How Can I Find the Right Digital Camera for My Microscopy Application?

Nowadays, image processing is found in a wide range of optical microscopy applications. Examples for this are medical and biological research, diagnostics, testing of medicinal products, or material sciences.
Microscopy provides direct access to studying the structure and function of the most diverse objects. However, using only one method it is only very rarely possible to obtain all the required information. Consequently, by and by many different microscopy technologies offering numerous presentation options and resolutions were developed.
This white paper illustrates the various aspects to consider when selecting a camera.

Content

To use image processing in microscopy the selection of the most suitable components for any microscopy application is crucial. This includes in addition to the microscope itself also the selection of the correct lenses, lighting, filter sets, and polarizers, depending on the dyes and contrasting methods applied. Ultimately, the special requirements of the respective application are decisive for the selection of the most suitable microscopy camera and compatible image processing software.

The digital microscopy camera provides a microscopic image in an optimal way using image data. This image data may be recorded, stored, printed, or embedded in documents. Using a PC or mobile device, the image data provided by the microscopy camera can be digitally processed and analyzed using special software. The images provided by the digital microscopy camera can easily be displayed on a large monitor and thus can readily be used for live demonstrations or observation purposes.

However, how do you find the right digital camera for your microscopy application and what aspects do you have to consider when making the selection?

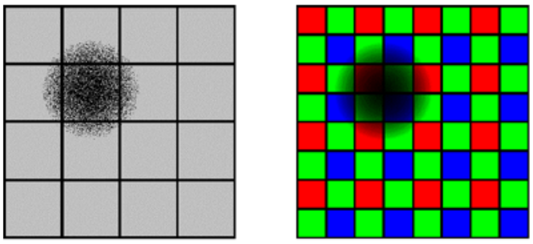
Typical microscopy workstation for routine laboratory applications with a binocular inverted microscope (front) and an upright microscope (in the background).

1. Monochrome or Color Camera?

Color cameras are very flexible in their application and are an integral part of the broad field of conventional light microscopy. Many routine microscopic applications in biomedical and clinical laboratory settings as well as in industry and material research are based on conventional light microscopy employing various lighting and contrasting methods. Additionally, special dyeing techniques are often used for microscopic specimens that allow for detailed analysis of otherwise low-contrast structures, for example as part of histopathological diagnostics of diseases and for monitoring disease progression.

Thus, for microscopy images color fidelity and color reproduction should be as accurate as possible to be able to differentiate reproducibly the finest structures in materials and biologic samples.

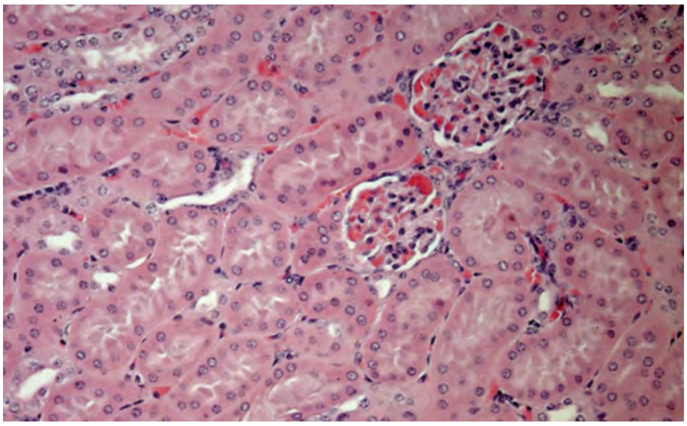
Digital color sensors in microscope cameras use differently colored filters, fitted to the sensor chip, for recording color information.



Monochrome sensor (left) and sensor with color filter in form of the Bayer pattern on a sensor chip (right). Frequently, smaller but more pixels are used for color sensors to achieve the same „real“ resolution. The black area symbolizes a dot in the reproduction.

Typically, color sensors record only one color per pixel, but most often all colors are emitted for every pixel. To achieve this, the information of the adjacent pixels is used as well for every pixel. To allocate complete color information to each sensor pixel, the gaps have to be filled with interpolated values for each of the three primary colors (“De-Bayering”). The De-Bayering may cause interpolation artefacts and the color filter reduces efficiency in light absorption, in particular under difficult light conditions.

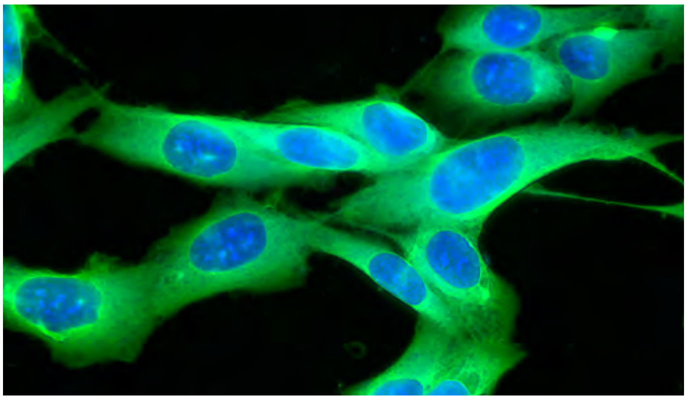
Nevertheless, thanks to color cameras with correspondingly high sensitivity, numerous applications can be covered using lower light intensity, as for example in standard fluorescence microscopy. Color cameras are ideally suited for microscopes that allow for working in light and dark fields, optionally in contrasting procedures and in fluorescence applications. They are also suitable for simultaneously monitoring and documenting several fluorophores using a multiband filters.



Light microscopic image of kidney tissue: to distinguish different cell and tissue structures a histological tissue section was dyed with hematoxylin and eosin (HE staining).

A monochrome camera delivers complete information about the brightness values on the image sensor, although without color information. Here the information for every pixel is available without further calculation. With monochrome cameras, there is no loss of intensity, as would be the case with color cameras due to absorption of light energy through the color filter. However, monochrome sensors often have a two-dimensional IR cut filter in front of the sensor, which negates the infrared emissions from the light source. In many applications, these infrared emissions do not contribute to the image information, but otherwise only reduce the image contrast.

Thus, if color information is not required, sensitivity and information content may be gained. Consequently, monochrome cameras are perfectly suitable for the application in sophisticated fluorescence microscopy. Exposure times may be reduced and thus sensitive fluorescence samples can be used considerately. Sensors that are more sensitive to light render low-noise images, even with weak light signals. Special NIR sensor variants offer an improved sensitivity range including the near-infrared range.



Fluorescence microscopic image of living human melanoma cells: following the fluorescence staining, certain cytoplasmic elements appear in green and the nucleus in blue fluorescence.

2. Sensor Types (CCD vs. CMOS), Shutter Options, Frame Rate

The next important step is selecting the most suitable sensor. From the technological perspective, one differentiates here between CMOS and CCD sensors. Next, the best frame rate, meaning the number of images that a camera must deliver per second to handle its task seamlessly, needs to be selected.

Sensor Types

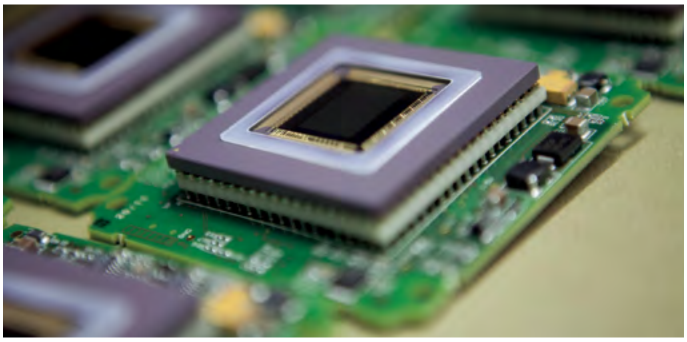
There is a wide range of CCD- (charge-coupled device) and CMOS (complementary metal oxide semiconductor) based sensors suitable as image sensors for microscopy cameras on the market. The technical design of these sensor types is fundamentally different, thus their characteristic properties differ as well. The selection of the right sensor type depends on the application.

Both CCD and CMOS sensors convert light (photons) into electrical signals. Noteworthy are their different characteristics and how these changed due to further technical development.

What are the notable characteristics of CCD sensors? Generally speaking, CCD sensors stand out for their low noise factor, high fill factor, the strong signal to noise ratio, and color fidelity for images, all at very high image quality. These properties make cameras using CCD sensors a good choice for low-light applications.

Today, CMOS sensors are at times better than many CCD sensors.

In recent years, they caught up with CCD sensors thanks to fundamentally new developments. Their high speeds (frame rate) and resolution (number of pixels), the lower power consumption and, most recently, improved noise characteristics, dynamics, quantum efficiency and color concepts have opened them up to applications previously reserved for CCD sensors. The same applies in microscopy, where in particular high speeds and the new color concepts of modern CMOS sensors deliver excellent live images.



CMOS area scan sensor.

Given the progress made in recent years, many more interesting new developments in CMOS technology can be expected down the road as well. The market trends suggest that the latest CMOS technology will largely replace CCD technology.

Frame Rate

The term is synonymous with ‘frames per second‘ or ‘fps‘. These terms describe the number of images that the sensor can capture and transmit per second.

The higher the frame rate, meaning the quicker the sensor, the more captured images per second and thus higher data volumes can be transmitted. The possible or necessary frame rate depends on the type of microscope system used with the cameras and on what the cameras in the microscope are required to record.

For many systems, viewing latency-free live images directly on the monitor is the main objective, which allows for seamless screening of specimens and quick focusing. The human brain detects approx. 14 to 16 images per second, this number is significantly higher for trained people. The frame rate of normal cinema movies is 24 fps, in more recent productions even 48 fps. Ideally, the frame rate for standard microscope cameras is within that range.

However, not only smooth playback, but also good image quality and sharpness of the moving images define comfort during live monitoring. Here progressive scan technology offers one decisive advantage.

For automated applications, that require in addition to high image quality also a high throughput, significantly higher frame rates could be important. - Thus, for example for the automated scanning of the sample range, for automated focusing or multiple images to reproduce the complete sample within the shortest possible time.

Shutter

Connected to the selection of the sensor is the selection of the shutter system.

The two major options are global and rolling shutters. The shutter protects the sensor within the camera against incoming light, opening only at the moment of exposure. The selected shutter or exposure time provides the right ‚dose‘ of light and determines how long the shutter remains open. CCD sensors always use global shutter, while CMOS sensors offer both model variants.

The difference between the two shutter variants is in the way the sensor exposes the images:

For global shutters the complete sensor is simultaneously exposed, meaning light strikes the entire surface of the sensor simultaneously.

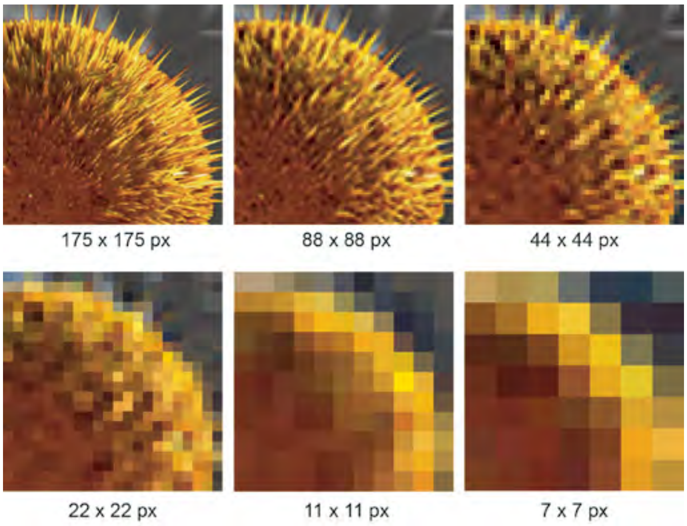
Sensors with rolling shutters, however, are exposed gradually cell by cell in a quick time sequence (within microseconds).The movement of the object during exposure may result in distortions depending on the selected exposure time and actual speed of the object. This effect is referred to as rolling shutter effect.

There are some situations in microscopy, for example during very fast movements of the sample/sample table or during the observation of highly dynamic processes, when this effect is undesirable. However, sensors with rolling shutters are well suited for most standard applications and they offer some benefits over global shutters. They impress with a significantly higher dynamic range thanks to their low readout-noise. High dynamics allow particularly high-quality recording and analysis of detailed structural information.

Additionally, it is possible to prevent the rolling shutter effect by using exposure times within specific limits. Some high-performance CMOS based cameras for scientific applications operate in rolling shutter mode.

3. Resolution, Sensor and Pixel Sizes

In digital image processing the term magnification, which in conventional microscopy is the result of lens and ocular magnifications, loses its original significance, as a digital image may be printed in varying sizes or reproduced on displays of different sizes. However, the optical resolution of the lens, mainly determined by the numerical aperture (NA) of the lens, remains important.



The same image recorded by a camera with a different number of pixels.

Resolution
In microscopy, the term resolution refers to the minimum distance dmin that two high-contrast objects may have to remain recognizable under the microscope as two individual objects without merging into one object in the image.

For the conventional light microscope under optimum lighting conditions the resolution is determined by the wavelength of the used light λ (for calculations 550 nm is generally used) and by the numerical aperture NA of the used microscope lens.

The Rayleigh criterion: $d_{min} = 0.61 \cdot \lambda / NA$ applies.

The term resolution is also used to describe digital camera sensors. It is often used to indicate the number of pixels on a sensor. A camera may, for example, have a resolution of 8 megapixels, meaning that the image, which the optics projects onto the sensor, is then resolved into 8 million pixels on the sensor. Applying this terminology sensors are specified using ‚resolutions‘ between 1 megapixel and 32 megapixels.

More meaningful is, however, the indication of the pixels resolution. If one traced the edge length of a camera pixel backwards through the optics onto the object, the pixel resolution indicates the corresponding length of a distance on the object that one camera pixel should ideally cover.

For a microscope camera, the pixel resolution for any of the used lenses in the microscope should be higher than the optical resolution of the lens. In fact, a pixel resolution at least three times as high as the optical resolution of the lens is recommended.

For microscope lenses numerical apertures range between 0.1 (typically for a 4 times magnifying lens) and 1.3 (typical for 100×) – corresponding to an optical resolution of 3.4 µm to 0.26 µm, for which the smallest optically resolved structures provide a sufficient number of pixels.

If for example you need lower magnifications, you will require a camera with a correspondingly high resolution to achieve an optimum recording and reproduction of all details of the microscopic image. Here resolutions of more than 5 megapixels may indeed be useful. For large magnifications, however, such a high resolution does often not deliver any additional image information due to the limitations of the optical system. Depending on the application, 3 to 5 megapixels are often quite sufficient.

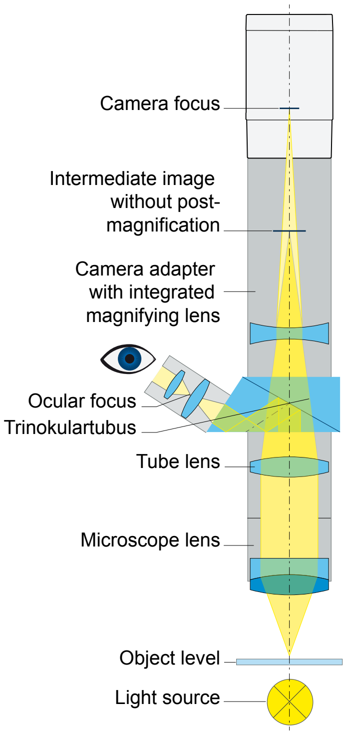
The pixel size also matters. For very small pixels, the optics has limitations regarding the smallest resolvable structures. For monochrome sensors pixel widths of 5 µm or more and for color sensors pixels starting with a side length of 2.5 µm are reasonable options.

Sensor Size and Field of View (FOV)

Is larger always better? An advantage of a large sensor is its large surface onto which more pixels can fit, which produces a higher resolution. The real benefit here is that the individual pixels are still large enough to ensure a good signal-to-noise-ratio – unlike on smaller sensors, where there is less space available and thus smaller pixels must be used (please refer also to section, Pixel size and sensitivity‘).

However, how large does the sensor actually need to be? Here, in addition to the required pixel size and number of pixels mainly the optimum reproduction of the field of view plays an important role.

How to adapt a digital camera to a microscope



Significantly simplified-schematic presentation of the adaptation of a digital microscope camera to a light microscope. Decisive for a perfect reproduction of the object are among others the correct position of the image level as well as the correct size of the field of view.

Depending on microscope type and camera, there are various different camera adapters on the market, for example to plug into the ocular shaft or as shown here to fit to the trinocular tube, typically for C-mount types, but also for CS mounts or bayonet fittings.

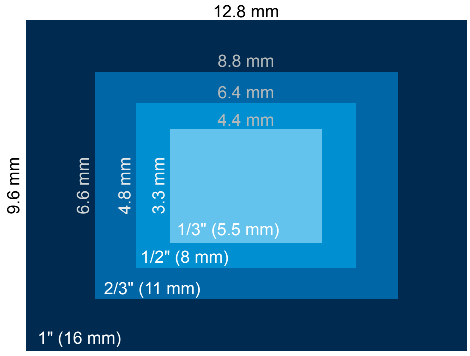
For digital microscope cameras, sensor sizes are typically between 1/3″ to 1″. Often an additional de-magnifying or post-magnifying intermediate optic integrated in the camera adapter is required to transfer the optimum image section.

Using a microscope, an image is created in two steps: the lens of the microscope creates – if necessary together with a tube lens in the microscope - an intermediate image. The user views this intermediate image through the ocular. The total magnification results from the magnification of the lens and that of the ocular. Due to physical and technical limitations, the lens is only able to create an intermediate image with full image sharpness in a limited area. The diameter of the round lens image, to which this is applicable, is called field of view width of the lens.

The image of the lens is again magnified by the ocular. The maximum diameter of an area, which the ocular is still able to magnify clearly, is called field of view of the ocular. The field of view of the ocular and field of view diameter of the lens should be adapted to each other to achieve the optimum reproduction performance of the ‚analog‘ microscope. The field of view of the ocular (field-of-view number) indicates how large the field of view of the lens for a specific microscope is. Common field of view numbers for oculars are for example 18, 20, or 22 mm. However, in practice fields of view diameters with more than 23 or 25 mm are also common.

By adapting a microscope camera to the microscope using a matching trinocular tube (mostly using a C mount connection) or the ocular holder, one bypasses the optics of the ocular and views the intermediate image of the lens directly through the camera.

Thus, the field of view diameter of the lens is the decisive value for the microscope camera, because the image created through the microscope lens is directly reproduced onto the sensor of the microscope camera. Consequently, the sensor size of the microscope camera should be optimally matched to the field of view diameter. – On the one hand to reproduce the maximum section of the round field of view onto the angular sensor, and on the other hand to prevent reproduction errors on the immediate edge of the field of view. This means, the image diagonal of the sensor should be a little bit smaller than the field of view diameter of the lens so that all areas of the sensor image may be reproduced with optimum sharpness.



Many sensors with varying image diagonals are available on the market. The illustration shows the most common sensor sizes. The size indication for sensors in inch is due to historical reasons and follows a convention that reflects the diameters of early image recording tubes. Following this convention, one inch does not equal 25.4 mm, but approx. 16 mm.

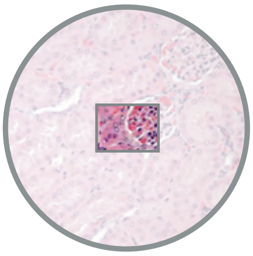
For many standard microscope optics, CCD and CMOS sensors with a size of 2/3″ offer a good compromise between magnification, resolution, and the size of the image section of the microscopic field of view.

Sensor size and field of view

Image section recorded using an adapted digital camera with defined sensor size compared to the image visible through the ocular:

- Lens magnification 10×
- Ocular magnification 10×
- Field-of-view number of the ocular 18
- C mount adapter with magnification factor 1

Under these conditions, the visible part of the intermediate image transmitted by a 1/3″ sensor is only 7%, whereas a 1″ sensor reproduces approx. 50% of the field of view.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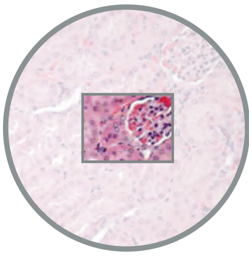
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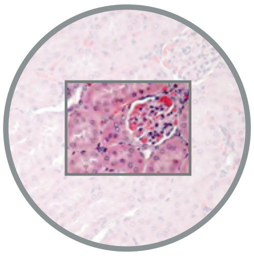
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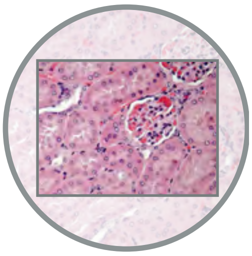
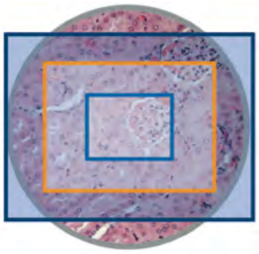

1"

If a significantly smaller or larger sensor is selected, the field of view of the lens needs to be adapted to the sensor using an adapter integrated in the intermediate optics, meaning de-magnifying or post-magnifying accordingly.

How to adjust sensor size and field of view



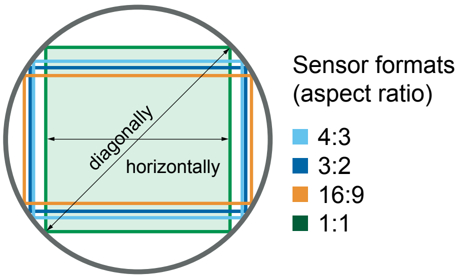
Optimizing and adjusting the sensor size and field of view using a C mount adapters with integrated intermediate optics:

- Lens magnification 10×
- Ocular magnification 10×
- Field-of-view number of the ocular 18
- Sensor sizes 1/2″ (inner image section) and 4/3″ (outer image section)

The percentage of the visible intermediate image transmitted by a 1/2“ sensor is without adaptation approx. 12 % (inner image section). The image section may be optimized (medium image section) accordingly using a C mount adapter with integrated de magnifying optics (0.65x or as in this case 0.5x). Sensors that are significantly larger than 1“ (outer image section) and extend beyond the edge of the field of view, may be adjusted to the field of view using a corresponding adapter with integrated magnifying optics (for example 1.2x or 1.6x).

Most common and perfectly suitable for microscopic applications are sensors with an aspect ratio of 4:3. Sensors with an aspect ratio of 1:1 reproduce the maximum section of the visually visible intermediate image of the lens. Other sensor formats such as 3:2 or 16:9 may also be used, but reproduce a smaller section of the field of view as they have a lower image height than 4:3 sensors, given the number of pixels is the same.

HD (high-definition) or Full HD formats, by now fully expected as standard for film and television, remain somewhat disputed for microscopy, but they are perfectly suitable, for example, to project live images to large monitors for discussion. Combining video technology and digital image processing, highly dynamic processes may be viewed, studied, and analyzed easily.



Pixel Size and Sensitivity
As previously discussed, the number of pixels or rather the resolution of an image sensor plays a key role for the information content and quality of the microscopic image. A general trend towards the maximum possible resolution is obvious, but a higher resolution also means smaller pixels. Generally, larger pixels are attributed a higher sensitivity than smaller pixels. Ultimately, the optimum combination of image noise, signal-to-noise ratio (SNR), saturation capacity, and the related dynamic range need to be selected specifically for every application.

Image Noise: Various noise effects, such as for example dark noise, photonic noise, or digitization noise may overlay the signal and thus have a negative effect on the digital image. In highly sensitive sensors, the image noise is particularly noticeable if the measuring signal is very low at low light. Here the detection limit is decisively determined by the dark noise that is caused by thermal effects, meaning the heat generation at the sensor. These thermal effects may be significantly reduced through passive or active cooling of the sensor, for example by using airflow, water, or a Peltier element (please also refer to section ‚cooled sensors vs. non-cooled sensors‘).

Saturation Capacity: The saturation capacity or also the full well capacity describes the maximum number of electrons that one individual pixel is able to absorb. Electrons are created by light striking the sensor.

SNR: Large pixels have a higher saturation capacity than smaller pixels. That means they have a larger capacity for electrons that are generated in the pixel by the light. This in turn means they are able to process more light.

A high saturation capacity means a better signal to noise ratio, which in term means the larger the pixel surface, the better the signal-to-noise ratio (SNR). The signal-to-noise-ratio indicates the factor by which the camera data share with the actual image content differs from the unwanted noise signal. A higher SNR translates into better image quality.

Simplified: The more pixels the sensor of your camera has for a given sensor size, the more unfavorable, meaning the smaller, the signal-to-noise ratio is.

Dynamic Range: After defining the lower and upper limits of the sensor responses to the light, we may finally explain the missing term ‚dynamic range‘ (DR): It is defined by the ratio of saturation capacity (full well capacity) to the detection limit.
Differently said: The dynamic range is the ratio of the lightest and the darkest values that a pixel is able to detect as a real response to incoming light. If your application requires a high dynamic range, a sensor with correspondingly larger pixels is recommendable.

Today, pixel, sizes between 3.5 µm and 6 µm offer the same output for what one needed 10 µm pixels before: a good compromise between light sensitivity and high resolution. High-resolution and favorable sensors have pixel sizes of 2.2 µm to under 1.4 µm. They offer a high pixel resolution but a lower light sensitivity due to their small surface.

The sensitivity of a sensor is particularly important for low-light applications, such as for example in fluorescence microscopy. Under such difficult lighting conditions, the camera needs to have a good signal-to-noise-ratio to still be able to detect very low light signals and nevertheless ensure a good image quality.

4. Cooled CCD/CMOS Sensors vs. Non-Cooled CCD/CMOS Sensors in Microscopy Applications

Any sensor generates a so-called dark noise or dark current. The heat generates electrons in the pixel on the sensor chip that mix with the electrons generated by light causing a disturbance to overlay the required image information and to distort the image content. Additionally, the dark current and the noise differ for non-cooled sensors depending on the sensor temperature. This causes fluctuations depending on the ambient and operating temperatures of the sensor, which heats up during operation. In case of long-term exposures, voltage and temperature of the sensor increase as well, which in turn is interpreted as pixels and visible as noise.

The dark current may be reduced by cooling the sensor. Reducing the sensor temperature by 7° Celsius compared to the ambient temperature reduces the dark current approx. by half, assuming the image signal remains the same.

Here new sensor technology promises significant advantages, this includes for example the new generation of CMOS sensors. A lower power consumption and optimized electronics mean these sensors offer a significantly improved noise performance.

Digital cameras with non-cooled sensors are perfectly suitable for many standard microscopy application delivering outstanding image quality and fulfilling all image processing and analysis requirements.

Cooled sensors are the right choice if particularly long exposure times are required under difficult lighting conditions or if particularly constant and reproducible results are required for image analysis or for further processing. Such requirements are prevalent in, for example, the sophisticated applications of modern fluorescence microscopy, in In vivo bioluminescence applications or in astronomy.

5. Interface

Generally, you as user of the camera often have the choice between several interfaces. The most important suitable interfaces are USB 2.0, USB 3.0, FireWire, Gigabit Ethernet, or Camera Link.

USB 3.0 stands out more and more as new simple standard for numerous applications. USB 3.0, also known as Super Speed USB, is the next generation of the popular “Plug & Play” Universal Serial Bus specification that builds on the strengths of USB 2.0 and irons out its weaknesses. Already today, USB 3.0 is available in standard PCs as standard interface without additional hardware. In the near future, USB 3.0 will displace FireWire and 2.0 from the range of available interfaces. FireWire is already no longer supported as standard by some operating systems. However, one will still be able to run a USB 3.0 camera on any commercially available PC without additional interface hardware in the future.

The quick interface is real-time compatible. Furthermore, the technical implementation of the USB 3.0 interface saves processor resources thanks to the direct transfer of image data onto the main memory. This free processor capacity can be used by the image processing application. This in turn allows for more complex processing steps and a quicker presentation of the results to the user.

A further strong point for the gigabit Ethernet interface comes into play when long cable lengths are required.

6. Summary

There is a wide product range to choose your microscopy camera for your specific application from. The range comprises both color and monochrome models with diverse sensor technologies, sensor sizes, resolutions, and frame rates.

In the future, cameras with CMOS sensors will play an important role in microscopy thanks to further development and a significantly improved performance. A CMOS sensor is not only cost-effective, compact and versatile, but also offers the benefits of a semiconductor sensor, without the occurrence of blooming or smearing effects. Additionally, newer CMOS sensors produce less noise. It is anticipated that CMOS sensors will replace CCD sensors in digital cameras in many application areas.

The following aspects may be important for the selection of the most suitable microscope camera:

- Authentic live images for smooth sample movement and easy focusing
- Highest image quality, color fidelity, and a good contrast range for documentation, image processing, and analysis.
- Excellent sensitivity for applications with low light levels
- Application characteristics determine potentially specific sensor types, for example: CCD, CMOS, NIR (near-infrared) optimized variants.
- The required pixel size and resolution for an optimum reproduction of the field of view affect the required sensor size.
- Size, weight, user comfort, compliance with software standards such as DirectShow, TWAIN, USB3 Vision, GenICam, depending on the predecessor system and intended universality.

Tips for Selecting the Camera:

- Color cameras may be used universally. If you want to use a color camera, make sure that the camera offers color profiling.
- For sophisticated fluorescence applications consider monochrome cameras as those generate less noise.
- Opt for a high resolution if you want to reproduce large sections of the sample with low magnification, for example, to use the images for documentation and archiving purposes.
- Select a suitable camera microscope adapter to make optimal use of the field of view.
- If your main objective is to view samples on a screen then the image quality of the live images and the speed of the camera are important.
- For standard microscopy applications, cameras with lower frame rates are often sufficient.
- Often the image quality may be significantly improved using suitable software. Always use the latest software version for your camera.

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Dr. Kristina Lotz is Market Manager Medical for Basler AG. Ms. Lotz graduated in molecular biology and began her career at Basler in Market Management in March 2013.

In her current position, she is responsible for the development of the vertical medical and life science market with its diverse applications and specific requirements. She looks after the Basler customers from this market and is responsible for the use of the broad camera portfolio in known application areas and the expansion into new segments.

About Basler

Basler is a leading manufacturer of high-quality digital cameras for applications in factory automation, medicine, and traffic monitoring. Product development is led by the demands of industry. The cameras offer simple integration, compact sizes, excellent image quality, and an outstanding price/performance ratio. Basler boasts more than 25 years of experience in image processing. The company is home close to 500 employees at its corporate headquarters in Ahrensburg, Germany and its subsidiaries in the USA, Singapore, Taiwan, China, Japan, and Korea.

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