Homework Set 7.1 – Assigned 2/20/2018, Due 2/27/18 by 10:30am

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Office hours – Broad 3rd floor kitchen – Friday 2/23 4:30-5:30pm, Monday 2/26 5-6pm or by appointment

Problem 1 (20 points) – Live cell light microscopy

You decide to image HeLa cells by live cell light microscopy. You are interested in observing clathrin-mediated endocytosis in cells that are migrating. You read that the cells crawl at a rate of 1 μm/minute and that clathrin-mediated endocytosis takes about 1 minute total (http://www.cell.com/cell/pdf/S0092-8674(16)30208-2.pdf).

Live cell fluorescence microscopy requires many extra considerations to keep the cells healthy (https://www.microscopyu.com/applications/live-cell-imaging/maintaining-live-cells-on-the-microscope-stage).

1.a. (6 points) You want to image your cells over several hours. Briefly discuss three factors you need to monitor to keep the cells alive during the experiment. (Note, there are more than three!)

1.b. (4 points) You want to image your cells over several hours. Discuss how you would adjust the data collection to reduce cell stress.

1.c. (5 points) Many mammalian cell culture media contain a pH indicator dye such as Phenol red. Why do many scientists suggest avoiding phenol red in a live cell imaging experiment?

1.d. (5 points) How can media pH be monitored in the absence of Phenol red indicator?

Problem 2 (35 points)– Objective lenses and aberrations

2.a. (5 points) Chromatic aberration can be longitudinal or lateral. Sketch and describe each type. Hint https://www.microscopyu.com/tutorials/chromatic

2.b. (5 points) In a few sentences, describe how apochromat and achromat objective lenses mitigate chromatic aberration.

The optical tube length of a microscope refers to the distance between the objective rear focal plane and the intermediate image plane. In finite tube length microscope systems, this distance is usually 160 mm, but some manufacturers sell a 170 mm version.

Examine the schematic of a compound microscope on the next page. On the right is a schematic of the microscope and on the left is a ray diagram. The orange box outlines the objective lens.

Image reproduced from http://microscopy.berkeley.edu/courses/tlm/cmpd/StandardLengths.html

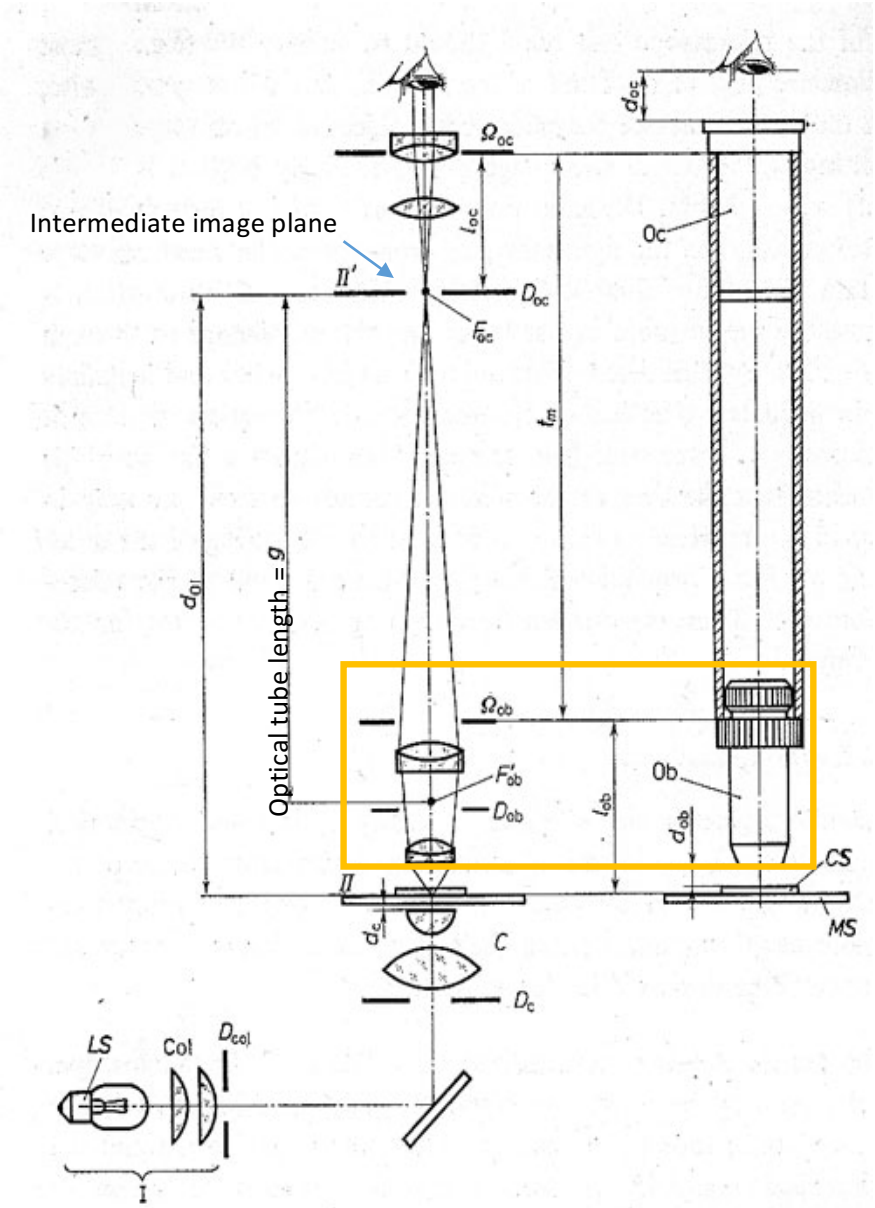


Fig. 2.41. Standard length parameters of microscope objectives and oculars.

2.c. (10 points) Let's say this microscope is designed for an optical path length of 170 mm. If a 160 mm objective lens is used in this microscope, how would the ray diagram change? Draw on the schematic and explain in a few sentences. Hint: three parameters will change

2.d. (10 points) Modern microscopes typically have infinity optical systems. Draw a diagram similar to the one above to explain the difference between infinity optical systems and finite tube length microscope systems.

2.e. (5 points) One common misconception is that the term infinity-corrected optics means that there is infinite space for additional elements (like filters, prism, polarizers) in the parallel optical path between the objective lens and the tube lens. What does the infinity designation actually refer to?

Problem 3 (20 points) – Numerical aperture

3.a. (3 points) What are the fundamental resolution limits for wide-field optical light microscopy?

3.b. (5 points) How does oil immersion increase the resolving power of a lens? Please draw a simple diagram to illustrate your answer.

3.c. (6 points) Your light microscope accepts three different objective lenses: an air objective lens (index of refraction n=1, half-angle θ=48˚), a water objective lens (index of refraction n=1.33, half-angle θ=58˚), and an oil immersion objective lens (index of refraction n=1.5, half-angle θ=68˚). Calculate the numerical aperture for each lens.

3.d. (6 points) What is the resolution of each lens? Assume a wavelength of 532 nm.

Problem 4 (25 points) – Digital image size

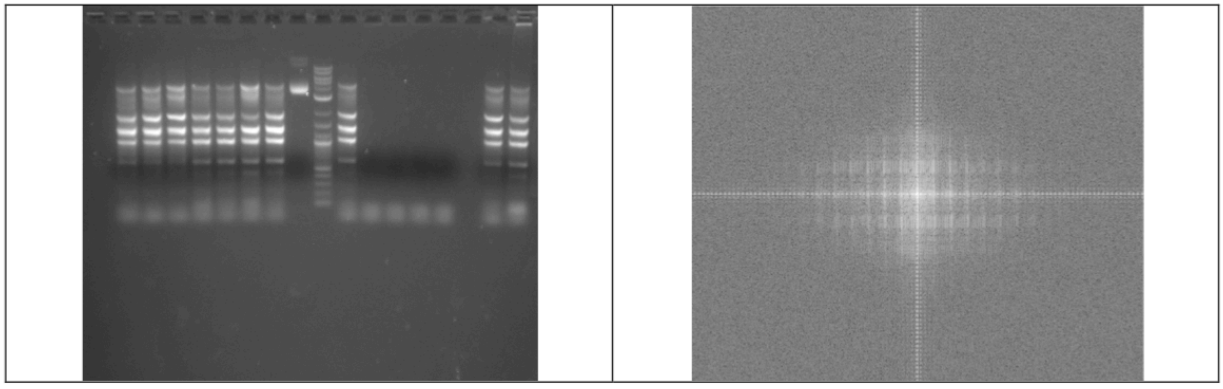
Your microscope has a 1024 by 1024 pixel sensor, and each image it takes has 16 bits of intensity information in each of the red, green, and blue bands.

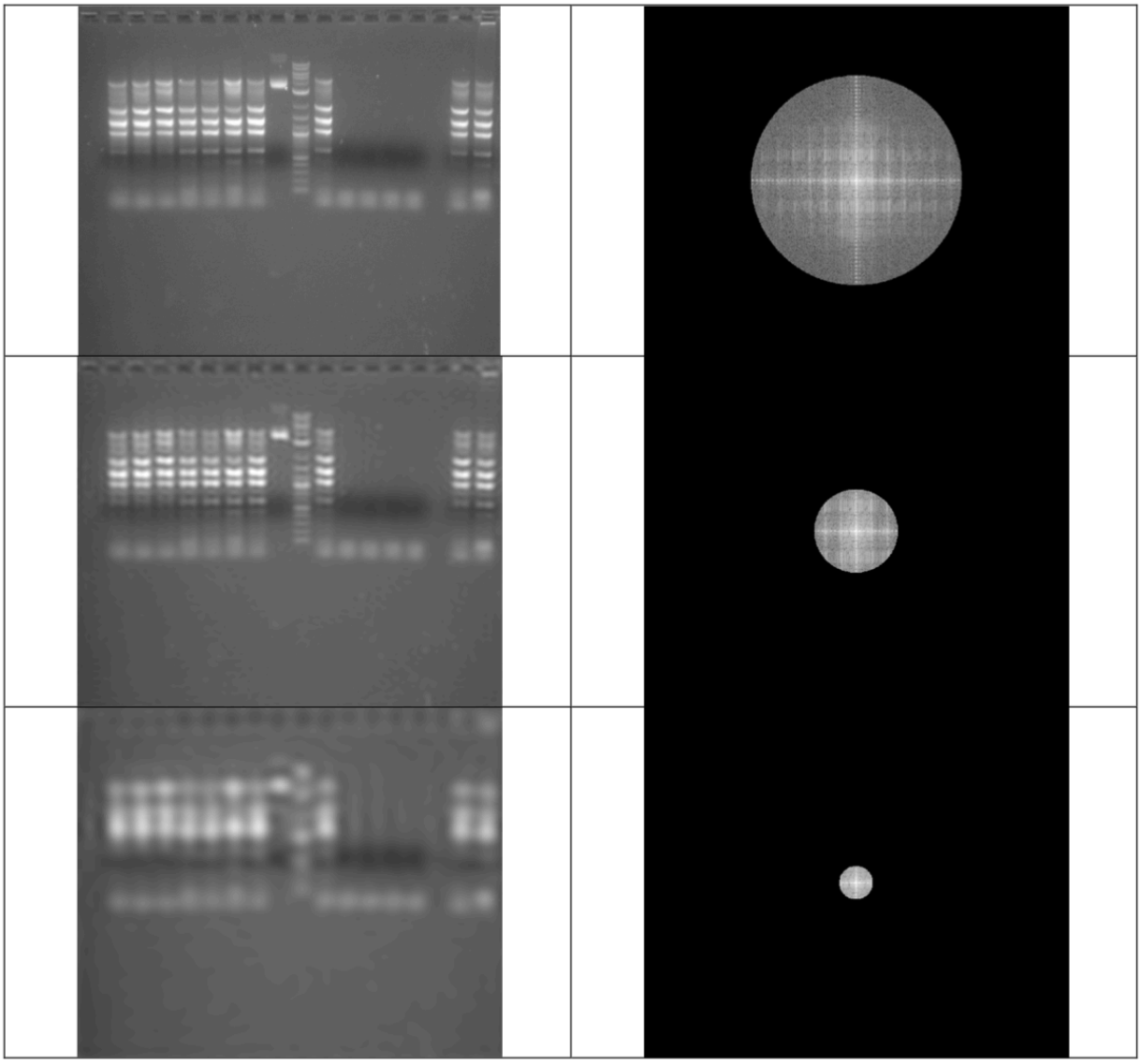
4.a. (5 points) How many bits is a single image? How many bytes are required to store the image?

4.b. (5 points) You want to do a 24-hour long time-lapse series in the microscope where you take one image at full resolution, full intensity information, with all three color bands. Your hard drive has 80 billion bits of space (9.3 gigabytes). How often can you take a picture without having the hard drive run out of space during the time-lapse series?

4.c. (5 points) How many fewer bits are required to store the image if you a) reduce the image's width and height by 50%, b) reduce the number of bits of intensity information to 8 bits, c) keep only one of the color bands, or d) do all simultaneously?

You have an image of a gel you want to send to your advisor. On the next page are the image and the Fourier transform of that image (note: these are much easier to view on the computer, when you print them they get much darker and harder to interpret).





If the frequency information is removed from outside a circle in the transform, low pass filtering the image, and the inverse Fourier transform is taken, we can see the effect on the reconstituted image.

4.d. (5 points) The circle's radius is 25%, 10%, and 4% of the width of the image. How many bits would it take to store just the data inside the circle, as opposed to storing the full Fourier transform? Assume that the source image is a 1024 x 1024 pixel square and has 8 bits of grayscale data per pixel. How does this compare to your previous answer?

4.e. (5 points) The correct term for the images to the right is a "power spectrum", which only displays the square of the amplitudes of the Fourier transform. What other information is needed to reconstruct the source image and how would it be used?