Exercise 01 - Solutions

1 Review Biomicroscopy I: Wide Field Microscopy

1.1 Microscope Comparison

For the two microscope systems given in Figure 1 answer the following questions:

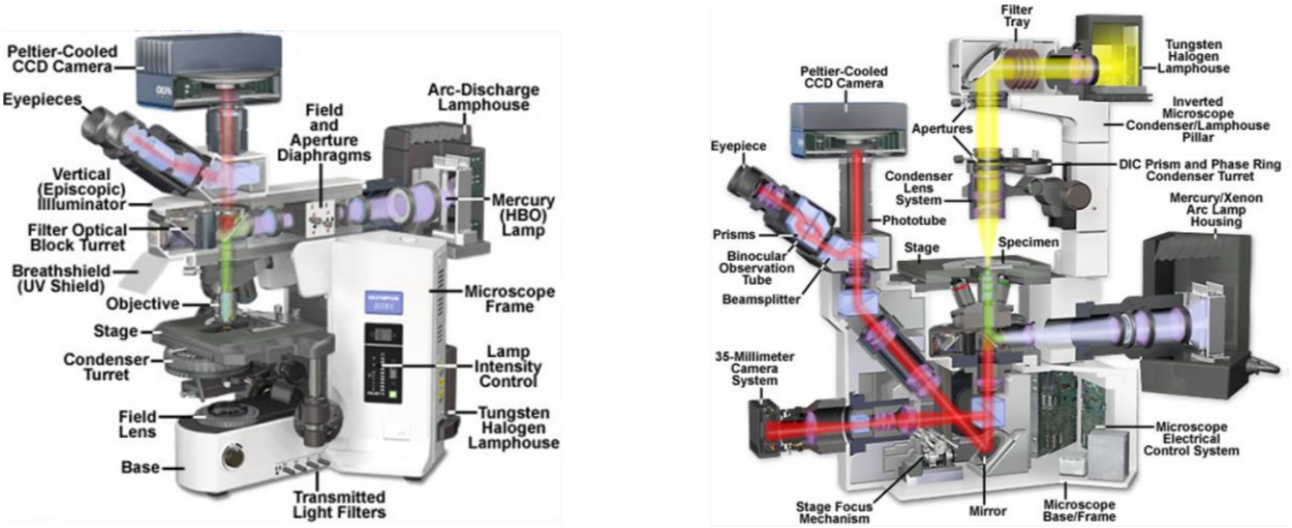

Figure 1: Cross-sectional schematic of two commonly used microscopes.

(a) The left one in Figure 1 is an upright microscope while the right is an inverted one.

(b) Refer to Figure 1.

(c) **For the upright microscope, the optical pathway for bright-field microscopy in transmission configuration is traced as below:** Illumination starts from the Tungsten Halogen Lamp, passes through multiple transmitted light filters, field lens and condenser, and then reaches the sample on the stage. Transmitted light is collected by the objective above the sample, and then to your eyes through the eyepieces or to the CCD camera.

For the inverted microscope, the optical pathway for bright-field microscopy in transmission configuration is traced as below: Illumination starts from the Tungsten Halogen Lamp on the very top, passes through filter tray, apertures and condenser, and then reaches the specimen on the stage. Transmitted light is also collected by the objective under the sample, reflected by the mirror and then goes to your eyes through the eyepieces or to the CCD camera.

(d) The focusing and movement of the sample is achieved by moving the stage for the upright microscope, while the movement is achieved by moving the objective lenses for the inverted one.

1.2 Optical Aberrations in Microscopy

(a) Chromatic dispersion, curvature of field and spherical.

(b) Chromatic dispersion comes from a frequency-dependent response of a material to waves. An optical lens has different refractive indices for different wavelengths of light, thus light rays of different wavelengths will refract to different degrees, leading to undesired chromatic aberration in a lens, such as the change of magnification and shift of focus.

(c) To add stops in the optical path, e.g. aperture stops.

1.3 Infinity Corrected System (ICS)

You are asked to build a basic ICS microscope. ICS microscope objective collimates rays from an object point. For this task you have following optical elements:

- An objective indicating "$50x/0.6$NA" and "∞" with a description indicating an ICS objective (design tube lens is 160 mm)
- An achromatic lens with $f = 160\,\text{mm}$
- An ocular (a.k.a. eyepiece) with magnification $M_{\text{oc}} = 10$

(a) The objective's magnification "$50\times$" means that $M_{\text{design}} = \left|\frac{f_{t(design)}}{f_o}\right| = 50$. Therefore, the objective's focal length is $f_o = \frac{160\,\text{mm}}{50} = 3.2\,\text{mm}$.

(b) In a similar fashion as in a), the eye piece's magnification "$10\times$" means that $f_e = \frac{250\,\text{mm}}{10} = 25\,\text{mm}$ ($250\,\text{mm}$ is the standardized object distance at which one can look with the eye).

(c)

$$M_{\text{design}} = \left|\frac{ft(design)}{f_o}\right| = \frac{160\,\text{mm}}{32\,\text{mm}} = 5$$

$$M_{\text{total}} = M_{\text{design}} \times M_{\text{ocular}} = \left|\frac{f_{\text{t(design)}}}{f_o}\right| \times M_{\text{ocular}} = \frac{160\,\text{mm}}{32\,\text{mm}} \times 10 = 50.$$

Note that a tenfold increase in the focal length of the objective results in a tenfold decrease of total magnification.

(d) A parallel light beam passes between the objective lens and tube lens in an ICS. The magnification does not change even if the distance between the objective lens and tube lens is changed. ICS allows introduction of other components, such as differential interference contrast (DIC) prisms, polarizers, and epi-fluorescence illuminators, into the parallel optical path between the objective and the tube lens with only a minimal effect on focus and aberration corrections.

1.4 Köhler illumination

(a) Köhler illumination provides most homogenous illumination and highest obtainable resolution. The light source is maximally out-of-focus, avoiding the overlap between light source image with the specimen. Köhler illumination is also minimizing the stray-light and unnecessary irradiation.

(b) On the illumination path given in the figure, Filament, Condenser Aperture Diaphragm, Objective Back Focal Plane and Eyepoint are conjugated.

2 Dark Field Microscopy

The NA of the objective lens should be less than the NA of the dark-field condenser ($\text{NA}_{\text{obj}} < \text{NA}_{\text{condenser}} = 0.9$), in order to avoid capturing the illumination beam by the objective lens. This will enable a successful dark-field imaging.