Conjugate Planes in Optical Microscopy

In a properly focused and aligned optical microscope, a review of the geometrical properties of the optical train demonstrates that there are two sets of principal conjugate focal planes that occur along the optical pathway through the microscope. One set consists of four **field** planes and is referred to as the field or **image-forming** conjugate set, while the other consists of four **aperture** planes and is referred to as the **illumination** conjugate set. Each plane within a set is said to be conjugate with the others in that set because they are simultaneously in focus and can be viewed superimposed upon one another when observing specimens through the microscope. **Table 1** lists the elements that make up each set of conjugate planes, including alternate nomenclature (listed in parentheses) that has often been employed and may be encountered in the literature.

APERTURE OR ILLUMINATING CONJUGATE PLANE SET	FIELD OR IMAGE-FORMING CONJUGATE PLANE SET
MICROSCOPE EXIT PUPIL: EYE IRIS DIAPHRAGM, RAMSDEN DISC, AND EYEPOINT	Retina of the Eye Camera Image Plane
OBJECTIVE REAR FOCAL PLANE (OBJECTIVE REAR APERTURE)	Intermediate Image Plane (Eyepiece Fixed Diaphragm)
CONDENSER APERTURE DIAPHRAGM (CONDENSER FRONT FOCAL PLANE)	Specimen Plane (Object Plane)
LAMP FILAMENT	Field Diaphragm (Field Stop) or (Köhler Diaphragm)

In normal observation mode (using the eyepieces), the conjugate set of object or field planes can all be simultaneously viewed when the specimen is in focus. This observation mode is referred to as the **orthoscopic** mode, and the image is known as the orthoscopic image. Observing the other conjugate set of aperture or diffraction planes requires the ability to focus on the rear aperture of the objective, which may be accomplished by using an eyepiece telescope in place of an ocular, or a built-in Bertrand lens on microscopes that are so equipped. This observation mode is termed the **conoscopic**, aperture, or diffraction mode and the image observed at the objective rear aperture is known as the conoscopic image. Although the terms orthoscopic and conoscopic are scattered widely throughout the literature, many microscopists favor using normal mode and aperture mode because the latter nomenclature more clearly relates to the operation of the microscope. Planes belonging to the pair of conjugate sets alternate in succession through the optical train from the light source filament to the final microscope image produced on the retina or the image plane of an electronic sensor. A thorough understanding of the relationships between these conjugate plane sets, and their location within the microscope, is essential in understanding image formation and carrying out correct adjustment of illumination.

The optical relationship between the conjugate plane sets is based upon the fact that, in the illuminating ray path, the spherical wave fronts converge and are brought into focus onto the aperture planes, while in the imaging ray path, the spherical waves converge into focused rays in the field planes. Light rays that are focused in one set of conjugate planes are nearly parallel when passing through the other set of conjugate planes. The reciprocal relationship between the two sets of conjugate planes determines how the two ray paths fundamentally interact in forming an image in the microscope, and it also has practical consequences for operation of the microscope.

The concept that specific planes in the optical path of the microscope are conjugate indicates that they are equal. That is, whatever appears in focus in one plane of a conjugate set will appear in focus in all the other planes belonging to the same set. On the other hand, the reciprocal nature of the two sets of microscope conjugate planes requires that an object appearing in focus in one set of planes, will not be focused in the other set. The existence of two interrelated optical paths and two sets of image planes characterize Köhler illumination and is the foundation that allows the various adjustable diaphragms and aperture stops in the microscope to be used to control both the cone angle of illumination, and the size, brightness and uniformity of illumination of the field of view. The planes belonging to the field set are sometimes referred to as the field-limiting planes because a diaphragm placed in any one of these planes will limit the diameter of the image field. The aperture planes may be considered aperture-limiting because the numerical aperture of the optical system can be controlled by fixed or adjustable (iris) diaphragms inserted at any of these positions.

A microscopist may not be aware that in the normal observation mode, the specimen image is actually a combined view of four in-focus conjugate image planes (including the specimen plane) whose optical characteristics are determined or modulated by another set of four out-of-focus aperture planes. In a properly adjusted microscope, this fact can be easily ignored. However, the mechanism of the image formation becomes much more apparent, and of immediate practical interest, if there is something seriously wrong with the image. Knowledge of the location and image-forming function of each of the conjugate image and aperture planes is critical in troubleshooting problems that arise in the microscope image.

One of the more common ways in which the conjugate nature of the various field planes (including the specimen) and aperture planes (including the illumination source) is exploited, is in the placement of filters and other illumination-modifying optical components. These optical elements are likely to be contaminated with dust or fingerprints, due to frequent handling, and are often not optically designed to be included with the objective, oculars, and other elements of the optical path that are intended to form the focused final image. Therefore, auxiliary components should never be placed in any plane conjugate with the specimen, because any defects, dust, or debris may appear in the final image of the specimen formed at the retina or at a camera imaging plane.