Infinity Microscope Basics

This page is still under serious construction, lots of sections need to be expanded

This overview is meant to complement other available resources online such as https://www.microscopyu.com/microscopy-basics/infinity-optical-systems. At ASI we make modular microscopes in arbitrary configurations, which has far more flexibility than standard “big 4” research microscopes, but doing so also sometimes requires deeper understanding of the underlying principles (i.e. not seeing a microscope as a “black box”).

What is an Infinity Microscope

At the core, an infinity microscope consists of a pair of lenses which magnify the image of the sample to an image sensor. The magnification is given by the ratio of effective focal lengths of the two lenses, which are designated the objective lens (near the sample, short focal length) and the tube lens (near the image sensor, longer focal length).

The “infinity” signifies that the distance between the two lenses is not important. Optically this is because because both the objective and tube lens have one side focused at infinity, unlike older objectives and tube lenses which needed to be mounted a certain distance from each other to yield the stated magnification.

The region between objective and tube lens is often called “infinity space,” but we prefer the term “collimated space” to signify that rays originating from a single point in the sample plane are collimated or parallel in this region. The distance occupied by collimated space doesn't affect the magnification. The exact length of collimated space usually does not matter, as long as it is short enough to avoid vignetting. Filters, polarizers, and other elements are usually placed in collimated space.

Internally, objective lenses have many individual elements (often more than 10) in order to sufficiently correct aberrations with a relatively short focal length (i.e. the light rays need to bend quite a lot and in specific ways). Usually microscope bodies are built to accommodate different objective lenses. The tube lens is usually mounted in the microscope, and optically are comparatively simple lens (usually just a few elements).

What is a Lens

A lens turns position into angles and angles into position at its focal plane. Mathematically one focal plane is the Fourier transform of the other focal plane.

Objective Lenses

Dry, dipping, immersion...

Field number/FOV

Tube Lenses

The nominal magnification of an objective assume the manufacturer's standard tube lens is used. Tube lens focal lengths are 200 mm for Nikon and Leica, 180 mm for Olympus, and 165 mm for Zeiss.

Flat-field Correction

Spherical Correction

Chromatic Correction

Chromatic correction is done independently in the objectives and tube lenses for Nikon and Olympus, meaning it is possible to mix and match objectives and tube lenses and even use a different focal length tube lens. However, Zeiss and Leica correct chromatic aberrations of their objectives using the tube lens, meaning that their particular tube lenses must be used (at the proper spacing) for optimum chromatic correction. [1)]

Vignetting

Recall that light coming from a point in the sample will be turned into a bundle of parallel rays coming out of the objective lens, and the further the point is from the optical axis the more tilted that bundle will be. That bundle of rays is collected and refocused to a point by the tube lens, **if** the whole bundle makes it to the tube lens (the tube lens is only a certain size, plus there may be spots in the microscope's optical path where rays may hit a side wall or miss a mirror en route to the tube lens). The further from center of the sample the point is, the more pronounced the angle and the more likely some of those rays won't reach the tube lens. This leads to vignetting or darkening around the edge of the image. Also increased distance between the objective and the tube lens (more “collimated space”) leads to increased vignetting. The larger the back aperture of the objective the more this is a concern.

The formula for vignetting-free distance, assuming everything is perfectly aligned, is:

$$\begin{equation} L_{coll} = (⌀_{TL} - ⌀_{BFP}) \times F_{TL} / ⌀_{sensor} \end{equation}$$

Where:

L_{coll} is the distance between the back focal plane and limiting aperture when vignetting just starts to happen

$ø_{TL}$ is the clear aperture of the tube lens (or whatever other element will clip the rays) (30-32 mm for most of ASI's tube lenses)

$ø_{BFP}$ is the diameter of the objective back focal plane, given by $2 \times NA_{obj} \times F_{obj}$.

F_{TL} is the tube lens effective focal length

$ø_{sensor}$ is the diameter of the image sensor (18.8 mm diagonal for standard sCMOS full-frame)

Rearranging this equation you can come to the following two equations in terms of the diameter of the vignette-free field of view at the sensor ($ø_{sensor,max}$) and sample ($ø_{sample,max}$):

\begin{equation} ø_{sensor,max} = (ø_{TL} - ø_{BFP}) \times F_{TL} / L_{coll} \end{equation}

\begin{equation} ø_{sample,max} = (ø_{TL} - ø_{BFP}) \times F_{obj} / L_{coll} \end{equation}

Collimated space starts at the back focal plane which falls inside the objectives, generally deeper inside for higher magnification. Olympus now specifies the location of the back focal plane and other manufacturers may provide the location if asked nicely, or you can measure it. [2)]

4f Spacing

The name “4f” suggests that lenses are placed so their focal planes are coincident. When two successive lenses are so arranged, the outer focal planes not only preserve position information but also angles as well (subject to the overall magnification). This can be important sometimes, e.g. on the illumination path of a light sheet microscope the galvo tilt is converted into a pure translation by placing the galvo at the focal plane of the scan lens. To keep that pure translation at the sample plane, the next lens and the objective lens need to form a 4f relay; if not then at the sample plane the galvo tilt will result in both translation as well as rotation of the input beam.

4f spacing is not needed on imaging path of most infinity microscopes because camera isn't sensitive to what angle the incoming rays arrive.

tech note, mim

1)

source: https://www.zeiss.com/microscopy/us/solutions/reference/basic-microscopy/microscope-objectives.html#correction

2)

To measure the position of the back focal plane of an objective: place an ideal lens outside the objective and adjust its position along the optical axis until collimated light into the pair of lenses in either direction results in collimated light coming out. The external lens is now focused at the back focal plane, so remove the objective lens and see where the external lens is focused.