Optics – Day 3
Kohler Illumination
(Philbert Tsai – July 2004)

Goal : To build an bright-field microscope with a Kohler illumination pathway

Prepare the Light source and Lenses

Set up Light source

Use 3" rail carriage

Determine Optical Axis. Level / Align five lens holders (on 1" rail carriages)

Set up the Microscope

Collector Lens

This lens relay images the lamp filament to a new location

Option 1: Double Lens Setup

Use 25mm Lens (KPX576) as Primary Collector Lens to pseudo-collimate the light

Use 75mm Lens (PAC046) as Secondary Collector Lens and form an image of the filament

Option 2: Single Lens Setup

Use 25mm Lens (KPX576) to form an image of the filament ~100mm from the lens

Aperture Diaphragm

This diaphragm controls the angle of rays (numerical apertures) illuminating the sample

Use 1" rail carriage

Place small iris at the image position of the filament

(This diaphragm therefore acts as a virtual light source for the sample)

Field Diaphragm

This diaphragm controls the area of illumination at the sample plane

Mount into X-Y translation square (Linos microbench)

Double Lens Collector Version:

Place Iris between the first and second Collector Lens

Single Lens Collector Version:

Place Iris roughly one focal length from the Collector Lens

Condenser Lens

This lens de-images the filament onto the sample to provide uniform illumination

Use 1" Carriage

Use 50.2mm Lens (KPX582C) to pseudo-collimate the light after the Aperture Diaphragm

Sample Stage

Use a 1" Carriage

Place a white index card in the sample holder

Close down the Field Diaphragm

Place the sample/card at the location where the Field Diaphragm is in sharp focus

Hint : if it is too small to see use another lens to magnify / relay image the sample to another card

Replace the white index card with a sample slide

Objective

This lens relay images / magnifies the sample to an intermediate plane

Use a 1" rail carriage

Use the 25mm Lens (PAC022) to project the image of the sample to an intermediate plane

~ 160mm away

Eye Lens

This lens projects the image to/beyond infinity [virtual] for viewing by a relaxed eye or camera

Use a 1" rail carriage

Use the 12.7mm Lens to project the image of the sample to infinity

You should be able to look at the image by eye or by camera.

Field Lens

This lens serves to gather light that would otherwise miss the Eye Lens and effectively magnifies the aperture of the objective to reduce clipping of the image

Use a 1" rail carriage

Try a variety of lenses (75mm, 100mm, 150mm)

Place the lens just in front of or behind the intermediate image plane of the objective

Camera

Use a 3" rail carriage

Focus the camera to infinity (focus it on a far away object)

Line the camera up behind the Eye Lens

Adjust the relative positions of the Field Lens, Eye Lens and Camera to obtain a good image

Magnification

Adjust the relative positions of the objective and the Eye Lens/Field Lens/Camera combination to obtain greater or lesser magnifications of the sample

Calculate the magnification of the system.

Kohler Illumination

Fully open both the field diaphragm and the aperture diaphragm.

Focus the sample in the microscope

Use neutral density (ND) filters to cut down the intensity appropriately

Q: Where is the worst place to put the ND filters? Where is the best place?

Q: Why not just turn down the current to the light source?

Close down the field diaphragm as small as it will go

Bring the edges of the field diaphragm into sharp focus by moving the condenser

Center the field diaphragm in the field of view using the X-Y knobs on the iris holder

Close down the aperture diaphragm to reduce the glare on the edges of the field diaphragm image

(reduce the glare until the black-to-white transition at the iris edge is very sharp)

The glare is due to scatter of large-angle light rays that "should" have missed the objective

Open up the field diaphragm until the iris edge is just outside the field of view

Q: What happens if you open the field diaphragm too wide?

Depth of Field

Close down the aperture diaphragm as far as it will go

(You may have to take out some of the ND filters to compensate for the reduced intensity)

Q: What has happened to the depth of field?

Hint: move the objective back and forth and see how quickly features go out of focus.

Compare this to the depth of field when the aperture is opened wider.

Q: What would happen if you used a point source (say an LED) to illuminate instead of a lamp?

Hint: recall that the aperture diaphragm is a virtual light source (a relay image of the filament)

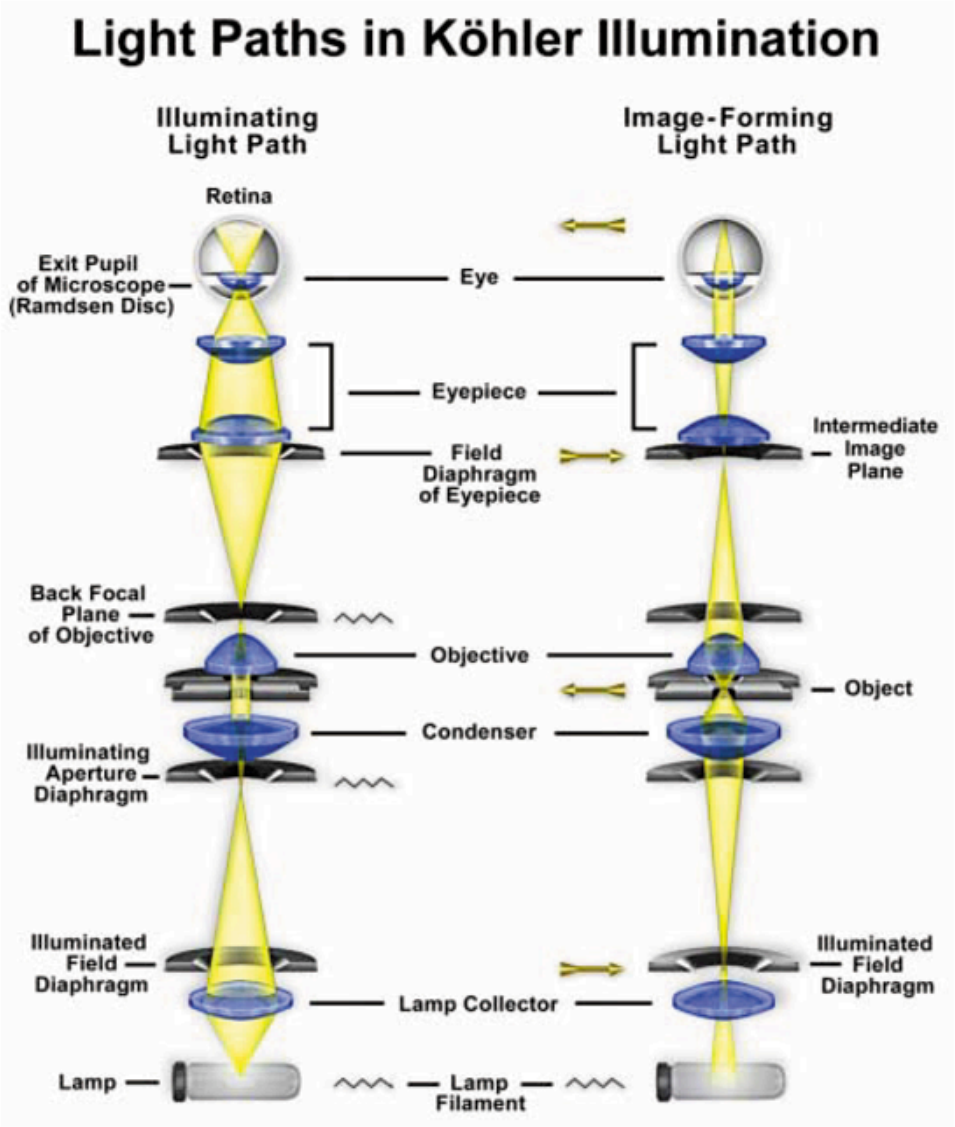

Figure 7. Light paths in Kohler illumination. The illuminating ray paths are illustrated on the left side and the image-forming ray paths on the right. Light emitted from the lamp passes through a collector lens and then through the field diaphragm. The aperture diaphragm in the condenser determines the size and shape of the illumination cone on the specimen plane. After passing through the specimen, light is focused at the back focal plane of the objective and then proceeds to and is magnified by the ocular before passing into the eye.

Figure taken from Optical Microscopy by Davidson and Abramowitz

HOW TO SET UP A MICROSCOPE PROPERLY FOR TRANSMITTED LIGHT ILLUMINATION:
KÖHLER ILLUMINATION

In order to get the best image possible from brightfield, phase contrast, differential interference contrast, or polarization optical setups with the light microscope, it is crucial that the light path be set up properly.

The method for doing this is called Köhler illumination after August Köhler, the man who invented it. It is also know as double diaphragm illumination because it employs both a field and an aperture iris diaphragm to set up the illumination. If the light path is set up properly, you will have the advantages of an evenly illuminated field, a bright image without glare and minimum heating of the specimen.

The following instructions apply to any microscope, upright or inverted which is equipped for transmitted light bright field illumination. Focussing of the field diaphragm as discussed here should be done for phase and differential interference optics as well.

To set up Köhler illumination:

1. Switch on the light source and make sure that light is coming through the field diaphragm at the base (upright microscope) or the top (inverted microscope) of the microscope stand. It may help to place a piece of paper over the field stop to see the light.
   Place your specimen on the stage and turn the nosepiece (which holds the objective lenses) to the 10X or 20X lens. Open the field diaphragm as far as it will go.
2. Notice whether or not your specimen is illuminated. It will help to place a piece of paper over the top of the specimen to see if light is getting through to it. If you are using the brightfield condenser stop, open the iris diaphragm (or aperture diaphragm) on the condenser turret (which contains the stops for brightfield and phase, etc) wide open to give the maximum illumination. If there is a swing-in front lens for the condenser (directly above (inverted) or below (upright) the specimen), you may need to swing it into the light path.
   The front lens should be about 1-3 mm above or below the specimen. There are condenser focussing knobs to do this.
3. Now bring your specimen into focus with the coarse and fine focussing knobs. the best way to do this is to rack the lens as close possible to the specimen watching the objective lens all the time(and NOT looking into the oculars) to make sure that the lens does not run into the slide. Then rack the lens away from the stage (or vice versa) while looking through the oculars to bring the specimen into focus (details are as sharp as they can be). If the light is too bright, reduce it with the rheostat on the light source.
4. When the specimen is in focus, start to close the field diaphragm and also begin to carefully move the condenser up and down with the condenser focussing knobs. Look for a sharp image of the edge of the field diaphragm. This may be a little with a long working distance condenser. Also, if the iris diaphragm in the condenser turret is open wide, the glare may obscure the edge of the field diaphragm silhouette somewhat. Furthermore, you may find that this edge is not centered.
5. When the edge of the field diaphragm silhouette is sharply defined, center it with the two knobs (usually knurled knobs) coming out diagonally from the condenser. Close down the field diaphragm most or all the way to get it centered properly. When it is centered, open the field diaphragm until its edge is outside the field. If you are doing brightfield or differential interference microscopy, do not yet open the field diaphragm.
6. As stated before, you may notice some glare around the edge of the field diaphragm, that the edge area outside the edge is not completely dark like the outer part of the whole field as you should see it now. This glare comes from light bouncing around in the light path and going in and illuminating the specimen in such a way as to obscure detail in the specimen. To reduce this glare, close down the iris aperture in the condenser turret until all of the dark area outside of the field stop silhouette is evenly dark. Now open up the field diaphragm until the edge of the diaphragm silhouette is outside the field of view. You should also now be able to turn up the light at the power source.
7. Your specimen should be properly illuminated and should give you a great image. If it does not, check to make sure your lenses and other optical components are clean. Then recheck to see that you have followed each step properly. If you still cannot get a good image and you are at UCLA, give me (Matt Schibler) a call at X59783 and I'll be glad to try to help you.

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