d) How should the grain boundary plane in (c) be oriented with respect to the incident electron beam? How is this achieved practically? (1p)

9. SPM (4p)

(a) How is the lateral movement of the STM scanner realized? Draw the schematics of the electrodes, piezoelectric tube and the way to apply a voltage to produce such movement. (1p)

(b) Name one of the AFM's spin-off technologies and describe the operation principle of the chosen technology. (1p)

(c) In AFM you could encounter artifacts in your images originating from either the tip or the scanner. One artifact related to the tip is the broadening effect. Picture that you are imaging a DNA strand in a contact mode on a flat surface (rigidly attached, double-helix axis parallel to the surface) with a tip that has a radius of curvature of 15 nm. If you approximate the DNA strand to a cylinder with a radius of 2 nm, how wide would it appear in your image? Further, how high would such DNA appear in the image? (2p)

Formula sheet

Element (A)	k_{ASi} (1) 100 kV
Na	5.77
Mg	2.07 ± 0.1
Al	1.42 ± 0.1
Si	1.0
P	
S	
Cl	
K	
Ca	1.0 ± 0.07
Ti	1.08 ± 0.07
V	1.13 ± 0.07
Cr	1.17 ± 0.07
Mn	1.22 ± 0.07
Fe	1.27 ± 0.07
Co	
Ni	1.47 ± 0.07
Cu	1.58 ± 0.07
Zn	1.68 ± 0.07
Ge	1.92
Zr	
Nb	
Mo	4.3
Ag	8.49
Cd	10.6
In	
Sn	10.6
Ba	

$\lambda = h / [2m_0eV(1 + eV/2m_0c^2)]^{1/2}$

$d_p = (d_g^2 + d_s^2 + d_d^2 + d_c^2)^{1/2}$

$r_{Sch} = 0.66\ C_s^{1/4}\ \lambda^{3/4}$

$n > (5/C)^2$

$2\ d_{hkl} \sin\Theta = n\lambda$

$b = 7.21 \times 10^5\ (\rho/A)^{1/2} t^{3/2}\ (Z/E_0)$

$$I \propto U\rho_s(E,r)e^{-2\frac{\sqrt{2m_e\phi}}{\hbar}d} \ with\ \phi = \tfrac{1}{2}\left(\phi_{sample} + \phi_{tip}\right)$$