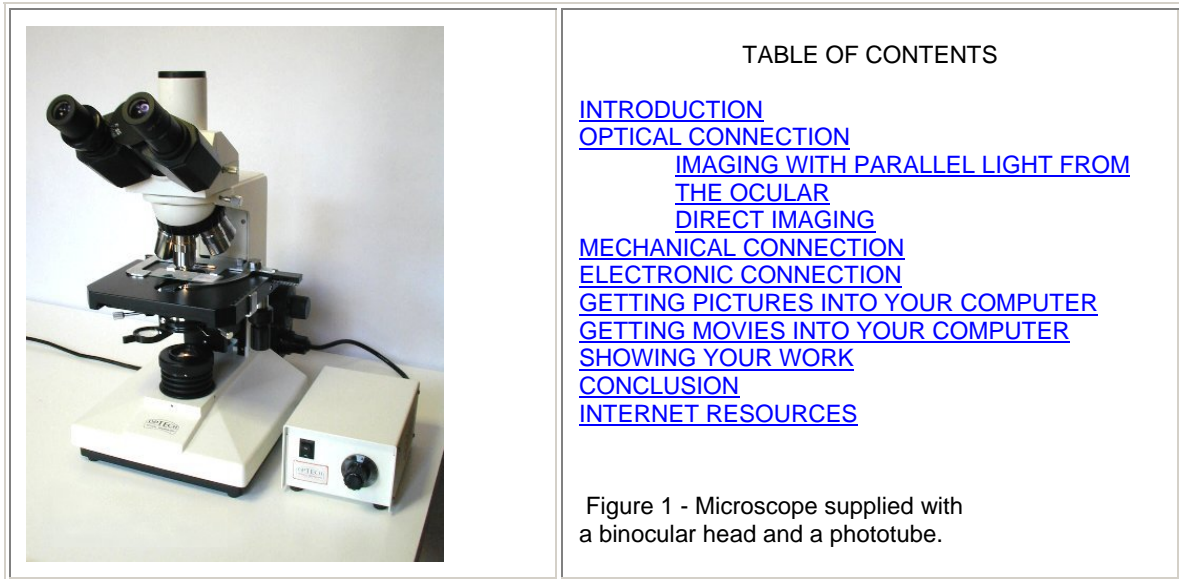


# PICTURES AND MOVIES WITH THE MICROSCOPE

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## INTRODUCTION

For many years photomicrographs were taken on glass photographic plates and large films, using cumbersome cameras. Those days are now a distant memory. Today, even the modern small film cameras are being replaced, as the techniques of digital photography are becoming more widespread every day. These instruments have made the imaging of microscopic objects much simpler and cheaper, and they have opened new horizons for editing and transmitting images and movies.

Those who have tried to make pictures through a microscope have probably already realized that it is not sufficient merely to have a microscope and a camera to obtain good images; it is also necessary to ensure proper optical, mechanical and electronic connections among the different devices involved in the process. In this article, we will provide some basic information on making still pictures and movies with the microscope, and especially the use of the digital imaging techniques that are now available to the professional and amateur alike. In addition, some of the information presented here is valid for photography through telescopes. In addition, some of the information here is valid for imaging through telescopes.

## OPTICAL CONNECTION

There are three main methods for taking still pictures or video through microscopes (figure 2). Normally, the light coming out of the eyepiece is in the form of parallel rays; however, by adjusting the focus, it is possible to make these rays convergent. The first method to obtain images uses this principle to form a real image of the object at a certain distance from the exit pupil of the ocular. By manipulating the focus, you can vary this distance and, if it is 250 mm, the image produced will have the nominal magnification you would expect from the objective and ocular you are using.

For example, if we use a 40 X objective and a 10 X eyepiece, at that distance we will obtain an image of the object magnified 400 times (400X). If, instead, we focus the image at half this distance -- that is, at 125 mm -- the image produced will be one-half of the nominal magnification. It is easy to understand that the images produced with this system are large, and suited to cameras using glass photographic slides or sheet films. Special adapters, however, can be used to reduce the size of the image and to fit it to the 24x36 mm frame of 35mm roll films. We will call this system "*imaging through the eyepiece in convergent light*" or "*projection from the ocular*". This system is not well suited to digital cameras, so we will not deal with it here.

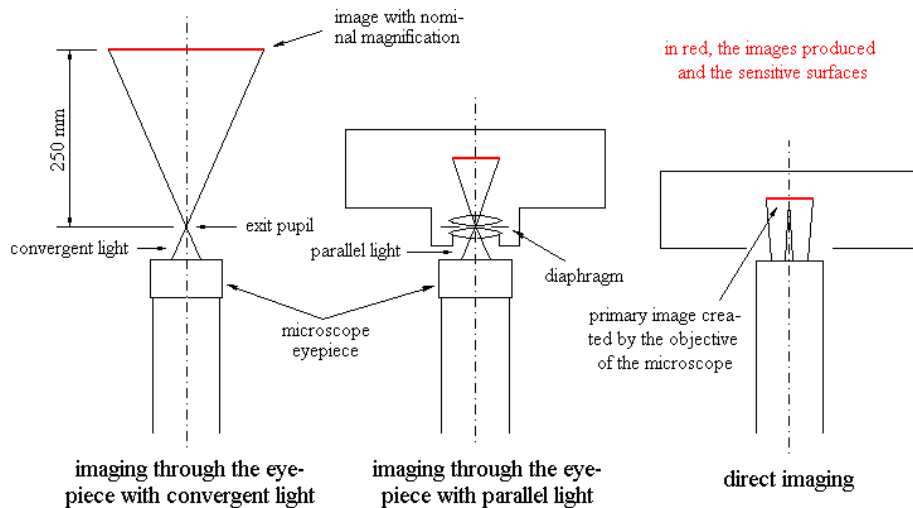


Figure 2 - The main methods for making still pictures and video with a microscope.

With the second method, the camera is placed very near the ocular of the microscope. This system is based on the fact that the imaging-forming light rays that come from the eyepiece is parallel and can be directly sent to an imaging apparatus that is designed to work in parallel light -- that is, a camera that is designed for ordinary photography of more or less distant objects. This system is usable with either common film or digital cameras, but it demands some "tricks," as we will soon see. We call this system "*imaging through the eyepiece with parallel light*", but it is also called the "*afocal method*."

The third method consists of projecting the primary image produced by the objective directly onto the sensitive surface of the photographic apparatus. To do this, it is necessary to remove both the ocular of the microscope and the lens of the camera. This system requires techniques that will be described later on. We call this system "*direct imaging*".

### IMAGING WITH PARALLEL LIGHT FROM THE OCULAR (OR AFOCAL METHOD)

In the normal visual use of the microscope, the light rays coming from the eyepiece are parallel and, in the same way that the eye is able to use this kind of light to focus the image on its retina, the camera with its normal lens is able to focus the image supplied by the microscope onto its sensitive surface.

By placing our camera very near the ocular of the microscope, we can shoot what it is showing. Even though it presents some difficulties, this method can work with photographic cameras. Unfortunately, it will seldom work with camcorders, unless the camcorder allows you to keep its diaphragm fully opened.

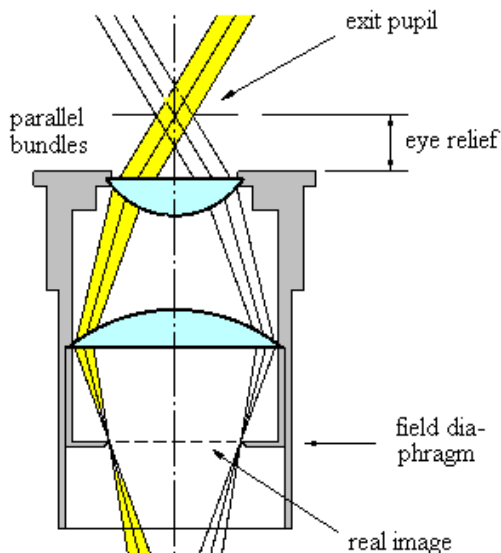


Figure 3 - Formation of the exit pupil in an eyepiece.

### The exit pupil of the eyepiece.

One of the first things you need to know when you are taking pictures through the microscope with this system is the position of the "exit pupil" of the eyepiece being used. The exit pupil is a small circular area through which all the light rays which form the image pass. As you see in figure 3, the exit pupil is located at the vertex of a cone of light that comes out from the eyepiece. If the diaphragm of the camera is not placed in the plane of the exit pupil, but instead, intercepts the cone, you will have a vignetting of the image. That is, the corners of the image will be dark.

Often, the camera is prevented from attaining the right position by the front surface of the camera lens, which touches the ocular before reaching the proper position. If the exit pupil is too close or too far away from the eyepiece, the vignetting that we mentioned before is certain to occur. If things work well, only the corners of the picture will be black; if things work less well your image will be reduced to a little disk. The ideal condition is when the circular field of the image is just outside the corners of the field of the camera. To obtain this condition, you can take advantage of the zoom lens provided on almost all digital cameras.

### Formation of the exit pupil.

Figure 3 illustrates the path of the marginal rays in an eyepiece. The objective of a microscope forms the real (or primary) image on the plane of focus of the eyepiece, which usually is located at its field diaphragm. As you can see in figure 3, the light coming from a point of this image passes through the lenses of the ocular and emerges from it as a narrow bundle of parallel light rays. This diagram shows the paths of the rays coming from two marginal points of the image, but the same is true for all other points. So, the light bundles that come from all image-points pass through the same circular area, which is also called the "Ramsden circle". This is the **exit pupil** of the eyepiece, or the **eyepoint**. Therefore, the whole of these parallel bundles forms a cone of light that passes through the pupil. As we have seen, the vertex of this cone is not a point, but a small disk.

### How to determine the eyepoint.

Switch the lamp of the microscope on and, with a frosted glass or a strip of translucent paper held horizontally, intercept the cone of light that comes from the ocular. Vary the distance of the frosted glass from the eyepiece; when the bright disk is at its minimum size, you will have located the exit pupil. The distance of the exit pupil from the eyepiece is the so-called **eye relief** of the eyepiece. The higher this value is, the less is the danger of vignetting. The eye relief should be at least 10 mm; in any case it has to be sufficient for the camera you want to use. Eyepieces that allow the viewer to wear glasses during observations through the microscope have a high eye relief, and they are marked by the symbol of a pair of glasses. Other eyepieces are specially designed for use in photography; they have a high eye relief and usually they also are marked with a pair of glasses (figure 5). Measure the eye relief of the eyepieces you have, as described above.

Although they are rare nowadays, please note that some photographic eyepieces designed for use with the old large cameras, such as the "Homals" of Carl Zeiss and the "Ampliplans" of Bausch & Lomb, are "negative" eyepieces and cannot be used for this purpose, although they gave beautiful results with the old large camera set-ups.

### Choosing the eyepiece.

In many fairly old microscopes, some corrections of the primary image are left to the eyepiece, which is designed for that purpose. For this reason, it is very possible that a photographic eyepiece, even though of

high quality, but not specially designed for your microscope, may interfere with the sharpness of the image. This disadvantage is more evident near the border of the field, where you will see chromatic aberrations, spherical aberrations and lack of flatness of the field -- that is, either the center or the edges of the field will be in focus, but not both at once. To avoid these problems, you must use an eyepiece suitable for your microscope. Before buying a new eyepiece, check the suitability of the eyepiece on the microscope itself. For our purposes, an eyepiece of 15 X, which is often among those supplied with the microscope and which is rarely used for visual purposes, could be very useful. In fact, sometimes these eyepieces have an eye relief greater than that of 10 X eyepieces. If one of these eyepieces allows you to avoid vignetting with the camera you want to use, you can obtain very good pictures or movies. If you want to buy an eyepiece which has not been designed for your microscope, you have to be very careful: try to compare the quality of the image you obtain with this eyepiece with that which you obtain with the eyepieces supplied with your microscope, paying close attention to the border of the field.

#### **Adjusting the camera.**

Before mounting the camera on the microscope, turn its automatic focusing off and adjust the camera lens to focus at infinity. To avoid vignetting caused by a small diaphragm setting (high f/ number) set the camera to work in diaphragm priority mode and open the diaphragm to the maximum (smallest f/ number). After adjusting the microscope, perform a "white balance" of the camera to assure proper color rendition.

#### **Adjusting the microscope.**

After you have located the object you wish to image and after you have selected the objective, you must set the illumination of the microscope to the best conditions for normal observation. Using the Köhler system of illumination, look for the best conditions of observation by adjusting the height of the condenser, the aperture diaphragm, the intensity of the illumination, etc.

Mount the camera and view the image on your computer monitor or a TV set. With the camera zoom, adjust the size of the image and then adapt the illumination of the microscope to the camera. Limit your adjustments to a few refinements. Avoid closing the substage diaphragm too much or varying the height of the condenser. If it is necessary to reduce the intensity of the light, use the voltage adjustment knob on the lamp's power supply. If you have placed the camera in the right position, you will need only a slight adjustment of the microscope's fine focus knob.

Often, cameras using a CCD sensor have an excessive sensitivity to visible light and to the infrared (IR). By lowering the intensity of the illumination, the color of the light becomes yellowish, so it is important to place a blue filter (i.e., Wratten 80A) on the exit port of the illuminator to raise the color temperature of the image. If the sensitive element of the camera is not well shielded from the IR, it is likely that you will not be able to accurately focus the image. To reduce this problem, it is useful to add an anti-IR filter like the IR-Cut Dielectric filter by the Baader firm, the Schott BG38 filter or the Hoya C-500S. You can also try adding a heat-absorbing glass like those used in slide projectors.

#### **Magnification on the sensitive surface.**

The magnification you obtain on the sensitive surface ( $M_{ss}$ ) is given by the following formula:  $M_{ss} = F_{op} * M_{ri} / F_{ey}$ , where:

$F_{op}$  is the focal length of the camera lens;

$M_{ri}$  is the magnification of the real image (also called the primary or intermediate image). It corresponds to the magnifying power of the objective of the microscope;

$F_{ey}$  is the focal length of the eyepiece, which you can calculate with this simple formula:  $F_{ey} = 250/M_{ey}$ , where  $M_{ey}$  is the power of the eyepiece.

If, for example, you use a camera with a normal lens of 50 mm focal length, a microscope with a 10 X objective and a 10 X eyepiece, the magnification on the sensitive surface will be equal to 20 X. Keep in mind that in these calculations all lengths have to be expressed in mm.

## **DIRECT IMAGING**

Another method of photography through the microscope is to let the primary image produced by the objective of the microscope fall directly on the sensitive surface of the camera. To do this, it is necessary to remove the lens of the camera and the eyepiece of the microscope (figure 2). Then, the camera must be placed on the tube of the microscope so that the sensitive surface is as close as possible to the plane where the primary image is normally formed in the microscope. This also allows you to maintain the same focus of the microscope when the eyepieces are used, which is especially convenient if photography will be alternated with direct visual observations.

### The relay lens.

In general, when still or video cameras with CCD sensors are used, a special optical adapter called a *relay lens* is placed between the microscope and the camera. The function of this device is to take the primary image of the microscope and make it available to the camera in a suitable way. In particular, these optical devices reduce the size of the image to best cover the field of the CCD sensors, which usually are small. Some relay lenses have the capability of continually varying the size of the image, thus working as a zoom. This is useful to better frame a microorganism and to fully exploit the sharpness of the objectives of the microscope. These optical adapters can also function as mechanical adapters. The Edmund company deals in these items, and its on-line catalog is useful to learn their features: <http://www.edmundoptics.com/>. Look for the term: "relay lens". Many other firms also supply this item.

The use of still or video cameras connected to a microscope with an optical/mechanical adapter that improves the direct imaging is the system usually utilized by professionals in microscopy to obtain pictures and movies. The cameras designed especially for microscopes have a lens that can be removed, or they lack a lens altogether and they also lack a recording apparatus. With direct imaging, you can obtain very good results, provided the video camera is designed for the scope used. Unfortunately, this type of apparatus is quite costly.

Today, you can purchase digital reflex cameras with removable lenses; several relatively inexpensive video cameras also allow you to remove their lens systems. Among them, there are many webcams, surveillance, and industrial video cameras. The "traditional" webcams supply 320x240 pixel movies and an analog signal. They are very inexpensive and they can be used to shoot movies through the microscope with fairly good results. Some of the webcams of the new generation are able to provide color video with 640x480 pixel resolution at 30 frames per second. To send this flow of data to a computer, these devices need to use a USB2 or Firewire connection. Usually, the normal lens supplied can be easily removed. Many amateur astronomers are already using these webcams with good results.

To seriously work in the field of imaging through the microscope, it is necessary to buy still or video cameras designed especially for microscopy and provided with a suitable relay lens. As we said, these are quite costly. The purchase of cheaper apparatus has to be done with caution, because it can turn out to be a pure waste of money. Anyway, improved digital cameras for still and video imaging, with promising features, are becoming readily available.

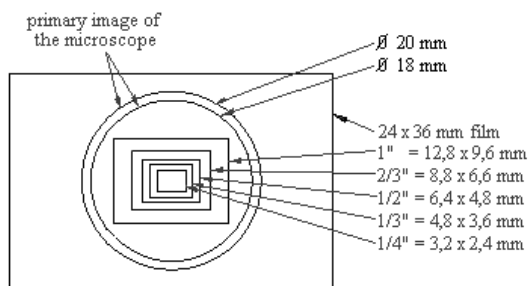


Figure 4 - Field of the primary image and size of the sensitive surfaces of the imaging devices.

The primary image yielded by a microscope has a useful diameter of about 18 - 20 mm (figure 4). With film cameras, this image will fully fill the frame, and it will have a circular shape. With digital cameras, which use small sized CCDs, a larger or smaller portion of the field will be recorded. As a consequence, you will have enlarged, and not very sharp, images. To avoid this disadvantage, it is necessary to use cameras with a sensor of 1/2" at least, or to use a relay lens.

There is no standard for the size of sensors. But, for reasons described in an article cited later on, the longer side of a sensor is about a half as long as the value of its diagonal (figure 4). To obtain further information about the sizes of the sensors, go to: <http://www.dpreview.com/news/0210/02100402sensorsizes.asp>

In order to perform direct imaging, it is better to use microscope objectives which provide a fully-corrected primary image, and which therefore do not need a compensating eyepiece. This detail has to be remembered when buying a microscope or an objective to be used for photography. The main problem which arises in using direct imaging is due to the high brightness of the image, which is not suited to the high sensitivity of the CCD to light, and in particular to the IR component. Usually, the sensors are provided with an anti-IR filter of their own, but it is usually not efficient enough to solve this problem. So, during the imaging you have to use another anti-IR filter. When you are buying a digital camera to be used without its lens, check to make sure the CCD is large enough, and that it is not too sensitive to visible light (>10 lux) and to the IR.

### Magnification on the sensitive surface.

For direct imaging, the magnification of the image on the sensitive surface should correspond to the magnification provided by the objective of the microscope. However, even if it is projected at close to the nominal distance, this image can be slightly larger than the value engraved on the objective. In fact, many eyepieces, including the very common Huygens, reduce the size of the primary image by means of their field lens. In addition, the relay lenses modify the size of this image. So, how can we determine the effective magnification in direct imaging?

For this purpose, you can use a special calibration slide, a "stage micrometer." It is a microscope slide on which is engraved a scale one millimeter long and divided into 100 parts. By placing this slide under the objective of the microscope, you will obtain a magnified image of the scale and, by means of a ruler, it will be easy for you to precisely determine its magnification not only on the sensitive surface, but also on prints, monitors and TV screens. This method is useful for all imaging systems and allows you to avoid the errors that are always possible with purely computational procedures.

### MECHANICAL CONNECTION

As we said, the camera has to be placed at a small distance from the eyepiece of the microscope or, for direct imaging, on the tube of the microscope. In trinocular microscopes, the camera has to be placed on the third tube, called the phototube.

To keep the camera in position it is necessary to use a **mechanical adapter**. It is often possible to buy adapters made specifically for your camera, which can be mounted on the microscope. There are also eyepieces provided with a thread (figure 5) that allows them to be directly mounted on some digital cameras (for example, the 28x0.75 thread is suited to the Nikon Coolpix). Adapters can be made on a lathe, but figure 6 shows an universal adapter that you can make with simple tools, avoiding machine work. For direct imaging, we suggest that you buy a relay lens, which will also work as a mechanical adapter.

According to the model of the camera, viewing and focusing are done by using a:

- small lateral telescope on the adapter (film camera, non-reflex);
- viewfinder (film reflex camera);
- LCD display (digital camera);
- TV screen or a monitor of a computer (digital camera).



Figure 5 - Eyepieces for photography. Notice the thread with which they are provided

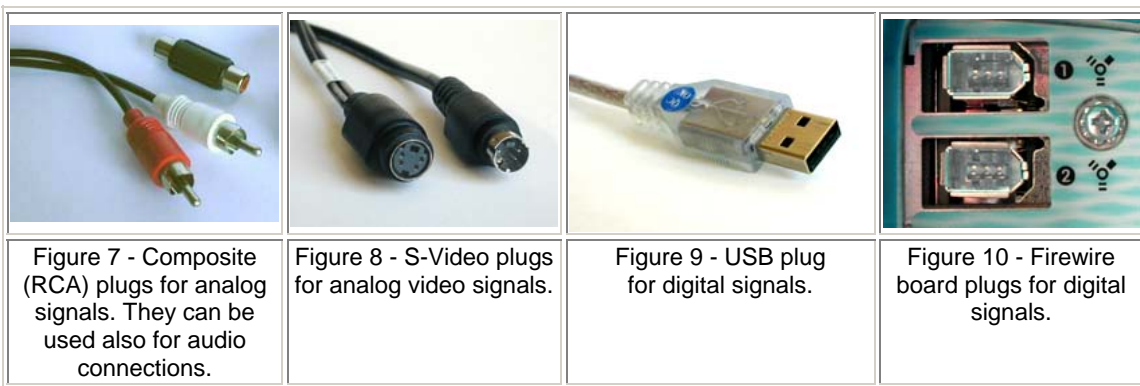


Figure 6 - Adapter you can make without using machine tools.

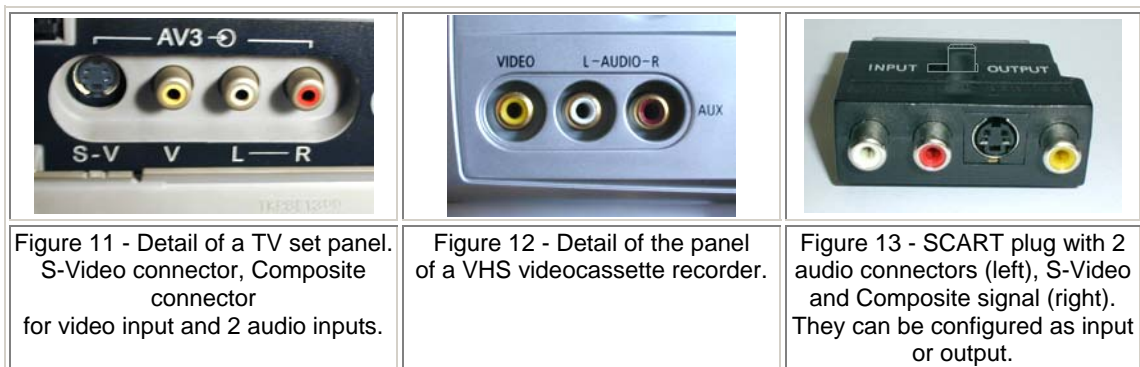
## ELECTRONIC CONNECTION

According to the type of signal produced, imaging equipment can be divided into **analog** and **digital** systems. Among the analog equipment, we have film cameras and conventional motion-picture cameras, also called cine-cameras; video cameras with analog recording systems; videocassette recorders; analog webcams and television sets. Digital equipment includes digital cameras, digital camcorders, DVD players and recorders, digital video recorders, digital webcams, computers and the relatively new digital television sets. At the moment, the recently available digital equipment is rapidly replacing analog technology. You can find many digital camera models on the market, and the first models of digital reflex cameras are becoming available. Within this group, there are many models of digital video cameras. DVD video recorders are already available, and while digital television sets are newcomers with respect to the other digital equipment, they are becoming fairly common. At this time, the two technologies are co-existing; therefore, digital equipment is often provided with analog inputs and outputs, so as to be compatible with analog systems.

Film cameras cannot, of course, be directly connected to videocassette recorders or TV sets. On the other hand, the electronic equipment is able to provide an analog or a digital signal, and they can be easily connected to TV sets, videocassette recorders and computers (figure 14).



Most electronic imaging devices available today can communicate with each other through analog electronic signals according to the Composite and the S-Video system standards. But ultimately, digital technology is destined to replace them. With the Composite signal, you can obtain a vertical resolution of 250 - 300 lines, typical of the VHS and Video8 systems. By using the S-Video signal, you can obtain better picture quality, attaining the 400 - 430 line resolution typical of the SVHS and Hi8 recording systems. In the Composite system, a single wire carries the luminance and chrominance signals, while in the S-Video (Separated Video) system, these signals run on distinct wires, assuring higher image quality. To be able to operate correctly, it is necessary that the signal, the cables, the connectors and the apparatus are appropriate and compatible. Figures 7 to 13 show the commonly used connectors for video and audio interconnection among different electronic devices. When you buy videocassette recorders or TV sets, determine that the proper connectors are present.



Usually, **digital cameras** are supplied with special cables or readers with which it is possible to download pictures recorded in a memory card to the hard disk of a computer. Not everyone knows that often, digital cameras also have an analog video output, so they can be used as video cameras. Unfortunately, in this use, they do not maintain their high resolution, as they usually work according to the Composite system.

Normally, to take motion pictures through the microscope, **video cameras** are used. Analog video cameras can supply a Composite or S-Video signal. The more modern video cameras record movies in digital format, and they provide a digital signal that, in most cases, follows the DV (**Digital Video**) format. This system provides 500 lines vertical resolution and produces sharper images with more faithful colors than the usual analog systems. Moreover, in making copies, the quality of digital movies does not deteriorate. Usually the DV format works with a resolution of 640x480 pixels. The **Mini-DV** format is derived from the DV, and it is different only in the size of the cassettes, which are smaller. Other digital formats, of higher quality, more costly and usually used in the professional field, are the **DVCPRO** and the **DVCAM**, which also utilize the DV format. Sony has created the **Digital Betacam** format, called also **Digi-Beta**, and it is suitable for those seeking high quality and who have no budget problems. The presence of an analog output slot in the digital video cameras allows the user to connect them to analog TV sets and videocassette recorders, while an analog input slot permits these video cameras to obtain an analog signal from TV sets and videocassette recorders and to digitize it.

To obtain the signal from an analog video camera, a computer must be provided with a **video capture card** with Composite and S-Video input connectors, which performs the analog/digital conversion of the signal. The card has to be able to capture the flow of data that is sent to it and, if possible, to also perform MPEG compression in real time. If the board has an insufficient data-processing capability, you will have "noise" in the video you are capturing, and it will be necessary to lower the definition of the frames. These cards can also receive the analog signal from TV sets and videocassette recorders. In most cases they also have an analog video output connector. Normally, the video capture cards are not able to process the audio signal. This function is left to an audio card, and this card can be used to add narration and music to the video.

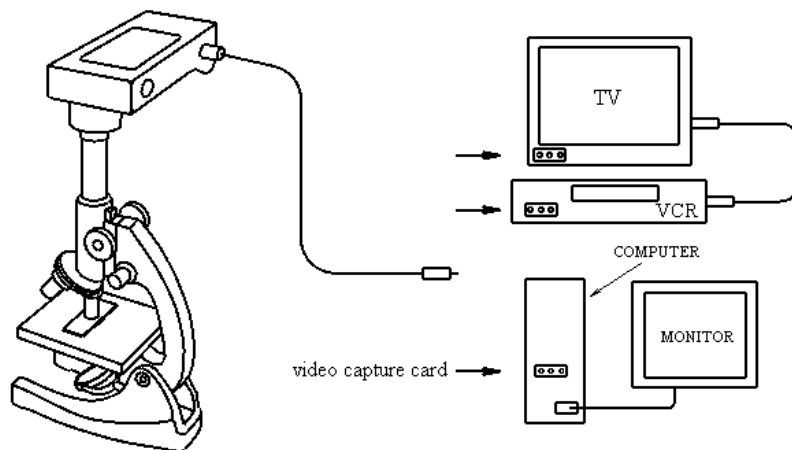


Figure 14 - Scheme of the connections among the imaging apparatus and TV set, videocassette recorder and computer.

Some **television sets** are provided with Composite and S-Video connectors (figure 11). Usually, these connectors only allow input of the signal. To extract the video signal, you can use a special SCART plug whose connectors can be configured as audio-video inputs or outputs (figure 13). The microscope/video camera/TV connection allows you to show many people at the same time what is happening under the microscope.

**Videocassette recorders** can also be supplied with Composite and S-Video connectors (figure 12), which usually allow the input of the signal only. To extract the signal, you can use the special SCART slot we mentioned. With videocassette recorders, you can take long movies (4 - 8 hours), which would consume a

lot of capacity on a hard disk. This is useful in following a microorganism for a long time, to be able to capture occasional events. Afterwards, you can choose and edit the best clips onto a new tape, or digitize them.

## LOADING PICTURES INTO YOUR COMPUTER

With film photography, you can obtain slides and prints. Unfortunately, to see if the shot is a good one, if the exposure is right, if the focus is correct, and if the object has not moved during exposure, you must wait until the film is processed and possibly printed. If you wish to send these pictures to other people, you can send a copy by the normal mail service. It is also possible to obtain copies of slides, but this is quite costly. You can even send pictures via the Internet, but only after they have been digitized using a scanner. For this purpose, you can use a photographic processing laboratory, which will take some time to do the work. You can also buy a digitizing scanner for slides or prints. Another solution is to digitize the slides by means of a digital camera.

In photography, digital technology has brought a true revolution. With this technology, you can obtain very sharp pictures that have very faithful colors. This is also due to the fact that digital imaging avoids the processing of the films by a photographic lab, which often deteriorates their quality, especially in accurately depicting true color. With digital imaging it is possible to immediately check the results of your shots; the convenience of this technique helps produce higher quality results.

Digital technology makes it easy to edit pictures, to send them to other people via the Internet, or to place them in web pages. It also allows the storage of the pictures without damage for a long time, avoiding scratches, dust and fingerprints. You can print your digital pictures with inkjet, laser and dye-sublimation printers. Photo labs can also print digital pictures on normal photographic paper, with good results and delivery in a few minutes. The resolution of inexpensive digital imaging systems is growing constantly, and it has now attained high levels. For many purposes, digital imaging is already competitive with film imaging, and it offers important advantages.

Usually, digital still cameras record images on a memory card. These images can be transferred to a computer by means of a special cable included with the camera, or by means of an external memory card reader that is seen by the computer as a normal diskette drive.

Once downloaded, the pictures can be retouched with image processing programs. Sometimes, they are saved in a non-compressed BMP or TIF format. Stored in these formats, the pictures occupy a lot of space on the HD. For example, a 1600x1200 pixel image with 16 million colors requires about 5.8 MB. As the memory cards have a limited capacity, the digital cameras perform compression that can reduce the same picture to a file size of approximately 250 KB, with a loss of quality that is hardly perceptible. Usually, these images are compressed using the JPEG system. Another format of compression is the GIF. It is best suited to drawings and to images formed by clean lines and fields of uniform hue, as is the case with line drawings and cartoons. On the other hand, when the image has many shades, as in the case of photographs, the compression system to be used is the JPEG.

## LOADING MOVIES INTO YOUR COMPUTER

**Analog signals:** To load analog movies, a computer has to be equipped with a **video capture card**. This card performs an analog/digital conversion and other operations such as resizing, compression and overlay. Usually, these cards are provided with their own software, including drivers. With these programs, you can set parameters such as:

- the size of the frames (320x240, 640x480, 720x576, 800x600 pixels);
- the frequency of the frames (10 fps, 25 fps, 30 fps);
- the number of colors (2 bytes/pixel= 65000 colors, 3 bytes/pixel= 16 million colors);
- the brightness and the contrast of the images (the possibility of performing these adjustments via software while you are shooting is important);
- the type of compression.

Clearly, if you choose high values of these parameters, you will demand a high power of expansion from the card. If the power requested is higher than the capabilities of the card, it will not be able to function correctly. Consequently, in the video you will have interruptions, frame jumping and other problems that will force you to lower the values of the parameters. Even after having lowered your specifications to the card, you will notice that a few seconds of video will occupy dozens of Mbytes of space on the HD. The

compression of the video will greatly reduce its size. Normally, the compression is performed in real time, but it is possible to do it after you have recorded the video.

**Digital signal:** To receive the signal from a digital video camera, your computer must have a USB port conforming to the 2.0 or higher versions in order to have sufficient data-transfer speed for smooth-running movies. A Firewire (IEEE-1394) port is even better; it can attain a transfer rate of 400 Mbit/sec. If your computer is not provided with these ports, you can buy a special card; there are cards with both these ports.

There are thousands of Compressors/Decompressors (**codecs**) for video. As codecs are in a constant state of evolution, most of them are out-of-date. This also means that a definitive and universal standard has not been established. Some codecs have been designed for cartoons, other for normal movies, still others for allowing the observation of continuous streaming data sent via the Internet.

How do you obtain the codecs? Some of the more recent codecs are supplied as a component of the latest computer operating systems, or are included with the video card's software. Still others come with movie viewing programs. Some codecs can be downloaded from the Internet or they are included in CDs enclosed in magazines devoted to digital movies. In any case, if you try to view a video for which your computer lacks a suitable codec, usually the system will ask you if you want to download it from the 'Net.

Video files can have different **extensions** such as AVI, MPG, MPEG, MJPG, MOV, etc. The extensions correspond to videos that have been taken with different codecs. Generally, they are proprietary compressors. For example, the AVI system was developed by Microsoft, and the MOV system by Apple. The MPEG format, however, is open-source. Within it, we find the MPEG-1, MPEG-2, and MPEG-4, of which the codecs DivX and Xvid are a part.

As a conclusion to this brief survey of ways to make images through the microscope, we want to emphasize that with a system based on a Composite signal you will obtain images of relatively small size (about 10 cm in horizontal dimension on the monitor), but it can be enough for amateur use. Working with the S-Video system allows you to obtain images of twice the linear size and of higher quality. With DV or Mini DV video cameras you will obtain even higher definition and quality. In any case, you will have to be careful that the entire system is compatible as to technology. In fact, if you pass an S-Video signal through Composite cables, the quality of the signal will be brought down to that of the lower-quality system.

The last step is to edit your video. With suitable software (video editors), you can cut different clips, connect them with dissolves and other effects, create titles, and add music and voice commentary.

## SHOWING YOUR WORK

Viewing personal movies is rewarding; as critics, we can forgive ourselves of many faults! Things change when we have to show our work in public. In that case, it would be really regrettable if, after having done so much hard work in preparing the video, you find that you are unable to show it because of some banal problem of electronic connection or interfacing. The main methods you can use to show your work are discussed later on. Whatever the procedure you have chosen, the habit of checking the connections and other arrangements well before the presentation is valuable. In this way, you will be able to check that you have everything necessary, and you will have time to obtain what you lack. For instance: have the suppliers provided the needed connectors? Are the cables long enough? You will also be able to place the chairs, the TV set or the screen in a suitable manner. You will be able to make sure you can darken the room, etc.

### From the video camera to the TV set in real time.

A video camera mounted on a microscope can send its analog signal directly to a TV set, and a group of persons can see in real time what is happening on the slide. This is particularly useful for a teacher, because it allows him or her to avoid having students look through the microscope one at a time. This will allow the instructor to show and explain, to all the class at once, what is happening under the microscope.

### From the videocassette to the TV set.

Viewing in real time has its attractions, but it is often difficult to be able to show precisely what you want. For example, if you want to speak about amoebae, you will not necessarily find one in the sample of pond water you brought with you to the classroom. But in a video you can collect clips that concern many different organisms, the fruits of days or months of observations. A simple way to prepare a video like this is to assemble the different clips on a VHS cassette. With a television set connected to the videocassette recorder by means of a SCART cable, you can show the video simply by pressing the "play" button. For

some TV sets, it is necessary to make certain its TV/AV button (or, in some instances, its channel selector) is set properly. In older TV sets, lacking a SCART connection, you will need to send the signal from the VCR to the TV set by means of the antenna cable, and you will need to tune the television set to the VCR channel, which usually is n 36 (3 or 4 in the U.S.A.). This operation is not always simple and it should be tested beforehand.

#### **From a disk to the monitor of a computer or to a TV set.**

Movies can be recorded on the HD of a computer, on a CD and even on DVD, as simple video files. Afterwards, you will be able to see these videos on a computer monitor by means of suitable viewing software, called "multimedia players" because they deal with the sound as well as the picture. Among the common programs of this type are: *Windows Media Player* (particularly suited for AVI movies), *Real Video* (created for radio/TV streaming), *Quick Time* (produced by Apple and suited for MOV movies). The viewers also use codecs to show the movies. In reality, the viewers do not limit themselves to handling their own types of files; they are able to show a variety of movies because they are designed with the more important codecs in mind. Some of these viewers are supplied with the operating system. These viewers and others can be downloaded from Internet.

It is possible to create videodisks such as VCD, S-VCD and DVD. They can be inserted in DVD readers for computers and the movies can be seen on the monitor. They can also be inserted into DVD home players and the movies can be seen on television sets.

The Video CD, or VCD, was introduced by Philips and Sony in 1993. It has a resolution of 352x288 pixels (PAL) and it is suitable for VHS. The movies are compressed with the MPEG-1 system. A VCD holds 70 minutes of audio/video material, and for a normal "feature-length" film 2 disks are needed. To write a VCD, you need a drive and a suitable program.

The DVD was introduced in 1997 and it is a further development of the Video CD. It is intended as a video device, but it is also used for music and data storage. DVD movies have a resolution of 720x480 or 720x576 pixels, higher than that of SVHS. This system uses MPEG-2 compression and each DVD can store more than 2 hours of high quality video or 8 hours of VHS video.

The Super Video CD, or S-VCD, is intermediate to the two preceding systems. The medium is a normal CD-Rom, but the movies are stored on it with an intermediate quality, similar to the SVHS system, and they are compressed according to the MPEG-2 format. Each S-VCD holds about 40 minutes of movie, and for a normal film 3 disks are needed.

The DivX is an MPEG-4 compression system that can be used on VCD, S-VCD and DVD. Essentially, it uses the same MPEG-1 and MPEG-2 compression algorithms, but it processes only the differences between one frame and the next. For the same image quality, this system attains a ratio of compression higher than that of the MPEG-2 and that of the preceding MPEG-4 codecs. It is used on readers for computers as well as on home players for TV sets. The Xvid system is an optimization of the DivX, created as open source. It is a compression system that has recently appeared and it is being widely adopted.

#### **Showing digital pictures and video.**

For several years, to show computer data and images on a screen, a projector was used which was made up of three small and very bright TV monitors; each of them was filtered with one of the three primary colors. These projectors produced images of very poor quality; the brightness was insufficient and the sharpness low. Recently, special projectors for digital pictures and movies have come on the market. They are able to project high-quality, very bright and sharp images on a screen large enough to be viewed by many people in a fairly good-sized room.

#### **Showing stereoscopic pictures and movies.**

The anaglyph technique, better known as the two-color technique, is the simplest one to utilize for projection of stereoscopic images. Probably the best method is to use the projector we mentioned before, which supplies images large enough to be seen with comfort by a good many people, something not possible using a TV set. The audience must wear two-color glasses. In another method, pictures and movies taken with two cameras separated by a precise distance can be sent to two projectors, one of which is horizontally polarized and the other vertically polarized, projected onto a metallic screen, which is not depolarizing. These images have to be observed with suitable glasses provided with polarizing filters. Yet a third technique entails the frames of a movie being seen alternately with the right and with the left eye. In this system, people are supplied with special glasses with liquid crystal lenses that become opaque or transparent in synchrony with the projector.

### **Sending pictures and movies on the Internet.**

Images and movies can be inserted into HTML pages, and they can thus be made available to a very wide public. A digital movie can also be sent by e-mail like any other file. When it has been received, it can be seen with the techniques we already have mentioned. Another method is to send a movie as a stream of data to be observed during the transfer. This method is used also for radio broadcasting over the Internet.

### **CONCLUSION**

Taking pictures or videos through the microscope requires the application of knowledge from several different disciplines. Among them, the study of the optics of the microscope and its illuminating system is of primary importance. Then, you need to know about the imaging devices and resources that allow you to record and to show pictures and movies. Knowledge of color vision theory and the way images are treated in the computer world is also very useful. Not least, knowledge of biology is of great importance because it allows you to single out interesting subjects to shoot.

We are fortunate that nowadays, digital still cameras and video cameras with good image quality and low prices are arriving on the market. They will allow people to produce images which, until short time ago, were available only to professionals. This innovation opens wide prospects to naturalists and to amateur microscopists, and it will also aid in spreading interest in science. Making images through the microscope can start as a pastime, but it can also lead a person who was simply keen on microscopy to accumulate a remarkable amount of knowledge that can be useful to many other people. Making pictures and short documentaries, combined with knowledge of biology, allows one to assist in popularizing science, and furthering science education at a local level in schools and among the general public. An interactive lesson in biology based on the use of the microscope, possibly assisted by a movie produced by local people, is much more effective in communication and educational terms than a "slick" documentary made by professionals, but shown in an anonymous way over TV. Moreover, quality projects can be proposed to publishers and television producers, and may thus gain an even larger audience.

We hope this information is useful to you, and we wish you all success in exploring the exciting new world of digital photomicrography!

### **FURTHER INTERNET RESOURCES**

- <http://www.microscopyu.com/articles/photomicrography/digital/index.html> Digital Imaging in Optical Microscopy.
- <http://www.olympusmicro.com/primer/photomicrography/errorindex.html> Troubleshooting Errors and Faults in Photomicrography
- [http://www.modernmicroscopy.com/article\\_pix/030902\\_coolpix/Evennett\\_digitalcamera.pdf](http://www.modernmicroscopy.com/article_pix/030902_coolpix/Evennett_digitalcamera.pdf) The New Photomicrography
- <http://www.barrie-tao.com/afocal.html> Afocal Photography
- <http://angelfire.com/ga2/photo/bracket.html> How to make a shutter release bracket for a camera lacking of it
- <http://www.moviecodec.com/> Information on the main codecs and their downloading
- <http://www.geocities.com/Athens/Forum/2496/vcdfaq.html> Information on the VCD
- <http://www.uwasa.fi/~f76998/video/svcd/overview/> Information on the SVCD
- <http://www.dvddemystified.com/dvdfaq.html> Information on the DVD
- <http://www.howstuffworks.com/projection-tv5.htm> How projection television with digital micromirror devices works

Internet keywords: digital photomicrography, videocamera firewire, webcam firewire

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