ARTICLE
Video Microscopy for Teaching: Optimizing the Field of View

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Whether teaching online or in person, video microscopy can demonstrate and document procedures such as dissection and electrophysiology. Such videos can streamline in-person lab work or make online material more interesting and lifelike. Microscope video can also be streamed live over Zoom or other services for live online demonstration. It can be difficult, however, to match a microscope and camera such that the field of view (FOV) captured by the camera encompasses the entire FOV seen by the microscope user. Standard recommendations usually give a camera FOV much smaller than the user's FOV. This paper explains how to work with three variables (camera sensor size, microscope coupler magnification, and ocular diameter) to achieve a good FOV match.

Key words: video, microscope, online

Video microscopy is a powerful teaching tool under any conditions (Wyttenbach, 2015). When in-person laboratory teaching is restricted and courses are moved online, video becomes even more valuable. My 2015 article discussed video planning, acquisition, and editing. This one discusses the equipment required to achieve a good match between the microscope view and the scene captured by video.

Ideally, a video would closely simulate the experience of looking through the microscope. This is especially true when the video demonstrates procedures that the viewer is expected to learn and do. Thus, our goal is to match the video field of view (FOV) as closely as possible to the microscope FOV.

THE GOAL
Optimizing the FOV involves an inherent tradeoff (Figure 1). The microscope FOV is circular, while camera sensors are rectangular. Capturing the entire circular FOV in a large rectangle wastes much of the view on blank space, while avoiding any blank space in the video requires capturing only a small central part of the microscope FOV. If video is to be edited and saved at lower than full resolution, a square crop may be optimal. If video is to be streamed live, the camera view cannot be cropped, and we must tolerate a small FOV or some blank space.

VARIABLES
The microscope FOV depends on the view diameter of the ocular and on magnification of the microscope body and objective. The camera FOV depends on the size of the camera's sensor, magnification of the camera coupler, and magnification of the microscope body and objective. Since the microscope body and objective are common to the two, they do not affect the relationship between microscope and camera FOV and are not considered further. These variables are the same whether using a phototube coupler or a trinocular head (Figure 2).

Ocular View Field
The relative FOV for the microscope user is determined solely by the view field of the ocular. This is usually printed on the ocular along with the magnification, such as 10×/22, 16×/14, or 20×/10. The second number is the view field diameter in mm. Perhaps counterintuitively, magnification is irrelevant to FOV, although it is a major determinant of the view field. To measure the view field of an unlabeled ocular, look at a ruler though the microscope and note the diameter of the view. Multiply that by the body (e.g., 2×) and objective magnification (e.g., 1×) to get the ocular view field. Figure 1A was taken with an iPhone through a 10×/23 ocular with objective and body magnifications of 0.75× and 1.33× respectively (total 1.0×), and shows a 23 mm diameter FOV, as expected.

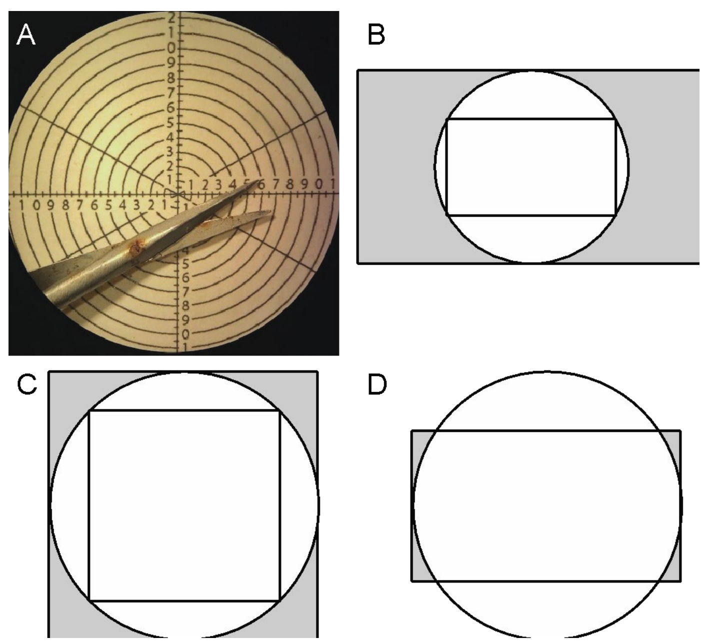

Figure 1. FOV tradeoffs. *A.* Photograph taken through the ocular, showing the circular FOV. *B.* Capturing the entire FOV (outer rectangle) vs. avoiding any blank space (inner rectangle); a 16:9 sensor aspect ratio is assumed. *C.* Cropping to a square can capture the entire field (outer square) or completely avoid blank space (inner square); an intermediate may be best. *D.* One possible compromise view for live streaming.

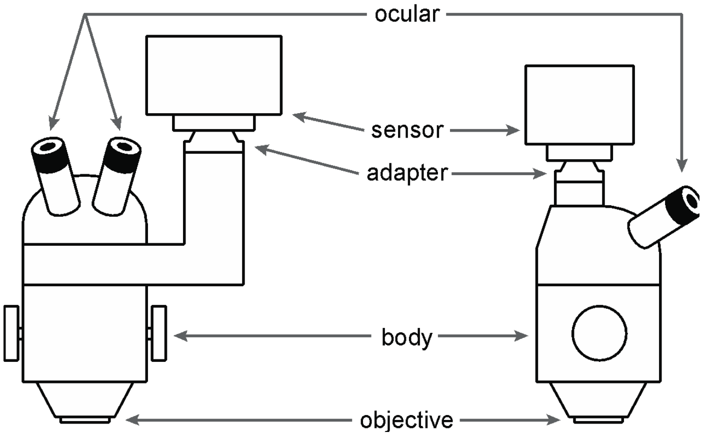

Figure 2. Variables affecting FOV. Ocular view diameter affects microscope FOV. Sensor size and coupler magnification affect camera FOV. Microscope body and objective magnification affect both views equally.

Coupler Magnification
Whether using a phototube (Figure 2, left) or a trinocular head (Figure 2, right), a coupler is required. This serves two functions. First, it physically connects the tube (which may have an inner diameter of 37, 38, or 40 mm) to the camera (which may use a C mount or manufacturer-specific bayonet mount). Secondly, it sets the correct distance to the sensor plane and focuses the image on the sensor. Depending on manufacturer, couplers are available in a limited set of magnifications, such as 0.33, 0.38, 0.50, 0.67, 0.75, 1.0, 1.2, 1.6, and 2.0×. In general, couplers in the 0.5-1.0× range are least expensive.

Sensor Size
Many ⅓" (diagonal) sensor cameras are marketed for microscopy. They can be fairly inexpensive. There are fewer ½", ⅔", and 1" microscope cameras and they are much more expensive (often optimized for low-light low-noise performance). Most consumer digital SLRs, however, are capable of video and can be fitted to microscopes; their sensors range from ⅔" to 1⅔" and they vary considerably in price. There are also small professional video cameras with sensors from ½" to 1", which generally cost more than comparable SLR options, although their video performance may be superior. The rest of this article focuses on ⅓" and digital SLR options, but the principles apply to all cameras.

The advertised sensor size of a camera may not be informative. Most sensors have an aspect ratio of 4:3 or 3:2 and use a smaller portion of the sensor to record videowith an aspect ratio of 16:9. Two cameras with the same sensor size may crop the sensor differently. The same camera may even crop differently for different video resolutions or frame rates. These measurements are reported in the user manual; most manuals can be found online before purchase. Table 1 shows some standard sensor sizes, as well as the areas used by some cameras in different circumstances. For example, the Nikon D810 and Z6 have full-frame (36×24 mm) sensors but crop differently for video. These cameras and many others allow one to select between FX (full-frame) and DX (crop mode). This flexibility is helpful when attempting to match FOVs.

Sensor or camera	Width	Height	Ratio
1/3”	4.8	3.6	4:3
1/2"	6.4	4.8	4:3
2/3"	8.8	6.6	4:3
1"	12.8	9.6	4:3
4/3" (micro four thirds)	17.3	13.0	4:3
APS-C (≈ full Nikon DX)	23.5	15.6	3:2
Full frame	36.0	24.0	3:2
Nikon D7500 (4K)	16.2	9.1	16:9
Nikon D7500 (HD 1.3)	18.0	10.0	16:9
Nikon D810 (cropped DX)	23.4	13.2	16:9
Nikon Z6 (cropped DX)	23.4	13.1	16:9
Nikon D810 (cropped FX)	32.8	18.4	16:9
Nikon Z6 (cropped FX)	35.9	20.1	16:9

Table 1. Sensor sizes. Width and height (mm) are for the sensor portion used for video. Ratio is the aspect ratio of this portion. The ⅓" sensors record video in a 4:3 aspect ratio, while others may crop to 16:9. Nikon measurements come from the user manuals. Others are standards found online; cameras may vary.

Phototube FOV
The phototube often transmits a larger image to the camera than is seen through even a wide-view ocular (Figure 3). This can be measured with a camera and low-magnification coupler (coupler magnification reduces the view) or may be visible by looking through the phototube by eye. It is relevant only in that areas not seen through the ocular may be picked up on camera. This may give a bit more latitude when cropping video.

OPTIMIZING FOV

Most microscope manufacturers sell camera couplers for their microscopes, as do many third-party dealers. All of them make essentially the same recommendation: for a camera sensor with a diagonal size of 1/N inches, use a coupler that magnifies by approximately 1/N. Thus, a ⅓"sensor requires a coupler with about 0.33× magnification (Best Scientific Couplers, 2015; Spot imaging, ND). This combination uses the optically best part of the microscope lenses, with the flattest field, and guarantees no vignetting (dark areas at the corners). It is apparently meant to optimize high-resolution photography of flat objects.

Video microscopy does not involve high resolution imaging of flat objects. Even 4K is far lower resolution than film or digital SLR. Teaching videos usually involve curved, rather than flat, objects, and vignetting at the corners is not objectionable. The 1/N recommendation imposes a cost: Camera FOV is much smaller than the microscope FOV (see Figure 4 for ⅓" and 0.33×). When working under the microscope, one must take care to keep the action in a small central part of the FOV, which can be difficult.

Video microscopy for teaching has different goals and will work best with a different match between sensor and coupler. Figure 4 suggests that we want a smaller coupler magnification than recommended. A 0.25× coupler would be ideal for a ⅓" sensor but does not exist.

The camera and microscope FOV are easily calculated from ocular view field, coupler magnification, sensor size, and microscope body and objective magnification:

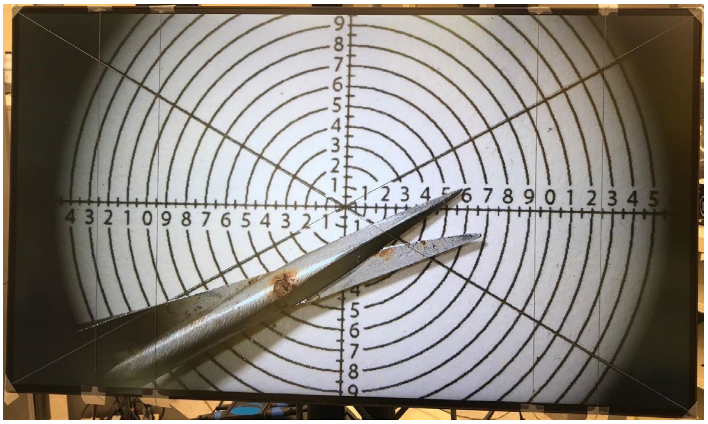

Figure 3. Phototube FOV. Photographed on a monitor connected to the camera via HDMI, this corresponds to the microscope ocular FOV in Figure 1A. The circular FOV is larger (28.5 mm diameter) than the ocular view field of 23 mm but is dim at the edges. Barely visible are threads taped to the monitor, showing square and 4:3 cropping.

$$microscope\ FOV\ (diameter) = \frac{ocular\ view\ field\ (diameter)}{body \times objective}$$

$$camera\ FOV\ (width\ or\ height) = \frac{sensor\ size\ (width\ or\ height)}{coupler \times body \times objective}$$

The Excel spreadsheet associated with this article uses these equations to graphically show the relative FOVs as these parameters vary. Use it to compare different sensor and coupler combinations (diagrams in Figures 4 and 5 come from the spreadsheet). This can be done to choose system components or can help configure the system if the camera has sensor crop options.

Editing vs. Streaming

If video is recorded to a computer and edited before use, there is a lot of flexibility. For the best use of space, a square crop can capture most of the microscope view with little vignetting (Figure 1C). One could even capture a much larger area at high resolution and then crop and re-center as needed. All current consumer-grade SLRs can record HD (1920×1080 pixels) and many can also record 4K (3840×2160). Either way, there is plenty of space to crop. For example, when producing dissection and electrophysiology video for online delivery recently, I recorded in HD, planning to crop to 1080×1080. That turned out to be larger than necessary or practical; I exported at 1080×1080 and 720x720 and ended up using the latter. This argues for a sensor/coupler combination that captures the entire circular microscope FOV, as in Figure 4 FX with 0.67 or 1.0× and APS-C with 0.67 or 0.50×.

Streaming services like Zoom do not allow one to crop the view. The image quality and resolution of streamed video are lower, but sufficient for basic demonstrations. If showing the entire microscope FOV is more important than high resolution, use a sensor/coupler combination such as that for FX and 1.0× (Figure 4). Alternatively, if detail in the central FOV is more important than a large field, choose a higher magnification relative to the sensor (close to the 1/N recommendation) and work to keep the important action in the center. This might be a view like that for APS-C with a

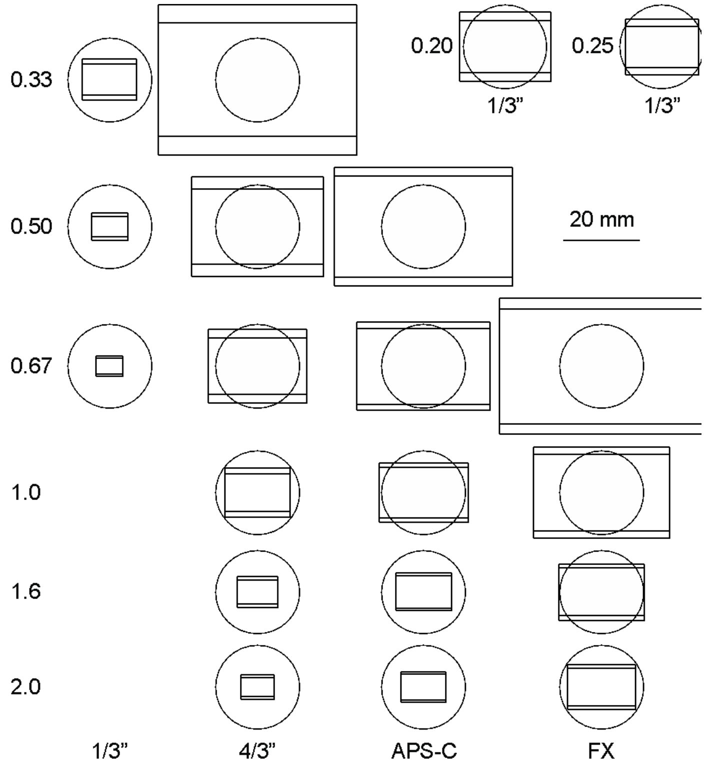

Figure 4. Calculated FOV for varied sensor/coupler combinations. Circles represent microscope FOV with a 22 mm ocular view field. In each case, the outer rectangle shows the area captured by the entire sensor, while the inner rectangle represents the largest 16:9 area that could be used for video. A ⅓" camera uses the entire sensor for video. FX cameras use a 16:9 crop for video. APS-C and 4/3" may capture full-sensor video or may crop. Nikon DX captures the same area as APS-C. Upper right shows FOV for a ⅓" sensor and 0.20 or 0.25× coupler (which do not exist).

1.6× coupler.

Ideally, a camera will offer different sensor crops for video. For example, a camera that switches between full frame and APS-C (FX and DX, for Nikon) can be optimized for either editing or streaming with a 1.0× coupler.

BUILDING A SYSTEM

Most of us are constrained by budget and already owning a microscope (the most expensive single piece). In that case, look for a phototube or trinocular that fits that model. Many phototubes and trinocular heads close one eyepiece and divert that image to the camera. This is not suitable for dissection, which requires stereoscopic vision. Be sure to find a phototube or trinocular head with a beam splitter. Unfortunately, these can be considerably more expensive. For any given microscope, there will probably be only one or two suitable phototubes. Phototube inner diameter may be 37, 38, or 40 mm, and the coupler must match. For any phototube, there may be only 3 or 4 available coupler magnifications but check third-party sellers on eBay as well as the microscope manufacturer. Use the provided spreadsheet to see the FOV provided by different combinations of camera and coupler. Coupler cost varies with magnification, so note the total cost (a more expensive camera with a cheaper coupler may give the lowest total).

Couplers generally adapt to C-mount, which is compatible with ⅓" cameras directly, but adapters from C-mount to bayonet mount are inexpensive and readily available on Amazon for all major camera brands.

If the microscope, phototube, and coupler are already present, sensor size is the only variable that can be adjusted. Look for cameras that can record HD video and can stream it via "clean HDMI" (the image straight from the sensor, without adding viewfinder information). Next, find the user manuals to determine exactly what portion of the sensor is used when recording video. Ideally, find cameras that allow selection of different sensor crops for video. Put all of these variables into the spreadsheet FOV calculator and select the best camera for your needs.

If the entire system is present, for example microscope with phototube, ⅓" camera, and 0.33× or 0.5× coupler, the FOV may not be favorable for video (Figure 4). However, there is a workaround. Rather than increasing the camera FOV to match the ocular view field, reduce the ocular view field with higher-magnification oculars (Figure 5). This can give a good match at low cost (used oculars are often sold on eBay). However, greater ocular magnification reduces the total FOV at low magnification. To restore the wide FOV, compensate with a magnification-reducing objective. This will increase the working distance, so the microscope stand may need to be reconfigured.

Finally, the camera and ocular views must be parfocal. To achieve this, focus the microscope body to give the sharpest view on an external monitor. Then adjust the oculars to focus the image for the microscope user.

USAGE

Most SLR cameras can save up to 30 minutes of H.264 compressed video onto a data card, which can be read into a computer via an inexpensive card reader for editing. While recording, they can simultaneously stream video to an external monitor via HDMI (a micro- or mini-HDMI to full-size HDMI cable is required). An external monitor is useful for checking the image while recording and can also be used to demonstrate to a class. Saving uncompressed video from the HDMI stream is also possible but beyond the scope of this article.

For streaming via Zoom or other service, HDMI output of the camera must be converted to USB and read directly on a computer. There are many options (search for "HDMI capture"). I have tried inexpensive (~$25) and expensive (~$140) ones and both work. However, the cheaper ones get hot after a few minutes of use, which may be a problem in the long run. In Zoom, select "USB Video" as the camera and turn off "mirror my video".

HDMI to USB video capture can also be used to record directly onto the computer. Apple computers can record via the QuickTime Player app, which allows one to select USB video as source. Comparable options are available for Windows.

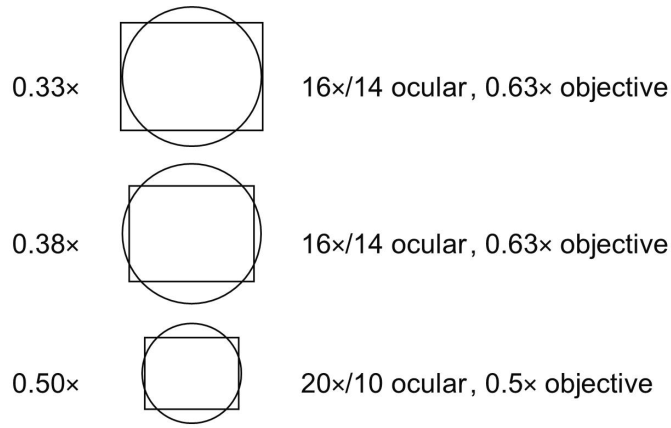

Figure 5. Compensating with smaller ocular view field. Circles and rectangles represent microscope and ⅓" camera views with the indicated coupler magnification (left) and ocular (right). Note that low-magnification objectives increase the working distance (double for 0.5x, 1.6x for 0.63x).

CONCLUSIONS

Modern digital cameras, combined with appropriate optical couplers, offer flexible options for video recording and live streaming. Matching the camera's field of view to that of the microscope is a challenge that can be surmounted with a few simple calculations.

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