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NightOWL II
LB 983 NC 100

NightOWL II LB 983 NC 100

Superiority in Molecular Optical Imaging

Inspired by nature

Bioluminescence imaging (BLI) and biofluorescence imaging (BFI) allow monitoring of gene expression in living organisms.

In 1989 BERTHOLD TECHNOLOGIES introduced its first low light imaging instrument – the LB 980 Luminograph. The first in-vivo gene expression experiments in plants and animals were performed on this instrument before 1993.

BLI utilizes light emitted by luciferase enzymes. Today bioluminescence markers can be tailored to any gene, enabling detailed research of gene function. BFI utilizes proteins, which fluoresce under illumination, either applied as exogenous reagents or endogenously expressed. Both BLI and BFI have contributed to the understanding of disease mechanisms and the development of new treatments.

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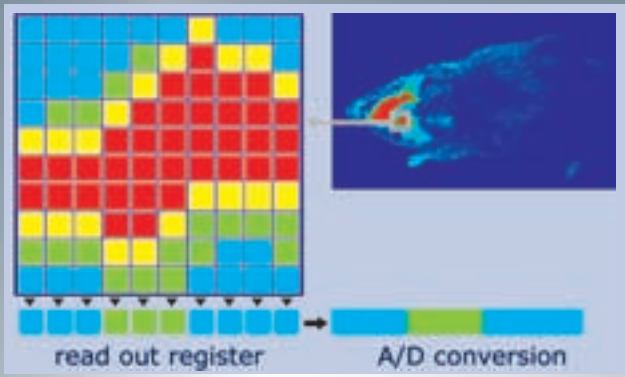
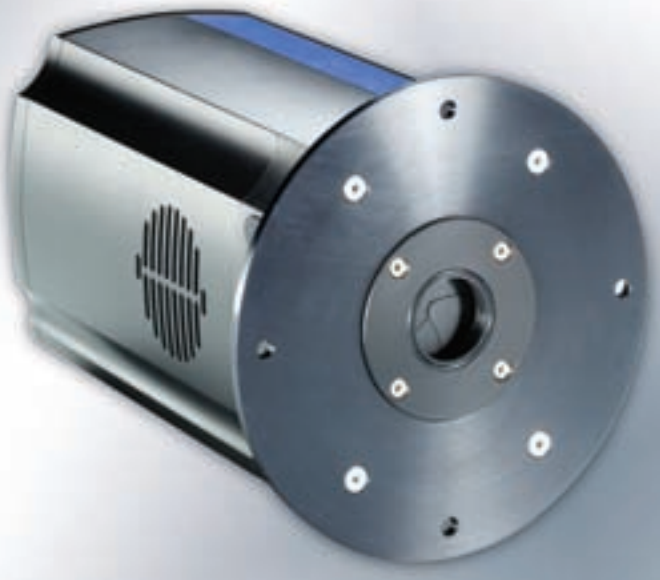
Full-frame CCD cameras

Images are optically projected onto the front of a parallel array acting as the image plane. The array takes the image information and partitions the image into discrete elements. Those elements are defined by the number of pixels thus "quantizing" the image. The resulting rows of image information are then shifted in a parallel fashion to the readout register that subsequently shifts the row of information to the output as a serial stream of data. The process repeats until all rows are transferred off chip.



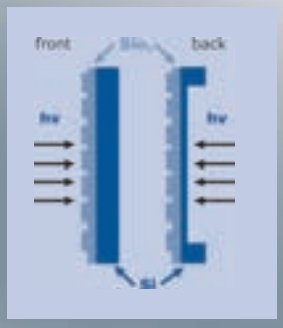
The image is reconstructed as dictated by the system. Since the parallel register is used for both image detection and readout, a mechanical shutter must be used to preserve image integrity.

This technology allows reliable image quantification, which is vital for comparative research.



NightOWLcam NC 100

is an ultra sensitive back-illuminated (or backlit) CCD camera with midband coating enhancing the quantum efficiency up to 90% in the spectral range between 500 – 660 nm, which is optimal for firefly luciferase, GFP and its derivatives.

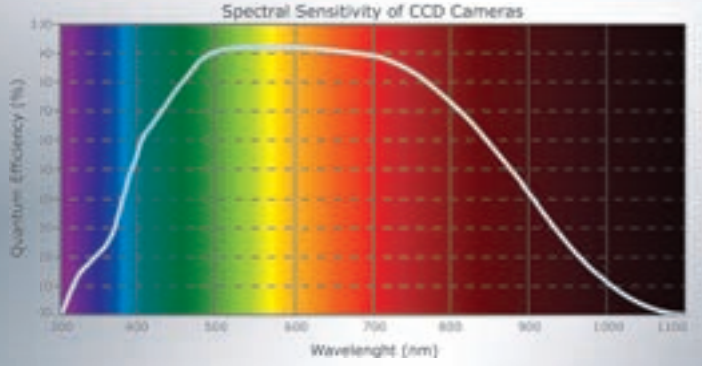


Back-illumination refers to a method of preparing the CCD sensor in a way that the photons directly strike the light-sensitive thinned back surface, in contrast to conventional CCDs where photons pass through non-light sensitive elements on the front of the CCD with a resulting loss of efficiency.

Today, these systems also have very low noise, and long exposures can therefore be used to integrate the signal over time and to obtain a usable signal.

Noise of a digital image consists of the signal noise and the camera noise which again comprises readout noise and dark noise, which is directly linked to the temperature. Efficient cooling of the array (absolute -80 to -90°C depending on the room temperature) ensures lowest light detection.

Camera	NC 100
CCD array type	back-illuminated
Grade	1
Sensitive area	13.3 x 13.3 mm
Pixel size	13 x 13 µm
Pixel resolution	1024 x 1024
Spectral range from	300 to 1100 nm
Max. quantum efficiency	90% at 620 nm
80 % quantum efficiency	460-770 nm
Full well capacity	100.000 e-/pix
Readout noise	<3 e- rms



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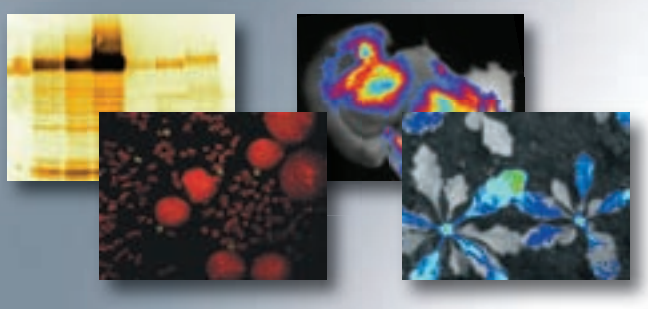
Versatility and flexibility

The conditions required to image living organisms can be very different. For example, today gas anaesthesia is used for small animals but is never used in plant imaging. For plants control of light, temperature or humidity are of more importance.

In the field of infectious diseases or food processing the study of bacterial growth is the objective. In dermatology and material science the very faint luminescence from free radical oxygen species (ROS) is measured. In life science, quality control or forensic studies you need a very sensitive instrument for Western, Southern and Northern blots.

To cover all these applications BERTHOLD TECHNOLOGIES provides the very flexible low light luminescence and fluorescence imaging system NightOWL and a wide variety of accessories:

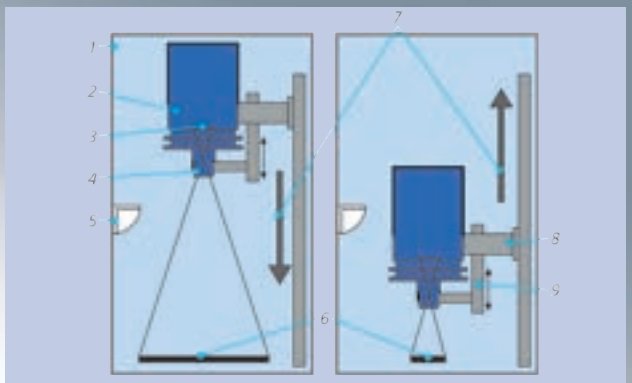
Moving of camera inside the cabinet	✓
Height correction in each position	✓
Large space inside the cabinet	✓
Easy exchange of camera	✓
Microscope and plant chamber adaption	✓
Power sockets inside the cabinet	✓
Control of interface inside the cabinet	✓
Positioning plates	✓
Macro table	✓
Flange	✓
Gas anaesthesia unit	✓
Workstation	✓
Fluorescence Reflectance Imaging	✓
Ring-light epi illumination	✓
Dual Line epi illumination	✓
Gooseneck spot illumination	✓
Transilluminators	✓
Orthogonal 3D-Imaging option	✓
Animal beds for multimodality imaging	✓



Cabinet

The NightOWL cabinet is extremely light-tight preventing any interferences from ambient light.

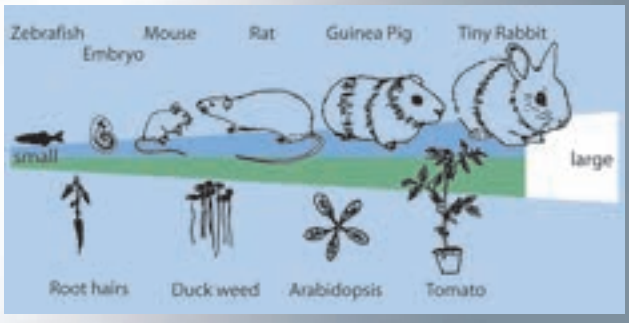
NightOWL is the first imager with a motor-driven camera inside the cabinet. Optimum resolution and focus of the sample is achieved by automatic positioning of the camera according to the actual sample size.



NightOWL dark cabinet

Light-tight housing (1) containing the Peltier cooled CCD camera (2) with a motor-driven vertical adjustment of magnification (7.8), CCD chip (3), the lens (4) with a second vertical precision drive for focus adjustment (9), a fluorescence light source (5) with exchangeable filters, the sample table (6) supporting 2D and 3D objects from 35 (right drawing) to 260 mm (left drawing).

The camera can be moved from a height of 50 mm to 725 mm allowing focussing on every sample size up to 250 mm. For close-ups a macro table can be used. The camera is set up with flat field and height correction. This calibration eliminates non-uniformities caused by variations in the optical path due to height, illumination or lens effects.



NightOWL II LB 983 NC 100

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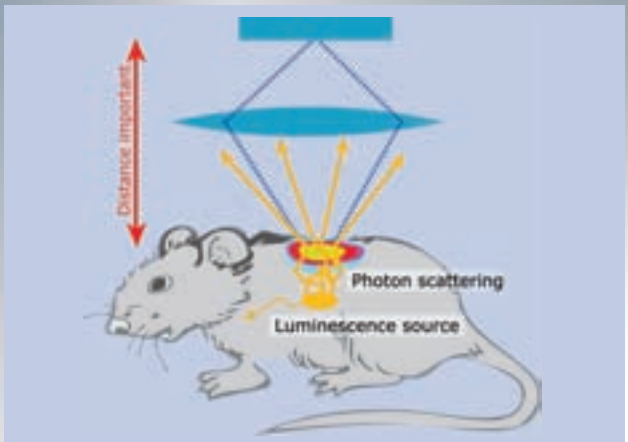
Resolution

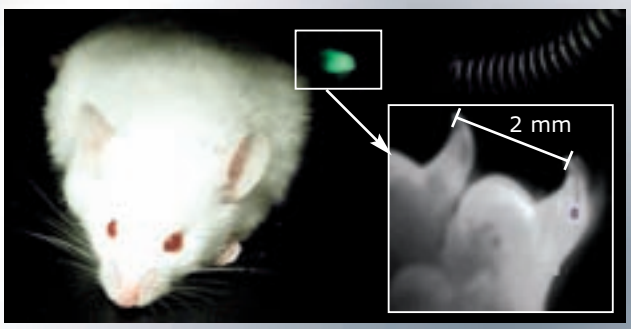
The total resolution of an image is a function of camera resolution, focal length and working distance. The software of NightOWL allows easy setting of sample size and sample height. Once the sample is defined the focus is set accordingly. The resolutions are therefore as follows, shown by some examples:

Sample size	Resolution
20 cm	200 µm
10 cm	100 µm
5 cm	50 µm
With macro table	
2 cm	20 µm
1 cm	10 µm

The closer the camera to the sample the more photons can be collected due to the spherical angle of the lens.



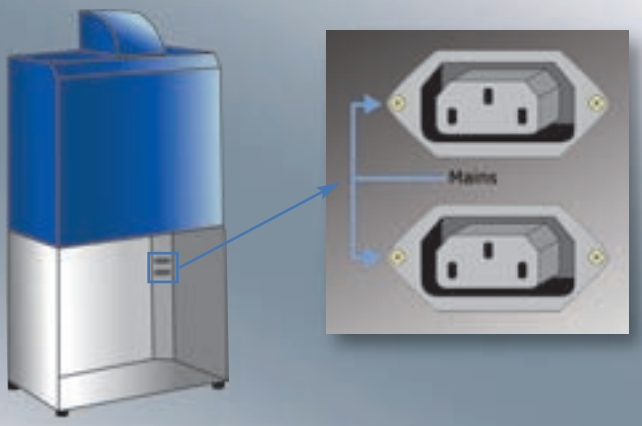
Sometimes small objects have to be aquired. With the macro table the magnification goes up to 5 fold. With another 5-fold digital zoom the overall magnification up to 25-fold can be possible.



The left hind leg of the anaesthetised mouse was illuminated with the gooseneck spot illumination. Only with flexible fibre optics it is possible to image the leg between macro table and camera. The distance between camera and object was only 2.5 cm (dsRed excitation at 525 nm and emission 605 nm; 3-fold digital magnification of the mouse claw).

Additional features

The NightOWL cabinet has enough space to install special light sources or to place transilluminators, heaters, coolers etc. These devices may even be switched on and off through the software and the built-in sockets. This possibility enables the researcher to add more features into the cabinet. Plant researchers often use special lamps or flash lights in their experiments. Researchers in material science sometimes need special heating devices. The transilluminators are also connected with mains.



The flange option provides light-tight access to the inner part for tubings, cables or even fibre optics, e. g. for special illumination of plants. Such modifications of the flange can be of course customized for special purpose. BERTHOLD TECHNOLOGIES will be pleased to quote for customized flanges.

NightOWL II is equipped with a telescopic table top for easier sample handling. It is very convenient to position and check the samples outside the cabinet and then to slide them inside for acquisition.

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Gas anaesthesia units

During the luminescence or fluorescence image acquisition rodents have to be anaesthesized. In principle, there are two ways: intraperitoneal injection of a liquid mixture of anaesthesia (e. g. ketamine / xylazine or tribromoethanol) or anaesthesia by gaseous isoflurane.

One of the benefits of gas anaesthesia is an increased luminescent signal in rodents by a factor of two compared to tribromoethanol anaesthesia. Breathing is normal, blood pressure and ATP levels are more stable. Gas anaesthesia is less harmful, so rodents can be anaesthesized for longer periods and more often per day, which is an important advantage.

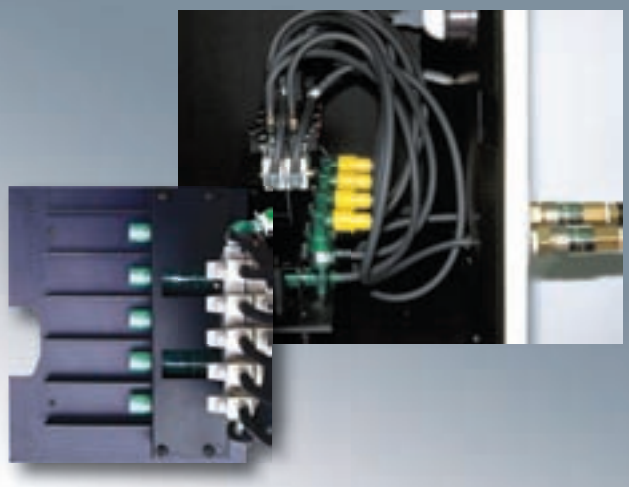
Unit for five rodents

The TEM gas anaesthesia system has been adapted to the NightOWL. The vaporizer system works with low pressure and low flow making sure no gas is leaking from the nozzles and reducing the throughput of isoflurane. A yearly calibration of the vaporizer ensures proper functioning.

The induction box can be used for both mice and rats. In collaboration with INSERM Unité 540, Montpellier, France, a special mouse tray has been developed. Up to five mice can be anaesthesized in parallel in this tray.

The tray is temperature controlled to ensure that body temperature is kept stable during imaging. To prevent crosstalk of light emission from one rodent to the other removable barriers separate five compartments.

The anaesthesia system comes complete, but if pressured air, oxygen line or scavenging line are installed in the lab, the anaesthesia system can be modified accordingly. If any gas anaesthesia unit is already present in the lab, only the inner tray has to be ordered.



Order information

Complete unit for 5 rodents, 220 Volt	41930
Complete unit for 5 rodents, 110 Volt	46238
Inner tray	45941

Single unit

The macro table for LB 983 is covered with a magnetic foil. A magnetic anaesthesia gas nozzle can be mounted in any direction for optimal mouse arrangement under the camera. This single gas nozzle is connected in the same way as the unit for 5 rodents, as is the induction box. Rats can be measured directly on the table since their body temperature is more stable.

Order information

Macro table with temperature control for mice	51578
Single gas nozzle for mice	53192
Single gas nozzle for rats	on request

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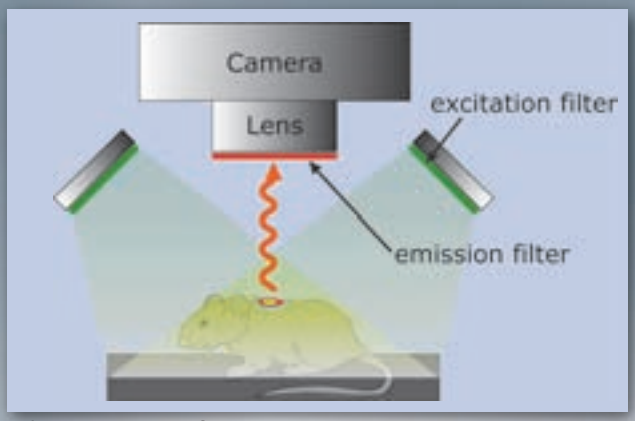
Fluorescence Reflectance Imaging (FRI)*

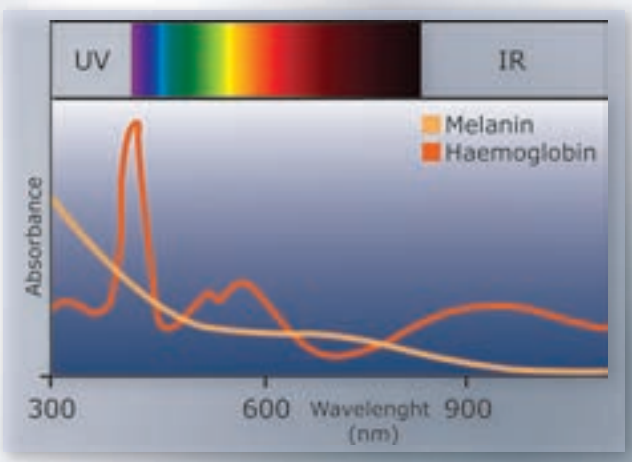
requires a range of illumination devices to excite the sample from above. The signals emitted are measured by a camera positioned above. To excite the fluophor and measure its emission, the proper illumination and set of filters have to be choosen. BERTHOLD TECHNOLOGIES offers a complete range of filters from 340 nm up to 1100 nm.



Schematic set-up of FRI

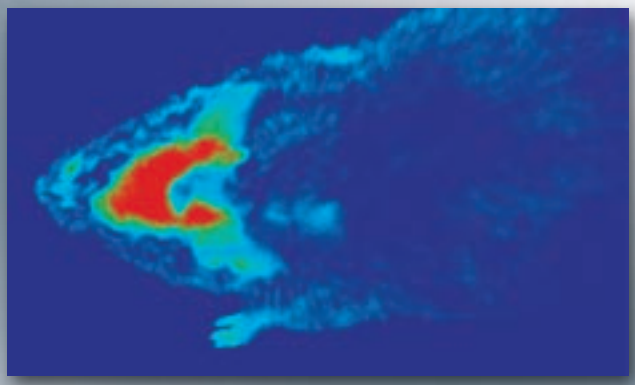
The fluorescence under illumination is either applied as exogenous agent or will be endogenously expressed.

In biofluorescence imaging (BFI) GFP and its derivatives YFP and dsRED are used. The excitation and emission optimum of these dyes are between 470, 500 or 550 nm for excitation and 530 up to 580 nm for emission. In this spectral region melanin in skin and haemoglobin in the blood vessels absorb very strong in animals. Therefore the signal intensity will decrease rapidly the deeper the fluorescent source is in the animal.



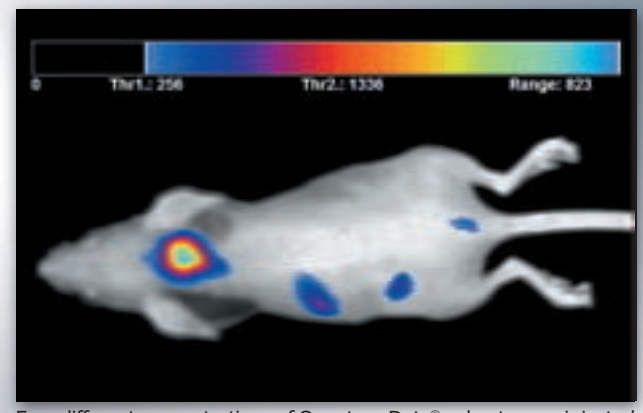
Spectral response of Melanin and Haemoglobin.

The best spectral range for penetrating an animal is between 600 nm and 900 nm. Therefore near infrared (NIR) fluorescence is a promising technique to get better signals from deep inside the animal. Researchers are developing different dyes for this application. For example Novartis in Switzerland showed the ability to bind oxazines to beta-amyloid deposits present in Alzheimer`s disease. Excitation of such dyes is done at 680 nm, emission is in the range of 720 nm.



Oxazine fluorescence in an Alzheimer disease mouse.

Another example of successful NIR fluorescence is the use of Quantum Dots® 700 or 800. The Stokes´ shift of these lanthanide complexes are very high (470 nm to 700 / 800 nm), additionally lifetime of emission is long (400 ns - 400 ms).

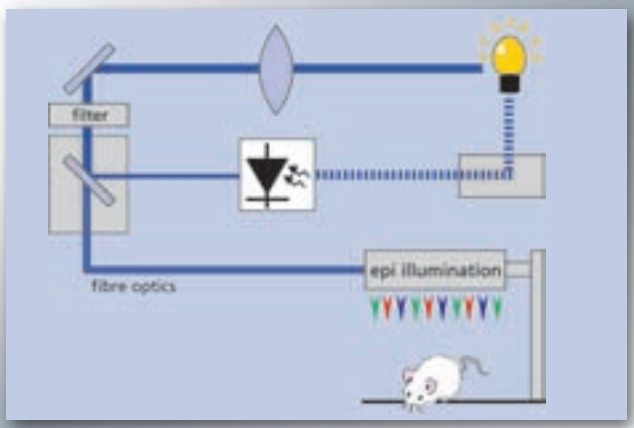


Four different concentrations of Quantum Dots® subcutanous injected

Another advantage of the IR-region is the low amount of autofluorescence. Other false positive signals can be detected, if too much chlorophyll (strong phosphorent substance) is in the food. Teeth also often glow due to incorporated phosphorus, as does paper, if treated with phosphate. If plastic devices are used e. g. Petri dishes, osmotic pumps etc. a special IR cut-off filter has to be used to inhibit autofluorescence at around 800 nm.

Optics and lamp control

The unique optical system from the LB 940 Mithras Multimode Reader has been integrated in the NightOWL II model. The light beam is kept constant for each fluorescent measurement, which is ideal with the ring-light epi illumination. If the ring-light is always set at the same height, the excitation energy on the sample will always be the same.



The lamp energy can be set by a lamp factor in the software. This allows calibration of the imaging system for each fluorophor. Comparison of the amounts of different fluophors in one sample becomes possible.

Fluorescence Illumination

Gooseneck spot illumination

An important requirement of plant researchers is to illuminate every leaf of a plant. With the gooseneck spot illumination and its flexible fibre optics BERTHOLD TECHNOLOGIES provides an excellent tool. It is possible to bend the fibre optics in every direction.

The gooseneck spot illumination is a perfect combination together with the macro table. The space between camera and object is very small, only the gooseneck spot illumination can bring light onto the imaged area (see example page 4).

Order information

Gooseneck spot illumination	29663

* Some techniques for generating and/or detecting light in biological subjects are patented and may require licences from third parties. Users are advised to independently determine for themselves whether their activities infringe any valid patent.

Ring-light epi illumination

The ring-light option for fluorescence illumination allows an even distribution of light upon the sample. The ring-light is mounted on an adjustable support stand allowing positioning at heights from 14 to 16 cm.

Order information

Ring-light epi illumination for LB 983	51685

Dual Line epi illumination

This epi illumination option is another alternative for fluorescence illumination to image rats or, if close-up images are required.

Order information

Dual Line epi illumination	52295
Dual Line epi illumination with temperature control	53109

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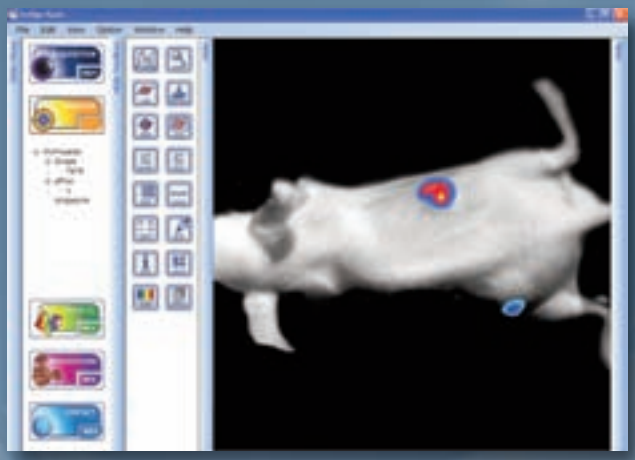
indiGO™ Software

The new easy-to-use indiGO™ software has been developed together with users. Well organized menus and dialogue boxes guide the user through camera set-up, image processing and image analysis.



- user defined
- quantitative analysis
- display of luminescence, fluorescence or photographic images
- contrast and image enhancement tools
- colour overlay e.g. photographic image with luminescence image, e.g. fluorescent gel with the hybridization signal, or of various fluorescent images
- line plot function
- surface plot function
- zoom function (up to 5-fold)
- definition of areas of interest and evaluation
- geometrical analysis
- arithmetic functions
- data export into spreadsheet
- raw data and processed data are filed separately (according to GLP rules)
- individual exposures or image sequences
- function to automate image processing steps
- image import and export (16-bit TIF file generated by indiGO™ can easily be processed by further software packages, e.g. for multimodality or co-registration)
- printing on any Windows printer via software
- version can be installed at instrument and office site
- remote control via internet for service and quick assistance

Software Options

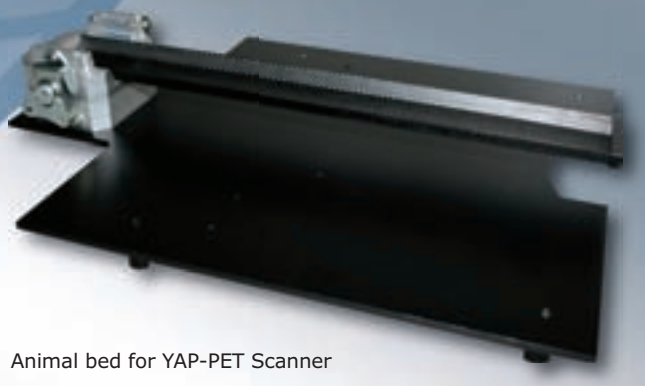
The newly developed indiGO™ software allows complete control over the hardware and offers all tools for image evaluation. There are special software needs which BERTHOLD TECHNOLOGIES offers as options, for example

- DICOM option
- 21 CRF part 11 option
- animal alignment option
- digital mouse atlas alignment option, etc.

Multimodality

NightOWL is prepared for the sequential modus of multimodal imaging. Several animal beds for this purpose have been developed, for example combination is possible with

- MR instruments of Bruker and Philipps
- PET/SPECT instruments by ISE
- X ray-CT instruments of Scanco Medical
- Ultrasound instruments of VisualSonic



Animal bed for YAP-PET Scanner

Multimodality software

Software packages to fuse MRI, PET, SPECT and X-ray CT data are available, since all these imaging technologies offer 3D-data. BLI and FRI data are the planar or 2D, so only the z-plane can be overlaid with the same z-plane of 3D-data. In case the orthogonal 3D-option is used, 3 z-planes can be overlaid. BERTHOLD TECHNOLOGIES will develop a solution to fuse BLI or FRI images with the other imaging technologies.

In case of the software package VINCI from Max-Planck-Institut, Cologne, fts-files can be already implemented.

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Applications*

Whole animals and plants can be imaged as well as blots, gels, microplates, cell culture dishes and arrays regardless of the luminescent or fluorescent markers used. Optical calibration ensures the comparability of all images captured with NightOWL.

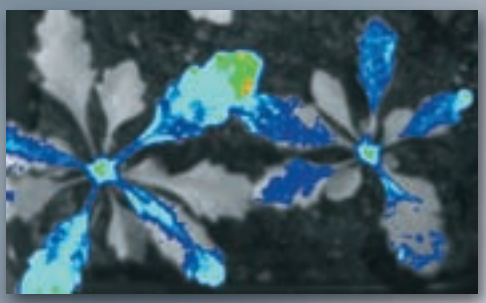
Detection of weak light signals with CCD cameras can be achieved with high quantum efficiencies and extremely low noise levels to enable long exposure times. The camera and cabinet design are the key to superior imaging performance, complemented by scientific evaluation software for quantification.

Application	NC 100
Biochip	+
Bioluminescence	+++
Blot documentation	++
Chemiluminescence	+++
Colony counting	++
Fluorescence	++
Gel documentation	++
In-vivo Imaging	+++
Microplates	+
Microscopy	++
Multi-label measurements	+++

+good performance ++superior performance +++excellent performance

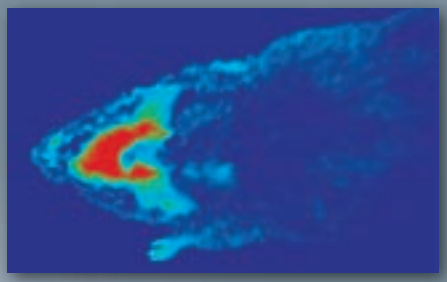
All applications and publications are presented on the web page: www.berthold.com/bio.

- In-vivo visualization of reporter gene expression in prokaryotic and eucaryotic cells, in living transgenic animals and plant.



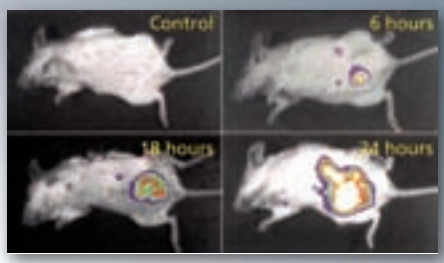
- Visualization of bacterial growth in food
- In-vivo visualization of skin diseases in dermatology
- Research and product optimisation in varnish, paint and pigment production
- Imaging of chemiluminescence of solid polymers
- Detection of ROS (reactive oxygen species)
- Forensic Science
- Imaging of microplates: immunoassays, reporter genes detection, gene probes and phagocytosis

- In-vivo visualization of fluorophors, e.g.



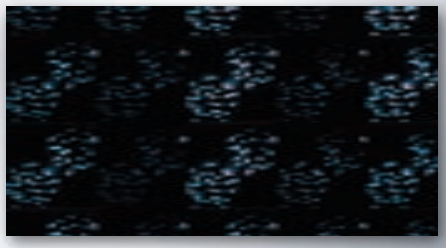
Oxazines bound to beta-amyloid deposits as present in Alzheimer`s disease.

- In-vivo visualization of infectious diseases



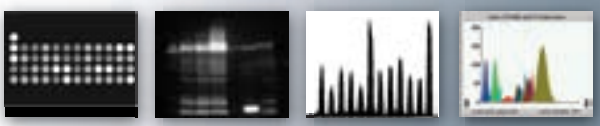
Intraperitoneally inoculated with Salmonella enteritidis carrying a lux Operon of Xenorhabdus luminescens; exposure time: 60 sec. (Courtesy: P. Hill, Nottingham, UK).

- Study of circadian rhythms via reporter genes in living transgenic plants.



The time-course follows the rhythm of transcription from the *CAB2* promoter over 48 hours.

- Gels and blots: imaging and measuring of chemiluminescent stained Southern, Northern and dot blots as well as Western blots.



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NightOWL II LB 983 NC 100

Technical Specification and Order information

NC 100	back illuminated, 1024 x 1024 pixel, quantum efficiency 90 % at 620 nm, sensitive from 300 to 1050 nm, dynamic range of 90 dB, cooling to absolute -80°C to -90°C depending on the room temperature.	
Resolution	Sample size	Resolution
	20 cm	200 µm
	10 cm	100 µm
	5 cm	50 µm
With macro table	2 cm	20 µm
	1 cm	10 µm
Exposure times	from 30 milliseconds to hours	
Pixel binning	variable to increase sensitivity	
Filters	4 excitation filters per slide 4 emission filters per wheel 340 nm up to 1100 nm additional filter slides/wheels available	
Light Source	75 W tungsten lamp	
Working distance	automated positioning of the camera allows working distances between 50 mm and 725 mm. For working distances below 50 mm the macro table has to be used. Connection to a microscope changes field of view also.	
Interfaces	to place transilluminators, heaters, coolers, light sources etc.	
Dimensions	122 x 60 x 40 cm (HxWxD)	
Weight	85 kg	
Regulations	CE, EN	

Laboratory environment	
Power Supply	110–240 V; 50/60 Hz; max 400 VA; minmum 3 sockets
Temperature Range	max 30°C
Humidity	10 – 80%, non condensing
PC Requirements	Pentium processor, 500 MHz (or better), CD ROM drive, 2 GB hard disk (or more), true colour 22" display, serial port, parallel port, free ethernet port (RJ-45 for service remote control), USB
Room	if gas anaesthesia is used room has to be ventilated; pressured air and scavenging line for surplus gas would be an asset.
Bench	stable to sustain 85 kg of the instrument; minimum size 120 x 50 cm (L x D)

Order Information	Order Number
NightOWL II LB 983 NC 100 complete incl. software	40508-30

For accessories please see separate brochure. For further information of available filters please see filter data sheet.

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Internet: www.Berthold.com/Bio

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