Adapting Consumer Digital Cameras for Photomicrography: Technical Aspects

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BIOGRAPHY

Jörg Piper obtained his MD from the University of Bonn, Germany. One of his scientific interests is light microscopical applications, especially reflection contrast, and he has created several models for 3D quantitative analyses of opaque cellular specimens. Since 1998 he has collaborated with the University of Oradea, Romania, as an associate and honorary professor. In Germany, he currently works as senior consultant for internal medicine, angiology and diabetology.

ABSTRACT

With regard to their value and qualitative aspects, high-level digital consumer cameras are attractive tools for photomicrography. Their images are comparable with analogue 35 mm film photographs. Compact and bridge cameras have several specific advantages such as less vibration associated with the shutter, display-based live imaging, and low or medium weight. Images taken with mirror-reflex cameras can be affected by lack of sharpness caused by vibration, dependent on their exposure and magnification, even when mirror lockup is used. Typically all automatic metering modes, including through-the-lens autoflash control, work properly. Furthermore, digital compact and bridge cameras can be successfully used for movies. This article describes methods for attaching these cameras to light microscopes and discusses some of the optical, mechanical and technical considerations.

KEYWORDS

light microscopy, photomicrography, consumer digital cameras, compact, bridge, mirror reflex, adapter, through-the-lens, flash

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INTRODUCTION

Manufacturers of light microscopes have for decades developed connection modules that allow their microscopes to be used with analogue cameras for photomicrography. These modules were constructed for professional use, mostly equipped with integrated 'compur' (iris diaphragm) shutters and systems for selective metering modes. However, for digital cameras, similar modules have not yet been widely developed. From an economic standpoint, consumer digital cameras are an attractive alternative solution for photomicrography as they are much cheaper and produced in high numbers. Moreover, high-end consumer digital cameras can produce high-resolution images, similar to analogue or film images.

However, not all digital cameras are suitable for photomicrography because of potential artifacts, vignetting effects, deficiencies of coverage, sharpness and several other other reasons [2,5]. In this article I present some technical solutions for digital photomicrography that have been rigorously and successfully tested in practice.

MATERIALS AND METHODS

Several models of current digital cameras with various designs (compact, bridge or prosumer, and single-lens reflex or mirror-reflex) were tested using conventional laboratory microscopes and stereomicroscopes equipped with monocular, binocular and trinocular tubes (viewing heads), and using several different illumination modes (bright and darkfield, phase and interference contrast, polarization, and reflected light). All aspects of mechanical and optical adaptation, exposure, manual focus, image control, potential artifacts, automatic and manual flash light control, and monochrome techniques were evaluated using several suitable digital cameras. The qualities of the respective images were visually compared using high-resolution monitor images and colour prints. The results of these tests are presented below: the major technical considerations are first described and then examples of several cameras that gave good results in practice are reported.

TECHNICAL BASICS

CCD sensor versus film

The areas of most CCD sensors are much smaller than that of the standard 35 mm photographic film (24×36 mm). Table 1 gives an overview of the geometrical dimensions of the sensors found in most digital consumer cameras [6,7]. The higher the area of a digital sensor and its pixels, the lower any artifacts may be, especially blooming and noise. Thus the maximum usable ISO speed equivalent of a digital camera is dependent on the dimension of its sensor. In low light situations such as fluorescence or polarisation microscopy, digital cameras with high sensor dimensions should be preferred.

The smaller the sensor area, the lower the recorded area of the microscopical image. So over-magnification can result when digital cameras with small sensors are adapted for microscopy in the same mechanical and optical ways as analogue cameras.

The standard colour CCD sensor used in consumer digital cameras consists of a single layer of photodiodes or pixels. For colour imaging each pixel is coated with a filter for one of the primary colours, red, green and blue (RGB) arranged in a pattern over the CCD according to the Bayer model. So each point in the colour image is formed from a quartet of pixels: two pixels are covered with green filters, and the two neighbouring pixels with red and blue filters. Thus, 50% of all pixels are sensitive only to green light, 25% to red, and 25% to blue

Figure 1:
Examples of ring-shaped adapters and photo-oculars.
(a) Promicron ring-shaped adapter for thread M52 with 28 mm (left) or 45 mm (right) internal thread.
(b) Leica Periplan GF ocular 10X/18 for spectacle wearers, equipped with 28 mm thread.
(c) Promicron photo-ocular 10X with 28 mm thread.
(d) Leica Vario photo-ocular 5.5-12.5X.

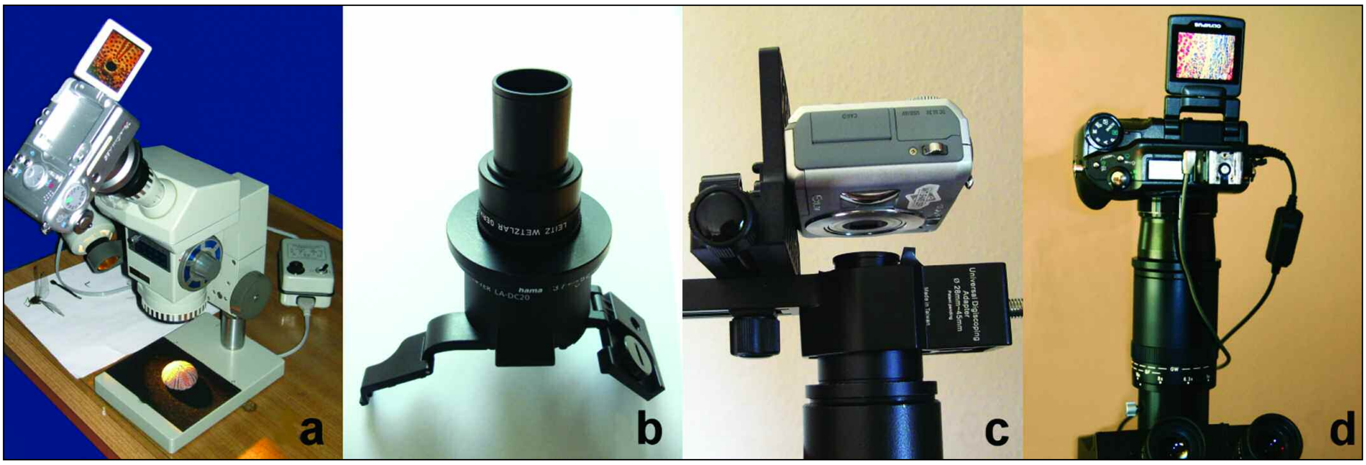

Figure 2:
Mountings of digital compact and bridge cameras. (a) Canon Powershot A 95 mounted on a stereomicroscope equipped with a Promicron photo-ocular 10X. (b) Completely mounted adapter for Canon Powershot S 80. Components: Canon conversion lens adapter LA-DC20; Hama stepping ring 37-52 mm; Promicron ring shaped adapter for thread M52; Leica Periplan ocular 10X/18 for spectacle wearers with 28 mm thread. (c) Casio Exilim EX-Z 110 with external Meade universal adapter, mounted on a monocular tube. (d) Olympus Camedia C 7070 with a Leica Vario photo-ocular, mounted on a Leica FSA phototube.

[1]. This means that when a monochrome (e.g. red or blue) specimen is photographed, only 25% of the pixels contribute to the digital image; the information in the other 75% of the pixels is interpolated electronically by the camera. Further artifacts can result from this arrangement such as lower contour sharpness, and stages and colour fringes in linear structures. So when red or blue specimens are to be documented, the number of sensor pixels can be an important determinant for the quality of the microscopic image.

The resolution of an analogue camera and analogue film is described by the maximum number of line pairs per millimetre (lppm). For consumer digital cameras a 5.5 megapixel (Mp) sensor can resolve 40 lppm, rising to 50 with 8.5 MP and 60 lppm with 12 Mp. Thus an 8 Mp sensor approximately corresponds to the average resolution of analogue film.

When a sensor is exposed by oblique incident light, it produces many more artifacts than film, especially vignetting, blooming artifacts and loss of sharpness [1]. Therefore, the simply constructed external camera adapters which can be used for analogue cameras are often insufficient for most digital cameras because of axial and centering deviations. Consequently digital cameras should preferably use adapters constructed with maximum optical and mechanical precision (Figure 1).

MECHANICAL ASPECTS

Using analogue-camera adapters

When adapters for analogue mirror-reflex cameras already exist (e.g. Leica Combiphot), digital mirror-reflex cameras can be used provided the adapters are compatible. For example, analogue Leica R-bodies can be replaced by digital Canon EOS mirror-reflex bodies.

Special solutions for digital cameras

When pre-existing analogue solutions are not available, digital cameras can be connected with a light microscope by various means.

Compact and bridge cameras must be connected together with their fixed zoom lens. The lens has to be centered directly over the ocular. The eyepoints of the respective lenses, the distances from lenses and sensors, the focal lengths of the lenses and the minimum realisable distances are not standardised. Therefore many compact and bridge cameras are not suitable for photomicrography, as mentioned above, so that individual tests have to be carried out to select practicable models.

The best results can be realised by properly centered bolted connections between the camera and eyepiece. The shutters of compact and bridge cameras (mostly compur shutters) are normally vibration free, so sharp images can be achieved for a complete range of speeds when a self timer, remote controller, remote switch or capture software are used. In most cases, the area of the image can be modified with help of the integrated zoom.

Digital mirror-reflex cameras can be adapted in different ways. As the camera lenses are removable, the camera body can be adapted without them. In this case, the lenses of the camera are replaced by the lenses of the microscope, i.e. the eyepiece and objective. If necessary, the eyepiece can be replaced by special photo-oculars offered by several manufacturers for different camera bodies.

Digital mirror-reflex cameras can also be adapted for use with a microscope in a similar manner to compact and bridge cameras without removing their lenses. In this case, the camera lens has to be bolted to the eyepiece as described above. When a camera is equipped with an APS sensor and a lens with a 35 mm focal length is used, the complete visual field of a conventional brightfield eyepiece can be used for imaging, so that the coverage and picture width correspond with the margins of the visual field. When lenses with 50 or 60 mm focal length are mounted, the resulting images approximately correspond with the central 60-70% of the visual field. In this way any lack of sharpness at the periphery caused by spherical aberration can be excluded. Zoom lenses should not be used, because lenses with constant focal lengths lead to higher optical quality in practice.

Even when the shutter works with mirror lockup and a remote switch is used, shutter vibration is more of a problem than with compact or bridge cameras. Vibrations can be reduced if the photo-ocular is fitted with soft grommets used as washers and dampers. As the intensity of vibration is dependent on the shutter speed, exposures lower than 1/15 s, or higher than 1/1000 s should be used. Vibration can also be avoided when a flash is used.

The Leica Vario photo-oculars are constructed for 46 and 48 mm shaft diameters, suitable for the respective Leica trinocular tubes. The 10× photo-oculars tested here are standardised for 23.2 mm shaft diameters.

Figure 3:
Canon EOS 350D, equipped with 35 mm lens, 10X photo-ocular and angle finder, mounted on a Leica FSA phototube.
KEY: 1. Leica FSA phototube. 2. Vertical connecting piece for photo-oculars, internal diameter: 23.2 mm. 3. Leica Periplan GF ocular 10X for spectacle wearers, screw thread: 28 mm. 4. Promicron ring-shaped adapter for thread M 58, bolted with a Hama stepping ring 55-58 mm. 5. Leica Summicron-R 1:2 / 35 mm. 6. Novoflex bayonet adapter EOS/LER. 7. Connecting cable of the Canon remote controller RS-60E3. 8. Seagull angle finder with integrated 2.5X loupe, combined with a Canon eyepiece extender EP-EX15.

They can be used with 30 mm or C-mount tubes using commercial mechanical adapters, such as the Promicron universal adapter set.

OPTICAL ASPECTS

Exposure

Usually, images are correctly exposed when the automatic metering modes of the digital camera are used (full aperture multi-zone or centre-weighted metering, partial metering at centre, and spot metering). If necessary, the exposure can be modified by an override (auto exposure bracketing (AEB), usual range: ±2 exposure value (EV)).

In special situations, the exposure should be controlled manually. The brightness can be modified and adapted to the specimen and camera by changing the microscope illumination. In most cases, ISO100 will lead to optimal results.

Image parameters

All adjustable parameters of the camera should be tuned for optimal quality (maximum image size, minimum compression, well balanced sharpness, contrast, colour saturation). In most cases, the automatic white balance (AWB) gives good results. In special situations, the settings may need to be changed.

Focusing

When the camera is adapted with its own lenses, the autofocus should be turned off. Usually, the lens should be focused to infinity. Images can be focused using the LCD display of compact and bridge cameras or in the viewfinder of mirror-reflex cameras. The ergonomics of mirror-reflex cameras can be improved if an angle finder display device is used. The precision of focusing can be improved when the image on the angle finder is observed using an integrated 2.5× loupe.

Compact and bridge cameras are commonly equipped with AV-based or USB-based video outputs, so that images can also be observed and focused using computer or TV monitors.

Image area

The area of the recorded image can be modified, in most cases, when compact or bridge cameras with zoom lenses or Vario photo-oculars are used.

The zoom factor of compact and bridge camera lenses should be low or moderate, e.g. 3-5×. Zoom lenses with higher factors, especially mega-zoom lenses, are not suitable for photomicrography because of their optical compromises and various resulting artifacts.

Flash light

When a flash can be adapted to the microscope and when the camera is equipped with a hotshoe and an off-camera shoe cord, images can be taken with ultra-short exposures, so that any lack of sharpness associated with vibration or movement is avoided.

The flash exposure can be modified manually by well known methods, which are in general also used for analogue cameras [3]. Moreover, automatic TTL flash exposure metering can lead to useful results.

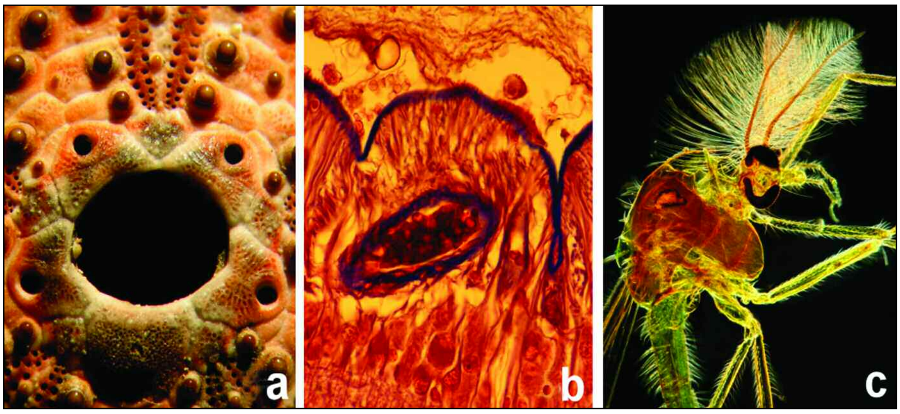

Figure 4:
Images from routine applications taken with various models of digital cameras. (a) Skeleton of a sea urchin, stereomicroscope, incident light, objective 1.6X, Promicron photo-ocular 10X, Canon Powershot A 95. Horizontal field width (HFW) = 9 mm. (b) Human retina, brightfield, objective 40X, Leica Periplan ocular 10X, Casio Exilim EX-Z 110. HFW = 0.14 mm. (c) Gnat, darkfield, objective 2.5X, Leica Vario photo-ocular 6.5X, Canon EOS 350 D, flash (Canon Speedlite 430 EX), automatic TTL metering, 1/2000 s. HFW = 4.1 mm. Photogrpahs taken by Timm Piper.

Illumination modes

The solutions described here are suitable for all standard illumination modes such as bright and darkfield, phase and interference contrast, polarization, epi-illumination, and fluorescence microscopy [4].

Monochrome techniques

When monochrome specimens are examined the quality of digital images can be improved if the specimen is illuminated by green light. In this way, 50% of all pixels in a colour CCD are directly exposed instead of 25%. Furthermore, when monochromatic light (540 nm) is used for illumination any unsharpness based on chromatic aberration is eliminated. The sensitivity of the human eye is optimized for green light, so that green images show maximum detail and tonal values (Figure 5).

ADAPTING DIGITAL CAMERAS: SOME EXAMPLES

Canon Powershot A95

This Canon compact camera can be connected to a microscope by a conversion lens adapter (e.g. Soligor for Canon A 80 adapter tube B-52), and a ring-shaped adapter with an external 52 mm screw thread and an internal 28 mm screw thread (e.g. Promicron adapter for filter thread M52). The ring-shaped adapter and the conversion lens adapter are bolted together. Conventional eyepieces for spectacle wearers, equipped with a 28 mm thread, can be bolted with the ring-shaped adapter (e.g. Leica Periplan GF ocular 10×/18 spectacle or Promicron photo-ocular). The weight of this construction is about 340 g (excluding batteries), so that it can be used for all varieties of tubes (monocular, binocular, trinocular). The shutter should be released by the self timer or the capture software so that vibration is avoided. Figure 2a shows this camera prepared for use on a microscope.

Canon Powershot S80

This Canon ultra-compact model (weight 225 g, excluding batteries) can be used in the same way as the Powershot A95. The conversion lens adapter (Canon LA-DC20) has a different external 37 mm screw thread. Therefore, an additional stepping ring 37-52 mm in diameter is necessary to connect the camera with the 52 mm ring-shaped adapter the eyepiece has to be bolted in. The Powershot S80 has a fixed display which is not swivel mounted. AC adapters or remote switches are not available. Figure 2b shows the adapter for this camera, connected with a Leica Periplan GF ocular for spectacle wearers.

Casio Exilim EX-Z 110

This Casio camera can be connected to a tube by simple external adapters (e.g. Meade Universal Digital Camera Adapter 49-14900, Figure 2c). Despite the specific disadvantages of such external adapters, already discussed, the sensor and lens of this camera do not lead to relevant artifacts when an external adapter is

Sensor and Camera	Width mm	Height mm	Diagonal mm	Area cm^2
35 mm photographic film	36	24	43	8.6
Leica Digital-Modul-R	26	18	32	4.7
APS-sensor (e.g. EOS 350D, 20D, 30D)	23	15	26	3.5
4/3 inch (e.g. Olympus mirror reflex)	17	13	21	2.2
2/3 inch (e.g. Leica Digilux 2)	9	6.5	11	0.6
1/2 inch (several compact cameras)	6.5	5	8	0.3

Table 1:
Geometric data of photographic film and typical digital camera sensors.
APS = advanced photo system. The inch size is a designation that relates back to the useable imaging area of video tube cameras.

used. Thus, this construction is the cheapest and most simple way to create photomicrographs. The camera has a very low weight (136 g, excluding batteries), but the weight of the Meade adapter is 350 g. Therefore, the Casio camera should preferably be fitted to monocular or trinocular heads. For still images the self timer should be used to avoid vibration.

Olympus Camedia C 7070

This high-quality Olympus 7.1 Mp bridge camera can be mounted in the same way as the Canon Powershot A95, using a suitable conversion lens adapter (e. g. King adapter for Olympus 48, 5-52) combined with the same ring-shaped adapter (52-28 mm) and the same oculars for spectacle wearers as mentioned above. Alternatively, this camera can also be used with a Leica Vario photo-ocular. As the Vario oculars have their own size of thread (45 mm), special ring-shaped adapters have to be made with an external 52 mm screw thread and an internal 45 mm screw thread, so that the Vario ocular can be bolted on instead of a conventional ocular for spectacle wearers. Such individual adapters can be specially manufactured by the Promicron company according to the customer´s needs. The weight of this camera is 433 g (excluding batteries), so that it should be used in combination with monocular or trinocular viewing heads.

When a compatible flash and off-camera shoe mount TTL flash cable are used (e.g. Olympus flash FL-36 or FL-20 and flash cable FL-CB05), automatic TTL flash light exposure metering is possible. The values of the ISO speed have to be shifted to achieve well-exposed results in most cases. When the camera works at ISO100, the ISO speed equivalent of the flash has to be switched to ISO400 or 800. Each commercial flash can be used with high speed synchronisation up to 1/2000 s in automatic or manual modes. The shutter works without vibration and can be controlled by a wireless remote control (Olympus RM-1). A high-power AC adapter is available (Olympus C-8AC). Figure 2d shows this camera, equipped with a Leica Vario photo-ocular, mounted on a trinocular viewing head.

Canon EOS digital cameras

According to the tests carried out, all Canon APS-chip models (~8Mp) can be used (EOS 350D, 20D, 30D). The shutter of the EOS 350D leads to less vibration because it does not work as hard as the shutter of the other bodies. Moreover, the EOS 350D has the lowest weight (485 g vs 700 g, excluding battery). In these respects, the EOS 350D is an ideal choice for microscopical purposes. The camera can be used with a remote switch (Canon RS-60E3, cable length: 65 cm) or wireless remote controller (Canon RC-1). The remote controller only works when the self timer is turned on; thus, the remote switch seems to be the more practicable option. The shortest shutter-curtain synchronisation is 1/200 s for normal flash modes and 1/4000 or 1/8000 s for high speed synchronisation, when a compatible flash is used (e.g. Canon Speedlite 430EX or 220EX), equipped with the attendant Canon Off-Camera Shoe Cord 2.

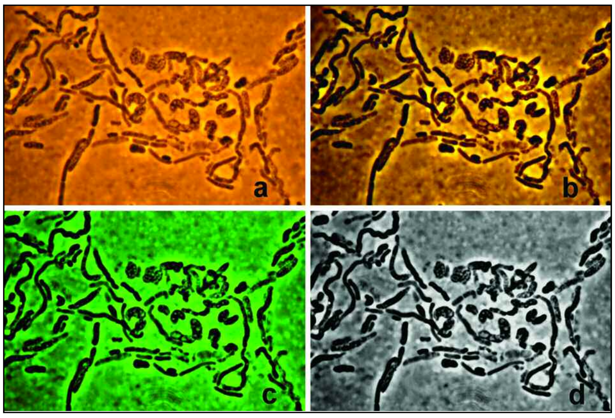

Figure 5:
Example of monochromatic techniques using images of Bacillus megatherium taken with 100X oil objective, Leica Vario photo-ocular 12.5X and Olympus Camedia C 7070 camera. HFW = 40 µm.
(a) Conventional phase contrast image.
(b) Conventional green filter. Image histogram equalized.
(c) Monochromatic green filter. Image histogram equalized.
(d) Monochromatic green filter. Image histogram equalized. Black and white image.

According to all tests carried out, the best optical results can be achieved when the Canon camera bodies are adapted with Leica-R lenses. These lenses with constant focal length are characterised by excellent optical and mechanical quality. Leica-R lenses can be adapted with Canon bodies by suitable bayonet adapters (e.g. Novoflex adapter EOS/LER). The annular ring adapters mentioned above, manufactured by Promicron, have to be bolted into the filter glass thread of the according Leica-R lens. Thus, the Canon body can be connected to conventional oculars for spectacle wearers as well as to Leica Vario oculars, when annular ring adapters are available for both types of oculars. When photographs are taken without a flash, the shutter speed should be lower than 1/15 s or higher than 1/500 s. Moreover, mirror lockup is necessary. Low weight conventional oculars for spectacle wearers should be used, fitted out with soft grommets as described above to reduce vibration. Figure 3 shows the assembly of a Canon EOS 350D equipped with an angle finder.

DISCUSSION

Several high-end consumer digital cameras, compact as well as bridge and mirror reflex, are suitable for photomicrographical purposes. When the resolution of the respective high-quality sensor is about 6 or 8 Mp, digital images can be recorded which are comparable with conventional analogue photographs taken on 24×36 mm film.

Digital cameras have various specific advantages, which are important for photomicrography. Images are immediately seen on the camera display and poor images can be retaken immediately. The ISO speed level can be changed for each image according to the brightness of the specimen – up to ISO400 when compact or bridge cameras are used, and up to ISO1600 or 3200 when a digital mirror-reflex camera is available.

Digital images can be optimized by post-processing with the help of standard image optimizing software. Thus, fundamental parameters which determine the image quality can be influenced (gradation, brightness, contrast, colour saturation, white balance, sharpness). Moreover, perturbing structures such as dust particles, artificial bubbles, etc. can be removed by software-based retouching.

The shutters of digital compact and bridge cameras work without any vibration. Microscopic life images can be permanently controlled by the camera display or by a TV or computer monitor. The weight of these cameras is lower than the weight of mirror reflex bodies. These facts might be regarded as fundamental advantages.

On the other hand, the sensors and pixels of compact and bridge cameras have smaller areas than those of most mirror-reflex cameras. Therefore, the maximum ISO speed level is lower and the noise level mostly higher than using mirror-reflex cameras. In practice, these technical aspects have just a low relevance, because the image processing algorithms, integrated in compact and bridge cameras, are permanently improved. Thus, in most situations, comparative microscopical images taken by mirror-reflex and non mirror-reflex cameras show no relevant visible differences in quality.

In conclusion, digital compact or bridge cameras can be used for most routine microscopical applications. In special situations, e.g. extreme low brightness or maximum print sizes, mirror-reflex cameras might be beneficial because of their lower noise and higher maximum ISO speed levels. A flash should be used with high-speed synchronisation whenever possible. In this way, any potential lack of image sharpness caused by vibration or movement can be prevented.

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