

## END SEM QUIZ LS 3104

19/11/18 [20 marks]

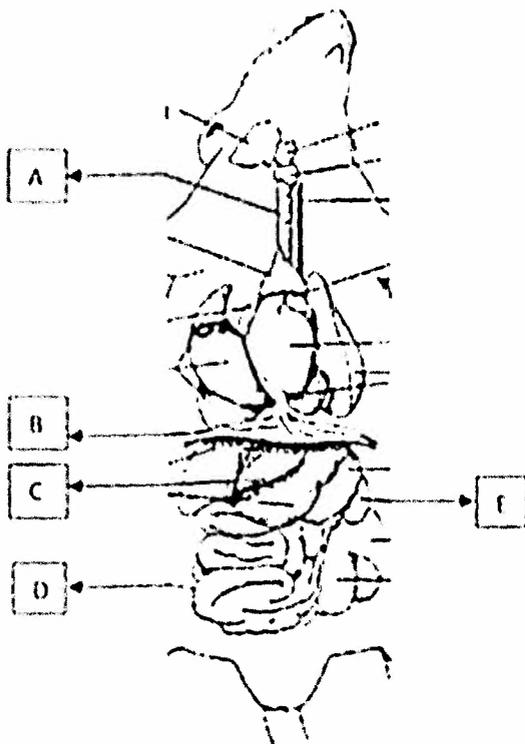
1. Draw a ray diagram to show how will you incident light at the back focal plane of the objective such that the light at the sample plane is focussed at the centre of the field of view. Assume the objective to be a convex lens. [2]
2. If a imaging system has a minimum resolvable distance of 300 nm, can it track a single bead attached to a motor protein an resolve the 30 nm steps. How would one design such an experiment? [2]
3. In what ways can we try to alter the PSF to enhance optical resolution? [1]
4. What is a "binary" image? [1]
5. Two objectives have the following specifications: A: 100x, 1.4 NA; B: 60x, 1.4 NA. Which one will yield a smaller "least resolvable distance"? [1]
6. Which of these (a - d listed below) will you use for the studies listed in i - v. Give one-line logic behind the choice. [10]
  - a. Electron Microscopy
  - b. fluorescent microscopy
  - c. immunofluorescence/ other fixed staining
  - d. others - specify
  - i. for studying role of flagella in cell motility
  - ii. for evaluating pili length distribution of a particular kind of bacteria
  - iii. evaluating step size of a molecular motor's walk (usually in tens of nms)
  - iv. evaluating colocalization of two proteins in a cellular compartment (say early endosome)
  - v. evaluating cell shape changes during development
7. Maximum intensity possible in a 16 bit image is: [1]
8. What should be the order of jobs for finding out how area of a cell evolves with time - while analysing a stack of images. Some example of "jobs" : make composite image, threshold, analyse particles with filter, etc. [2]

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1. In indirect immunohistochemistry,
  - a. The secondary antibody is tagged with DAB
  - b. The secondary antibody is tagged with HRP
  - c. The primary antibody is tagged with HRP and there is no secondary antibody
  - d. There is no primary antibody and the secondary antibody detects the proteins.



Identify A, B, C, D and E (5)

2. Pick the correct statement regarding PFA preparation,
  - a. Heat paraformaldehyde solution to 100C and it is ready to be used.
  - b. Heat paraformaldehyde to 65°C and add a few drops of NaOH to clear the solution.

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- c. Heat paraformaldehyde to 100°C and add a few drops of NaOH to clear the solution
  - d. Heat paraformaldehyde to 65°C and add a few drops of NaCl to clear the solution.
3. Alcohols being protein denaturants are the best fixatives for tissue preservation.  
True or False.
4. For antigen retrieval to unmask epitopes, the following can be used,  
a. Heat      b. Proteinase K      c. Trypsin      d. All of the above.
5. Pick the right order,  
a. Fixation, sectioning, staining, embedding, mounting  
b. Staining, Embedding, fixation, sectioning, mounting  
c. Fixation, embedding, sectioning, staining, mounting  
d. Sectioning, fixation, embedding, staining, mounting.
6. Which of the following steps is NOT a correct part of the process of "progressive" staining?  
a. Hematoxylin is used first for staining  
b. Hematoxylin is overstained  
c. No differentiator is used  
d. Eosin is used as the counterstain
7. Which cation allows Hematoxylin to bind to and stain negatively charged molecules in cells and tissues?  
a.  $\text{Fe}^{3+}$   
b.  $\text{Zn}^{2+}$   
c.  $\text{Na}^+$   
d.  $\text{Al}^{3+}$
8. Differentiators reduce the ability of hematoxylin to bind to tissue sites by  
a. Reducing the pH  
b. Increasing the pH  
c. diluting the concentration of hematoxylin  
d. increasing the concentration of hematoxylin
9. Which of the following amino acids may provide binding sites for eosin in cytoplasmic and connective tissue proteins?

- a. Leucine
- b. Lysine
- c. Methionine
- d. Glutamic acid

10. What causes eosin staining to reduce and become murky as pH of staining solution reduces below 4?

- a. The net negative charge of proteins increase
- b. Eosin precipitates
- c. Acidic groups of eosin get protonated and eosin becomes neutral
- d. Eosin interacts with negatively charged proteins

11. Overstaining with eosin may lead to

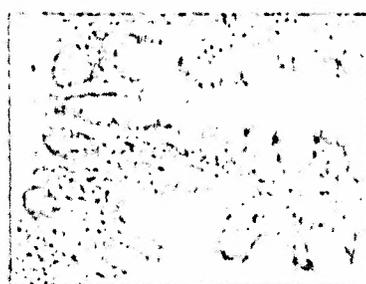
- a. Purple nuclei
- b. Blue nuclei
- c. Purple cytoplasm
- d. Blue cytoplasm

12. In the following slides, the dark staining is of hematoxylin while the light staining is of eosin. Which of the slides represent staining at a lower pH?

a.



b.



13. Identify the following hematoxylin-eosin stained tissue sections:

