Two Basic Methods for Photomicrography

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This paper is intended as a basic explanation of two methods used to place the image formed by a compound microscope onto the sensor or film of a camera. It is primarily directed at those wishing to do photomicrography with cameras they might already own and use for general photography. It does not deal at all with dedicated microscope cameras. A more detailed look at the necessary sensor specifications, along with a useful spreadsheet can be found here.

Preliminary considerations

First, a couple of concepts need to be discussed. A distinction needs to be made between a **real** image and a **virtual** image. A **real** image is one that can be placed directly upon a piece of film, or a camera sensor. For many, it is easiest to envision a photographic slide or movie projector. If we place a screen at the appropriate distance in front of the lens of the projector, the image that previously existed in "space" is now placed upon the screen surface and can be seen. We are dealing with a real image. In the microscope, a real image of the subject is formed by the objective. If you could hold a piece of ground-glass, or a piece or semitransparent paper at the proper location you would be able to see the image formed on its surface.

A **virtual** image is one that cannot be projected. It requires a lens (like the lens in your eye) to form, and then project, a real image onto the light sensing material… your retina, film, or a camera sensor. The place we encounter a virtual image in a microscope is directly above the viewing eyepiece(s). The eyepiece creates a virtual image made of parallel rays and formed at infinity. The addition lens of the eye, or a camera, is needed to produce a real image from this virtual image.

Techniques for photomicrography will differ depending on the camera used, and whether the image "intercepted" for photography is a real or virtual image.

Another important consideration is the basic type of optical system used in the light microscope. Two types of optical systems are typically encountered -- **finite** tube length and **infinity-corrected.** Almost all microscopes currently produced by the major manufacturers today utilize what is commonly referred to as **infinity-corrected** optics. Many previous models used **finite** optics. If you don't know what you have, take a look at the markings on the objectives. If you see something like 160/0.17 you have a finite optical system, with a mechanical tube length of 160mm (160mm, 170mm and 210mm were common tube lengths). If you see the infinity symbol (∞), something like ∞/0.17, then your microscope uses an infinity-corrected optical system.

The compound microscope objective forms a circular image of the subject -- a real image -- that is located in "space" just below the eyepieces. (Generally it is 10mm below the edge of the eyepiece tube). This is called the intermediate image. This intermediate image occurs at this location with both finite and infinity-corrected systems. There is however one very crucial difference. With infinity optical systems there is an additional lens inside the microscope body between the objective and the intermediate image. It is most commonly known as the **tube lens**. With finite optical systems there is no additional lens between the objective and the intermediate image. Microscopes that were made using a finite optical system were, almost universally, designed so that some final important optical corrections were to be made with the viewing or photographic eyepieces. The most common aberration corrected by eyepieces was CDM (chromatic difference in magnification). In some cases the eyepieces also corrected for spherical aberration and image field curvature. (A notable exception would be Nikon CF series, introduced in 1976 which were of finite 160mm tube length, but were "fully" chromatically corrected in the objective. However the eyepieces did provide some field flattening in this series). Unfortunately, there was no standardization between manufacturers as to the exact amount and type of correction to be accomplished by the eyepiece. So the "safest" approach is to use the eyepieces and photo-eyepieces that each manufacturer designed to be used with their objectives. This means that in a microscope with finite optics we have a real "intermediate" image available, but in most cases we cannot think of it as "finished", or ready to be recorded until it has passed through an appropriately correcting ("compensating") eyepiece.

In microscopes that use an infinity-corrected optical system, these final corrections, if needed, are made by the internal tube lens. Thus, the intermediate image can be considered "finished" for all practical purposes. No addition correction need be made to improve the image quality.

On microscopes with trinocular heads this same intermediate image can also be directed so that it exists in the trinocular tube.

The diameter of the useful part of the intermediate image circle is around 20mm. In newer microscope optical systems it can have a diameter of 25mm or more, with some older objectives it may be 18mm or less. Most 10X wide-field viewing eyepieces have a field number (FN) of around 20mm, so for our purposes here we will use that number as the amount of the intermediate image with which we intend to work. (The field number of a viewing eyepiece designates the diameter of the part of the intermediate image that it "sees").

Now we need to consider the camera that will be used. We can break them down into two groups. The first would be cameras with a non-removable, attached lens. The second would be cameras that have no lens attached, allowing direct access to the film or sensor. A single lens reflex camera (SLR) can be used either with, or without an attached lens.

Since cameras in the first group must be used with their attached lens, you will need to work with a virtual image from the microscope, such as the image formed above a regular viewing eyepiece. Cameras in the second group will require a real image from the microscope, an image that will be "projected" and placed directly onto the film/sensor. In this second group it is possible to encounter a huge variety of film/sensor sizes that need to be accommodated. Some small "C- mount" lens-less digital cameras have sensors as small as 3x4mm, while film was commonly used up to 4x5inch in dimension. These days the 35mm format will be, by far, the most common film size, with frame dimensions of 24x36mm. Fixed-lens cameras will also have different sized sensors/film, but as will be seen below, the "normal" focal lengths of the attached lens will vary with format, and do most of the work when it comes to proper image sizing.

Now let's consider the proper "fitting" of the intermediate image onto the sensor /film. Let's look at three different camera sensors. A popular CCD sensor in fixed-lens digital cameras has dimensions of 7.2x5.3mm, with a diagonal of about 9mm. (This is also a fairly common sensor size for scientific and surveillance type "C-mount" cameras that are sold with no lens attached). A common sensor size for digital SLRs is about 23.7x15.5mm, with a diagonal of 28mm. A "full frame" 35 mm film camera or DSLR has a sensor size of 36x24mm with a diagonal of 43mm. If our desire is to record as much as possible of the view seen through a pair of 10X viewing eyepieces, we will need to enlarge or reduce the size of the 20mm diameter intermediate image to make it "fit" onto sensors with diagonals of 9mm, 28mm, and 43mm. If we do not do this, we will record only a small section of the field that is seen when using the smallest sensor; have modest vignetting with the medium sensor camera; and experience rather severe vignetting with the largest sensor.

If, with a 9mm diameter sensor, we want to record the entire 20mm field seen through the eyepieces, the intermediate image will need to be reduced by a factor of 9/20, or .45x. The DSLR with a sensor diameter of 28mm requires the intermediate image to be enlarged by a factor of 28/20, or 1.4X. The "full-frame" sensor, with a 43mmm diameter will require a magnification of the intermediate image by 43/20, or 2.15X. In each case the same 20mm field of view will be recorded, but there is a significant difference in the amount of reduction or enlargement needed to accomplish this with the different sized camera sensors.

Fixed-lens Cameras

Let's address the method used for a fixed-lens camera first. This is often referred to as the "afocal" method. As discussed above, a regular "viewing" eyepiece will be used in the microscope in order to obtain the "virtual" image needed for this type of camera. That eyepiece should ideally be one that the manufacturer designed to be used with the objectives on your microscope. **This would obviously be particularly important with older, finite optical systems that required compensating eyepieces to completely correct the image.**

Using your eye (not the camera) the subject is brought into focus as seen through that eyepiece. The camera, with the lens focus set manually to "infinity" is held directly over the eyepiece, and a picture is taken. The eyepiece should be a "high-eyepoint" type (often marked with an eyeglasses symbol). The camera aperture should be set to the largest opening (lowest f-number). If the settings are available on the camera, this is best accomplished by using aperture priority auto-exposure, or full manual settings.

One major problem often encountered is vignetting of the view in the camera image. This can be severe… resulting in a small circular image surrounded by black; or it can be modest… just a slight darkening of the corners of the image. The only way to know if this will be a problem is to give it a try. Sometimes vignetting can be minimized by zooming to a longer focal length. Carefully move the camera back and forth while you observe for any changes in vignetting, and be sure you have tried it as close as possible to the eyepiece. If possible, use an eyepiece with a higher eyepoint, and/or a larger field number. The best eyepieces for this use will have the longest possible eye relief. This is the distance from the surface it the eyepiece to the exit pupil, or "eyepoint"". At this location is a circular area, the "Ramsden disk", through which all light rays from the eyepiece will pass. In a typical high eyepoint eyepiece this distance may be around 18 to 20mm. Ideally, the camera will be positioned so that the Ramsden disk will fall inside the camera lens and coincide with the entrance pupil of the lens. This works quite nicely with many cameras that have small lenses, but many of the fixed-lens cameras being made today have lenses that are too large to position properly to avoid vignetting. If you have a fixed-lens camera and the vignetting problem is too severe, you might want to consider special camera adapters that are manufactured to "couple" these cameras to a microscope. Some of these adapters are, in essence, custom made extra large "eyepieces" with longer eyepoints, and sometimes larger Ramsden disks. A few places to look for these are:

Diagnostic Instruments Inc	http://www.diaginc.com
Martin Microscopes	http://www.martinmicroscope.com
Perspective Image LLC	http://www.perspectiveimage.com/index.php
Zarf Enterprises	http://zarfenterprises.com/index.html

Scoptronix http://www.scopetronix.com/mvp.htm
Microscope World http://www.microscopeworld.com/MSWorld/camera_chart.aspx

While some of these may be able to solve a vignetting problem they can be relatively expensive, and will not provide the any optical compensation that microscope manufacturers may have built into their eyepieces.

It should be mentioned that the compensation provided by "compensating" or "corrective" eyepieces is an important issue. But realistically some people are not too troubled by the aberrations that occur when no eyepiece compensation is used. It is more apparent (and problematic) with certain objectives than with others. There are software solutions that can help remove some of the chromatic aberrations that might occur in digital images. So while you should be aware of the need for compensating eyepieces with many microscopes that have finite optical systems, and it is best to use the optics as the designers intended, you may be able to get results that you find satisfactory without them. On the other hand, if you spend $400 on an adapter that does not provide the proprietary correction your objectives need, you may be dissatisfied with the results.

While this technique is most commonly used with cameras where the lens is permanently attached, it is also possible to use this method with a SLR camera. In that case the focal length of the lens used on the camera should be typically of "normal" to slightly "long" for the format of the camera. As discussed above, vignetting may be an issue. If your objectives provide poor results without the compensation provided by the manufacturer's corrective eyepieces, and no suitable projection eyepieces can be found, then this may be the best approach.

It is not absolutely necessary to read and understand this next section to be successful with the fixed-lens camera approach… but it is worthwhile to understand the following points. The mathematical relationships below should not be considered "exact", but they do provide very good guidance when contemplating the equipment to use.

When viewing through the microscope we use the product of the objective magnification and the eyepiece magnification to arrive at the viewing magnification. So if you have a 40X objective and 10X eyepieces, you are viewing the subject at 40*10 = 400X. But if you were to hold a camera with a lens up to the eyepiece and take a photograph you would not be recording the image on the sensor at a magnification of 400X. It would be recorded on sensor at a considerably lower magnification. If this were not the case you would be recording just a very tiny section from the middle of the field of view.

In order to determine the magnification that will be recorded on the sensor, you can consider the microscope eyepiece and the camera lens as a single magnification "unit". The magnification this "unit" provides can then be multiplied by the objectives magnification to arrive at the actual recorded magnification on the sensor.

The magnification provided by the combination of eyepiece and camera lens ("M_{unit}") can be determined by the following relationship:

$$M_{unit} = (FL/250) * (\text{Eyepiece Magnification})$$

Where FL is the actual focal length, in millimeters, of the lens on the camera

Knowing the magnification provided by the combination of the camera focal length and eyepiece magnification (**M_{unit}**), the magnification recorded on the sensor is then determined by the product of this value and the objective magnification:

$M_{recorded}$ = (Objective Magnification)*(M_{unit})

It is interesting to note that when a focal length that is considered "normal" for the format (such as a 50mm lens on a 35mm full frame camera) is used with a 10X eyepiece, the amount of the intermediate image that is recorded is very close to the 20mm field of view observed through 10X eyepieces. Let's see this with the three formats we have considered.

With the 7.2x5.3mm sensor, the focal length of a "normal" lens would be about 11mm. So:
M_{unit} = (FL/250)* (Eyepiece Magnification)
M_{unit} = (11/250)* (10)
M_{unit} = 0.44

If a 40X objective were used, the magnification recorded on the sensor would be:
$M_{recorded}$ = (Objective Magnification)*(M_{unit})
$M_{recorded}$ = 40*0.44 = 17.6X

You can determine the amount of the intermediate image that will be recorded by dividing the sensor format diagonal by the magnification provided by the combination of eyepiece and camera lens:

FNOS = (format diagonal)/ M_{unit}

where FNOS refers to the "field number of sensor", the size of the portion of the intermediate image that is placed onto the sensor.

So for this small sensor:
FNOS= 9/.44 = 20.5
The field recorded on the sensor would be just over 20.5mm diagonally.

With the 23.7x15.5mm sensor the focal length of a "normal" lens would be about 35mm.
M_{unit} = (35/250)* (10)
M_{unit} = 1.4
If a 40X objective were used, the magnification recorded on the sensor would be:
40*1.4 = 56X
The field recorded on the sensor would be just over 20mm diagonally

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And with the 36x24mm sensor the normal lens focal length is about 50mm.
M_{unit} = (FL/250)* (Eyepiece Magnification)
M_{unit} = (50/250)* (10)
M_{unit} = 2
If a 40X objective were used, the magnification recorded on the sensor would be:
40*2 = 80X
The field recorded on the sensor would be just over 21mm diagonally.

10X eyepieces are the ones most commonly used for viewing. For photomicrography the majority of people find they want to record most of what they see through the eyepieces, but not necessarily right up to the very edges. When 35mm film was widely used for photomicrography, the most commonly used photo-eyepiece provided a magnification of 2.5X. This recorded a field of view (diagonally) of 17.3mm, and many found this a very comfortable arrangement. Personal tastes and needs vary, and some might prefer a tighter "crop" of the image seen through the viewing eyepieces. And naturally, if higher power viewing eyepieces with lower field numbers are used, adjustments should be made to the camera focal length and/or trinocular eyepiece so that an appropriate size field is recorded on the sensor.

Cameras with no attached lens

In this case we need a real image that will be projected onto the sensor. We know that there is a real image formed by the objective – the intermediate image. If it were possible to position the camera with the sensor at the exact location of the intermediate image we could successfully take pictures. While it is not always physically possible to position a camera at the correct location, this "direct projection" is actually a viable choice with some microscopes using infinity-corrected optical systems where the intermediate image can be accessed. It is sometimes possible to do this with a microscope using finite optics as well, but in this case the intermediate image may still be in need of some optical corrections that would be provided by the proper eyepiece. In either case, the size of the sensor placed thusly will determine the field number recorded. Ideally, it would be great to have a sensor with a format diagonal of about 17mm placed to receive a fully corrected intermediate image. Most of the time however, our camera sensor will be either considerably smaller or larger (and/or the intermediate image may need final correction). In these cases we need to employ optics that will magnify or reduce the intermediate image so that we get a good "fit" onto the sensor, recording the desired amount of the observed image

The amount of reduction or magnification needed will vary with the sensor size, and the amount of the field of view that we desire to record. Let's consider the same three sensor sizes used above. For this example we will also use 10X viewing eyepieces with a field number of 20mm to view the subject. Furthermore, we will say that we wish to record a 17mm diagonal section of the intermediate image with the sensor (As above, we will refer to this as the FNOS, "field number of sensor"). It then becomes straightforward to calculate the magnification that is required by the optics we will employ.

$M_{projected}$ = (format diagonal)/FNOS

For 7.2x5.3mm sensor (diagonal 9mm)
$M_{projected}$ = 9/17 = .53x

For 23.7x15.5mm sensor (28mm diagonal)
$M_{projected}$ =28/17 = 1.65x

For 24x36mm sensor (43mm diagonal)
$M_{projected}$ =43/17 = 2.5x

Re-arranging the same relationship we can see what happens if we use inappropriate photo-eyepieces for the camera format in use.

FNOS = (format diagonal)/M*projected*

So if we were to use an Olympus NFK 3.3x photo-eyepiece with a Nikon D200 DSLR we would be recording a section of the intermediate image as follows:

FNOS = 28/3.3 = 8.5mm

This is probably too "tight", and too large a crop of the viewed image for most people's tastes.

If the fairly common NFK 2.5X photo-eyepiece were to be used with a camera with the small sensor, things would be far worse:

FN = 9/2.5 = 3.6mm

We would record only the central 18% of what we observe through the eyepieces!

But the 2.5X used on a DSLR with a format diagonal of 28mm, we would get the following:

FN = 28/2.5 = 11.2mm

This is a little more than ½ of the observed field. This may be too much of a crop for some, but many could live with this arrangement. Some might actually prefer it.

A 1.67X used on a DSLR with a format diagonal of 28mm gives the following:

FN = 28/1.67 = 16.8mm

This is a nearly ideal fit for many people. You would record about a 17mm width of a 20mm field.

So the desirability of having an appropriate sized image projected into the camera body is obvious.

The ideal photo-eyepiece to use for this purpose is one specifically designed for this purpose. This is a much simpler quest for users of currently manufactured microscopes using infinity-corrected optics. Being in current production, and addressing the need to accommodate the cameras currently used, these optics are readily available from the microscope manufacturer. And remember, with infinity-corrected optics there is no need for additional proprietary correction of the intermediate image. So it is possible for other manufacturers (such as Diagnostic Instruments) to manufacture optics that can be used on different brands of microscopes. They need only be of the appropriate magnification and have high quality optics.

Users of older microscopes that incorporate finite optics may have a tougher job finding the appropriate projective eyepiece. When finite optics was the norm, images were typically recorded on film or Polaroid material. 4x5" was a common format, and 35mm, with frame dimensions of 24x36mm, was the small format. As a result, the commonly seen photo-eyepieces had magnifications ranging from 2.5X - 10X to magnify the intermediate image produced by the objective onto the desired film format. There was certainly no need to accommodate sensor sizes of 5x7mm and even smaller. The most commonly found projective photo-eyepieces for microscopes with finite optics are those made by Olympus and Nikon.

The Nikon CF PL ("**P**rojection **L**ens") series included a 2X, 2.5X, 4X and 5X, and were made to be used with their CF series objectives. They are of high quality, and provide no additional chromatic aberration correction. Note that these are different from the CF PLI series, which are used with the newer infinity-corrected systems. (See the bottom of this link).

https://www.nikon-instruments.jp/eng/page/products/list12.aspx

Olympus manufactured a FK series, and then a NFK series. (See this reference).

http://www.alanwood.net/photography/olympus/photo-eyepieces.html

These were “corrective”. The NFK series was designed to be used with the Olympus “LB” objectives and included a 1.67X, 2.5X, 3.3X, 5X, and 6.7X. Noteworthy was the availability of the NFK 1.67X photo-eyepiece. As it happens, this is just about the perfect magnification for the most common sensors used in DSLRs. Unfortunately, this is an item that can be difficult to locate, and fetches a price 2 or 3 times that of the more common NFK 2.5X. While the corrections in the NFKs are made to match up with Olympus objectives, they are of excellent quality, and will often provide very good results with other manufacturers objectives that normally need some eyepiece compensation.

Closing notes:

The procedure I use to make my microscopes parfocal with the camera can be found here.

The NFK eyepieces were designed to be used with a distance of 125mm from the shoulder of the eyepiece to the plane of the sensor. As an eyepiece is “pulled” further out from the trinocular tube, the camera will need to be lowered (moved closer to the eyepiece) and the projected magnification decreases. I have often thought that since the NFK 1.67X eyepiece is so difficult to track down, it might be possible to use the more common 2.5X with the eyepiece slightly “raised” to provide a magnification close to the 1.67X. Some would find this magnification preferable. I will probably test this and compare results to those obtained with the 1.67X. It may turn out to be a viable option to users of DSLRs wanting to record nearly all of the field of view. I’ll update this piece to reflect my results when this is finished.