# Köhler Illumination Microscopy

**Aim**: To demonstrate the setup and alignment of a Köhler illumination using optical components.

**Apparatus:** Lamp, Collector lens, Field stop (iris), Flip mount filters, ND filters wheel, Condenser, Sample stage, Objective, camera

**Theory:** Köhler illumination provides uniform illumination across the entire field of view, which is essential for producing high-quality images. This uniformity eliminates shadows and bright spots, ensuring that the specimen is illuminated evenly.It is a method of specimen illumination in microscopy that creates parallel light rays to pass through the specimen, eliminating the image of the light source filament. It was developed by August Köhler in 1893 . Köhler illumination uses various optical components including a collector lens, field diaphragm, condenser diaphragm and condenser lens to produce evenly illuminated, high-contrast images without shadows or glare. It is used in various types of microscopy including brightfield, phase contrast, darkfield and polarized light microscopy.

**Method:** The microscope illumination system is quite subtle and sophisticated, but also relatively simple. However, the simplicity is masked at first by the number of unfamiliar parts.
the list of things we need to control in the illumination path:

1. Intensity (brightness)
2. Spectrum (color)
3. Location (what part of the sample is illuminated)
4. Uniformity (even illumination across the sample)

The first two items are relatively straightforward and should be familiar.
For an incandescent lamp, the spectrum does change with the temperature of the bulb filament as you turn down the power, the filament gets cooler and the spectrum of the light emitted shifts toward the red. In order to control the intensity separately from the spectrum of the light, we will use neutral density (effectively, gray) filters to control the intensity without adjusting the power to (and hence the temperature and color of) the filament. Furthermore, since a hot filament emits light at all wavelengths, we can use a color filter to select only the wavelength region we want. As an example, a green color filter (like the one you have) is often used to limit the range of wavelengths one is imaging with and hence also to eliminate chromatic aberration. Green is usually chosen because it is near the peak sensitivity for the human eye. Conveniently many silicon-based cameras (e.g. CCD or CMOS) are also fairly sensitive in the green. The remaining properties, location, uniformity are more complicated. It makes sense to address these in order:

There are actually two components to location: where the light source is and what part of the sample is illuminated. The location of the light source is simple; it is generally not convenient to put the actual light source up against the sample, especially if the light source is a hot filament. However, when the light source is far from the sample the light gets quite dim, this problem is especially problematic for small or mostly transparent samples, which scatter very little of the incident light. The obvious thing to do is to use a lens to collect the light from the filament and focus it toward (or onto) the sample. Not surprisingly, the lens used to do this is called the "collector lens.

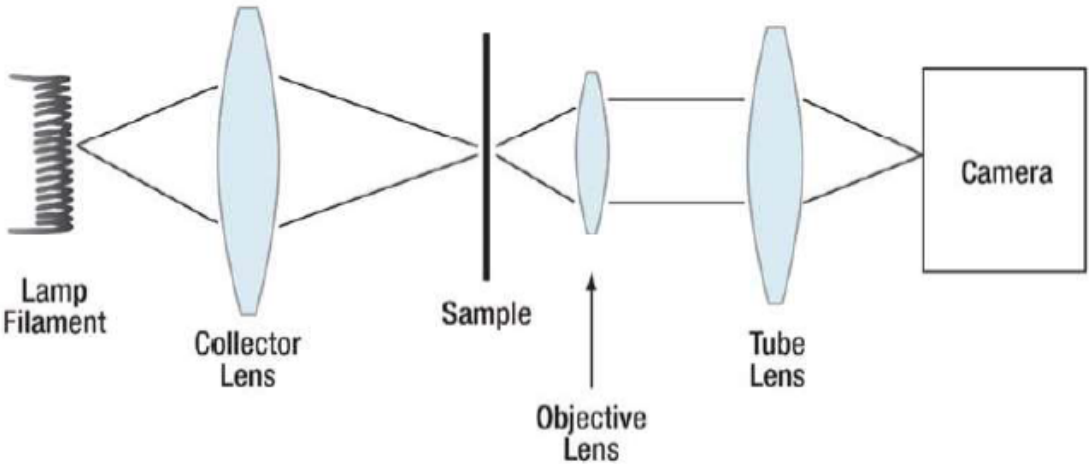


Figure 1: Critical Illumination

In addition, the collector lens is positioned such that it images the lamp filament onto the sample. Of course the sample is imaged onto the camera (by the objective and tube lens), so the filament must also be imaged onto the camera.

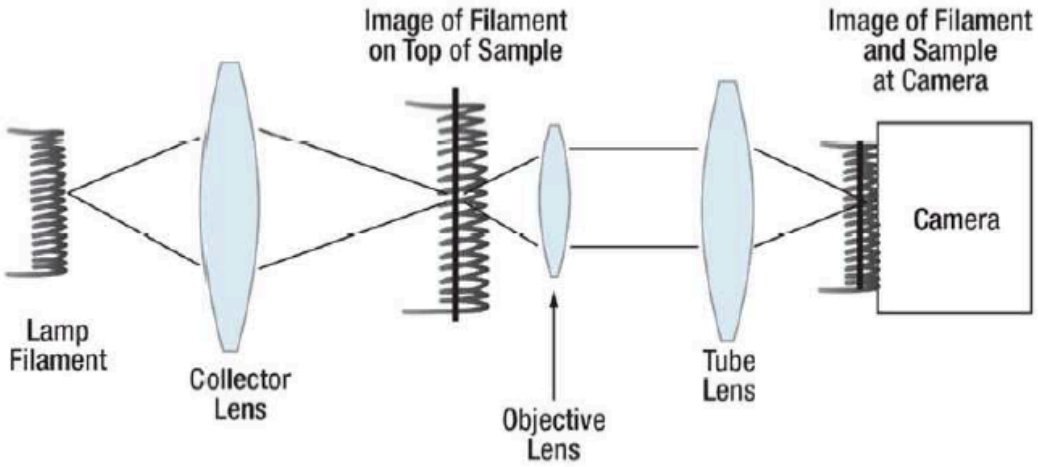


Figure 2: Conjugate Planes: The filament, sample, and camera face are all imaged onto each other, i.e. are "conjugate"

The term for two planes that are imaged onto each other is conjugate. Conjugate planes are very important in microscopy. The most obvious conjugate planes are the sample plane and the camera. If the image is in focus then those two planes are conjugate. This concept can be extended further: for instance, if you look through a microscope eyepiece and see the sample, then your retina and the sample plane are conjugate.

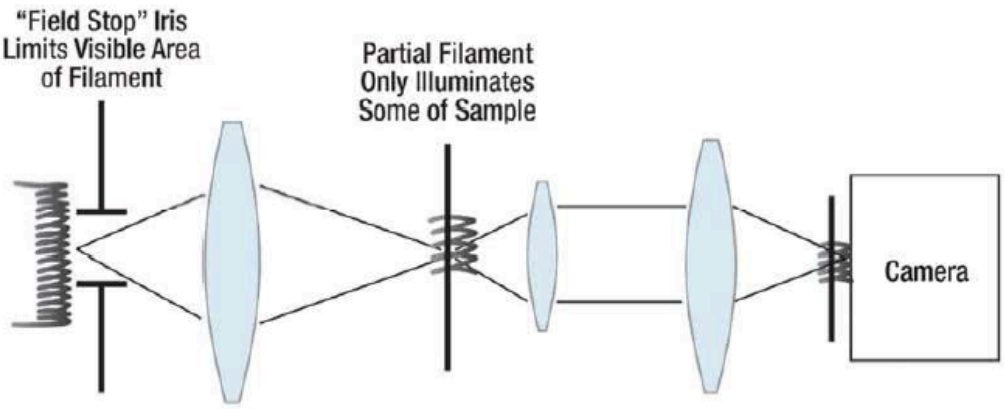


Figure 3: Field stop limits area of sample that gets illuminated. Compare this image to fig 2

A good way to make the filament look like it is infinitely far away without having to put it a long distance off (and so losing a lot of intensity) would be to put the filament one focal length behind a lens so that the filament would be focused at infinity on the other side. Even better, if we put the sample exactly one focal length on the other side of the lens then each point in the filament would illuminate the entire sample resulting in complete uniformity.
Since optimizing complete setup is somewhat complicated, and there is an accepted standard for how it is done in typical microscope systems.In our lab experiment we need to arrange the optical components in a systematic way,as shown below.

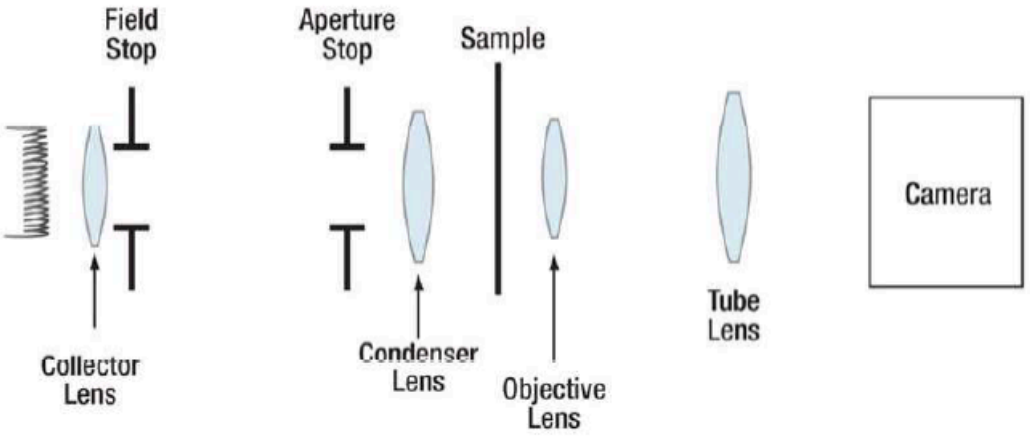


Fig4.All the parts of the microscope, in order.

## Alignment technique:

1. Place an optic in a rail carrier in front of the camera, so the optic is up against the end of the camera lens tube.
2. Get the height correct by looking to see if the camera tube and the new optic are flush (or feeling around the edge with your fingers to verify  be sure to wear gloves if doing this!)

3. Lock down the rail carrier set screw to fix the height.

4. Use a post collar to fix the height of the post against the rail carrier.

5. Remove the rail carrier from the rail, and reattach it wherever you want the optic to be. This maintains both the correct height and correct angle for the optic.

6. Alternatively, loosen the rail carrier thumbscrew, remove the optic, and put it in a different rail carrier. This maintains height but not angle.

## Optics setup:

### (A) Collection optics setup

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1. Make sure that all lenses are at the same height; otherwise getting an image will be complicated, and images themselves will be poor.

2. You will do best to start with the camera at the right height, then carefully match the height of all your lenses (objective, condenser, and collector) to that.

3. If the camera tube lens center is ~9.5 cm above the center of the top rail (so ~ 1 mm of post is projecting below the rail carrier), you will have enough leeway to adjust the resolution target height as needed.

## (B) Illumination optics setup

1. Place your lamp at the far opposite end of the rail from the camera (lamp body should not extend off the rail; keep it ~2.5 cm inside the end of the rail.)

2. Place the f = 35 mm PCX lens (as your collector lens) close to the lamp (~1" or 2.5 cm away.)
a. Remember: this lens needs to be the same height as the lens on the camera.
b. Place the condenser in the sliding rail carrier on the lamp side of the sample;
c. Remember, the lens must be the same height as the lens on the camera.

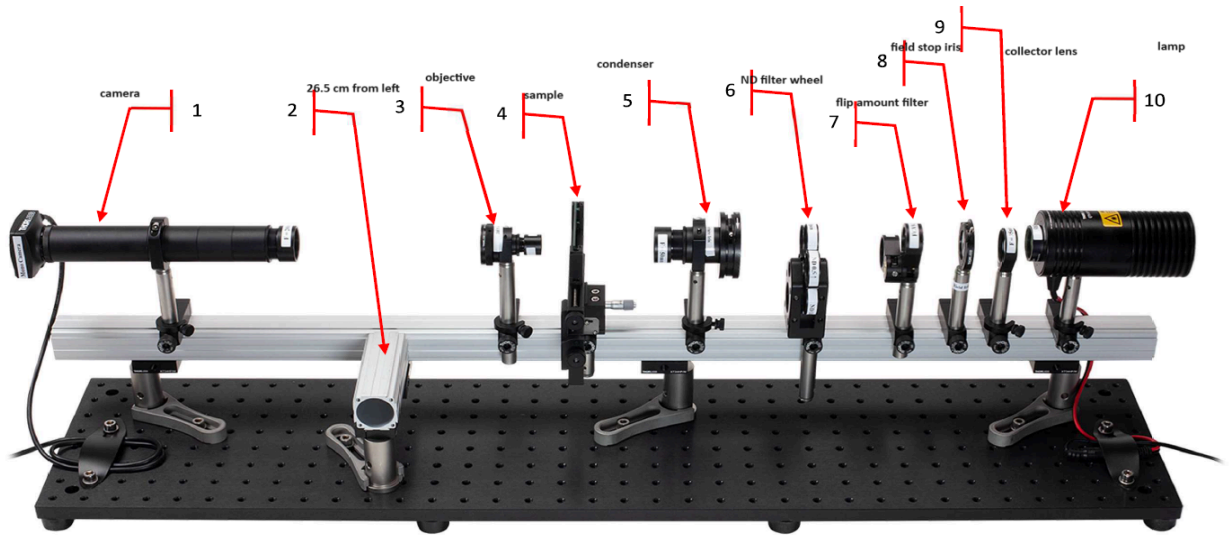


Fig.5 Optical components for Kohler setup in an ordered way.

## Takeaway:

After performing this experiment,

(a) You will get familiar with the different optical components
(b) You will learn about the focusing of image using lens and other optical components
(c) You will learn importance of microscopy and kohler illumination
(d) You will be able to align the setup for kohler illumination microscopy

## Write up:

1. Describe what you see when you examine the light in the optical path in the following places. Does it makes sense
Explain why or why not ?
a. Between the collector lens and the aperture stop.
b. Between the condenser lens and the sample.
c. In the objective back focal plane.

2. Make a table showing:
a. Your resolution in white light with the aperture stop open to the same height as the filament image.
b. Your resolution with the IR filter in place.
c. Your resolution with the green filter in place.

How do these compare to the Rayleigh resolution you expect?
i. What wavelength seems to make sense to choose for white light if you want to match the Rayleigh resolution?

3. Use a spreadsheet to plot the relative image intensity vs. the objective NA

| Iris Setting, mm | Calculated NA | Pixel Avg. | Background Avg. | Exposure | Brightness |
|---|---|---|---|---|---|
| 1.0 | | | | | |
| 1.5 | | | | | |
| 2.0 | | | | | |
| 2.5 | | | | | |
| 3.0 | | | | | |
| 3.5 | | | | | |