Composition and specification of product Cellgraph

Product Name Cellgraph **Model Number** AB-3000B

Cooling CCD Camera

CCD type : Back- illuminated EMCCD
Pixels : 512 X 512
Pixel Size : 16 um
AD resolution : 14 , 16 bit
Cooling temperture : -80 (air cooling), -90 (water cooling, option)
Objective lens x 4 , x 10, x 20 (Option)

Stage

X-Y-Z axis : Manual
Z axis : Motorized (external control)

Sample installation part

Sample Holder : 35mm culture dish
Constant temperature function : Room temperature +5 to 45 degrees, 0.1 degree step

CO incubation

Humidifying Unit
CO gas mixer

Lighting

Bright field : White LED with dimming function
Fluorescence : Blue LED with dimming function
Filter : 515nm , 580nm, 620nm Long pass filter

Control Program

Imaging modes : Live/ Interval/ Stage control/ Combination/ Background
Filter Switch : Automatic control of set filter
Exposure time : 30msec to 90 minutes
External control of lighting : Available
Image save format : 16 bit TIFF
Taken image check function : Present

Analysis Program

ROI setting : square, spline, polygon, grid, circle
Signal calculation : sum of the ROI
Average signal per pixel of ROI
Result data : Savable in CSV format
Graph display
Creating time lapse animation file : Entire sequence or specified sequence is saved in avi format
Image data processing : pseudo color display / hot pixel delete
Size(main body) : 430 mm(W) x 600 mm(D) x 650 mm(H)
Weight : Approx. 35kg (only main body of Cellgraph)
Power supply(main body) : AC100-240V 106VA(varies according to the components in the whole system)

ATTO Cellgraph for single cell imaging

Cell friendly real-time cell imaging using bioluminescence

Cellgraph

The mutual adjustment of the expression of various genes within a cell is a fundamental control process at work within all living bodies, which is to say that there is a gene expression network, and for analysis of these gene expressions, fluorescent proteins such as GFP and bioluminescent proteins such as firefly luciferase are used. Although cellular damage caused by excitation from exposure to light might become a problem when using a fluorescent protein as a reporter, there is no such concern with luciferase. Moreover, luciferase is not an endogenous protein such as Alkaline Phoshatase and Peroxidase, and D-luciferin for which cell toxicity is considered relatively small is its luminescent substrate, so it is a reporter suitable for experiments to assay the transcriptional activity of the promoter in real-time for several days (“real-time reporter assay”).

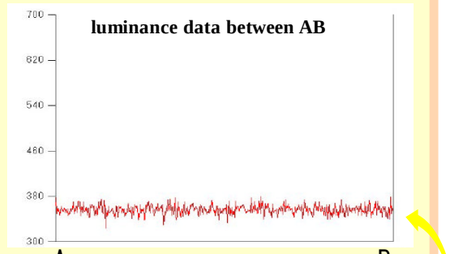
AB-300B type Cell graph(Cellgraph) is an imaging system developed to detect low light of a single cell. The detection of this low light is achieved by employing an optical system with high condensing efficiency and a cooled CCD with the highest level of absolute sensitivity. In addition, cell and tissue culture for an extend period is enabled using the standard equipment of the incubator and continuous measurement of the promoter activity is enabled at the single cell level using the genes for bioluminescent proteins such as the firefly luciferase.

High-end, ultrasensitive CCD camera and high condensing efficiency optical system

Cellgraph

A highly sensitive CCD with EM function is installed, which achieves absolute sensitivity of single photon/count

A highly sensitive back illuminated cooled CCD that achieves absolute sensitivity 1 count/photon (534 nm) is installed. The Electron Multiplying (EM) function can also be set, so it is possible to capture an image with higher sensitivity.
Noise range of this CCD is about 10 counts (at conversion of 16bit AD) and the detection limit when S/N is 2 is about 20photon/pixel. There is no problem with exposures of about 60min by thermoelectric cooling down to -90 degrees with vaccum sealing off.



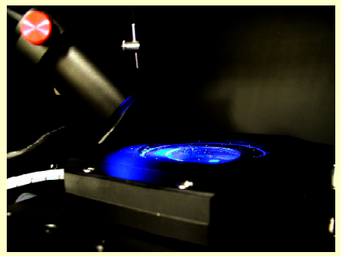
Dark image of exposure for ten minutes

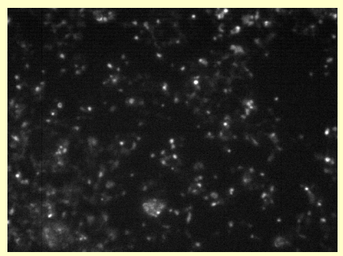
Bright Optical system

Numerical aperture (NA) of the general 4x object lens is about 0.1 to 0.2. In Cellgraph, the 4x objective lens has achieved NA=0.53. An object lens for higher magnification with large NA can also be used.

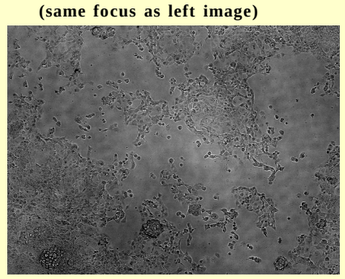
Focus adjustment in fluorescent image

In addition to lighting for bright field, transmitted illumination of 480 nm is attached. When GFP, etc. is transfected at the time, it becomes easy to set the target.





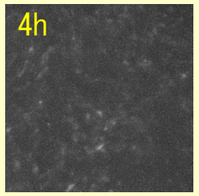
Bright feild image
(same focus as left image)



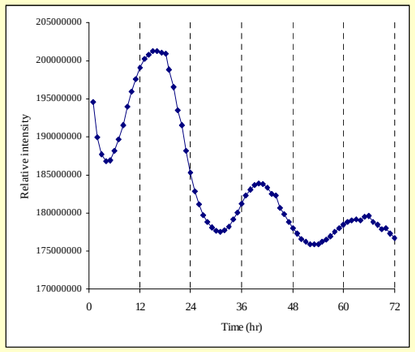
Imaging of stable cell line

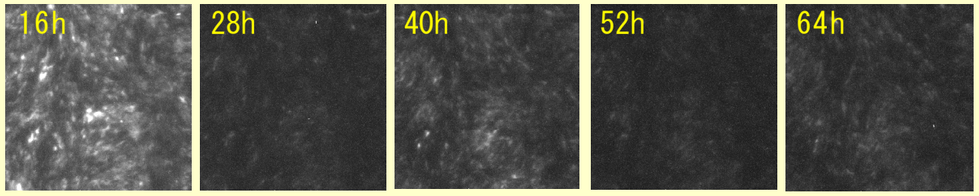
Cellgraph

This is a series of images taken every 12 hours after Dexamethasoue stimulation of a stable expression cell line of Rat 1 cells into which the promoter of Bmall (One of the clock genes) with firefly luciferase gene added had been introduced. The magnification is 5.2 times, and the exposure time is 20 minutes. The images show that periods of luminescence and dark were repeated, and when the amount of total luminescence over the entire image sequence is calculated from all images and graphed, the circadian variation becomes clear.



When the sum total of the brightness value is obtained from the entire time sequence of images and the value is plotted, the change in the Bmal1 promoter revitalization can be confirmed. The image can be divided into height and width, and the sum total brightness of each area can be obtained.

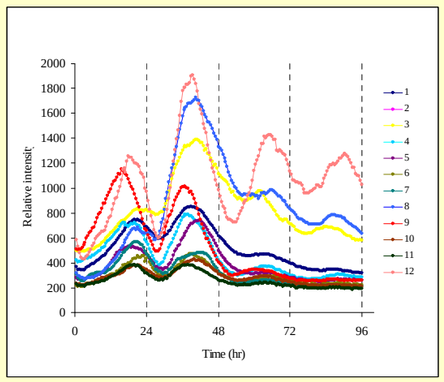




< Data support > Hokkaido University graduate school medicine,Laboratory of Integrative Physiology Prof. Kenichi Honma

Analysis of intercellular gene expressions in culture cells 250cells available / bioluminescence

Cellgraph



As shown in the Fig. on the right, when measuring the amount of luminescence of an individual cell from the time series image, it was confirmed that the promoter activity of Bmal1 was rhythmical almost synchronous.

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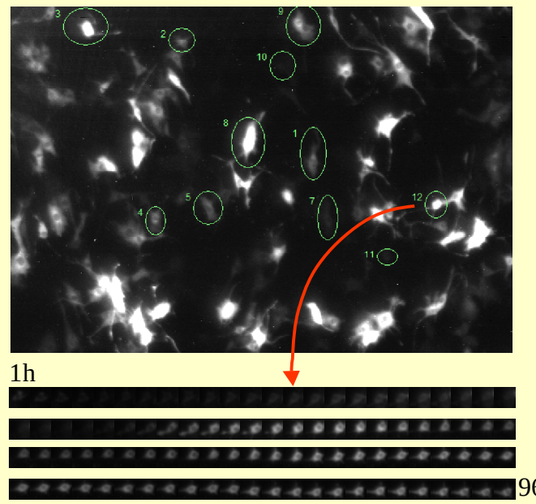
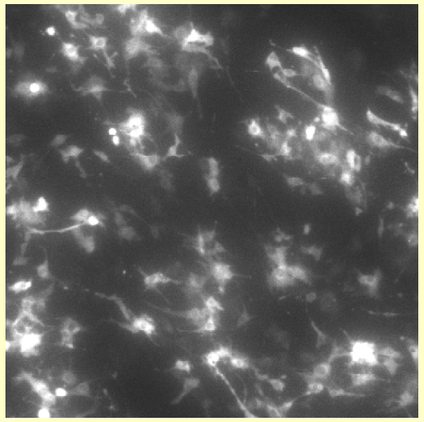
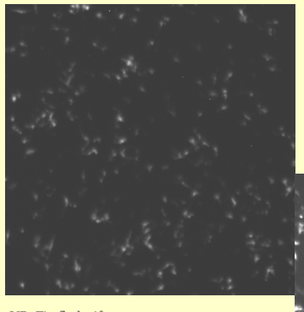


Expression vector 2 ug, in which luciferase cDNA is inserted downstream of the Bmal1 promoter, is transfected by the lipofection method into mouse derived fibroblast NIH3T3 seeded in a glass bottomed dish. After stimulating with dexamethasone, it is transferred to DMEM medium including 25mM HEPES, 10% FBS and 200 u M D-luciferin potassium, and an image is taken at an exposure time of 20 minutes at 5, 6x objective lens.

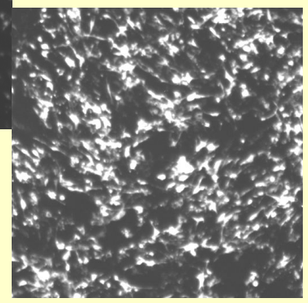
Luciferase with high luminescence efficiency

Cellgraph





When you use luciferase with high luminescence efficiency, it is also possible to perform imaging by the exposure for a few minutes at about ten times the magnification.



UP :Firefly luciferase
RIGHT:Luciferase with high luminescence efficiency, Emerald Luc(TOYOBO)

Expression vector 2 ug, in which luciferase cDNA is inserted downstream of CMV promoter, is transfected into NIH3T3 cells seeded in a glass bottomed dish by the lipofection method. After culturing for 24 hours, it is transfered to DMEM medium including 200 uM D-luciferin and 10% FBS. Luminescence was imaged using 10x objective lens at an exposure time of 3 minutes.

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National Institute of Advanced Industrial Science and Technology, cell engineering research div. Cell dynamics research group Prof.. Yoshihiro Nakajima

Relatedproduct

Luminometer for Real-time Reporter Assay in culture cells

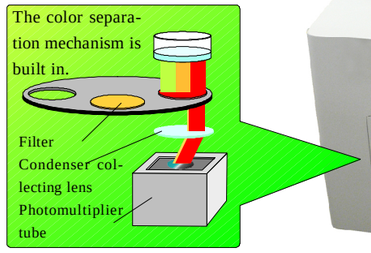
Cellgraph

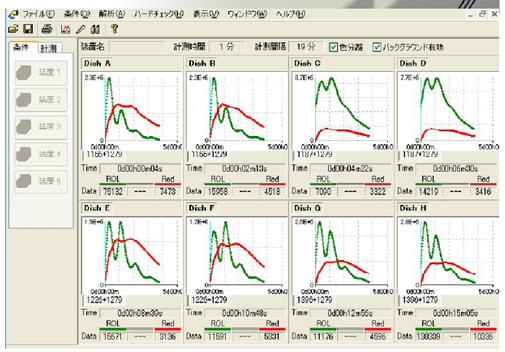
This is the optimum device to monitor gene expression of established cell culture and tissue slice culture at fixed intervals over a few hours to a few days. For the long term cultures, it is possible not only to control temperature in the cabinet by air circulation but also to introduce CO . Moreover, an optical filter is built in , so it is possible to monitor up to three kinds of gene expressions using luciferase with different colors of luminescence.

Up to eight 35mm culture dishes can be set at a constant temperature. They are arranged in such a manner that no stray light occures between these dishes. Because dishes can be easily replaced by opening the top door, the stimulatory effect of drugs can easily be simulated. After the door is closed, temperature rises promptly, so stable assay results are obtained.

The CO density can be maintained at 5% using CO internal regulator and sensor.

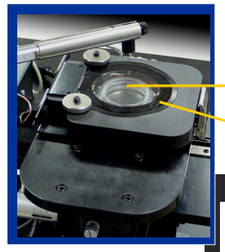
Internal filters are changed over one by one and luminescence permeating through the filter is measured by a photomultiplier tube.



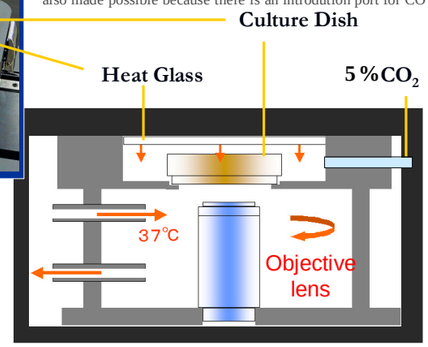


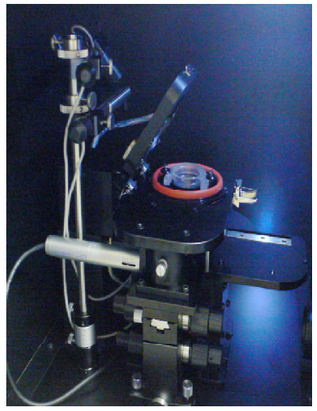
Incubation function enabling long term measurement

Cellgraph



The temperature of the 35mm culture dish can be kept constant at 37 degrees. The capability to continue the culture for an extended period is also made possible because there is an introdution port for CO .





Dual reporter assay for single cell imaging

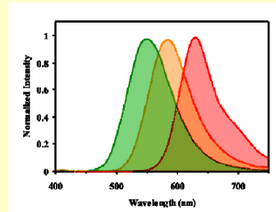
Cellgraph

In the case of the firefly luciferase the emission spectrum changes with depend on the pH. This characteristics is not suitable for a reporter assay in a live cell. Recently luciferases have been cloned that has a different emission color though its substrate is the same D-luciferin. Becasue the emission spectrum of these luciferases are pH- independent, the real-time reporter assay can be made dual if the luminescence can be distinguished.

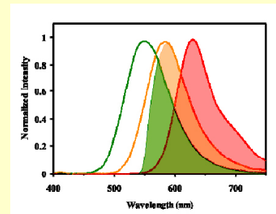
発光タンパク質 Luciferase	基質 Substrate	極大波長 Max. Wavelength	pH-dependent emission pHによる波長変化 Wavelength shift
Firefly (*Photinus piralis*)	D-Luciferin	560～620nm	pHにより変動 YES
Railroad worm	D-Luciferin	620nm	安定 no
Brazilian Click Beetle (*Phrophourus plagiopthalamus*)	D-Luciferin	540nm	安定 no
Firefly (*Rhagophthalmus ohbai*)	D-Luciferin	560,580nm	安定 no
Renilla	Colenterazine	470nm	no

The best luminescence color separation method for low light signal

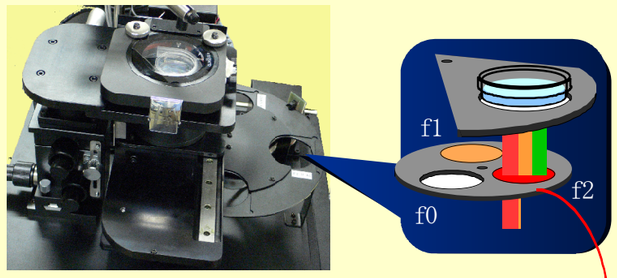
This is a technique that can efficiently separate low light signals with adjacent luminescence wavelengths. This example shows separation of three elements, where the filter transmittance of each element is determined beforehand. Next the amount of luminescence of the sample is measured (F) in the case where a filter is not used (F0) and the case where each filter (f1, f2) used (F1, F2). It is easy to calculate each luminescence element because the relationship of the following formula (F) can be established as the relationship between the actual measured value(F0-2) and transmittance(k). Even if the separation of the luminescence color with an individual filter is imperfect, this formula is suitable for the measurement of low light signal because a filter with high transmittance can be used.

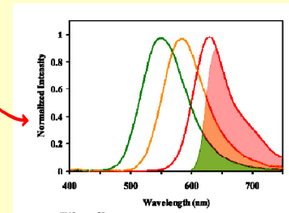


No filter



Filter f1





Filter f2

Y. Nakajima *et al* (2005) Multicolor luciferase assay system: one-step monitoring of multiple gene expressions with a single substrate , BioTechniques , **38** (6) , 891-894

$$\begin{pmatrix} F0 \\ F1 \\ F2 \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 \\ \kappa f1r & \kappa f1g & \kappa f1b \\ \kappa f2r & \kappa f2g & \kappa f2b \end{pmatrix} \begin{pmatrix} G \\ O \\ R \end{pmatrix}$$

F : Measurement value
κ : Transmittance of each color light in each optical filter
G, O, R : Each color light

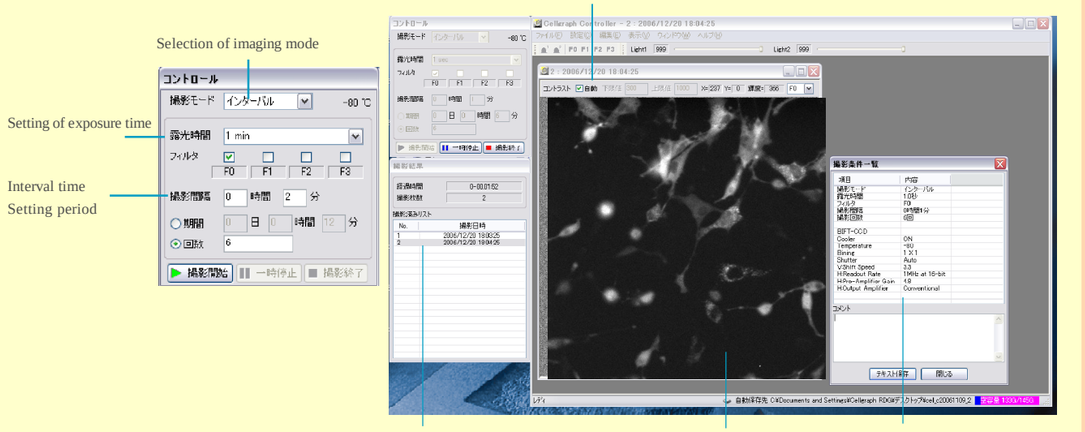
Separation formula of luminescence element

Various imaging modes

Cellgraph

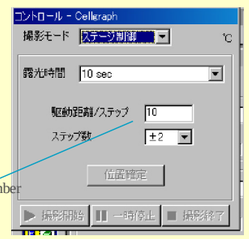
Interval imaging

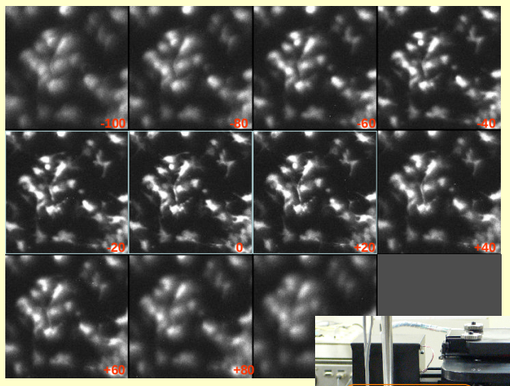
This mode enables taking time-lapse image.



Stage control Imaging

In case where it is difficult to specify the optimum focus of luminescence of the target, there is a mode that enables imaging while changing the stage position (Z axis) automatically. The optimum position can be assessed from the series of images taken in this mode.





NIH3T3 cells
objective 20X lens
3 min exposure
20 Step

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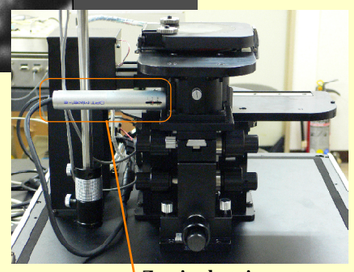
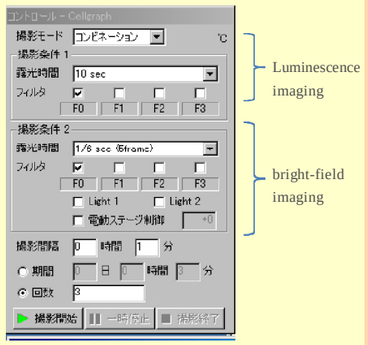


Combinations imaging

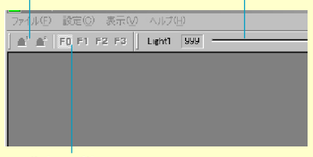
It is possible to alternately take an image of the bright field after taking the luminescence image



Bright field imaging

Turning lighting on and off of the bright field imaging

Brightness of lighting

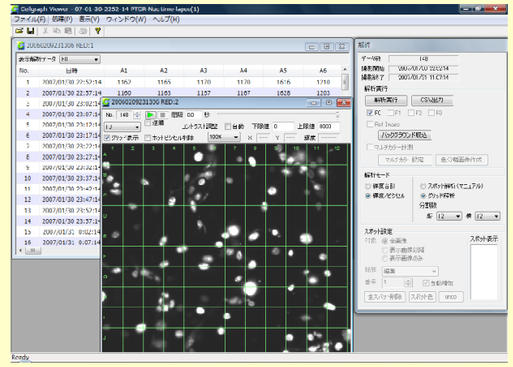


Analytical edit function

Cellgraph

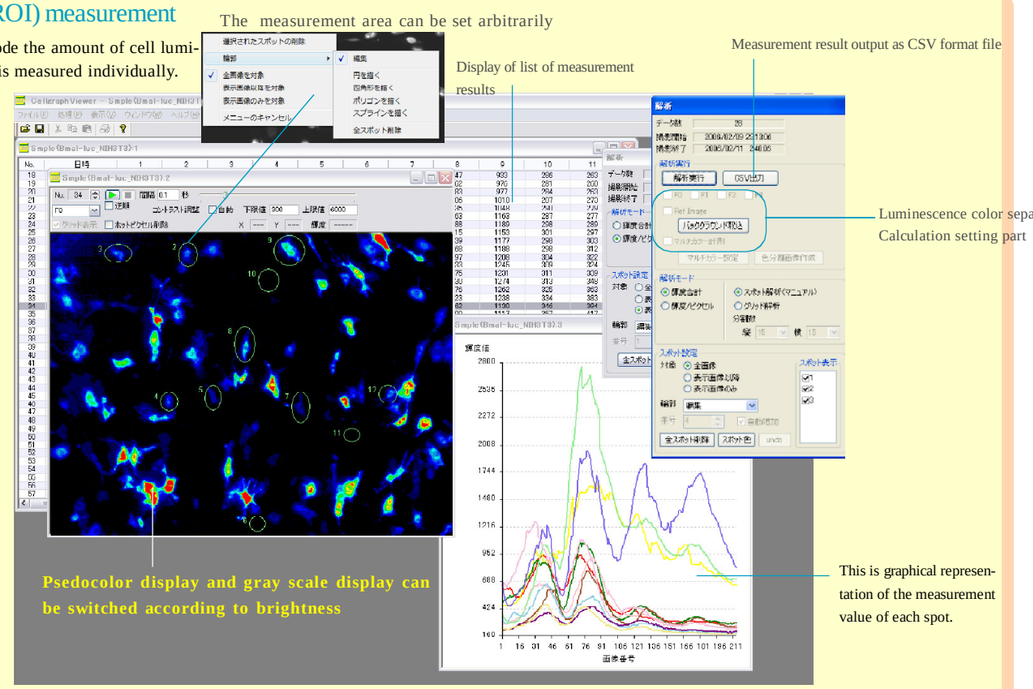
Grid measurement

In case where it is difficult to examine the difference in local expressions or when it is difficult to recognize individual cells such as in a stable expression cell line, a grid measurement mode can be used, which measures the amount of luminescence by dividing an arbitray area into a Grid.



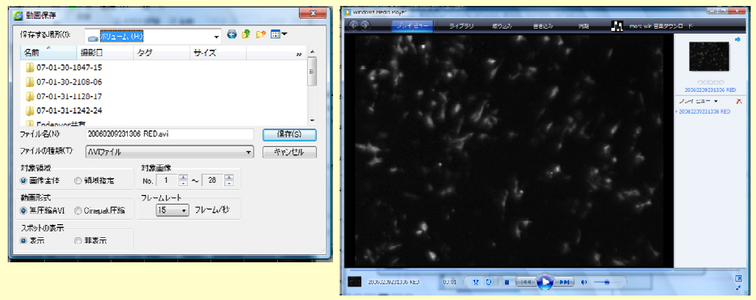
Spot (ROI) measurement

In this mode the amount of cell luminescence is measured individually.



Creating an animation

Entire sequence of time lapse images or images of a specified sequence can be saved as an animation in an avi format file.



Playback by Windows Media Player
Windows Media Playere is a registered trademark of Microsoft corporation