Exercise 02

1 Phase Contrast Microscopy

1.1 Optical Path length and Relative Phase Shift

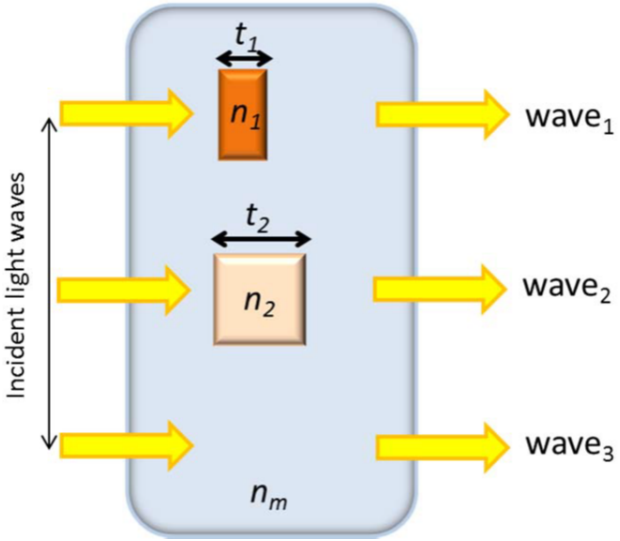


Figure 1: Example for calculating optical path lengths.

Figure 1 shows schematic of two biological specimens in medium, incident waves and interacting waves with each specimen (wave 1 and wave 2), and non-interacting wave with any specimen (wave 3). Assume that the refractive indices and thicknesses for specimen 1 and specimen 2 are ($n_1 = 1.83$, $t_1 = 500\,\text{nm}$) and ($n_2 = 1.58$, $t_2 = 1\,\mu\text{m}$), respectively. The medium is water with an index of $n_m = 1.33$. Assume that illumination is a monochromatic light with a wavelength of $\lambda = 500\,\text{nm}$.

(a) Calculate the optical path length of each specimen.

(b) Calculate the magnitude of phase induced on wave 1 and wave 2 by the corresponding specimen.

(c) Calculate the optical path length difference between the waves interacting with the first specimen (i.e. wave 1) and surrounding (i.e. wave 3).

(d) Calculate the optical path length difference between the two waves interacting with the second specimen (wave 2) and surrounding (wave 3).

(e) Calculate the phase shift between wave 1 and wave 3 . Calculate the phase shift between wave 2 and wave 3 .

(f) Comment in a few very short sentences whether (and why) the contrast of specimens 1 and 2 will be same or different when imaged with a phase contrast microscope.

1.2 Phase Contrast Microscopy

Considering the optical layout of the phase contrast microscope shown in Figure 2 as well as the formula and assumptions below, answer the questions (a-d):

- In the center of the back-focal plane of the microscope is a "small" phase plate shifting the phase by $\frac{\pi}{2}$
- A phase-shift of φ is expressed by a multiplication of the field by $e^{i\varphi}$
- Assume that the diameter of apertures, objective, lens is infinite
- In Biomicroscopy I, we learned about 2-f and 4-f systems. In Figure 2, sample and phase-plate planes (as well as phase-plate and detector planes) are related to each other by Fourier transform. If the input at the sample plane is $U_1(x_1)$, then the response at the phase-plate plane (i.e. at the Fourier plane) is given by:

$$\hat{U}_2(x_2) = \frac{1}{i\lambda f}\Im\left\{U_1(x_1)\right\}(p)\,, \quad \text{where } p = \frac{x_2}{\lambda f}$$

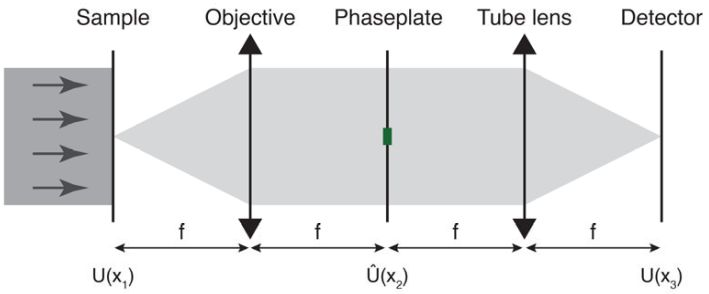


Figure 2: Optical layout of a microscope with a phase plate in the back-focal plane.

- Dirac function $\delta(x)$ is used in optics to represent a very small object.
- Fourier Transform of Dirac Function is uniform such that $\Im\{\delta(x)\}(p) = 1$
- Remember Euler's formula: $e^{ix} = \cos(x) + i\sin(x)$
- Use Taylor expansion for trigonometric functions

(a) At the sample plane we have a Dirac-sample acting purely as a transparent phase object[1]. The optical field of this sample can be described as:

$$U(x_1) = e^{i\delta(x_1)\varphi}$$

Assume that the phase introduced by this sample is small, i.e. $\varphi \ll 1$. Simplify the description of the field $U(x_1)$.

(b) What is the field $\hat{U}(x_2)$ at the back focal plane of the objective for this sample with small phase?

(c) **Without phase plate**: What is the field $U(x_3)$ on the detector plane? If you measure the intensity using a camera, what would you observe?
Hint: Since φ is small you can assume φ^2 and higher orders are negligible.

(d) **With phase plate**: The phase filter is shifting the central part of the frequencies by $\frac{\pi}{2}$. What is the field $U_{\text{ph}}(x_3)$ directly behind the phase plate? What is the field on the detector plane if you use the phase-plate? If you measure the intensity using a camera, what would you observe this time?

[1]Pure phase object: This sample does only alter the phase of the light that passes through it, but not its amplitude. If you "look" at the sample you will see nothing. For example, calculating the intensity of the sample with field $U(x_1)$ would lead to $I = UU* = 1$.

2 Polarization Optics

2.1 Intensity Control with a Polarizer-Analyzer Pair

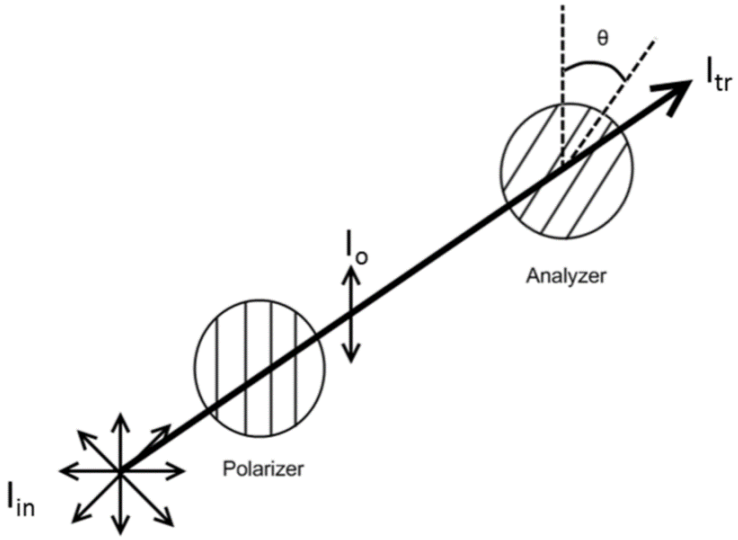


Figure 3: Schematic of a polarizer-analyzer setup.

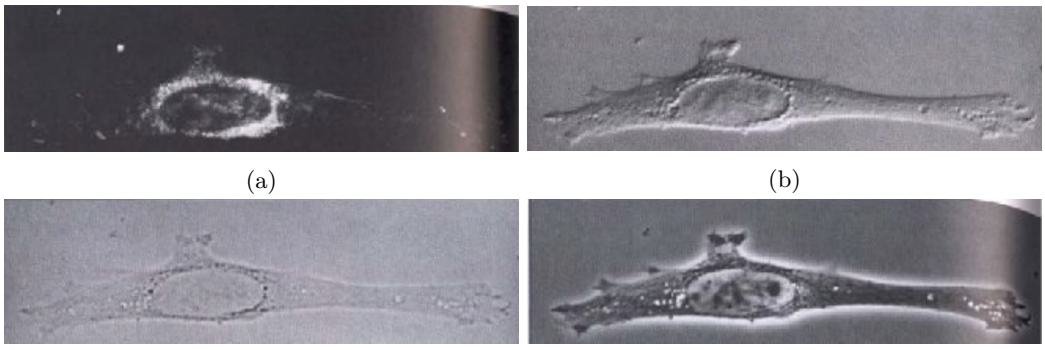
In the setup shown in Figure 3 unpolarized light I_{in} with $\lambda = 500\,\text{nm}$ is transmitted through a pair of polarizers called Polarizer-Analyser pair. The analyzer can be rotated around the optical axis. Calculate the intensity of the light in each section as a function of the relative orientation Θ of the two polarizers:

Section 1: linearly polarized light (I_0) after first polarizer

Section 2: transmitted light (I_{tr}) through both polarizers

3 Identifying Microscopy Techniques

Figure 4 showcases the same sample processed using different imaging techniques operating at similar resolution. Assign the following techniques to each image: *Bright-field microscopy*, *Dark-field microscopy*, *Phase-contrast microscopy*, *Differential interference contrast (DIC) microscopy*. Which cues allow you to assign modalities to each image?



(a) (b)

(c) (d)

Figure 4: Sample imaged under various microscopy techniques. Images from *Friedrich-Schiller-Universität Jena*