

**BMB/Bi/Ch 173 – Winter 2017**

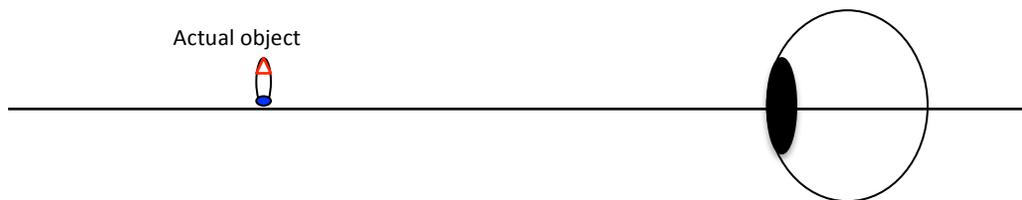
**Problem Set 7.1: Light Microscopy Basics – Assigned 2-21-17. Due 2-28-17 by 10:30am**

TA: Wen Zhou (201 Kerckoff, office hour: Fri 2/24 5-6pm, Mon 2/27 5-6pm or by appointment)

**90 points total**

**1. (34 points) Microscopy and Lens Basics**

- a. (10 points) You have a cell with two stains at the two poles. Draw the components of a compound microscope between the object and the eyeball, and the ray diagram. Be sure to label all the lenses, and their corresponding focal points. Draw the real image projected through objective lens and on retina, and the virtual image observed through the eyepiece. Be sure to label the locations of the dyes.



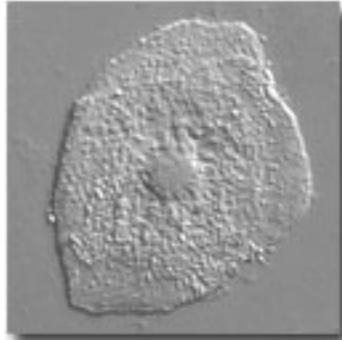
- b. (6 points) What are the fundamental resolution limits for wide-field optical light microscopy?
- c. (10 points) How does oil immersion increase the resolving power of a lens? Please draw a simple diagram to illustrate your answer.
- d. (8 points) You are using a lens without oil immersion (index of refraction in air  $n = 1$ , half-angle  $\theta = 48^\circ$ ). What is the numerical aperture of the lens? If you are using light with a wavelength of 532 nm, what is the resolution of the lens?



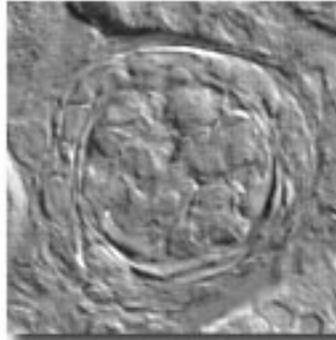
3. (30 points) Below shown are 3 different biological samples imaged with phase contrast and DIC.

human buccal mucosa  
epithelial (cheek) cell

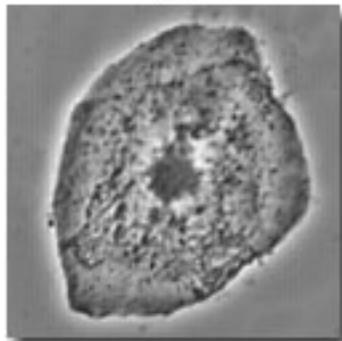
A thick section of mouse  
kidney tissue



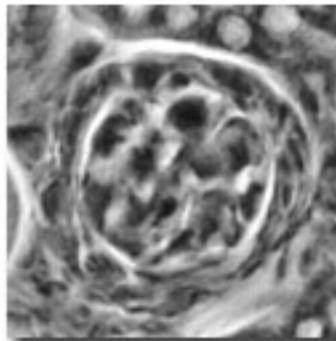
(a)



(c)



(b)



(d)

- a. Using diagrams, briefly describe how contrasts were generated with the phase contrasts and DIC.
- b. Among images (a)-(d), which are images acquired with Phase Contrast mode, which are images acquired with DIC. How can you tell?

- c. There are certain biological content that are more clearly visible in one mode than the other. Fill out the table below with “V” or “X” or “Maybe”, to mark what you can see in each mode

	Phase contrast	DIC
Nucleus of human cheek cell		
Numerous bacteria on the surface of the human cheek cell		
Bundles of cells enclosed within a tubule in kidney section		
Some nuclei in the kidney section		

In a few sentences (3 max), briefly compare the information observed in the two modes. What biological contents are more visible in each mode, and why?