Microscopy Problem Set

1. Which of the following does not describe NA
    - Numeric aperture
    - The size of a pinhole necessary for two-photon microscopy
    - Characterizes the range of angles over which the system can accept or emit light.
    - *n* sin α where n is the incidence of refraction of the media and α is the maximal half-angle of the cone of light that can enter or exit the lens

2. Background signal (out of focus light) is typically a problem with which microscopy technique and why?

    - epifluorescence
    - two-photon
    - confocal
    - TIRF
    - light sheet

3. Match the concept with its key feature:

TIRF	absorption
confocal	virtual pinhole
AiryScan	evanescent field
dichromatic mirror	pinhole

4. You've mounted a tissue sample that expresses a GFP-tagged protein in glycerol (refractive index, n = 1.45). If the angle of emitted light from a GFP tagged protein is 31°, what is the refracted angle through the glass coverslip (n = 1.5)?

Fluorophore	**Excitation**	**Emission**
BFP	378	440
CFP	435	475
GFP	399	510
YFP	512	527
RFP	553	573

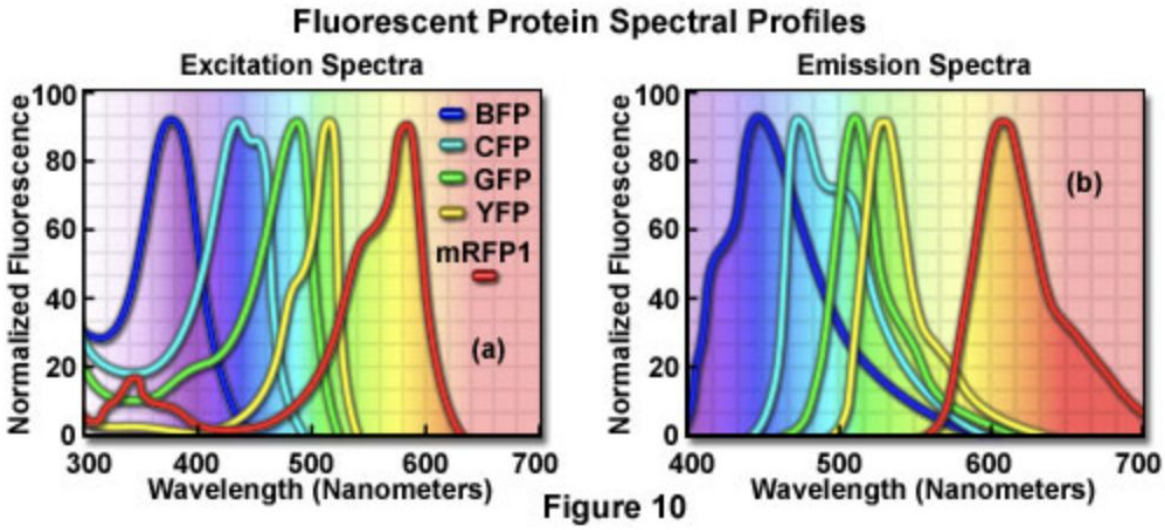

Figure 10

5a. Which fluorophore pair constitutes a 'good' FRET pair, and why:

- CFP-YFP
- GFP-BFP
- BFP-GFP
- CFP-GFP
- GFP-YFP
- BFP-RFP

5b. If you're trying to visualize two proteins, which fluorophore pair might you want to avoid, and why:

- GFP/RFP
- CFP/YFP
- BFP/CFP
- BFP/RFP
- GFP/YFP

6. What is the resolution of a RFP-tagged protein (see table of ex/em wavelength) examined with a 63x, oil-immersion 1.3 NA lens.

6b. What is the z-resolution of this protein if the refractive index of oil used is 1.5?

7. Match the technique to one of the proposed studies. (While there might be several techniques that each experiment might use, for these purposes, each technique will be used only once).

A. 2-Photon

B. Epifluorescence

C. FRAP

D. FRET

E. TIRF

1. You want to examine the potential of exocytosis of glutamatergic neurotransmitter vesicles in cultured astrocytes (flat glial cells.) You have a GFP-tagged vesicular glutamate transporter 1 (vGLUT1).
2. You want to examine if dopamine neurons in the substantia nigra also express the ionotropic AMPA receptor subunit GluR2. You have a very good chicken antibody to the dopamine transporter (DAT) and a rabbit antibody against GluR2.
3. You want to examine the fluidity of NMDA receptor subunits in the soma of cultured glutamatergic neurons. You have a functional GFP-tagged NMDA receptor subunits.
4. You are interested in the dynamics of dendritic spines in the hippocampus. He wants to see if spine formation and loss follows any rhythmicity. You have a transgenic mouse that expresses CFP.
5. You want to know if your newly discovered protein, Dice, directly interacts with the sodium channel, NaV1.1. You have a CFP-tagged DICE and an YFP-tagged NaV1.1.