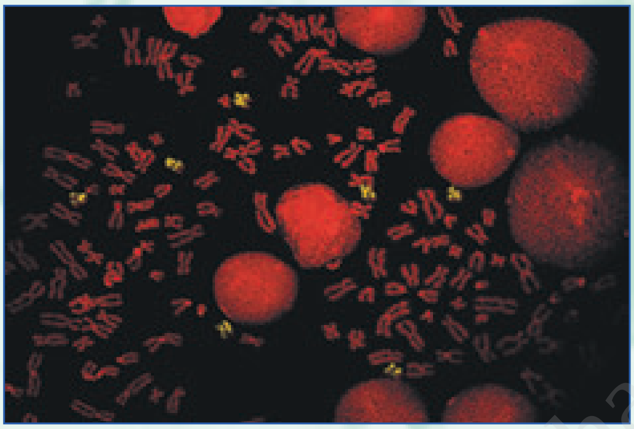
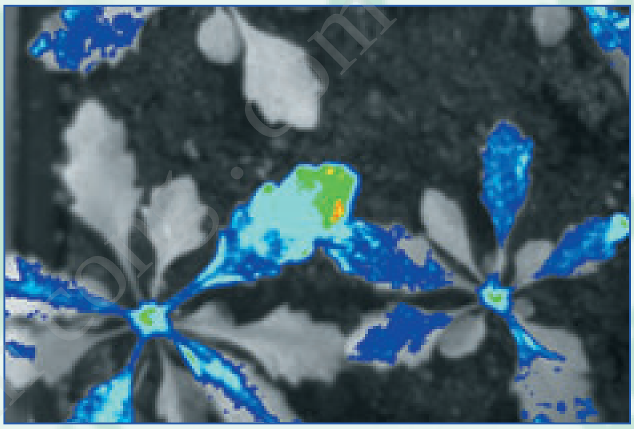
BERTHOLD TECHNOLOGIES

detect and identify





Glossary

Optical Digital Imaging

Glossary Optical Digital Imaging

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A

A/D conversion
see → **Bit Depth of Camera Data**

Animal bed
is needed to fix and anesthetize an animal in such a way to perform → **multimodality imaging** in different scanners.

Aperture
see → **f-stop**

B

Back-thinned or back-illuminated CCD chips
are a rather expensive and delicate type of chips for high-end scientific-grade → **slow scan CCD**, → **iCCD** and → **ebCCD** cameras. By etching down to about 10 – 15 µm the illuminated back is thinned so that it becomes transparent. These sensors have a substantially improved sensitivity over the entire spectral range as compared to standard or front-illuminated CCD chips. Between 450 and 650 nm the quantum efficiency may exceed 90 %.

A substantial downside of this chip type is a readout noise that is usually considerably higher than that of standard chips even at slow digitization speed. The dark noise can be higher in cases as well.

Bandpass filters
transmit with high efficiency a particular wavelength band while rejecting by absorption or reflection the out-of-band energy. They are the simplest and most cost-effective way to transmit a well-defined band of light. Compared with a grating monochromatic system, the cost of bandpass filters is usually significantly lower, and performance is comparable, if not better, in terms of in-band transmission and out-of-band rejection.

Binning
is the combination of neighbouring CCD pixels during readout. The result is an increased signal and thus an improved sensitivity and a better signal-to-noise ratio, however, at the cost of loss in spatial resolution. The binning factor is adjustable and usually square (2x2, 4x4...) to avoid image distortion.

BLI
stands for **B**io**L**uminescence **I**maging, where the reaction is catalyzed by luciferases, an enzyme class, which can be coexpressed in a reporter gene assay. In contrast chemiluminescence are those reactions which are catalyzed by a chemical reaction.

BFI
stands for **B**io**F**luorescence **I**maging and uses the natural occurring fluorescence proteins like GFP, eGFP or YFP, which can be coexpressed in a reporter gene assay. In contrast chemifluorescence is done with all the non-protein labels (see also → **fluorescence reflectance imaging**).

Blooming
occurs, if the illumination exceeds the → **full-well capacity** of the photodiodes in bright areas of the image. Excess charges will overflow into adjacent wells and erroneously cause high intensities of the corresponding image pixels. This is a clear hint that the image is overexposed.

Bit Depth of Camera Data
refers to the digitization of the analog light signal by the analog-to-digital (A/D) converter of the camera, which is the binary range of the possible grayscale values of the produced images. Common bit depths are 8 to 16 bit, which equals 2^8 = 256 to 2^{16} = 65536 gray levels.

The human scotopic vision (night vision) can distinguish in the order of 50 gray scales. Computer monitors are able to display 8 bit digital grayscale images. 16 bit images are reduced to 8 bit upon display.

Bit Depth of Digital Images
gives the number of possible gray scales (intensities) an image file format allows. An 8 bit number comprises 8 digits that are either "0" or "1". Up to 256 different gray values can be listed with 8 bit numbers because with each bit having one of the two values there are 2^8 = 256 possibilities. In the decimal system they represent the values 0 - 255. Similarly, in 16 bit format 2^{16} = 65536 different gray values can be stored.

C

Camera Resolution

is obviously an important parameter for the selection of a CCD camera. Digital images consist of → **pixels**, which represent miniature photodiodes on the CCD chip. Each photodiode integrates the intensity of its area. It is easy to understand that the image resolution depends on the total number of pixels it is composed of and corresponds to the number of photodiodes on a CCD chip of a certain size.

It should be noted very clear, that even if small CCD pixels improve the resolution, they also reduce the → **dynamic range** of the device, because the → **full-well capacity** depends also on the size of the photodiodes.

Furthermore, the average signal per pixel is directly dependent on the size (area) of the pixels. This is the reason why luminescence and fluorescence applications can not be optimized with one type of CCD camera, since big pixels are best for luminescence, small pixels best for fluorescence.

Camera Noise

comprises → **dark noise** and → **readout noise**.

CCD

stands for "charge-coupled device".

CCD Chip Types

are manufactured nowadays in a broad variety for different purposes and applications, see therefore
→ **Back-thinned CCD chips**
→ **Full-frame CCD chips**
→ **Frame-transfer CCD chips**
→ **Interline-transfer CCD chips**
→ **Electron Multiplying CCD (emCCD) chips**

CCD Chip Size

is often given in an inch measure: 1/2" chip, 2/3" chip, etc. This does not refer to the chip diagonal in inch but has historical reasons. It relates the field-of-view of the camera to that of former tube cameras.

CMOS Chips (APS)

In an (Active Pixel Sensor) **C**omplimentary **M**etal **O**xide **S**emiconductor chip each photodiode (pixel) is coupled to an individual amplifier. Also there is one A/D converter for each column of pixels so that the data of a row of amplifiers can be read out in parallel. The individual amplifiers lead to an overall reduction of noise, however, fixed pattern noise artefacts are induced, at least in early generation CMOS sensors, because of variations in amplifier gain and offset across the chip.

Advantages of these devices are the possibility of pixel-wise gain manipulation and true region-of-interest readout. A fundamental difference to CCD chips is further that here incoming photons remove electrons from charged capacitors instead of creating electrons in empty ones. A consequence is relatively high noise at low light levels which is one of the reasons for which CMOS cameras are not widespread in this field.

C-mount

is a mechanical adapter between camera and lens. The mechanical definition of a C-mount is a 1 inch hole with 32 TPI (turns per inch) threading, female on the camera side, male on the microscope side. The optical definition of a C-mount is that the image reaches the → **focal plane** of the CCD at 17.5 mm beyond the shoulder of mounting ring.

Each microscope needs a special C-mount male adapter piece offered by the manufacturer. Cameras are always equipped with the same female adapter port for easy exchange.

Color Images

produced by computer monitors and TV screens are generated by pixelwise mixing of the three primary colors red (R), green (G) and blue (B).

This is called additive mixing of light. Digital images where the color information is encoded by intensities of the primary colors are commonly referred to as RGB images. The three light components are called color channels.

Contrast

is the variation of signal intensity relative to the average intensity (Δ I/I). Together with resolution it is the most important factor determining the quality of an image. Contrast and resolution are linked by the Contrast Transfer Function.

Conversion Factor

The A/D converter divides the electronic charges of the CCD pixels that are readout from the chip by a certain factor, the conversion factor, thus delivering a digital signal measured in counts (or gray levels). The maximum possible conversion factor would be the full-well capacity divided by the bit depth. However, often the conversion factor is somewhat lower than this.

Some cameras provide the option to reduce the conversion factor for low light level experiments, refered as change of gain. This way saturation is reached faster while the light-to-gray scale digitization is finer (fewer electrons per count) and the resulting intrascene dynamic range of the image is improved.
An example: A 12 bit camera with a full-well capacity of 18,000 electrons might have a standard conversion factor of 4. That means, saturation (4095 counts) is reached with 16,380 electrons; this would be the effective full-well capacity.

With a conversion factor of 2 the limit would be 8190 electrons, or half the signal. Consider an image with a background intensity of 1000 electrons and a maximum signal of 5000 electrons. With a conversion factor of 4 the resulting image would have an intensity spectrum of 1000 gray levels after background subtraction but 2000 if the factor is 2. The intrascene dynamic range would be twice as high.

CW fluorescence intensity

stands for Continuous Wave (CW) fluorescence intensity, sometimes referred as multi wavelength fluorescence imaging, but is identical with → **fluorescence reflectance imaging**.

D

Dark Noise (Thermal Noise)

is caused by current fluctuations on the photodiodes in the absence of light arising from thermally generated electrons. The dark noise is the geometric mean of dark current and integration time. It is temperature dependent, this is why many CCD cameras are actively cooled, for example by Peltier elements. Roughly, a 6-8 °C decrease in temperature reduces the dark current by about half.

Data Readout Rate

is usually the rate limiting step in analog-to-digital signal conversion. The conversion rate ranges somewhere between 1 and 20 MHz (pixels per second) for 10 - 16 bit converters (16 bit data) in most cameras. For very low → **readout noise**, the data readout rate is sometimes set to 100 kHz.

Depth of Field or Depth of Focus (DOF)

is defined as the difference between the closest and farthest distances an object may be shifted before an unacceptable blur is observed. DOF should not be confused with → **working distance**.

Digital Image files

are based on tables that list the intensities, or "counts", of all pixels. The values are stored in the binary system as 8 or 16 bit numbers; see also → **Bit Depth**. Which pixel count gets which gray value or color in the image display assigned, is defined in look-up tables that are edited upon changing the contrast or blanking out background etc.

Displaying Digital Images

The on-screen appearance of digital images, the way the data is displayed normally, is defined by the display palette or look-up table. It relates the different gray scales or colors to the range of pixel intensities that occur in the image.

Most image processing programs offer an autoscale function which adjusts the gray scale palette so that the darkest pixel of an image is set to black and brightest to white. The contrast of the image display can further be optimized by changing the slope of the gradient or any background intensity be blanked out by shifting the black threshold upwards.

Monochrome images can also be displayed in pseudo-colors, for example in order to appear as observed through the eyepiece of a microscope. Instead of a black and white palette, for example a black and green palette can be used.

Often false color palettes are used because of the increased contrast they offer. This is due to the ability of the human eye to better distinguish colors than gray scales. A popular such palette is the black-blue-green-red pseudo-rainbow. Note that with the same slope of the gradient as above, the weaker details of the image are better visible.

Distortion

is an optical error (aberration) in the lens that causes a difference in magnification at different points, within the image.

Dynamic Range

of a CCD camera (DR) is the maximum signal (which is directly related to the → **full-well capacity** (FWC)) divided by the → **camera noise** (CN), the combination of the dark and readout noises: DR = FWC/CN. The higher the dynamic range the more reliable is the quantification of differences between the dimmest and the brightest intensities of an taken image.

The dynamic range is sometimes measured in decibels (dB) (DR = 20 x log (FWC/CN).

The dynamic range of acquired image data is maximum intensity divided by total noise. At high light levels it is practically limited by the signal noise, that is the square root of the maximum signal of the image (prior to digitization).

The intrascene dynamic range of an image (intensity range, difference between brightest and dimmest pixel) is determined by camera gain, exposure time, sample staining, microscope settings, background intensity, display palette and more variables and can be represented by the intensity histogram.

E

Electron-bombarded CCD (ebCCD) cameras

is a type of intensified cameras that is kind of an intermediate between CCD and ICCD cameras. Like ICCDs they contain a photocathode to generate photoelectrons but do not feature an MCP.

The photoelectrons are accelerated by a high voltage gradient (1.5 - 2 kV) before "bombarding" a back-thinned CCD chip. Unlike photons, which, depending on the QE, are converted into one electron at the most when collected by a photodiode of a CCD chip, the impact of an accelerated electron generates up to several hundred new charges.

This is only a modest gain if compared with iCCDs but in general ebCCDs have a higher spatial resolution and a better signal-to-noise ratio.

Electron Multiplying CCD (emCCD) cameras

This is a new on-chip gain technique that can be applied to all current chip types. Such chips feature an additional gain register inserted between shift register and output amplifier through which all electrons are moved serially upon data readout.

In each charge, transfer step electron multiplication occurs upon impact ionization caused by electrodes with higher voltage amplitude than is necessary for the transfer alone. While the multiplication factor per step might be low, the huge number of steps during serial readout leads to a significant gain.

For example, 0.5% gain per step leads to a 165-fold signal increase for 1024 pixels per line. Besides voltage and number of transfer steps, the gain factor is also dependent on the chip temperature. The lower the temperature the more probable is the generation of secondary electrons.

The fundamental difference to intensified CCDs is that the photoelectrons are multiplied prior to reaching the CCD chip, while here the gain is achieved on-chip. Because it is done before readout the read noise is not affected and consequently the signal-to-noise ratio enhanced significantly.

On the other hand, dark charges are multiplied together with the photoelectrons, however, the cooling of the CCD keeps this factor low. A certain additional noise factor arises from the probabilistic nature of the secondary electron generation and the uncertainty that goes along.

An advantage over intensified CCDs is that there is no risk of hardware damage due to overexposure. The spatial resolution is the same as for an analogous standard CCD and is not reduced by a photocathode or MCP.

Entocentric Lenses

are designed to enhance perspective, i.e. close objects appear larger than distant objects.

F

Field of View

is the area of the object that will be viewed on the monitor.

Fill Factor

is a measure of the space between the pixels, which are not photon-sensitive. Todays full-frame chips are close to 100 percent, which means there is nearly no "empty space" between the diodes and no incoming photons are lost.

Filters

are often used in imaging for different purposes and applications. Refer therefore for

→ **Bandpass filters**
→ **IR-cut off filters**

FireWire

see → **IEEE-1394**

Fluorescence Molecular Tomography (FMT)

FMT, supported by the company VisEn, images the animal, collects data sets from multiple points of view and captures on the order of 50,000-100,000 source-detector pair measurements within a scan time on the order of 2-4 minutes. Due to such data set the light source is reconstructed by software showing the size and the depth of the light source.

Fluorescence Reflectance Imaging

is the typical set of illumination to excitate the sample from above. The signals which are emitted from the sample are measured by a camera also positioned above the sample. The proper set of filters have to be choosen to excite the fluophor and measure its emission.

F-mount

is another mechanical adapter between camera and lens. The mechanical definition of a F-mount is that of Nikon lenses. The optical definition of a F-mount is that the image reaches the focal plane of the CCD like in Nikon SLR-cameras beyond the shoulder of mounting bajonett.

Focal length

is defined as the distance between lens and focal plane when the lens is set to infinity.

Focal plane

is the distance between shoulder of mounting ring and CCD chip. There are a variety of mechanical adapters or → **Optical Interfaces**.

Frame-transfer CCD chips

use two-part chips in which one half is exposed and collects photons while the other is used for temporary data storage only and masked to protect it from incoming light. During the exposure of an image, the data of the previous image are readout from the storage array via the serial shift register through an output amplifier and A/D converter.

Once exposure and readout are completed the newly accumulated charges are very rapidly moved from the light sensitive half to the emptied storage array; this is termed the "frame transfer". Afterwards the cycle can be repeated and the next image acquired. A disadvantage of this principle is the possibility of charge smearing during the parallel transfer if the light influx is continuous throughout.

F-stop (f/#)

also called → **aperture**, is a measure of the light gathering ability of a lens, which is the diameter of lens divided by the focal length. The smaller the f/#, the "faster" the lens. The smallest f/# and the → **focal length** specifies a lens.

Full-frame CCD chips

consist of a high-density array of photodiodes that convert the incoming photons into electrical potentials. The → **fill factor** is close to 100 percent, which means there is nearly no "empty space" between the diodes and no incoming photons are lost. After image exposure the data readout is performed by shifting the charges in a parallel fashion one row at a time to the serial register.

The serial register then shifts each row of information sequentially to an output amplifier before it is directed to an A/D signal converter.

Unless mechanical shutters or synchronized illumination is used for exposure, smearing artifacts occur because the photodiodes are being continuously illuminated during parallel register readout.

Full-Well Capacity

is the maximum amount of charges (electrons) the photodiodes of a CCD chip can accommodate. It is also called well depth and usually ranges from about 10,000 to 350,000.

The linear full-well capacity is the point where the linear relationship between the amount of incident light and the charges it originates is lost, i.e., when the pixel well nears saturation resulting in a degradation of the signal.

G

Gray scale

refers to the number of possible intensities (gray values) an image file format allows. With an 8 bit A/D conversion the gray scale include values between 0 - 255, because with each bit having one of the two values there are $2^8 = 256$ possibilities. Similarly, in 16 bit format $2^{16} = 65536$ different gray values can be stored.

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H

Histogram

A histogram is the graphical version of a table which shows what proportion of cases fall into each of several or many specified categories.

The categories are usually specified as non overlapping intervals of some variable.

I

IEEE-1394

also called FireWire, is a modern plug-and-play interface between PC and camera and allows the connection of several cameras to one board without the need of a frame grabber. It is sort of a high-speed serial memory bus capable of operating at 400 Mbit/s. 1394 refers to the total bus bandwidth.

The bandwidth being 30 times larger than that of USB makes it more suitable for the high-speed data transfer desirable for digital imaging.

Image File Formats

There is a vast variety of data formats, some of which are relatively universal, others are specific for certain software programs or operating systems. The most widespread format may be the "tagged image file format" or TIFF (*.tif). There are many derivatives of this format which quite often causes the problem that a software is unable to open a file that was created with a different program.

Other common formats are bitmap (*.bmp) and windows metafile (*.wmf). Postscript (*.ps) is a page description format with the necessary information for printers. Encapsulated postscript (*.eps) may contain CMYK, gray scale, vector or compression data and consists of a postscript code that is independent from the type of output device. Such files can be read by many programs but often not further processed.

Certain formats (*.gif, *.jpg, *.png) are used to compress images in order to reduce storage space and download time for use in the world wide web. These formats employ different algorithms to store sequences of pixels with repeating patterns as one piece of information instead of pixel by pixel as in the uncompressing formats.

The "graphic interchange format" (GIF) can render the entire color range of 24 bit RGB images but one image may only contain 256 colors at the most (8 bit). Otherwise the compression occurs without information loss. It is most useful for graphics with sharp contours. A useful feature is that one color can be set transparent. The format was invented for the online service CompuServe and there are patent issues to be considered.

The JPEG format ("joint photographers expert group") changes the colors of the individual pixels assuming that the eye is unable to resolve certain details anyway. The compressing factor is adjustable so that the user has direct influence on the quality of the image - and on the data size. Transparency of colors is not possible.

Many consumer cameras offer this format as an option. The compression is very effective but because of the loss of information this file type is not useful for quantitative imaging but probably serves best for standard photography.

The "portable network graphic" (PNG) was developed especially for the use in websites as a patent free alternative to GIF. PNG supports not only truecolor and reyscale images (like JPEG) but also palettized images (similar to GIF). This means that an individual 8 bit palette is stored along with the image and each pixel refers to one of the colors in this palette.

Thus not each pixel has to carry three-channel information and the overall amount of data is reduced. Shades of transparency can be achieved via storage of an additional alpha channel whereas GIF supports only binary transparency.

The compression is without loss of information for truecolor images which however renders it less effective with respect to data reduction as compared to the "lossy" JPEG compression.

This is especially the case for landscape photography, for example, while for graphics and rather uniform images the compression factor tends to be higher.

The "fts" format was developed by NASA to save more information with the image. Since there is no editor to manipulate the pictures, this file-format is very save due to GLP.

Intensified CCD Cameras

are divided in two general types of cameras used for image intensification. In the most common intensified CCD cameras, the iCCDs, an intensifier unit is optically coupled to a CCD chip via relay lenses or fiber-optic tapered bundles.

The second type are the → **electron-bombarded CCD cameras (ebCCD)** where the photoelectrons are merely accelerated (but not multiplied) before reaching the CCD chip. iCCD cameras are basically full-performance CCD cameras optically coupled in two possible ways (see below) to an intensifier.

A so-called proximity-focused intensifier (or wafer tube) consists of an entrance window, a photocathode, a microchannel plate (MCP) electron multiplier and a phosphorescent output screen. The photocathode converts the photons into electrons via the photoelectric effect.

The quantum efficiency of the conversion is an important parameter and depends on the coating material which differs in the different generations of intensifiers (see below). The photoelectrons are driven to the MCP which is set under a field of several hundred volts. The MCP contains millions of parallel channels with a diameter of about six micrometers in the newest generations.

The channels are coated with a secondary electron emitter which generates more electrons when hit by passing electrons. The intensification gain caused by the avalanche effect of multiple collisions is adjustable over a wide range up to several 10,000. The electrons are accelerated by a voltage of several kV upon exiting the MCP before reaching the phosphor screen where they are converted back into photons with an additional multiplication factor. The screen output light is then relayed to the CCD chip either by a lens or fiber-optic coupling.

The advantage of relay lens coupling is the possibility of constructing removable intensifiers that enable to easily convert the iCCD camera reversibly into a standard CCD camera or retrofit an existing camera. However, the → **light efficiency** is limited and causes a significant loss of signal and a reduced signal-to-noise ratio.

A much more efficient method to optically couple intensifier and CCD chip is through a fiber-optic taper which, however, requires a far more sophisticated manufacturing process.

Such a taper is a bundle of microscopic fibers (2 - 3 microns in diameter) that guides the light from the fluorescent screen to the CCD chip. There are up to nine fibers per pixel usually machined directly onto the diode array. Each microfiber has a cladding to maximize light transmission and a stray-light absorbing coating to contain leakage and prevent the resulting contrast reduction.

The signal-to-noise ratio of iCCD cameras is usually lower than that of standard CCD cameras because thermal noise from the photocathode and multiplication noise from the MCP are additional contributions to the overall camera noise.

The low light detection abilities of intensified cameras also allow a very high time resolution. This is due to the fact that the devices can be switched on and off very fast. This so called gating is necessary to provide the time needed to move the data off the CCD chip while the influx of photoelectrons is temporarily stopped.

The switchable voltage difference between photocathode and MCP enables this. If the voltage at the MCP is more positive than accelerated at the photocathode, the photoelectrons are toward the MCP; if the MCP is more negative, the electron influx is stopped and the intensifier is gated off.

The relatively high resistance of the photocathode material does not allow gating faster than about 25-50 ns.

For gating faster than 2 ns, a nickel underlayer can be deployed to reduce the resistance, which, however, lowers the transmission and consequently reduces the QE significantly. With high-performance fiber-coupled iCCDs, single photon detection is possible. At higher light levels the gain can be adjusted to increase the dynamic range.

Main disadvantages of intensified cameras are the lower → **quantum efficiency** of the photocathode as compared to that of the CCD chip alone. Another disadvantage is increased noise and a limited intrascene dynamic range of the images because the wells fill much faster upon intensification due to the electron multiplication. iCCD cameras furthermore have a reduced spatial resolution caused by the microchannel plate.

Interline-transfer CCD chips

On interline-transfer chips each column of individual photodiodes has a light-shielded (masked) vertical transfer shift register directly adjacent to it. The parallel photodiode registers and interline masks are separated by transfer gates.

Similarly to frame-transfer CCDs, there are cycles of simultaneous photodiode exposure and charge transfer channel readout followed by very rapid interline charge transfer from the photodiodes to the emptied shift registers.

Interline-transfer chips are usually equipped with microlense arrays. There is a lens for every pixel to collect photons that would otherwise remain undetected by hitting the interline masks or transfer gates. These lenslet arrays increase the so-called photodiode fill factor by more then a factor of three.

These devices also include an "electron drain" to prevent electron overflow into neighbouring pixels by overexposure and the resulting blooming artifacts in the images. Furthermore, electronic shuttering is possible by switching the photodiodes voltage in order to prevent photoelectrons that are generated during off-times from reaching the transfer registers.

IR-cutoff filters

are used to cut autofluorescence of plastics e. g. in the range of 800 nm.

L

Lenses

project optically the image onto the front of a parallel CCD array.

Lenslets

see → **Microlenses** (on chip)

Light efficiency

is a function of transmission and inversely of the square of → **magnification** and → **lens f-stop**.

Look-up table (LUT)

By use of a Look-Up Table (LUT), image values are mapped to greyscale. The top of the LUT is initialized to white and the bottom to black, with greyscale linearly distributed in between.

M

MACU

stands for **M**ultimodal **A**nimal **C**arrier **U**nit, a special → **animal bed**.

Magnification (System)

is the total magnification from the object to the image on the monitor, which is the product of the → **primary magnification** and the camera-to-monitor magnification (the ratio between the monitor size and the sensor size).

Microlenses (on chip)

also called lenslets, are tiny little lenses above each pixel to collect photons that would otherwise remain undetected by hitting the interline masks or transfer gates. These lenslet arrays increase the so-called photodiode fill factor by more then a factor of three.

Multimodality imaging

is widely considered to involve the incorporation of two or more imaging technologies (modalities), for example, optical and nuclear medicine reporter agents or by performing on the same objekt optical imaging together with MRI, PET, SPECT or x-ray CT.

N

Noise

of a digital image consists of the → **signal noise** and the camera noise which again comprises → **dark noise** and → **readout noise**. The dark noise is usually very low and comparable in size with the readout noise only in uncooled cameras.

In case of Peltier-cooled cameras its contribution can be ignored even for very long exposure times. In general, the dominating noise factor in most experiments is the unavoidable signal noise, only for very low light intensities does the camera noise significantly influence the image quality.

O

Object Distance

also called working distance, is the measurement from objective lens to the object.

Optical Imaging

is used for those methods where a lens for imaging is needed, in contrast to MRI, PET, SPECT, CT or ultrasonic.

Optical Interface

describe the mechanical adaptation between camera and lens. The mechanical definition is various, but the optical definition is always connected with the → **focal plane**. Optical interfaces are standardized nowadays:

→ **C-Mount**: 1-32UN2A
NF-Mount: M 17x0,5
L-Mount: M 39x1/26"
→ **F-Mount** (Nikon lens bayonet)
U-Mount (M42x1)

P

Perspective Errors

also called parallax, is a phenomenon in conventional or → **entocentric lenses**, which causes a change in magnification as it moves in and out from best focus. Closer objects appear larger than objects further away. → **Telecentric lenses** optically correct for this occurrence.

Pixels

or picture elements are tiny squares on the CCD chip. Each pixel represents an area of the image and its intensity is linearly dependent on the charges generated by the incident photons in the corresponding photodiode of the parallel shift array of the CCD chip.

The more pixels in a given image area the higher the resolution of the image is. If a digital image is increasingly enlarged there will come a point, when the individual elements can be seen as separate dots – similar to graining in a silver halide photography – and the more pixels an image contains per area, the more it can be enlarged, before the separate pixels start to show.

The size of the image can be described by its dimensions in numbers of pixels, for example, 1024 x 1024 pixels.

Primary Magnification

is defined as the ratio between the sensor size and the → **Field of View** (FOV), which is correlated with the focal length of the lens and object distance.

Q

Quantum Efficiency (QE) of the camera

is a measure that describes the ability of the CCD to convert the photons that are collected by the photodiodes of the chip into electronic charges, usually given in percent. The QE is a function of the wavelength. Current high resolution chips peak around 550 nm (yellow-green) with about 85% QE equipped with → **lenslets** (on chip microlenses). Back-illuminated chips even top this with more than 90 %.

Quantum Efficiency (QE) of the reaction

The efficiency of a bioluminescent reaction is also described by the quantum efficiency. In this case the ability is meant to convert substrate molecules into photons, given in percent. The bioluminescent reaction of American firefly luciferase and luciferin has a quantum efficiency of 91%, where as bacterial luciferases can have as less as 15%.

Quantum Efficiency (Total)

is defined as the multiplication of → **quantum efficiency of the reaction** and → **quantum efficiency of the camera**.

R

Readout Noise

is the noise caused by the camera electronics, mostly by the on-chip preamplifier, upon quantification of the signal but also dependent on the A/D conversion rate. It is of significance only in low light applications.

Resolution (Total)
of an image is a function of → **camera resolution**, → **focal length** and → **working distance**.

S

Shading
is gradual noise and/or sensitivity variations across the CCD chip and the corresponding inherent intensity gradients in the images. The cause is a gradual mismatch of photodiodes and on-chip microlenses.

Signal Noise (Photon Noise, Shot Noise)
Light has an inherent noise that derives from the stochastic nature of the photon flux and equals the square root of the intensity. For a signal of 100 photons a noise of of 10 photons or 10% results, while for 10,000 photons it is only 1% (100 photons).

Since this noise is caused by the natural statistical variation of the light, it is not influenced by the camera design. Binning reduces the signal noise.

Slow-Scan CCD Cameras
CCD cameras are often termed slow-scan cameras because their standard frame rate is usually less than that of a video camera. The "scan" derives from the fact that the data readout is performed pixel row by pixel row.

System Magnification
is the total magnification from the object to the image on the monitor, which is the product of the → **primary magnification** and the camera-to-monitor magnification (the ratio between the monitor size and the sensor size).

T

TD optical molecular imaging
stands for Time Domain (TD) optical technology. In TD optical imaging, photons are classified based on the time at which they emerge from the tissue.

The generated time-of-flight distribution (generally referred to as a TPSF or temporal-point-spread-function) can be thought of as a statistical distribution of all possible photon paths between the point of illumination and the point where the light exits the tissue. This distribution can be used to recover the optical characteristics of the specimen and thus discriminate scattering from absorption.

Telecentric Lenses
are parallax-free, that is, the size of an object in the image does not vary with its distance from the lens. For this reason, telecentric lenses find ready application in inspection systems for gauging measurements of objects which may be positioned at variable positions from the camera.

The only difference in the appearance of objects will be how far they are in or out of focus, depending on the depth of focus currently set. Telecentric lenses always have a fixed working distance and field of view.

TWAIN
The TWAIN Working Group is a not-for-profit organization which represents the imaging industry. TWAIN's purpose is to provide and foster a universal public standard which links applications and image acquisition devices.

U

USB (Universal Serial Bus)
is a common I/O interface used to connect computers with all kinds of low-speed (1.5 Mbit/s) and full-speed (12 Mbit/s) peripheral devices (up to 127 at a time) including many cameras. However, for applications requiring high-speed data transfer such as fast imaging it is inferior to the IEEE-1394 (FireWire) standard. The new version 2.0 with 480 Mbit/s (high-speed) is 40 times as fast as USB 1.1.

V

Voxel
is defined as a picture element in 3-dimentional view.

W

Well Depth
see → **Full-Well Capacity**

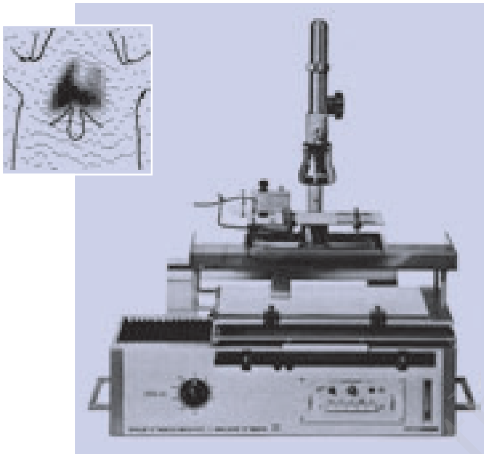
Working Distance
see → **Object Distance**

BERTHOLD TECHNOLOGIES

detect and identify

BERTHOLD TECHNOLOGIES, one of the pioneers of Molecular Imaging

BERTHOLD TECHNOLOGIES GmbH & Co. KG is located in Bad Wildbad, Germany. The company was founded in 1949 by Prof. Dr. Rudolf Berthold and was named "Laboratorium Prof. Dr. Rudolf Berthold". At the end of the 70ies BERTHOLD developed an animal imager based on a TLC scanner. The results are very rough due to poor resolution.



Since Siemens, Germany, developed a high sensitive intensified CCD camera, and Prof. Szalay became aware of it, the idea was born to monitor non-invasivly gene expression in living organisms.

BERTHOLD developed a cabinet for such a camera and the imaging software. In 1989 BERTHOLD introduced its first low light imaging instrument - the LB 980 Luminograph. The first in-vivo gene expression experiments in plants and animals performed on this instrument date back to the year 1993.

It turned out, that intensified CCD cameras had major problems in linearity and dynamic range, so, BERTHOLD introduced slow scan CCD cameras. NightOWL was launched in 1996 as the most sensitive molecular imaging system known for a long time. With NightOWL BERTHOLD made a substantial contribution to the breakthrough of this technology.

Today, a new generation of slow scan CCD cameras is developed based on enhanced sensitive chips. Furthermore, based on the microplate reader technology of BERTHOLD, in LB 983 NightOWL II fluorescence can be performed much better and easily due to high sophisticated lamp and beam control and automated filter changing.

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