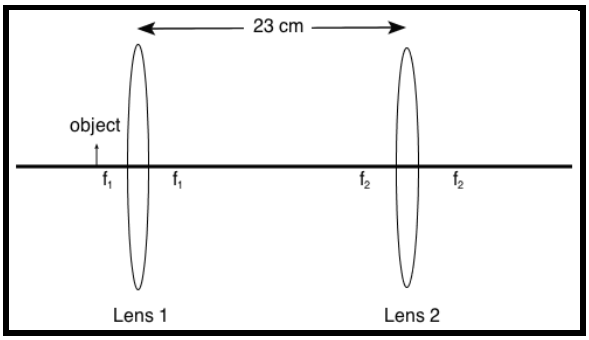
Physics 390 Practice Midterm

1. Consider the configuration shown below. Lens 1 has a focal length $f_1 = 3$ cm, and lens 2 has a focal length of $f_2 = 4$ cm. The center-to-center separation between the lenses is 23 cm. The object is 3.5 cm from Lens 1.



   (A) Determine the location and type of the final image using the thin lens equation. Also determine the magnification generated by the first lens using the transverse magnification equation.

   (B) Sketch (to reasonable accuracy) the ray trajectories through both lenses.

2. You are using a microscope and a grating as a specimen.

   (A) Your grating has 1000 lines/mm. Compute the spacing between lines, d, and use the grating equation, $m\lambda = d \sin\theta$, to predict the angles at which the grating will diffract light when illuminated by light with λ = 500 nm.

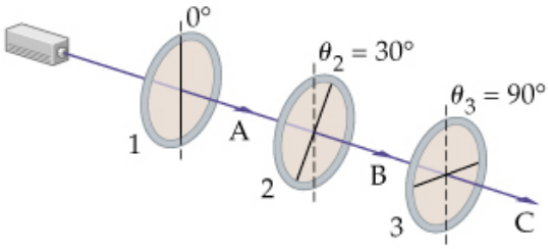
   (B) Your air objective has NA = 0.6. How many diffraction orders will the objective capture? According to Abbe, will you see an image of the grating? Justify your answer!

3. Sketch the vector (Z, T, D) diagrams describing:

   (A) Brightfield and negative phase contrast imaging.

   (B) For each case, comment briefly on the relative lengths of the Z and T vectors and what these lengths imply about contrast.

4. Consider the polarization configuration at right. The laser emits unpolarized light of intensity 100 W/m^2.

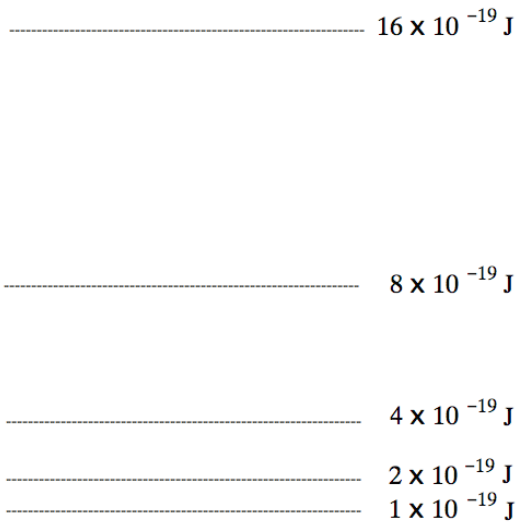


   (A) Calculate the intensities at positions A, B, and C.

   (B) What is the intensity at C if polarizer 2 is removed?

   (C) Use the results in (A) and (B) to explain the essence of polarization microscopy, where polarizer 2 is the analog of a birefringent specimen.

5. Consider a hypothetical atom with five quantized energy states, as shown.



(A) How many lines would be present in the emission spectrum?

(B) What is the shortest wavelength photon emitted by this atom?

6. Fill in the blanks by choosing the technique that best suits your needs. Your technique options are:

Brightfield, Phase Contrast, Darkfield, Polarization, Wide-field Fluorescence, LSCM, SDCM (spinning disk), and Deconvolution

You want to:

(A) Obtain a blur-free fluorescence image using a software approach _____

(B) Image a crystal _____

(C) Image a fixed cell that is intrinsically absorbing _____

(D) Obtain a blur-free fluorescence image using a hardware approach _____

(E) Generate a multi-color image of specific structures in living cells without correcting for blur _____

7. Fill in the blanks by choosing the technique that provides the best answer. Your technique options are:

Two-photon, TIRFM, STED microscopy, SIM, and PALM

You want to:

(A) Generate a super-resolution image without implementing any post-acquisition processing _____

(B) Achieve two-fold resolution enhancement using standard fluorophores _____

(C) Image single-molecules _____

(D) Image about a millimeter into the cortex of a mouse brain _____

(E) Image movement of a protein at the surface of a cell growing on glass _____

8. Order the steps in setting up Köhler illumination (some options may be irrelevant):

A. Center the condenser/field diaphragm	E. Insert polarizer
B. Adjust the field diaphragm to match the field of view	F. Focus on the sample
C. Align the condenser annulus over the phase plate	G. Look in the back focal plane
D. Focus on the field diaphragm	H. Adjust contrast with the condenser diaphragm

9. Match the techniques below to their optical basis.

Brightfield	A. Rejection of out-of-focus light
PALM	B. Birefringence
Polarized light	C. Light absorption
LSCM	D. Stochastic switching
	E. Superposition of slightly sheared beams

10. Which of the following components are in conjugate planes?

(A) Light source and condenser diaphragm/condenser FFP

(B) Objective BFP and retina

(C) Field diaphragm and intermediate image

(D) Sample and condenser diaphragm/condenser FFP

11. List the image-forming conjugate planes in the Köhler pathway.

12. The Nyquist theorem states that the optimal sampling rate is slightly less than:

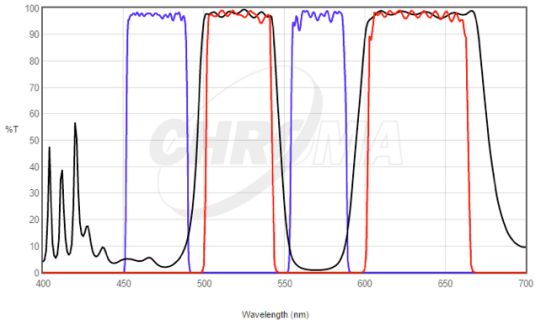
(A) $\frac{1}{10}\times$ smallest dimension that you want to resolve

(B) $\lambda/2NA$

(C) $\frac{1}{2}\times$ smallest dimension that you want to resolve

(D) $2\times$ smallest dimension that you want to resolve

13. The diagram at right describes the spectral characteristics of a two-color filter set.

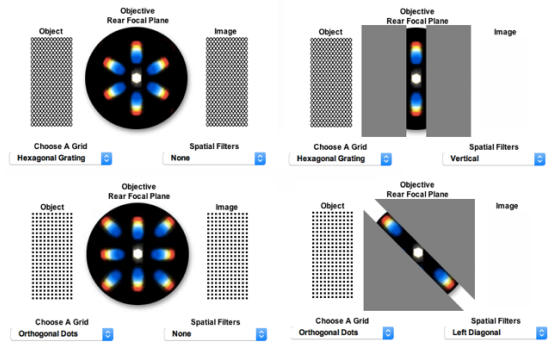


(A) What are the excitation ranges for the fluorophores? _____

(B) What are the emission ranges for the fluorophores? _____

(C) Which color describes the dichromatic mirror? _____

14. Sketch the image that is produced by the spatial filtering operations shown in the figure below.



Directions: Indicate if the following statements are true or false by writing T or F in the space provided.

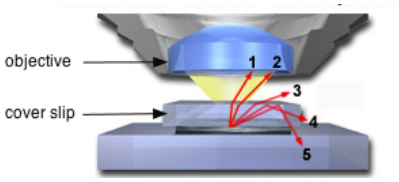
1. _____ Cells typically exhibit good contrast under Brightfield illumination.

2. _____ In a compound microscope, the specimen is placed at a distance, o, that is a little greater than the objective focal length, f.

3. _____ In two-photon microscopy, a fluorophore absorbs two photons essentially simultaneously, and this confines excitation and emission to a thin region where the illumination is very intense.

4. _____ In Köhler's method, uniform illumination is created by focusing the filament onto the specimen plane.

5. _____ The diagram at right most probably depicts an oil immersion objective, which has oil between the (glass) specimen cover slip and the objective.



6. _____ An optical microscope will generate <u>*no*</u> image of an isolated object that is smaller than $\sim \lambda/2$ because its size is smaller than the resolution limit.

7. _____ The $m = 0$ (central) diffraction spot in the back focal plane of a lens encodes a uniform background in the image plane.

8. _____ A handy rule of thumb is that the resolution of a conventional optical microscope is $\sim \lambda/4$.

9. _____ According to Ernst Abbe, the objective must capture at least one of the non-zero diffraction spectra to generate a resolved image.

10. _____ In Darkfield microscopy, oblique illumination enters the objective in the absence of a specimen and is diffracted away from the objective in the presence of the specimen.

11. _____ You want to manipulate the image of a mesh using spatial filtering and create an image consisting of a series of horizontal lines. Your filter should pass the central line of horizontal spots in the back focal plane of the lens.

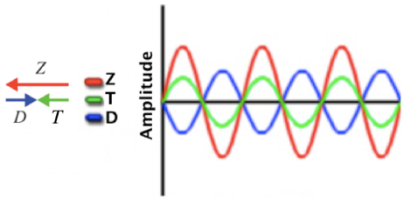
12. _____ In standard (one-photon) fluorescence microscopy, $\lambda_{\text{excitation}} > \lambda_{\text{emission}}$.

13. _____ The excitation band of a fluorophore should overlap with a region of low transmission by the dichromatic mirror.

14. _____ In phase contrast microscopy, the cell produces amplitude variations in the electric field that then are converted into phase variations in the electric field using spatial filtering.

15. _____ When the object distance, o, is $2f$, the ***magnitude*** of the transverse magnification is 1.

16. _____ The vector and interference diagrams at right show how the undiffracted (*Z*), total (*T*), and diffracted (*D*) waves relate in a method of image formation. For the case shown, the contrast is likely to be very poor.



17. _____ Super-resolution in optical microscopy can be achieved by viewing two closely spaced objects sequentially so that their diffraction patterns do not overlap during image acquisition.

18. _____ A thin, transparent specimen creates a diffracted (*D*) wave that is ~180° out of phase with the undiffracted (*Z*) wave.

19. _____ In laser scanning confocal microscopy, a pinhole in the back focal plane of the objective preferentially passes only light originating from in-focus parts of the specimen.