Live-cell Imaging System

Single cell Imaging

# Cellgraph

Superior performance for biological variation research
with high sensitivity, accuracy and resolution
by ATTO imaging system


Since 1964
ATTO
Made in Tokyo
SupportingLifeResearch

# Capturing extremely weak bioluminescent signals in living cells

Cellgraph AB-3000B is an imaging system developed to detect low-level light emission in a single living cell using a highly sensitive EM-CCD camera.

The detection of low-level light emission has been achieved by employing an optical system with high condensing efficiency and a cooled EM-CCD camera with the highest level of absolute sensitivity. By using a temperature and $CO_2$ gas concentration* control system and a humidifying unit, the atmosphere inside the sample holding chamber is the same as that of inside $CO_2$ incubators. This incubation system enables long-term observation of cultured cells and tissue slices in the living state. The Cellgraph system also includes a color separation mechanism with built-in optical filters, enabling multicolor reporter gene assay using multiple luciferases. The accompanying analysis software makes it possible to measure the intensity of the light emitted from individual cells on the acquired images, and to collect continuous data of the bioluminescence intensity on a cell-by-cell basis. The compact design system blocks ambient light completely, so that a dark room is not required. Imaging conditions, such as the selection of filters, the adjustment of lighting, and the duration of imaging, as well as the condition of the sample chamber and the focusing, are fully controlled by a PC with dedicated software.

The Cellgraph is an ideal imaging system to observe faint bioluminescence emitted by cells and tissue for an extended period of time while keeping them alive.

* The $CO_2$ gas injection unit is an optional accessory.

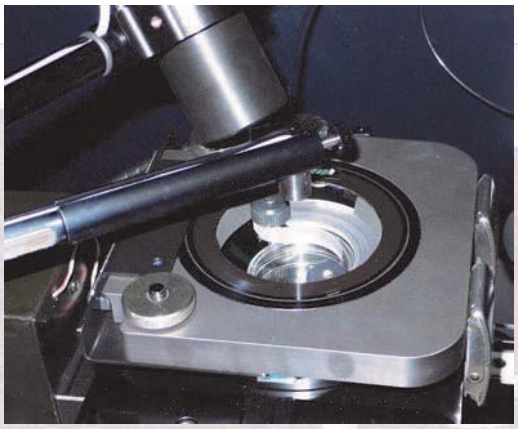
## Z-axis Motorized Stage

When the bioluminescence of the target is too dim and it is too difficult to focus on it, stage control mode is available. With this imaging mode, serial images are acquired as the stage moves along its z-axis automatically, thus making it easy to find the optimum stage position.


## Ultra Sensitive EM-CCD Camera

An ultra sensitive CCD Cooled camera with Electron Multiplying (EM) gain function, which achieves absolute sensitivity of a single photon/count, is mounted.

**Bright optical system by allowing light to travel for more sensitive &**



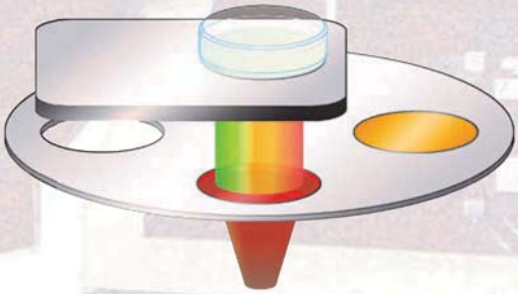
## Lighting

Apart from a white LED illumination for bright field application, a transmitted blue LED illumination with 480 nm wavelength is installed, enabling measurement of GFP fluorescence.

## Culture Chamber

Inside the chamber where the culture dish is placed, the temperature can be maintained at 37 °C and the air can be humidified and injected with $CO_2$ , enabling long-term culture.

* The $CO_2$ gas control and injection unit is an optional accessory.



## Optical Filters

With ATTO's unique technology for light separation, each bioluminescence component can be visualized and quantified after it is isolated through highly transmissive optical filters.

## Objective lenses

Depending on your sample of interest and purpose, a wide variety of objective lenses from 4x to 60x magnification are available.

**that minimize light loss**
**the shortest distance**
**clearer visualization**

# The ultimate system for bioluminescence imaging

-Acquire images of cultured cells or tissue samples on a φ 35 mm dish in the living state

-Provide a $CO_2$ incubator equivalent atmosphere by controlling the temperature, $CO_2$ gas concentration and the humidifying

-Highly sensitive and accurate detection of faint bioluminescence with an EM-CCD camera

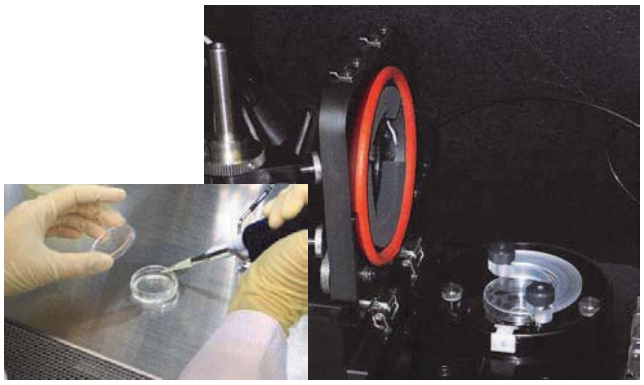
-Isolates and captures multicolor faint bioluminescence

-Easy operation via a user-friendly interface

-Full control from a PC with dedicated software

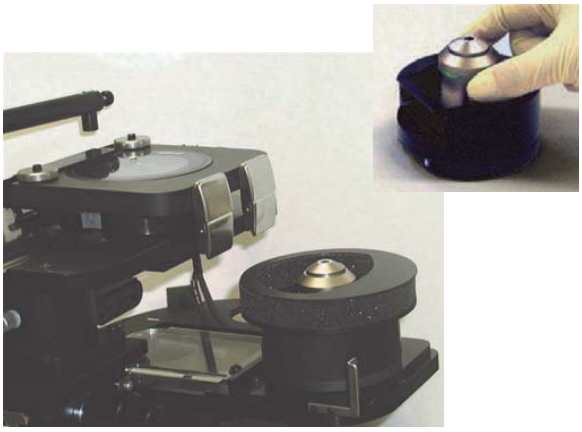
-Completely lightproof, compact design

# Simple and Easy to Use Workflow



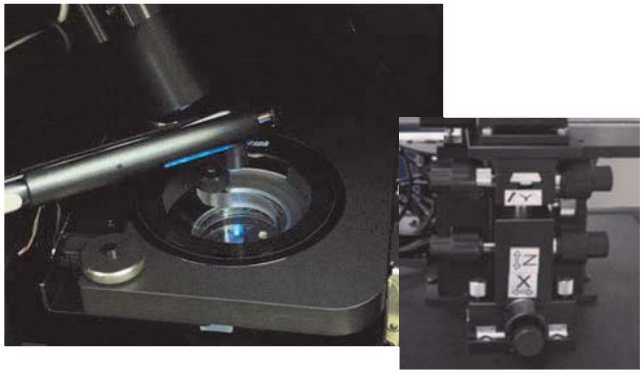
## 1. Setting the sample

Place the sample with a bioluminescent substrate such as luciferin in the chamber.



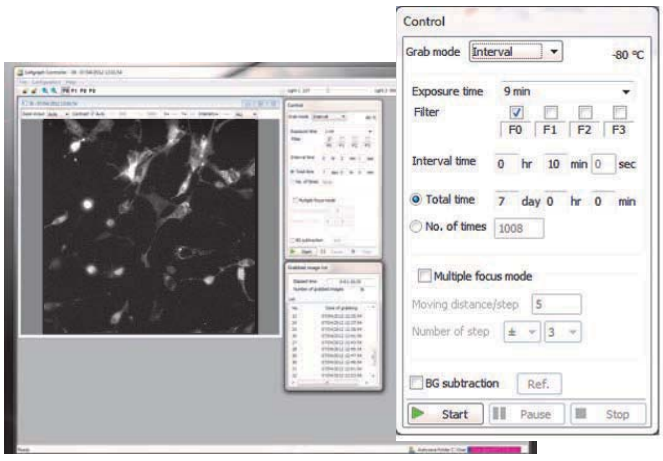
## 2. Setting the objective lens

Place the objective lens in the lens holder.



## 3. Setting the illumination and focusing

After setting the lighting, adjust the field of view and focus the camera while looking at the display monitor of the PC.




## 4. Starting image capturing

Set the capturing condition and click “Start-button.” The system will start the imaging procedure.

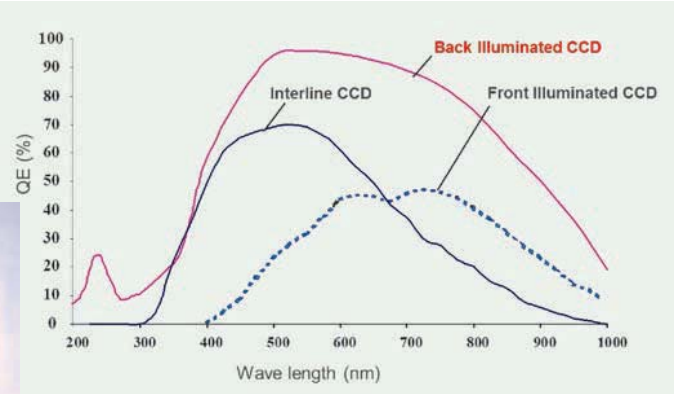
# A bright optical system delivers the best performance

## Ultra-sensitive EM-CCD camera

An ultra-sensitive cooled and back-illuminated CCD that achieves absolute sensitivity of single photon/count (at 535 nm) is mounted. The absolute sensitivity is calibrated by ATTO's unique method using a laser light source. This absolute sensitivity test enables measurement of total bioluminescence intensity at the focus point as photon counts (Japanese patent number: 3585439).

By amplifying the luminescent signal by setting the EM (Electron Multiplying) gain, images can be acquired with a shorter exposure time.

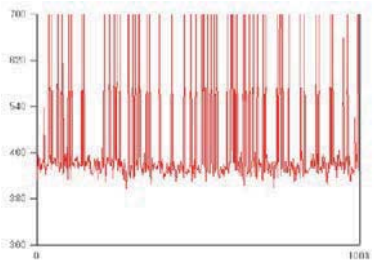
Active pixels: 512 x 512
Pixel size: 16 (W) x 16 (H) mm
Image area: 8.2 x 8.2 mm
Quantum efficiency: Over 90%

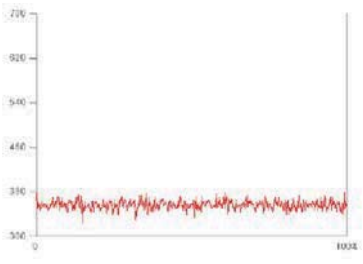



*CCD cameras can be selectable

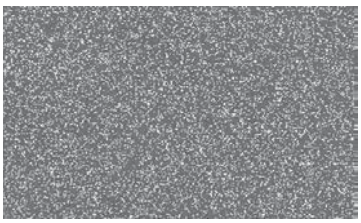
## Extra-low Noise Floor with -90°C Cooling

Noise level





Dark image



Normally used CCD camera

Cellgraph's EM-CCD camera

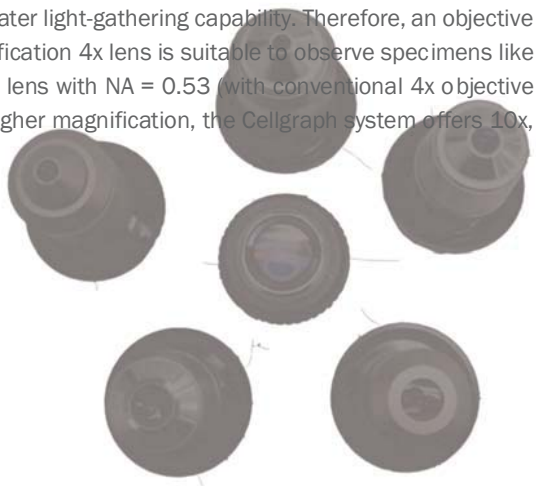
With a CCD camera, the longer the exposure time, the higher the background noise accumulates. The figure shown left indicates a dark image captured by Cellgraph with an exposure time of 10 minutes. Compared to a conventional CCD camera it has an extremely low background noise. With the Cellgraph system, the background noise is minimized by cooling the EM-CCD camera to -90°C with a water-cooling system (to -80°C with an air-cooling system), enabling a prolonged exposure time of 60 minutes.

*The water-cooling system is an optional accessory.

## Bright low-magnification objective lens

When considering which objective lens to choose, the most critical aspect is the numerical aperture (NA). When comparing lenses with the same magnification, a lens with a larger NA value has higher resolution and greater light-gathering capability. Therefore, an objective lens with a large NA value is needed to detect faint bioluminescence. A low magnification 4x lens is suitable to observe specimens like cultured tissue slices. The Cellgraph system employs a 4x magnification objective lens with NA = 0.53 (with conventional 4x objective lenses; NA = 0.1 to 0.2), which provides bright images at low magnification. For higher magnification, the Cellgraph system offers 10x, 20x, 40x and 60x objective lenses with large NA values.

The 4x objective lens for the Cellgraph system

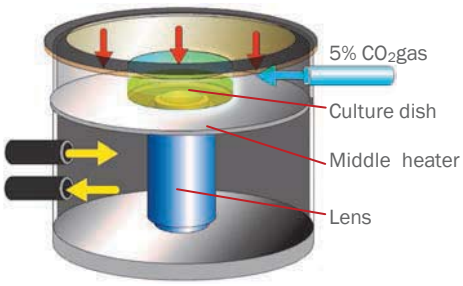


# Optimum environmental designing for live cell imaging

## Cell-friendly incubation function

The Cellgraph system has the same incubation function as commonly used $CO_2$ incubators in the laboratory for cell culture. The system stably provides an atmosphere (controlled temperature, $CO_2$ gas concentration and humidifying) that is suitable for the long-term observation of living cells or tissues.

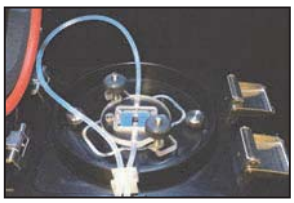
The inner structure of Cellgraph chamber is illustrated in the figure on the right. The temperature is controlled by an air conditioning unit and a heat glass. When the room temperature is 20°C, the temperature of the sample-holding chamber can be set and maintained at 25-45°C for a long period of time. The $CO_2$ gas injection unit* keeps the $CO_2$ gas concentration at 5% and humidifies the air before injecting. Other available optional units include an injection unit that allows you to inject reagents such as stimulant drugs during observation and a perfusion unit that flows the culture medium.




The temperature in a lower chamber is regulated with warm air that is blown by a heater

(※)$CO_2$has injection unit

Injection Unit

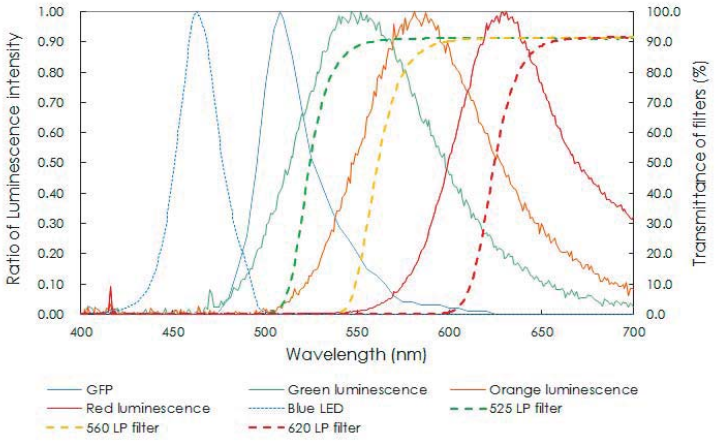


Perfusion Unit

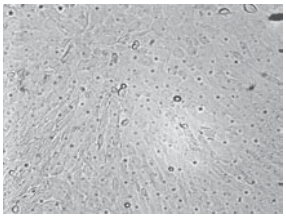

For culturing and observing tissue slices, Cell Culture Inserts (Millicell, PICM ORG 50, Millipore) is used.

## Filter system that captures light of various wavelengths

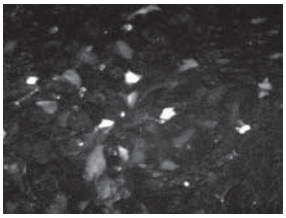
The Cellgraph system is suitable for continuous, real-time monitoring of faint bioluminescence (up to three colors), fluorescence (GFP), and bright field images of cells or tissue slices cultured on a φ 35 mm culture dish for several hours to several days. ATTO's color separation function uses fewer optical filters compared to conventional methods. As a result, the signal loss through the optical filters is minimized, and the captured images are close to the actual signals. The system also employs long path filters with excellent light permeability of over 90%, making it suitable to capture faint bioluminescence (Japanese patent number: 4052389). The graph shown on the right indicates the emission spectrum and the wavelengths and transmittance of the color separation filters with the Tripluc multi-color luciferases (TOYOBO).




Up to two imaging conditions can be set. Dual imaging with a combination of bright-field and bioluminescence, or fluorescence and bioluminescence, is possible. The acquired images can be edited and analyzed by specialized software called "Cellgraph Viewer." Using this software, it is easy to quantify the amount of bioluminescence, or to create movie files, montage of images, merged images, etc.

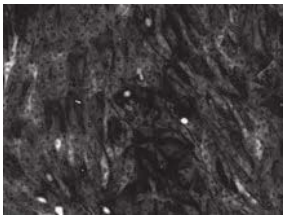


Bright field image

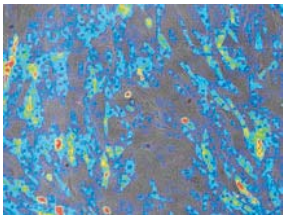


GFP image

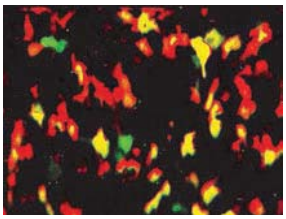
The light source for GFP (Blue LED)



BioLuminescence image



Merged image of bright field and bioluminescence
(shown in pseudo color)

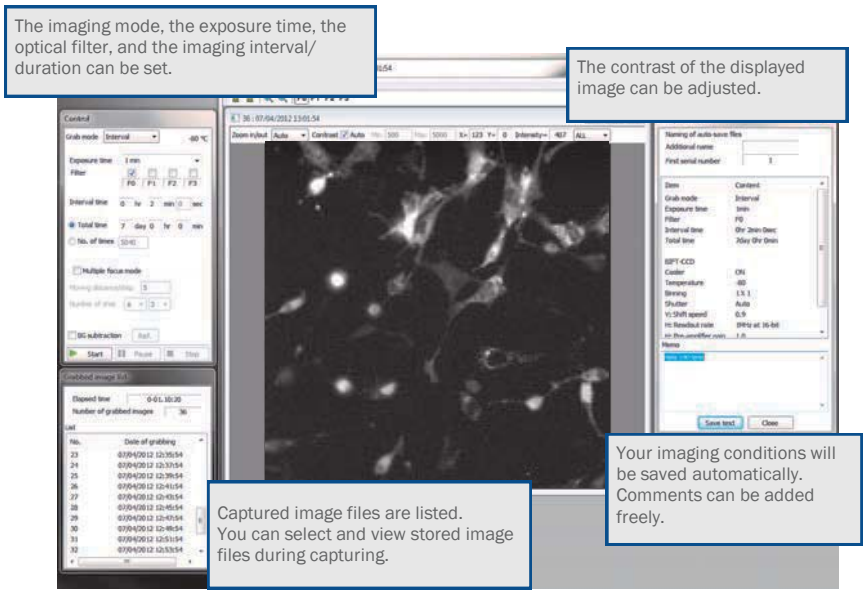


Two-color bioluminescence

# Various imaging modes that capture any biological event

## Various imaging modes

The Cellgraph system offers imaging modes that are suitable for various situations.




### Live mode

Shows the capturing image in real time. Used to adjust the position of sample and focusing while looking at the bright field image.

### Interval mode

Captures images at a regular interval, one by one, for a specified period of time. Used to create time-lapse images of bioluminescence.

### Stage control mode

Captures serial images while moving the motorized z-axis stage. Used for fine adjustment of the focus.

### Combination mode

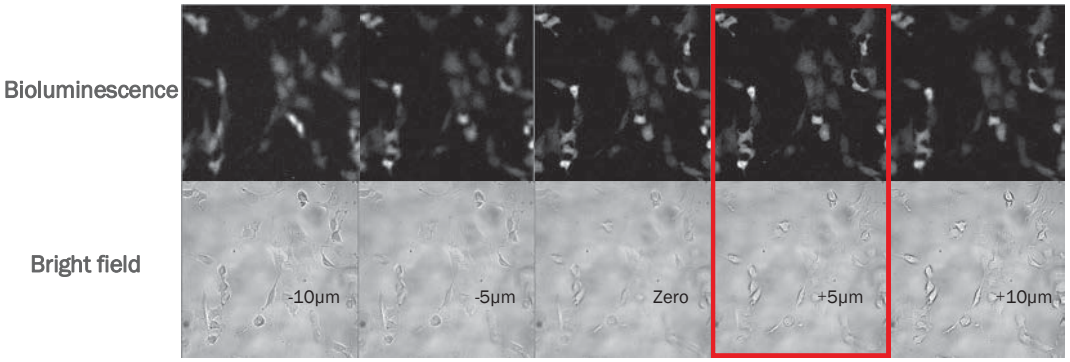
Captures time lapse images of bioluminescence and bright field images (or fluorescence images) simultaneously.

### Background mode

Captures the background images. The captured images will be used for background subtraction of bioluminescence imaging.

## A stage control function for easy focusing

To capture faint bioluminescence, a long exposure time is needed. In such cases, it is difficult to adjust fine focusing manually. The Cellgraph system offers Stage control mode, during which serial images are acquired as the stage automatically moves along its z-axis. After setting the moving distance per step and the number of steps, the system will automatically captured each focus image so that you can find the best focus point. All you need to do is to wait while engaged in other tasks.

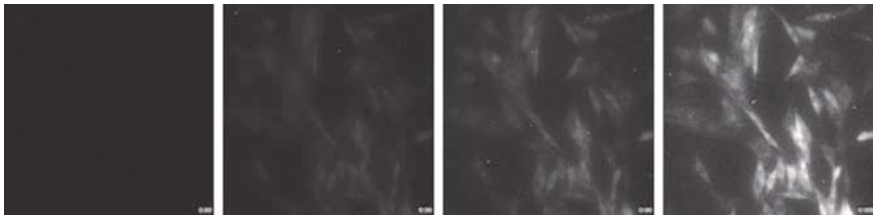




Images of NIH3T3 cell lines stably expressing luciferase. Those images were captured by Stage control mode, with a 40x objective lens, exposure time of 10 minutes, moving distance per step at 5 µm, and number of steps at ± 2. The image framed with a red line is the one with the best focus.

## Signal amplifying of the CCD camera

The Cellgraph system has various amplifying modes for the CCD camera to secure capturing of faint bioluminescence images.



without gain | EM gain (MAX) | EM gain (MAX) + Pre-amplifier gain (middle) | EM gain (MAX) + Pre-amplifier gain (MAX)

### Binning

1x1, 2x2, 4x4, and 8x8 binning modes are available.

### Pre-amplifier gain

Amplifies extra-low signals before A/D converting..

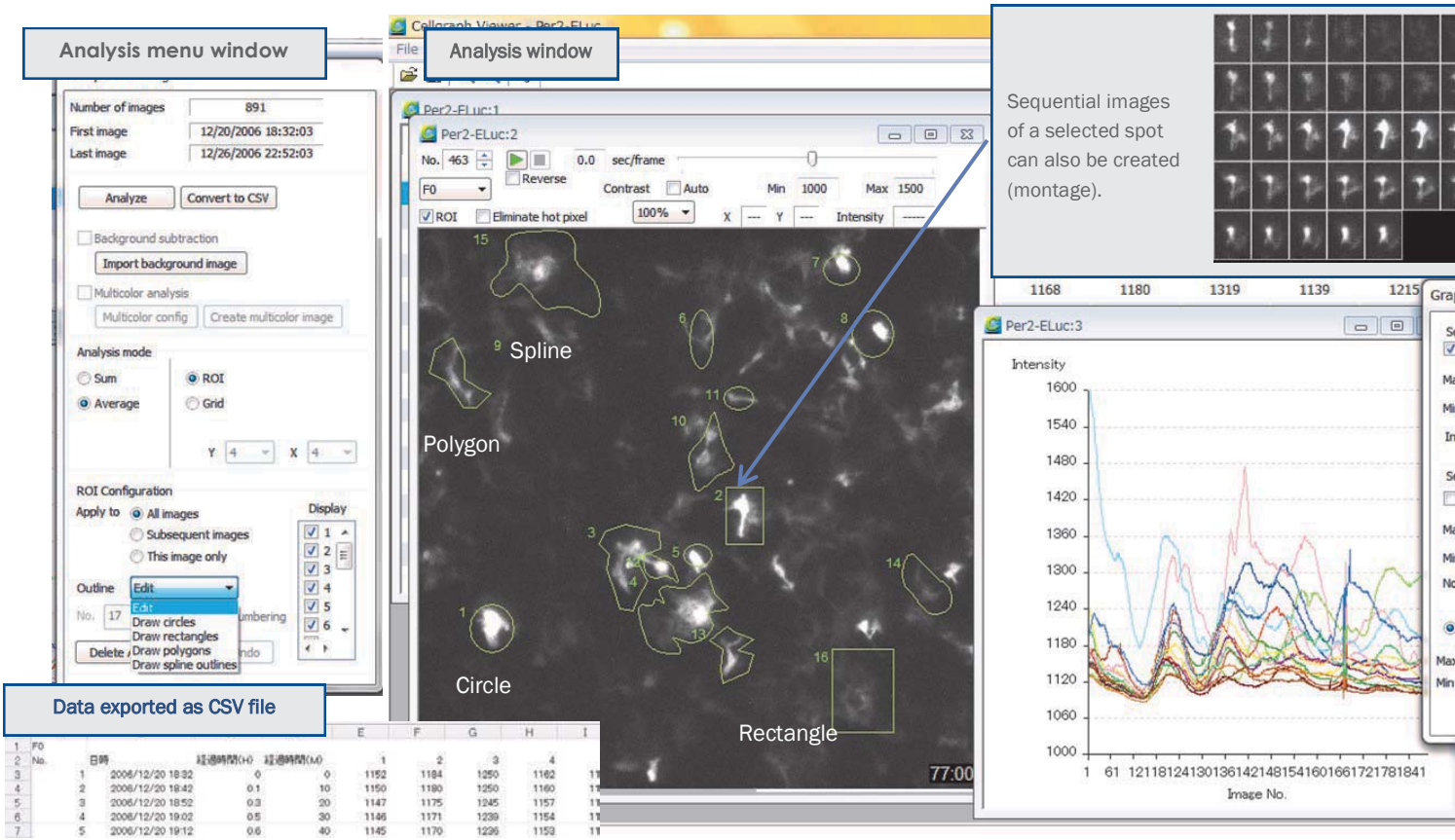
### Electron multiplier gain

The signals are amplified by EM (electron multiplier) gain while the electric charge of CCD is transferred. The procedure reduces background noise, and captures images with an excellent S/N ratio within a short exposure time. Available from x1 to x1,000.

# CellGraph Viewer, simple and easy to use analysis software

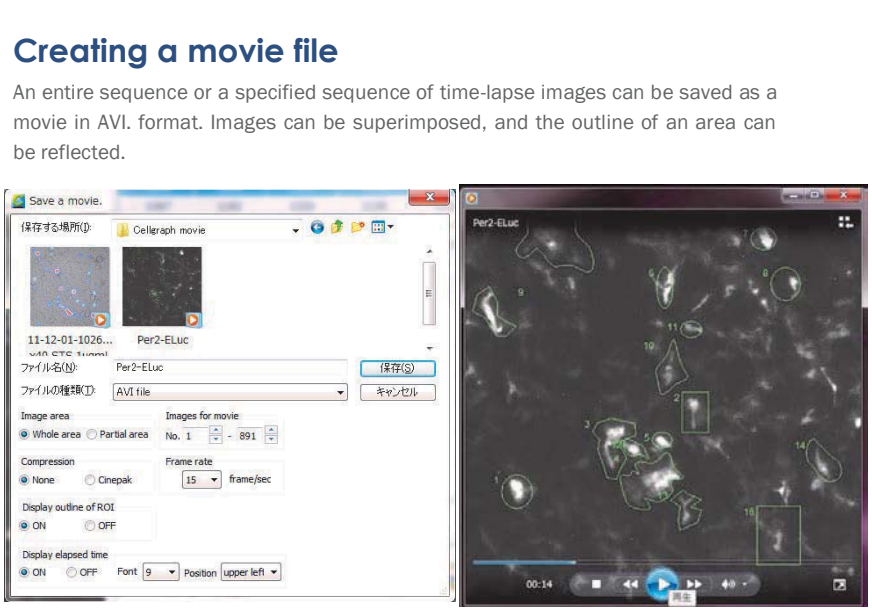
## Spot (ROI) measurement

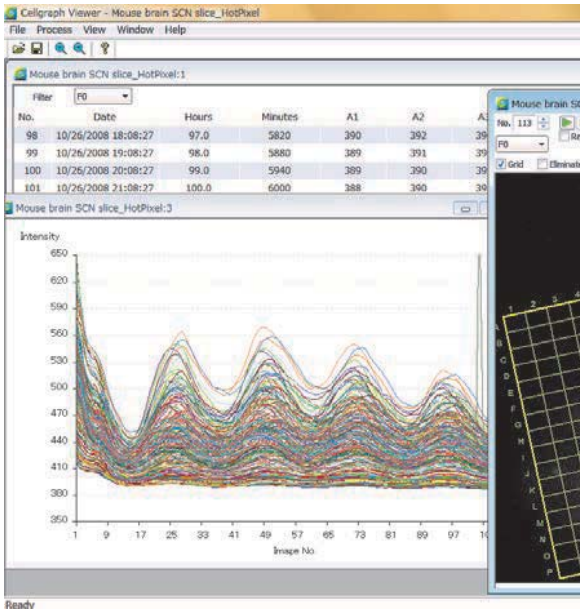
In this mode, bioluminescence can be measured individually in any given region of interest; for example, each cell in the area. You can select regions simply b clicking on the image. The intensity of the bioluminescence in the selected area will be quantified, and its corresponding graph will be plotted automatically. Th result of analyzed bioluminescence intensity data can be exported as CSV format files. There are four outline tools: circles, rectangles, polygons and splin (encircled by a curved line).



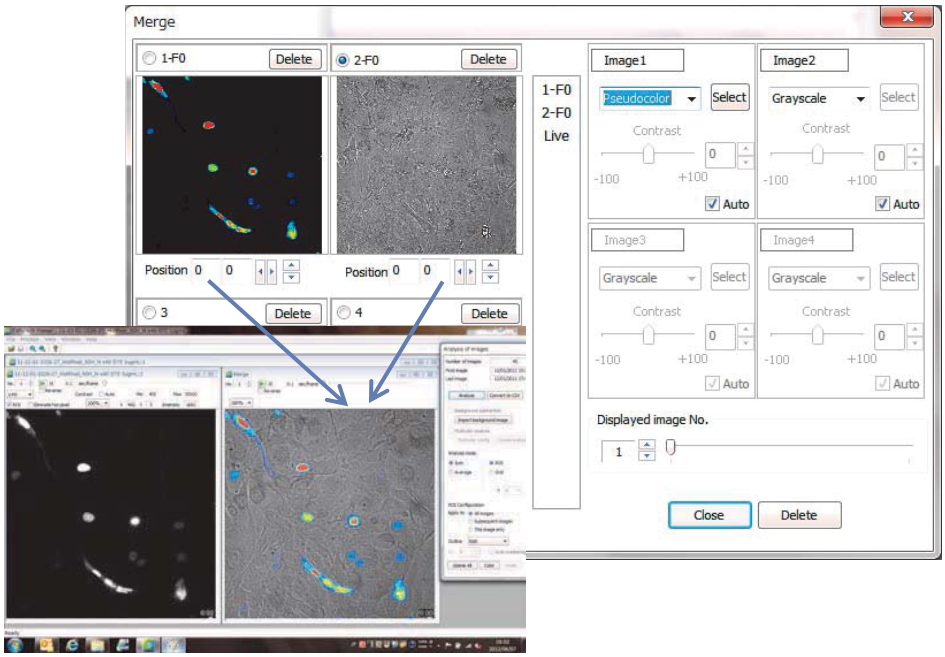

## Creating a movie file

An entire sequence or a specified sequence of time-lapse images can be saved as a movie in AVI. format. Images can be superimposed, and the outline of an area can be reflected.



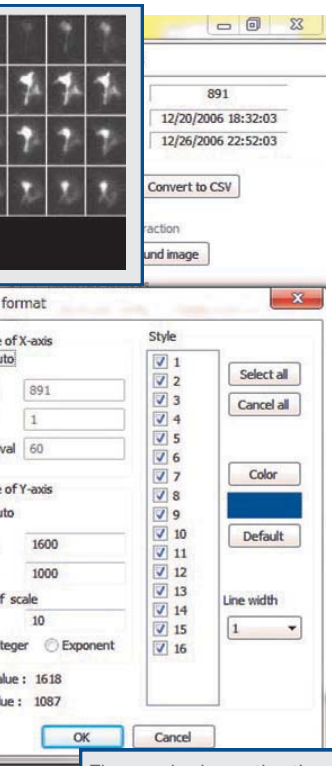




The graph shows the time course of bioluminescence value (brightness value) of each grid block. The y-axis can also be displayed on a logarithmic scale.




## Superimposition of acquired images

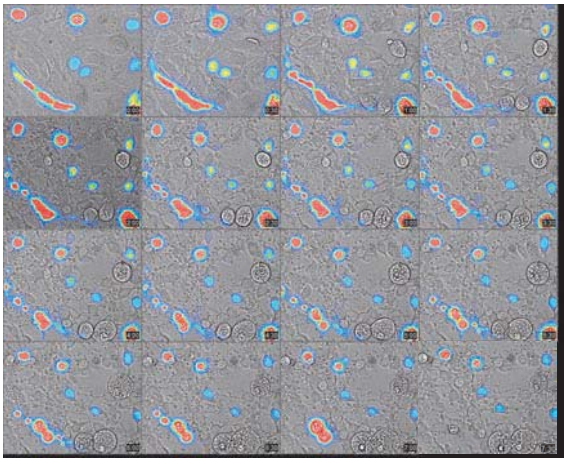
Images acquired through each optical filter can be superimposed and edited. When superimposing images, the display mode of each image can be selected from the gray scale, color scale, or pseudo color. The contrast or position of each image can be adjusted as well. Sequential images or a movie can also be created from the superimposed images.

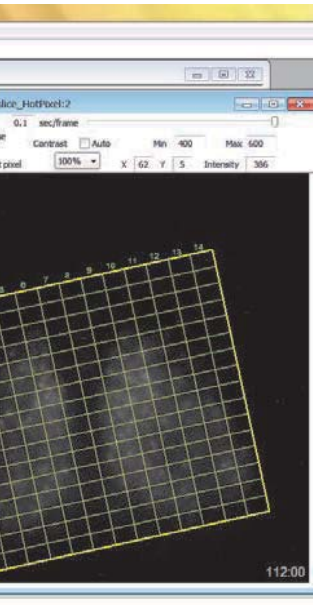



The graph shows the time course of the bioluminescence signal intensity in each selected area. The y-axis can also be displayed on a logarithmic scale.

## Creating sequential images

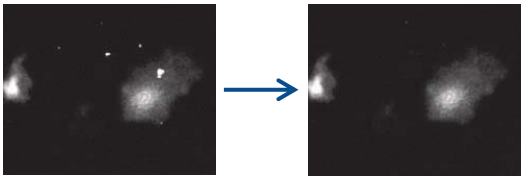
The time course changes can be displayed as a sequence of images (montage). Montage sequencing can be created in spot display, pseudo color display, or superimposed display mode.






## Grid measurement

In this mode, a grid is set on an image and the signal intensity in each compartment of the grid will be measured. The size and the number of elements of the grid can be set freely, and the grid can be rotated in any given angle.



## Removal of hot pixels

Removes dot-like noise on images. The signal intensity of the removed pixel will be substituted with the mean brightness value of adjacent pixels.

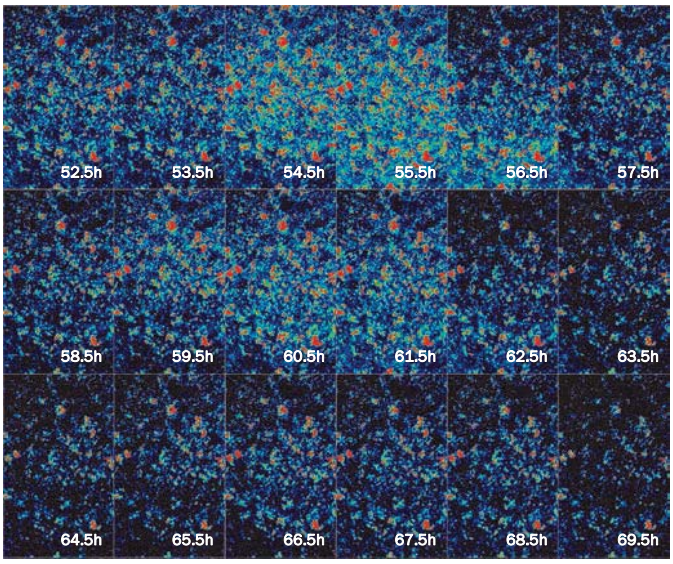
# Analysis tool for various biological phenomena

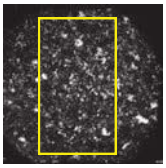
- **Real time reporter gene assay**
  - Transcriptional factor activity
  - Clock gene (chronopharmacology)
  - Gene expression (Transfection, RNAi,,,),,,
- **Analysis of cell responses**
  - Drug response (Anti-cancer drug, DDS,,,)
  - Stress response (Hormone, Inflammation, Anti-Oxidant)
  - Cytotoxichology,,,
- **Analysis of signal pathway**
  - Signal cascade (Calcium)
  - Apoptosis,,,

| | Bioluminescence | Fluorescence |
|---|---|---|
| Probes | Luciferase, Aequorin,,,,others | GFP, YFP, RFP,,,,others |
| Emission of light | Enzyme substrate (luciferin,,,etc.)<br>Unnecessary external light | Excitation light<br>Unnecessary substrates |
| Advantages | Low background<br>Quantitative analysis<br>No-damage to cells<br>Long-term analysis | High resolution<br>High intensity |
| Disadvantages | Low intensity | High background<br>Photo-bleaching<br>Damage to cells |
| | Quantitative analysis<br>Long term dynamics | Intracellular localization<br>Short term dynamics |

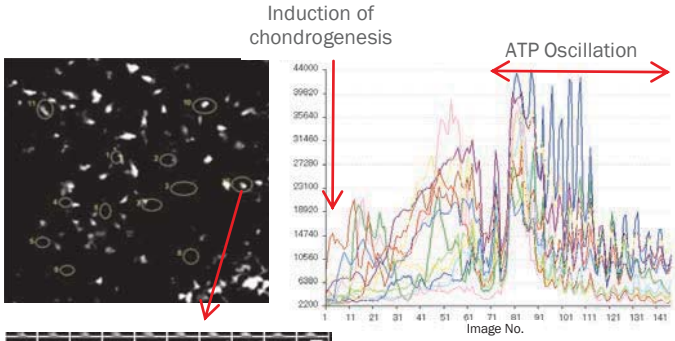
## Visualization of ATP oscillations in the early stage of chondrogenesis

Cellular condensation in embryonic limbs that occurs in the early stage of chondrogenesis is considered to play a critical role in the secretion of adhesion molecules and extracellular matrixes. However, the mechanisms that regulate the secretion of these factors remain uncertain. The following data were collected with the Cellgraph system. It shows the visualization of ATP oscillation after the induction of chondrogenesis in ATCD5 cells transfected with an ATP-dependent Phyxothrix hirtus luciferase gene. Blockade of the ATP oscillation prevented cellular condensation. The degree of cellular condensation also increased with the frequency of ATP oscillations. These results suggest that ATP oscillations play a critical role in the early stage of chondrogenesis. As demonstrated in this study, the Cellgraph system is an effective tool for examining intracellular metabolic mechanisms.



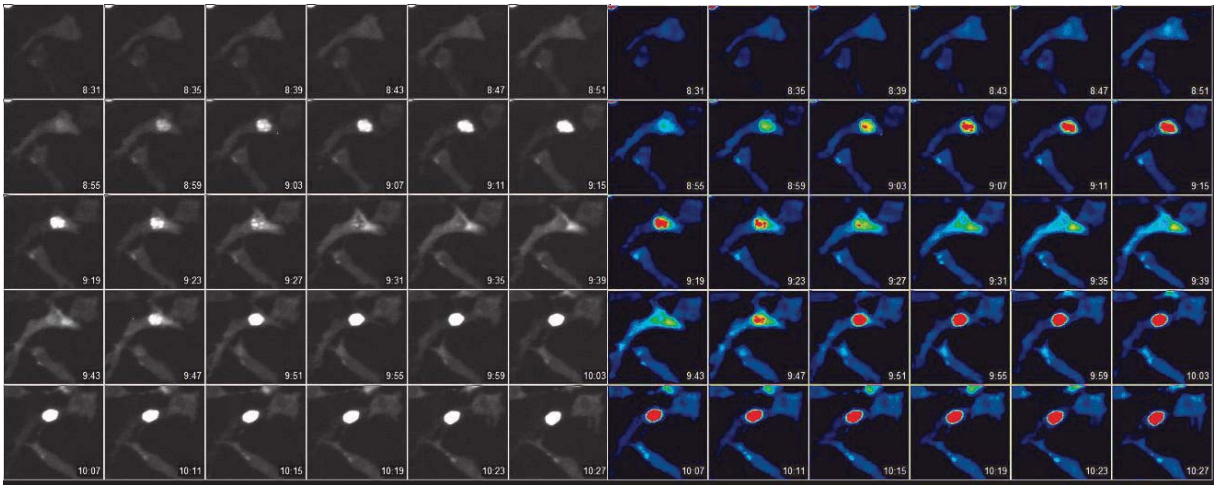
The figure above indicates the sequential images of ATP oscillation observed at low magnification. They are displayed in pseudo color. This area corresponds to the area framed by yellow lines in the figure at the bottom. The figure shows ATP signals propagating as a wave from the top to the bottom.




Shown in the graph above are the bioluminescent intensities of individual cells circled with yellow lines in the image on the left. The data were analyzed by the Cellgraph's imaging software. The image on the bottom left is a montage sequence put together from a series of images of a single cell among these.

**Data Supported:** Dr. HJ.Kwon, Hokkaido Univ., JAPAN
**Reference:** HJ.Kwon *et. al.*, Cell Death and Disease, Vol.3 (2012)

## Time-lapse imaging of intracellular trafficking of importin α

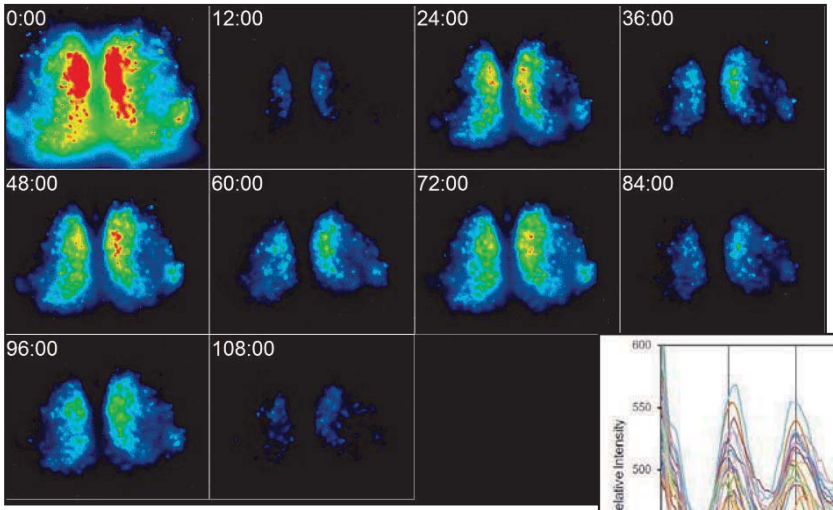


Bioluminescent image　　Pseudo color image

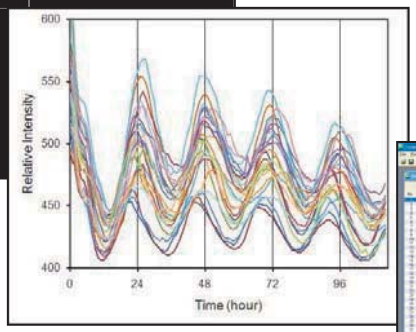
Visualization of nucleocytoplasmic shuttling of importin α by the Cellgraph system. In this study, importin α gene fused with luciferase was expressed in NIH3T3 cells. The time-lapse images were acquired using three minutes exposure time at intervals of four minutes with a 40x objective lens without binning. The bioluminescence signal was initially detected in the cytosol, then in the nucleus. After that, the bioluminescence signal in the nucleus gradually increased. As shown above, the Cellgraph system is an ideal tool for observing biological events such as trafficking of proteins that occur over a prolonged period of time.

**Data Supported:** Dr. Y. Nakajima, AIST, JAPAN
**References:** Y. Nakajima *et al*. *PLOS ONE*, Vol. 5 (2010)

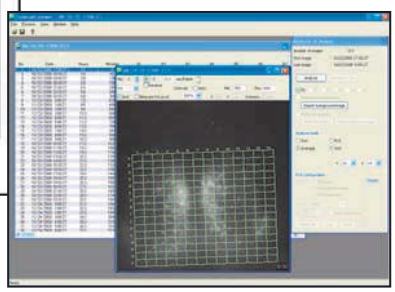
## Bioluminescence imaging of a brain tissue slice containing the mouse hypothalamic superachiasmatic nucleus (SCN)




The Brain were removed and sectioned into 100 µm thick slices using a Microslicer, each of which was then placed in a culture insert (Millipore). The left figure shows the time-lapse images of a SCN section acquired over a period of five days using the Cellgraph system. Using the grid measurement function, the bioluminescence intensity in each area was analyzed and quantified. The results are shown in the graph on the left.




Using the Cellgraph system, a brain tissue slice from a transgenic mouse that express luciferase under control of the clock gene promoter were analyzed.

# Specifications

| Model No./Name | AB-3000B /Cellgraph |
|---|---|
| Cooled CCD Camera | CCD type: Back-illuminated EMCCD<br>Active pixel: 512 x 512 pixel<br>Pixel size: 16 x 16μm<br>AD Resolution: 14/16 bit<br>Cooling temperature:<br>-80°C with an air-cooling system when the air temperature is 25°C, -90°C with a water-cooling system* (when the water temperature is 25°C)<br>-85°C with an air-cooling system when the air temperature is 20°C, -100°C with a water-cooling system* (when the water temperature is 16°C)<br>*The water-cooling unit is an optional accessory. |
| Objective Lens | 4x (NA 0.53)（Other magnification lenses are also available as optional accessories） |
| Stage | X-Y-Z axis manual stage, Z-axis motorized stage |
| Culture dish | 35mm culture dish |
| Constant temperature function | （Room temperature +5°C) to 45°C, 0.1°C step |
| Lighting | White LED with dimming function　Blue LED with dimming function |
| Optical Filter | Up to three filters can be set (525LP/560 LP/620 LP are standard equipment) |
| Exposure time | 30 milliseconds to 90 minutes |
| Imaging interval | Indefinite |
| Control software<br>System requirement | OS : Windows 7/Vista/XP<br>RAM: 1 GB or more (512 MB or more for XP)<br>Hard Drive: 30 GB or more free disk space required<br>Interface: Full size PCI slot x1, serial port x1, USB port x1 |
| Size（main body） | 430 mm (W) x 600 mm (D) x 650 mm (H) |
| Weight （main body） | Approx. 40kg |
| Power （main body） | AC100-240V 106VA（varies according to the components of the whole system） |

| Optional Accessories | |
|---|---|
| CCD camera water-cooling unit | Constant-temperature water circulating system, heat-resistant tube for circulation |
| $CO_2$ gas injection unit | $CO_2$ gas-mixer |
| $CO_2$ gas humidifying unit | Bubbling system for gas humidification |
| Perfusion culture chamber unit | Chamber for perfusion |
| Objective lenses | 2x, 10x, 20x, 40x, 60x |

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3. Y. Nakajima, T. Yamazaki, S. Nishii, T. Noguchi, H. Hoshino, K. Niwa, V. R. Viviani and Y. Ohmiya, Enhanced beetle luciferase for high-resolution bioluminescence imaging. *PLoS One*, **5(4)**, e10011 (2010)
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**AB-2270 Luminescencer OCTA**
**Tube type luminometer**

**AB-2350 PHELIOS**
**96/384 well plate luminometer**


**AB-2550 KronosDio**
**Luminometer for live-cell & tissues**


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