Mating Cameras To Microscopes

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I. Introduction

The compound microscope forms images in two stages. The objective (first stage) projects a real image of the specimen into the intermediate image plane (IIP) of the microscope at a ratio of magnification (*M*). The resolution in the IIP is typically between 40 and 100 line pairs per millimeter (lp/mm). Since the resolution of the human eye is of the order of 5 lp/mm, additional virtual magnification (*V*) by the eyepiece (second stage) is necessary to match the resolution of the eye to that of the microscope. The total microscope magnification (V_{TOTAL}) is the product of *M* x *V*. The designation V_{TOTAL} is used because the eye views a virtual image in the microscope.

The diameter, in millimeters, of that part of the intermediate image that is utilized by the eyepiece is called the field of view number (FOV). Depending on the numerical aperture (nA) and magnification of the objective and the FOV number of the eyepiece, microscope images may contain up to five million image elements, referred to as pixels. Video cameras can resolve only a fraction of this available information due to the inherent resolution limits of the camera detectors. Thus, when mating video cameras to microscopes, choices need to be made whether to sacrifice some of the field of view in the interest of matched resolution or vice versa. This article discusses how best to mate video cameras to microscopes at magnifications which preserve specimen image resolution when necessary. This paper will also consider what needs to be done to maintain the optical corrections of objective and eyepiece in the video image.

The microscope's resolution (*r*) depends on the numerical aperture (nA) of the objective. It is commonly calculated at:

$$r = \frac{1.22 \times \text{lambda}}{nA_{OBJ} + nA_{COND}}$$

where *r* is the minimum spacing of specimen detail, lambda is the wavelength of the illuminating light, and nA_{OBJ} and nA_{COND} are the numerical apertures of objective and condenser, respectively. In the case of green light (lambda 0.55 µm) and a condenser numerical aperture equal to that of the objective one can state the resolution simply as:

$$r(\mu m) = \frac{1}{3 \times nA}$$

or, converted to line pairs per millimeter (lp/mm):

$$r(\text{lp/mm}) = nA \times 3000.$$

Resolution usually refers to distances in the object plane. In the context of this article, the term circle of confusion (Z) Is sometimes used to signify resolution in planes other than the object plane: for example, the intermediate image plane, where a point source appears as a confused circle of radius $Z = M \times r$.

Magnification is a means of diluting the density of the image information to suit the limited resolution of the sensing structure of the receiver. The human eye, for example, typically has a resolution of 0.2 mm at a viewing distance of 25 cm, the so-called conventional viewing distance (CVD). At a magnification V_{TOTAL} = nA X 600, $Z = V_{TOTAL} \times r$ and becomes 0.2 mm: thus, the instrument and the eye are matched in regards to resolution. A somewhat broader range of magnifications from V_{TOTAL} = nA x 500 to V_{TOTAL} = nA x 1000 are considered reasonable if the eye is to appreciate all the spatial information an objective produces. This is called the range of useful magnifications. At V_{TOTAL} < nA x 500 there is information overload and the eye cannot detect the finest specimen detail resolved by the microscope. At V_{TOTAL} > nA x 1000 no information is revealed that was not already apparent at lower magnifications; we have entered the zone of empty magnification $Z = r \times V_{TOTAL}$ (see Table I), where *r(µm)* = 1/3 nA, lp/mm = nA x 3000 and *w* = the angle subtended by two image points spaced by the distance Z (tan *w* = Z/CVD)

$$\text{Total Pixels} = \frac{\text{lp/mm} \times \text{FOV no.}}{(2 \times M\,\text{IIP})} \text{ ^2 x pi}$$

In mating video cameras to microscopes the resolution limits of a video system need to be taken into account just as those of the human eye. In fact, we have to concern ourselves with both. First we have to determine at what magnification, expressed in multiples of the aperture, we need to project the image on to the faceplate or the video camera in order to resolve specimen detail on the monitor screen. Next we need to consider the maximum permissible viewing distance from the screen at which our eye can still fully appreciate all the displayed information.

Table I

Range of Useful Microscope Magnifications for the Human Eye

Z (µm) at CVD Of 25	Z (lp/mm)	Angle	Product (nA x V_{TOTAL})	Pixels in millions at FOV	
100	10-0	1'23"	300	4.01	▯
112	8.9	1'32"	336	3.91	Zone of
125	8.0	1'43"	375	3.14	lost
140	7.1	1'56"	420	2.50	information
160	6.3	2'12"'	480	1.92	▯
175	5.7	2'24"	525	1.60	▯
200	5.0	2'45"	600	1.23	Range of useful
220	4.5	3'02"	660	1.01	magnifications
250	4.0	3'26"	750	0.79	(nA x 500 to
280	3.6	3'51"	840	0.63	nA x 1000)
320	3.1	4'24'	960	0.48	▯
350	2.9	4'49"	1050	0.40	▯
400	2.5	5'30"	1200	0.31	Zone of empty
440	2.3	6'30"	1320	0.25	rnagnification
					▯

The answer to the first part of the question can be worked out reliably in an empirical way: A test object close to the resolution limit of the lens is chosen, such as the diatom *Pleurosigrna angulatum* in the case of an objective of 40 x magnification with an nA of 0.65 (40/0.65). This diatom shows a periodic structure of dots spaced by 0.65 µrn in hexagonal arrangement. Our objective will just comfortably resolve this structure. If the dots are clearly resolved *on* the screen, the video system takes full advantage of the resolution available from the microscope.

What one will find, typically, for an ordinary video camera of 2/3-inch tube diameter is a minimum magnification at the faceplate of the camera of 130:1 which translates, more generally, into nA x, 200 to be necessary in order to match the resolution of the camera to the microscope. Thus, between the intemediate image plane and the faceplate of the video camera additional magnification of 3.25/1 is needed in the case of the 40/0.65 objective. How much of the fleid of view will be captured under these conditions? Division of the faceplate diagonal (11 mm) by the magnification occurring between the IIP and the camera faceplate (325:1) yields 3.4 mm. Of the 25 mm field of view, customary in today's microscopes, 98% of the area had to be sacrificed to fully match the resolving power or the objective lens. This is the worst-case scenario but even with the best video technology we would be lucky to keep the lost area to 80%.

To reconcile the demand for imaging large fields for surveying purposes at one time and small fields to preserve resolution at other times the use of a pancratic (zoom) type eyepiece is helpful. A zoom range or 1:6 will comfortably cope with both extremes and for many samples lesser zoom ranges may be perfectly adequate. The magnification changers built into many microscopes serve the same purpose. It is worth remembering that the light intensity changes inversely to the square or the magnification and sometimes higher intensity may have to be given priority over resolution as, for example, in fluorescence applications.

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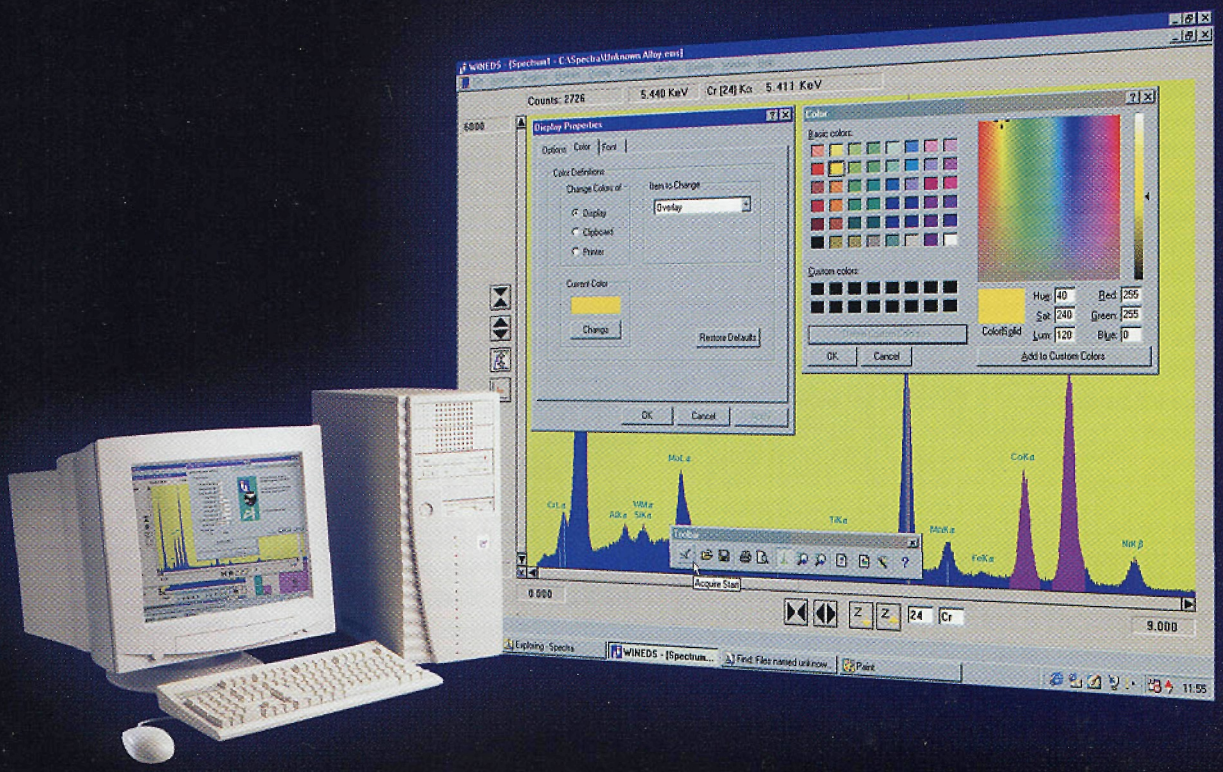

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Now to the second part of the question. A structure clearly resolved on the screen must be viewed from the proper distance. If we move too close to the screen the TV lines become annoyingly apparent. That tends to happen at viewing distances of less than five screen diagonals. If we move too far away the eye's resolution may become the limiting factor. To address this concern in a rational way we return to the earlier statement that the range of useful magnifications lies somewhere between the extremes of nA x 500 and nA x 1000. This rule holds for the TV screen as well. How do we calculate the magnification? The magnification of real images such as the intermediate image in the microscope or the image on the TV screen can be measured with a ruler or a scale if the dimensions of the object are known. This we call the ratio of magnification. To distinguish it from virtual magnification we say "ten to one" and write 10:1. In this article the letter *M* is used to signify the ratio of magnification.

Virtual images. on the other hand. are formed, for example, by magnifying glasses (simple microscopes). The image exists only in the plane of our retina and is thus inaccessible to measurement. We define the magnification of such images by relating the retinal image size to what it would have been if the object were viewed from a distance of 25 cm without any optical aids. This reference distance of 25 cm is, by agreement, the closest accommodation distance of the average human eye and called the conventional viewing distance (CVD). From a distance of 25 cm we see things at 1x magnification. A magnifying glass of *f* = 12.5 cm permits the infinity-accommodated eye to cut the conventional viewing distance effectively by halt. Therefore the magnification. being reciprocal, is called "two times" and written as 2x.

The ratio of magnification on the monitor screen is the product of several factors:

M (objective) x *M*(magnification changer) x [*M*(video adapter) or *V*(eyepiece)] *x V*(camera lens)(=*f* lens/25 cm) *x* Electronic magnification = *M* screen

"Electronic magnification" is the ratio of the screen diagonal over the camera faceplate diagonal. For a 15 inch screen and a 2/3 inch TV tube the electronic magnification is 35:1.

In the case of an objective 40/0.65, an eyepiece 10X, a camera lens 0.32X, and electronic magnification of 35:1 we have a total screen magnification of *M* = 4480:1. If viewed from a distances of 25 cm we aiso have a magnification of V = *4480x.* From viewing distance of 50 and 100 cm the magnification shrinks to *2240*>< and 1120x, respectively, or generally;

$$V = \frac{M \text{ screen x } 25 \text{ cm}}{d} \text{ , or } d = \frac{M \text{ screen x } 25 \text{ cm}}{V}$$

where *d* is the distance in centimeters from the screen to the observer.

For *V* to be in the range of useful magnifications of 500 to 1000 times the objective nA. we ca:cuiate the minimum and maximum distances for an objective nA of 0.65:

$$d_{MIN} = \frac{M \text{ screen x } 25 \text{ cm}}{\text{nA x } 5000} = \frac{112000 \text{ cm}}{325} = 345 \text{ cm}$$

Thus. within a range of viewing distances from 345 to 690 cm we will be ablle to appreciate the full resolution of the optics. Had we chosen a lesser M_{SCREEN}, then d_{MIN} soon becomes less than five screen diagonals and some of the information literally falls through the cracks of the screen's line raster

II. Optical Considerations

Classification of microscope objectives into Achromats, Fluorites, and Apochromats refers to their color and spherical corrections. In a perfect objective the image does not change its axial location with wavelength. It may, however, change in size. A lateral chromatic difference of magnification (CDM) of 1% across the extremes of the visible spectrum is not unusual. Color fringes, increasingly apparent the larger the FOV number, result from this condition if left uncorrected. For many decades CDM was successfully eliminated by the use of compensating eyepieces. Some of these eyepieces may additionally contribute to improved flatness of field.

C-Mount adapters connect cameras with standardized C-mounts to the photo tube of the microscope. The simplest kind of C-mount adapters just place the camera target into the IIP and then have 1/X magnification. Theoretically, the image is marred by CDM yet in practice the shortcomings of the TV technology are even more severe and tend to mask the image defects, which are minor anyway across a typical FOV of 11 mm.

The shrinking size of camera chips requires proportionally reduced magnifications for the C-mount adapters. Magnifications of 0.63:1, 0.5:1, and so on demand appropriate internal optics with properties similar to the compensating eyepieces.

In recent years the chromatic aberration correction philosophy of most manufacturers has changed. Nikon and Olympus eliminate CDM entirely in the objective. Leica and Zeiss prefer to assign the correction of CDM to the tube lens which is a necessary part of all infinity-corrected microscope optical systems which are now ubiquitous. Either way the intermediate image is free of CDM; thus the optics of the C-mount adapters need to be chromatically neutral. To use the same adapter for various makes of microscopes continues to be elusive because of mechanical constraints as well as different approaches used to achieve flatness of field. It Is important therefore to use the proper adapter both in terms of vintage of the optical system and the manufacturer of the microscope

Table II

Matching Projection Magnification to Camera Faceplate Size

Class	Dimensions and diagonal of faceplate area (mm)	M projective*
1-inch TV tube	8.9 x 11.9 (d 14.9)	1.25 : 1
2/3-inch TV tube	6.6 x 8.8 (d 11)	1.00 : 1
1/2-inch TV tube	4.85 x 6.5 (d 8)	0.63 : 1
1/3-inch TV tube	3.6 x 4.8 (d 6)	0.50 : 1

* The magnification of projectives is stepped so that a similar FOV number of 11 to 12 is obtainable with all TV tubes.

A lens system designed to transfer the intermediate image onto the faceplate of a camera is sometimes called a projective. Standard C-mount adapters (with the exception of the lensless 1x adapter) are projectives. A different approach has bean taken traditionally in microscope cameras made for use with films. Here a standard eyepiece is used and the rays are brought into focus by an optically neutral camera lens. Such an arrangement is not without merit for video microscopy, either. It assures proper correction of CDM and other lens aberrations: it permits the change of the final magnification by changing the eyepiece power. Furthermore, eyepieces which contain a reticle for length measurements are usable. If the eyepiece is of the pancratic type its magnification is adjustable to the size of the camera faceplate even beyond the zoom range, by the proper choice of focal length for the camera lens.

It is perhaps worthwhile to add at this point that a system rather immune to vibration problems can be created if the video microscope is arranged in two units. One consists of the microscope including the photo eyepiece. The camera with its lens forms the second. No direct mechanical connection exists between the two and the camera is supported independently of the microscope, for example, by a copy stand. Any motion between the two, as long as it results only in a parallel displacement of the optical axis, will not affect the image at all. This strategy was indispensable in the era of microcinernatography and may serve a useful purpose even today when heavy video cameras are used, the weight of which, when directly borne by the microscope stand, make it much more susceptible to externally generated vibrations. ■

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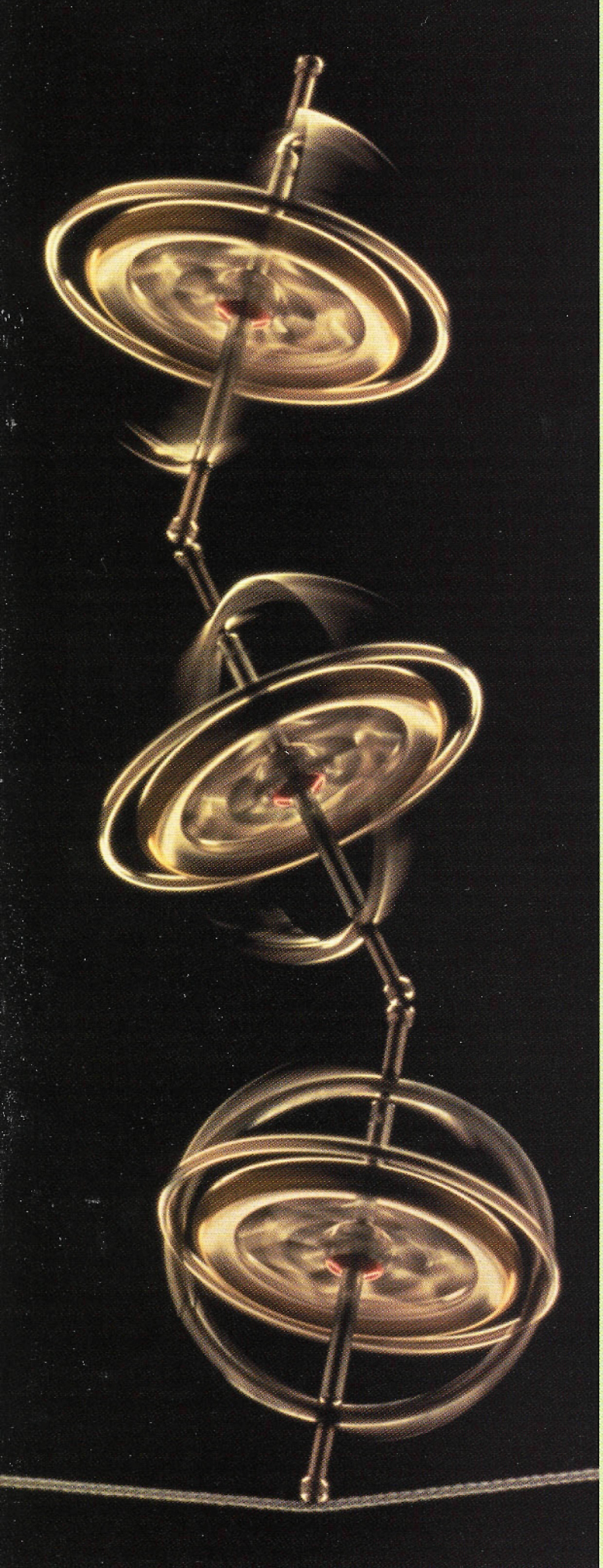

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