

VisiLuxx-II Imaging System

VisiLuxx-II Imager

State-of-the-Art System for Luminescence- and Fluorescence Imaging

The new VisiLuxx-II Imager is the best solution for macroscopic in-vivo imaging of luminescence- and fluorescence signals in living animals, plants and bacteria. The flexibility of our system and the use of the latest camera technologies open up a wide variety of applications. Increased resolution, high dynamic range and lowest noise offer the best platform for all kind of spatial and quantitative analysis. Time dependent luminescence and fluorescence can be recorded and analyzed.



VisiLuxx-II Imager system

Technical specifications:

- thermoelectrically cooled CCD camera system down to -90°C
- high quantum efficiency (up to 95 %)
- from 512 x 512 pixel up to 2048 x 2048 pixel resolution
- standard or EM CCD technology
- low readout noise
- extremely low dark charge
- up to 16 bit dynamic range
- compact and functional design
- programmable LED for reflected light illumination
- special selected optics
- field of view e.g. 2 x 2 cm up to 20 x 30 cm

Optional:

- heated table up to 40°C
- fluorescence excitation: filterwheel with up to 10 fluorescence filters
- spectral analysis module with up to 6 barrier filters
- Gas anesthesia system for different laboratory animals
- motorized sample stage

Applications:

Bioluminescence:

- Luciferase-reporter gene (luc, ruc, lux) active in mammals and plants or bacteria's e.g. gene expression and virus activation in tumors,
- Monitoring of in vivo activation of transcription factors in mice
- Phytochrome-expression in plants, promotor-assays in bacteria
- BRET: bioluminescence resonance energy transfer of e.g. luciferase to YFP for the detection of protein interaction

Chemiluminescence:

- Detection of Southern-, Northern-, Western- and Ligand-Blots
- Promotor assays e.g. with lacZ as a reporter gene
- Colony hybridization e.g. with digoxigenin-labeled cDNA
- DNA microarrays e.g. with biotinylated cDNA

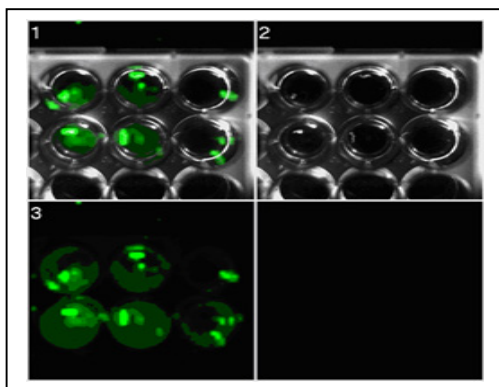
Chemifluorescence:

- Detection of Southern-, Northern- and Western-Blots with fluorescence excitation of enzymatically generated fluorescent products

Fluorescence:

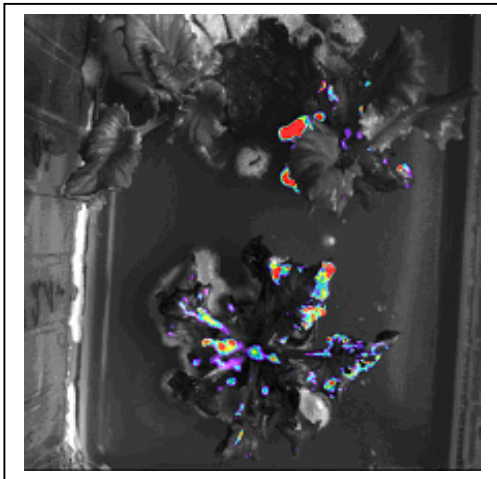
- GFP-fusionprotein expression and localization
- GFP as a reporter gene e.g. instead of lacZ
- DNA-microarrays with fluorescence-labelled cDNAs
- Antibody-microarrays e.g. with Cy3 labelled protein
- Detection of DNA/RNA with SYBR dyes and EtBr
- Protein detection with SYPRO-dyes and fluorescence-conjugated antibodies

Microtiter Analysis

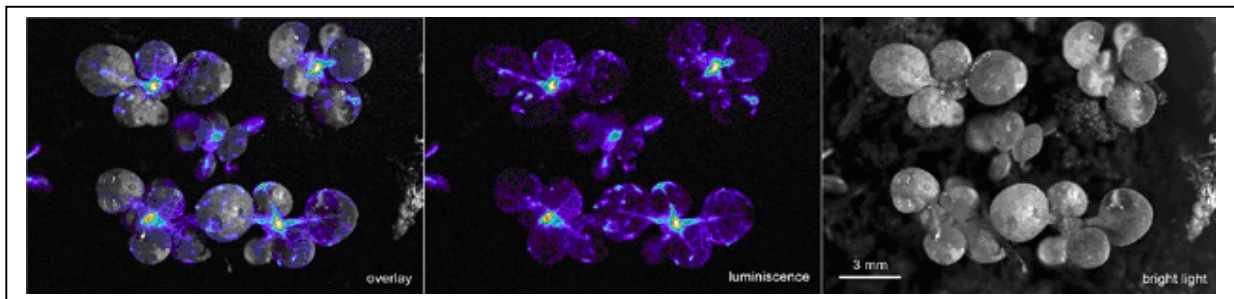


Detection of luminescence assays on microplates is useful for kinetics, different concentrations and if experiment requires long time integration. Quantifying luminescence reaction in e.g. drug screening and measurement of oxygen species are typical applications.

In Vivo Plant Analysis

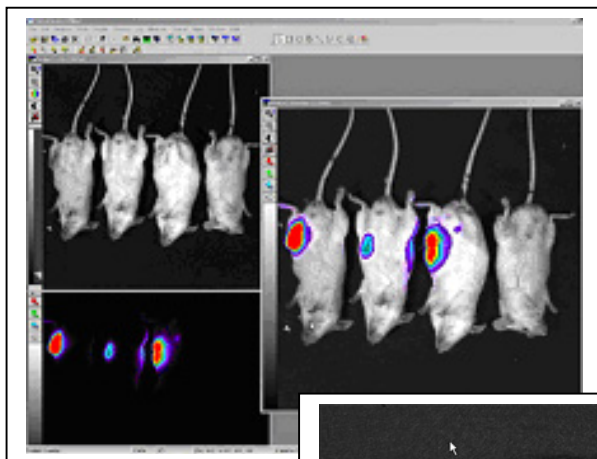


Visualization of reporter gene by luminescence monitoring. Figure 1 shows leaves of an Arabidopsis plant. Luciferase reporter gene have been squeezed. Light emitting after 5 minutes of integration time directly after treatment.

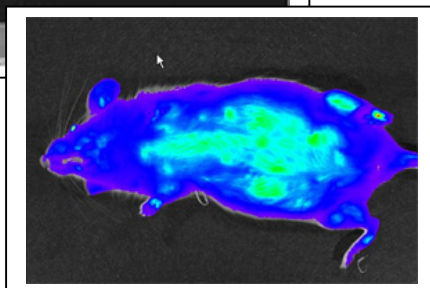


Luciferase measurement in plants VisiLuxx-II, Dr. Greb, Academy of Science

In Vivo Mouse Analysis



Detection of reporter genes expressed from internal tissues. The detection of weak light signals with scientific CCD camera requires extremely low noise level. Images show overlay of bright field images with luminescence image.



Specific cells in living animals are labelled by fluorescence light emitting proteins. Example with GFP labelling, tumor and auto-fluorescence is visible.

Setup and Design

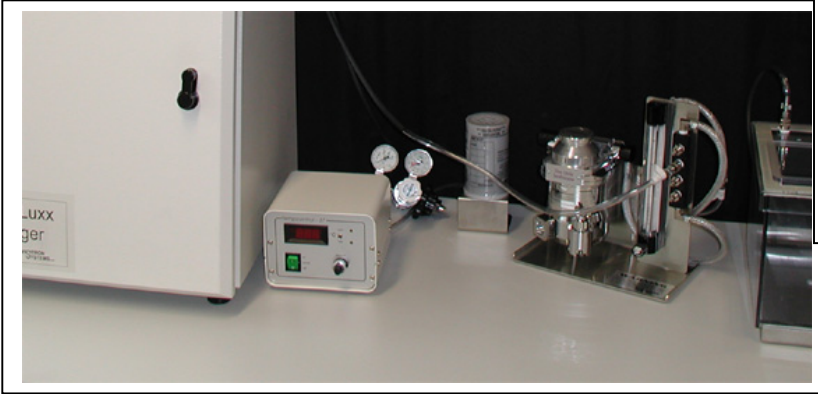
The VisiLuxx-II imager allows to image faint luminescent and fluorescent signals. The versatile darkbox is absolutely light tight and the field of view can be easily selected with the enclosed height adjustable stage. All systems are prepared for the extension with our spectral analysis and fluorescence option, so that the new VisiLuxx-II imager can be upgraded at any time to perform multi-wavelengths experiments in fluorescence and luminescence.

Scientific Camera Options for VisiLuxx-II Imager:

- **Standard camera 2.0 MP**
 - 1600 x 1200 pixel, 7,4 x 7,4 μm pixel
 - regulated peltier cooling to about -23°C
 - 14 bit 20MHz capture time
- **Deep cooling camera 1.4MP**
 - 1392 x 1040 pixel, 6,45 x 6,45 μm pixel
 - regulated peltier cooling to about -44°C
 - 14 bit 20MHz capture time
- **Deep cooling camera 4 MP**
 - 2048 x 2048 pixel, 7,4 x 7,4 μm pixel
 - regulated peltier cooling to about -44°C
 - 14 bit 20MHz capture time
- **EM/CCD 512backilluminated**
 - combined with high sensitive backillumination 95% QE
 - 512 x 512 pixel, 16 x 16 μm , frame transfer CCD
 - 16 bit dual ADC, 10 Mhz and 1 Mhz operation
 - thermoelectrically cooling down to -80°C
- **EM/CCD 1024 backilluminated**
 - combined with high sensitive backillumination 95% QE
 - 1024 x 1024 pixel, 13 x 13 μm , frame transfer CCD
 - 16 bit Dual ADC, 10 Mhz and 1 Mhz operation
 - thermoelectrically cooling down to -60°C
- **CCD 512 backilluminated**
 - backilluminated CCD Chip, 16 bit dynamic
 - 512 x 512 pixel, 24 x 24 μm
 - high quantum efficiency of about 95 %
 - thermoelectrically cooling down to about -75°C
- **CCD 1024 backilluminated**
 - backilluminated CCD Chip, 16 bit dynamic
 - 1024 x 1024 pixel, 13 x 13 μm
 - high quantum efficiency of about 95 %
 - thermoelectrically cooling down to about -75°C

GAS Anesthesia Option for In-Vivo Application in Mice

The VisiLuxx anesthesia is very safe for small animals. There is minimal threat of animal loss resulting from the system. It is simple to use and virtually eliminates human error. The system is totally integrated turnkey design that includes all hardware components required for anesthesia. The system includes mouse induction chamber, oxygen regulation, mouse nosecone and filters.



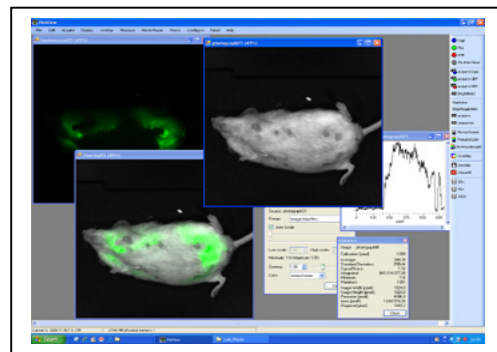
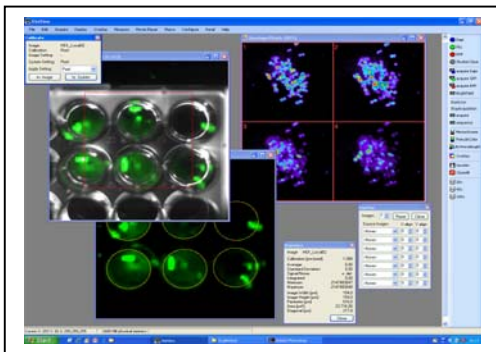
VisiLuxx Fluorescence Option

- programmable HBO / halogen light source
- including fast shutter
- special illumination through light guide
- excitation filter wheel with 6 positions
- emissions filter wheel with 6 positions
- light guide
- filter set for dyes: e.g. GFP, DsRed, Cy5.5



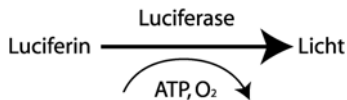
VisiLuxx Camera Control and Image Analysis Software

Complete image acquisition and optional device control is done via the VisiLuxx imaging software. All camera parameters like exposure time, binning or region of interest are accessible. A convenient live mode allows precise focusing of the specimen. Customized protocols are recordable and repetitive tasks can be performed by pushing a single button on a user-defined toolbar.



Quantitative Bioluminescence In-Vivo Imaging

The macroscopic observation of luminescence is a frequent used technique for medical or biological applications (Toth et al., 2001; Sölling und Rainov, 2003; Sato et al., 2004). It takes advantage of the biochemical reaction of the firefly:



In presence of ATP the enzyme Luciferase catalyses the oxidation of luciferin and emits light. Thus, living mammalian cells, which express Luciferase e.g. because of a gen-tag, will emit light if the organism has been treated with luciferin.

As the organism is not injured during the imaging process, this technique is suitable to study the influence of drugs on the same individual animal. This increases statistics and minimizes the number of necessary animals. The technique is not restricted to measurements close to the animal's surface but can also detect light coming from viscera and even from the brain since light can penetrate mammalian cells by several centimeters.

To measure the very faint light signals an absolute light proof dark chamber is necessary. The signals are acquired with a high sensitive camera with specifications depending on the needs of the application.

There are two key numbers very important: the quantum efficiency (i.e. the sensitivity) of the CCD-chip and the cooling of the CCD-chip. The cooler the CCD-chip the better is the signal noise ratio because the thermal noise

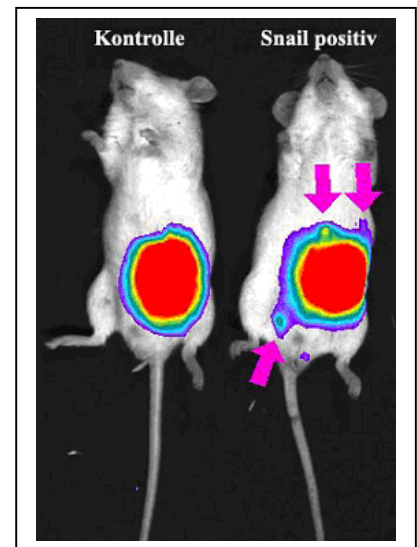


becomes smaller. For luminescence applications the cooling is usually exceeding the capability of regular fluorescence cameras.

Advanced multi-level peltier elements offer thermoelectrical cooling down to -90°C . Using liquid nitrogen, it can go down to -120°C .

The VisiLuxe II Imager by Visitron Systems GmbH is a system solution which offers all the necessary features for in-vivo luminescence measurements.

As an application example Dr. Strand and co-worker examined the influence of the transcription factor *Snail* on the development of metastases in mice. Hek293 cells have been transfected with the luciferase gen either with



an empty vector (control) or with a vector of *Snail* cDNA (Snail positiv). The transfected cells have been injected subcutaneously and imaged in the VisiLuxe II after 21 days. Strong light emission is clearly visible in both animals (control and Snail positive). However, in contrast to the control the *Snail* transfected animal exhibits additional growth of the tumor which has been marked with arrows.

Visitron Systems GmbH 2008

Sato A et al. *In vivo bioluminescence imaging*. Comp. Med. 2004. Vol. 54, pp 631.

Sölling A, Rainov NG, *Bioluminescence imaging in vivo – application to cancer research*. Expert Opin. Biol. Ther 2003. Vol. 3, pp 1163

Strand D., Kashyap A., I. Medizinische Klinik und Poliklinik, Johannes-Gutenberg Universität, pers. Kommunikation 2006

Toth R, et al. *Circadian Clock-Regulated Expression of Phytochrome and Cryptochrome Genes in Arabidopsis*. Plant Phys, 2001, Vol. 127, pp 1607.