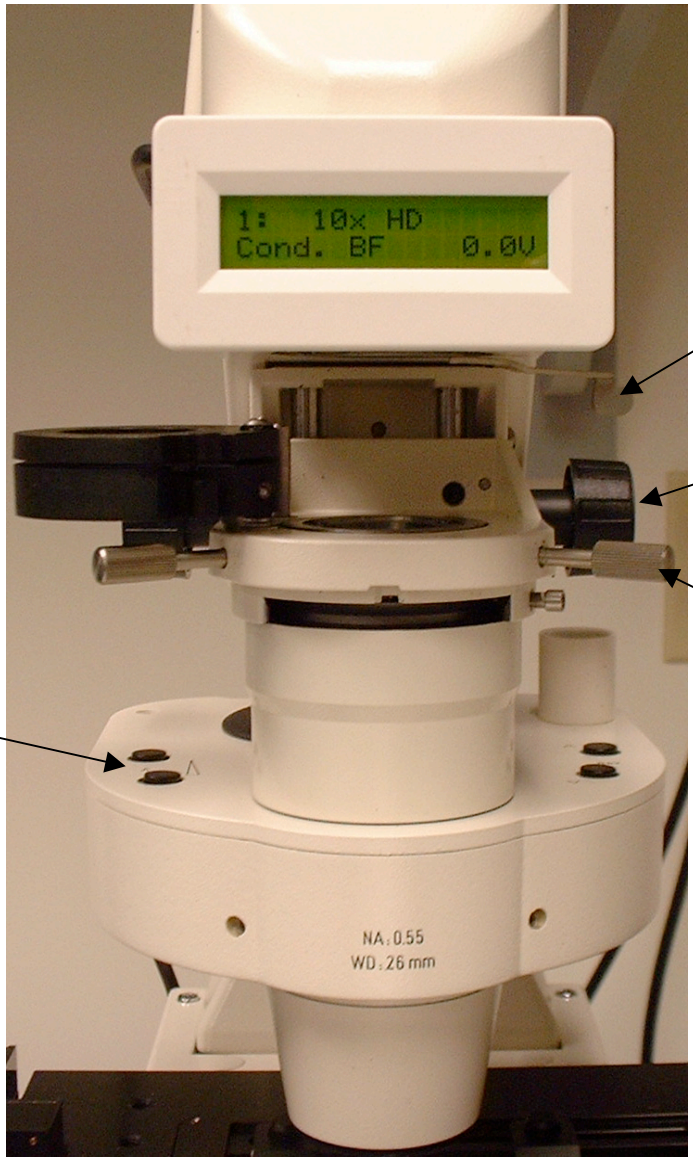
Koehler Illumination

Introduction

Koehler illumination is a critical determinant of optical performance in light microscopy. When a microscope has been properly adjusted for Koehler, each source point from a lamp contributes equally to illumination in the specimen plane. Thus, variations in intensity in the image can be attributed to the object and not to irregular illumination from the light source. The following tutorial describes the process of setting Koehler illumination on a Zeiss 200M research microscope.

Adjusting the microscope for Koehler illumination

Figure 1

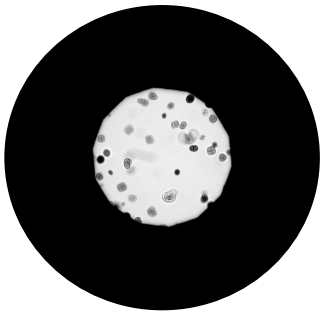


1. Place a specimen slide on the stage of the microscope.

2. Turn on the microscope lamp and focus on the specimen. At this point, the field diaphragm and the condenser front aperture diaphragm (figure 1) can be fully opened to ensure that the sample is illuminated.

3. Focus and center the condenser. With the specimen in focus, close down the field diaphragm completely. Now while examining the specimen through the eyepiece, focus on the angular outline of the diaphragm using the condenser's focusing knobs. If there is no light after closing the field diaphragm, the condenser diaphragm may be out of the field of view. In this case use the condenser positioning screws to move the condenser diaphragm into the field of view (it may be helpful to open the field diaphragm slightly thereby producing a larger target to find). After focusing the image of the field diaphragm in the eyepiece, bring it into the center of the field of view using the condenser positioning screws. Figure 2 shows a picture of a properly focused and centered field diaphragm.

Figure 2



4. Open the field diaphragm until it is almost as large as the field of view in the eyepiece. By constantly keeping a small amount of the field diaphragm visible during imaging, the degree of Koehler illumination can be easily determined. If the image of the field diaphragm appears blurry of off centered at any time, simply readjust the condenser position as described above to achieve even illumination. When imaging through the camera, opening the field diaphragm to a point where it is only slightly larger than the field of view of the camera may yield the best results.

5. The microscope is now adjusted for Koehler illumination. As a final adjustment, the numerical aperture of the condenser can be changed using the two buttons located on the left side of the condenser diaphragm. This adjustment will affect the resolution of the microscope, change the contrast of the image, and establish the depth of field. It is not possible to optimize the condenser aperture position for both contrast and resolution and the final setting is usually based on the inherent contrast of the specimen.

Useful references

Inoué, S., and Spring, K. R. (1997). Video microscopy : the fundamentals. (New York, Plenum Press).

Murphy, D. B. (2001). Fundamentals of light microscopy and electronic imaging. (New York, Wiley-Liss).

“Molecular Expressions Optical Microscopy Primer” website at Florida State University. http://micro.magnet.fsu.edu/primer/index.html