How to choose a microscope for immunofluorescene

Amit K. Dinda MD, PhD
Renal Pathology Laboratory,
Department of Pathology
All India Institute of Medical Sciences
New Delhi

Immunofluorescence is an essential component of diagnostic renal pathology. Buying a microscope for a diagnostic set up is an important issue considering the diverse range of microscopes available from various manufacturers. Cost effective investment is also a major concern specially in a private set up. This brief article is aimed to address the question of "How to choose a microscope for immunofluorescence?".

Any good basic model of a bionocular microscope may be selected with a facility to attach a digital capture device. Even if the digital capture device is not needed for routine immunofluorescence study of renal biopsies, it is a good idea to archive the images of immune-complex deposit in a particular case for review at a later date as well as comparison with the light microscopic and other clinical or laboratory findings.

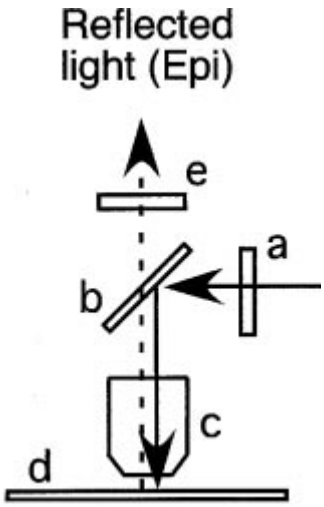

Fig.1. Schematic diagram of optical pathway of typical epifluoresceobnce microscope. (a – excitation filter, b – dichromatic mirror, c – objective lens, d – specimen, e – emission filter)

Choosing an objective lens according to the requirement and budget is a critical factor for the price of the microscope. One should know about different types of objectives available for light microscopy before choosing one as mentioned in the following table:

Table 1. The types of objective lenses and their significance for use

Type	Significance for use
Plan	Lens is corrected to allow the whole field to be in focus
Achroplan	Best for transmitted light
Epiplan	Designed for reflected light use
Achromat	Have good color correction for two wavelengths of light
Planachromats	Achromats with correction for flatness of field
Plan-Neofluar or Plan-Fluotar	Semiapochromatic lenses, have good color correction for at least three wavelengths and also have the all around flatness of field, excellent for polarization microscopic techniques, excellent lenses for all types of fluorescence microscopy.
Fluar	Any lens with the term "fluar" has fluorite elements in it and all of these are very good for fluorescence work
Apochromatic	The most highly color corrected objectives: they are corrected for four wavelengths and are top of the line in objective lenses.

The apochromatic or planapochromatic lenses are the top of the line lenses most often with highest numerical apertures. One has to be careful in choosing these lenses for fluorescence as they do not transmit UV light. They work very well for visible light excitation in the blue and green ranges. So for best use with a lower budget Plan-fluotar group of semiapochromatic lenses can be selected.

The numerical aperture of the objective lens is also important for the brightness of the fluorescence image. After the imprint of the magnification on any quality objective lens, there is usually a slash followed by a number which may be anything from 0.035 to 1.4. This number is the numerical aperture (N.A.) of the lens. This number is directly related to the resolution and for those doing fluorescence microscopy, it is related to the amount of brightness of the specimen. The higher the N.A. of a lens the better its resolving power and the brighter the image it can produce.

Now a days, for all standard fluorescence microscopes filter blocks or cubes are available which consists of emission or barrier and excitation filters with dichromatic (or dichroic) mirror. The filter block may be chosen according to the fluorochrome to be used. For usual diagnostic immunofluprscence of kidney and skin biopsy FITC is used. So a diagnostic set up can be started with single filter block for FITC. However for research purpose one should go for at least three filter blocks covering UV range and red range fluorochrome. For buying the filter blocks selecting high quality of the filters and dichroic mirror is very important. Earlier the fluorescence filters were usually constructed from dyed glass or gelatin sandwiched between two glass plates. Now a days high-resolution filters are made with interference optics for excitation filters to pass or reject wavelengths of light with a great specificity and high transmission. Dichromatic beamsplitters are specialized interference filters designed to reflect or pass light of specific wavelengths when placed into the light path at a 45-degree angle. Barrier filters are fabricated with both colored glass or interference coatings (or a combination of the two).

Abbreviations employed by manufacturers to identify the properties of their excitation filters as follows:

1. UG = ultraviolet glass
2. BG = blue glass
3. SP/KP = Shortpass filters (K is an abbreviation for kurz, which means "short" in German)

Abbreviation for barrier or emission filters are as follows:

1. LP or L = longpass filters
2. Y or GG = yellow or gelb (German) glass
3. R or RG = red glass
4. OG or O = orange glass
5. K = kante, a German term for edge
6. BA = barrier filter

When the filter type is designated with a number, such as BA515, that designation refers to the wavelength (in nanometers) at 50-percent of its maximum transmission.

Numerous interchangeable abbreviations are used for describing the dichromatic beamsplitter such as CBS for a chromatic beam splitter, DM for dichroic mirror, TK for "teiler kante", German for edge splitter, FT for "farb teiler" (German for color splitter), and RKP for reflection short pass. The filter combinations are produced by “hard coat sputter” or “soft coating” techniques. The filter composite produced by soft coat techniques exhibit higher blocking value optical densities and provides greater ease of fine-tuning specific wavelength bands. The soft coats are more susceptible to humidity and heat degradation, and must be handled more carefully than hard coat

As a light source the most common lamps used are mercury burners, ranging in wattage from 50 to 200 Watts, and the xenon burners that range from 75 to 150 Watts. The microscope arc-discharge lamp external power supply is usually equipped with a timer to track the number of hours the burner has been in operation. Arc lamps lose efficiency and are more likely to shatter if used beyond their rated lifetime (200-300 hours). Recently LED based long life light sources are introduced in Indian market that produces good intensity of light for several thousand hours with least maintenance. These light sources do not produce much heat, so the protective lamp housing is not needed and the current consumption is also low. It can also work on battery backup.

A routine diagnostic setup does not need the digital capture device (camera) with the fluorescence camera. However for achieving of images a capture device should be installed with the microscope. There is a wide range of photomicrography systems available in the market. The one with more than 10 megapixel sensitivity and user-friendly software with ease of capturing should be chosen than those with more complicated control systems. The image size should not be very big so that it can be handled, achieved and transported easily.

The innovations in science and technology of imaging are replacing the old systems with more efficient user-friendly devices which are cheaper and cost effective. Before buying a fluorescence microscope the latest developments in the market should be studied with review of the new products and new companies.