Labs 3-4. MOST DIFFICULT TOPICS (preparation for Final Exam)
Confocal microscopy offers several advantages over conventional widefield optical microscopy, including (1) the ability to control depth of field, (2) elimination or reduction of background information away from the focal plane (that leads to image degradation), (3) the capability to collect serial optical sections from thick specimens.

The basic key to the confocal approach is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus.

http://www.olympusfluoview.com/theory/confocalintro.html
Confocal microscopy provides only a marginal improvement in both axial (z; along the optical axis) and lateral (x and y; in the specimen plane) optical resolution, but is able to exclude secondary fluorescence in areas removed from the focal plane from resulting images. Even though resolution is somewhat enhanced with confocal microscopy over conventional widefield techniques, it is still considerably less than that of the transmission electron microscope. In this regard, confocal microscopy can be considered a bridge between these two classical methodologies.
Pollen Grain Serial Optical Sections by Confocal Microscopy

Figure 6

Three-Dimensional Volume Renders from Confocal Optical Sections

(a) (b) (c) Figure 7
What is a fluorescence lifetime? If a fluorescence lifetime is $\tau$, how will a transition intensity change in time?

The fluorescence lifetime refers to the average time the molecule stays in its excited state before emitting a photon. Fluorescence typically follows first-order kinetics:

$$\frac{dN(t)}{dt} = -\Gamma_{tot} N(t) = -\frac{1}{\tau} N(t)$$

so $N(t) = N_o \exp(-t/\tau)$

where $N(t)$ is the concentration of excited state molecules at time $t$, $N_o$ is the initial concentration and $\Gamma_{tot}$ is the decay rate or the inverse of the fluorescence lifetime $\tau$.

Fluorescence intensity $F(t)$ is proportional to the excited state population $F(t) = \Gamma_{rad} N(t) = 1/\tau_{rad} N(t)$.

Various radiative and non-radiative processes can de-populate the excited state. In such case the total decay rate is the sum over all rates: $\Gamma_{tot} = \Gamma_{rad} + \Gamma_{nrad}$

where $\Gamma_{tot}$ is the total decay rate, $\Gamma_{rad}$ the radiative decay rate and $\Gamma_{nrad}$ the non-radiative decay rate.

For commonly used fluorescent compounds, typical fluorescence lifetimes are within the range of 0.5 to 20 ns.

The lifetime is an average value of the time spent in the excited state. For a single exponential decay 63% of the molecules have decayed prior to $\tau$ and 37% decay at $t>\tau$. (see J.R. Lakowicz, Fluorescence spectroscopy, Plenum Press, NY (1983).
CdSe quantum dots
ex=395nm
cm=470nm

τ₁ = 3.4ns (41%)
τ₂ = 19.1ns (34%)
τ₃ = 85.8ns (17%)
τ₄ = 200.2ns (8%)

Chi-sq=0.88

CdSe quantum dots - ESM distribution analysis

Amplitude

3.4ns
20.2ns
83.1ns
18.8ns

Lifetime (ns)
What are photonic bandgap and photonic crystal materials?

*Photonic band gap* (PBG) materials are a new class of dielectrics which are the photonic analogues of semiconductors. The photonic band gap is a wavelength (frequency) interval over which linear electromagnetic propagation effects have been turned off (light does not go through).

Photonic bandgap materials are dielectric structures with a periodically varying refractive index with the periodicity of the order of the light wavelength.

Use photonic crystal terminology only for 2D and 3D periodic structures that have substantial index contrast.
Bragg scattering

Regardless of how small the reflectivity $r$ is from an individual scatter, the total reflection $R$ from a semi infinite structure:

$$R = r e^{-ikx} + re^{-2ika} e^{-ikx} + re^{-4ika} e^{-ikx} + ... = r e^{-ikx} \frac{1}{1 - e^{-2ika}}$$

Diverges if

$$e^{2ika} = 1 \quad \Rightarrow \quad k = \frac{\pi}{a}$$

Bragg condition

Light can not propagate in a crystal, when the frequency of the incident light is such that the Bragg condition is satisfied

Origin of the photonic band gap
LIQUID CRYSTALS:

(1) What type of liquid crystal should you take to obtain selective reflection?
(2) Describe the procedure for obtaining selective reflection from the liquid crystal layer.
(3) What parameters of liquid crystal define the wavelength of a maximum of a selective reflection band?
(4) What parameters of liquid crystal define the spectral bandwidth of a selective reflection curve?
(5) Make a plot of a selective reflection curve of the liquid crystal sample that you prepared.

**Crystal phase**
- Centers and directions both aligned

**Nematic (liquid crystal) phase**
- Centers random, directions approximately aligned

**Isotropic (liquid crystal) phase**
- Centers and directions random

Cholesteric (chiral nematic)
1) What type of liquid crystal should you take to obtain selective reflection?

Cholesteric (chiral nematic)
CHIRALITY

An object that cannot be superimposed on its mirror image is called chiral

Chiral objects
- Nonsuperimposable
  - mirror images

Nonchiral objects
- Superimposable
  - mirror images
(2) Describe the procedure for obtaining selective reflection from the liquid crystal layer.

Planar alignment by unidirectional motion of substrates.
3) What parameters of liquid crystal define the wavelength of a maximum of a selective reflection band?

\[ \lambda_o = n_{av} P_o, \]

where pitch \( P_o = 2a \)
(a is a period of the structure);
\[ n_{av} = (n_e + n_o)/2 \]

(4) What parameters of liquid crystal define the spectral bandwidth of a selective reflection curve?

\[ \Delta\lambda = \lambda_o \Delta n/n_{av} \quad \Delta n = n_e - n_o. \]

5) Make a plot of a selective reflection curve of the liquid crystal sample that you prepared.
where for most CLC media with rod-like molecules, the principal
dielectric constants can be written as $\varepsilon_1 = n_e^2, \varepsilon_2 = n_o^2, \varepsilon_3 = n_o^2$.

$$\varepsilon(z) = R(-\varphi)\varepsilon_{princ}R(\varphi)$$

$$R(\varphi) = \begin{pmatrix} \cos \varphi & \sin \varphi & 0 \\ -\sin \varphi & \cos \varphi & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

$$\varepsilon(z) = \begin{pmatrix} n_{av}^2 + \Delta n_{av}\cos(2\pi z / p) & \pm \Delta n_{av}\sin(2\pi z / p) & 0 \\ \pm n_{av}^2\sin(2\pi z / p) & n_{av}^2 - \Delta n_{av}\cos(2\pi z / p) & 0 \\ 0 & 0 & n_o^2 \end{pmatrix}$$

A period of dielectric properties of a CLC coincides with a half-pitch $p/2$ of
the helix since the anisotropy directions are rotated by a half-turn and 180
degree rotation is physically identical to 360 degree rotation.
Selective reflection curves of 1-D photonic bandgap planar-aligned dye-doped cholesteric layers (mixtures of E7 and CB15)
The values of a second order correlation function $g^{(2)}(0)$

Colloidal semiconductor quantum dots in a cholesteric liquid crystal photonic bandgap microcavity

$g^{(2)}(0) = 0.18 \pm 0.03$

$g^{(2)}(0) = 0.11 \pm 0.06$

Colloidal semiconductor quantum dots in a cholesteric liquid crystal photonic bandgap microcavity

NV color centers in nanodiamonds (~25 nm monocrystals)

$g^{(2)}(0) = 0.54 \pm 0.06$

For antibunched light $g^{(2)}(0) < 1$; $g^{(2)}_{\text{max}}(\tau) = 1$.

For coherent light $g^{(2)}(\tau) = 1$ for all values of $\tau$ including $\tau=0$.

For bunched light $g^{(2)}(0) > 1$ and $g^{(2)}(0) > g^{(2)}(\tau)$. 