Introduction to Optical Microscopy (2nd Edition)
Problem Set

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Chapter 1

Introduction

Problem 1

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

a) Calculate the 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.

b) Calculate the ABCD transfer matrix when $s_0 = f_0$ and $s_1 = f_1$? This is called a 4f, or telecentric, imaging system. What is the resultant magnification?

c) Calculate the ABCD transfer matrix when $s_0 = f_0$ and $s_1 = f_1 + \delta z$? What happens to magnification for a beam parallel to the optical axis?

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 $\varepsilon \ll f_0 < f_1$).

a) 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?

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

Problem 3

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. Insert an intermediate lens of focal length f a distance f_0 behind lens f_0.

a) Where is the new image plane?

b) What is the magnification at this new image plane?

Problem 4

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).

a) Calculate the ABCD transfer matrix from the first conjugate plane to the last conjugate plane. What is the net magnification? Is the imaging perfect (i.e. telecentric)?

b) 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?

c) At what value of f is there no cross-talk between ray position and angle?

d) 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)?

Problem 5

In the thin lens formula, distances s_0 and s_1 are measured relative to the lens. Consider instead measuring distances relative to the lens front and back focal planes. That is, write $z_0 = s_0 - f$, and $z_1 = s_1 - f$. Show that the thin lens formula can be expressed equivalently by the so-called Newtonian lens formula $z_0 z_1 = f^2$.

Chapter 2

Monochromatic wave propagation

Problem 1

a) Derive Eq. 2.24 from Eqs. 2.13 and 2.23.
b) Derive Eq. 2.25 from Eqs. 2.14 and 2.23.

Problem 2

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

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

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

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

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

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

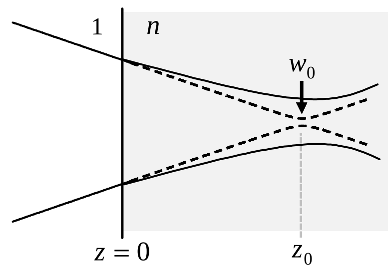
This equation is called the paraxial Helmholtz equation.

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

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

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.

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



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

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

$$\left(\pi\kappa_\perp^2 - i\kappa\frac{\partial}{\partial z}\right)\mathcal{A}(\vec{\kappa}_\perp; z) = 0. \tag{2.1}$$

d) 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 3

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

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

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

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

This is called the transport of intensity equation.

Problem 4

When a field is focused into a glass slab, the refraction at the slab interface produces aberrations in the field that cause the focus to distort. These aberrations are commonly characterized by their effect on the phase of the radiant field. Specifically, consider focusing a Gaussian field into a glass slab of index of refraction n, where the slab interface is located at $z = 0$ (see figure). When no slab is present ($n = 1$), the Gaussian field produces a focus of beam waist w_0 (see Eq. 2.59) at a distance z_0. When the slab is present, the focus is both distorted and axially displaced.

a) Start by calculating the radiant field incident on the slab interface at $z = 0$, bearing in mind that, by symmetry, transverse momentum $\vec{\kappa}_\perp$ must be conserved. That is, $\vec{\kappa}_\perp$ must be the same on both sides of the interface. (Hint: use the Rayleigh-Sommerfeld transfer function).

b) Next, calculate the radiant field inside the slab. You should find that the phase of the radiant field is given by

$$\phi\left(\vec{\kappa}_\perp; z\right) = 2\pi\left(z\sqrt{n^2\kappa^2 - \kappa_\perp^2} - z_0\sqrt{\kappa^2 - \kappa_\perp^2}\right).$$

c) While there are different ways to define the location of the new beam focus, one way is where $\phi\left(\vec{\kappa}_\perp; z\right)$ is as flat as possible about $\vec{\kappa}_\perp = 0$. Find the axial displacement of the new focus. (Hint: expand $\phi\left(\vec{\kappa}_\perp; z\right)$ in orders of $\kappa_\perp/\kappa$).

Problem 5

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).

a) 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 $x = z$?

b) 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*)

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

Chapter 3

Monochromatic field propagation through 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.

a) Derive $H(\rho)$ in the case where an obstructing disk of radius $b < a$ is inserted into the aperture.

b) Derive $H(\rho)$ in the case where the disk is transmitting but produces a phase shift of $90°$.

c) Derive $H(\rho)$ in the case where the disk is transmitting but produces a phase shift of $180°$.

d) 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 null 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 H for this imaging system. (Hint: use the Fourier shift theorem).

Problem 3

Consider imparting a spiral phase onto an otherwise azimuthally symmetric pupil. That is, if the pupil coordinates are $\vec{\xi} = (\xi \cos\varphi, \xi \sin\varphi)$, then the pupil function is given by $P\left(\vec{\xi}\right) = P(\xi)\, e^{im\varphi}$, where m is a positive integer. Assume unit-magnification 4f imaging, with lenses of focal length f. Derive a general expression for the amplitude point spread function $H(\vec{\rho})$ associated with this spiral-phase pupil. (Hint: make use of the Fourier transform properties of separable functions in cylindrical coordinates found in Appendix A. Your result should be in the form of a simple integral containing J_m.)

Problem 4

Derive Eqs. 3.16 and 3.17 from Eq. 3.6.

Problem 5

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

$$\int d^2\vec{\rho}_c \, H(\vec{\rho}_c + \tfrac{1}{2}\vec{\rho}_d) H^*(\vec{\rho}_c - \tfrac{1}{2}\vec{\rho}_d) = H(\vec{\rho}_d).$$

b) 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. 4.6. (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 $\delta\rho_{\mathrm{Rayleigh}}$ such that the maximum of the $\mathrm{PSF}(\rho)$ of one point lies at the first zero of the $\mathrm{PSF}(\rho)$ of the other point. That is, $\delta\rho_{\mathrm{Rayleigh}}$ is defined as the minimum distance such that $\mathrm{PSF}(\delta\rho_{\mathrm{Rayleigh}}) = 0$.

a) Derive $\delta\rho_{\mathrm{Rayleigh}}$ in terms of λ and NA (you will have to do this numerically).

b) 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 $\mathrm{PSF}_{\mathrm{annular}}(0)/\mathrm{PSF}_{\mathrm{circular}}(0)$?

c) Provide a numerical plot of $\mathrm{PSF}_{\mathrm{annular}}(\Delta\kappa_\perp\rho)$ and $\mathrm{PSF}_{\mathrm{circular}}(\Delta\kappa_\perp\rho)$ 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?

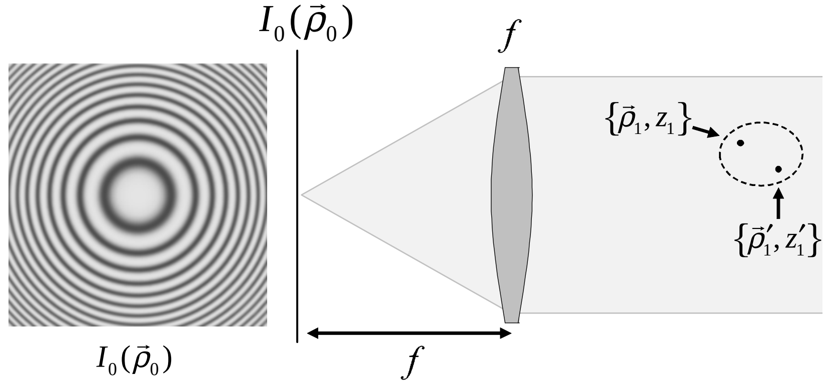
Problem 3

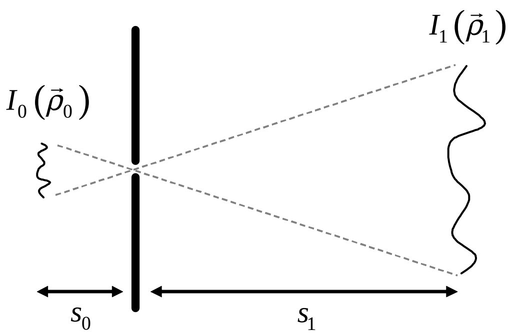
The 3D coherence function of Köhler illumination is given by Eq. 4.63. Sketch the range of 3D spatial frequencies $\{\vec{\kappa}_\perp, \kappa_s\}$ spanned by this coherence function. This is called the frequency support associated with Köhler illumination.

Problem 4

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

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





as illustrated in the figure.

a) You will find that $\mu_1(\vec{\rho}_{1d}, z_{1d})$ (Eq. 4.63) 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?

b) Draw a sketch for what happens to the coherence peaks when the pattern in $I(\vec{\rho}_0)$ is shifted upward.

Problem 5

Consider a pinhole camera, as shown in the figure. A 2D incoherent intensity distribution $I_0(\vec{\rho}_0)$ is projected through a pinhole of pupil function $P\left(\vec{\xi}\right)$ and creates an image $I_1(\vec{\rho}_1)$. The object and image planes are distances s_0 and s_1, respectively, from the pinhole plane. Show that, under the Fresnel approximation,

$$\mathcal{I}_1\left(\frac{1}{M}\vec{\kappa}_\perp\right) = \frac{1}{s_0^2}\mathcal{I}_0(\vec{\kappa}_\perp)\int \mathrm{d}^2\vec{\xi}_c\, P\left(\vec{\xi}_c + \frac{s_0}{2\kappa}\vec{\kappa}_\perp\right) P^*\left(\vec{\xi}_c - \frac{s_0}{2\kappa}\vec{\kappa}_\perp\right) e^{i2\pi(1-1/M)\vec{\xi}_c\cdot\vec{\kappa}_\perp}$$

with magnification $M = -\frac{s_1}{s_0}$. Note that the OTF here is not simply the pupil autocorrelation function, as it is for a 4f system. (Hint: one can proceed by making use of Eq. 2.50 to propagate $E_0(\vec{\rho}_0)$ to $E_1(\vec{\rho}_1)$, and then calculate $I_1(\vec{\rho}_1)$. The replacement $\langle E_0(\vec{\rho}_0)\, E_0^*(\vec{\rho}_0')\rangle \rightarrow \kappa^{-2} I_0(\vec{\rho}_0)\,\delta^2(\vec{\rho}_0 - \vec{\rho}_0')$ from Eq. 4.43 then leads to the above result).

Chapter 5

3D Imaging

Problem 1

a) Derive Eq. 5.24.

b) 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

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

Based on the Fresnel approximation, derive analytically:

a) $\mathcal{H}_+(\kappa_x, 0; 0)$ and $\mathcal{H}_+(0, 0; z)$

b) $H_+(x, 0, 0)$ and $H_+(0, 0, z)$

c) $\mathrm{PSF}(x, 0, 0)$ and $\mathrm{PSF}(0, 0, z)$

d) $\mathrm{OTF}(\kappa_x, 0; 0)$ and $\mathrm{OTF}(0, 0; z)$.

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

Note also, $\mathcal{H}_+$ 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

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

where I_0 is a constant.

a) Based on Eq. 5.42, derive an expression for the imaged intensity distribution. This expression should look like

$$I_1(x_1, y_1) \propto (1 + M(q_x, z_s) \cos(2\pi q_x x_1)) .$$

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

b) 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).

Problem 4

a) Verify that the second moment of an arbitrary function $F(\vec{\rho})$ is given by

$$\int d^2\vec{\rho}\,\rho^2 F(\vec{\rho}) = -\frac{1}{4\pi^2}\nabla^2\mathcal{F}(0)$$

where $\mathcal{F}(\vec{\kappa}_\perp)$is the 2D Fourier transform of $F(\vec{\rho})$.

b) For a cylindrically symmetric imaging system whose OTF has a cusp at the origin, what does the above result tell you about the dependence of PSF on $|\vec{\rho}|$ for large $|\vec{\rho}|$? Verify this dependence for the case of an unobstructed circular pupil.

Problem 5

a) Derive Eq. 5.8 from 5.7.

b) Use this to re-express the amplitude point spread function $H_+(0, z)$ for a Gaussian pupil (Eq. 5.23) in a similar form as Eq. 5.21 for a circular pupil. For equal $\Delta\kappa_\perp$, which has the more rapidly varying carrier frequency? Which has the first zero-crossing?

Chapter 6

Radiometry

Problem 1

a) Derive Eq. 6.11 (i.e. $I_\infty(\vec{r}) = \left(\frac{1}{r}\right)^2 \mathcal{R}_0\left(\frac{\kappa}{r}\vec{\rho}\right)$) using the Fraunhofer approximation given by Eq. 2.62.

b) Verify Eq. 6.16, using the paraxial approximation.

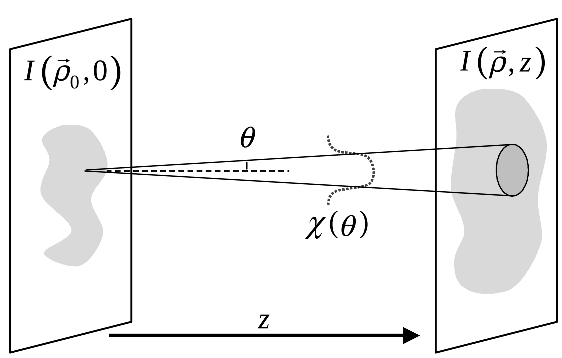
Problem 2

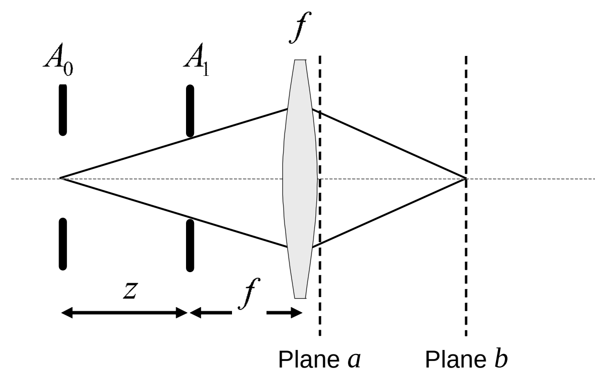
Assume that light emanating from an intensity distribution $I(\vec{\rho}_0, 0)$ obeys a paraxial angular distribution $\chi(\theta)$ everywhere (see figure). Based on purely geometrical arguments, one may write the convolutions

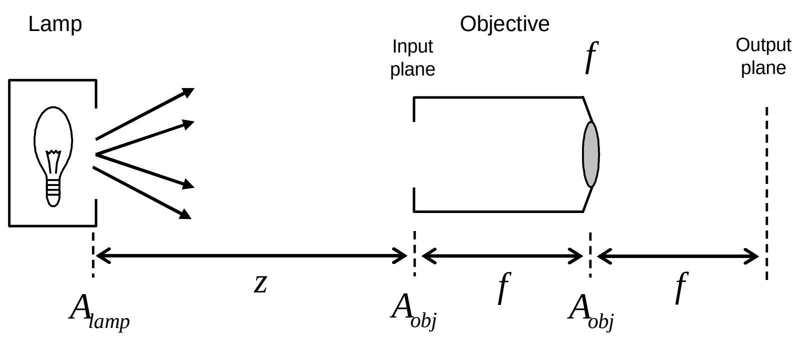
$$I(\vec{\rho}, z) = \frac{1}{z^2}\int d^2\vec{\rho}_0\, \chi\left(|\vec{\rho} - \vec{\rho}_0|/z\right) I(\vec{\rho}_0, 0)$$

$$\vec{\Theta}_\perp(\vec{\rho}, z) = \frac{1}{z^3 I(\vec{\rho}, z)}\int d^2\vec{\rho}_0\, (\vec{\rho} - \vec{\rho}_0)\, \chi\left(|\vec{\rho} - \vec{\rho}_0|/z\right) I(\vec{\rho}_0, 0)\,.$$

Show that $I(\vec{\rho}, z)$ and $\vec{\Theta}_\perp(\vec{\rho}, z)$ constructed in this manner obey the transport of intensity equation (Eq. 6.21).







Problem 3

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

a) Calculate the throughput of this system using the recipe outlined in Section 6.5.1, treating plane a as the output plane. Identify the aperture and field stops.

b) 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 output plane.

Problem 4

A lamp in a housing emits incoherent light through an aperture of area A_{lamp} (see figure). The emitted light power is Φ_{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.

a) 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.

b) 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 output plane? What is the coherence area of the beam at the output plane? Estimate the beam spot size at the output plane.

c) 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 5

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

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

(Note: this differs from the single-mode Gaussian beam described by Eq. 6.34 in that $w_c > w_d$).

a) Calculate the number of modes in this beam.

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

c) 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.

d) 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. 2.45 and 2.47) can be re-written in the form

$$E(\vec{\rho}, z, t) = -i\bar{\kappa} \int \mathrm{d}^2\vec{\rho}_0 \, \frac{\cos\theta}{r} E(\vec{\rho}_0, 0, t - r/c)$$

which is valid for narrowband fields whose wavenumber is centered around $\bar{\kappa}$ (assuming propagation in vacuum). This expression can be simplified using the Fresnel approximation (Section 2.4.1). 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

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

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

$$\mu(\rho_d) = e^{-\rho_d^2/2\rho_\mu^2}$$
$$\gamma(\tau) = e^{-i2\pi\bar{\nu}\tau} e^{-\pi\tau^2/2\tau_\gamma^2}$$

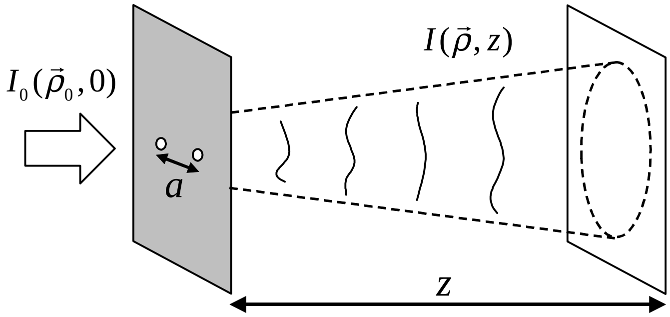
where $\bar{\nu} = \bar{\kappa}c$.

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

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

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

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



b) 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?

c) 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.

d) 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

Consider a light beam that randomly switches between two states of intensities I_A and I_B. Let $P_A(t)$ and $P_B(t)$ be the probabilities that the beam is in states A or B respectively, such that

$$\frac{d}{dt} P_A(t) = -\lambda P_A(t) + \mu P_B(t)$$

where λ and μ are switching rate constants. Such a beam is called a random telegraph wave.

a) Show that the mean intensity of the beam is given by

$$\langle I \rangle = \frac{\mu I_A + \lambda I_B}{\mu + \lambda}.$$

b) Show that the intensity variance of the beam is given by

$$\sigma_I^2 = \frac{\mu\lambda (I_A - I_B)^2}{(\mu + \lambda)^2}.$$

Problem 3

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 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.

a) 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.

b) Verify that when $\tau_\gamma \ll T$ the contrast obeys the relation given by Eq. 7.50.

Problem 4

Consider the intensity distribution $I(\vec{\rho})$ 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 camera, which consists of a 2D array of detectors (pixels), each of area $A = L \times L$. As a result, $I(\vec{\rho})$ becomes integrated upon detection, and then sampled. The detected power, prior to sampling, can thus be written as

$$\Phi_A(\vec{\rho}) = A \int \mathrm{d}^2\vec{\rho}\,'\, R_A(\vec{\rho} - \vec{\rho}\,')I(\vec{\rho}\,').$$

a) Provide expressions for $R_A(\vec{\rho})$ and its Fourier transform $\mathcal{R}_A(\vec{\kappa}_\perp)$.

b) 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 Eq. 4.62) that the coherence function of a such a speckle pattern is given by

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

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 camera in terms of $\mathcal{R}_A(\vec{\kappa}_\perp)$, $\mathrm{OTF}(\vec{\kappa}_\perp)$ and $\mathrm{OTF}_s(\vec{\kappa}_\perp)$.

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

Problem 5

Derive Eqs. 7.35 and 7.36 from Fig. 7.10.

Chapter 8

Detector Noise

Problem 1

For photoelectron arrival times governed by Poissonian statistics, it can be shown that the wait time τ between successive photoelectrons is governed by the probability distribution (see Eq. 7.10):

$$p(\tau) = \frac{1}{\bar{\tau}} e^{-\tau/\bar{\tau}}$$

where $\bar{\tau}$ is the average wait time. It can also be shown that, starting at an *arbitrary* time, the wait time for the next photoelectron is given by the same probability distribution with the same $\bar{\tau}$. The same is true for the "wait time" (going backward in time) for the previous photoelectron. But from these last two statements, it appears that the average wait time between successive photoelectrons should be $2\bar{\tau}$ and not $\bar{\tau}$. How can one reconcile all these statements? This is a classic problem in probability theory. (Hint: the arbitrary start time is more likely to fall within photoelectron intervals that are large.)

Problem 2

a) Show that if the instantaneous power Φ 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

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

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

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

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

$$\sigma_K^2 = \langle K \rangle + \langle K \rangle^2 .$$

Note: for part (b), you will find the following identity to be useful:

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

Problem 3

Derive Eq. 8.26 from Eq. 8.23.

Problem 4

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.

a) 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?

b) 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 5

Consider a 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 accommodate 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.

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

b) 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 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

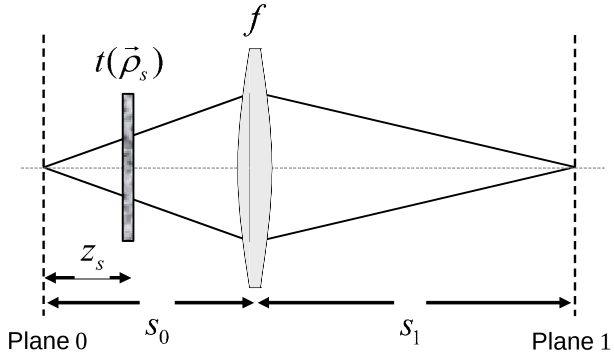
Absorption and scattering

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 image 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 source plane (without the sample), followed by a forward projection to the image plane. This last projection is given by Eq. 3.17.



Problem 2

Show that if a sample δn is so weak that multiple scattering can be neglected, and the fields are mostly forward directed (i.e. paraxial), then the beam propagation method yields identical results as the Born approximation.

Problem 3

Consider illuminating a sample with a plane wave directed along $\hat{z}$, and recording the resultant scattered far field in the transmission direction within a cone of angle $\theta_{\max}$. Based on the information provided by this scattered far field, how well can a sample $\delta\varepsilon$ be axially resolved if the sample is

a) a thin, uniform plane?

b) a point?

Use the Fourier diffraction theorem and provide an estimate as a function of $\theta_{\max}$.

Problem 4

A wave traveling through a slowly spatially varying index of refraction $n(\vec{r})$ can be written as

$$E(\vec{r}) = A(\vec{r})\, e^{i2\pi\bar{\kappa}W(\vec{r})}$$

where $\bar{\kappa}$ is the average wavenumber associated with $\bar{n}$. This expression is similar to the Rytov approximation except that $A(\vec{r})$ does not represent the incident field, but rather represents a slowly varying amplitude (real). Surfaces of constant $W(\vec{r})$ are called wavefronts of the field.

Show that when the above expression is inserted into the Helmholtz equation (Eq. 9.20), and in the geometric-optics limit where the light wavelength $\bar{\lambda}$ becomes vanishingly small, one arrives at the so-called Eikonal equation:

$$\left|\vec{\nabla}W(\vec{r})\right|^2 = |n(\vec{r})|^2 .$$

This equation serves to define $\vec{\nabla}W(\vec{r})$, which can be interpreted as a light ray direction in geometrical optics.

Problem 5

Derive Eqs. 9.71 and 9.73.

Chapter 10

Widefield microscopy

Problem 1

Consider a thin sample that induces both phase shifts $\varphi(\vec{\rho}_0)$ and attenuation $\alpha(\vec{\rho}_0)$. The local sample transmittance can then be written as $t(\vec{\rho}_0) = e^{i\phi(\vec{\rho}_0)}$, where $\phi(\vec{\rho}_0) = \varphi(\vec{\rho}_0) + i\alpha(\vec{\rho}_0)$ is a generalized complex phase function ($\varphi(\vec{\rho}_0)$ 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 $I^{(\psi)}(\vec{\rho})$.

a) 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

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

we obtain

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

where $I_i = |E_i|^2$.

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

b) 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 10.1.3 (all other imaging conditions being equal).

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

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

Problem 3

a) Derive Eq. 10.30 from Eqs. 10.28 and 10.29.

b) Rewrite Eq. 10.30 in terms of local tilt angles $\Theta_i(\vec{\rho}_{0c})$ and $\Theta_0(\vec{\rho}_{0c})$ going into and out of the sample (see Eq. 6.20 for definition of tilt angles). Express $\vec{\nabla}\phi(\vec{\rho}_{0c})$ in terms of $\Delta\Theta(\vec{\rho}_{0c}) = \Theta_0(\vec{\rho}_{0c}) - \Theta_i(\vec{\rho}_{0c})$.

Problem 4

Write Eq. 10.31 in terms of fields rather than radiant fields, and use this to derive Eq. 10.40 more directly (at least, for thin samples that are in focus).

Problem 5

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

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

Show that the relation between $\Delta\theta$ and ϕ is given by $\Delta\theta = 2\phi + \frac{\pi}{2}$.

Chapter 11

Interference microscopy

Problem 1

Equations 11.23 and 11.27 are idealized in that they consider the integration over $\vec{\rho}$ to be infinite. In practice, the integration can only be performed over the area of the camera, which has a finite size $L_x \times L_y$. Derive the effect of this finite size on the transverse spatial resolution of the reconstructed sample $\delta\varepsilon(\vec{\rho}_0)$. In particular...

a) Show that in the case of lensless Fourier holography (Fig. 11.3), this resolution is given by $\delta x_0 = \frac{\lambda}{2n\theta_x}$ and $\delta y_0 = \frac{\lambda}{2n\theta_y}$, where $\theta_x = \frac{L_x}{2z}$ and $\theta_y = \frac{L_y}{2z}$. (Assume δx_0 and δy_0 are small).

b) Show that in the case of Fourier holography with a lens (Fig. 11.4), this resolution is given by $\delta x_0 = \frac{\lambda}{2n\theta_x}$ and $\delta y_0 = \frac{\lambda}{2n\theta_y}$, where $\theta_x = \frac{L_x}{2f}$ and $\theta_y = \frac{L_y}{2f}$.

Note the analogy of these results with the standard resolution criterion given by Eq. 3.36. In performing these calculations, you will run into sinc functions. Define the width of $\mathrm{sinc}(ax)$ to be $\delta x = \frac{1}{a}$.

Problem 2

Consider the three lensless digital holographic microscopy configurations shown in Figs. 2,3 and 9, and assume all parameters in these configurations are the same, and that the camera has sensor size L and pixel size p (both square).

a) What camera parameter defines the transverse spatial resolution δx_0 and δy_0 of the reconstructed sample in all three configurations?

b) What camera parameter defines the transverse field of view Δx_0 and Δy_0 of the reconstructed sample in all three configurations? (where field of view corresponds to the maximum transverse extent of the sample, assumed centered on axis).

c) Provide estimates for Δx_0 and Δy_0 in all three configurations, assuming these are smaller than L and much smaller than z (roughly as depicted in the the figures), and assuming the reference-beam tilt angle θ in the case of off-axis Fresnel holography is in the x direction only.

Hint: the sample and reference beams interfere at the camera, and produce fringes. These must be properly sampled according the Nyquist sampling criterion.

Problem 3

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

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

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

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

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.

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

Problem 4

Consider the technique of phase-stepping, as described in Section ???.

a) Why are a minimum of three phase steps required to determine $\widetilde{I}_{sr}(\vec{\rho})$?

b) The phase steps need not be circular. For example, show how one can recover the amplitude and phase of $\widetilde{I}_{sr}(\vec{\rho})$ using the phase sequence $\phi_k = \left\{0, \frac{\pi}{2}, \pi\right\}$.

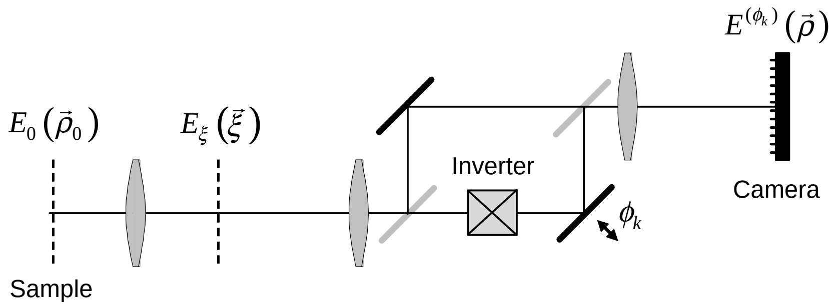
c) Consider a reference beam that is frequency shifted (as opposed to phase shifted) relative to the sample beam, such that $E_r(\vec{\rho}) \rightarrow E_r(\vec{\rho})e^{i2\pi\delta\nu\, t}$. Assume that the camera frame rate is $3\delta\nu$, and the camera exposure time is $(3\delta\nu)^{-1}$. Write an algorithm to recover $\widetilde{I}_{sr}(\vec{\rho})$ from a sequence of three camera exposures.

Problem 5

Most widefield microscopes are based on 4f configurations. Here we consider a 6f configuration. In particular, a 2f system projects an in-focus sample field $E_0(\vec{\rho}_0)$ onto a Fourier plane, where it is denoted by $E_\xi\left(\vec{\xi}\right)$. The Fourier field is then re-imaged with a unit magnification 4f system that separates and re-combines the field through two paths, one on which is inverting, as shown in the figure. That is, the output field is given by

$$E^{(\phi_k)}(\vec{\rho}) = \frac{1}{2}\left(E_\xi(\vec{\rho}) + E_\xi(-\vec{\rho})\, e^{i\phi_k}\right)$$

where ϕ_k is a controllable phase shift that can be applied to the inverting path. Phase-stepping interferometry then allows one to synthesize the complex intensity



$$\widetilde{I}(\vec{\rho}) = \left\langle E_\xi(\vec{\rho}) E_\xi^*(-\vec{\rho}) \right\rangle .$$

a) Derive an expression for $\widetilde{I}(\vec{\rho})$ in terms of the radiant mutual intensity at the sample plane (adopting the usual coordinate transformation of Eq. 4.17). Hint: use results from Chapter 4

b) Assume that the intensity distribution at the sample plane is spatially incoherent, meaning that the mutual intensity at this plane can be expressed in the form of Eq. 4.43. Derive an expression for the sample intensity $I_0(\vec{\rho}_0)$ in terms of the above radiant mutual intensity.

c) Show that even if the sample is displaced away from the focal plane by a distance z_0, the sample intensity $I_{z_0}(\vec{\rho}_0)$ remains equal to $I_0(\vec{\rho}_0)$, independently of z_0. That is, the 6f system described here provides extended-depth-of-field imaging, where the recovered image remains in focus independently of the axial location of the (spatially incoherent) sample.

Chapter 12

Optical Coherence Tomography

Problem 1

Derive Eq. 12.15.

Problem 2

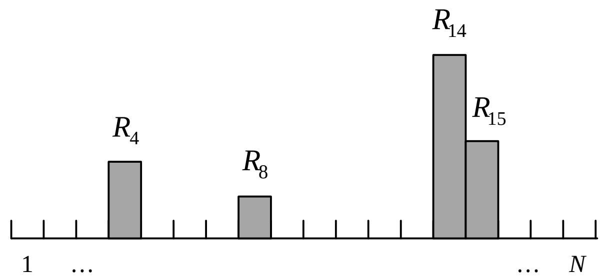
Figure 12.6 provides a plot of $\widetilde{I}_{sr}(z_r;\nu_n)$ as a function of ν_n for a given value of z_r. What does this plot look like if the sample consists of a single reflecting plane located at depth z_s? What happens when $z_s = z_r$? When $z_s > z_r$? When $z_s < z_r$?

Problem 3

Show that the axial range of swept-source OCT scales inversely with $\delta\nu$. Specifically, consider a swept-source laser that produces a spectrum $S(\nu_n)\, e^{-(\nu-\nu_n)^2/\delta\nu^2}$, where $S(\nu_n)$ is the envelope of the frequency scan. As in the text, treat ν_n as a continuous variable and assume that $S(\nu_n)$ is slowly varying on the scale of $\delta\nu$. Show that $\widetilde{I}_{sr}(z_r - z_n)$, as defined by Eq. 12.29, is confined to within a range of z_n's defined by a Gaussian envelope. What is the extent Δz_n of this axial range? That is, what is the width (waist) of this Gaussian envelope?

Problem 4

Our goal in this problem is to compare direct versus multiplexed signal acquisition, under conditions of constant illumination intensity I_i and equal total measurement time Δt. Consider objects of reflectance strength R_n distributed along a line at positions indexed by n, where $n = 1...N$ (as shown in figure), such that the power reflected from each object is given by $\Phi_n = R_n I_i$. These objects may be imaged directly, by illuminating each position one after the other in sequence, and recording the reflected power from each position with a single detector. Alternatively, the objects may be imaged in a multiplexed manner by illuminating the objects in parallel with a sequence of quasi-uniform intensity patterns (of average intensity I_i), and detecting the total reflected power for every illumination pattern with the same single detector. In both cases, N measurements are made, each of duration $\delta t = \Delta t/N$. In the case of multiplexed acquisition, the final image must be reconstructed



numerically based on some kind of demultiplexing algorithm. Assume that each object signal adds "coherently" upon reconstruction (i.e. scales with the number of measurements N), and is given by $NR_nI_i\delta t$. Assume also that the noise adds "incoherently" upon reconstruction (i.e. scales as $\sqrt{N}$ times the noise associated with each measurement).

a) Consider a sparse distribution of objects, namely a single object of reflectance strength R located at arbitrary position m. Assume a noiseless detector of unity gain and take into account only shot noise. Compare the SNR's associated with this object for direct versus multiplexed acquisition. Which is best? Specifically, derive $\mathrm{SNR}_{\mathrm{dir}}/\mathrm{SNR}_{\mathrm{mul}}$.

b) Consider a dense distribution of objects, each of equal reflectance strength R, such that an object is located at every position n. Derive $\mathrm{SNR}_{\mathrm{dir}}/\mathrm{SNR}_{\mathrm{mul}}$ for any given object. You should find that the multiplex (or Fellgett) advantage has disappeared.

c) Consider the same dense distribution of objects, except that one object located at arbitrary position m has reflectance strength $\frac{1}{10}R$. Derive $\mathrm{SNR}_{\mathrm{dir}}/\mathrm{SNR}_{\mathrm{mul}}$ for this weaker object. You should find that you are better off using direct acquisition. This difficulty with multiplexed acquisition is generally referred to as the multiplex disadvantage.

d) Repeat calculations **(a)**-**(c)** taking into account a detector readout noise of standard deviation σ_r. Assume that σ_r is so large that shot noise can be neglected. Does the sample sparsity matter in this case? Note that this scenario is similar to the scenario of FD-OCT.

e) Consider now the condition of constant illumination power Φ_i rather than constant illumination intensity. In other words, in the case of direct acquisition, the illumination intensity sequentially delivered to each location n is given by $I_i = \Phi_i/\delta A$, where δA is the focused illumination area. In the case of multiplexed acquisition, the illumination is spread over an area $\Delta A = N\delta A$ spanning all N locations, and delivers instead an average illumination intensity given by $I_i = \Phi_i/\Delta A$. Are there any cases where multiplexing is advantageous?

Problem 5

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

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

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.

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

b) Consider two noise sources: shot noise and dark noise. The latter is modeled as producing background photoelectron counts obeying Poisson statistics. Let $\langle N_S \rangle$, $\langle N_R \rangle$, and $\langle N_D \rangle$ 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

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

(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 $\langle N_R \rangle \gg \langle N_S \rangle$ and $\langle N_R \rangle \gg \langle N_D \rangle$, then $\sigma_{\varphi_N} \to \sqrt{\frac{1}{2\langle N_S \rangle}}$, 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.

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

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

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

a) Show that

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

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.

b) Show that, based on our simple model,

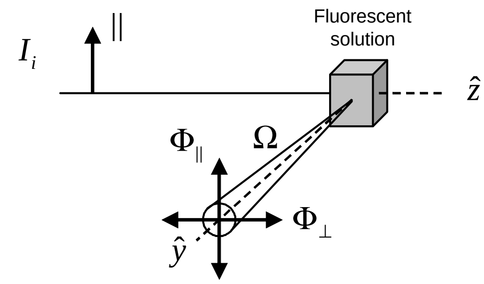
$$\frac{\Phi_f}{\Phi_f^{(Q)}} \leq \frac{\tau_e}{\tau_e^{(Q)}}$$

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 $\Phi_{\parallel}$ and $\Phi_{\perp}$ respectively. It can be shown that these powers are given by



$$\Phi_{||}(t) = \Omega\sigma_f \int K_{||}(t-t')I_i(t')dt'$$
$$\Phi_{\perp}(t) = \Omega\sigma_f \int K_{\perp}(t-t')I_i(t')dt'$$

where

$$K_{||}(t) = \tfrac{1}{3}\left(1 + 2R(t)\right)K(t)$$
$$K_{\perp}(t) = \tfrac{1}{3}\left(1 - R(t)\right)K(t)$$

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

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

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

The measured fluorescence anisotropy is defined by

$$r(t) = \frac{\Phi_{||}(t) - \Phi_{\perp}(t)}{\Phi_{||}(t) + 2\Phi_{\perp}(t)}$$

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

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

This is known as the Perrin relationship. In deriving this relationship, bear in mind that the denominator of $r(t)$ remains constant over time.

b) Denote Φ_f as the total *emitted* fluorescence power in all solid angles. Derive an expression for the total *measured* fluorescence power $\Phi_{||}(t) + \Phi_{\perp}(t)$ when $\tau_e/\tau_\theta \to 0$ (i.e. the rotation is slow compared to the excited state lifetime). When is this measured fluorescence power equal to $\Omega\Phi_f$?

c) Derive an expression for the total *measured* fluorescence power when $\tau_e/\tau_\theta \to \infty$ (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 $\Omega\Phi_f$.

Problem 3

Consider using a microscope to image a sample containing N different fluorescent species of unknown concentrations $C_1, ..., C_N$. Each of these species emits fluorescence with a different spectrum. Accordingly, the microscope is equipped with N different spectral channels. The microscope has been pre-calibrated so that a unit concentration of species j is known to produce a signal M_{ij} in channel i. When the unknown sample is measured, signals $S_1, ..., S_N$ are obtained in each channel.

a) Derive a general "spectral unmixing" formula to deduce $C_1, ..., C_N$ from the measured signals. Hint: your formula should be in terms of the matrix of cofactors of $\mathbf{M}$.

b) Consider using a two-channel microscope to look at a fluorescent molecule [1]. The introduction of a non-fluorescent binding agent $[x]$ leads to an interaction $[1] + [x] \to [2]$, where the species [2] produces fluorescence that is spectrally different from [1]. The efficiency of the interaction is defined by

$$q_{\text{int}} = 1 - \frac{C_1'}{C_1}$$

where C_1 and C_1' are concentrations of species [1] before and after the response.

Show that

$$q_{\text{int}} = \frac{-M_{22}\Delta S_1 + M_{21}\Delta S_2}{M_{22}S_1 - M_{21}S_2}$$

where $\Delta S = S' - S$ and $\mathbf{M}$ is assumed to be non-singular.

Problem 4

Frequency-domain FLIM provides a measurement of $\overline{\mathcal{R}}(\nu)$, as defined by Eq. 13.27, for a given modulation frequency ν.

a) Consider a solution containing only a single fluorescent species. Draw a plot of $\mathcal{R}(\nu)$ in the complex plane as a function of increasing ν. This is called a "polar" or "phasor" plot of the fluorescence response, where $\text{Re}[\mathcal{R}(\nu)]$ and $\text{Im}[\mathcal{R}(\nu)]$ are the in-phase and quadrature components respectively. At what value of ν is the quadrature component peaked? For any given ν, where does a small fluorescence lifetime place $\mathcal{R}(\nu)$? Where does a large fluorescence lifetime place $\mathcal{R}(\nu)$?

b) Consider two fluorescent species of known cross-sections and lifetimes. Arbitrarily choose locations for $\mathcal{R}^{(1)}(\nu)$ and $\mathcal{R}^{(2(}(\nu)$ on the plot you have drawn above (for a given modulation frequency). Now consider making a measurement of $\overline{\mathcal{R}}(\nu)$ for a mixture of these two species of unknown concentration fractions. Where must $\overline{\mathcal{R}}(\nu)$ be located relative to

$\mathcal{R}^{(1)}(\nu)$ and $\mathcal{R}^{(2(}(\nu)$? From a single measurement, can you infer the concentration fractions of the two species?

c) Consider a solution containing an unknown number of fluorescent species of responses characterized by Eq. 13.25. What can you say about the resulting $\overline{\mathcal{R}}(\nu)$? Specifically, where must $\overline{\mathcal{R}}(\nu)$ be located for low, high, and mid-range modulation frequencies?

Problem 5

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.

a) Define a corresponding probe volume V_ψ.

b) Show that $\Gamma_f(\tau) = \frac{1}{\langle N \rangle}\left(1 + \frac{2D\tau}{w_0^2}\right)^{-3/2}$, 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. 14.5 it is clear that a purely phase-shifting point object produces no discernible change in detected intensity in a transmission confocal microscope. That is, if $\delta\varepsilon$ is real then $I(\vec{\rho}_s, z_s)$ is independent of $\delta\varepsilon$ 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 amplitude-PSFs are identical and Gaussian (Eq. 5.21). Follow these steps:

a) Calculate E_b.

b) Calculate $E_s(\vec{\rho}_s, z_s)$. For simplicity, neglect scanning and set $\vec{\rho}_s$ and z_s to zero.

c) From the resulting $E(0,0) = E_b + E_s(0,0)$, derive the detected intensity $I(0,0)$ and show that this depends on (real) $\delta\varepsilon$ to first order (neglect any higher order dependence on $\delta\varepsilon$).

Problem 2

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 (assume that the beam is blocked beyond the annulus). A transmission detector records the power Φ_T transmitted through the pinhole. A reflection detector records the power Φ_R reflected from the annulus. The confocal signal is then given by the difference of these recorded powers, namely $\Delta\Phi = \Phi_T - \Phi_R$.

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

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

Hint: 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.

Problem 3

Consider a fluorescence confocal microscope where the illumination and detection PSF's are the same and Gaussian, as defined by Eq.5.34, and the pinhole is small.

a) Imagine dithering the pinhole of this microscope in the transverse direction by small amounts $\pm\frac{1}{2}\delta\vec{\rho}_p$, and demodulating the acquired image at the dither frequency. This is essentially equivalent to acquiring two images I_+ and I_- with the pinhole located at $+\frac{1}{2}\delta\vec{\rho}_p$ and $-\frac{1}{2}\delta\vec{\rho}_p$, respectively, and then subtracting, obtaining a final image given by $\Delta I = I_+ - I_-$. Derive the effective confocal PSF, or $\Delta\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$, of this instrument to first order in $\delta\vec{\rho}_p$. Express your answer in terms of the conventional (undithered) PSF, or $\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$. Discuss some features of $\Delta\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$, such as its axial profile and its response to a laterally uniform sample.

b) Now consider acquiring a conventional image with this instrument, and simply calculating the gradient of this image along the direction $\delta\vec{\rho}_p$ (or, more precisely, the difference image using the same transverse shift $\delta\vec{\rho}_p$) . Your answer should be the same to within a scaling factor. What is this scaling factor?

Problem 4

Consider the same as Problem 3, except that now the pinhole is dithered by small amounts $\pm\delta z_p$ in the axial direction. Derive the effective confocal PSF, or $\Delta\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$, of this instrument to first order in δz_p. Express your answer in terms of the conventional (undithered) $\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$. Qualitatively describe what sample features this instrument is sensitive to. Show that $\Delta\mathrm{PSF}_{\mathrm{conf}}(0, z)$ decays more rapidly than $\mathrm{PSF}_{\mathrm{conf}}(\vec{\rho}, z)$ by a factor of $|z|^{-1}$ for large $|z|$.

Problem 5

Consider a fluorescence confocal microscope where the illumination and detection PSF's are the same and Gaussian, as defined by Eq.5.34 (though re-expressed in terms of their field waists w_0 – see Eq. 5.23). Let the pinhole also be a Gaussian, defined by $A_p(\vec{\rho}) = e^{-\rho^2/w_p^2}$. Derive an expression for the normalized axial profile of the confocal PSF, namely $\mathrm{PSF}_{\mathrm{conf}}(0, z) / \mathrm{PSF}_{\mathrm{conf}}(0, 0)$. Qualitatively describe the $z$dependence of this profile. Plot this profile for $w_0 = \lambda = 1\mu\mathrm{m}$, and for the different ratios $w_p/w_0 \approx 0, 1$ and 2.

Chapter 15

Structured illumination microscopy

Problem 1

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

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

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.

a) 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.

b) 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.

c) 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.

d) 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?

e) 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

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

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?

Problem 4

SIM strategies involving random illumination patterns, such as DSI, generally require the calculation of signal means and variances, defined respectively by

$$\mu = \frac{1}{N}\sum_{i=1}^{N} x_i$$
$$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N} (x_i - \mu)^2$$

where x_i are independent signal realizations.

a) Show that σ^2 can be written equivalently as $\sigma^2 = \frac{1}{N}\sum\limits_{i=1}^{N} x_i^2 - \mu^2$

b) ...and again as $\sigma^2 = \frac{1}{2N^2}\sum\limits_{i,j=1}^{N} (x_i - x_j)^2$

c) ...and yet again as $\sigma^2 = \frac{1}{2(N-1)}\sum\limits_{i=1}^{N-1} (x_{i+1} - x_i)^2$. This last representation is particularly insensitive to slow signal fluctuations that may be due to extraneous instrumentation noise.

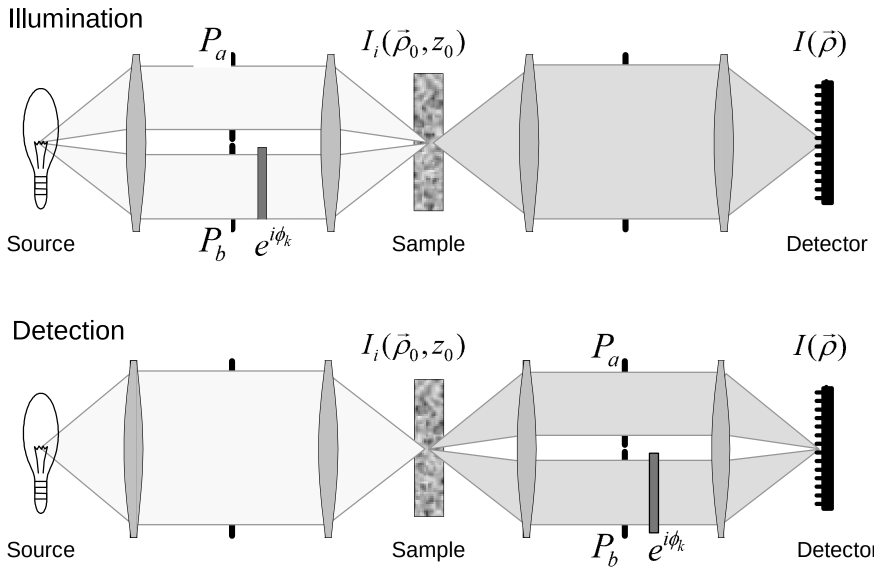
Problem 5

In Section 15.5, it was stated that SIM can thought of in the context of pupil synthesis. The basic idea of pupil synthesis is to construct an effective (synthesized) pupil from a sequence of images obtained from multiple pupil configurations. For example, two-pupil synthesis can be performed either in the illumination or detection paths of a generic widefield fluorescence microscope, as shown in the figure. In either case, it is assumed that one pupil, P_b, is phase-shifted relative to the other pupil P_a with a circular phase sequence ϕ_k.

a) Consider illumination synthesis (top). Light from any source point can equally travel through both illumination pupils, producing a field incident on the sample given by the coherent superposition

$$E_i^{(k)}(\vec{\rho}_0, z_0) = E_i^{(a)}(\vec{\rho}_0, z_0) + e^{i\phi_k} E_i^{(b)}(\vec{\rho}_0, z_0).$$

Show that conventional phase stepping leads to the synthesis of a complex image given by Eq. 15.6, with an effective complex illumination distribution given by Eq. 15.7.



b) Consider detection synthesis (bottom). Show that conventional phase-stepping leads to the synthesis of a complex image with an effective complex PSF. Derive corresponding expressions for $\widetilde{I}(\vec{\rho})$ and $\widetilde{\mathrm{PSF}}(\vec{\rho}, z)$, where the latter is in terms of amplitude point spread functions. Assume that the source intensity is uniform.

Chapter 16

Multiphoton microscopy

Problem 1

Consider a collimated beam that undergoes both one- and two-photon absorption as it propagates within a sample. That is, the beam intensity obeys the relation

$$\frac{dI}{dz} = -\alpha_1 I - \alpha_2 I^2.$$

a) Show that the resulting intensity is given by

$$I(z) = \frac{\alpha_1 I(0)\, e^{-\alpha_1 z}}{\alpha_1 + \alpha_2 \left(1 - e^{-\alpha_1 z}\right) I(0)}.$$

b) In the limit $a_2 \to 0$, the above result reduces to the familiar Lambert-Beer law. What does it reduce to in the limit $\alpha_1 \to 0$?

Problem 2

The fluorescence power emitted by a molecule under continuous two-photon excitation 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.

a) 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).

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

$$\frac{\langle \Phi_f \rangle}{h\nu_f} = \frac{\xi}{\tau_l}\left(\frac{1 - e^{-\tau_l/\tau_e}}{1 - e^{-\tau_l/\tau_e} + \xi e^{-\tau_l/\tau_e}}\right)$$

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

c) 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 3

In the case of two-photon excitation, show that if the sample is a thin uniform plane at a defocus position 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 illumination beam cross-sectional area $A_i(z_s)$, independently of the shape of the illumination PSF_i. Is this also true of three-photon excitation?

Hint: define cross-sectional area in a similar manner as Eq. 6.22.

Problem 4

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

a) Show that the three-photon excitation volume is given by $V_{3f} = \frac{32}{105}\pi^2 w_0^2 z_R$ and the volume contrast is given by $\gamma_{3f} = \frac{35}{128}$.

b) 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 in the case of two-photon excitation, and it scales as w_0^{-2} in the case of three-photon excitation.

Problem 5

Consider a laser whose average power is a constant $\langle \Phi_i \rangle$ but whose repetition rate $R = \tau_l^{-1}$ can be varied. This laser is used to perform multiphoton excitation in a thick sample of extinction coefficient μ_e. But there is a problem. The maximum peak intensity that the sample can tolerate at the laser focus is $\hat{I}_{\max}$. Derive a strategy of adjusting the repetition rate to maximize the fluorescence produced at the beam focus, for arbitrary depth within the sample. That is, find an expression for the optimal $R\,(z_s)$.

Chapter 17

Multiharmonic microscopy

Problem 1

Consider a pulsed laser beam whose power is written as

$$\Phi_l(t) = U_l \sum_{n=0}^{N} \delta(t - n\tau_l)$$

where U_l is the energy per pulse and τ_l is the pulse period. Now consider that each pulse is subject to a temporal jitter $\delta\tau_l$, which may be considered a random Gaussian variable. Show that the Fourier transform of $\Phi_l(t)$, averaged over large N, can be written as

$$\left\langle \hat{\Phi}_l(\nu) \right\rangle = U_l e^{-2\pi^2\nu^2\sigma_{\tau_l}^2} \frac{\sin(\pi N \bar{\tau}_l \nu)}{\sin(\pi \bar{\tau}_l \nu)}$$

where $\bar{\tau}_l$ is the mean pulse period, and $\sigma_{\tau_l}^2$ is the variance of the temporal jitter.

Hint: you may want to use a result from Appendix B.5.

Problem 2

The second harmonic tensorial product $\vec{P} = \epsilon_0 \vec{\chi}^{(2)} : \vec{E}\,\vec{E}$ (see Eq. 17.24) can be expanded as

$$P_i = \epsilon_0 \sum_{j=1}^{3} \sum_{k=1}^{3} \chi_{ijk}^{(2)} E_j E_k.$$

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.

a) Defining $\mathbf{R}(\theta, \varphi)$ to be the rotation matrix linking the molecule system to the laboratory system (see Eq. 17.14), show that

$$P_l^{(L)} = \epsilon_0 \sum_{m=1}^{3} \sum_{n=1}^{3} \chi_{lmn}^{(L)} E_m^{(L)} E_n^{(L)}$$

where

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

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

b) 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,

$$\vec{P}^{(L)} = \epsilon_0 \chi_{rrr}^{(2)} \left(\hat{r} \cdot \vec{E}^{(L)} \right)^2 \hat{r}.$$

Problem 3

It can be shown that the susceptibility tensor $\chi^{(3)}$ responsible for third-harmonic generation in a homogenous isotropic medium can be written as

$$\chi_{klmn}^{(3)} = \chi_0 \left(\delta_{kl}\delta_{mn} + \delta_{km}\delta_{ln} + \delta_{kn}\delta_{lm} \right).$$

Show that no THG can be produced in such a medium if the driving field is a circularly polarized plane wave.

Problem 4

Consider generating SHG with a focused beam as in Fig. 17.4, 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.

a) Use the 3D Gaussian approximation (Eq. 17.26) to derive the field $E_{2\nu_i}^{(2)}(\vec{r})$ produced by the two membranes. Express your answer in terms of $E_{2\nu_i}^{(1)}(\vec{r})$, the field produced by a single membrane (i.e. Eq. 17.36).

b) As in Fig. 17.4, the SHG is emitted in two off-axis lobes at $\cos\theta \approx 1 - \frac{\delta\kappa_i}{\kappa_i}$ and $\varphi \approx [0, \pi]$. Plot the intensity ratio $\frac{I_{2\nu_i}^{(2)}(\vec{r})}{I_{2\nu_i}^{(1)}(\vec{r})}$ in the lobe directions, as a function of $\frac{\Delta x_0}{w_0}$ (hint: use Eq. 17.22). At approximately what value of $\frac{\Delta x_0}{w_0}$ is this intensity ratio peaked?

Problem 5

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

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

Assume a focused illumination beam and use the 3D-Gaussian illumination profile given by Eq. 17.26. Express your result in terms of r, θ and φ.

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

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

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

Chapter 18

Pump-probe microscopies

Problem 1

Derive Eqs. 18_PP and 18_PVV

Problem 2

CARS microscopy is performed with Gaussian pump and Stokes pulses defined by $E_p(t) = E_p \exp\left(-t^2/\Delta t_p^2\right) \exp\left(-i2\pi\nu_p t\right)$ and $E_s(t) = E_s \exp - \left(t^2/\Delta t_s^2\right) \exp\left(-i2\pi\nu_s t\right)$, which overlap in time.

a) The spectral resolution $\Delta\nu_{\mathrm{CARS}}$ of a CARS microscope can be defined as the half-width at $1/e$-maximum of $\left|\mathcal{P}_{\mathrm{CARS}}(\nu)\right|^2$. Show that this spectral resolution is defined by the spectral width of the pump beam alone. What is this spectral resolution?

b) Consider adding a frequency chirp to the pump pulse, but not the Stokes pulse. The chirp rate is b. That is, the pump field and its Fourier transform are given by

$$E_p(t) = E_p e^{-t^2/\Delta t_p^2} e^{-i2\pi(\nu_p + bt)t}$$
$$\mathcal{E}_p(\nu) = \frac{1}{\sqrt{\pi}\Delta\nu_p'} E_p e^{-(\nu-\nu_p)^2/\Delta\nu_p'^2}$$

where $\Delta\nu_p' = \Delta\nu_p\sqrt{1 + i2\pi\Delta t_p^2 b}$ and $\Delta\nu_p = \frac{1}{\pi\Delta t_p}$.

What is the new spectral resolution of the CARS microscope?

c) The pump pulse, in addition to being chirped, is also temporally broadened to a width $\Delta t_p' > \Delta t_p$. Again, calculate the CARS spectral resolution. What is the maximum chirp rate allowed for this new resolution to be better (narrower) than the original resolution calculated in part (a)?

Problem 3

Most commonly, two techniques are used to obtain a CARS spectrum. The first makes use of picosecond pump and Stokes beams (that is, both are relatively narrowband). A spectrum can then be obtained by scanning the frequency of one of the beams, and acquiring data sequentially. This has the advantage that only a single detector is required.

Alternatively, the Stokes beam can be femtosecond (i.e. broadband), and a spectrum can be obtained by recording the CARS frequencies in parallel using a grating and line camera (called a spectrograph). This has the advantage that it does not require frequency scanning.

Alternatively still, both the pump and Stokes beam can be femtosecond and chirped, with the *same* chirp rate (see Problem 2). For this last scenario, describe a technique to obtain a CARS spectrum without modifying the laser parameters and using only a single detector. Qualitatively, what happens if the chirp rates are not the same?

Problem 4

Equation 18_chi32 suggests that CARS microscopy cannot provide a direct measure of $\operatorname{Im}\chi_r^{(3)}(\nu)$. But consider the case where a measurement of $\left|\chi^{(3)}(\nu)\right|^2$ is obtained over a large range of frequencies, much larger than the Raman features of interest. Assume also that $\chi_{nr}^{(3)}$ is much greater than $\chi_r^{(3)}(\nu)$, as is common in practice. Can you devise a numerical technique to estimate $\operatorname{Im}\chi_r^{(3)}(\nu)$ from your measurement?

Hint: use Fourier transforms.

Problem 5

Derive Eq. ???. Note that this problem is rather lengthy and can benefit from the aid of software such as *Mathematica.*

Hint: Use spherical coordinates, and integrate over φ_0, then r_0, then θ_0. Also helpful is the identity

$$J_0(2\pi\kappa a) = \frac{1}{2\pi}\int_0^{2\pi} e^{-i2\pi\kappa a\cos(\varphi-\varphi_0)}d\varphi_0.$$

Chapter 19

Superresolution

Problem 1

The pupil and point spread functions of a microscope are denoted by $P(\vec{\xi})$ and $\mathrm{PSF}(\vec{\rho})$ respectively. 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

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

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. 19.3 ???.

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

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

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

$$\phi(\vec{\rho}) = \alpha \xi^2(\vec{\rho})$$

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 channels – see Eq. 13.8 ???).

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

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

c) By using appropriate demodulation, assume that the components of $\phi_{\text{mod}}(\vec{\rho}, t)$ oscillating at the first (Ω) and second (2Ω) harmonics can be isolated. Use the technique employed in Section 19.2.2 (???) to compare the curvatures of $\phi_{\Omega}(\vec{\rho})$ and $\phi_{2\Omega}(\vec{\rho})$ to the curvature of $\phi(\vec{\rho})$ (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 $\phi(\vec{\rho})$'s to the same peak height before comparing their curvatures.

Problem 3

Assume a molecule is imaged onto a unity-gain 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

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

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 $\frac{d\chi^2(x)}{dx} = 0$.

a) 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

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

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

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

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

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

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

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. 19.34?

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

Problem 4

The purpose of this problem is to compare STED microscopy with continuous versus pulsed beams. The case of continuous-wave beams was treated in the chapter, where it was found in Eq. ??? that the time averaged fluorescence rate per molecule was

$$\langle\phi\rangle_{\mathrm{cw}} = \frac{\langle\alpha\rangle\, k_r}{k_r + \sigma_{se} \langle I_{se}\rangle}$$

where σ_{se} and $\langle I_{se}\rangle$ are the stimulated-emission cross-section and STED beam illumination intensity, respectively ($k_{se} = \sigma_{se} \langle I_{se}\rangle$ and assume $\langle\alpha\rangle \ll k_r$ and $k_{nr} = 0$).

Consider now using pulsed beams. The excitation beam has pulse period τ_l and infinitely narrow pulse duration. The STED beam has pulse period τ_l and pulse duration τ_p. Assume that the onsets of the STED pulses immediately follow the excitation pulses. Assume also that the molecule is excited with probability ξ at each excitation pulse, such that the average excitation rate is $\langle\alpha\rangle = \xi/\tau_l$, where $\tau_l \gg k_r^{-1}$. Finally, for fair comparison, assume that $\langle a\rangle$ and $\langle I_{se}\rangle$ are the same in both pulsed and continuous cases.

a) Derive an expression for $\langle\phi\rangle_{\mathrm{pb}}$ when using pulsed beams. Hint: this involves solving for the excited-state probability $e\,(t)$, and integrating.

b) Show that STED is most efficient (i.e. $\langle\phi\rangle_{\mathrm{pb}}$ is smallest) when $\tau_p \to 0$. What is $\langle\phi\rangle_{\mathrm{pb}}$in this case?

c) Using your result from (b), show that pulsed-beam STED is always more efficient than continuous-wave STED (i.e. $\langle\phi\rangle_{\mathrm{pb}} < \langle\phi\rangle_{\mathrm{cw}}$), even for arbitrarily small values of $\langle I_{se}\rangle$, provided $\tau_l > k_r^{-1}$.

Problem 5

Consider a distribution of N point-like molecules located at positions $\vec{\rho}_n$, each fluorescing with time-dependent intensities $f_n\,(t)$. These are imaged by a standard widefield microscope. The resulting intensity at the camera is

$$I\,(\vec{\rho},t) = \sum_{n=1}^{N} \mathrm{PSF}\,(\vec{\rho}-\vec{\rho}_n)\, f_n\,(t)$$

a) Show that if the time-dependent intensities $f_n(t)$ are *independent* of one another, then an image constructed by the temporal variance at each position $\vec{\rho}$ is given by

$$\sigma_I^2(\vec{\rho}) = \sum_{n=1}^{N} \mathrm{PSF}^2(r - r_n)\,\sigma_n^2$$

where σ_n^2 is the temporal variance of $f_n(t)$. This is an unusual type of imaging since it provides a representation not of average fluorescence levels but rather of variances of fluorescence levels. Nevertheless, this can lead to superresolution imaging (called Superresolution Optical Fluctuation Imaging – or SOFI), by exploiting a priori knowledge of fluorescence statistics and the fact that PSF^2 is narrower than PSF.

b) Consider molecules that produce fluorescence with Gaussian statistics (see Section ???). Rewrite the above result in terms of the average fluorescence levels $\langle f_n \rangle$.

c) Convince yourself that you would not achieve the same result by simply squaring the initial image. In other words, compare the above result with $\langle I(\vec{\rho}) \rangle^2$.

Chapter 20

Imaging in scattering media

Problem 1

Consider Fig. 20.1 in the limit of small-angle scattering. A light ray enters a scattering medium from a perpendicular direction (as shown). After several scattering events, the probability distribution for the ray position is spread over transverse area of width $w(L)$, where L is the penetration depth. Use a simple model where the transverse position of the ray undergoes a random walk as the ray propagates into the sample. That is, at each scattering event, the ray takes a step of size $l_s\theta$ in the transverse plane, with arbitrary direction. Provide rough scaling laws for $w(L)$. Specifically, how does $w(L)$ scale with L? With μ_s? With $(1-g)$?

Compare your result with the spread incurred by a Gaussian focus (Eq. 20.47).

Problem 2

In Chapter 9 we considered the Rytov solution for field propagation in an inhomogeneous medium (see Section ???), given by

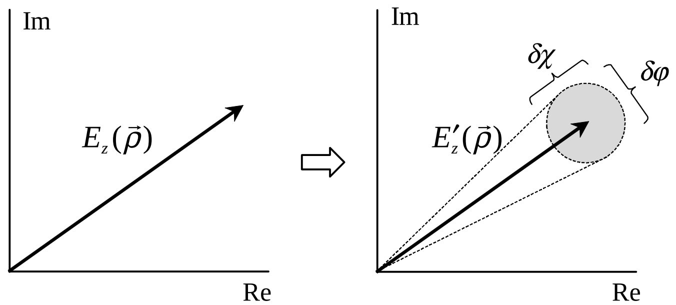
$$E(\vec{r}) = e^{i\Psi(\vec{r})} E_i(\vec{r})$$

Decompose $\delta\Psi(\vec{\rho})$ into real and imaginary components, such that $\Psi(\vec{\rho}) = \varphi(\vec{\rho}) + i\chi(\vec{\rho})$. Assume that both $\varphi(\vec{\rho})$ and $\chi(\vec{\rho})$ obey Gaussian statistics. That is, they obey the probability distributions given by

$$p_\varphi(\varphi) = \frac{1}{\sqrt{2\pi}\sigma_\varphi} e^{-\varphi^2/2\sigma_\varphi^2}$$

$$p_\chi(\chi) = \frac{1}{\sqrt{2\pi}\sigma_\chi} e^{-(\chi-\bar{\chi})^2/2\sigma_\chi^2}$$

where σ_φ and σ_χ are standard deviations (not to be confused with cross-sections), and a bias $\bar{\chi}$ is introduced to take into account a mean attenuation of the field.



Derive the corresponding probability distributions for the field amplitude $A = |E|$ and intensity $I = |E|^2$ (in terms of σ_φ, σ_χ and $\bar{\chi}$). Your results should be examples of what are known as log-normal probability distributions.

Hint: remember that probability distributions transform as $p_Y(Y) = p_X(X)\,|dX/dY|$.

Problem 3

We return here to the Beam Propagation Method described in Chapter 9. Imagine that each phase screen imparts spatially random phases, such that the field just after the screen is given by $E'_z(\vec{\rho}) = e^{i\delta\psi(\vec{\rho})} E_z(\vec{\rho})$, where $E_z(\vec{\rho})$ is the field just before the screen. As illustrated in the figure, we can decompose $\delta\psi(\vec{\rho})$ into real and imaginary components, such that $\delta\psi(\vec{\rho}) = \delta\varphi(\vec{\rho}) + i(\bar{\chi} + \delta\chi(\vec{\rho}))$, where $\delta\varphi(\vec{\rho})$ and $\delta\chi(\vec{\rho})$ are (real) phase and amplitude variations, both centered on zero so that $\delta\varphi(\vec{\rho}) = \delta\chi(\vec{\rho}) = 0$, and a bias $\bar{\chi}$ is introduced to account for any mean amplitude reduction (note that $\delta\varphi(\vec{\rho})$ and $\delta\chi(\vec{\rho})$ are not the same as in Problem 2). Moreover, assume that the phase and amplitude fluctuations are uncorrelated, and their variances $\left\langle \delta\varphi(\vec{\rho})^2 \right\rangle$and $\left\langle \delta\chi(\vec{\rho})^2 \right\rangle$ are independent of $\vec{\rho}$, meaning that the phase screen is statistically homogeneous.

The effect of the phase screen on the mutual intensity can be written as

$$J'_z(\vec{\rho}, \vec{\rho}\,') = K_{\delta z}(\vec{\rho}, \vec{\rho}\,')\, J_z(\vec{\rho}, \vec{\rho}\,').$$

a) Show that, with our usual coordinate transformation,

$$K_{\delta z}(\vec{\rho}_d) = \exp\left(-2\bar{\chi} + 2\left\langle \delta\chi^2 \right\rangle - \left\langle \delta\chi^2 \right\rangle (1 - \gamma_{\delta\chi}(\rho_d) - \left\langle \delta\varphi^2 \right\rangle (1 - \gamma_{\delta\varphi}(\rho_d))\right)$$

where $\gamma_{\delta\chi}(\rho_d)$ and $\gamma_{\delta\varphi}(\rho_d)$ are normalized autocorrelation functions of $\delta\chi(\vec{\rho})$ and $\delta\varphi(\vec{\rho})$, respectively.

Hint: Identities from Appendix ??? are useful here.

b) Assume that the phase and amplitude variations obey similar statistics, as suggested in the figure. That is, assume $\left\langle \delta\varphi(\vec{\rho})^2 \right\rangle = \left\langle \delta\chi(\vec{\rho})^2 \right\rangle \equiv \frac{1}{2}\left\langle |\delta\psi(\vec{\rho})|^2 \right\rangle$, and $\gamma_{\delta\chi}(\rho_d) = \gamma_{\delta\varphi}(\rho_d) \equiv \gamma_{\delta\psi}(\rho_d)$. Also, use the a priori knowledge that $K_{\delta z}(0) = \exp(-\mu_a \delta z)$, and $\left\langle |\delta\psi(\vec{\rho})|^2 \right\rangle = \mu_s \delta z$.

Show that $K_{\delta z}(\vec{\rho}_d)$ simplifies to

$$K_{\delta z}(\vec{\rho}_d) = \exp\left(-\mu_e \delta z + \mu_s \delta z\, \gamma_{\delta\psi}(\rho_d)\right).$$

This model for small propagation distances can be compared with the more exact Eq. 20.23 for larger propagation distances.

Problem 4

Problem 3 introduced the complex phase variations $\delta\psi\left(\vec{\rho}\right)$ imparted by a phase screen in the Beam Propagation Method (Chapter 9). Here a link will be established between $\gamma_{\delta\psi}\left(\rho_d\right)$ and $\gamma_{\delta n}\left(r_d\right)$, where δn are index-of-refraction variations of the sample, assumed to be isotropic and spatially invariant, with normalized autocorrelation function given by Eq. ???.

a) In accord with the Beam Propagation Method, neglect diffraction effects when considering transmission through the phase screen. That is, write $\delta\psi\left(\vec{\rho}\right) = 2\pi\kappa \int_0^{\delta z} \mathrm{d}z\, \delta n\left(\vec{r}\right)$.

Show that

$$\gamma_{\delta\psi}\left(\rho_d\right) = \frac{\int_{-\infty}^{\infty} \mathrm{d}z_d\, \gamma_{\delta n}\left(\rho_d, z_d\right)}{\int_{-\infty}^{\infty} \mathrm{d}z_d\, \gamma_{\delta n}\left(0, z_d\right)}.$$

Hint: make use of the following trick (similar to the trick used with Eq. ???)

$$\int_0^{\delta z} \mathrm{d}z \int_0^{\delta z} \mathrm{d}z' = \int_0^{\delta z} \mathrm{d}z_c \int_{2|z_c-\delta z/2|-\delta z}^{\delta z-2|z_c-\delta z/2|} \mathrm{d}z_d \approx \int_0^{\delta z} \mathrm{d}z_c \int_{-\infty}^{\infty} \mathrm{d}z_d$$

where the second approximation makes the assumption that the characteristic size of the index-of-refraction inhomogeneities is much smaller than δz, allowing the integration limits for $\mathrm{d}z_d$ to be extended to infinity without significant error.

b) Convince yourself that the first equality in the above trick is true.

Hint: make a sketch of the integration area spanned by $\mathrm{d}z_c$ and $\mathrm{d}z_d$.

Problem 5

Consider a sample where the index-of-refraction variations obey Gaussian statistics given by

$$\gamma_{\delta n}(r_d) = e^{-r_d^2/l_n^2}$$

where l_n is the index-of-refraction correlation length.

a) Show that, in the small-angle approximation (equivalent to $\kappa l_n \gg 1$), the corresponding phase function is given by

$$p\left(\theta\right) \approx \frac{1}{2\pi\left(1-g\right)} e^{-\theta^2/2(1-g)}$$

where

$$g \approx 1 - \frac{1}{2\pi^2\kappa^2 l_n^2}.$$

b) Verify that Eq. 20.57 is satisfied.