

Biomicroscopy I - Solutions Exercise Sheet 6

October 28, 2025

1 Köhler illumination

- Köhler illumination provides a more homogeneous illumination and higher resolution. The light source is maximally out-of-focus, avoiding the overlap between the light source image and the specimen. Köhler illumination is also minimizing the stray-light and unnecessary irradiation.
- The elements shown in the schematic are light source, collector lens, field stop, aperture stop, condense lens, object plane and objective. Therefore, the diaphragm 1, which is the field stop, is used to control the illumination area/field (option **b.**).
- The diaphragm 2, which is the aperture stop, is used to control the numerical aperture of the illumination and the illumination intensity (options **c.**, **e.**).
- The object plane is conjugated with the field stop diaphragm 1, the light source plane is conjugated with the aperture stop diaphragm 2.

2 Achromatic doublet

- Assuming that the lenses are thin we can find the matrix by multiplying three matrices corresponding to the boundaries between materials (two curved and one flat, with accurate signs for the radii) followed by free propagation matrix (minding multiplication order):

$$\begin{aligned}
 M &= \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -\frac{n_0 - n_2}{n_0 R_3} & \frac{n_2}{n_0} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -\frac{n_2 - n_1}{n_2 (-R_2)} & \frac{n_1}{n_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -\frac{n_1 - n_0}{n_1 R_1} & \frac{n_0}{n_1} \end{bmatrix} = \\
 &\stackrel{n_0=1}{=} \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & n_2 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -\frac{n_2 - n_1}{-n_2 R_2} & \frac{n_1}{n_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ -\frac{n_1 - 1}{n_1 R_1} & \frac{1}{n_1} \end{bmatrix} = \begin{bmatrix} 1 + d \left(\frac{n_2 - n_1}{R_2} - \frac{n_1 - 1}{R_1} \right) & d \\ \frac{n_2 - n_1}{R_2} - \frac{n_1 - 1}{R_1} & 1 \end{bmatrix}
 \end{aligned}$$

- Focusing parallel rays corresponds to $A = 0$. Therefore we can find the equation for the focusing distance d :

$$1 + d \left(\frac{n_2 - n_1}{R_2} - \frac{n_1 - 1}{R_1} \right) = 0 \quad (1)$$

$$d = \frac{R_1 R_2}{R_2 (n_1 - 1) - R_1 (n_2 - n_1)}$$

C. Equation 1 should be fulfilled for both wavelengths (achromat is designed to focus both wavelengths at the same point). Therefore, we can write the following system of equations out of it:

$$\begin{cases} \frac{n_1^{400}-1}{R_1} - \frac{n_2^{400}-n_1^{400}}{R_2} = \frac{1}{d} \\ \frac{n_1^{700}-1}{R_1} - \frac{n_2^{700}-n_1^{700}}{R_2} = \frac{1}{d} \end{cases}$$

where $n_{1,2}^{400}$ and $n_{1,2}^{700}$ are the refractive indices of materials for 400 and 700 nm respectively and $d = 500$ mm. By solving this system one can get the radii $R_1 \approx 66.6$ mm and $R_2 = 200$ mm.

3 Interference of waves

Consider interference of two plane waves incident at angles α and β (see Fig. 1).

$$\mathbf{E}(\mathbf{r}, t) = \text{Re} \{ (\mathbf{E}_1 e^{i\mathbf{k}_1 \cdot \mathbf{r}} + \mathbf{E}_2 e^{i\mathbf{k}_2 \cdot \mathbf{r}}) e^{-i\omega t} \}$$

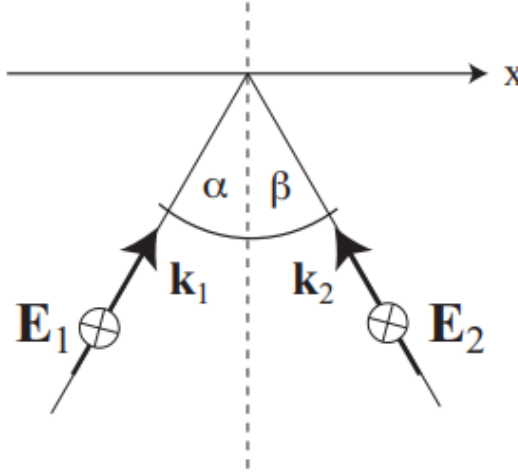


Figure 1: Interference of two plane waves incident at angles α and β .

Let's consider two monochromatic plane waves of the same frequency ω , same polarization, and wavelength λ , coming at angles α and β with respect to some axis (say the y-axis, dashed lines). Assume both waves propagate in the x-y plane. Then the wave vectors are:

$$\mathbf{k}_1 = k(\sin(\alpha)\hat{x} + \cos(\alpha)\hat{y})$$

$$\mathbf{k}_2 = k(-\sin(\beta)\hat{x} + \cos(\beta)\hat{y})$$

where $k = \frac{2\pi}{\lambda}$

We need to calculate the intensity of the total field, which is simply given by the sum of \mathbf{E}_1 and \mathbf{E}_2 :

$$\mathbf{E}(\mathbf{r}, t) = \{ (\mathbf{E}_1 e^{i\mathbf{k}_1 \cdot \mathbf{r}} + \mathbf{E}_2 e^{i\mathbf{k}_2 \cdot \mathbf{r}}) e^{-i\omega t} \}$$

We can evaluate this field along x-axis and get:

$$\mathbf{E}(x, t) = \{ (\mathbf{E}_1 e^{ikx\sin\alpha} + \mathbf{E}_2 e^{-ikx\sin\beta}) e^{-i\omega t} \}$$

Then we obtain the intensity:

$$\begin{aligned}
I(x) &= \frac{1}{2} \sqrt{\frac{\varepsilon_0}{\mu_0}} |(\mathbf{E}_1 e^{ikx \sin \alpha} + \mathbf{E}_2 e^{-ikx \sin \beta})|^2 \\
&= I_1 + I_2 + \frac{1}{2} \sqrt{\frac{\varepsilon_0}{\mu_0}} [\mathbf{E}_1 \mathbf{E}_2^* e^{ikx(\sin \alpha + \sin \beta)} + \mathbf{E}_1^* \mathbf{E}_2 e^{-ikx(\sin \alpha + \sin \beta)}] \\
&= I_1 + I_2 + \sqrt{\frac{\varepsilon_0}{\mu_0}} \operatorname{Re} \{ \mathbf{E}_1 \mathbf{E}_2^* e^{ikx(\sin \alpha + \sin \beta)} \}
\end{aligned}$$

This expression works for any complex vectors \mathbf{E}_1 and \mathbf{E}_2 . We assume that these vectors are real and that they are polarized along z -axis as it is shown in the Figure. Then we obtain:

$$I(x) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(kx(\sin \alpha + \sin \beta))$$

The only term oscillates in this expression is $\cos(kx(\sin \alpha + \sin \beta))$. It gets values between -1 and +1. Hence, the biggest and smallest signals are $I_1 + I_2 \pm 2\sqrt{I_1 I_2}$. We can introduce visibility as:

$$\eta = \frac{I_{max} - I_{min}}{I_{max} + I_{min}},$$

which has a maximum value of $\eta = 1$ for $I_1 = I_2$. The interference period can easily be found to be $\Delta x = \lambda/(\sin \alpha + \sin \beta)$, and it is shortest for $\alpha = \beta = \pi/2$ equal to $\Delta x \lambda/2$.