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ATTO AB-2500, AB-2550 Kronos

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ATTO AB3000B Cellgraph

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Bioluminescence analysis Tools from ATTO

-Application data -

AB-2550

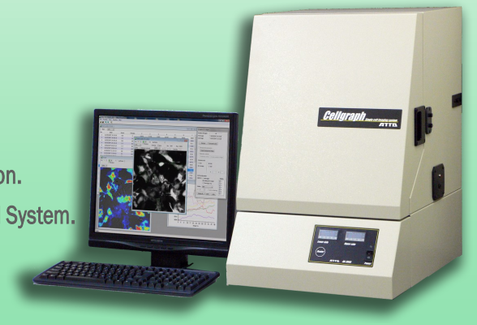
Photon Countable Incubator
KronosDio

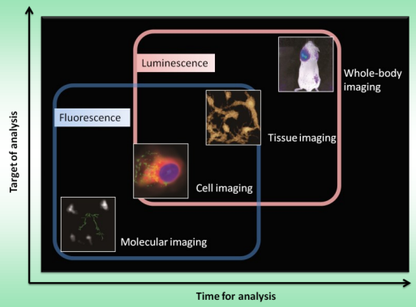
Luminescence Measurement of Live-cells under Fully Controlled Environment.
Real-time Reporter Assay of up to Three Genes by Individual Luminescence Color.
Photomultiplier-Based High Sensitivity Measurement System.
Highly Applicable to Long Term and Various Luminescence Assay.

AB-3000B

Single cell Imaging
Cellgraph

Real-TimeLive-cell Imaging using Bioluminescence in Complete Incubation.
High-Ultrasensitive CCD Camera and Highly Effective Condensing Optical System.
Dual Reporter Assay for Single Cell Imaging.
Various Imaging Mode and Analytical Edit Function.





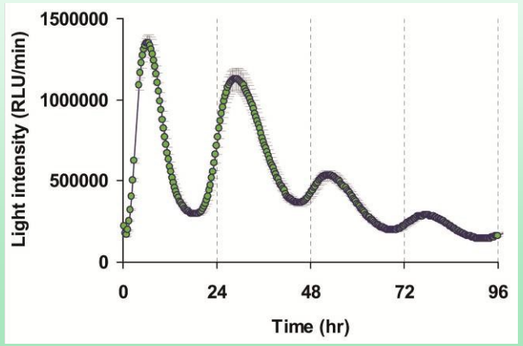
Making full use of the properties of the luminescence assay system

The “luminescence” assay system has excellent advantages different from those of the “fluorescence” assay system using GFP. A strong advantage of the fluorescence assay is that fluorescence can be detected even at the level of a single molecule simply by applying a strong excitation light. That is not the case in the luminescence assay, because the light emitted from a single molecule is too weak to detect. On the other hand, the luminescence system is an extremely non-invasive method suitable for long-term observation. Thinking of the hierarchy of biological units (i.e., molecules, cells, tissues, organs, and the whole body) in the field of life-science research, the fluorescence system may cover the structural units from molecules to tissues, while the luminescence system is capable of imaging the higher-level structural units, from cells to the whole body.

Real-time Reporter Assay of Live-cell

Bioluminescence reporter assays are used in various studies of biological functions, such as gene expression, signal transduction, post-translational modification, protein interaction,,,etc. Our improved technologies of detection system for Luciferase assays achieved the quantitative visualization of gene expression and monitoring of very weak luminescence signals in live-cell for long-term.

Real-time Monitoring for Luciferase expression as a reporter in live-cells

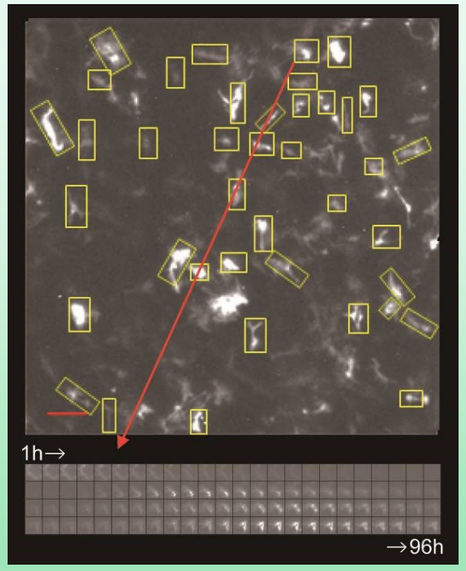


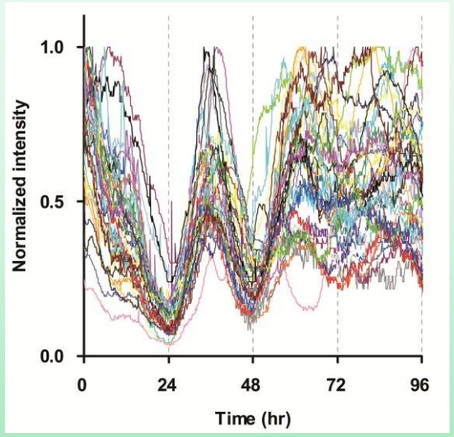
NIH3T3 cells expressed Bmal1 promoter fused Luciferase gene as a reporter gene was stimulated synchronization of circadian rhythm by adding dexamethasone. Monitoring of luminescence in cultured NIH3T3 cells with Kronos-Dio were started by adding of 200 μM D-luciferin potassium salt.

Measurement time: 60 sec
Experiment time: 4 days

Data Supported: Dr. Y. Nakajima, AIST, JAPAN

Single-cell imaging





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

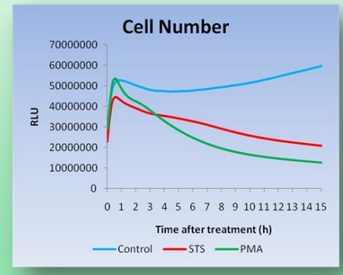
Long-term single cell imaging of transcriptional oscillation in living cells using ELuc.

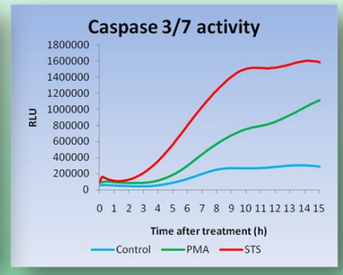
Representative CCD image of *mPer2* promoter-driven ELuc luminescence in rat primary astrocytes (scale bar, 100 mm) and measurement analysis of luminescence from 40 individual cells (graph). Images were acquired using 9 min of exposure time at intervals of 10 min with a 4x objective lens (numerical aperture (NA), 0.5). Signals were normalized to maximum count.

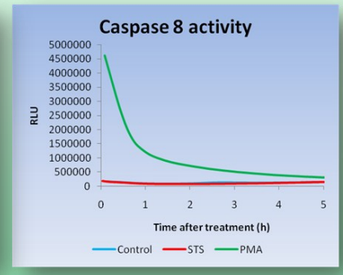
Analysis of Apoptosis

Analysis of apoptosis

Kronos Dio can examine apoptotic events continuously by using luminescence probes. Luciferase gene stably expressing NIH3T3 cells were treated with apoptosis inducing agents, Staurosporin (STS) or Phorbol 12-myristate 13-acetate (PMA). After adding 0.2mM Luciferin, Caspase-Glo® 3/7 substrate (Promega) or Caspase-Glo® 8 substrate (Promega) to the medium, luminescence was measured with Kronos Dio for 1 min of exposure time at interval of 10 min.



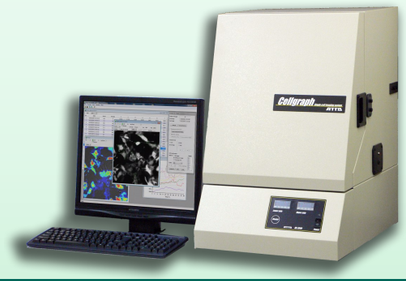




AB-2550 Kronos Dio

Specification

Measurement Container : 35mm dish
Number of Samples: 8 dishes Max.
Temperature: Peltier device and air circulation
room temperature -5°C to 45°C
CO_2 Gas: 5% in density.
Control with sensor and regulator
Detector: Photomultiplier Tube
Measurement Method: Photon counting
Wave length: 350nm to 670nm
Time: 1 to 60sec / from 1 to 60 min
Filters: F0 (none), F1 (560nm), F2 (620nm)



AB-3000B Cellgraph

Specification

Detector: Back- illuminated EMCCD (-80°C)
Up to -90°C (water cooling, optional)
Pixels: 512 × 512
Objective lens: ×4, (optional ×10, ×20)
Stage : X-Y-Z axis: Manual
Z axis: Motorized (external control)
Sample container : 35mm dish
Temperature: room temperature +5°C to 45°C
CO_2 (option): Humidifying Unit, CO_2 gas mixer
Lighting: Bright field: White LED
Fluorescence: Blue LED
Filter: 515, 580, 620 nm long pass filter
Imaging modes: Live/Interval/Stage control/
Combination/Background
Filter Switch: Automatic control of set filter
Exposure time: 30 msec to 90 minutes

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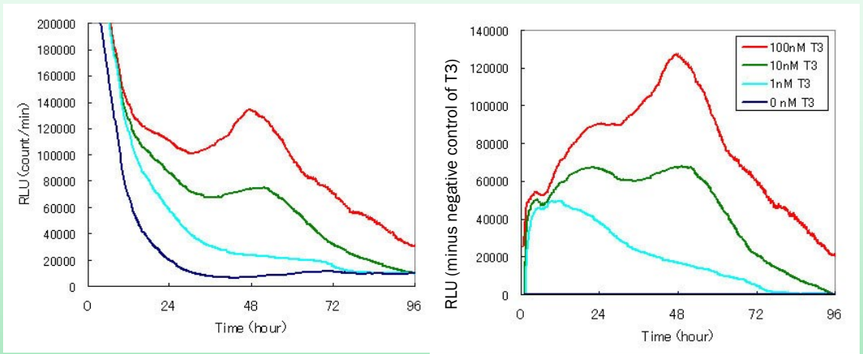
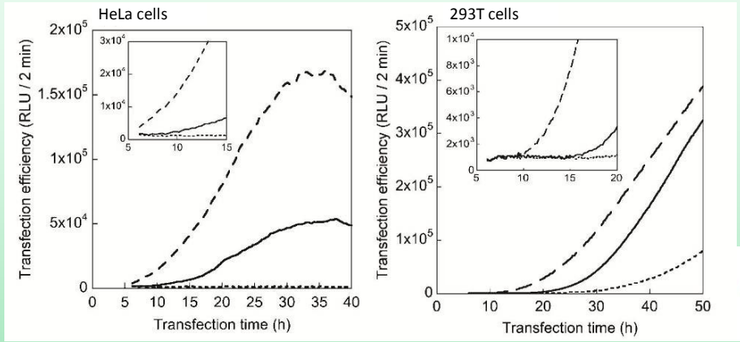
Analysis of DDS & Drug's effect

Drug stimulation response



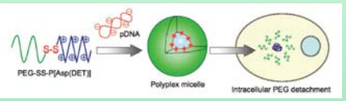
Binding of thyroid hormone (T3) to nuclear receptors influences the transcriptional rate of specific genes. The carboxylic acid, sodium butyrate , is known to reduce nuclear binding of T3 in GH3 rat pituitary cels.
The reporter vector including the firefly luciferase gene with the downstream of growth hormone (GH) promoter was transfected into GH3 cells. Cells were exchanged culture medium to OPTI-MEM and cultured for three hours, and then thyroid hormone (T3) is added. The transcription activity of GH promoter was real-time monitored as their luminescence intensity with Kronos Dio. Thus Kronos Dio is useful tools to monitor sequential biological responses against drugs, chemicals, and other substances.

Time-dependent profiles of transfection efficiency



DDS carriers

PEG-SS-P[Asp(DET)]
P[Asp(DET)]
PEG-P[Asp(DET)]



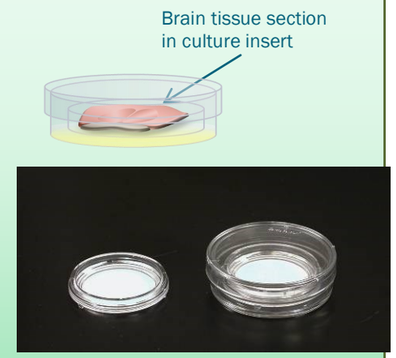
Time-dependent profiles of transfection efficiency with novel DDS carriers against HeLa and 293T cells.
The cells were incubated with each polyplex in DMEM containing 10% FBS for 6 h, followed by incubation in DMEM containing 10% FBS and 100 μM D-luciferin in the absence of polyplexes. The time shown in the *x*-axis started from the addition of polyplex solutions and the measurement started from 6 h. The inserts are expanded figures from 5 to 15 h.

Data Supported: Dr. K. Itaka, Tokyo univ., JAPAN
Reference: S. Takae *et al*., *J.Am.Chem.Soc*., 130 (2008)

Real-time Reporter Assay of Tissue Section

Kronos Dio and Cellgraph can be analyzed bioluminescence of tissue section.
Our application data will be described below.

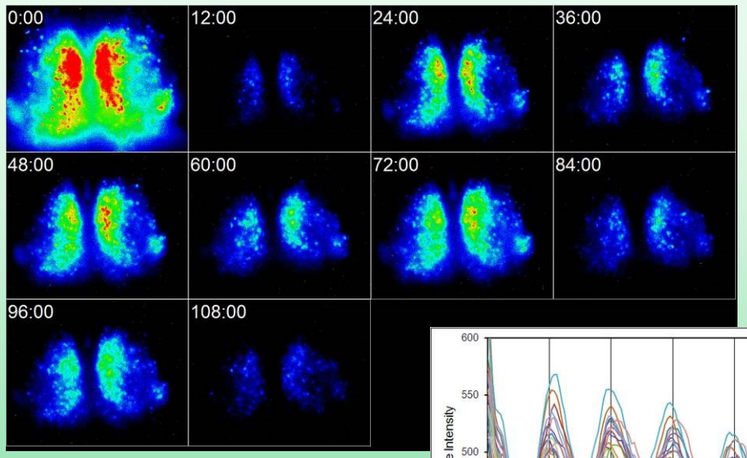
Real-time reporter assay of mouse SCN secction



A transgenic mouse including luciferase gene with a promoter of clock gene (Per 1) related to circadian rhythm was added with luciferase gene was kept with the period from 06:00 hrs to 18:00 hrs as the light period, and its suprachiasmatic nucleus (SCN) was cut into 300 μm slices using a micro slicer and the slices were placed on culture inserts (Millicell CM) and monitored for 11 days. Extremely stable circadian rhythm was detected.

Data Supported: Dr. K. Honma, Dept. of physiology, Hokkaido University
Reference: S. Nishide et al. *Gene to Cells,* Vol. 11 (2006)

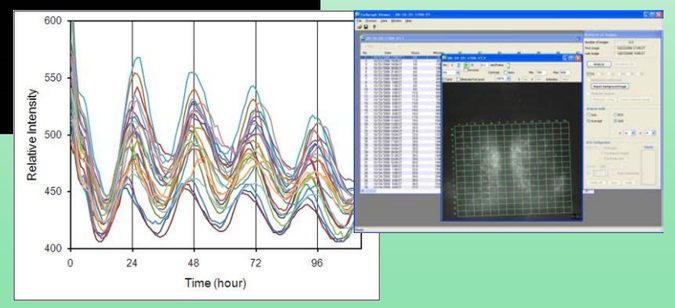
Bioluminescence imaging of mouse SCN section



Brain tissue section from transgenic mouse expressing clock gene promoter fused to luciferase gene as a reporter was analyzed by Cellgraph.

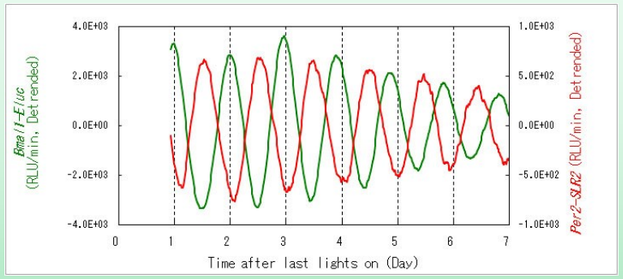
Excised brain tissue was sliced with microslicer in 100 μm thickness, and tissue section was placed on culture insert. Time-lapse bioluminescence image of culturing brain tissue (suprachiasmatic nucleus: SCN) was photographed by Cellgraph for 5 days.

The difference in local luminescence intensity of tissue section which is hard to recognize individual cells can be analyzed by a grid measurement mode. This mode analyzes luminescence intensity by dividing an arbitrary area into a grid.



Real-time Reporter Assay with Multi-Color

Simultaneous monitoring of multiple circudian gene expression



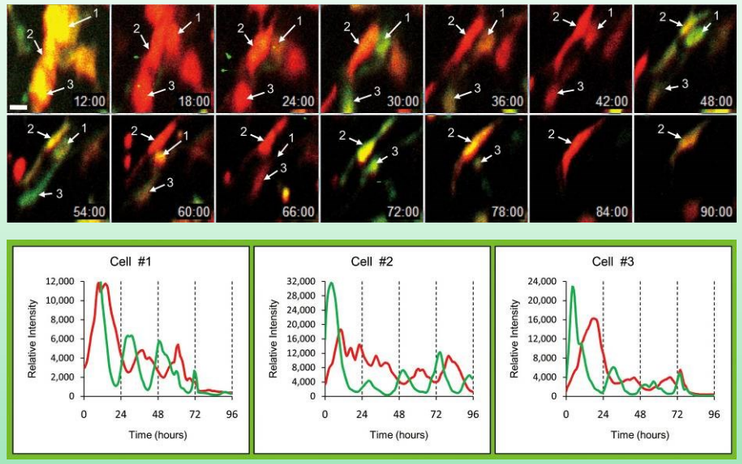
Multiple luciferase gene expression can be separated by differences in individual luciferase luminescence color and monitored them simultaneously for long-term with Kronos Dio.

Dual color luciferase assay of brain tissue section from transgenic mice expressing circadian gene promoter fused to luciferase, *Bmal1*-driven ELuc (Green luminescence) and *Per2*-driven SLR2 (Red luminescence) is shown. The bioluminescence of *Bmal1*-driven ELuc (cpm) is plotted with green lines and shown on the left y axis. The bioluminescence of *Per2*-driven SLR2(cpm) is plotted with red lines and shown on the right y axis. The x axis shows the time after last lights-on for the animals. The expressions of *Bmal1* and *Per2* in tissue section were stable for long-term and showed antiphase. Graph data were smoothed and detrended.

Data Supported: Dr. Y. Nakajima and Dr. Y. Ohmiya, AIST, JAPAN
Reference: T. Noguchi *et al*. *Biochemistly,* Vol.49 (2010)

Bioluminescence imaging of dual gene expression in single live-cell

The dual-color imaging of NIH3T3 cells cotransfected with the Bmal1-pSLR plasmid (red) and the Per2-pEluc plasmid (green), showing circadian rhythms of green and red luminescence, respectively. Representative circadian bioluminescence rhythms from individual NIH3T3 cells co-transfected with Bmal1-pSLR plasmid (red) and Per2-pEluc plasmid (green) for 3–4 days. Each graph represents real-time analysis data of quantitative bioluminescence intensity.



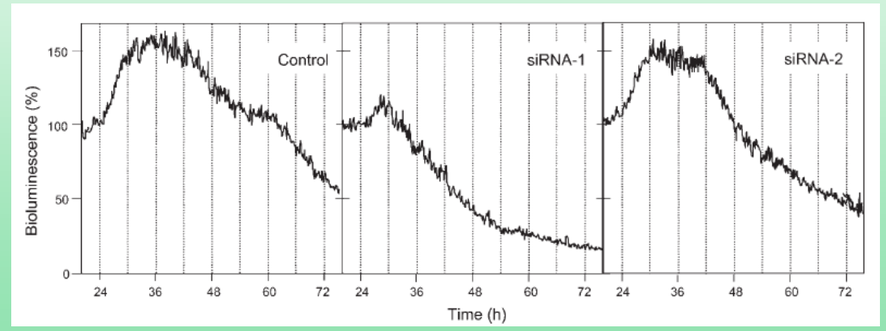
Green and red bioluminescences from target cells are divided into two color components by the dichroic mirror. The green light goes through the dichroic mirror, and the red light is reflected. Introduction of two dichroic and reflective mirrors into the optical system can be achieved that two images with individual color component from single cell are analyzed simultaneously by one CCD camera system.

Data Supported: Dr. HJ.Kwon, Hokkaido Univ., JAPAN
Reference: HJ.Kwon *et. al*., BioTechniques Vol48 (2010)

Analysis of RNA expression & Protein transport

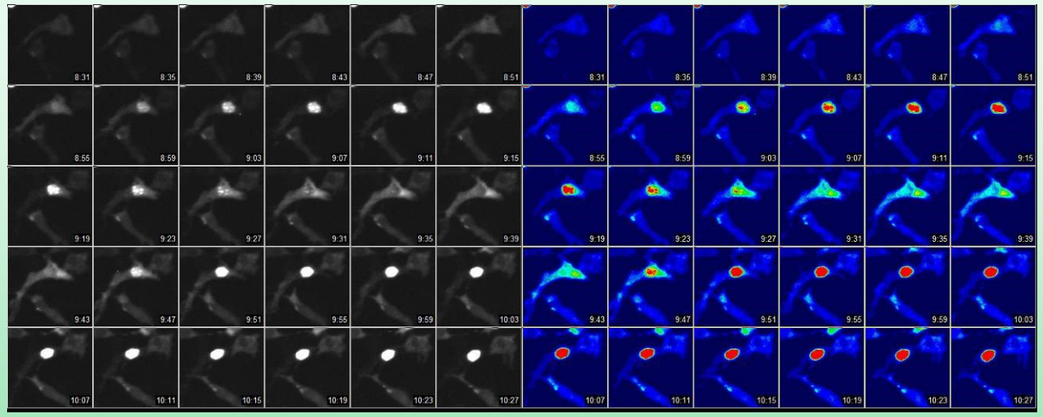
Measurement of RNA interference (siRNA) effect

The inhibitory effect of gene expression by siRNA were examined with Kronos. The effect of RNA interference is maintained for a long time (>1 week). Kronos Dio can be easily monitored the inhibitory effect of gene expression by using luciferase as a reporter gene.
Myostatin (GDF-8) is known to negatively regulate skeletal muscle mass in myogenesis and belongs to TGFβ family. Myoblast was co-transfected $(CAGA)_{10}$ sequence, which is DNA binding site of GDF8, fused to luciferase gene, with or without siRNA-1 (or negative control siRNA-2). After 20 h of siRNA introduction, the photon counts were measured 1 min intervals. Data are normalized to the values at 20 h.



Data supported: Dr. M. Hattori, Kyushu Univ., JAPAN
Reference: F. Sato *et. al.*, *Am J Physiol Cell Physiol*, 291(2006)

Time-lapse imaging of intracellular trafficking of importin α



Time-lapse luminescence imaging of the nucleocytoplasmic shuttling of ELuc::importin α in NIH3T3 cells. The expression plasmid carrying ELuc::importin α was transiently transfected into NIH3T3 cells. Images were acquired after 3 h of transfection using Cellgraph for 3 min of exposure time at intervals of 4 min with a 40x objective lens without binning. The luminescence signal was detected initially in the cytosol, and then the signal increased gradually in the nucleus. Thus Cellgraph is powerful tool for cellular imaging at single-cell level over the long term.

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

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