These questions are designed to solidify and test your knowledge, and to give you representative examples of the types of questions that might appear on exams. Most essay type questions generally require **at most** one or two sentences to be answered correctly. During the midterm and final exams, you will be given a sheet with formulas or equations, so these do not have to be memorized (though some are pretty easy to remember with a little practice). Keep checking this Virtual Homework document on the class web site, as new questions will generally be added after each lecture.

I. THE MICROSCOPE (Section A)

1. *Escherichia coli* bacteria (or mitochondria) are about 1 μm in size. A typical liver cell is about 10 μm in dimension, whereas ribosomes are about 20 nm in diameter. How large would each of these objects appear to be when viewed in the following microscopes?
   a. A 10X magnifying glass
   b. A light microscope operated at 100X magnification
   c. A light microscope operated at 1000X magnification
   d. A transmission electron microscope operated at 10,000X magnification
   e. A transmission electron microscope operated at 50,000X magnification

2. Why do microscopists care that imaging electrons are accelerated to very fast speeds (nearly the speed of light) in a TEM? (Answer all that correctly apply; wrong answers would result in points being deducted on an exam)
   a. At such speeds electrons have very short wavelengths (thousandths of nanometers), which means that diffraction limited resolution in electron images is very poor.
   b. At such speeds electrons have very long wavelengths (several millimeters), which means that diffraction limited resolution in electron images is very poor.
   c. At such speeds electrons have very short wavelengths (thousandths of nanometers), which means that diffraction limited resolution in electron images is very good.
   d. At such speeds electrons have very long wavelengths (several millimeters), which means that diffraction limited resolution in electron images is very good.
   e. I have no clue why anyone except someone like Einstein or DeBroglie might care.

3. Assume for the sake of this question that the human eye is a **perfect** lens with a semi-angular aperture of 0.45° and a refractive index of about 1.35 (n=1.35). Using these values, what would the **theoretical** resolving power of the eye **lens** be according to the Rayleigh criterion for photons with a wavelength of 400 nm? If your answer is much different than the 70 μm value quoted in the lecture notes as the **actual** resolving power of the eye, then explain what accounts for the difference.

4. Under **optimal** viewing conditions the eye is able to just resolve 1' of arc (i.e. one minute or 1/60th of a degree). What is the smallest size object that can be seen with the naked eye if the object is at the following distances from the eye? Hint: you do **not** need to consider the effects of wavelength or aperture angle or refractive index to answer this question.
   a. At 100 meters
   b. At 1000 meters
   c. On the moon (about 385,000 km away from Earth)

5. Some formerly, very near-sighted (i.e. myopic) persons (like one professor who teaches this class) could at one time see objects held about 100 mm in front of either eye. Under "ideal" conditions, what is the smallest object that could be detected from this distance? Hint: the same logic used in the last question will help you solve this one.

6. Nearsighted people can hold objects closer to their unaided eyes without eyestrain than a person with "perfect" vision. Which person is able to see finer details in objects and why is this? Is the focal length of the eye of the nearsighted person longer or shorter than the eye of the person with perfect vision? What consequence does this have in terms of where the nearsighted person forms an image of an object at infinity?

7. According to **ideal** lens theory, each point in an object is imaged as a single point in the image plane. In reality, even if we neglect the effects of lens aberrations (these will be discussed in later lectures), the image formed in the image plane of a lens is imperfect. Why is this? What does the image of an object point look like?

8. We know that the Abbe "simple ½ the wavelength" rule gives a reasonable estimate of the resolving power of light microscopes but severely overestimates the resolving power in a TEM. What important factor does Abbe's simple rule neglect that accounts for this discrepancy?

9. Using the principles of **geometrical** lens theory, as an object is brought closer to the front focal plane of a converging lens from a point to the left of the front focal plane, the image formed will (**grow to a larger size/ shrink to a smaller size / stay the same size**) and appear (**behind / in front of / at**) the (**front / back**) focal point and will be (**real / virtual**) and (**erect / inverted**). Circle all choices that make the entire statement correct.

10. Assume you have a standard glass converging lens of focal length 1.333 cm and place an object 2.0 cm in front of the lens. Where will the image form and what will its magnification be? Is the image real or virtual and is it erect or inverted? Draw a sketch of this optical setup that is accurate enough to demonstrate your understanding of the principles of image formation according to simple geometrical theory.

11. Imagine you were asked to build a two-imaging-lens microscope with a total distance from the specimen to the final image plane of exactly 1 meter (= 1000 mm = 100 cm). Assuming you could only afford to design an instrument in which the focal lengths of both imaging lenses were equal, and each lens magnifies *its* object to the same extent, what focal length would be required to achieve a final image magnification at this distance equal to 10,000 times?

    For the purposes of answering this question, assume that you have cleverly designed an instrument that is always perfectly in focus and thus requires no focusing adjustments. Where would the lenses have to be placed relative to the specimen plane? Note: each lens is a converging lens and forms a real, inverted image.

12. What is the primary difference between the way light is refracted through glass and the way electrons are refracted through an electromagnetic lens?

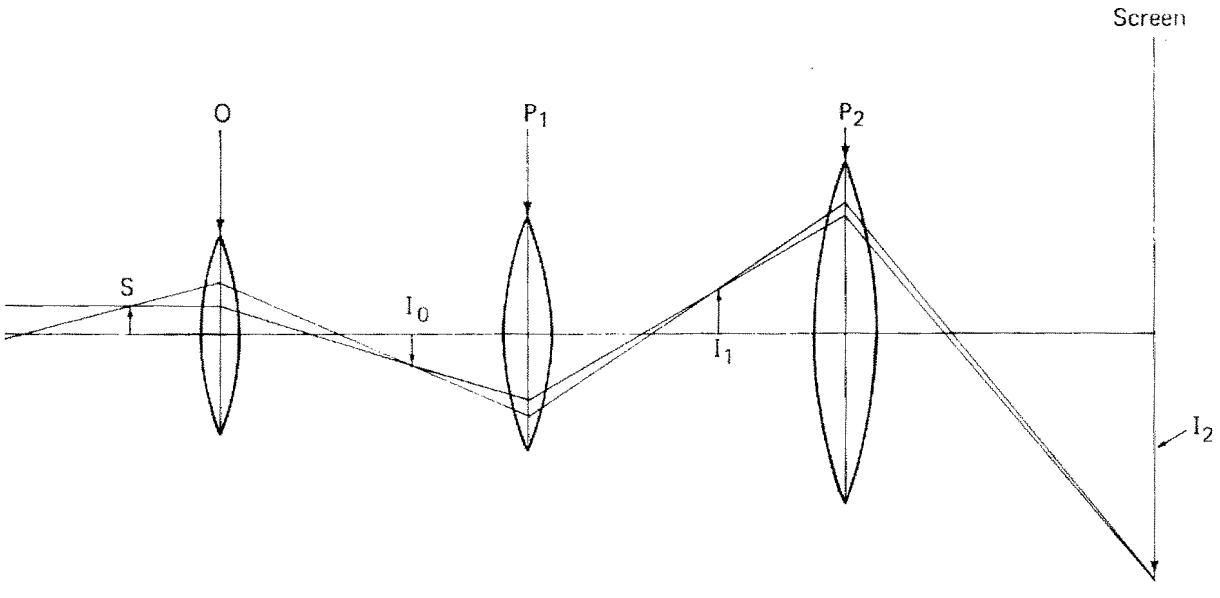
13. The focal length of an electromagnetic lens (**gets longer / gets shorter / stays the same**) as the velocity of the imaging beam of electrons decreases.

14. Electromagnetic lenses are (**converging / diverging / both converging and diverging**) whereas electrostatic lenses are (**converging / diverging / both converging and diverging**). (Circle only one word or phrase for each of the two fill ins).

15. The focal length of an electromagnetic lens depends on which of the following:
    a. Accelerating voltage of the microscope
    b. Speed of the beam electrons
    c. Current passing through the lens
    d. Electron wavelength
    e. All of the above (a-d)
    f. None of the above (a-e)
    g. Only (a) and (c)

16. In **classical geometrical** light optics, rays of light are brought to focus as a result of the refraction of light at the surfaces of suitably shaped lenses made of material whose refractive index is substantially different from that in air. What are the two major, fundamental differences between light and electron optics with regard to the principles of the focusing action of light optical and electromagnetic lenses?

17. The following figure from your lecture notes (p.23; Fig.1.31) is a highly schematic diagram to illustrate high magnification mode of operation in a three lens TEM. However, it is not an accurate drawing. For example, assuming the positions of the object (S) and first image (I_0) are correct, what must be wrong with the depiction of I_0? Note: completely ignore the presence of P1 and P2 and any further errors in that part of the diagram.



I. THE MICROSCOPE (Section B)

1. The table below (column #1) lists the three primary types of electromagnetic lenses used in TEMs. In each of the three remaining columns, circle just **one** phrase or value in each box that **best** describes each lens.

Lens	Distance from electron source	Function	# found in modern TEM
Objective	nearest farthest intermediate	beam focusing image forming	0 1 2 or more
Condenser	nearest farthest intermediate	beam focusing image forming	1 2 3 or more
Projector	nearest farthest intermediate	beam focusing image forming	0 1 2 or more

2. In the absence of lens asymmetry, the aberration or property of the objective lens that most limits TEM resolving power is (**chromatic aberration / spherical aberration / diffraction / distortion**). Circle the correct answer.

3. Spherical aberration in electromagnetic lenses is a primarily a result of:

   a. Defects in manufacture of electromagnetic lenses
   b. Gradual increase in lens field strength at increasing distance from the optic axis
   c. Lack of rotational symmetry in the lens field
   d. Differences in wavelengths of beam electrons
   e. Differences in wavelengths of irradiating photons

4. The "circle of least confusion" is:

   a. The smallest disc image that can be obtained when placing a viewing screen behind a lens to form the sharpest possible image of a point in an object.
   b. A place where octapoles are used to eliminate or reduce lens astigmatism
   c. The place where two line foci superimpose in an astigmatic lens.
   d. A group of ultra brainy students sitting in a circle.
   e. A large Airy disc image of an object point.

5. In a TEM, image resolution is limited by: (answer all that correctly apply; points would be deducted for wrong answers)

   a. Chromatic aberration in the objective lens
   b. Astigmatism in the objective lens
   c. Radiation damage to the sample
   d. Spherical aberration in the objective lens
   e. Magnification on the recording device (film or CCD or DDD)
   f. None of the above, as the manufacturer of the TEM specifies the resolving power of the particular instrument installed in a specific location

6. The objective lens is said to be the most important lens in the TEM. Give three reasons why this statement is true.

7. Which of the following statements **best** describes the function of the anticontaminator?

   Device to reduce leaks in the vacuum system near the specimen.

   Device to burn off the contamination layer that builds up on the specimen.

   Device that holds liquid nitrogen and keeps the objective lens super cold, thereby reducing the effects of thermal drift and vibration.

   Device to help trap residual gases in the EM column near the specimen that might arise, for example, as a result of a careless operator (*e.g.* one who just finished eating some "finger-lickin' good", extra crispy fried chicken just before loading a sample into the specimen holder).

   Device that produces a clean vacuum in the gun chamber, thereby increasing filament lifetime.

   Device that holds liquid nitrogen and keeps the specimen holder super cold, thereby reducing the effects of thermal drift and vibration.

8. Four different optical setups are listed in the table below. In all instances, dimensions are given in mm. The objective lens is a converging lens that follows the laws of geometrical optics for a thin lens. For each example, provide the image distance, whether the image formed is REAL or VIRTUAL, and what the image magnification would be. Which of the following situations **BEST** represents the conditions for image formation by the OBJECTIVE lens of a typical transmission electron microscope? For each setup, **briefly** indicate your rationale for or against designating it as "BEST". **HINT**: If you are tempted to use graph paper and drawings to solve this problem (which can work but would be tedious), you are making this more difficult than it needs to be.

	Focal length (objective)	Object distance	Image distance	Real or Virtual?	Magnification
a.	5.0	5.02			
b.	2.5	2.40			
c.	1.0	1.10			
d.	2.0	2.04			

9. Depth of focus in the transmission electron microscope is large enough such that the final image to be recorded appears essentially unchanged (except for magnification) over a considerable distance (meters). (**True / False**)

10. Depth of field in the light microscope is such that the final image contains information from all depths in the specimen, at the same level of focus, projected in the micrograph. (**True / False**)

11. Because the depth of focus in a TEM is so large (meters) the microscopist does not have to be too concerned about the precise focus setting of the objective lens. (**True / False**)

12. Imagine walking in from bright sunlight conditions into a rather dark room indoors. Once your eyes have had time to readjust to these new conditions, will your depth of field be larger or smaller than it was outdoors? Or will it be the same? Explain your answer.

13. Of what importance is it to know that the resolution of a photographic emulsion or of a CCD camera is several times greater than the resolution of the fluorescent viewing screen on the microscope?

14. Reasons for maintaining a good vacuum in the electron microscope include which of the following?

a. Reducing contamination of the specimen
b. Increasing the mean free path of the imaging electron beam
c. Reducing etching of the specimen
d. Increasing filament lifetime
e. All of the above (a-d)
f. None of the above

I. THE MICROSCOPE (Section C)

1. The probability of electron scattering increases with increasing (**specimen density / specimen thickness / specimen mass thickness**). Circle the correct answer or answers, or, if none are correct, supply the correct answer.

2. Some biological electron microscopists refer to elastic and inelastic scatter as 'good' and 'bad' scatter, respectively. Explain the rationale for such a statement.

3. For each item listed below, indicate the contrast mechanism (scattering/interference) that is dominant and in which direction (up/down) contrast changes. Note: assume in each instance that the microscopist has only made the stated change.

a. Increase voltage from 60kV to 100kV
b. Underfocus objective lens slightly from "in-focus" position
c. Reduce the size of the objective aperture from 50 μm to 25 μm
d. Examine 60 nm thick specimen instead of one that is 30 nm

4. To optimize interference contrast without sacrificing resolution in studying most biological specimens, it is best to adjust the strength of the objective lens to a position that is which of the following? Circle the **best** answer.

a. Slightly over focus
b. Well under focus
c. Close to "near" or "true" or "exact" or "dead" focus
d. Slightly under focus
e. Well over focus

5. Where do unscattered electrons appear in the back focal plane of the objective lens in the transmission electron microscope? Where do these electrons appear at the viewing screen?

6. The contrast transfer function (CTF) of the TEM is: (circle ALL correct answers)
a. In part a property of the objective lens
b. In part influenced by the microscopist
c. Totally under the control of the microscopist
d. Only a property of the TEM and not influenced by the microscopist
e. When expressed as an equation it describes the relative contrast in images as a function of spatial resolution in the object
f. A mystery to all but a few Caltech grads.

I. THE MICROSCOPE (Sections D and E)

1. Which two points generally define the optic axis of the TEM? Circle the best answer.

   a. Electron gun crossover and the center of the objective lens
   b. Center of the objective lens and the center of the viewing screen
   c. Electron gun crossover and the center of the viewing screen
   d. Center of the condenser aperture and the center of the specimen
   e. Center of the condenser aperture and the center of the objective aperture
   f. Center of the viewing screen and the center of the Wehnelt aperture

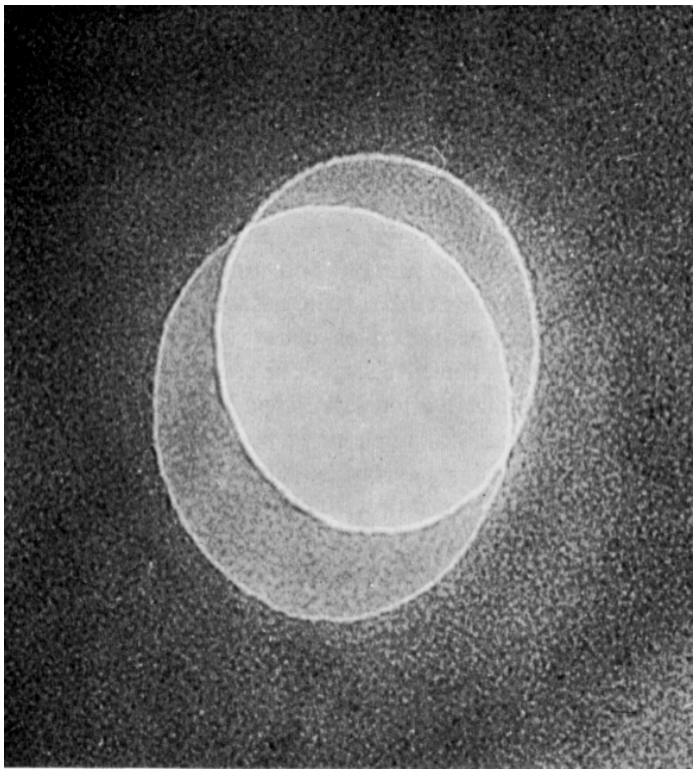
2. A primary goal when the microscopist aligns a transmission electron microscope is to make sure that the optical axes of all electromagnetic lenses coincide with an axis that intersects the center of the fluorescent viewing screen. What principle electron-optical property of electromagnetic lenses and electrons is exploited to help make alignment of the imaging lenses relatively straightforward? Note: the principle differs from what occurs in glass optics and photons.

3. You seem to be having a real bad day at the microscope. You just can't seem to get any images to the "true" or "in focus" setting. The local "expert" tells you that the microscope was just fully aligned, so that can't be a source of the problem. You notice that as you change the objective lens current the image doesn't change dramatically in terms of its contrast no matter where you focus. You know the problem can't be related to contamination because you are religious about making use of the anticontaminator. What is the likely source of the problem you are experiencing and what is the solution? How will you know when you have solved the problem?

4. The following double-exposure micrograph, which you have seen in lecture and in the notes, shows the effects of both drift and contamination in the electron microscope.

   a. Assuming the micrograph magnification shown here is 300,000 times, and that the time between exposures was 1 minute, what is the measured drift rate in nm/sec? Hint: depending on how you make your measurement, you may need to correct for the effect of contamination.

   b. What is the contamination rate in nm/sec?



5. For each of the following situations, which way would the accelerating voltage in the TEM have to be adjusted (**higher / lower / doesn't matter**) to achieve the stated result? For all examples, assume that NOTHING other than the accelerating voltage is changed.

   a. Increase scattering/aperture contrast
   b. Increase microscope resolving power
   c. Decrease specimen radiation damage
   d. Study thicker biological specimens
   e. Increase inelastic scattering
   f. Decrease effects of stray electric or magnetic fields
   g. Decrease chromatic aberration effects
   h. Decrease elastic scattering
   i. Correct for image astigmatism (*i.e.* to stigmate the image)

6. Assuming all other microscope parameters are held constant, use of a <u>larger objective</u> lens aperture will result in which of the following? Circle the correct answer or answers.
   a. Increased effects of spherical aberration
   b. Decreased illumination of the specimen
   c. Decreased specimen irradiation
   d. Decreased beam coherency
   e. Decreased interference contrast
   f. Decreased scattering contrast
   g. Decreased aperture contrast
   h. All of the above
   i. None of the above

7. What is the main function of the objective aperture? What is one **benefit** as well as one **drawback** to using a very small size (*i.e.* <20 μm) objective aperture?

8. For accurate and optimal focusing in the TEM, it is common practice to initially focus on the specimen support film and to try and make it disappear. Why is this? (One or more answers may be correct but points would be deducted for wrong answers)

   a. Background support film is generally a constant, familiar 'object' and therefore it is easier to assess the actual defocus level.
   b. Making the film background disappear is one way to reduce spherical aberration in the objective lens.
   c. The best way to set a specific level of focus is to first find the objective lens setting that generates a 'true' or 'near' focus image and then defocus the objective lens by the amount specified by the TEM manufacturer for the microscope you are using to achieve the desired defocus.
   d. Making the film background disappear is one way to help correct for astigmatism in the objective lens.
   e. Statement is untrue. Microscopist should always focus on the specimen and try to maximize contrast even if this seriously sacrifices resolution.

9. What is the best way to assess whether details one sees in recorded images of a specimen are genuine or merely artifacts of the phase contrast granularity of the support film?

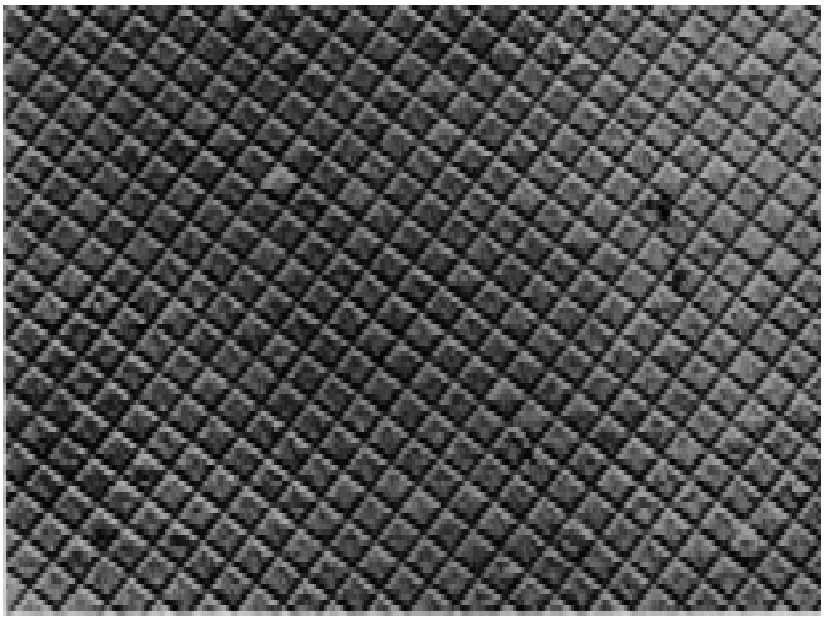
10. For a standard 8 x 10 cm piece of photographic film, what size region ("field of view") of the specimen will be imaged on the piece of film if the image is recorded at a magnification of 100,000X? What would the field of view be if the micrograph were recorded at a magnification of 10,000X?

    a. 800 Å by 1000 Å
    b. 0.8 μm by 1.0 μm
    c. 8.0 μm by 10 μm
    d. 80 nm by 100 nm
    e. 8.0 mm by 100 mm

11. In an "ideal" electron optical instrument (*i.e.* one in which the imaging lenses have no aberrations and infinitely large apertures, and no need for an objective aperture), an electron image of a thin, unstained biological specimen recorded at "near" or "true" focus would, by definition, appear completely featureless because there would be no scattering and no interference contrast. In such an "ideal" situation ALL scattered rays would be captured by the lens and focused at the image plane 'in phase' with the unscattered rays. In the real world, however, even an image recorded at the "in focus" setting shows a small amount of interference contrast. What is the main source of this small interference contrast? HINT: Do NOT consider any effects of scattering.

12. Under focusing most affects (**amplitude / aperture / interference / scattering**) contrast. Circle the correct answer or answers. If a slight amount of under focus is a good thing when imaging in the TEM, why not under focus a bit more?

13. Assuming the image of the cross-ruled replica grating (2160 lines/mm) shown below is an accurate, direct copy of a micrograph, which of the following magnifications comes closest to matching that of the original micrograph? 1000X, 2,000X, 5,000X, 10,000X, 20,000X, 50,000X Circle the correct answer and **show the calculations you used to arrive at that answer**.



14. Photographic emulsions are said to be nearly perfect recorders of electron images. Why is this?

15. For a standard 1024 x 1024 pixel CCD camera with 15 μm pixels, what is the effective size of each pixel at the specimen if an image magnification of 50,000X is used? What is the effective size of each pixel if the micrograph were recorded at a magnification of 15,000X? What is the Nyquist limit for this CCD when recording images at 50,000X magnification?

a. 3 Å
b. 10 nm
c. 2 nm
d. 10 Å
e. 0.6 nm

I. THE MICROSCOPE (Section F) For CHM 265 and BGGN 262

1. The electron diffraction pattern is first formed at the back focal plane of the objective lens **(TRUE / FALSE)** and is then magnified by the projector lenses **(TRUE / FALSE)** onto the fluorescent screen of the TEM **(TRUE / FALSE).**

2. With everything held constant except the accelerating voltage, the distance between spots (Bragg reflections) in an electron diffraction pattern of a crystalline specimen decreases as the voltage is increased. **(TRUE / FALSE)**

3. The central region of the electron diffraction pattern from a crystalline biological specimen is often the most dominant feature and arises mainly from the unscattered and inelastically scattered electrons. **(TRUE / FALSE)**

4. Why do dark field images require much longer photographic exposure times than typical bright field images?

5. Contrast in dark field images of biological specimens is (**lower than / higher than / the same as**) bright field images. (Circle correct answer and explain).

6. In dark field imaging the elastically scattered electrons are blocked by the objective aperture and contrast arises from interference of the elastic and inelastically scattered electrons. **(TRUE / FALSE)**

7. Contrast in dark field images of very thin biological specimens is lower than in bright field images. **(TRUE / FALSE)**

8. Is radiation damage an important consideration in imaging of biological specimens using dark field? Explain.