Köhler illumination

Köhler illumination is a method of specimen illumination used in transmitted- or reflected-light microscopy[1] . It was designed by August Köhler in 1893, and overcame the limitations of previous techniques of sample illumination (ie: critical illumination). Prior to the advent of Köhler illumination, the filament of the bulb used to illuminate the sample could be visible in the sample plane. This created what is known as a filament image. Various techniques were used to remove the filament image, for example lowering the power of the light source, using an opal bulb, or placing an opal glass diffuser in front of the light source. However, all these techniques, although effective in reducing the filament image to a certain degree, had the effect of reducing the quality and uniformity of light reaching the sample. Reducing the power of the light source and introducing an opal bulb both caused a reduction in the spectrum of incident light. For transmitted-light microscopy wide spectrum white light is desirable in order to realize the maximum amount of contrast. Further, adding an opal glass diffuser will cause the light reaching the sample to be uneven. Uniformity of light is essential to avoid shadows, glare, and inadequate contrast when taking photomicrographs. Köhler illumination overcomes these limitations by creating parallel light rays to pass through the specimen. Because the light rays that pass through the specimen are parallel they will not be in focus when creating the image of the specimen thus eliminating the image of the filament.

Principles

Condenser Optical Components

- High intensity bulb
- Collector lens
- Field diaphragm
- Condenser diaphragm
- Condenser lens

Setting Up Köhler Illumination

1. Focus on the specimen.

2. Close the field diaphragm to its most closed state so that you can see the edges of the diaphragm (may be blurry) in the field of view.

3. Use the condenser focus knobs to bring the edges of the field diaphragm into the best focus possible.

4. Use the condenser-centering screws to center the image of the closed field diaphragm in the field of view.

5. Open the field diaphragm just enough so that its edges are just beyond the field of view.

6. Adjust the condenser diaphragm to introduce the proper amount of contrast into your sample. The amount of contrast added will depend on the sample, however too much contrast can introduce artifacts into your images.

7. Adjust the light intensity as necessary. To adjust light intensity it is best to use a neutral density filter rather than increasing or reducing the supply of power to the lightsource. Neutral density filters block all wavelengths of light equally, while changing the power to the light source will alter the balance in the spectrum of incident light giving a yellow/brown appearance to the image.

Used In

- Bright field microscopy
- Phase contrast microscopy
- Dark field microscopy
- Differential interference contrast microscopy
- Polarized light microscopy
- Hoffman modulation contrast microscopy

See also

- Optical microscopy
- August Köhler

External links

Köhler illumination setup instructions [2] from berkeley.edu.

References

[1] Olympus Microscopy Resource Center - Köhler Illumination (http://www.olympusmicro.com/primer/anatomy/kohler.html)
[2] http://microscopy.berkeley.edu/courses/TLM/condenser/kohler.html

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