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Lecture Notes

Koehler Illumination

I. INTRODUCTION

August Koehler introduced his method in 1893. His objective was to obtain, within the optical microscope, a uniform illumination on the analyzed sample, despite the eventual use of non-homogeneous sources (like the electrical lamps).

As described in more detail below, the Koehler illumination setup presents two main characteristics:

- Creates an evenly illuminated field of view
- The working NA of the condenser and the size of the illuminated field can be regulated independently

These two important characteristics are clearly identified in the optical ray tracing through the microscope setup. Indeed, a set of "illuminating apertures" A_1, A_2, A_3, A_4, and another of "illuminated field diaphragms" naturally stand out from the ray tracing diagrams (they are schematically shown below but will be explained in more detail in the following sections.)

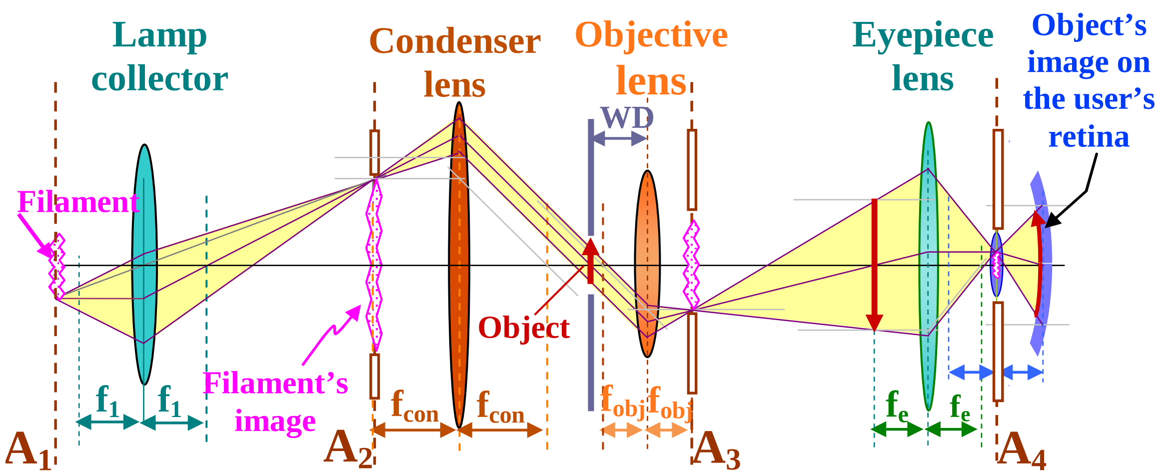


Fig. 1 Illuminating apertures diaphragms A_1, A_2, A_3, A_4. The A_2 diaphragm controls the light intensity reaching the sample.

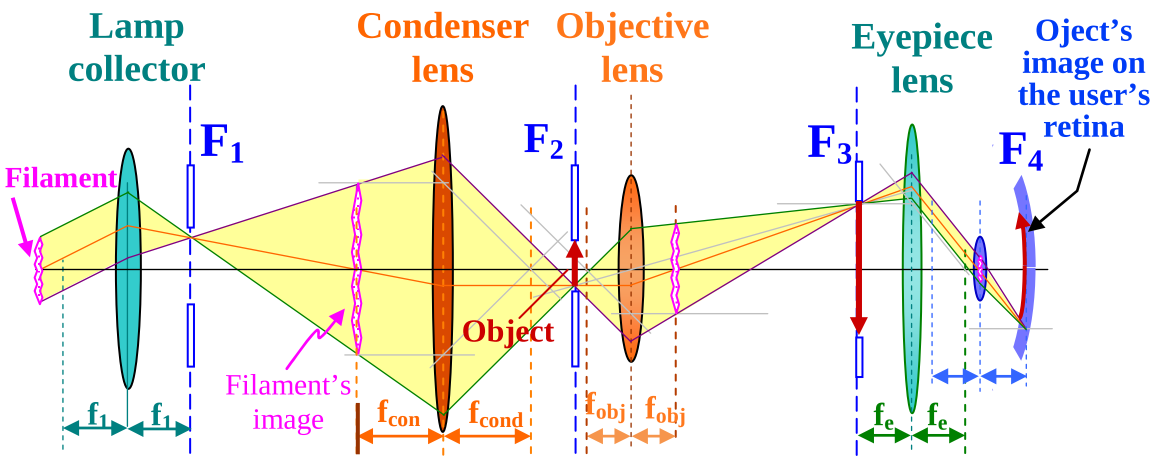


Fig. 2 Illuminated field diaphragms F_1, F_2, F_3, F_4. The F_1 diaphragm controls the size of the illuminated sample area.

II. UNDERLYING BACKGROUND

The description that follows assumes a prior knowledge of a conventional optical microscope set up, namely:

> **an objective lens forms a first image at the front focal plane of the eyepiece, and the latter forms a virtual image at infinity. This implies that a bundle of parallel rays reaches the user's eye, and a real final image is formed at the user's eye retina.**

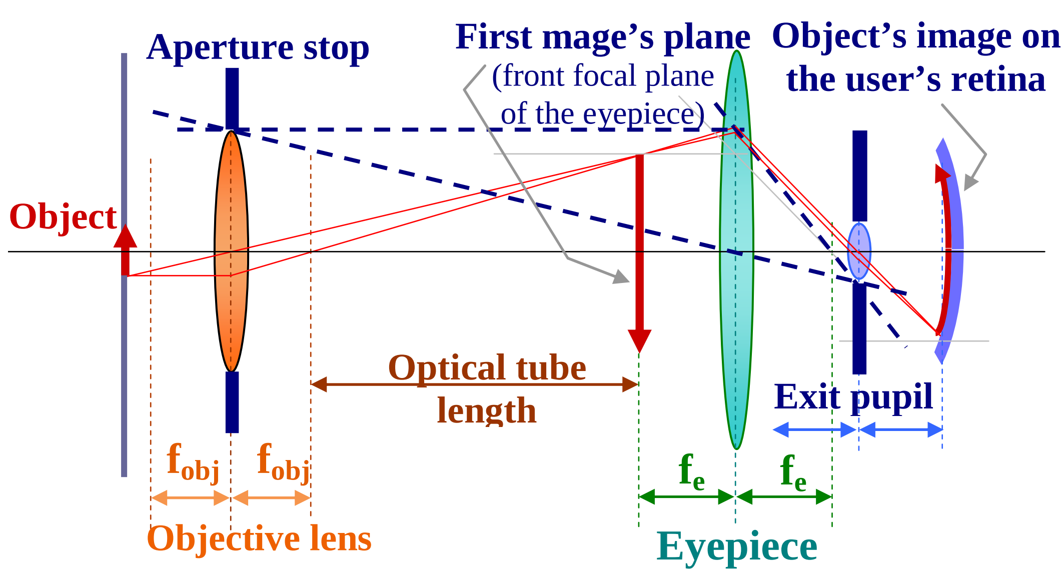


Fig. 3 Basic components of an optical microscope

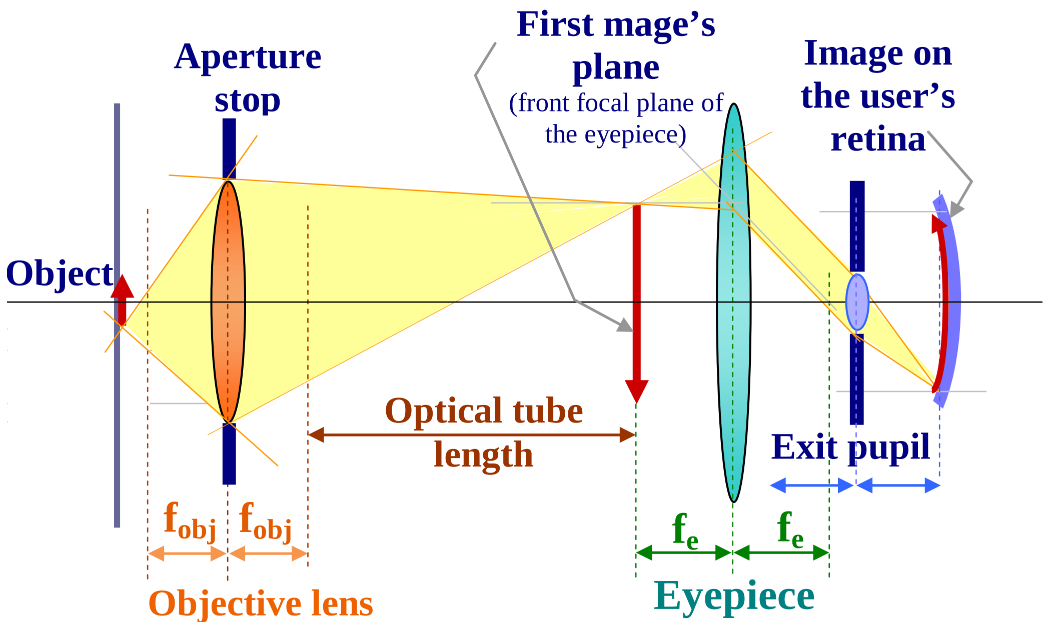


Fig. 4 Basic components of an optical microscope

It is in this optical microscope setup that we are going to introduce a proper illumination, the Koehler illumination, to *a*) fully exploit the maximum resolution of the microscope objective (by being able to select the proper numerical aperture of the condenser lens as to match that of the objective lens), *b*) obtain an uniform illumination over the sample despite using lamps of non-uniform brightness, while *c*) minimizing vigneting (the filament is imaged at the user's eye lens.)

III. DESCRIPTION

First we describe the Koehler illumination setup the way it should stand for its proper use within an optical microscope (we start from the illumination section towards the sample stage and then to the ocular eyepiece). In the second part we will outline some hands on "tricks" on how to actually implement the Koehler illumination (we'll see that the order of descriptions reverses, starting rather from the sample stage and continues with the illuminating lamp set up.

III.1 Filament and lamp collector

- **The purpose is to place the image of the filament at the back focal plane of the condenser lens**
- **Notice the magnification of the filament's image changes with the filament's position.**

In practice, since the illumination set-up (i.e. filament's position) is rarely changed, the lamp position is chosen at the very beginning of the setup such that the filament's image fills the largest diameter of the aperture diaphragm A_2. (This results in a photo-metrically inefficient use of the lamp's light when working at lower openings of the aperture diaphragm A_2.)

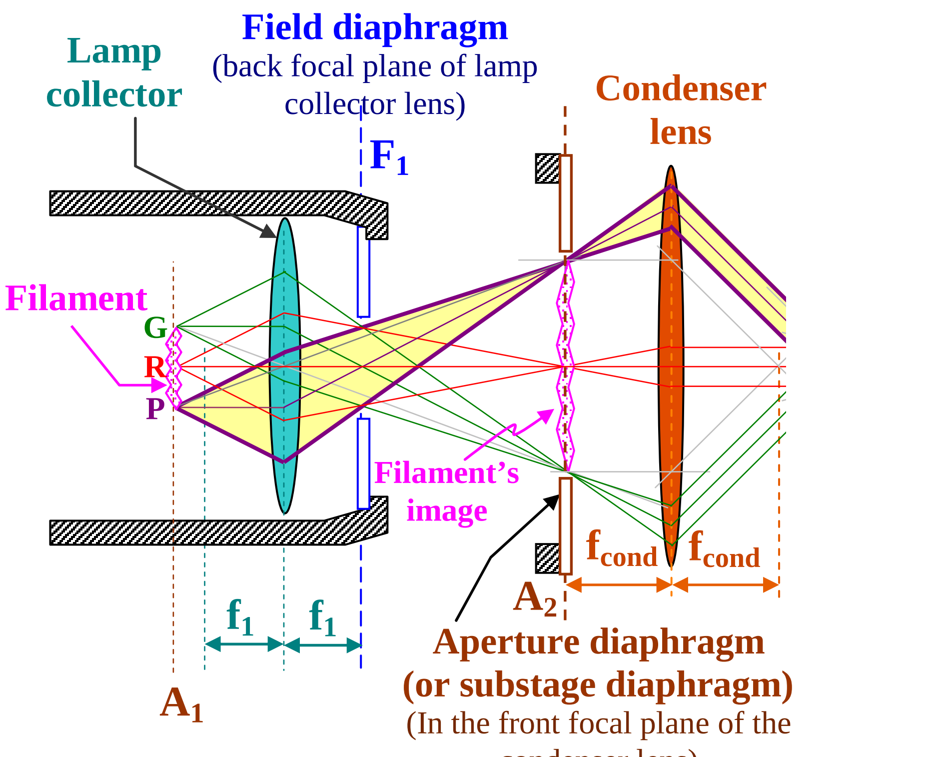


Fig. 5 Filament imaged at the front focal plane of the condenser lens.

- **Notice that the field diaphragm F_1 (located at the back focal plane of the lamp collector) is uniformly illuminated**

Indeed, notice in the graph above that it doesn't matter whether or not the point sources G, R, and P at the filament are of equal intensity. If, for example, one of those sources were off, the overall illumination would certainly be dimmer, but the intensity across the field diaphragm would still be uniform.

III.2 The condenser lens

- **One of the roles of the condenser lens is to image the field diaphragm (F_1) into the object's plane (F_2)**

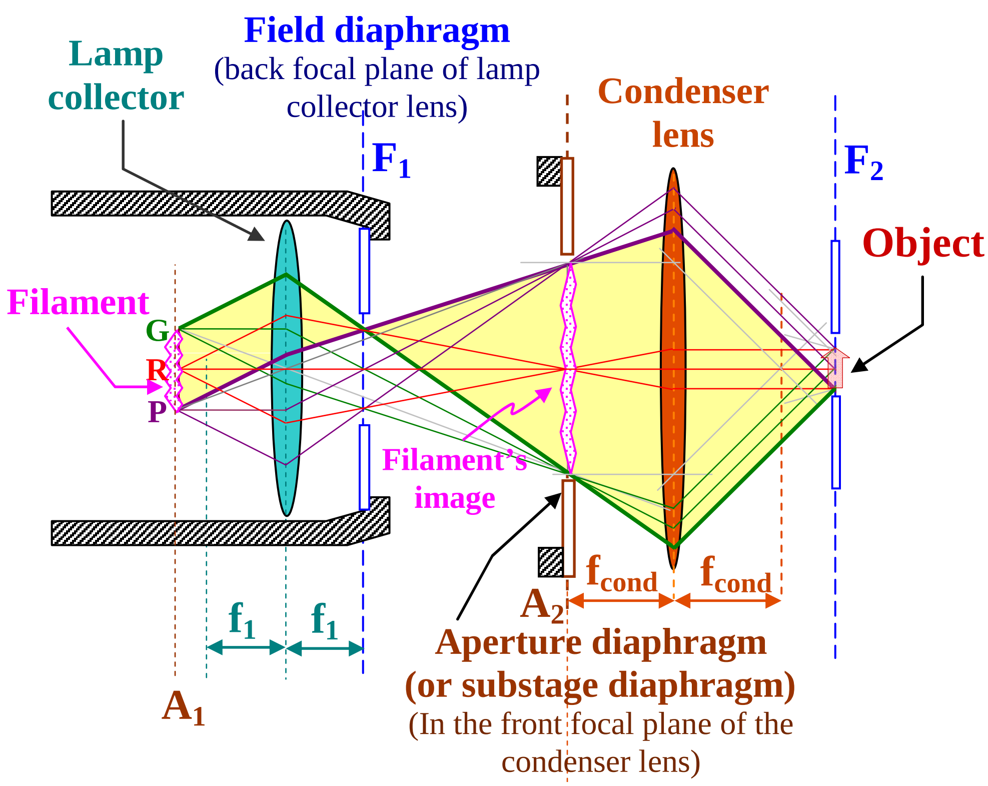


Fig. 6 Field diaphragm F_1 imaged at the object's plane F_2.

- **The aperture diaphragm A2 controls the numerical aperture of the condenser lens**

Notice in the figure below that the smaller aperture A_2 the smaller the angle θ.

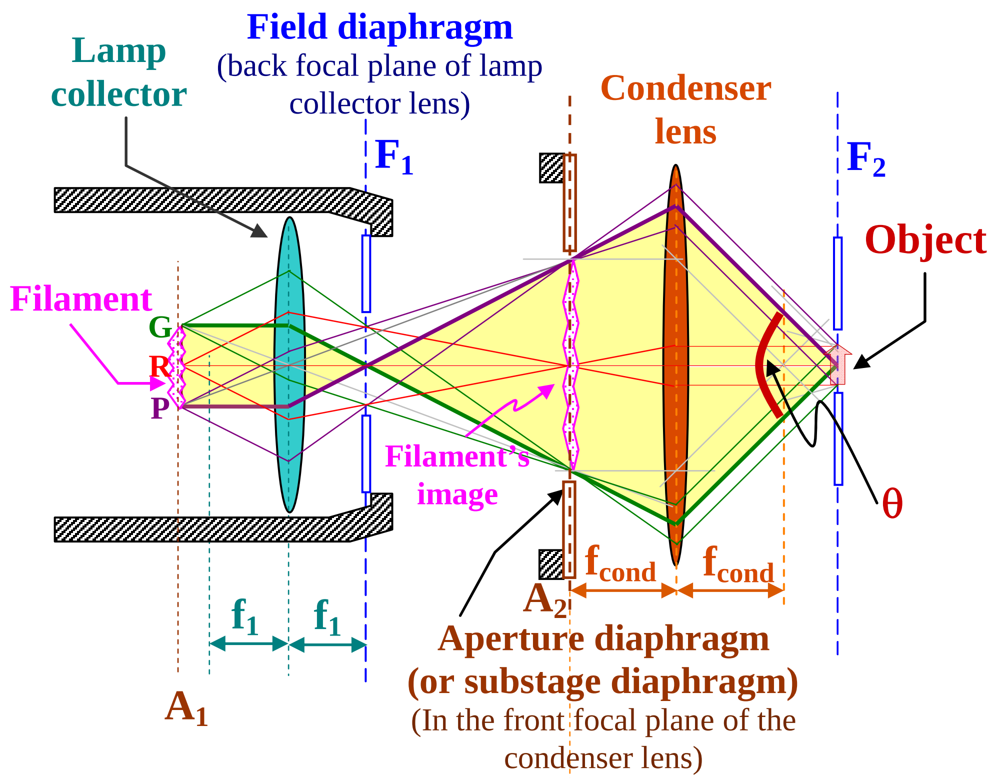


Fig. 7 The size of the aperture diaphragm A_2 determines the condenser's numerical aperture.

III.3 Independent control of the illuminated field area and the condenser's numerical aperture

The diagrams in the Figs 6 and 7 above highlight the main characteristics of the Koehler illumination

- **The field diaphragm (F_1) controls the size of the illuminated area on the object's plane (F_2).**

Indeed, notice in Fig. 6 that if we decrease the size of the field diagram (F_1) then the rays will illuminate a smaller area of the specimen.

- **A decrease in the field diaphragm (F_1) does not affect the light intensity (or power density Watt/m^2) received at the specimen.**

The size of the illuminated area decreases, but the local intensity received at each site of the object is not affected.

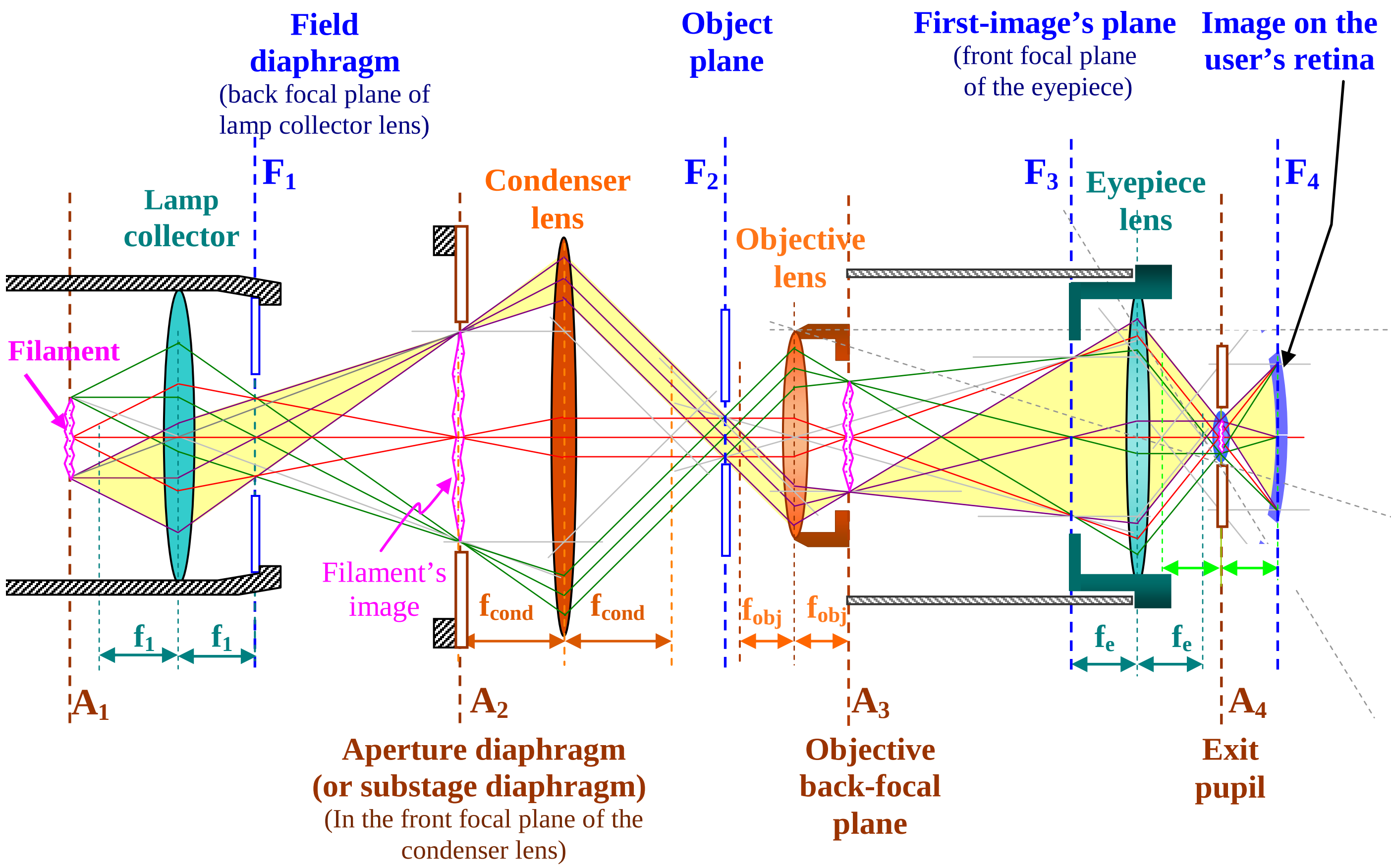
- **That is, the aperture diagram (A_2) and the field diaphragm (F_1) affect independently the optical setup.**

The aperturediagram (A_2) controls the light intensity reaching the object, but without affecting the size of the illuminated area. If we decrease the size of the aperture diagram (A_2) then a smaller area of the filament contributes to the illumination of the object, but this does not affect the size of object illuminated area.

Viceversa, a decrease in the field diaphragm (F_1) affects the size of the illuminated area in the object plane, but the local light intensity received by each point of the object is not affected.

III.4 Complete optical setup

The above description constitutes the expected arrangement in an Koehler illumination setup. The resulting full ray tracing in the optical microscope is shown below.



IV. IMPLEMENTATION

Starting with the objective lens and the eyepiece in their proper position,

- **image the field diaphragm F_1 in the object's plane**

For that purpose, have the field diaphragm quite open and try to obtain a sharp image of the borders. You achieve that placing the condenser lens in the proper position.

- **Image the filament in the front focal plane of the condenser lens.**

You can obtain this by placing a piece of aper in the back focal plane of the objective lens. A clear image of the filament should be obtained there.

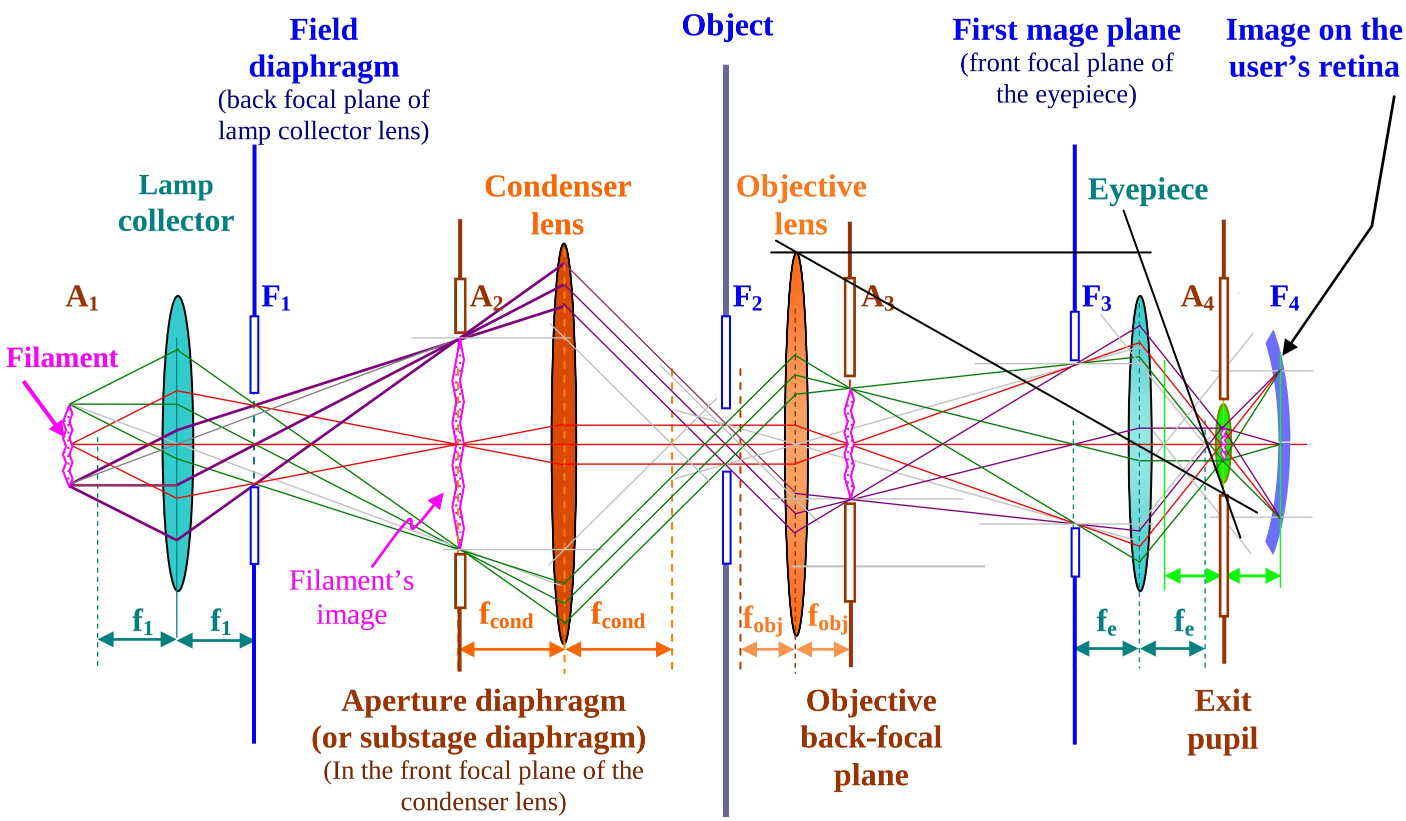
V. REFERENCES

C. Hammond, "Symmetrical Ray Diagrams of the Optical Pathways in Light Microscopes" *The Americas Microscopy and Analysis*, Sept Issue, p. 5-8 (2006).
The article considers infinity corrected optics

C. Hammond, “A symmetrical representation of the geometrical optics of the light microscope,” *Journal of Microscopy*. **192**, 63–68 (1998).

Shinya Inoue, K. R. Spring, “*Video Microscopy, the Fundamentals*” 2nd Ed. Plenun Press (1997).

Backup figure



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