**Homework #8: Optical Microscopy**

*Chem 6614 Chemical Instrumentation Due Wed 23 April*

*Your name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_answers\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_/20 pts*

8.1 Which lens combination produces the shortest focal length ?

a) plano-plano b)concave-convex c)double convex d) double concave e) plano-convex

8.2. Material X of unknown refractive index nd(x) receives 40o air

a beam of sodium D light at the incident angle shown.

a) Given the refracted angle shown (25.27o), what is the value material X

of nd(x) ? Show work. 25.27o

nair ~1.0003 nair sin(40) =nxsin (25.27)=> 1.003\*sin (40)= nx sin(25.27)

nx = 1.0003 sin(40)/sin(25.27)=1.0003\*0.643/0.4268 =1.508 ~1.51

nd(x) = \_\_\_1.51\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b) what material is X most likely made of ? \_\_\_\_\_\_window glass\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

8.3. Why is both the simple magnifier image and the compound microscope image referred to as a

`virtual image’ ? *Because the image you `see’ is a mental projection (hence virtual, not real) of the real image painted on your retina created by the illusion that the real image is actually ~10 inches away-the default distance assumed by the eye since the real image falls inside your eye’s minimum focal distance.*

8.4. What is the difference between a phase object and an amplitude object ? (2 pts)

**Phase object has ~ same nd as medium (e.g. it wasn’t stainable)**

**Amplitude object’s nd significantly different that medium (or is stainable)**

8.5. What is the `default’ distance for the human eye ? \_\_\_\_10\_\_\_\_ inches or \_\_\_25\_\_\_\_\_\_ cm

8.6 What prevents us from serially placing many objectives between an object and the eyepiece to

produce unlimited magnification ? In other words, why can’t we see atoms with a typical optical

microscope ? (2 pts)

***lenses are not perfect…with each bearing a host of aberrations (chromatic, spherical etc.) causing loss of signal***

8.7a. Of the objectives listed below, which is the most expensive (=best) lens system? \_\_b\_\_\_\_\_\_\_

8.7b. Of the objectives listed below, which is the least expensive (=crappiest) lens system?\_\_\_\_c\_\_\_\_\_\_

8.7c. Which objective below is spherically-corrected at the Na-D line and chromatically corrected at Na-D

and either red or blue wavelengths ? (=> only one wavelength corrected for & plan) \_\_\_a,d\_\_

besides Na-D e.g. achromat

a)D-plan-achromat b) S-plan-apochromat c) E-achromat d) S-plan-achromat

8.8. What is the role of oil in an oil immersion objective ? increases numerical aperature by `sucking in’ more light

8.9. What two inventions located in ASC’s BH-2 Olympus Phase scope allow resolution of phase objects ?

Phase annulus, phase ring

(below stage), (above objective)

8.10 The numeric apertures of higher magnification objectives are always higher than lower magnification

Objectives. Why don’t we just use the higher magnification objectives since their light gathering power

is greater ? (Hint: what physical trade-off occurs as we move to higher magnification?)

*higher magnification objectives must be focused closer to stage, reducing the area of light collection and reducing total light input, even though NA goes up*

8.11 The individual particles of silica packing of an HPLC column are said to be separated by ~0.2 μm.

Assuming you are working with an objective with NA=0.60, and with orange light with λ=500 nm,

can you resolve the particles in the packing ? (2 pts show work) YES **NO**

Why: ( a calculation) R= 0.61\*λ/NA = 0.61\*500/0.6=508 nm=508\*10-9 m =0.508 μm

This is larger than the 0.2 μm separation needed, so we could not resolve

Particles in packing

8.12. You wish to observe an un-stainable strain of *staph aureus fongmoronicus* whose diameter is roughly

0.5 μm. Given that the human eye can distinguish objects down to about 0.1 mm, and given that

you’ve got a 10X eyepiece , suggest :

Magnification ~NA

1. An objective and likely NA to use (check BH-2 for likely NA) \_\_\_\_40\_X \_0.65\_\_\_\_

1 pt 1 pt

1. Would you want to use a phase scope ? **Yes** No explain why or why not below (1 pt)

***Staph is unstainable so you need phase contrast to see***

0.5 μm = 0.5\*10-6 m=0.5\*10-3 mm = object size

0.5\*10-3 mm (object size) = 1

0.1 mm eye limit Me\*Mo

Me\*Mo > .1/.0.0005= 200

10\*Mo > 200

Mo =40 NA~0.65