Biophysics 210

Discussion Section 4: Brightfield Contrasting Techniques

Useful equations: $R = 1.22 * \lambda / (NA_{objective} + NA_{condenser})$

1. Which of the following applications would darkfield microscopy be a useful technique?

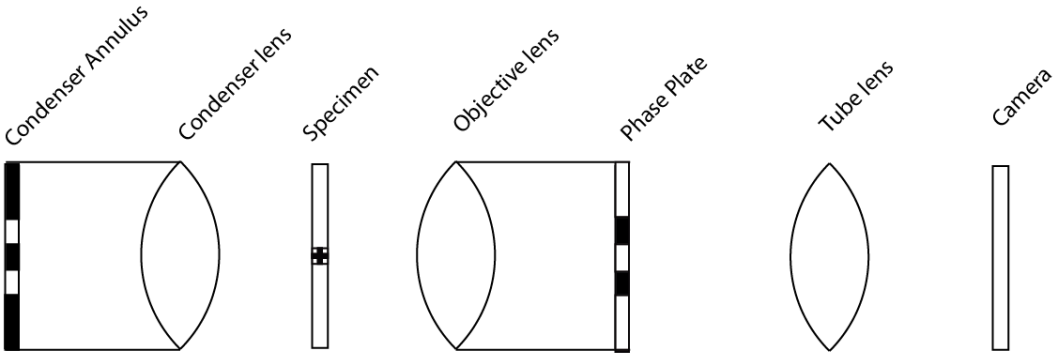
    a. Visualizing the nucleolus in normal and cancer human cell lines.

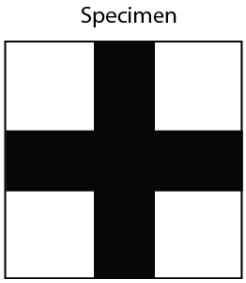
    b. Studying flagellar movement in the protist *Euglena gracilis*.

    c. Studying a highly photosensitive behavior in *Caenorhabditis elegans*.

2. You have just purchased a new darkfield condenser with an NA of 1.25 to use with your lab's microscope. This microscope comes equipped with the following objectives: 10x/0.45NA, 20x/0.75NA, 40x/1.3NA, and 100x/1.4NA. What is the largest magnification you will be able to achieve for your darkfield imaging? Draw ray diagrams to support your answer for an objective that will work and one that won't work.

3. The setup below describes a simple configuration to perform phase contrast microscopy. Assume the condenser annulus is 100% opaque in the areas colored black. The phase plate is 50% opaque and retards light by a phase of 90 degrees. Assume that like most cells, the black areas in the specimen are of greater refractive index that the surrounding media and retard light by lambda/4. Assume Kohler illumination. Starting at the condenser annulus, (a) draw ray diagrams following light that is undiffracted and diffracted by the specimen as it moves toward the detector. (b) Include close-ups of the both the undiffracted and diffracted rays as they pass through the specimen and phase plate (represent the rays as simple sine waves). Finally, (c) depict in general terms how the specimen will look at the image plane of the camera, paying attention to areas of relative brightness and relative darkness. Repeat steps (b) and (c) for a phase plate that advances undiffracted light by lambda/4.




4. Qualitatively, what effect will rotating your sample on the stage have on the output image in DIC? In phase contrast?

5. You notice something has been removed from the light path of your Smith DIC microscope when you sit down to use it; when you put your specimen on, instead of seeing a grey field with a light and dark edge on either side of each object, you see mostly blackness with bright outlines on the edges of your objects. What piece has been removed? If you had a similar image on a Nomarski DIC microscope, what component would you adjust to get a "standard" looking DIC picture?

6. What is the maximum resolution you can obtain (assuming 500nm light) using the 40x/1.3NA objective using
   a) a 1.3NA brightfield condenser
   b) the brightfield condenser+DIC and
   c) a 0.85NA phase contrast condenser?
   What must you use with the brightfield condenser to ensure you're getting the maximal NA? What should you do when sitting down to your microscope to get the maximal achievable NA under <u>all</u> of these conditions?

7. Assuming the setup from question 6, would you want to use the brightfield condenser, the brightfield condenser+DIC, or phase contrast to image the following samples? (Some samples may have multiple correct answers)

   a) changes in nuclear morphology in unstained HeLa cells grown on glass coverslips(nuclear size =10μm)

b) changes in nuclear morphology in unstained HeLa cells grown on (birefringent) plastic tissue culture dishes

c) strong histological staining of protein accumulation in dendrites in a 0.5mm thick brain slice (dendrite diameter=0.25μm)