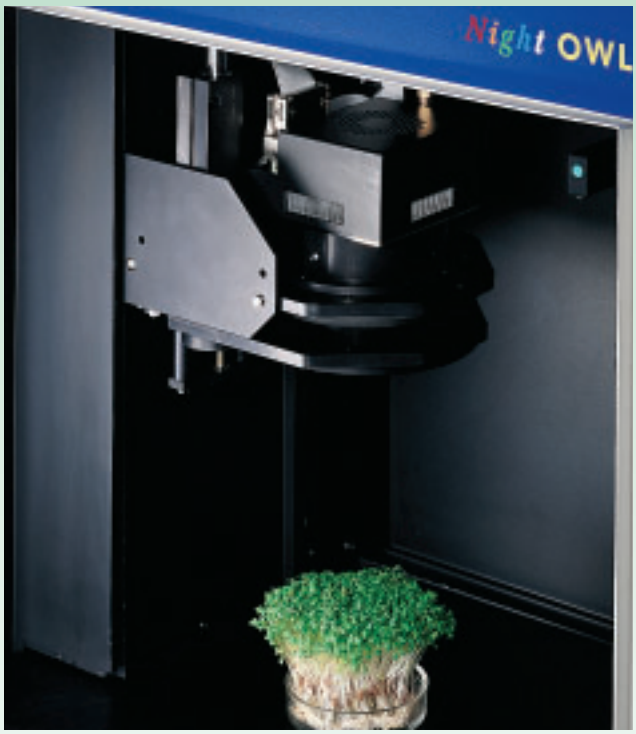
NightOwl
is the key to luminescence and fluorescence imaging

The BERTHOLD NightOWL is a versatile imaging system for measurement of 2D and 3D samples. Both microscopic and macroscopic sized samples can be investigated. Whole animals or plants can be imaged as well as blots, gels, microplates, petri dishes or macro (dot) arrays. Adaption of the CCD camera to a microscope enables the investigation of cells, tissue sections from insects, animals and plants. NightOWL is designed to meet the needs of scientists measuring weak light signals as they are emitted from luminescent as well as fluorescent probes.

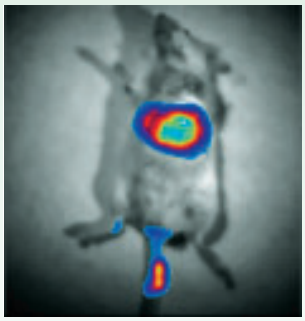
The instrument operates under direct computer control. Images can be acquired and processed using the WinLight32 software or additional image processing packages.



The basic features of NightOWL can be summarized as follows:

- weak light detection
  luminescence and fluorescence
- macroscopic imaging
  optimized sample resolution in dark box
- sample format
  2D and 3D samples can be measured
- microscopic imaging
  additional increase in sample resolution using microscopes

NightOWL cabinet with CCD camera



Detection of reporter genes expressed from internal tissues of lab animals

A growing demand for visualization of gene expression and protein subcellular localization has created the need for a sensitive and versatile imaging system such as NightOWL.
The detection of weak light signals with CCD cameras demands extremely low noise levels to enable long exposure times. The camera design is the secret to imaging performance.

Key applications

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Macroscopic imaging
in vivo visualization of reporter gene expression in prokaryotic and eucaryotic cells, in living transgenic animals and plants

■

study of circadian rhythms via reporter genes in living transgenic plants

■

in vivo visualization of skin diseases in dermatology

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research and product optimation in varnish, paint and pigment production

■

imaging chemiluminescence of solid polymers

■

imaging of microplates: immunoassays, luciferase detection, gene probes and phagocytosis

■

gels and blots: imaging and measuring of chemiluminescent stained Southern, Northern and dot blots as well as Western blots

■

Microscopic imaging
in vivo imaging of reporter gene expression in single cells for studying gene expression, transfection efficiency, protein targeting, protein localization and living cells

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ATP measurement

■

visualization of cellular luminescence

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FISH imaging

■

BRET

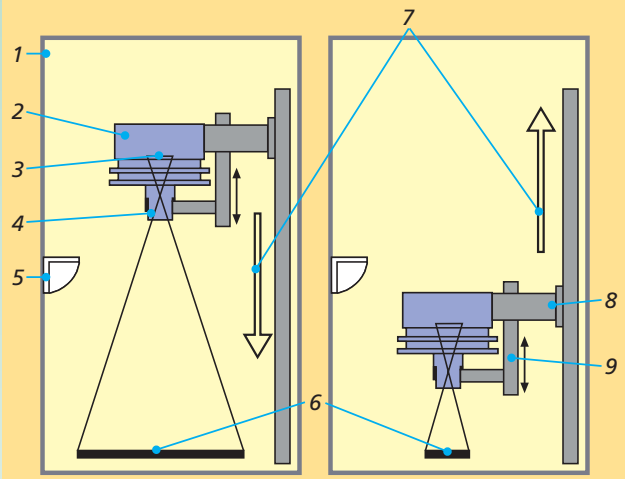
■

imaging of immunfluorescence stained samples

■

monitoring of Ca^{2+} fluctuations

Camera design is the secret to imaging performance



Scheme of the NightOWL dark cabinet. Light-tight housing (1) containing the -70° C 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 weak (5) and fluorescence light source with inter-changeable filters, and the sample table (6) supporting 2D and 3D objects from 35 (right drawing) to 300 mm (left drawing).

The NightOWL system enables exposure times from 30ms to many hours, with a measurement dynamic range of more than 5 decades, enabling detection of almost every photon emitted from the sample.
There are two main sources of noise, dark noise and readout noise:

Dark noise

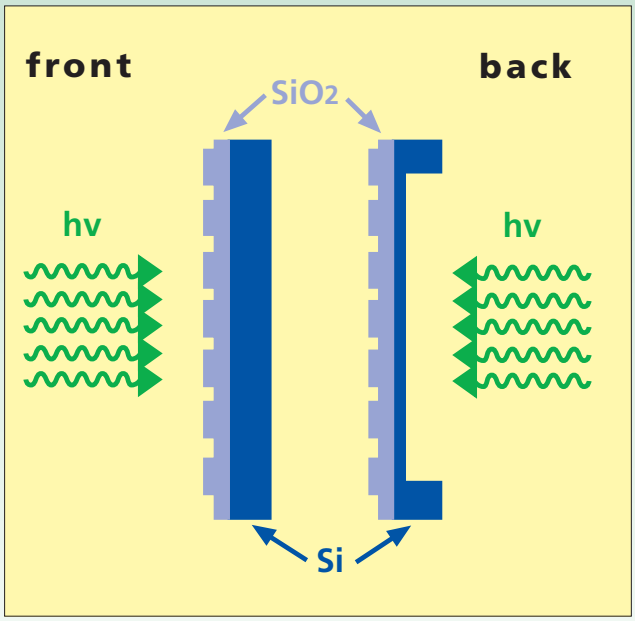
is generated thermally by leakage currents within the CCD and is accumulated even in the dark. This noise can not be differentiated from the signal generated charge. The background level is, therefore, increased leading to a reduction of sensitivity and dynamic range. The CCD temperature is minimised to lower the dark current: the dark current is reduced by a factor of 3 to 4 for each 10°C fall in temperature. Therefore, the NightOWL CCD chip is cooled down to –70°C by a four-stage Peltier device within the ultra high vacuum of the camera housing. Additionally, the NightOWL CCD chip is equipped with multi-pinned-phase (MPP) device that again significantly lowers the dark current.

Readout noise

Is only significant at low signal levels such as those generated by weak luminescence reactions. It arises due to statistical fluctuations that are present on every signal read out from the CCD. The readout noise is proportional to the square root of the readout rate. Therefore, NightOWL operates at a slow scan rate of 50 kHz, i.e. 50 000 pixels per second, to reduce the readout noise to <7 electrons RMS. This noise is indicated as “electrons RMS”, i.e. Root Mean Square, standard deviation.

Two CCD cameras available

For NightOWL two 16 bit CCD chips are available: a so called standard chip that is illuminated from the front and a back thinned chip that is illuminated from the rear.



The back illuminated chip (see scheme) has an increased quantum efficiency, because the incoming photons are not absorbed by the silicon dioxide layer of the chip. Therefore, quantum efficiencies of up to 80% at 650 nm are obtained, whereas the standard chip has an efficiency of about 40% at 650 nm. To increase the sensitivity in the short wavelength range, the standard chip is covered with an UV-to-VIS converter. Due to the higher quantum efficiency of the back thinned CCD chip, less light is required to achieve the same single-to-noise ratio.

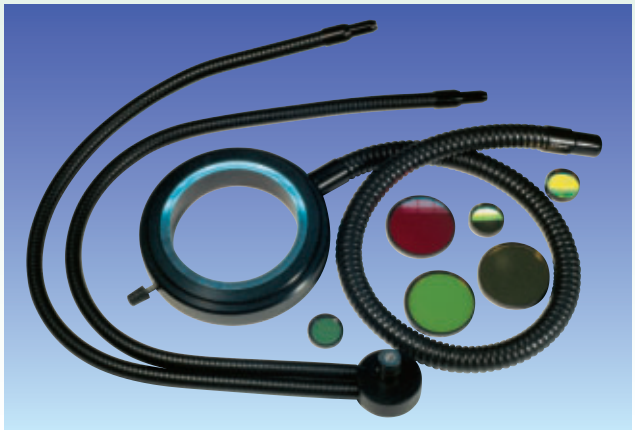
Furthermore the signal-to-noise ratio can be increased by pixel binning. Combining single pixels of the CCD to "super pixels" increases this ratio, because a readout noise charge is generated per readout of each pixel or super pixel. For example the pixel binning of 3 x 3 pixels has one "unit" of readout noise per 9 pixels compared to one "unit" per individual pixel when no binning is used. The signal-to-noise ratio is 1:9 instead of 1 to 1 without 3 times 3 pixel binning. For blot or gel detection flexible binning in the x-direction fits better to the band's shape, e.g. 1x10.

Optimized sample resolution

For optimal resolution of the sample the cooled CCD camera is automatically positioned dependent on the actual sample size. The complete sensitive area of the CCD is therefore always used for the measurement resulting in sample resolutions ranging from 600 µm to 70 µm per pixel. Adaption of the CCD camera onto a microscope increases sample resolution dependent on the magnification settings.

Options

There is enough space inside the NightOWL cabinet to install special light sources or to place transilluminators, heaters, coolers etc. Such devices could be switched off and on by software, when connected to the builtin sockets.

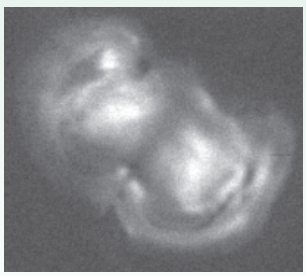


To measure fluorescence different lighting systems such as gooseneck fiber optics or ringlights with the corresponding filters are available. A tungsten halogen lamp (340-700 nm) with 90 W is used for illumination.

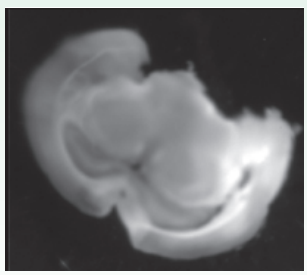
Applications

Imaging of reporter gene expression

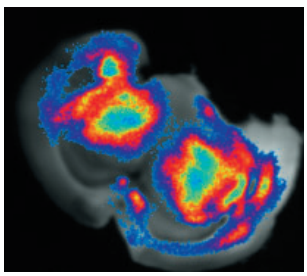
Reporter genes have become an invaluable tool in studies of gene expression. With the powerful overlay technique, luminescence signals can be superimposed onto the brightfield image, to localize gene expression.
First an illuminated or transilluminated image is acquired with a high resolution (no pixel binning) and a short exposure time of 20 msec. Then the luminescent image is acquired in the dark using low resolution (pixel binning) and long exposure times of up to hours to enhance the sensitivity. Using image processing the luminescent signals are colour coded according to intensity and then overlaid on the b/w bright field image. The resulting image combines both spatial and luminescent information.



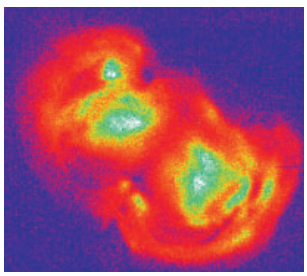
luminescence signal



transmitted image



color overlay



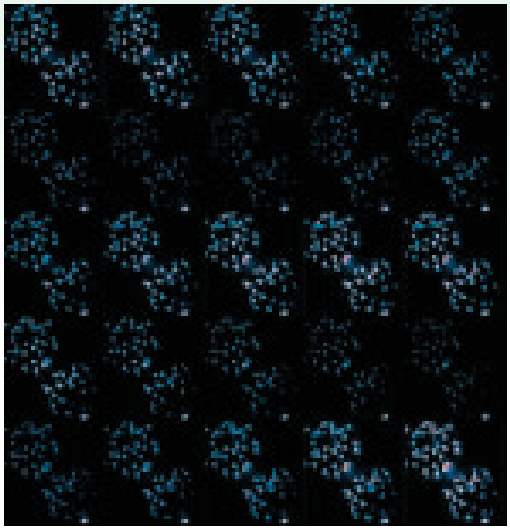
pseudocolor presentation

Imaging of luciferase expression in tissue sections of the brain of a transgenic mouse.
(Courtesy B. Hengerer, H. Berns, Ciba-Geigy, Swiss).

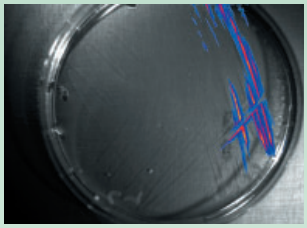
Macroscopic imaging

A recent trend in reporter gene analysis has been towards chemiluminescent and bioluminescent assays for reporter gene products. Because of the sensitivity of this type of detection technique.

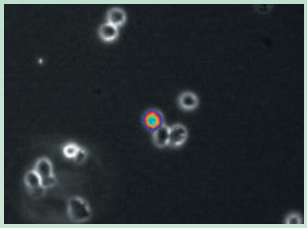
The firefly luciferase in particular has found widespread applications to monitor gene expression in bacteria, plants and mammalian cells. Recently, several genes have been adapted as indicators of transcriptional activity: bacterial luciferases from various sources, b-galactosidase or phosphatase, for example. Imaging of gene expression to gain spatial and temporal information about gene regulation is particular useful and opens a new dimension in gene expression studies.



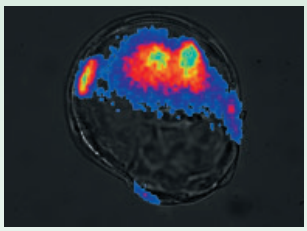
Time-course showing the circadian rhythm of bioluminescence in a population of *Arabidopsis thaliana* seedlings expressing the firefly luciferase gene under the control of the *Arabidopsis CAB2* promoter. The time-course follows the rhythm of transcription from the *CAB2* promoter over 48 hours. Two circular 10 cm petri dishes of seedlings are represented for each time-point. (Courtesy BERTHOLD TECHNOLOGIES, Redbourn, Hertfordshire, UK).



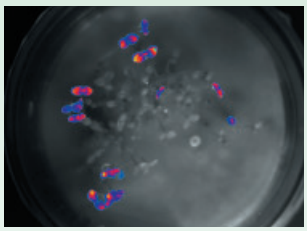
Visualization of Rhizobium leguminosarum-viciae marked with the luxAB gene from Vibrio harveji. Bacteria expressing the luxAB coded luciferase gene emitted light after the addition of substrate (Courtesy W. Lotz, Univ. of Erlangen).



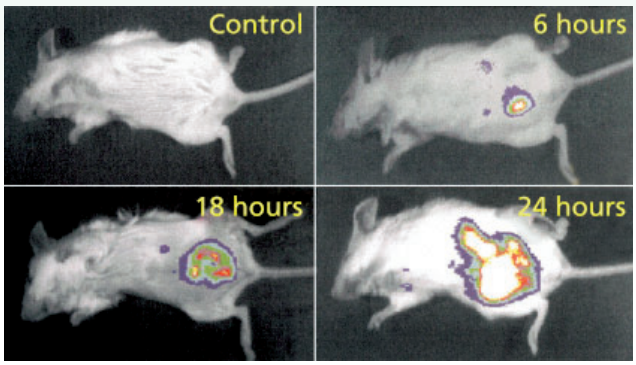
Monitoring of transient expression of firefly luciferase in mammalian cells. Cells transfected with a LUC expression vector were assayed in vivo after addition of luciferin. Overlay of luminescence and brightfield image.



Bioluminescence emitted from living cells carrying a mouse-promotor-LUC fusion gene after addition of luciferin were monitored (Courtesy Thompson, INRA, Paris).



Visualization of bioluminescent tobacco seedlings carrying a plant-promotor-LUC fusion gene. Seedlings were analyzed in the presence of luciferin.

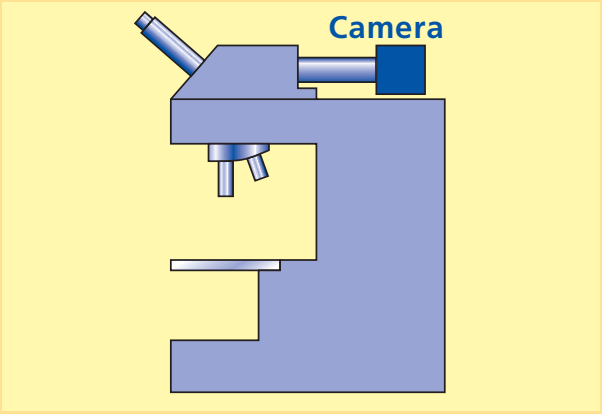


Intraperitoneally inoculated with Salmonella enteritidis carrying a lux Operon of Xenorhabdus luminescens; exposure time: 60 sec. (Courtesy: P. Hill, Nottingham, UK).

Applications

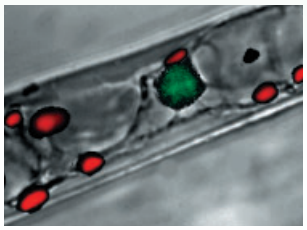
Microscopic imaging (C-mount attachment to microscope)

The NightOWL is available as an attachment to a microscope via a standard C-mount. This version can be purchased as a seperate model without the dark cabinet.
For these users who have both microscopic and macroscopic applications the camera is easily removed from the dark cabinet and attached to the microscope, connected to the electronics by an extension cable.

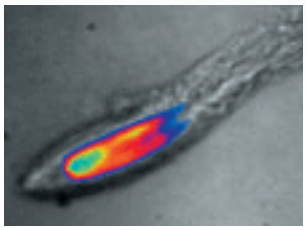


Fluorescence imaging

The green fluorescent protein (GFP) from the jellyfish Aequorea victoria has proved to be a versatile tool for studies of gene expression and targeting in a variety of different organisms. GFP fluorescence is species independent and does not require any cofactors, substrates or additional gene products. Because the gene product is easily detected the GFP gene has become a unique reporter system in procaryotic and eucaryotic cells, in transgenic plants and animals.



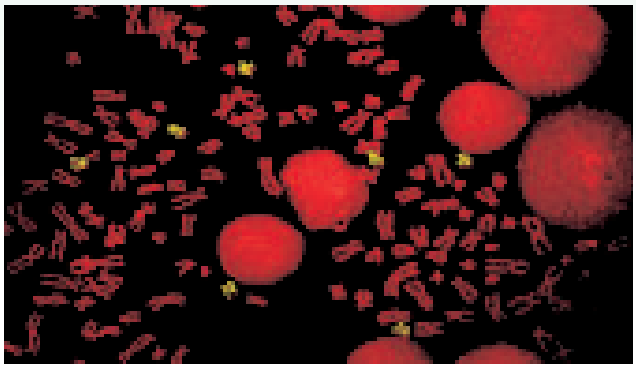
Visialization of GFP expression in tobacco roots. With the powerful overlay technique localization of fluorescent signals in tissues, in individual cells and organells is possible.



Monitoring of GFP localization in a leafhair cell of a transgenic tobacco plant. GFP cDNA was modified to include a targeting sequence to localize GFP selectively in the nucleus of the cell.

FISH imaging

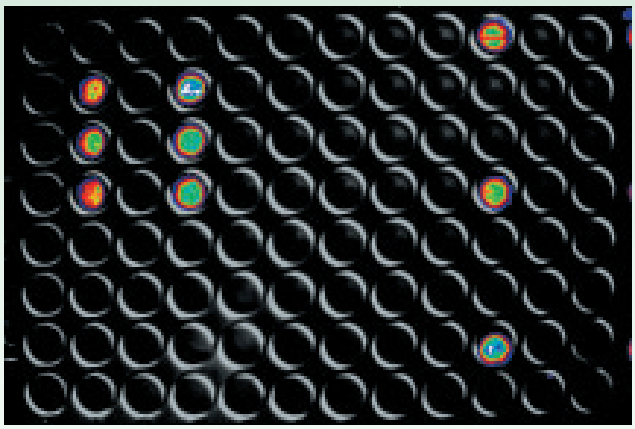
Fluorescence in situ hybridization (FISH) has found widespread applications in the analysis of human chromosomes. A variety of labeled DNA probes and fluorescence based detection methods are available for detection of specific nucleic acid sequences on chromosomes. The use of cooled CCD cameras has provided further improvement of signal detection in FISH analysis.



Imaging of Fluorescence in situ hybridization results. A digoxigenin labeled probe specific to chromosome 18 were used. Visualization of hybridization results was performed with FITC-labeled anti-DIG antibodies. For counterstaining of the chromosomes propidium iodide was used (Courtesy S. Tsui, Univ. Hongkong).

2D luminometer microplate assays

Many assays are based on measurement of luminescence in microplates. The parallel detection of microplates with an imaging system enables the user to measure a large amount of ultra weak samples at once. Glow type luminescence reactions in a microplate can be measured simultaneously, e.g. to follow up kinetics. An enormous increase in sample throughput compared to current systems is achieved.



Measurement in pW and cm, related to 560 nm
Measuring: Mean Grey [pW/cm2]

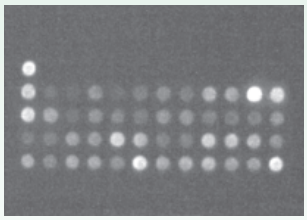
	1	2	3
A	0.099698	0.00031970	0.15041
B	0.11117	0	0.14665
C	0.098325	0.0011111	0.14605

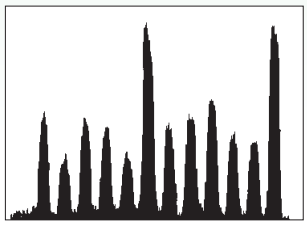
Signal intensity can be measured in pW/cm² and Photons. Measured data can be easily transferred to EXCEL.

Blot and gel detection

For detection and quantification of specific DNA or RNA probes in Southern and Northern blots as well as Western blots non-radioactive chemiluminescent detection methods are becoming increasingly popular. The range of light intensities that can be differentiated by autoradiography is severely limited by X-ray films non-linear response range. In contrast, the NightOWL imaging system offers the advantage of an exceptionally higher dynamic range. Evaluation of high and low concentrations can be performed by taking one image with a shorter exposure time as compared to autoradiography.

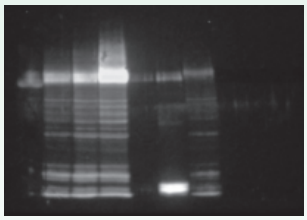
Imaging of a DNA dot blot and measuring of signal intensities

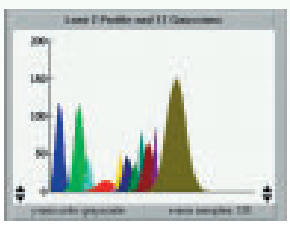




2D-Plot of signal intensities

Western blot for protein detection

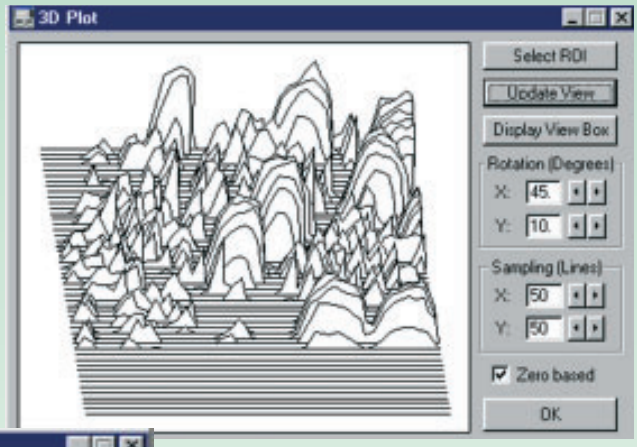


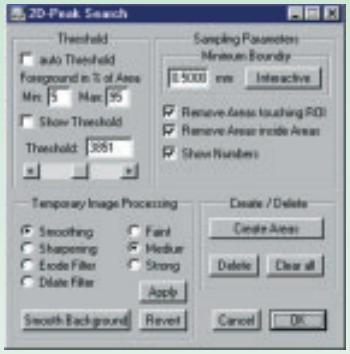


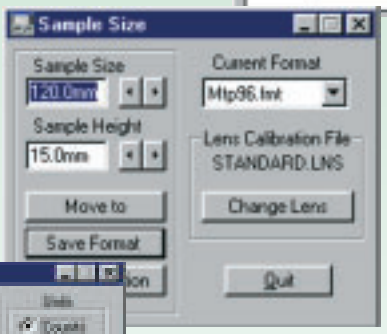
2D-Plot of peak-height

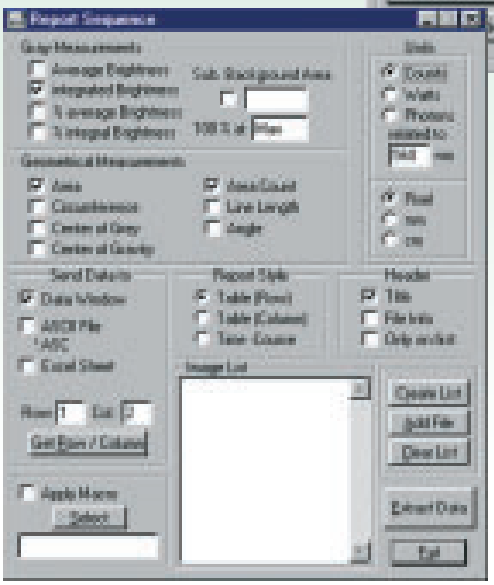
Software

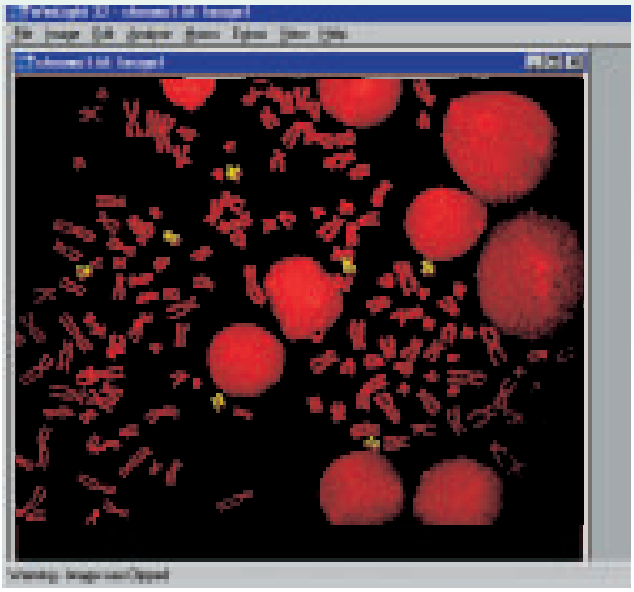
The easy to use WinLight software has been developed using customers input and covers a broad range of laboratory situations. Well organized menus and dialogue boxes help you to move easily through experiments.











WinLight is a 32 bit-software and runs with Windows™ 95, 98, NT 4.0 and Windows™ 2000.

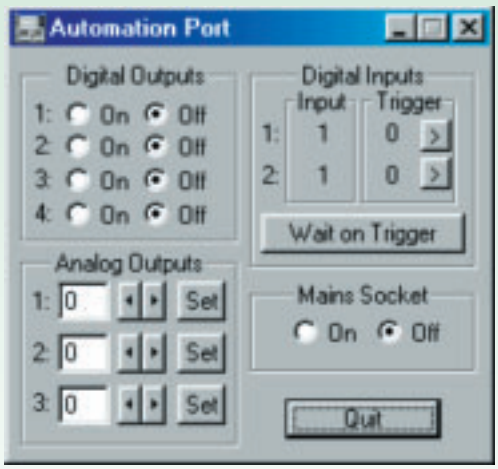
WinLight for NightOWL

- presentation of luminescence, fluorescence, or illuminated pictures in black and white or pseudocolor
- many contrast and image enhancement tools
- color overlay of several files (such as illuminated image with luminescence image, or of the fluorescent gel with the hybridization signal, or of various fluorescent images)
- line plot functions
- 3D-presentation
- zoom function
- gel evaluation functions
- automatic evaluation of predefined areas
- data export into spreadsheet or graphical software packages
- raw data and processed data are filed separately (according to GLP rules)
- hardware control makro function to automate image processing
- individual exposures or image sequences
- macro function to automate image processing steps
- import and export in a variety of file formats

  The tif-file generated by WinLight could be easily processed by further software packages e.g. Image Pro, Media Cybernetics.
- printing on any windows printer

All under PC control

Via software, additional devices inside the NightOWL cabinet could be switched off and on (4 digital outputs, 3 analog outputs 1 mains socket)



NightOWL LB 981 UltraSens
Specifications and ordering informations

CCD Camera		4-stage Peltier-cooled monochrome CCD slow scan camera, front illuminated (fi) or back illuminated (bi), cooled 100° C below ambiente temperature
A/D conversion		16 bits per pixel
Active pixels	fi:	578 V x 385 H pixels, 223K pixel area
	bi:	512 H x 512 V pixels, 262K pixel area
Readout noise	fi:	8 e- rms
	bi:	3 e- rms
Dynamic range	fi:	92 dB
	bi:	98 dB (20log Nsat/Readout noise at nominal operating frequency and 25°C)
Dark current	fi:	0,001 e-/pixel/s at -70° C
	bi:	0,042 e-/pixel/s at -70° C
Spectral response	fi:	400 - 1100 nm
	bi:	200 - 1100 nm
Quantum efficiency	fi:	45% at 650 nm
	bi:	70 - 80 % at 650 nm
Exposure times		from 30 milliseconds to hours
Pixel binning		variable in x and y to increase sensitivity
Working distance		Automated positioning of the camera allows working distances between 35 mm and 725 mm. For working distances below 35 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.
Fluorescence		up-gradable, see options
Dimensions		102 x 60 x 40 cm (H x W x D)
Weight		85 kg

Order Guide

NightOWL LB 981 UltraSens front illuminated complete 230V version	981-27837-10
NightOWL LB 981 UltraSens back illuminated complete 230V version	981-27837-11
NightOWL LB 981 UltraSens front illuminated complete 115V version	981-27837-20
NightOWL LB 981 UltraSens back illuminated complete 115V version	981-27837-21
Flange option for lighttight ports	981-40275
Microscope option	981-27739
Macro table	981-40112
Additional Licence WinLight32 Software	981-35072
Upgrade to WinLight32 Software; requires return of old Dongle	981-35073
Gel-Pro 4.0 evaluation software for gels, blots and colony counting	981-40555
Image-Pro software	981-40558
Fluorescence Light Source option for manual filter changing complete	981-29624
Emission filter slide incl. filter holder	981-40219
Filter holder for filter slide	981-40225
Dual gooseneck spot illumination	981-29663
Ringlight epi illumination	981-39741
Dual line epi illumination	981-39759
NiR-Cutoff Filter	981-40341

We offer the full range of bandpass filters. For excitation you need the F-size, for emission the R-size of filters.

Recommended minimum Data system:

Pentium 200 MHz, 32 MB RAM, 2 GB hard disk, True Colour 17" display, Windows™ 98, NT 4.0, 2000 or XP

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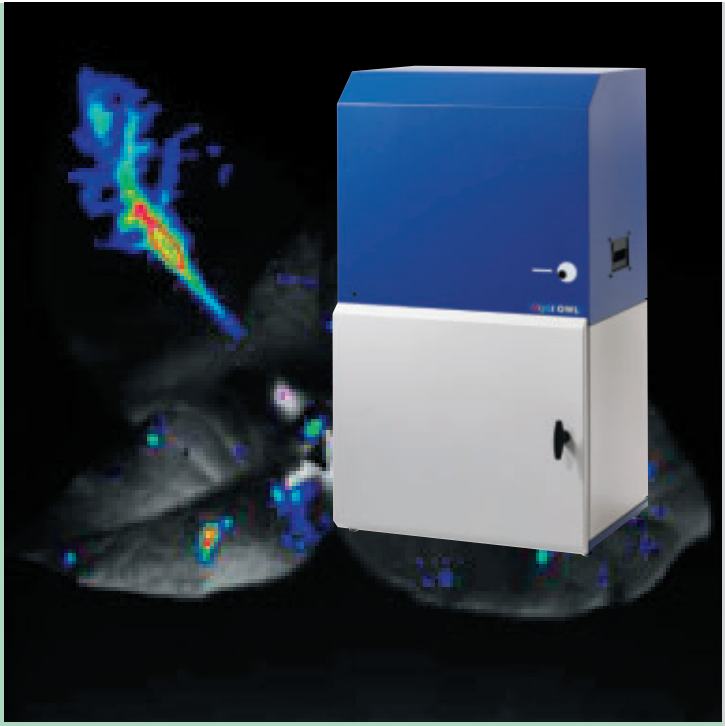
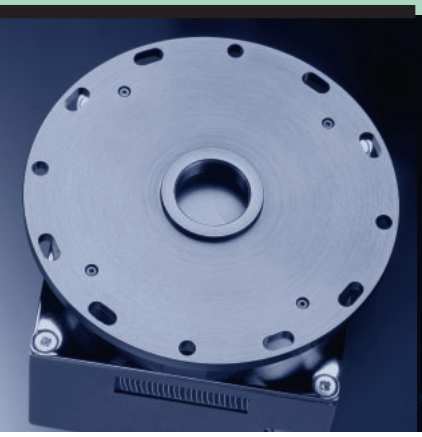
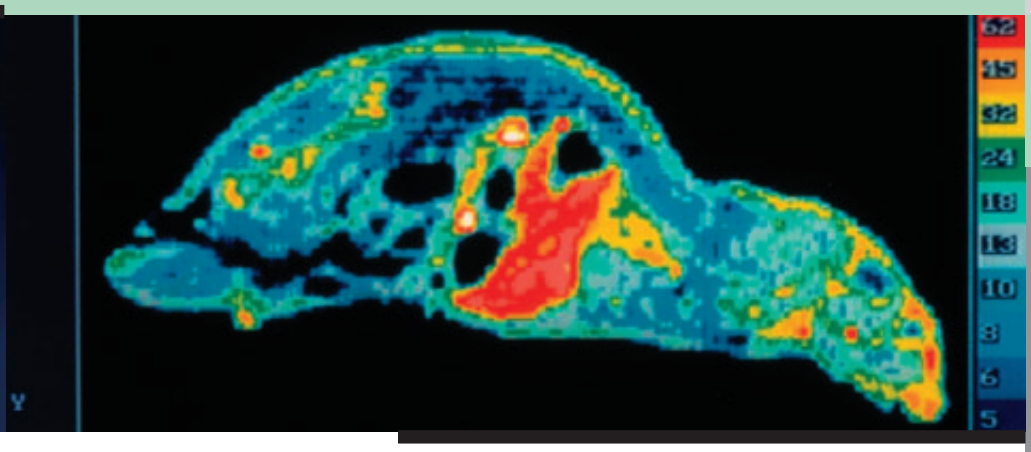


BIOANALYTICAL INSTRUMENTS

Ultra Sensitive
Whole Sample Imaging

NightOWL LB 981 UltraSens





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