

Media Cybernetics White Paper

Koehler Illumination

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Introduction

A good microscope illumination system should fulfill three criteria. First, it should provide even illumination of exactly the field of view. Illuminated specimen areas outside the field of view can potentially contribute stray light which reduces image contrast. Next it should uniformly illuminate the object field even when inhomogeneous light sources are used such as coiled filament or arc lamps. Finally, it should provide a variable illuminating aperture, adjustable from a maximum aperture matching that of the highest power objective to a minimum aperture equal to about one half the aperture of the lowest power objective. This is required because the relationship of objective to condenser aperture controls resolution, contrast, and depth of field. The setting of the condenser aperture also influences the image brightness but that is not its purpose. The rheostat setting or the use of neutral gray filters regulate the brightness without affecting other image characteristics.

In epi-fluorescence microscopy the aperture diaphragm has no impact on resolution and depth of field because no coherency exists between illuminating and imaging rays. Here the aperture diaphragm may legitimately be used to regulate the image intensity.

The Illumination Path

Conceptually one might picture the illumination part of the microscope as a second, identical microscope which is arranged on a common axis and in mirror symmetry with the image forming microscope. The counterpart to the objective is the condenser, the counterpart to the eyepiece field diaphragm is the luminous field stop and the counterpart to the eyepiece plus eye is the lamp collector. In the illuminating microscope the light source takes the place of the retina. In such an arrangement the illumination is necessarily restricted to the field of view and the condenser aperture equals the objective's aperture yet can be made variable by fitting an iris diaphragm in the condenser's rear focal plane.

This is the concept of critical or Nelsonian illumination. Since the light source is imaged into the object plane critical illumination is restricted to the use of homogeneous light sources such as the center portion of the flame of a kerosene lamp or a ribbon filament lamps (historic).

Koehler illumination takes this idea one step further. It leaves all components in the same place as in critical illumination except the lamp filament which is moved closer to the adjacent lens, the collector, so that the image of the light source moves into the condenser rear focal plane (Figure 1).

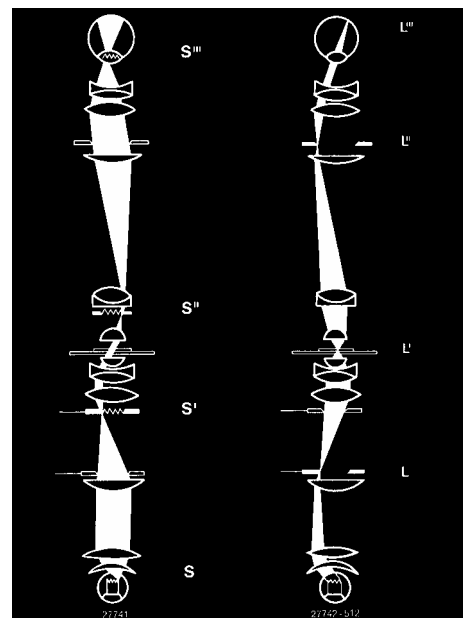


Figure. 1- The Ray Path. On the left the *illuminating* path with its four conjugate image planes S, S', S'', S''' is shown. Tracing one point on the source through the system shows how it ends up being spread across a sizeable area of the retina.

On the right the *imaging* path with its four conjugate image planes L, L', L'', L''' is shown. Tracing one image point, originating on the retina, backwards through the system shows that the light originated from a sizeable area of the source.

In critical illumination the illuminating and the imaging path share conjugate planes and the microscopical image is marred by the superimposed image structure of the light source. The distinguishing feature of Koehler illumination is the projection of the source into the condenser back focal plane S' instead of the plane of the luminous field stop L , whereby the evenness of illumination is much improved.

Tracing the cone of light issuing from a point on the light source we see it change to parallel rays in the space between condenser and objective and then come into focus again in the rear focal plane of the objective and finally in the exit pupil of the eyepiece. From here the cone of light spreads to illuminate a large area of the retina. This is the principle of Koehler illumination

Light that originated at a single point on the source serves to form an image across the retina. Conversely, if we trace a point on the retina backwards we find that it covers a substantial area of the source. No longer do we see the image of the object and the source simultaneously. Illuminating and imaging ray path each show sets of four conjugate planes which do *not* coincide and thus we are free to choose light sources according to their intensity and spectral characteristics rather than their homogeneity. By substitution of the fixed luminous field stop for an iris diaphragm in the illuminating microscope we add some flexibility to the illuminating microscope which can then serve several objectives (Figure 2).

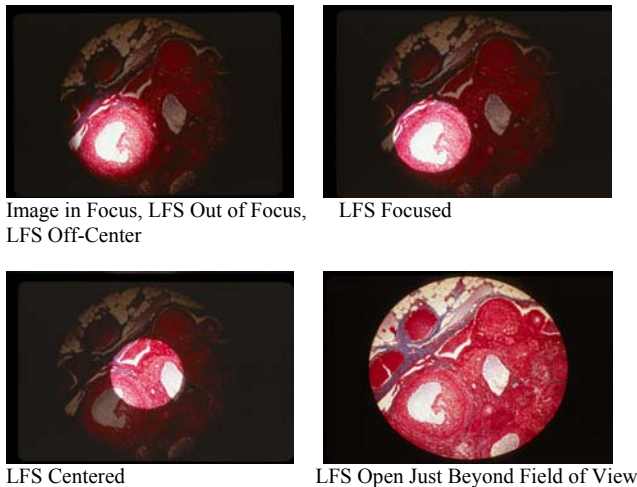


Figure 2. Adjustments to the condenser and the luminous field stop (LFS for Koehler illumination.

Light Sources

Are we free to choose any kind of light source? Not quite. Not only does the evenness of the field illumination matter but that of the objective back focal plane as well. Otherwise we would exclude some zones of the objective from participating fully in the image formation. The maximum resolution of the system could then not be realized and if the illumination were additionally distributed non-symmetrically, artifacts would mar the image. From this follows, that the image of point-like light sources must be magnified to fill the condenser back focal plane evenly with light. Similarly, the pattern of alternating bright and dark zones as produced by tungsten halogen bulbs must be homogenized for careful microscopical work, for example by the use of specially grooved disks.

The advantages of Koehler illumination are not always immediately apparent. In fact, if they are in the brightfield microscopy of moderately absorbing objects, the microscope or its optics are probably poorly baffled. With highly absorbing objects which happen to just fill the field or in fluorescence and DIC microscopy, the gain in contrast between a carefully set and an excessively opened luminous field stop are readily apparent. Either way, it is good practice to set the microscope carefully for Koehler illumination because it defines the proper condenser settings, especially its height, unambiguously.