

Exercise 02 - Solutions

1 Phase Contrast Microscopy

1.1 Optical Path length and Relative Phase Shift

(a) Optical path length can be formulated as:

$$\text{OPL} = d \times n \quad \text{with distance } d \text{ and refractive index } n$$

For first and second specimens:

$$\text{OPL}_1 = 0.5 \text{ } \mu\text{m} \times 1.83 = 0.915 \text{ } \mu\text{m}$$

$$\text{OPL}_2 = 1 \text{ } \mu\text{m} \times 1.58 = 1.58 \text{ } \mu\text{m}$$

(b) The phase induced through OPL is,

$$\varphi = \frac{2\pi}{\lambda} \text{OPL}$$

$$\varphi_1 = \frac{2\pi}{0.5 \text{ } \mu\text{m}} \times 0.915 \text{ } \mu\text{m} = 3.66\pi$$

$$\varphi_2 = \frac{2\pi}{0.5 \text{ } \mu\text{m}} \times 1.58 \text{ } \mu\text{m} = 6.32\pi$$

(c) Optical path difference of the surrounding for a distance of $t_1 = 0.5 \text{ } \mu\text{m}$:

$$\text{OPL}_3^{t_1} = 0.5 \text{ } \mu\text{m} \times 1.33 = 0.665 \text{ } \mu\text{m}$$

$$\Delta\text{OPL}_{31} = \text{OPL}_1 - \text{OPL}_3 = 0.25 \text{ } \mu\text{m}$$

(d) Optical path difference of the surrounding for a distance of $t_2 = 1 \text{ } \mu\text{m}$:

$$\text{OPL}_3^{t_2} = 1 \text{ } \mu\text{m} \times 1.33 = 1.33 \text{ } \mu\text{m}$$

$$\Delta\text{OPL}_{32} = \text{OPL}_2 - \text{OPL}_3 = 0.25 \text{ } \mu\text{m}$$

(e) The phase induced through the distances t_1 and t_2 on the surrounding is:

$$\varphi_3 = \frac{2\pi}{0.5 \text{ } \mu\text{m}} \times \text{OPL}_3$$

$$\varphi_3^{t_1} = \frac{2\pi}{0.5 \text{ } \mu\text{m}} \times \text{OPL}_3^{t_1} = 2.66\pi$$

$$\varphi_3^{t_2} = \frac{2\pi}{0.5 \text{ } \mu\text{m}} \times \text{OPL}_3^{t_2} = 5.32\pi$$

$$\Delta\varphi_{31} = \varphi_3 - \varphi_3^{t_1} = \pi$$

$$\Delta\varphi_{32} = \varphi_3 - \varphi_3^{t_2} = \pi$$

(f) Since the phase shifts resulted from the both specimens have the same value of π , the specimens will have the same contrast when imaged with a phase contrast microscope.

1.2 Phase Contrast Microscopy

(a) Under the assumption of $\varphi \ll 1$ the field becomes:

$$\begin{aligned} U(x_1) &= e^{i(\delta(x_1)\varphi)} \\ &= \cos(\delta(x_1)\varphi) + i \cdot \sin(\delta(x_1)\varphi) \\ &\stackrel{\varphi \ll 1}{=} 1 + i \cdot (\delta(x_1)\varphi) \end{aligned}$$

(b) Using Fraunhofer approximation we find:

$$\begin{aligned}\hat{U}(x_2) &= \frac{1}{i\lambda f} (\delta(p) + i\varphi) \\ &= \frac{1}{i\lambda f} \left(\delta \left(\frac{x_2}{\lambda f} \right) + i\varphi \right)\end{aligned}$$

(c) **Without phase plate:** Using Fraunhofer approximation we find:

$$U(x_3) = \frac{-1}{\lambda^2 f^2} (1 + i\varphi\delta(x_3))$$

To find the intensity we calculate:

$$\begin{aligned}I(x_3) &= |U(x_3)|^2 = U(x_3) \cdot \overline{U}(x_3) \\ &= \frac{(-1)^2}{\lambda^4 f^4} (1 + i\varphi\delta(x_3))(1 - i\varphi\delta(x_3)) \\ &= \frac{1}{\lambda^4 f^4} (1 + \varphi^2 \delta^2(x_3)) \\ &\stackrel{\varphi \ll 1}{=} \frac{1}{\lambda^4 f^4}\end{aligned}$$

So we find a continuous signal without any dependency on x_3 : without phasefilter we do not see our sample on the detector plane.

(d) **With phase plate:** Now the signal behind the phase plate experiences a phase shift for all central frequencies and hence:

$$\begin{aligned}\hat{U}_{\text{PhP}}(x_2) &= \frac{1}{i\lambda f} (e^{i\frac{\pi}{2}} \delta(p) + i\varphi) \\ &= \frac{1}{i\lambda f} \left(i\delta \left(\frac{x_2}{\lambda f} \right) + i\varphi \right)\end{aligned}$$

Where we have neglected the phase shift for the central frequencies of the continuous signal on the right hand side of the equation¹. Thus the field on the detector plane is:

$$U_{\text{PhP}}(x_3) = \frac{-i}{\lambda^2 f^2} (1 + \varphi\delta(x_3))$$

And we find for the intensity:

$$\begin{aligned}I_{\text{PhP}}(x_3) &= |U_{\text{PhP}}(x_3)|^2 = U_{\text{PhP}}(x_3) \cdot \overline{U}_{\text{PhP}}(x_3) \\ &= \frac{-i^2}{\lambda^4 f^4} (1 + \varphi\delta(x_3))^2 \\ &= \frac{1}{\lambda^4 f^4} (1 + 2\varphi\delta(x_3) + \varphi^2 \delta^2(x_3)) \\ &\stackrel{\varphi \ll 1}{=} \frac{1}{\lambda^4 f^4} (1 + 2\varphi\delta(x_3))\end{aligned}$$

So we find a dirac peak at the center of the detector. Hence, our “invisible” phase-object becomes “visible” in terms of intensity through the phaseplate.

¹This is correct if we assume that we have an infinitely small phase plate

2 Polarization Optics

2.1 Intensity Control with a Polarizer-Analyzer Pair

When polarized light hits a polarizer, it can be treated as two components: one component in the same orientation as the polarizer, which will be transmitted, and another component perpendicular to the polarizer, which will not be transmitted.

The intensity of the transmitted component can be calculated by Malus' Law, where α is the relative angle between the two polarizations:

$$I' = I \cos^2(\alpha)$$

The unpolarized light I_{in} has a uniform distribution of polarizations for all polarization angles α . Upon interaction with the first polarizer, the intensity is reduced by a factor of $\frac{1}{2}$, because the average of $\cos^2(\alpha)$ over all angles α is:

$$\frac{I_{\text{in}}}{2\pi} \int_0^{2\pi} \cos^2(\alpha) d\alpha = \frac{1}{2} I_{\text{in}}$$

Hence: $I_0 = \frac{1}{2} I_{\text{in}}$

For calculating the transmission of I_0 through the analyzer we can apply Malus Law another time. We get:

$$I_{\text{tr}} = I_0 \cos^2(\Theta) = \frac{1}{2} I_{\text{in}} \cos^2(\Theta)$$

3 Identifying microscopy techniques

- Image (a) corresponds to dark-field microscopy. The extremely dark background is the clearest cue.
- Image (b) corresponds to DIC microscopy. The sample **looks like** it has been illuminated with a light source located at the bottom of the image. This gradient hints that the technique is DIC.
- Image (c) corresponds to bright-field microscopy. While there is no specific distinguishing cue, the level of detail of the image is clearly the smallest among all of them.
- Image (d) corresponds to phase-contrast microscopy. The halo-like white texture appearing around the sample is the clearest hint of phase-contrast microscopy.