Introduction to Optical Microscopy (1st Edition)
Problem Set

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Chapter 1

Introduction

Problem 1

Consider an "afocal" arrangement where the lenses are separated by distance $f_0 + f_1$

1) Calculate ABCD transfer matrix between plane 0 located a distance s_0 in front of lens 0, and plane 1 located a distance s_1 behind lens 1.

2) What happens when $s_0 = f_0$ and $s_1 = f_1$? (This is called a 4f, or telecentric, imaging configuration)

How does 4f imaging compare with single-lens imaging? (e.g. which is better?)

Problem 2

Consider a 4f imaging arrangement of the type described in problem 1. That is, two lenses of focal lengths f_0 and f_1 are separated by distances $f_0 + f_1$. The object plane is located a distance f_0 in front of the lens 0. The corresponding image plane is located a distance f_1 behind lens 1. Consider a slight error such that lens 1 is displaced a distance ε from its nominal 4f position (where $\varepsilon \ll f_0 < f_1$).

1) Derive the imaging transfer matrix for the case where the object plane remains at it's initial position? What is the magnification? Why is this magnification not well defined?

2) Where should the imaging plane be for the magnification to be well defined?

Problem 3

Consider two single-lens imaging systems with lenses f_0 and f_1 and magnifications M_0 and M_1 respectively. Place these two imaging systems in tandem (i.e. 3 conjugate planes).

1) Calculate the ABCD transfer matrix from the first conjugate plane to the last conjugate plane. What is the net magnification? Is the imaging perfect?

2) Now place a lens f exactly at the middle conjugate plane (this is called a field lens). Re-calculate the above ABCD matrix. Has the net magnification changed?

3) At what value of f is the imaging perfect?

4) A field lens is also useful for increasing the field of view. That is, given that lenses have finite diameters, a field lens can allow the imaging of bigger objects. Can you explain why (qualitatively)?

Chapter 2

Monochromatic wave propagation

Problem 1

A paraxial wave propagating in the z direction may be written as

$$E(\vec{r}) = A(\vec{r})e^{i2\pi\kappa z} \tag{2.1}$$

where the envelope function $A(\vec{r})$ is slowly varying. The conditions for $A(\vec{r})$ to be slowly varying are

$$\lambda\frac{\partial A(\vec{r})}{\partial z} \ll A(\vec{r}) \tag{2.2}$$

$$\lambda\frac{\partial^2 A(\vec{r})}{\partial z^2} \ll \frac{\partial A(\vec{r})}{\partial z}. \tag{2.3}$$

1) Show that in free space (no sources), the envelope function of a paraxial wave satisfies a simplified version of the Helmholtz equation given by

$$\left(\nabla_\perp^2 + i4\pi\kappa\frac{\partial}{\partial z}\right)A(\vec{r}) = 0. \tag{2.4}$$

This equation is known as the paraxial Helmholtz equation.

2) The Fresnel free-space propagator may be written as a paraxial wave, such that

$$H(\vec{\rho}, z) = H_A(\vec{\rho}, z)e^{i2\pi\kappa z} \tag{2.5}$$

where $H_A(\vec{\rho}, z) = -i\frac{\kappa}{z}e^{i\pi\frac{\kappa}{z}\rho^2}$ is the associated envelope function. Show that $H_A(\vec{\rho}, z)$ satisfies the paraxial Helmholtz equation.

3) The radiant field associated with a paraxial wave may be written as

$$\mathcal{E}(\vec{\kappa}_\perp; z) = \mathcal{A}(\vec{\kappa}_\perp; z)e^{i2\pi\kappa z}. \tag{2.6}$$

Show that $\mathcal{A}(\vec{\kappa}_\perp; z)$ satisfies a mixed-representation version of the paraxial Helmholtz equation given by

$$\left(\pi\kappa_\perp^2 - i\kappa\frac{\partial}{\partial z}\right)\mathcal{A}(\vec{\kappa}_\perp; z) = 0. \tag{2.7}$$

4) Finally, show that a field that satisfies the Fresnel diffraction integral also satisfies the paraxial Helmholtz equation (hint: this is much easier to demonstrate in the frequency domain).

Problem 2

Let $A(\vec{r})$ be the envelope function of a paraxial wave, as defined in Problem 1. That is, $A(\vec{r})$ satisfies the paraxial Helmholtz equation. In general, $A(\vec{r})$ is complex and can be written as

$$A(\vec{r}) = \sqrt{I(\vec{r})}e^{i\phi(\vec{r})} \tag{2.8}$$

where $I(\vec{r})$ is the wave intensity and $\phi(\vec{r})$ is a phase, both real-valued.

Show that $I(\vec{r})$ and $\phi(\vec{r})$ satisfy the equation

$$2\pi\kappa\frac{\partial I(\vec{r})}{\partial z} = -\vec{\nabla}_\perp \cdot I(\vec{r})\,\vec{\nabla}_\perp\phi(\vec{r}). \tag{2.9}$$

This is known as the intensity transport equation.

Problem 3

Consider two point sources located on the x_0 axis at $x_0 = \frac{d}{2}$ and $x_0 = -\frac{d}{2}$. Use the Fresnel and Fraunhofer diffraction integrals to calculate the resultant fields $E_{\text{Fresnel}}(x, 0, z)$ and $E_{\text{Fraunhofer}}(x, 0, z)$ obtained after propagation a large distance z. Derive the corresponding intensities $I_{\text{Fresnel}}(x, 0, z)$ and $I_{\text{Fraunhofer}}(x, 0, z)$ (note: these are observed to form fringes).

1) Derive the fringe envelope functions of $I_{\text{Fresnel}}(x, 0, z)$ and $I_{\text{Fraunhofer}}(x, 0, z)$. In particular, what is the ratio of these envelope functions at the location $x = z$?

2) Derive the fringe periods of $I_{\text{Fresnel}}(x, 0, z)$ and $I_{\text{Fraunhofer}}(x, 0, z)$. In particular, what is the ratio of these periods at the location $x = z$? (note: the periods may vary *locally*)

3) Which approximation, Fresnel or Fraunhofer, is better off axis?

Chapter 3

Monochromatic field propagation through a lens

Problem 1

Consider a 4f imaging system of unit magnification (i.e. both lenses of focal length f), with an unobstructed circular aperture of radius a.

1) Derive CSF(ρ) in the case where an obstructing disk of radius $b < a$ is inserted into the aperture.

2) Derive CSF(ρ) in the case where the disk is transmitting but produces a phase shift of 90°.

3) Derive CSF(ρ) in the case where the disk is transmitting but produces a phase shift of 180°.

4) Consider imaging an on-axis point source of light with either of the above systems. Compared to the unobstructed aperture system, is it possible to obtain an increase in the image intensity on axis? If so, under what conditions? Is it possible to obtain a nul in the image intensity on axis? If so, under what conditions?

Problem 2

Consider inserting a thin wedge into an otherwise unobstructed circular pupil of radius a of a 4f imaging system (both lenses of focal length f). The wedge induces a phase shift that varies linearly from 0 at the far left to 2ϕ at the far right of the aperture. Derive the CSF of this imaging system. (Hint: use the Fourier shift theorem).

Problem 3

1) Show that if $P(\vec{\xi})$ is binary (i.e. $P(\vec{\xi}) = 0$ or 1), then

$$\int \mathrm{CSF}(\vec{\rho}_c + \frac{1}{2}\vec{\rho}_d)\mathrm{CSF}^*(\vec{\rho}_c - \frac{1}{2}\vec{\rho}_d)d^2\vec{\rho}_c = \mathrm{CSF}(\vec{\rho}_d). \tag{3.1}$$

2) What is the implication of the above relation? In particular, what does it say about the imaging properties of two identical, unit-magnification, binary aperture imaging systems arranged in series?

Chapter 4

Intensity propagation

Problem 1

Derive the variable change identity given by Eq. 4.5. (Hint: use a Jacobian).

Problem 2

For a circular pupil imaging system, an alternative definition of resolution is given by what is known as the Rayleigh criterion. This criterion states that two point objects are resolvable if they are separated by a minimum distance $\Delta\rho_{\text{Rayleigh}}$ such that the maximum of the $\text{PSF}(\rho)$ of one point lies at the first zero of the $\text{PSF}(\rho)$ of the other point. That is, $\Delta\rho_{\text{Rayleigh}}$ is defined as the minimum distance such that $\text{PSF}(\Delta\rho_{\text{Rayleigh}}) = 0$.

1) Derive $\Delta\rho_{\text{Rayleigh}}$ in terms of λ and NA (you will have to do this numerically).

2) Consider a circular pupil imaging system where the pupil is partially obstructed by a circular opaque disk (centered) whose radius is η times smaller than the pupil radius ($\eta < 1$). Derive the PSF for this annular pupil system. What is the ratio $\text{PSF}_{\text{annular}}(0)/\text{PSF}_{\text{circular}}(0)$?

3) Provide a numerical plot of $\text{PSF}_{\text{annular}}(\Delta\kappa_\perp\rho)$ and $\text{PSF}_{\text{circular}}(\Delta\kappa_\perp\rho)$ for $\eta = 0.9$ (normalize both plots to unit maximum). What does the Rayleigh resolution criterion say about the resolution of the annular pupil system compared to that of the circular pupil system? Would you say the annular system has better or worse resolution?

Problem 3

1) Consider the propagation of incoherent light through a lens of focal length f. The lens is situated a distance f from the light source, whose 2D intensity distribution is given by $I_0(\vec{\rho}_0)$. Use the Fresnel approximation to derive a resultant 3D coherence function at an arbitrary position $\{\vec{\rho}_{1c}, z_{1c}\}$ beyond the lens. In particular, show that this 3D coherence function is given by

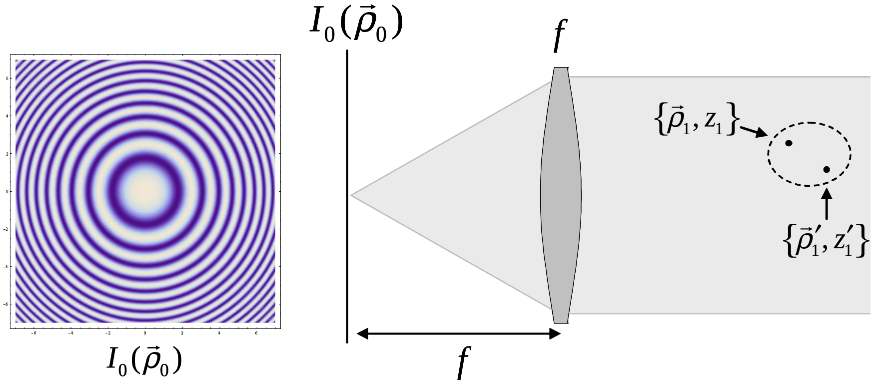
$$\mu_1(\vec{\rho}_{1d}, z_{1d}) = \frac{1}{W} e^{i2\pi\kappa z_{1d}} \int I_0(\vec{\rho}_{0c}) e^{-i2\pi\frac{\kappa}{f}\vec{\rho}_{1d}\cdot\vec{\rho}_{0c}} e^{-i\pi\frac{\kappa}{f^2}z_{1d}\rho_{0c}^2} d^2\vec{\rho}_{0c}. \tag{4.1}$$

Hint: you may find it helpful to start with Eq. 3.6.

2) Consider the specific example where the intensity distribution of the incoherent source is given by

$$I_0(\vec{\rho}_0) = \frac{1}{2} I_0 \left(1 + \cos(2\pi\rho_0^2/a^2)\right) \tag{4.2}$$

as illustrated in the figure. You will find that $\mu_1(\vec{\rho}_{1d}, z_{1d})$ is peaked when $\{\rho_{1d}, |z_{1d}|\} \rightarrow \{0, 0\}$, as expected; but it is also peaked for another value of $\{\rho_{1d}, |z_{1d}|\}$. What is this value?



Chapter 5

3D Imaging

Problem 1

1) Derive Eq. 5.22.

2) What is the implication of the above relation? In particular, what does it say about the imaging properties of two identical, unit-magnification, binary-aperture imaging systems arranged in series?

Problem 2

Consider a unit-magnification 4f imaging system (all lenses of focal length f) with a square aperture defined by

$$P(\xi_x, \xi_y) = \begin{cases} 1 & |\xi_x| < a \text{ and } |\xi_y| < a \\ 0 & \text{elsewhere.} \end{cases} \tag{5.1}$$

Based on the Fresnel approximation, derive analytically:
1) $\mathrm{CTF}(\kappa_x, 0; 0)$ and $\mathrm{CTF}(0, 0; z)$
2) $\mathrm{CSF}(x, 0, 0)$ and $\mathrm{CSF}(0, 0, z)$
3) $\mathrm{PSF}(x, 0, 0)$ and $\mathrm{PSF}(0, 0, z)$
4) $\mathrm{OTF}(\kappa_x, 0; 0)$ and $\mathrm{OTF}(0, 0; z)$.

It will be convenient to define a bandwidth $\Delta\kappa_\perp = 2\kappa\frac{a}{f}$.

Note, CTF and OTF are in mixed representations. You will run into special functions such as sinc(...) and erf(...). As such, this problem is best solved with the aid of integral tables or symbolic computing software such as *Mathematica*. Be careful with units and prefactors. For example, make sure the limits $x \to 0$ and $z \to 0$ converge to the same values!

Problem 3

Consider a unit-magnification 4f imaging system (of spatial frequency bandwidth $\Delta\kappa_\perp$) with a circular aperture. A planar object at a defocus position z_s emits a periodic, incoherent intensity distribution (per unit depth) given by

$$I_{0z}(x_0, y_0, z_0) = I_0\left(1 + \cos\left(2\pi q_x x_0\right)\right)\delta(z_0 - z_s) \tag{5.2}$$

where I_0 is a constant.

1) Write Eq, 5.39 in terms of intensity spectra and an OTF, all in mixed representation.

2) Based on your result above, derive an expression for the imaged intensity distribution. This expression should look like

$$I_1(x_1, y_1) \propto \left(1 + M(q_x, z_s)\cos\left(2\pi q_x x_1\right)\right). \tag{5.3}$$

In other words, the imaged intensity is also periodic, but with a modulation contrast given by $M(q_x, z_s)$. What is $M(q_x, z_s)$?

3) In the specific case where $q_x = \frac{1}{2}\Delta\kappa_\perp$, what is the modulation contrast when the object is in focus? At what defocus value does the modulation contrast fade to zero (express your result in terms of λ, n and NA)? What happens to the modulation contrast just beyond this defocus? (Hint: use the Stokseth approximation).

Please note: there is an error in the expression for the Stokseth approximation (Eq. 5.35). The factor of 4 in the jinc function is erroneous and should be omitted.

Chapter 6

Radiometry

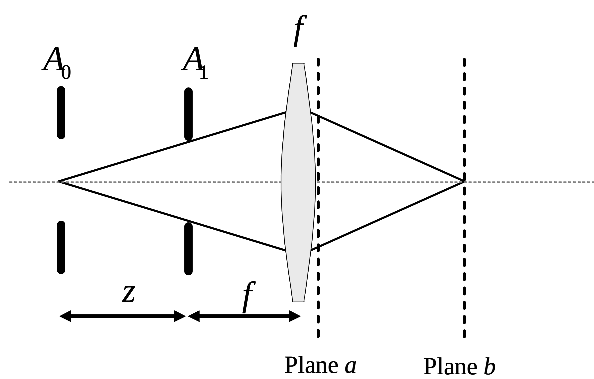
Problem 1

Consider the following single-lens imaging system, of arbitrary magnification M, which obeys the thin-lens formula. Assume the lens is large and $A_0 \approx A_1$.

1) Calculate the throughput of this system using the recipe outlined in Section 6.3.1, treating plane a as the output plane. Identify the aperture and field stops.

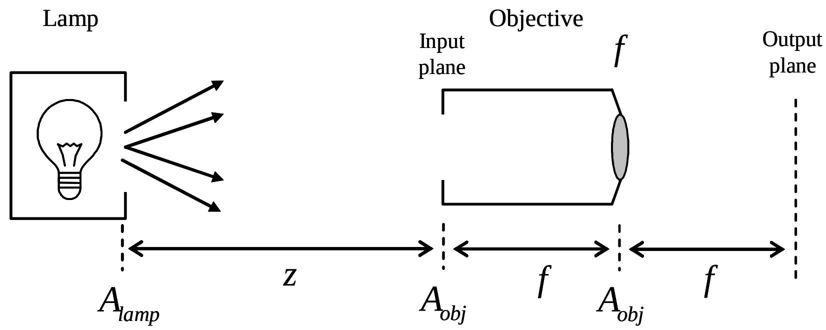
2) Now do the same, but this time treating plane b as the output plane. Are the aperture and field stops the same?

Note: you should find that the throughput is independent of which plane a or b is treated as the ouput plane.



Problem 2

A lamp in a housing emits incoherent light through an aperture of area A_{lamp} (see figure). The emitted light power is W_{lamp}. This light illuminates an objective comprising a lens and an aperture at the back focal plane, both of area A_{obj} (assume $A_{obj} \lesssim A_{lamp}$). The lens has focal length f_{obj}. A variable distance z separates the lamp and the objective.



1) In the case where the lamp touches the objective (i.e. $z = 0$), estimate the number of modes (coherence areas) that enter the objective at the input plane. What is maximum power of the beam at the output plane (i.e. the objective "front" focal plane)? What is the coherence area of the beam at the output plane? Estimate the beam spot size (total beam area) at the output plane.

2) In the case where the lamp separated a large distance z from the objective, estimate the number of modes that enter the objective at the input plane. What is the maximum power of the beam at the ouput plane? What is the coherence area of the beam at the output plane? Estimate the beam spot size at the output plane.

3) At what value of z does the beam at the output plane become a diffraction-limited spot (i.e. single mode)? At this value, what is the number of modes that enter the objective at the input plane?

Note: perform rough estimates only – that is, angular spreads of 2π steradians can be approximated as angular spreads of 1 steradian.

Problem 3

Consider a more general Gaussian-Schell beam whose mutual intensity is given by

$$J_0(\vec{\rho}_{0c}, \vec{\rho}_{0d}) = \left(I_0 e^{-2\rho_{0c}^2/w_c^2}\right)\left(e^{-\rho_{0d}^2/2w_d^2}\right). \tag{6.1}$$

(Note: this differs from the single-mode Gaussian beam described by Eq. 6.14 in that $w_c > w_d$).

1) Calculate the number of modes in this beam.

2) Calculate the area and coherence area of this beam upon propagation a large distance z. Show explicity that the number of modes is conserved.

3) Consider using a lens of numerical aperture NA_i to focus this beam. If the beam just fills the lens (roughly speaking), estimate the size the the resultant focal spot.

4) If instead the beam overfills the lens such that only 1% of the beam power is focused, estimate the size of the resultant focal spot.

Chapter 7

Intensity fluctuations

Problem 1

Non-monochromatic fields can be described by explicitly taking into account their time dependence. It can be shown that when the time dependence of a field is made explicit, the radiative Rayleigh-Sommerfeld diffraction integral (Eq. 2.21) can be re-written in the form

$$E(\vec{\rho}, z, t) = -i\kappa_0 \int \frac{\cos\theta}{R} E(\vec{\rho}_0, 0, t - R/c) d^2\vec{\rho}_0 \tag{7.1}$$

which is valid for narrowband fields whose wavenumber is centered around κ_0 (assuming propagation in vacuum). This expression can be simplified using the Fresnel approximation (Section 2.3). Based on this expression, evaluate the intensity distribution $I(\vec{\rho}, z)$ a distance z from two pinholes irradiated by a beam $I_0(\vec{\rho}_0, 0)$ that is partially coherent both in space and time. In particular, assume that the irradiating beam is both quasi-homogeneous and quasi-stationary, with a separable mutual coherence function given by

$$\Gamma(\vec{\rho}, \vec{\rho}'; t, t+\tau) = \langle I_0 \rangle \, \mu(\rho_d)\gamma(\tau) \tag{7.2}$$

where $\rho_d = |\vec{\rho} - \vec{\rho}'|$, and $\mu(\rho_d)$ and $\gamma(\tau)$ are Gaussian. That is, we have

$$\mu(\rho_d) = e^{-\rho_d^2/2\rho_\mu^2} \tag{7.3}$$

$$\gamma(\tau) = e^{-i2\pi\nu_0\tau} e^{-\pi\tau^2/2\tau_\gamma^2} \tag{7.4}$$

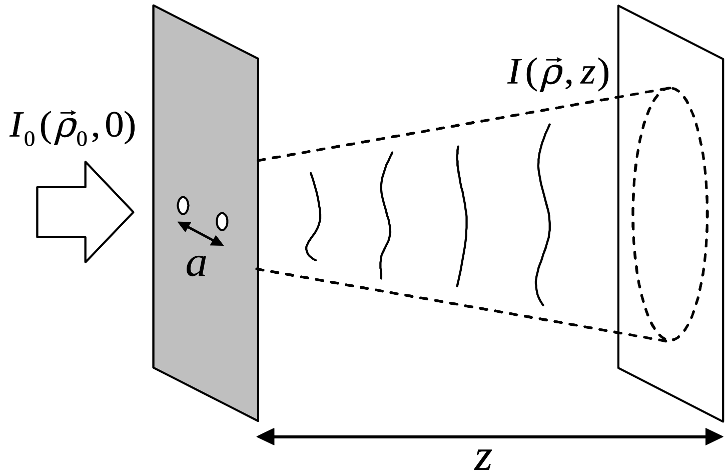
where $\nu_0 = \kappa_0 c$.

The pinholes are separated by a distance a along the x direction (see Figure).

1) Consider only the x direction and derive an expression for $I(x, z)$. Your expression should look something like

$$I(x, z) \propto \frac{1}{z^2} \langle I_0 \rangle \left(1 + M(x) \cos 2\pi x/p\right) \tag{7.5}$$

representing a fringe pattern of modulation $M(x)$ and period p.



2) What is the maximum modulation strength $M(x)_{\max}$? What happens to this strength as ρ_μ or τ_γ tends toward infinity? Does this strength depend on z?

3) What is the period p of the fringes? Express your answer in terms of λ_0 and $\theta = \frac{a}{z}$, corresponding to the angle subtended by the pinholes.

4) How far do the fringes extend in x? Specifically, at what value $x_{1/e}$ does the modulation strength decrease by a factor of $1/e$ relative to its maximum? Express your answer in terms of θ and the coherence length $l_\gamma = \tau_\gamma c$. Does $x_{1/e}$ depend on ρ_μ?

Problem 2

A technique of laser speckle contrast analysis can be used to assess blood flow within tissue. In this technique, laser light is back-scattered from tissue, and a CCD camera is used to record the resultant speckle pattern (assumed to obey circular Gaussian field statistics). Any motion in the tissue causes the speckle pattern to fluctuate in time. By measuring the contrast of these fluctuations as a function of the camera exposure time T one can deduce a temporal coherence time τ_γ. The local blood flow velocity can then be inferred from τ_γ, provided one is equipped with a theoretical model relating the two.

1) The coherence function of light scattered from randomly flowing particles is often assumed to obey the statistics of a phase-interrupted source (see Eq. 7.11). Derive the expected contrast of the measured speckle fluctuations as a function of τ_γ and T.

2) Verify that when $\tau_\gamma \ll T$ the contrast obeys the relation given by Eq. 7.48.

Problem 3

Consider the intensity distribution $I_1(\vec{\rho}_1)$ at the image plane of a unit-magnification imaging system whose point spread function is written $\mathrm{PSF}(\vec{\rho})$. This intensity distribution is detected by a CCD camera, which consists of a 2D array of detectors (pixels), each of area $A = L \times L$. As a result, $I_1(\vec{\rho}_1)$ becomes integrated upon detection, and then sampled. The detected power, prior to sampling, can thus be written as

$$W_A(\vec{\rho}_1) = A \int D_A(\vec{\rho}_1 - \vec{\rho}_1^{\,\prime}) I_1(\vec{\rho}_1^{\,\prime}) d^2\vec{\rho}_1^{\,\prime}. \tag{7.6}$$

1) Provide expressions for $D_A(\vec{\rho})$ and its Fourier transform $\mathcal{D}_A(\vec{\kappa}_\perp)$.

2) Let the intensity distribution at the object plane $I_0(\vec{\rho}_0)$ be a "fully developed" speckle pattern produced by incoherent light. It can be shown (e.g. see Section 17.3) that the coherence function of a such a speckle pattern is given by

$$|\mu_0(\vec{\rho}_{0d})|^2 = \frac{\mathrm{PSF}_s(\vec{\rho}_{0d})}{\mathrm{PSF}_s(0)} \tag{7.7}$$

where PSF_s is the point spread function associated with the speckle generation (not necessarily the same as PSF).

Express the spatial contrast of the imaged speckle pattern recorded by the CCD camera in terms of $\mathcal{D}_A(\vec{\kappa}_\perp)$, $\mathrm{OTF}(\vec{\kappa}_\perp)$ and $\mathrm{OTF}_s(\vec{\kappa}_\perp)$.

3) What happens to the above contrast as the size of the CCD pixels becomes much larger than the spans of both $\mathrm{PSF}(\vec{\rho})$ and $\mathrm{PSF}_s(\vec{\rho})$?

Chapter 8

Detector Noise

Problem 1

1) Show that if the instantaneous power W of a light beam obeys a negative-exponential probability density, then, upon detection, the number of photoelectron conversions per detector integration time T obeys a probability distribution given by

$$P_K(K) = \frac{1}{1+\langle K\rangle}\left(\frac{\langle K\rangle}{1+\langle K\rangle}\right)^K \tag{8.1}$$

where $\langle K\rangle = \frac{\eta}{h\nu}\langle W\rangle T$.

This is called a Bose-Einstein probability distribution (in probability theory it is called a geometric distribution).

2) Based on the above result, verify that the variance in the detected number of photoelectron conversions is

$$\sigma_K^2 = \langle K\rangle + \langle K\rangle^2 . \tag{8.2}$$

Note: for part 2, you will find the following identity to be useful:

$$\sum_{k=0}^{\infty} k^n \gamma^k = \begin{cases} \frac{1}{1-\gamma} & (n=0) \\ \frac{\gamma^n (n+1)!}{(1-\gamma)^{n+1}} \sum_{m=0}^{n-1}\sum_{j=0}^{m+1} (-1)^j \frac{(m-j+1)^n}{j!(n-j+1)!}\gamma^{-m} & (n\geq 1) \end{cases} \tag{8.3}$$

Problem 2

Consider a detector voltage measured through an impedance $R = 10^5\ \Omega$ (this is a typical value). Assume that the detector is at room temperature, but that dark current is negligible. The charge of a single electron is 1.6×10^{-19}C.

1) Let's say a single photoelectron is generated at the detector cathode (i.e. input). What is the minimum detector bandwidth B required for the measurement of this photoelectron to be shot-noise limited?

2) The bandwidth derived above is found to be unrealistic. In fact, the detector bandwidth is known to be 10 MHz (also a typical value). What is the minimum current preamplification M required for the measurement of the single photoelectron to be shot-noise limited? (assuming this preamplification to be noiseless).

Problem 3

Consider a CCD camera with a 12-bit dynamic range and a pixel well capacity of 10,000e^-. Assume that the camera gain G is properly set to accomodate these ranges. The camera amplifier produces a readout noise of 10e^- (i.e. $\sigma_r = 10$; note that the readout noise is in units of *number* of electrons as opposed to electron charge). Assume the illumination light is stable (i.e. exhibits no classical fluctuations). Dark noise and Johnson noise are negligible.

1) What is the minimum average readout value $\langle N \rangle$ for the measured signal to be shot-noise limited?

2) This is not good enough. Let us say we want to measure a signal as low as $\langle N \rangle = 1$. To do this, we will incorporate an electron multiplication stage in our CCD camera. What electron multiplication gain M is required to guarantee that the measurement will be shot-noise limited even at this low signal? (consider the electron multiplication stage to be noiseless).

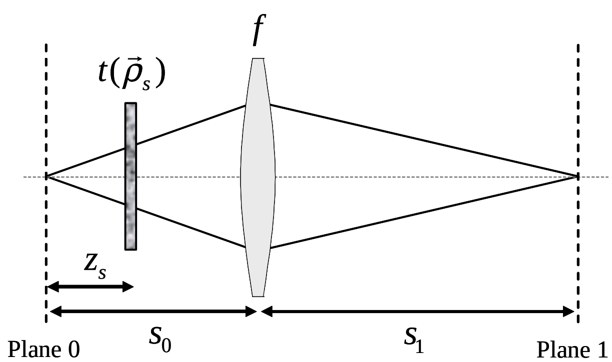
Chapter 9

Absorption and scattering

Problem 1

We have seen that when a plane wave is sent through a thin transmitting sample, the scattered field far from the sample (Eqs. 9.7 or 9.16) is not quite a perfect Fourier transform of the sample transmittance function (absorption or phase). The problem is that there remains a residual, spatially-dependent phase prefactor $e^{i\pi\frac{\kappa}{z}\rho^2}$ in the scattered field.

Show that by using point-source illumination and a single lens, this residual phase prefactor can be eliminated for a particular sample location z_s (see figure). That is, the field at the image plane of the source is given by the perfect Fourier transform of the sample transmission function $t(\vec{\rho}_s)$. What is this sample location z_s and what is the resulting field at the illumination plane? Use the Fresnel approximation and assume that s_0 and s_1 obey the thin-lens formula.



Note: There are several ways to solve this problem. Use the fact that a forward projection of the field from the sample plane to the image plane is equivalent to a backward projection of this field to the illumination plane (without the sample), followed by a forward projection to the image plane. This last projection is given by Eq. 3.15.

Problem 2

The scattering cross section of a small dielectric sphere can be calculated exactly by matching solutions of the field inside and outside its boundary. This is the well-known Clausius-Mossotti boundary-value solution, which is generally presented in the literature as

$$\sigma_{\mathrm{scatt}} = \frac{8\pi}{3} k^4 a^6 \left| \frac{n_r^2 - 1}{n_r^2 + 2} \right|^2 \tag{9.1}$$

where a is the radius of the dielectric sphere ($a \ll \lambda$), n_r is the ratio n_s/n (where n_s and n are the index of refraction of the dielectric sphere and surrounding medium respectively), and k is the angular wavenumber of the incident light in the medium.

Show that, to lowest order in $\delta n/n$, the scattering cross sections given by the Clausius-Mossotti solution and by Eq. 9.65 are identical.

Problem 3

Derive Eqs. 9.67 and 9.69.

Chapter 10

Phase contrast

Problem 1

Consider a thin sample that induces both phase shifts $\phi(\vec{\rho}_0)$ and absorption $\alpha(\vec{\rho}_0)$. The local sample transmittance can then be written as $t(\vec{\rho}_0) = e^{i\widetilde{\phi}(\vec{\rho}_0)}$, where $\widetilde{\phi}(\vec{\rho}_0) = \phi(\vec{\rho}_0) + i\alpha(\vec{\rho}_0)$ is a generalized complex phase function ($\phi(\vec{\rho}_0)$ and $\alpha(\vec{\rho}_0)$ are real). Show that this complex phase function can be effectively imaged with a modified Zernike phase microscope.

Specifically, consider a Zernike phase contrast microscope whose pupil function can be controlled so that

$$P(\xi) = \begin{cases} e^{i\psi} & \xi \leq \varepsilon \\ 1 & \varepsilon < \xi \leq a \\ 0 & \xi > a \end{cases}$$

where ψ is an adjustable phase shift that is user-defined (assume $\varepsilon \ll a$).

The sample is illuminated with an on-axis plane wave of amplitude E_i. The resultant intensity recorded at the image plane, for a given ψ, is written as $I_1^{(\psi)}(\vec{\rho}_1)$.

1) Show that by acquiring a sequence of four images with $\psi = \left\{0, \frac{\pi}{2}, \pi, \frac{3\pi}{2}\right\}$, and by processing these four images using the algorithm

$$\widetilde{I}_1(\vec{\rho}_1) = \frac{1}{4}\left[\left(I_1^{(0)}(\vec{\rho}_1) - I_1^{(\pi)}(\vec{\rho}_1)\right) + i\left(I_1^{(\pi/2)}(\vec{\rho}_1) - I_1^{(3\pi/2)}(\vec{\rho}_1)\right)\right]$$

we obtain

$$\widetilde{I}_1(\vec{\rho}_1) = iI_i \int \mathrm{CSF}(\vec{\rho}_1 - \vec{\rho}_0)\widetilde{\phi}(\vec{\rho}_0)d^2\vec{\rho}_0$$

where $I_i = |E_i|^2$.

That is, the constructed complex "intensity" $\widetilde{I}_1(\vec{\rho}_1)$ is effectively an image of the complex phase function of the sample, from which we can infer both $\phi(\vec{\rho}_0)$ and $\alpha(\vec{\rho}_0)$. The imaging response function is given by the microscope CSF. Use the weak phase approximation and assume unit magnification.

2) Derive a similar algorithm that achieves the same result but with a sequence of only three images.

Problem 2

Consider a modified Schlieren microscope where the knife edge, instead of blocking light, produces a π phase shift. Compare this modified Schlieren microscope with the standard Schlieren microscope described in Section 10.2.2 (all other imaging conditions being equal).

1) Which microscope is more sensitive to samples that are purely phase shifting? (Assume weak phase shifts.)

2) Which microscope is more sensitive to samples that are purely absorbing? (Assume weak absorption.)

Problem 3

In DIC microscopy, a bias is used to adjust the relative phase between the cross-polarized fields. Such a bias can be obtained by introducing a quarter wave plate (QWP) between the Nomarski prism and the polarizer in the DIC detection optics. When the fast axis of the QWP is set to 45° from vertical (or horizontal), then the bias phase $\Delta\theta$ can be adjusted by rotating the polarizer angle ϕ. The Jones matrix for a QWP whose fast axis is aligned in the vertical direction is given by

$$\mathrm{M}_{\mathrm{QWP}}^{(0^\circ)} = e^{i\pi/4} \begin{pmatrix} 1 & 0 \\ 0 & -i \end{pmatrix} . \tag{10.1}$$

What is the relation between $\Delta\theta$ and ϕ?

Chapter 11

Holographic microscopy

Problem 1

Equations 11.11 and 11.13 are idealized in that they consider the integration over $\vec{\rho}_h$ to be infinite. In practice, the integration can only be performed over the area of the CCD camera, which has a finite size $L_x \times L_y$. Derive the effect of this finite size on the spatial resolution of the reconstructed field $E_0(\vec{\rho}_0)$. In particular...

1) Show that in the case of lensless Fourier holography (Fig. 11.2), this resolution is given by $\Delta x_0 = \frac{\lambda}{2n \sin\theta_x}$ and $\Delta y_0 = \frac{\lambda}{2n \sin\theta_y}$, where $\sin\theta_x = \frac{L_x}{2z_d}$ and $\sin\theta_y = \frac{L_y}{2z_d}$. (Assume Δx_0 and Δy_0 are small).

2) Show that in the case of Fourier holography with a lens (Fig. 11.3), this resolution is given by $\Delta x_0 = \frac{\lambda}{2n \sin\theta_x}$ and $\Delta y_0 = \frac{\lambda}{2n \sin\theta_y}$, where $\sin\theta_x = \frac{L_x}{2f}$ and $\sin\theta_y = \frac{L_y}{2f}$. (Assume, for simplicity, that $z_c = f$).

In perfoming these calculations, you will run into sinc functions. Define the width of $\text{sinc}(ax)$ to be $\Delta x = \frac{1}{a}$.

Problem 2

Consider performing digital holography with a CCD camera of size $L_x \times L_y$ comprising square pixels of size $\delta L \times \delta L$. Calculate the maximum sample size (or field of view) $\Delta x \times \Delta y$ allowed in each of the following microscopy configurations, such that the Nyquist sampling criterion is obeyed:

1) On-axis Fourier holography with a lens (assuming $z_c = f$).

2) On-axis lensless Fourier holography (assuming small angles and a distance z_d between the sample and camera).

3) On-axis Fresnel holography (assuming a distance z_d between the sample and camera). Derive a condition for the minimum z_d allowed.

4) Off-axis Fresnel holography (assuming a distance z_d between the sample and camera, and a reference-beam tilt angle θ in the x direction only). Derive a condition for the minimum z_d allowed.

Hint: You will find Fig. 11.9 to be highly useful in this exercise.

Problem 3

1) On-axis digital holography is performed with circular phase stepping. Consider an arbitrary CCD pixel and assume a camera gain of 1 (i.e. the CCD directly reports the number of detected photoelectrons). The phase stepping algorithm applied to this pixel may be written as

$$\widetilde{N} = \frac{1}{K}\sum_{k=0}^{K-1} e^{i\phi_k} N^{(\phi_k)} \tag{11.1}$$

where $N^{(\phi_k)}$ is the pixel value recorded at reference phase ϕ_k (for a given intergration time). Neglect all noise contributions except shot noise. Show that the variances of the real and imaginary components of $\widetilde{N}$ are given by

$$\mathrm{Var}\left[\widetilde{N}_{\mathrm{Re}}\right] = \mathrm{Var}\left[\widetilde{N}_{\mathrm{Im}}\right] = \frac{1}{2K^2}\left\langle N_{\mathrm{total}}\right\rangle \tag{11.2}$$

where $\langle N_{\mathrm{total}}\rangle$ it the *total* number of pixel values accumulated over all phase steps.

Hint: Start by writing $N^{(\phi_k)} = \langle N\rangle + \delta N^{(\phi_k)}$, where $\delta N^{(\phi_k)}$ corresponds to shot noise variations in the number of detected photoelectrons. Use your knowledge of the statistics of these variations.

2) What happens to the above result if the camera gain is G?

Chapter 12

Optical Coherence Tomography

Problem 1

Widefield phase-sensitive OCT is performed with circular phase stepping (4 steps). Consider an arbitrary CCD pixel and assume a camera gain of 1 (i.e. the CCD directly reports the number of detected photoelectrons). The phase stepping algorithm applied to this pixel may be written as

$$\widetilde{N} = \frac{1}{4}\sum_{k=0}^{3} e^{i\phi_k} N^{(\phi_k)} \tag{12.1}$$

where $N^{(\phi_k)}$ is the pixel value recorded at reference phase $\phi_k = \frac{2\pi k}{4}$ (for a given integration time T). Our goal is to determine the phase of r_z recorded by this pixel. To do this, we must determine the phase of $\widetilde{N}$, which we denote here by φ_N.

1) Derive an expression for φ_N in terms of the four measured pixel values $N^{(\phi_k)}$.

2) Consider two noise sources: shot noise and dark noise. The latter is modeled as producing background photoelectron counts obeying Poisson statistics. Let N_S, N_R, and N_D be the average pixel values obtained from separate measurements of the sample beam, the reference beam, and the dark current respectively, using a *total* integration time required for all four steps (i.e. $4T$).

Show that the error in the determination of φ_N has a standard deviation given by

$$\sigma_{\varphi_N} = \sqrt{\frac{1}{2N_S}\left(1 + \frac{N_S}{N_R} + \frac{N_D}{N_R}\right)}. \tag{12.2}$$

(Without loss of generality, you may set the actual φ_N to be any arbitrary value – in particular, you may assign it to be equal to zero.)

Hint: Start by writing $N = \langle N \rangle + \delta N$, where δN corresponds to shot noise variations in the number of detected photoelectrons. Use your knowledge of the statistics of these variations.

Observe that when the reference beam power is increased to such a point that $N_R \gg N_S$ and $N_R \gg N_D$, then $\sigma_{\varphi_N} \to \sqrt{\frac{1}{2N_S}}$, meaning that the phase measurement accuracy becomes limited by sample-beam shot noise alone (i.e dark noise becomes negligible). This is one of the main advantages of interferometric detection with a reference beam.

Problem 2

Consider performing FD-OCT without phase stepping. That is, in Eq. 12.22, replace $\widetilde{W}_{\text{fd}}(z_r;\kappa)$ directly with $W_1(z_r;\kappa)$ (obtained from Eq. 12.2). For simplicity, assume that $\Delta\kappa$ is so large that $\text{sinc}(\Delta\kappa\, z)$ may be approximated as a delta function $\delta(\Delta\kappa\, z)$.

1) Derive an expression for $\mathcal{W}_{\text{fd}}(z_r;z_\kappa)$. This expression should contain four terms.

2) Show that it is possible to disentangle the contributions from the four terms provided we have *a priori* knowledge about $r_z(z_0)$. In particular, assume that $r_z(z_0)$ is so small that the second term arising from W_{ss} can be neglected (see Eq. 12.2). Moreover, adjust z_r such that $r_z(z_0)$ is known to vanish when $z_0 < z_r$ (for example, z_r can be adjusted to lie just outside the sample volume). Without loss of generality, define this z_r to be 0.

Derive an expression for $\mathcal{W}_{\text{fd}}(z_r = 0; z_\kappa > 0)$, making use of the above *a priori* knowledge. This new expression should contain only a single term.

Problem 3

In deriving Eq. 12.23, we assumed that $\widetilde{W}_{\text{fd}}(z_r;\kappa)$ was a continuous function of κ. This is an idealization. In practice, $\widetilde{W}_{\text{fd}}(z_r;\kappa)$ must be a *sampled* function of κ. Denote the sampling interval as $\delta\kappa$. That is, in Eq. 12.22, make the replacement

$$\widetilde{W}_{\text{fd}}(z_r;\kappa) \to \delta\kappa \sum_n \widetilde{W}_{\text{fd}}(z_r;\kappa)\delta(\kappa - n\delta\kappa) \tag{12.3}$$

where n is an integer.

1) Show when we take sampling into consideration, the coherence gating envelope $\hat{G}(\hat{z}) = \text{sinc}(2\Delta\kappa\hat{z})$ in Eq. 12.23 becomes instead

$$\hat{G}(\hat{z}) = \frac{\delta\kappa}{\Delta\kappa}\frac{\sin\left(2\pi\left(\Delta\kappa + \delta\kappa\right)\hat{z}\right)}{\sin(2\pi\delta\kappa\hat{z})} \tag{12.4}$$

with the shorthand notation $\hat{z} = z_0 - z_r + z_\kappa/2$.

2) Provide a plot of $\hat{G}(\hat{z})$ using the arbitrary values $\delta\kappa = 1$ and $\Delta\kappa = 100$. You will observe that $\hat{G}(\hat{z})$ is peaked at several values of $\hat{z}$ (as opposed to a single value in Eq. 12.23). What are these values?

3) What is the problem with $\hat{G}(\hat{z})$ being peaked at multiple values? Can you define a maximum axial range Δz_κ associated with the reconstruction of $r_z(z_\kappa)$? What happens if the actual $r_z(z_\kappa)$ extends beyond this range? (Recall z_r is fixed).

Note: for part 1, you will find the following identity to be useful:

$$\sum_{n=a-b/2}^{a+b/2} e^{i2\pi nc} = e^{i2\pi ca}\frac{\sin\left(\pi c\left(b+1\right)\right)}{\sin(\pi c)} \tag{12.5}$$

Chapter 13

Fluorescence

Problem 1

Consider a solution of two-level fluorescent molecules such as the one depicted in Fig. 13.1(b). The fluorescence from this solution is decreased by the addition of a quencher Q. The effect of this quencher is to induce an additional non-radiative decay of the excited state such that

$$|e\rangle + Q \xrightarrow{k_q} |g\rangle \tag{13.1}$$

where k_q is the quenching rate constant, in units $s^{-1}M^{-1}$ (M = molar concentration).

1) Show that

$$\frac{\tau_e}{\tau_e^{(Q)}} = 1 + \tau_e k_q[Q] \tag{13.2}$$

where $\tau_e^{(Q)}$ and τ_e are the excited state lifetimes with and without the presence of the quencher, and $[Q]$ is the molar concentration of the quencher.

Such quenching is said to obey a Stern-Volmer relationship.

2) Show that, based on our simple model,

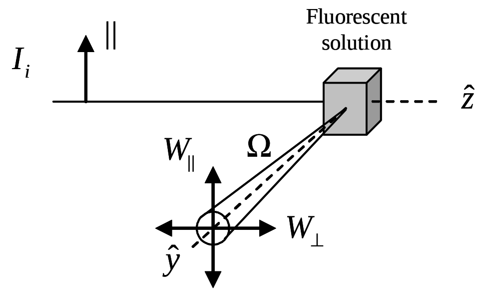
$$\frac{W_{\mathrm{f}}}{W_{\mathrm{f}}^{(Q)}} \leq \frac{\tau_e}{\tau_e^{(Q)}} \tag{13.3}$$

where the equality holds only in a particular limit. What is this limit?

Problem 2

Molecules in solution undergo both translational and rotational diffusion. A method for characterizing rotational diffusion is by measuring fluorescence anisotropy. This can be done using the standard configuration shown below.

An illumination beam of intensity I_i is vertically polarized (x direction). The resultant fluorescence emission power is measured in the y direction within a small solid angle Ω. A



polarizer is used to distinguish the measured vertical and horizontal powers, denoted by $W_{\|}$ and $W_\perp$ respectively. It can be shown that these powers are given by

$$W_{\|}(t) = \Omega\sigma_{\mathrm{f}} \int K_{\|}(t-t')I_i(t')dt' \tag{13.4}$$

$$W_{\perp}(t) = \Omega\sigma_{\mathrm{f}} \int K_{\perp}(t-t')I_i(t')dt' \tag{13.5}$$

where

$$K_{\|}(t) = \frac{1}{3}\left(1+2R(t)\right)K(t) \tag{13.6}$$

$$K_{\perp}(t) = \frac{1}{3}\left(1-R(t)\right)K(t) \tag{13.7}$$

where σ_{f} is the fluorescence cross section, $K(t)$ is given by Eq. 13.26 (assume a single two-level fluorescent species), and $R(t)$ comes from rotational diffusion. In particular, if the rotational diffusion is isotropic, then

$$R(t) = r_0 e^{-6D_\theta t} = r_0 e^{-t/\tau_\theta} \tag{13.8}$$

where D_θ is a rotational diffusion constant and, concomitantly, τ_θ is a rotational diffusion time.

The measured fluorescence anisotropy is defined by

$$r(t) = \frac{W_{\|}(t) - W_{\perp}(t)}{W_{\|}(t) + 2W_{\perp}(t)} \tag{13.9}$$

1) Show that if the illumination intensity is constant, then the steady-state fluorescence anisotropy is given by

$$\langle r\rangle = \frac{r_0}{1+\tau_e/\tau_\theta}. \tag{13.10}$$

This is known as Perrin's relationship.

2) Denote W_f as the total *emitted* fluorescence power in all solid angles. Derive an expression for the total *measured* fluorescence power when $\tau_e/\tau_\theta \rightarrow 0$ (i.e. the rotation is slow compared to the excited state lifetime). When is this measured fluorescence power equal to ΩW_f?

3) Derive an expression for the total *measured* fluorescence power when $\tau_e/\tau_\theta \rightarrow \infty$ (i.e. the rotation is fast compared to the excited state lifetime). In the case, the molecule orientation is essentially randomized before fluorescence emission can occur. Explain why the measured fluorescence power in this case is smaller than ΩW_f.

Problem 3

Consider performing FCS with a solution of freely diffusing fluorescent molecules and a 3D Gaussian probe volume defined by $\Psi(\vec{r}) = \exp(-r^2/w_0^2)$. The average concentration of molecules is $\langle C \rangle$. Their diffusion constant is D.

1) Derive $G_f(\tau)$.

2) Verify that $G_f(\tau \rightarrow 0) = \frac{1}{\langle N \rangle}$, where $\langle N \rangle$ is the average number of molecules in the probe volume.

Chapter 14

Confocal microscopy

Problem 1

From the result in Eq. 14.23 it is clear that a purely phase-shifting point object produces no discernable change in detected intensity in a transmission confocal microscope. That is, if ϕ is real then $I_1(\vec{\rho}_s, z_s)$ is independent of ϕ to first order. This result is based on the assumption that the microscope is well aligned.

Consider now a transmission confocal microscope that is misaligned. In particular, consider displacing the pinhole out of focus by a distance Δz_p. Show that this misaligned transmission confocal microscope now becomes sensitive to a phase-shifting point object. For simplicity, assume that the illumination and detection CSFs are identical and Gaussian (Eq. 5.31). Follow these steps:

1) Calculate E_{1B}.

2) Calculate $E_{1S}(\vec{\rho}_s, z_s)$. For simplicity, neglect scanning and set $\vec{\rho}_s$ and z_s to zero.

3) From the resulting $E_1(0,0) = E_{1B} + E_{1S}(0,0)$, derive the detected intensity $I_1(0,0)$ and show that this depends on ϕ to first order (neglect any higher order dependence on ϕ).

Problem 2

Consider a fluorescence confocal microscope with different illumination and detection PSFs, and a pinhole of arbitrary radius a.

1) Derive a general expression for the effective confocal point spread function, $\mathrm{PSF}^{\mathrm{conf}}(\vec{\rho}_s, z_s)$. Do not concern yourself with prefactors or normalization.

2) What happens to $\mathrm{PSF}^{\mathrm{conf}}(\vec{\rho}_s, z_s)$ when $a \to 0$?

3) What happens to $\mathrm{PSF}^{\mathrm{conf}}(\vec{\rho}_s, z_s)$ when $a \to \infty$? How does this compare to the PSF of a standard widefield microscope?

4) Derive a general expression for the effective optical transfer function $\mathrm{OTF}^{\mathrm{conf}}(\vec{\kappa}_{\perp s}; z_s)$ in terms of OTF_i and OTF_0. Again, do not concern yourself with prefactors or normalization.

Problem 3

Consider a fluorescence confocal microscope equipped with a reflecting pinhole, that is a pinhole of radius a surrounded by a reflecting annulus of outer radius b and inner radius a (asume that the beam is blocked beyond the annulus). A transmission detector records the power W_T transmitted through the pinhole. A reflection detector records the power W_R reflected from the annulus. The confocal signal is then given by the difference of these recorded powers, namely $\Delta W = W_T - W_R$.

1) Calculate $\Delta W(z_s)$ if the sample is a thin uniform fluorescent plane located at a defocus position z_s. For simplicity, assume that $\mathrm{PSF}_0 = \mathrm{PSF}_i \equiv \mathrm{PSF}$ (and hence $\mathrm{OTF}_0 = \mathrm{OTF}_i \equiv \mathrm{OTF}$). Express your result in terms OTF and omit extraneous prefactors.

2) Show that for a particular ratio b/a, the optical sectioning strength of this microscope is greater than that of a standard confocla microscope. In particular, show that $\Delta W(z_s) \propto |z_s|^{-3}$ when $|z_s|$ is large, for a particular ratio b/a. What is this ratio?

Note: to solve this problem recall that $\mathrm{OTF}(\vec{\kappa}_{\perp}; z_s)$ scales as $|z_s|^{-3/2}$ when $\kappa_{\perp} \neq 0$ and $|z_s|$ is large.

Chapter 15

Two-photon microscopy

Problem 1

The fluorescence power emitted by a molecule under continuous illumination is given by Eq. 13.9. This equation is no longer valid in the case of pulsed illumination. In particular, consider pulsed illumination with a pulse period τ_l and a pulse width τ_p. Assume $\tau_p \ll \tau_e$ such that, at most, only one excitation can occur per pulse. Define g_p to be the probability of finding the molecule in the ground state at the *onset* of every pulse (in steady state). Moreover, define ξ to be the probability of excitation per pulse provided the molecule is in the ground state.

1) Derive an expression for the average fluorescence power emitted by a molecule under pulsed illumination, in terms of g_p. (For simplicity, assume that the molecule is a simple two-level system with a radiative quantum yield equal to 1).

2) Derive an expression for g_p in steady state and show that

$$\frac{\langle W_{\mathrm{f}} \rangle}{h\nu_{\mathrm{f}}} = \frac{\xi}{\tau_l} \left(\frac{1 - e^{-\tau_l/\tau_e}}{1 - e^{-\tau_l/\tau_e} + \xi e^{-\tau_l/\tau_e}} \right) \tag{15.1}$$

where τ_e is the excited state lifetime. Hint: to solve this problem, start by deriving the probability e_p of finding the molecule in the excited state at the *onset* of a pulse. To achieve steady state, this probability must be in balance with the residual probability from the previous pulse

3) Let α be the excitation rate (two-photon or otherwise) during each pulse, and assume that the pulse width is so short that $\alpha\tau_p \ll 1$. Derive an expression for e_p when the repetition rate of the illumination becomes so high that the illumination becomes effectively a continuous wave (i.e. when $\tau_l \to \tau_p$). How does this expression compare with Eq. 13.8?

Problem 2

One- and two-photon excited fluorescence are specific cases of a more general n-photon excited fluorescence, where the power emitted by a molecule is written as

$$W_{\mathrm{f}} = \sigma_{n\mathrm{f}} I_i^n \tag{15.2}$$

where $\sigma_{n\mathrm{f}}$ is a generalized n-photon excited fluorescence cross section (for example, compare with Eqs. 15.1 or 13.2).

When using pulsed illumination with pulse period τ_l and pulse width τ_p, derive an expression for $\langle W_{\mathrm{f}} \rangle$ in terms of $\langle I_i \rangle$.

Problem 3

A Gaussian-Lorentzian focus is used to produce two-photon excited fluorescence.

1) Show that if the sample is a thin uniform plane at a defocus postion z_s, with concentration defined by $C(\vec{r}) = C_\rho \delta(z - z_s)$, then the total generated fluorescence power is inversely proportional to the beam cross-sectional area $A(z_s)$. Hint: define cross-sectional area in a similar manner as Eq. 6.2.

2) Show that if the sample is a volume of uniform concentration C, then the total generated fluorescence is independent of the beam waist w_0.

Note: it may be helpful to rewrite Eqs. 15.16 and 15.17 in a more general form

$$\Psi(\vec{r}) = \frac{\mathrm{PSF}_i^2(\vec{r})}{\mathrm{PSF}_i^2(0)} \tag{15.3}$$

$$\phi_f = \Omega_0 \sigma_{2\mathrm{f}} W_i^2 \mathrm{PSF}_i^2(0). \tag{15.4}$$

Chapter 16

Coherent nonlinear microscopy

Problem 1

The second harmonic tensorial product $\vec{S} = \vec{\chi}^{(2)} : \vec{E}\,\vec{E}$ (see Eq. 16.17) can be expanded as

$$S_i = \sum_{j=1}^{3}\sum_{k=1}^{3} \chi_{ijk}^{(2)} E_j E_k. \tag{16.1}$$

This product depends on the coordinate system in which it is evaluated. The two relevant coordinate systems for this problem are the fixed laboratory system (denoted by L) and the molecule system (denoted by M), which may be arbitrarily oriented relative to the laboratory system.

Consider a uni-axial molecule oriented along $\hat{r}$, illuminated by a field given by $\vec{E}^{(L)}$ in the laboratory system.

1) Defining $\mathrm{R}(\theta,\varphi)$ to be the rotation matrix linking the molecule system to the laboratory system (see Eq. 16.5), show that

$$S_l^{(L)} = \sum_{m=1}^{3}\sum_{n=1}^{3} \chi_{lmn}^{(L)} E_m^{(L)} E_n^{(L)} \tag{16.2}$$

where

$$\chi_{lmn}^{(2)(L)} = \sum_{i=1}^{3}\sum_{j=1}^{3}\sum_{k=1}^{3} R_{i,l}(\theta,\varphi) R_{j,m}(\theta,\varphi) R_{k,n}(\theta,\varphi) \chi_{ijk}^{(2)(M)}. \tag{16.3}$$

Hint: recall that $\mathrm{R}(\theta,\varphi)$ is orthogonal.

2) For simplicity, assume that all components of the molecule second-order susceptibility $\chi_{ijk}^{(2)(M)}$ are zero, except for $\chi_{111}^{(2)(M)} \equiv \chi_{rrr}^{(2)}$. Show that, in this case,

$$\vec{S}^{(L)} = \chi_{rrr}^{(2)} \left(\hat{r}\cdot\vec{E}^{(L)}\right)^2 \hat{r}. \tag{16.4}$$

Problem 2

Consider generating SHG with a focused beam as in Fig. 16.4, but with two labeled membranes separated by a distance Δx_0. Each membrane exhibits identical, uniform second-order susceptibility $\chi_\rho^{(2)}$, but their markers are oriented in opposite directions.

1) Use the 3D Gaussian approximation (Eq. 16.19) to derive the field $E_{2\nu}^{(2)}(\vec{r})$ produced by the two membranes. Express your answer in terms of $E_{2\nu}^{(1)}(\vec{r})$, the field produced by a single membrane (i.e. Eq. 16.29).

2) As in Fig. 16.4, the SHG is emitted in two off-axis lobes at $\cos\theta \approx 1 - \frac{\delta\kappa_\nu}{\kappa_v}$ and $\varphi \approx [0, \pi]$. Plot the intensity ratio $\frac{I_{2\nu}^{(2)}(\vec{r})}{I_{2\nu}^{(1)}(\vec{r})}$ in the lobe directions, as a function of $\frac{\Delta x_0}{w_0}$ (hint: use Eq. 16.15. Please note that there is an error in this equation – on the left hand side $\delta\kappa_m$ should be $2\pi\delta\kappa_m$).

At approximately what value of $\frac{\Delta x_0}{w_0}$ is this intensity ratio peaked?

Problem 3

1) Calculate the third-harmonic intensity pattern produced from a localized 3D-Gaussian susceptibility distribution given by

$$\chi^{(3)}(\vec{r}_0) = \chi^{(3)} e^{-r_0^2/w_\chi^2}. \tag{16.5}$$

Assume a focused illumination beam and use the 3D-Gaussian illumination profile given by Eq. 16.19. Express your result in terms of r, θ and φ.

2) Derive an expression for the backward/forward ratio of THG intensities emitted along the $\hat{z}$-axis. That is, derive an expression for

$$\frac{I_{\text{backward}}}{I_{\text{forward}}} = \frac{I_{3\nu}^{(\theta=\pi)}(\vec{r})}{I_{3\nu}^{(\theta=0)}(\vec{r})}. \tag{16.6}$$

What does this ratio tend toward as $w_\chi \to 0$?

Chapter 17

Pupil synthesis

Problem 1

Consider performing coherent structured illumination microscopy with a modulated field source (as opposed to a modulated intensity source). That is, start with

$$E_{\mathcal{L}}(x_l, y_l) = E_{\mathcal{L}} \left(1 + \cos(2\pi q_x x_l + \phi)\right) . \tag{17.1}$$

Such a field can be obtained, for example, by sending a plane wave through a sinusoidal amplitude grating. This field is imaged into the sample using an unobstructed circular aperture of sufficiently large bandwidth to transmit q_x.

1) Derive an expression for the resulting intensity distribution $I_i(x_0, y_0, z_0)$ in the sample You will note that this distribution exhibits different modulation frequencies at different defocus values z_0.

2) At what values of z_0 does $I_i(x_0, y_0, z_0)$ correspond to an exact image of the source intensity $I_{\mathcal{L}}(x_l, y_l)$? These images are called Talbot images.

3) At what values of z_0 does $I_i(x_0, y_0, z_0)$ correspond to the source intensity image, but with an inverted contrast? These images are called contrast-inverted Talbot images.

4) At defocus planes situated halfway between the Talbot and the contrast-inverted Talbot images, $I_i(x_0, y_0, z_0)$ exhibits a new modulation frequency. What is this modulation frequency? What is the associated modulation contrast?

5) Your solution for $I_i(x_0, y_0, z_0)$ should also exhibit a modulation in the z_0 direction. What is the spatial frequency of this modulation? Note: there is no control of the phase of the z_0-direction modulation (i.e. there is no equivalent of ϕ in the z_0 direction). Devise an experimental strategy to gain phase control in the z_0 direction.

Problem 2

Show that the absolute value of the complex intensity $\widetilde{I} = \frac{1}{K}\sum_{k=0}^{K-1} e^{i\phi_k} I_k$ obtained from phase stepping can be rewritten as

$$\left|\widetilde{I}\right| = \frac{1}{3\sqrt{2}}\sqrt{\left(I_0 - I_1\right)^2 + \left(I_1 - I_2\right)^2 + \left(I_2 - I_0\right)^2} \tag{17.2}$$

when $K = 3$.

Problem 3

Consider performing SIM with a coherent fringe pattern of arbitrary spatial frequency $\vec{q}$. Calculate the resulting sectioning strength when the detection aperture is square (as opposed to circular). That is, calculate how the signal from a uniform fluorescent plane decays as a function of defocus z_s (assumed to be large). Specifically, consider the fringe frequencies $\vec{q} = \{q_x, 0\}$ and $\{q_x, q_y\}$. Are the sectioning strengths for these two frequencies the same?

Chapter 18

Superresolution

Problem 1

The pupil and point spread functions of a microscope are denoted by $P(\vec{\xi})$ and $\mathrm{PSF}(\vec{\rho})$ repectively. Consider introducing phase variations (or aberrations) in the pupil function, such that $P_\phi(\vec{\xi}) = e^{i\phi(\vec{\rho})}$, leading to $\mathrm{PSF}_\phi(\vec{\rho})$. A standard method for evaluating $\mathrm{PSF}_\phi(\vec{\rho})$ is with the Strehl ratio, defined by

$$S_\phi = \frac{\mathrm{PSF}_\phi(\vec{0})}{\mathrm{PSF}_0(\vec{0})} \tag{18.1}$$

where $\mathrm{PSF}_0(\vec{\rho})$ is the theoretical diffraction-limited PSF obtained when the pupil is unobstructed (i.e. $P_0(\vec{\xi}) = 0$ or 1). The larger the Strehl ratio, the better the quality of PSF_ϕ. Show that the introduction of aberrations can only lead to a degradation in the point spread function (i.e. $S_\phi \leq 1$). Proceed by first verifying Eq. 18.3.

Hint: You will find the Schwarz inequality to be useful here, which states:

$$\left| \int X(\vec{\kappa}_\perp) Y(\vec{\kappa}_\perp) d^2\vec{\kappa}_\perp \right|^2 \leq \left(\int |X(\vec{\kappa}_\perp)|^2 \, d^2\vec{\kappa}_\perp \right) \left(\int |Y(\vec{\kappa}_\perp)|^2 \, d^2\vec{\kappa}_\perp \right) \tag{18.2}$$

where X and Y are arbitrary complex functions.

Problem 2

Consider a confocal microscope whose illumination and detection PSFs are identical. The detected power from a simple two-level molecule can be written in a simplified form as

$$w(\vec{\rho}) = \alpha \xi^2(\vec{\rho}) \tag{18.3}$$

where α is the molecule excitation rate exactly at the the focal center, and $\xi(\vec{\rho}) = \frac{\mathrm{PSF}(\vec{\rho})}{\mathrm{PSF}(0)}$. The above expression is valid in the weak excitation limit, namely $\alpha \ll k_r$ (equivalent to $\langle e \rangle \approx \frac{\alpha}{k_r}$ – see Section 13.1.1). In the strong excitation limit, then this expression must be modified to take into account saturation. In particular, we must write $\langle e \rangle = \frac{\alpha}{\alpha + k_r}$ (neglecting non-radiative decay chanels – see Eq. 13.8).

1) Derive an expression for $w_{\mathrm{sat}}(\vec{\rho})$ taking saturation into account (for simplicity, only keep terms to first order in $\frac{\alpha}{k_r}$). Note that $w_{\mathrm{sat}}(\vec{\rho})$ corresponds to an effective confocal PSF, which is now saturated.

2) Now consider modulating the excitation rate such that $\alpha(t) = \alpha\,(1 + \cos(2\pi\Omega t))$. Correspondingly, $w_{\mathrm{mod}}(\vec{\rho}, t)$ also becomes modulated, and exhibits harmonics. Derive an expression for $w_{\mathrm{mod}}(\vec{\rho}, t)$.

3) By using appropriate demodulation, assume that the components of $w_{\mathrm{mod}}(\vec{\rho}, t)$ oscillating at the first (Ω) and second (2Ω) harmonics can be isolated. Use the technique employed in Section 18.2.2 to compare the curvatures of $w_{\Omega}(\vec{\rho})$ and $w_{2\Omega}(\vec{\rho})$ to the curvature of $w(\vec{\rho})$ (unsaturated and unmodulated). That is, derive approximate expressions for $\Delta\rho_{\Omega}$ and $\Delta\rho_{2\Omega}$. In particular, show that the effective first harmonic PSF exhibits sub-resolution while the effective second harmonic PSF exhibits superresolution.

Note: remember to normalize all $w(\vec{\rho})$'s to the same peak height before comparing their curvatures.

Problem 3

Assume a molecule is imaged onto a unity-gain CCD camera with unity magnification. Use maximum likelihood to estimate the error in localizing a molecule. That is, begin by defining a chi-squared error function given by

$$\chi^2(x) = \sum_i \frac{\left(N(x_i) - \bar{N}(x_i; x)\right)^2}{\sigma_N^2(x_i; x)} \tag{18.4}$$

where i is a pixel index, $N(x_i)$ is the actual number of photocounts registered at pixel i, and $\bar{N}(x_i; x)$ and $\sigma_N^2(x_i; x)$ are the expected mean and variance, respectively, of the photocounts at pixel i for a molecule located at position x. Assume the photocounts obey shot-noise statistics alone. For simplicity, consider only a single dimension (the x axis).

The estimated position of the molecule $\hat{x}$ is obtained by minimizing $\chi^2(x)$. That is, $\hat{x}$ is a solution to the equation $\frac{d\chi^2(x)}{dx} = 0$.

1) Show that the error in the estimated molecule position, defined by $\delta x = \hat{x} - x_0$, where x_0 is the actual molecule position, has a variance given by

$$\sigma_x^2 \approx \left(\sum_i \frac{1}{\bar{N}(x_i; x_0)} \left(\left. \frac{\bar{N}(x_i; x)}{dx} \right|_{x_0} \right)^2 \right)^{-1} \tag{18.5}$$

Hint: to obtain this result, it is useful to first solve for δx by writing

$$N(x_i) = \bar{N}(x_i; x_0) + \delta N(x_i; x_0) \tag{18.6}$$

$$\bar{N}(x_i; x) \approx \bar{N}(x_i; x_0) + \delta x \left.\frac{d\bar{N}(x_i; x)}{dx}\right|_{x_0} \tag{18.7}$$

and keeping terms only to first order in $\delta N(x_i; x_0)$ and δx. Note that $\sigma_x^2 = \langle \delta x^2 \rangle$.

2) Derive σ_x^2 for the specific example where the PSF at the camera plane has a normalized Gaussian profile given by

$$\bar{N}(x_i; x) = \frac{N}{\sqrt{2\pi} w_0} \int_{|x_i - x| - a/2}^{|x_i - x| + a/2} e^{-x'^2/2w_0^2} dx' \approx \frac{Na}{\sqrt{2\pi} w_0} e^{-(x_i - x)^2/2w_0^2} \tag{18.8}$$

where w_0 is the Gaussian waist and a is the camera pixel size (assume $a \ll w_0$).

How does your solution compare with Eq. 18.35?

Hint: approximate the summation with an integral. That is, for an arbitrary function $f(x_i)$, write $\sum_i f(x_i) \approx \frac{1}{a} \int f(x_i) dx_i$.