Optical Methods in Neurobiology
BIOL 6222
Final Exam
April 27, 2008

Answer each of the following 15 questions. Do so in YOUR OWN WORDS. Be concise, but answer each question completely. You may use drawings to supplement your answers, but they must be accompanied by a verbal explanation. You may work together on your quest for information, but you must write your own answers, in YOUR OWN WORDS. You may use any source of information, including the lecture notes, online resources, and textbooks. You must submit your answers as MS Word documents (NOT pdf files) by email (mrea@uh.edu) no later than 11:59 pm on Tuesday, May 6, 2008.

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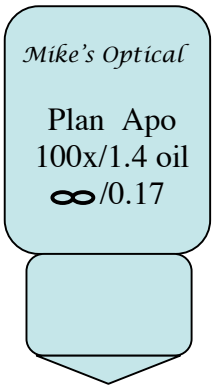
1. Describe the process of refraction of a planar monochromatic wave front at the linear boundary between two transparent media of differing refractive index. Why does refraction of the wave front occur? How is this phenomenon exploited in optical microscopy.

2. Consider the objective lens drawn on the right. → Identify **and define** each visible designation on the lens.



3. What is chromatic aberration and why does it occur?

4. Describe 3 different experimental situations in which you would choose differential interference contrast over phase contrast microscopy, and 2 situations in which you would not.

5. Describe the light path of the imaging rays in a light microscope. Where are the conjugate planes of focus?

6. Describe the process of achieving Koeller illumination. Where are the conjugate planes of focus of the illuminating rays in a compound microscope after establishment of Koeller illumination.

7. What is/are the principal advantage(s) of infinity corrected optics?

8. What is total internal reflection, and how is it useful in optical microscopy? Describe two situations in which total internal reflection microscopy would be a useful experimental tool.

9. Discuss the fluorescence characteristics of any 3 of the following fluorescent proteins: PA-GFP, Kaede, Dronpa, PS-CFP, Kindling

10. Choose three fluorophores for a hypothetical triple label colocalization experiment using a confocal laser scanning microscope equipped with 3 PMT detectors and the following five laser excitation lines (405 nm, 488 nm, 568 nm, 543 nm, 633 nm). Describe the reasons for your choices, and the characteristics and locations of the filters and dichroic mirrors in the optical path.

11. Why do fluors photobleach? Describe three strategies for reducing the rate of photobleaching during fluorescence microscopy.

12. What is Foerster resonance energy transfer (FRET), and how is it useful in optical microscopy? Describe 3 distinct situations in which FRET would be a useful experimental tool.

13. How do lasers work, and what are the special characteristics of the light that is produced?

14. Describe the advantages and disadvantages of the following light sources for optical microscopy: tungsten-halogen lamp, mercury vapor lamp, xenon lamp, argon laser.

15. Light is said to display particle- and wave-like properties. Describe three experimental situations in which the wave-like properties of light are apparent.