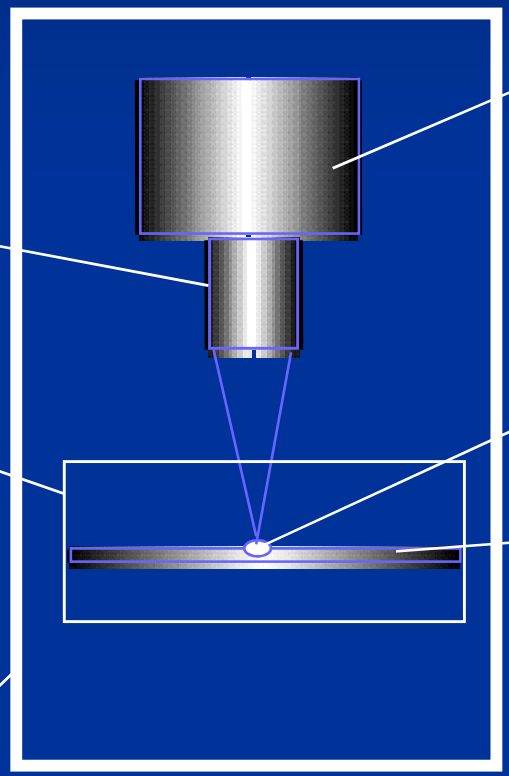
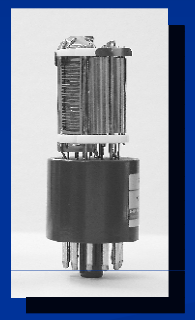
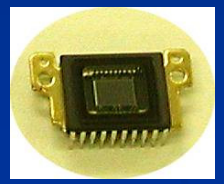
Real-time Monitoring of Gene Expression in Living Cell by Bioluminescent Detection System

Basic Structure of Light detecting equipment



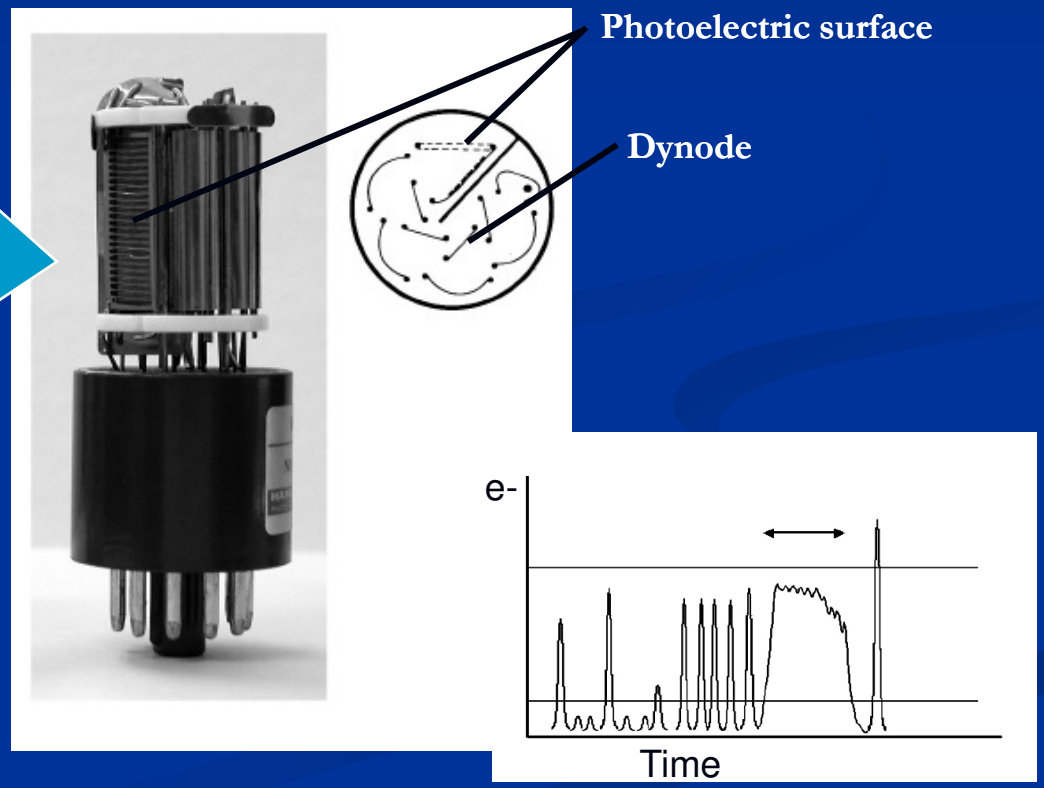




Photon counting

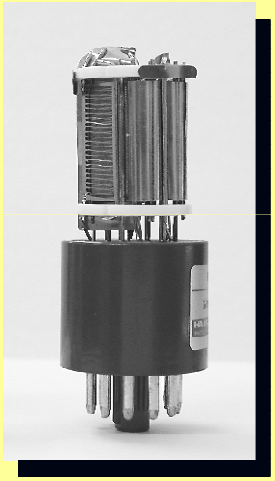
Luminometer

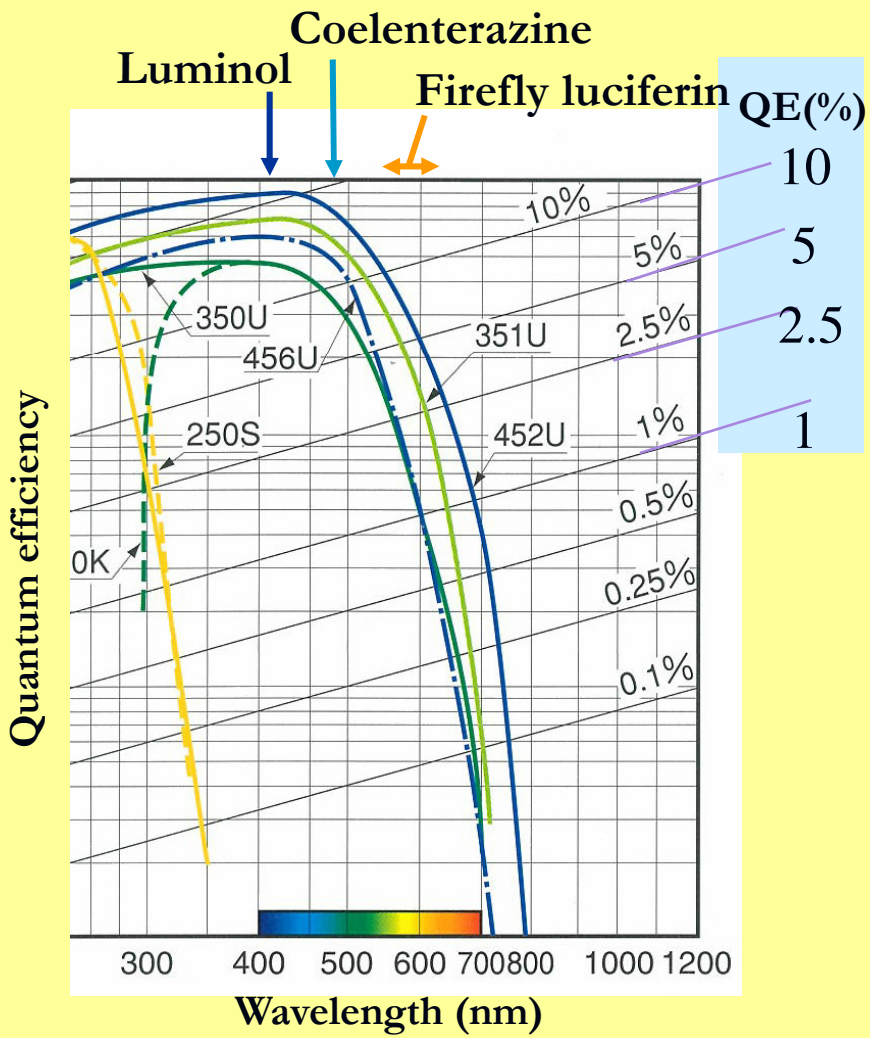
Photomultiplier tube (PMT)



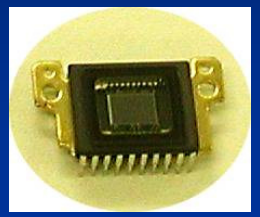
Quantum efficiency of PMT

Quantum efficiency (QE) = electrical signal / incident photon

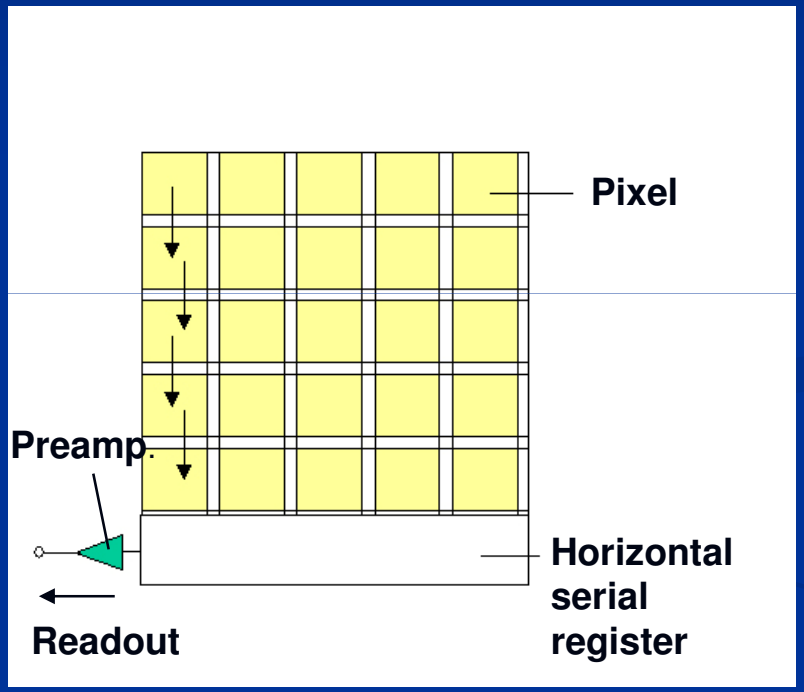




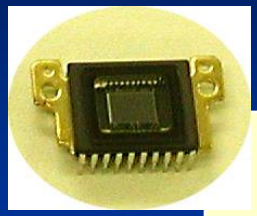
Charged Coupled Device (CCD)

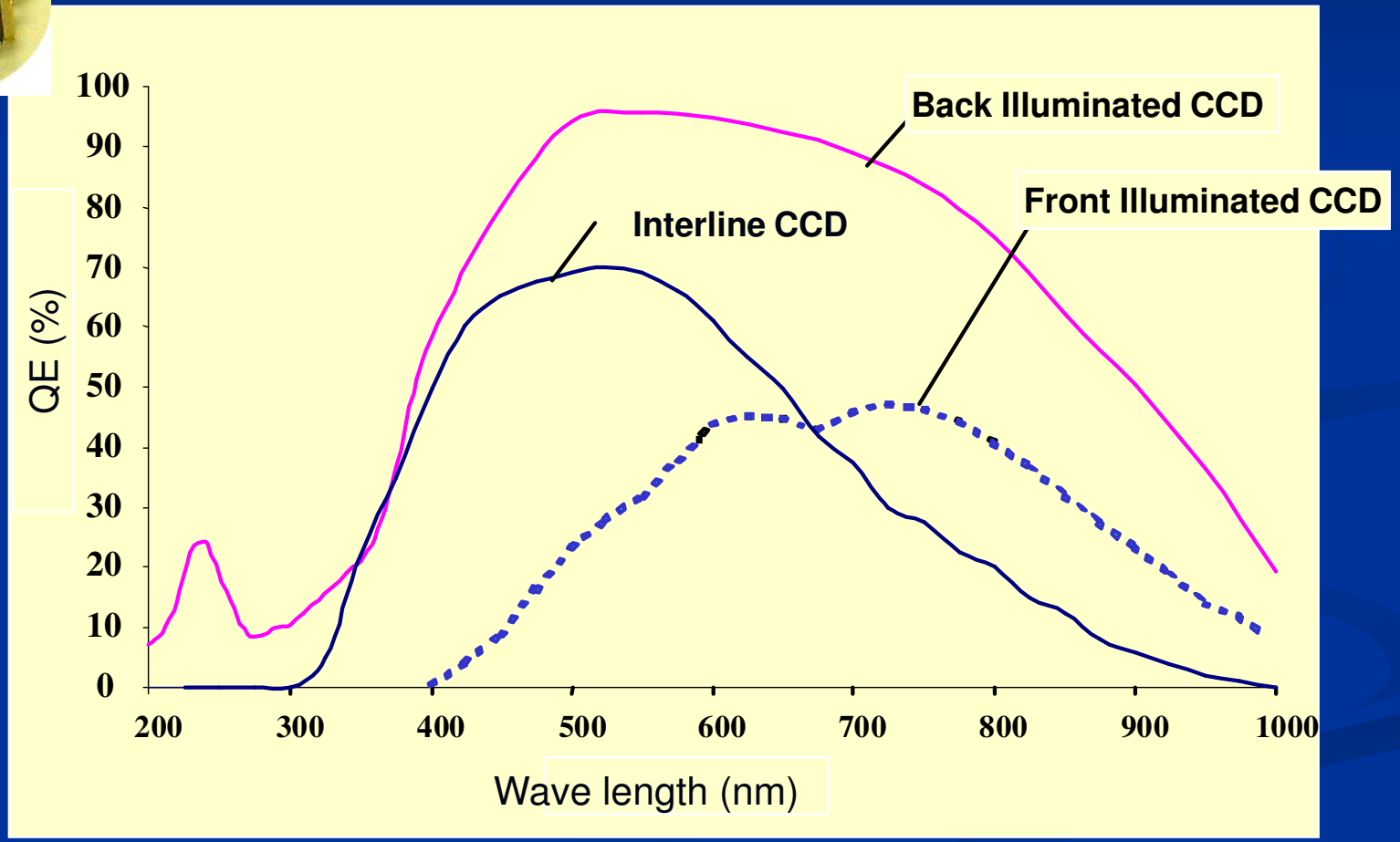


CCD

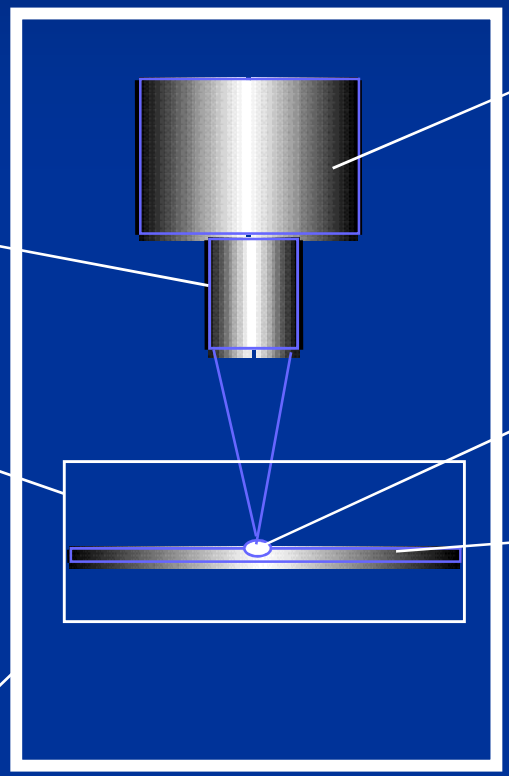


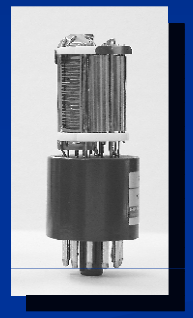
Quantum Efficiency of CCD

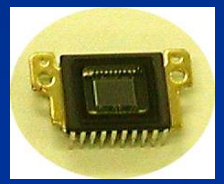




Basic Structure of Light detecting equipment



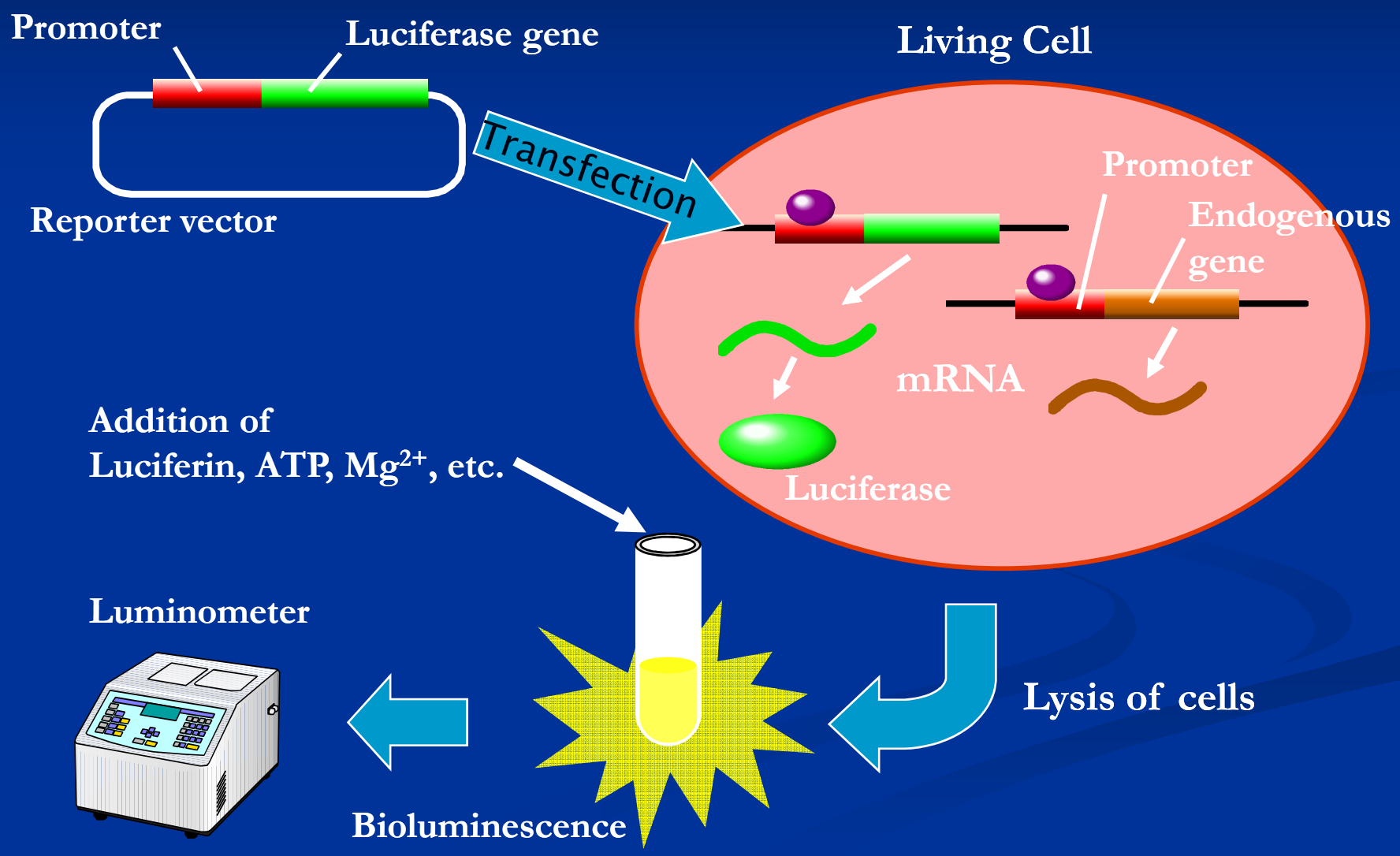




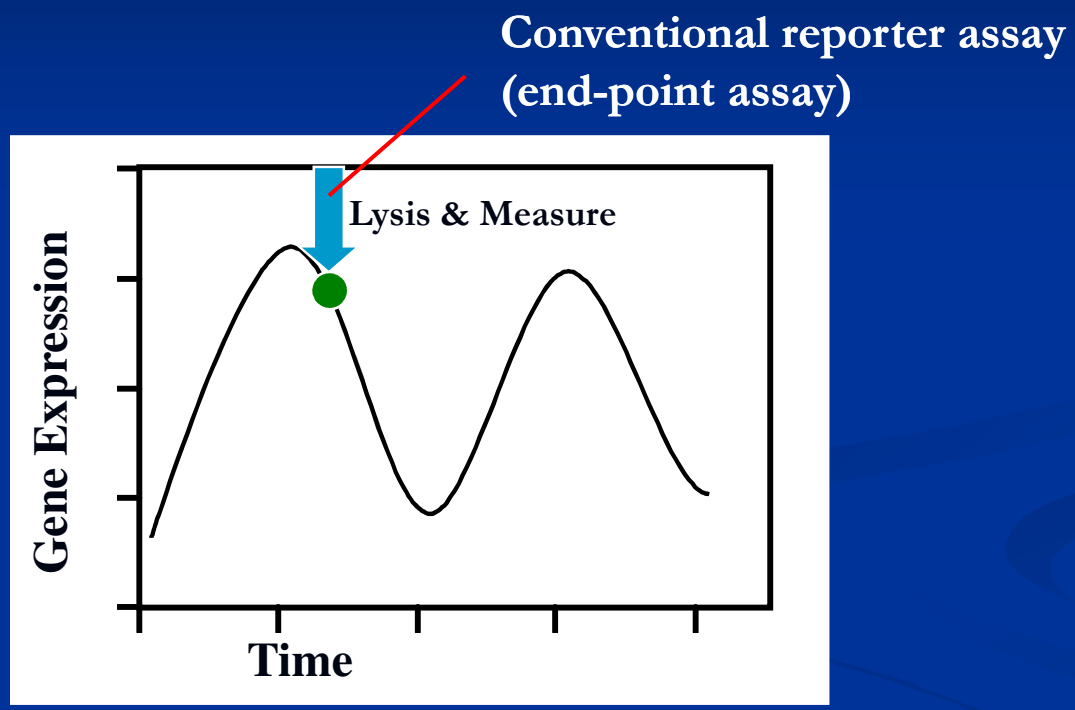
; Living cell

Real-time Reporter Assay

Analysis of Gene Expression by Reporter Assay

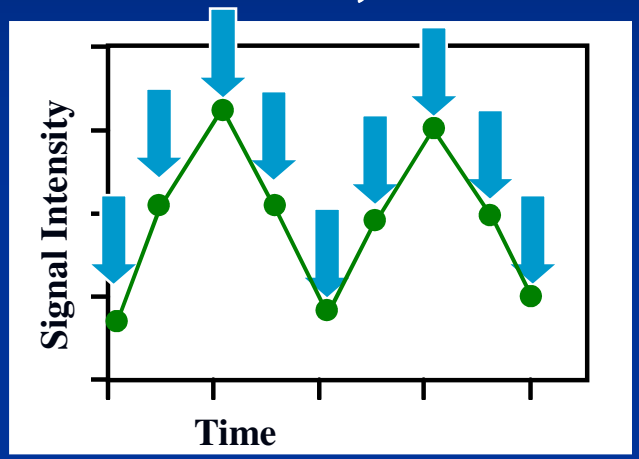


Monitoring of Time Course

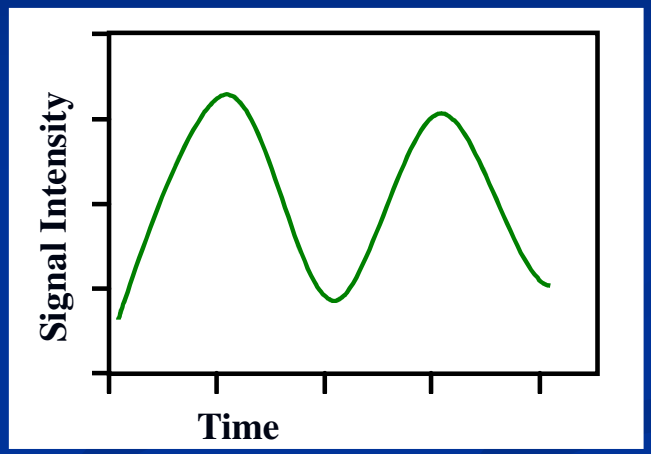


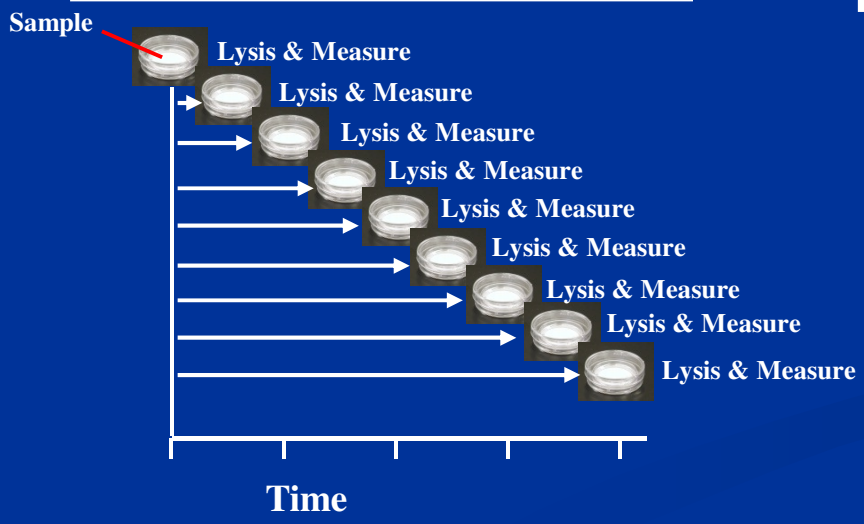
Monitoring of Time Course

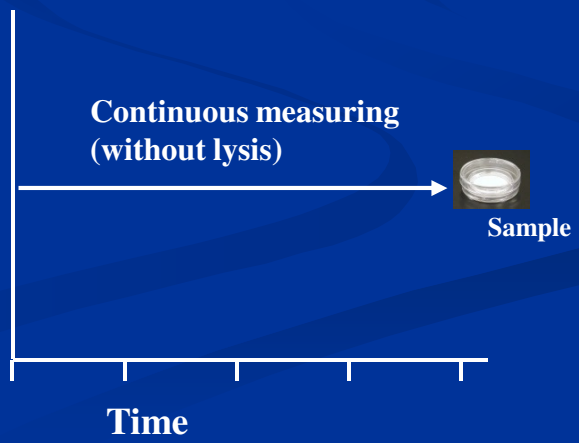
Conventional reporter assay
Real-time PCR, Northern blot



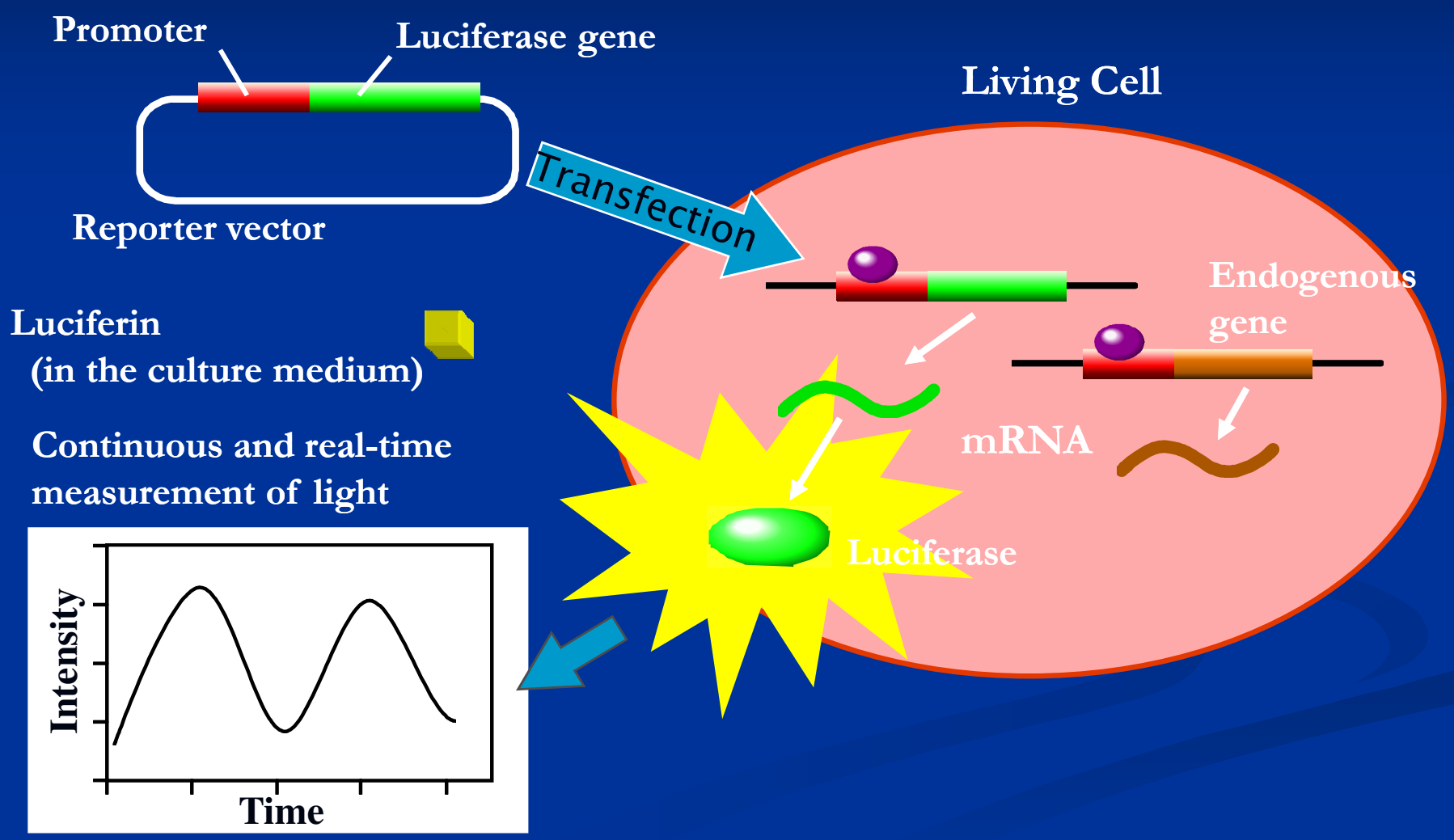
Real-time reporter assay



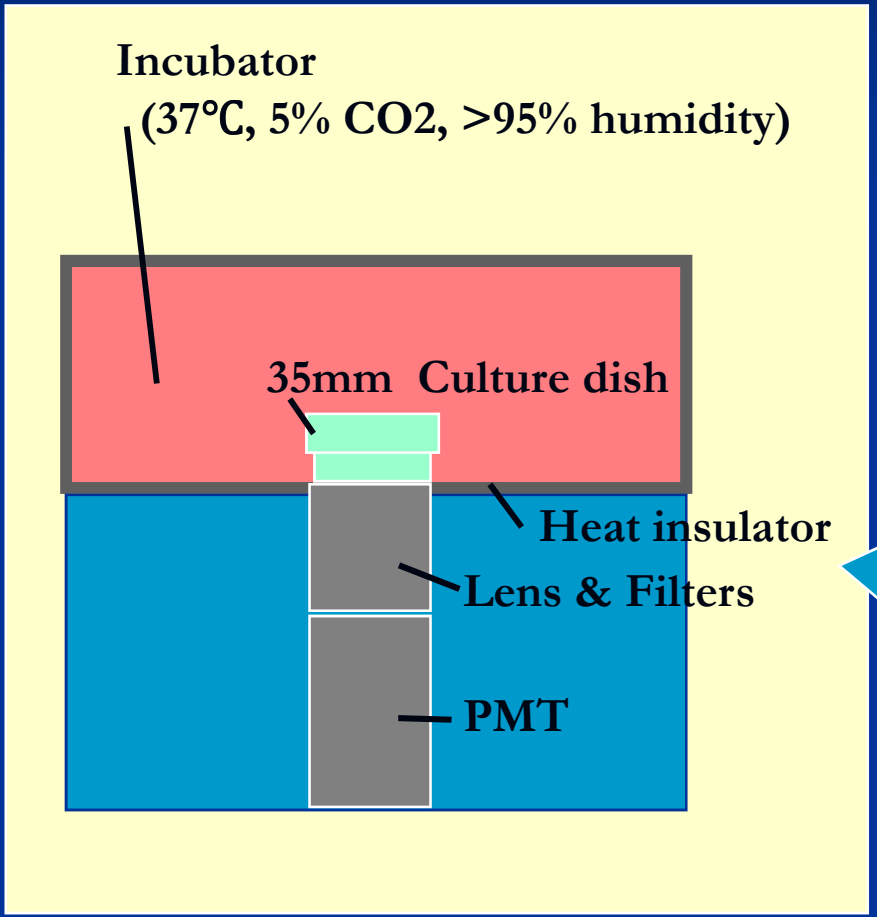




Real-time Reporter Assay

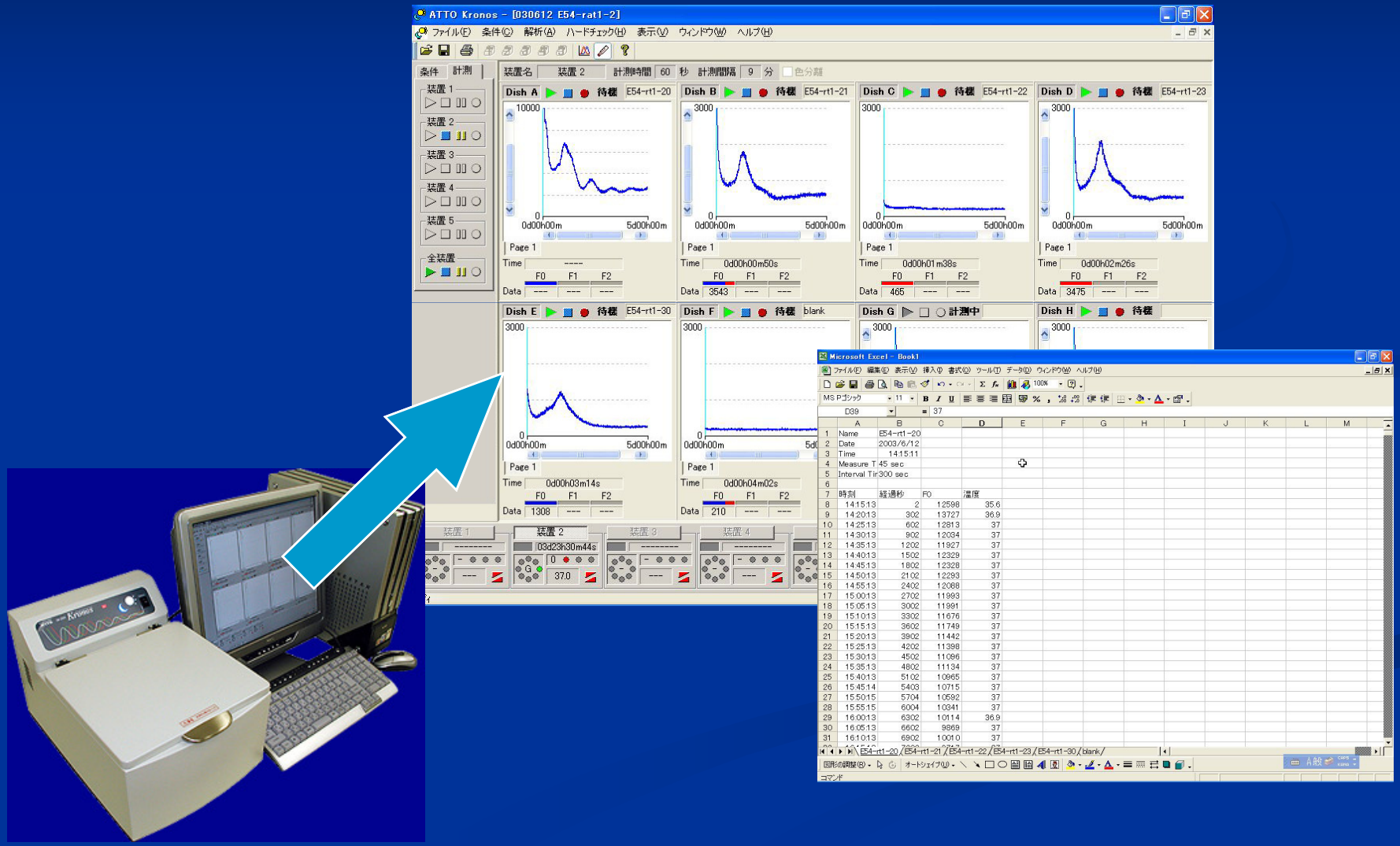


Luminometer for Real-time Reporter Assay in Living Cell

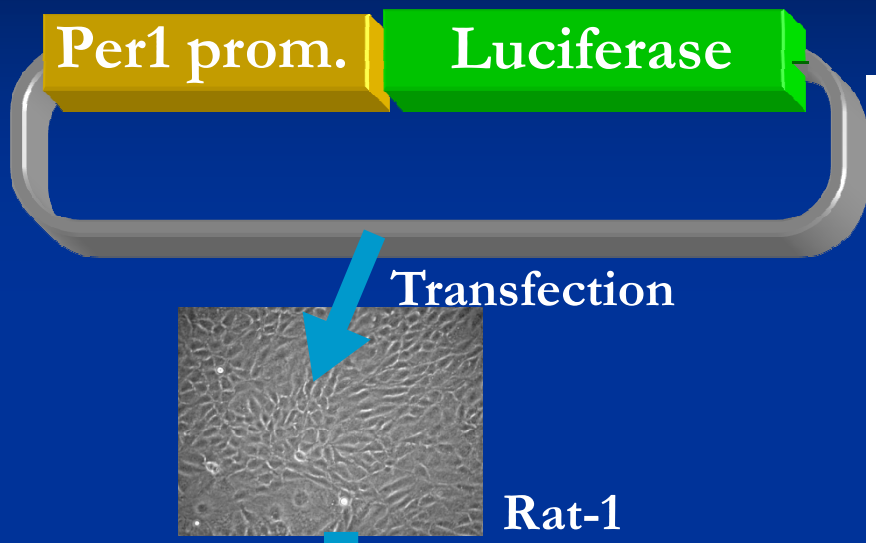


"Kronos" (AB-2500)

Data Display on PC



Circadian Rhythm Monitoring in Culture Cell

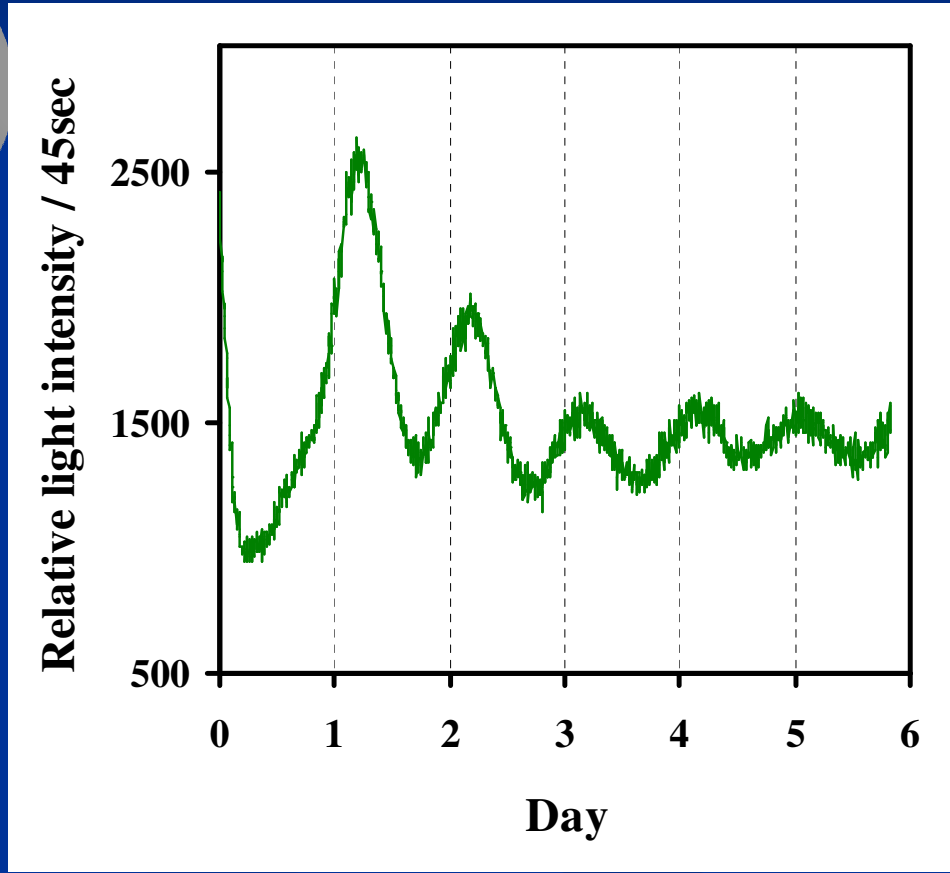


Rat-1 fibroblast cell

Stimulation

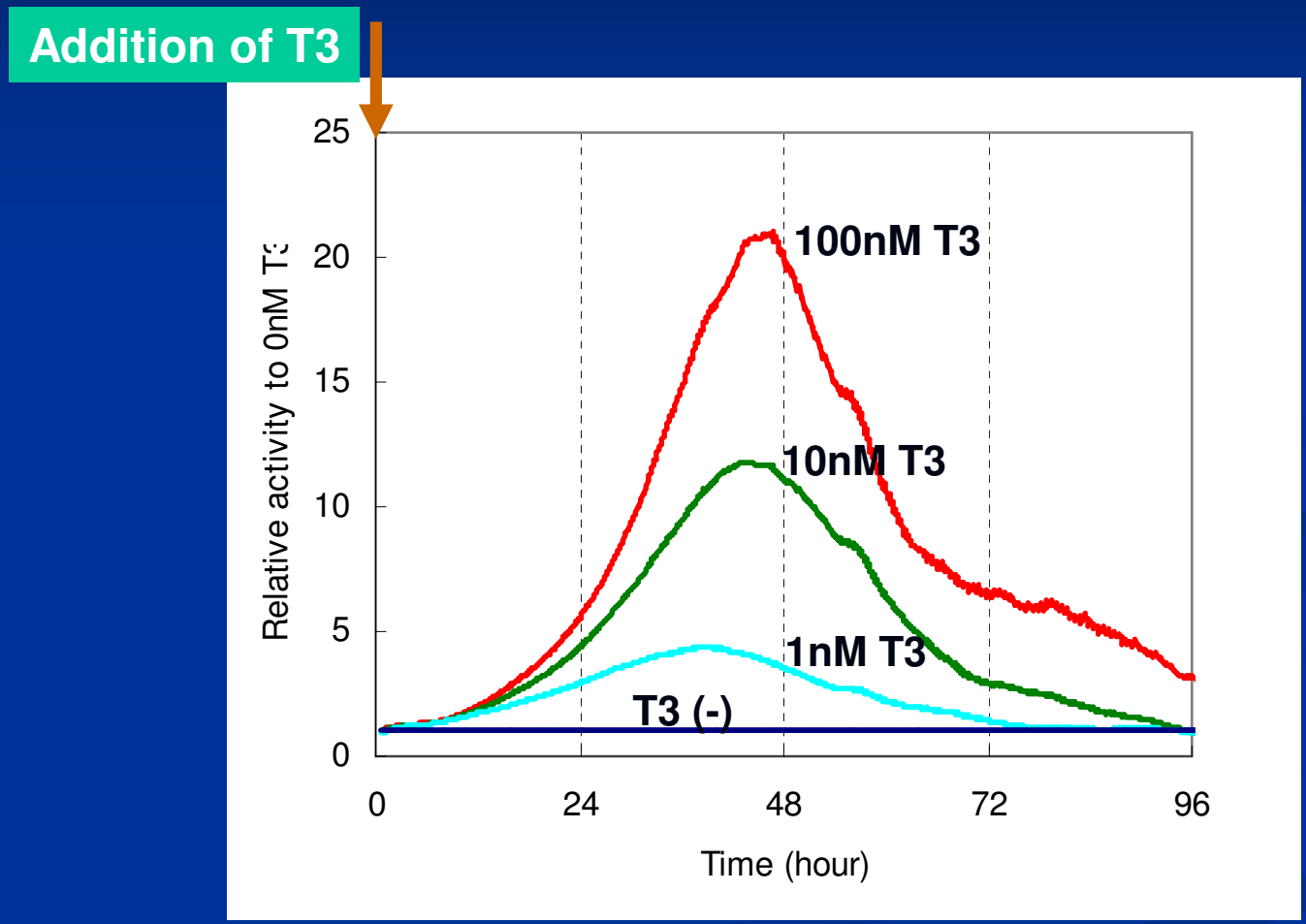
Medium change and Addition of Luciferin

Measurement



Y. Nakajima,
National Institute of Advanced Industrial Science and Technology (AIST), Japan

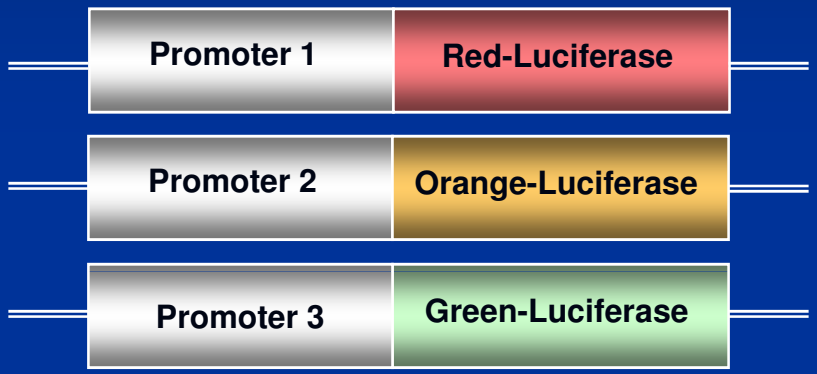
Effect of Thyroid Hormone(T3) to Growth Hormone Promoter Activity

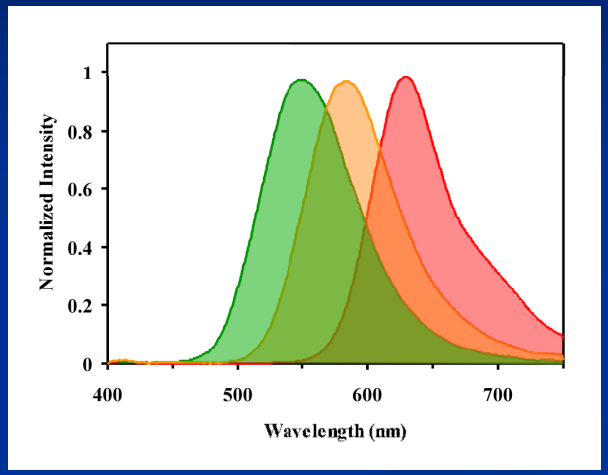


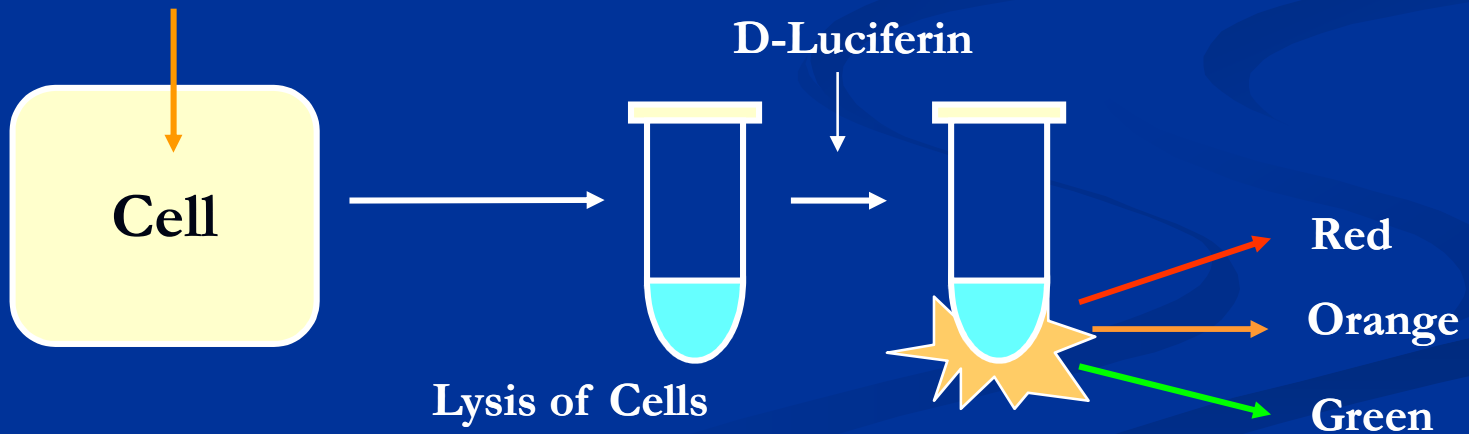
Cell : GH3 (rat pituitary adenoma cell)
Reporter : Growth hormone promoter - Luciferase

Multi-color detection

Multi-Color Reporter Assay

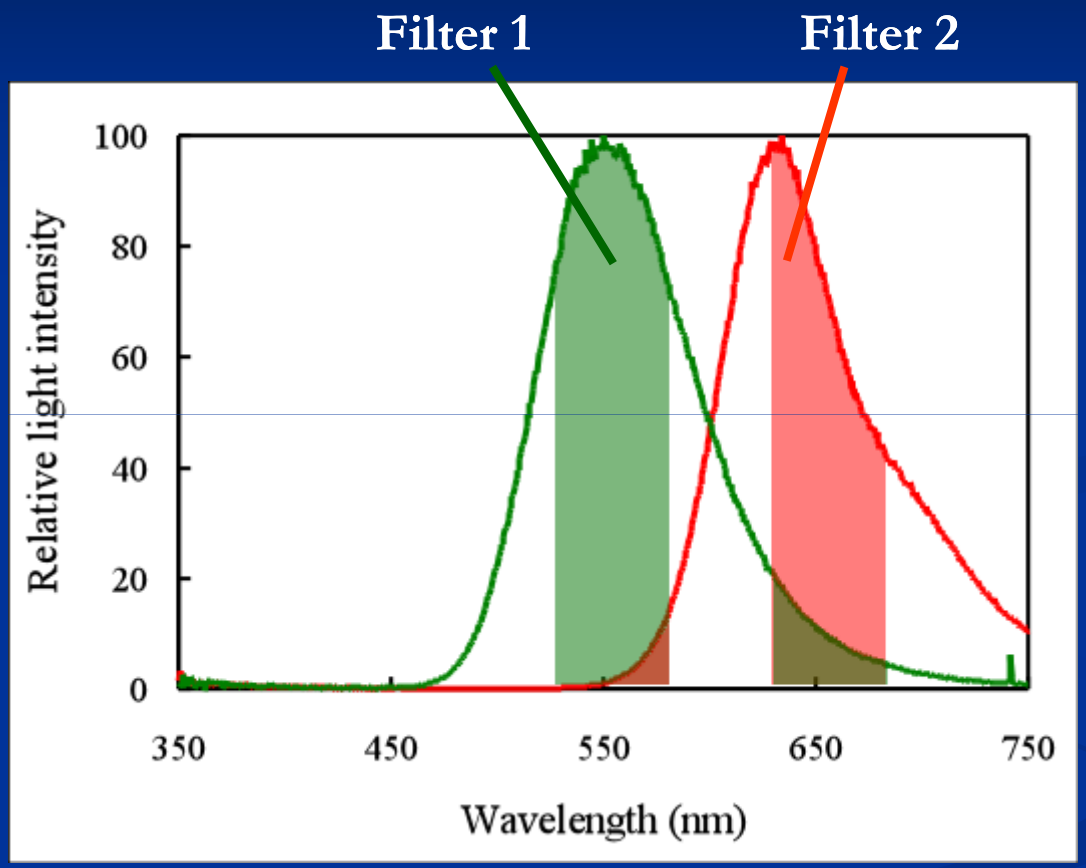






Y. Nakajima, *et al.*, *FEBS Lett. 565, p122-126 (2004)*
Bio Techniques 38, p891-894 (2005)

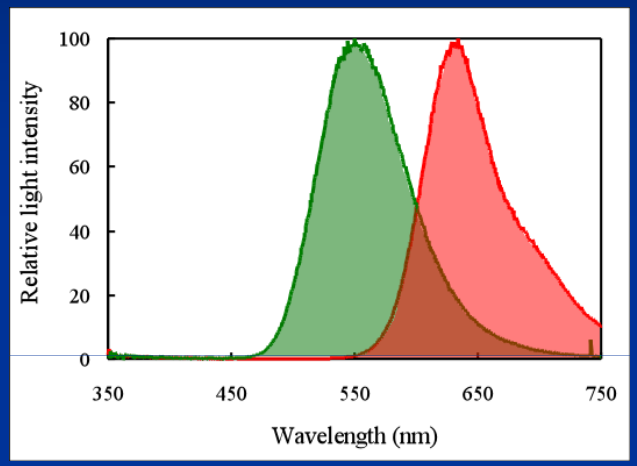
Conventional Multi-color Detection



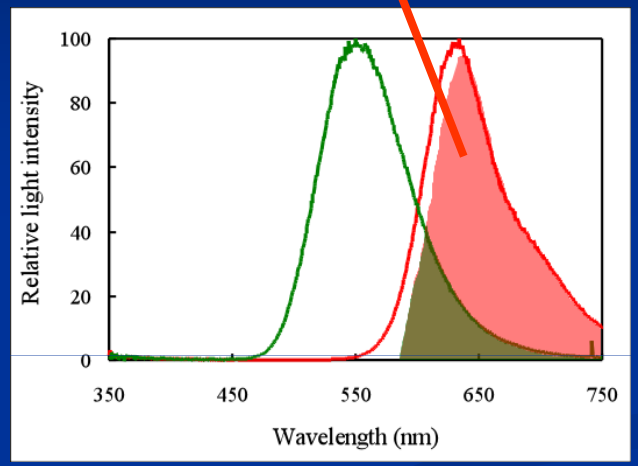
The loss of luminescence by filter is high.

New Method of Multi-color Detection

No filter



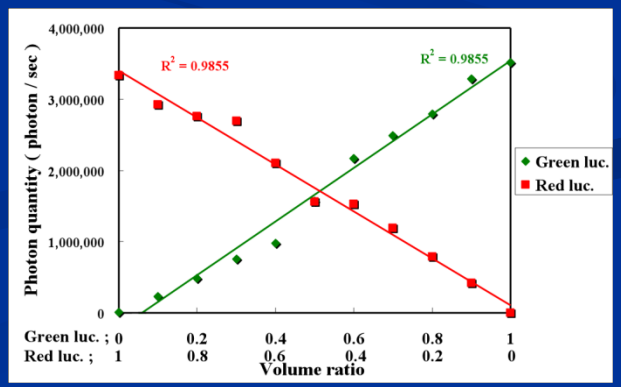
Filter 1



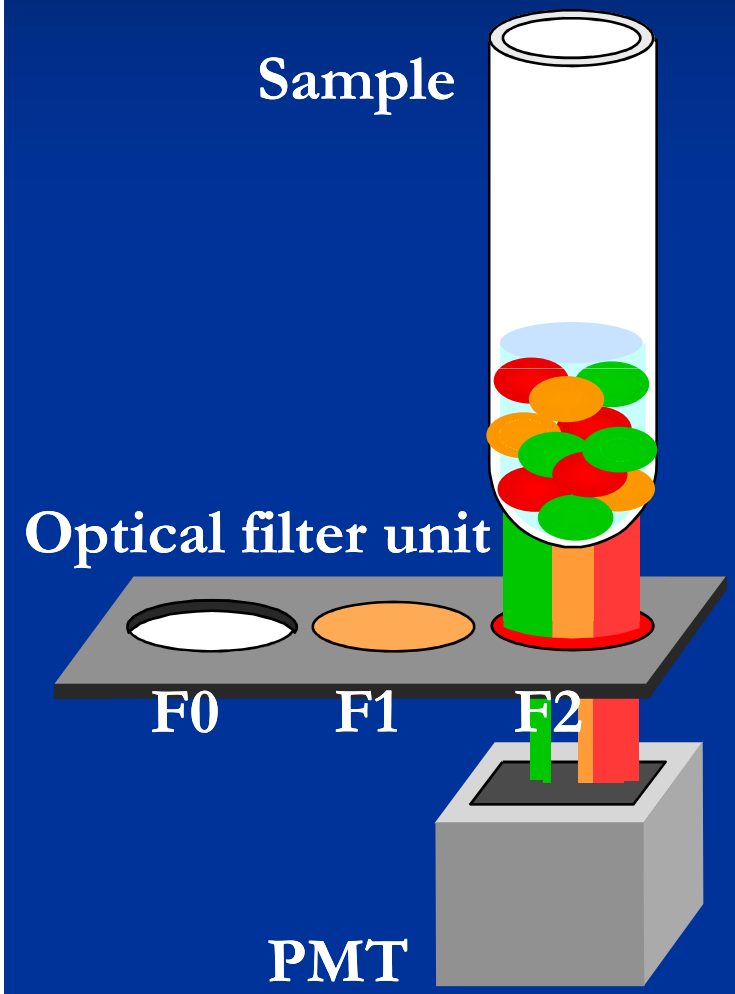
The loss of luminescence by filter is very low.

Calculations

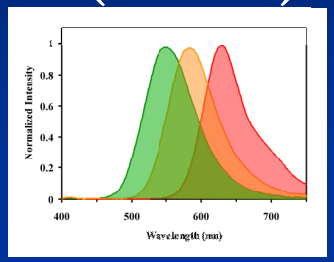
The calculated values were proportional to concentrations of each luciferase.



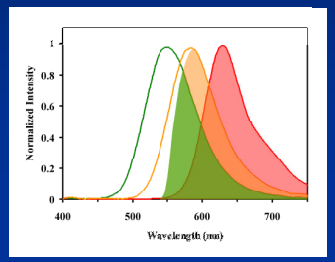
Advanced Method of Quantitative Color Detection



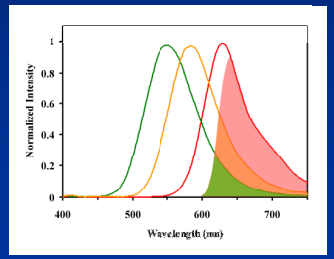
F0 (no filter)



F1



F2



$$\begin{pmatrix} F0 \\ F1 \\ F2 \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 \\ \kappa_{f1g} & \kappa_{f1o} & \kappa_{f1r} \\ \kappa_{f2g} & \kappa_{f2o} & \kappa_{f2r} \end{pmatrix} \begin{pmatrix} G \\ O \\ R \end{pmatrix}$$

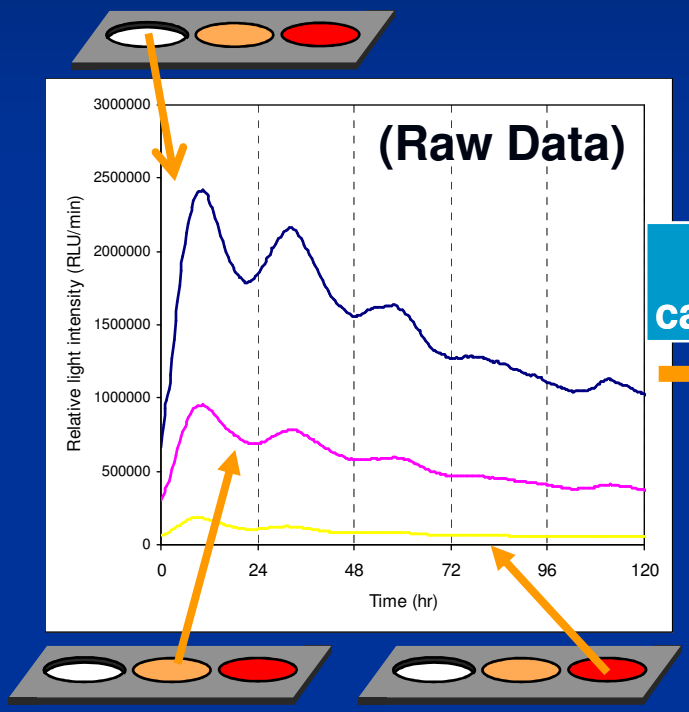
F : Measurement value

κ : Transmittance of each color light in each optical filter

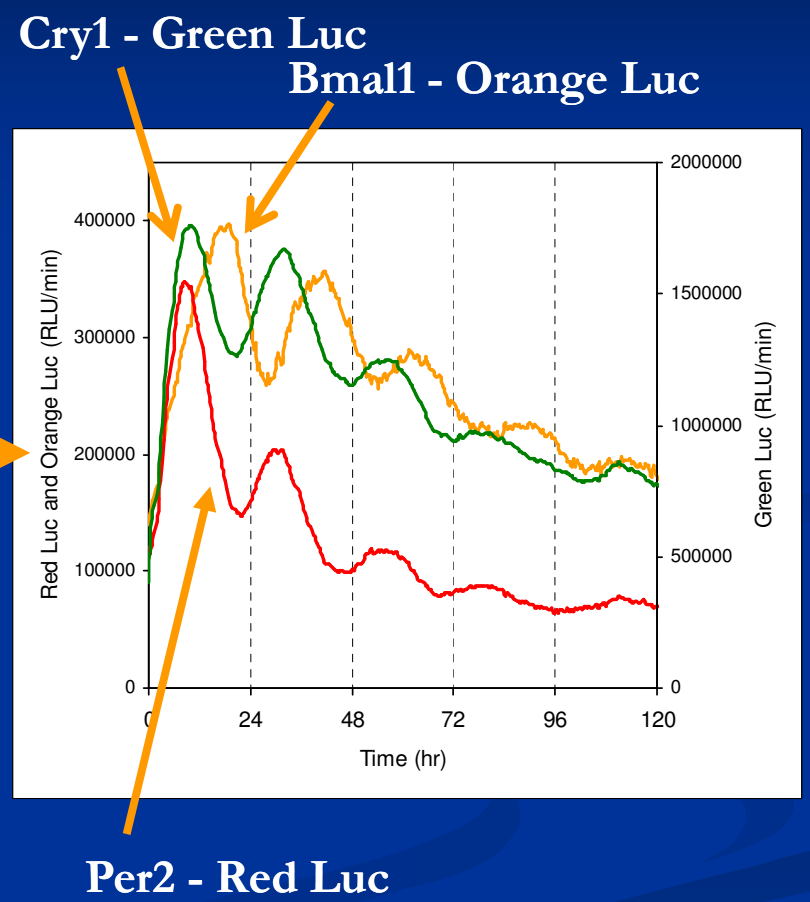
G, O, R : Each color light

Real-time Multi-color Reporter Assay

Real-time Multi Color Reporter Assay



matrix calculation



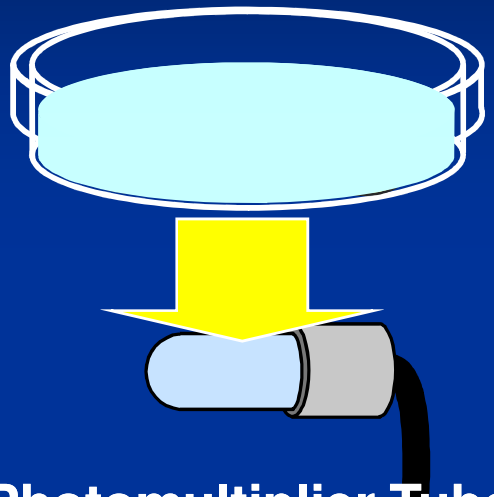
Cell :NIH3T3
Reporter : Cry1 promoter-ELuc (Green luciferase),
Bmal1 promoter-SLO (Orange luciferase),
Per2 promoter-SLR (Red luciferase)

Y. Nakajima, AIST, Japan

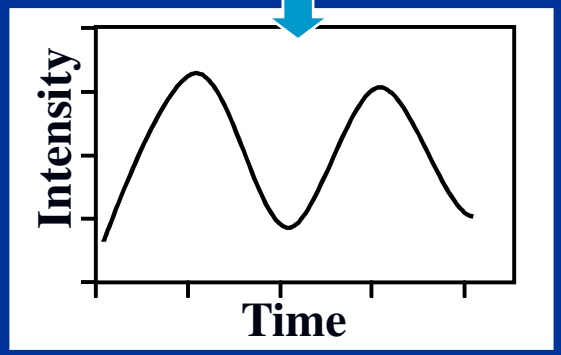
Bioluminescent imaging in living cell

Mass ? or Individual ?

Luminometer

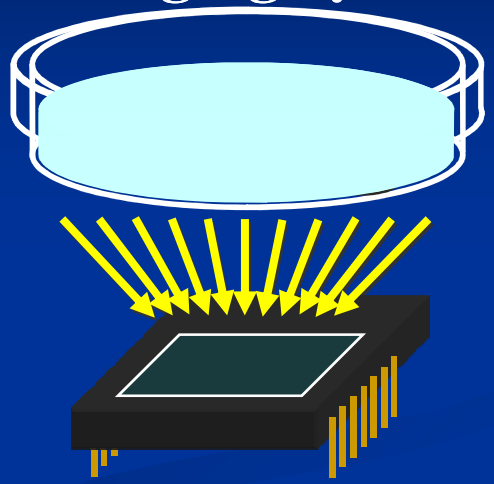


Photomultiplier Tube

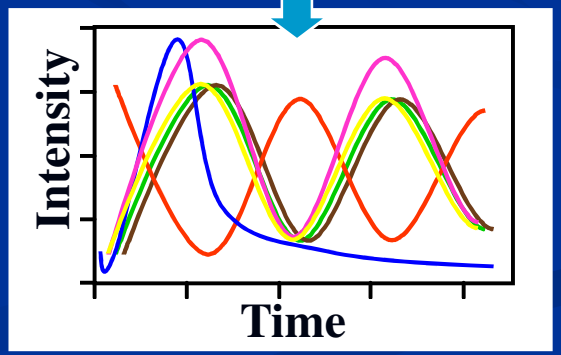


Monitoring of Total Cells

Imaging System



Cooled CCD



Monitoring of Individual Cell

Bioluminescent Imaging System Key Point

(1) High sensitivity

(2) Blocking of Outside light

(3) Long term cell culturing

Bioluminescent Imaging System
Key Point

(1) High sensitivity

Detector (; CCD camera)

S/N Signal ; Quantum efficiency

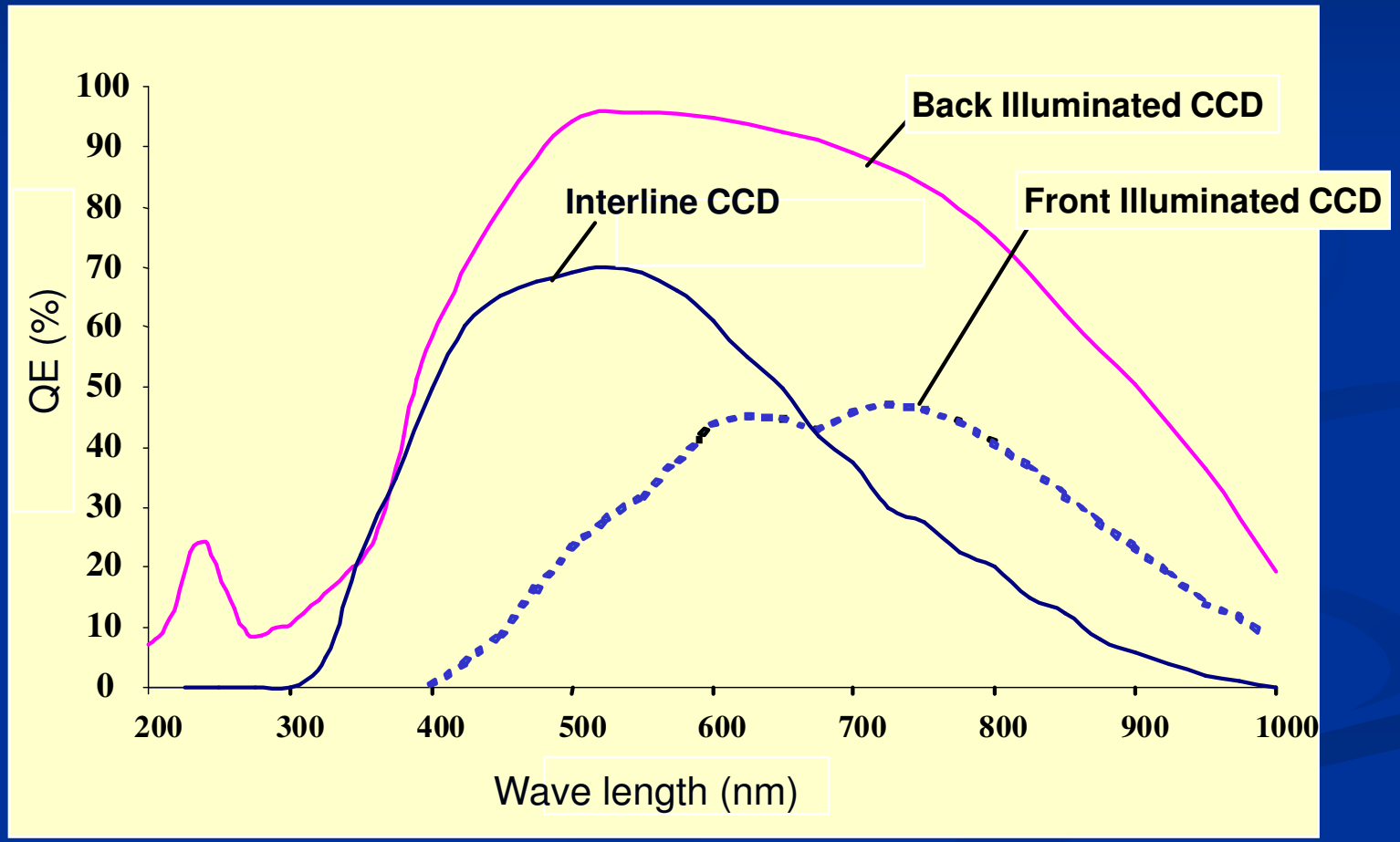
Noise ; Thermal noise, Readout noise, etc.

Optical system

NA (numerical aperture)

Optical transparency

Quantum Efficiency of CCD

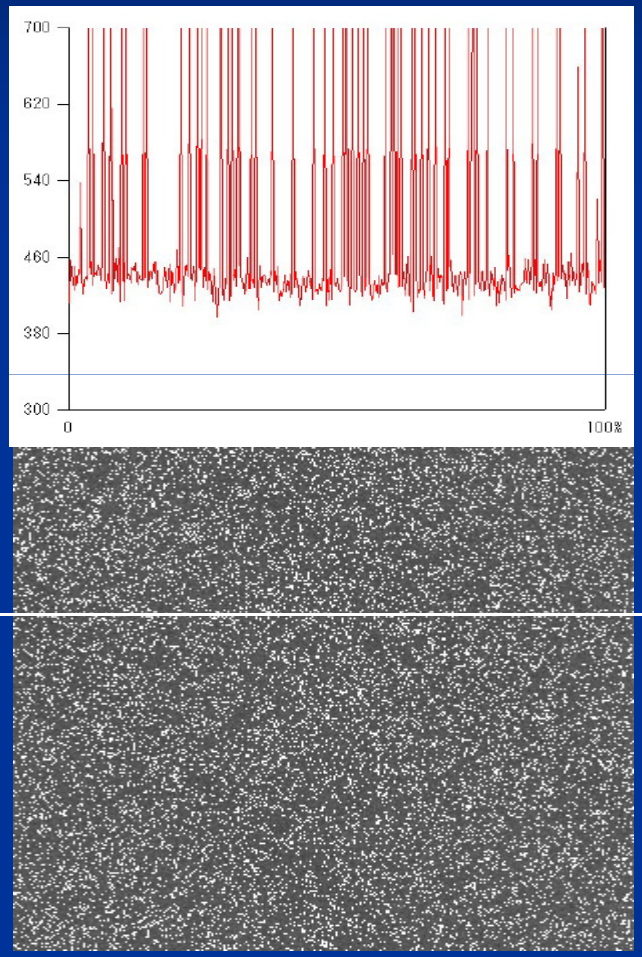


Reduction of Thermal Noise

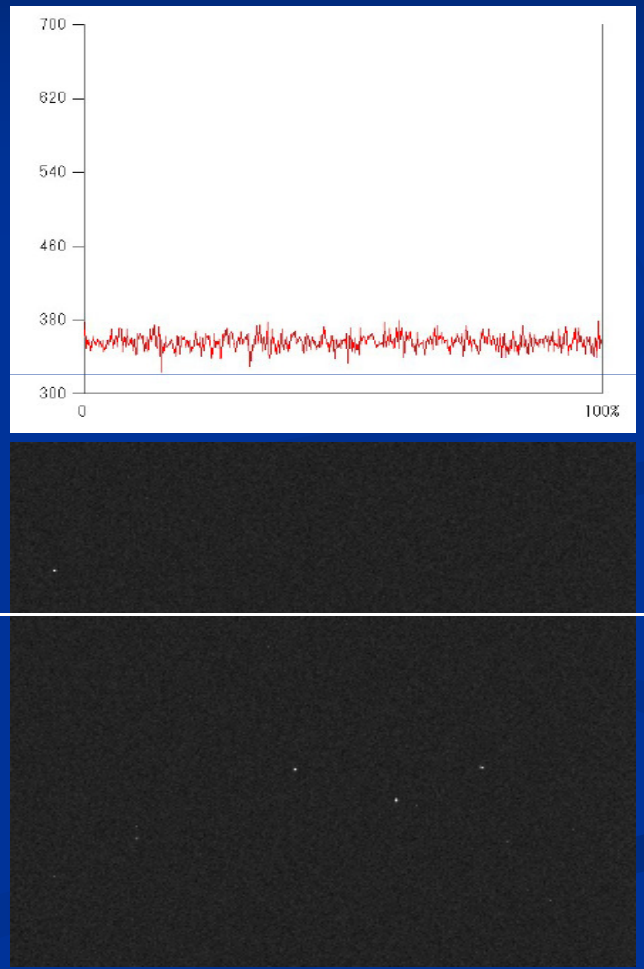
Image brightness on the drawn line

Dark Image

-30°C

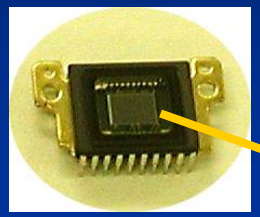


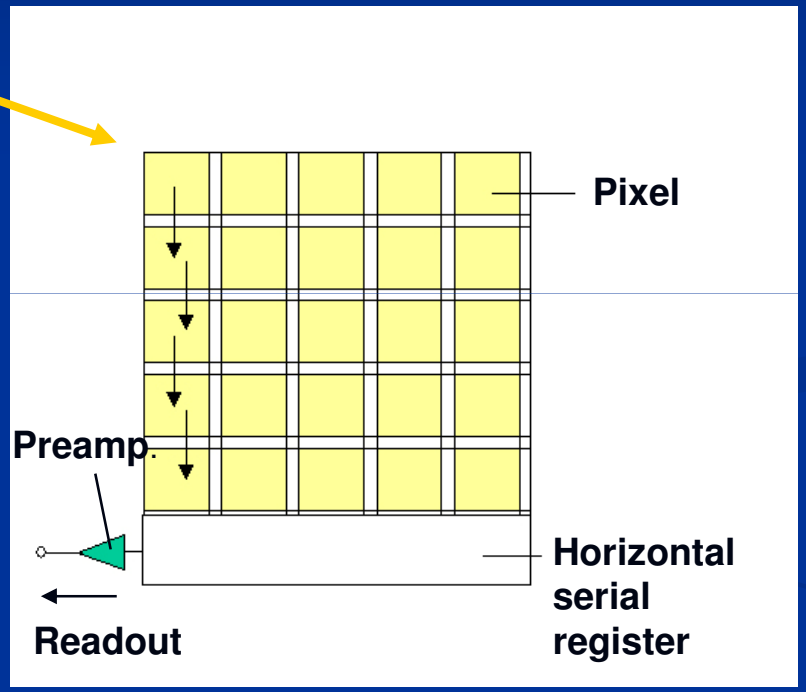
-70°C



Readout Noise

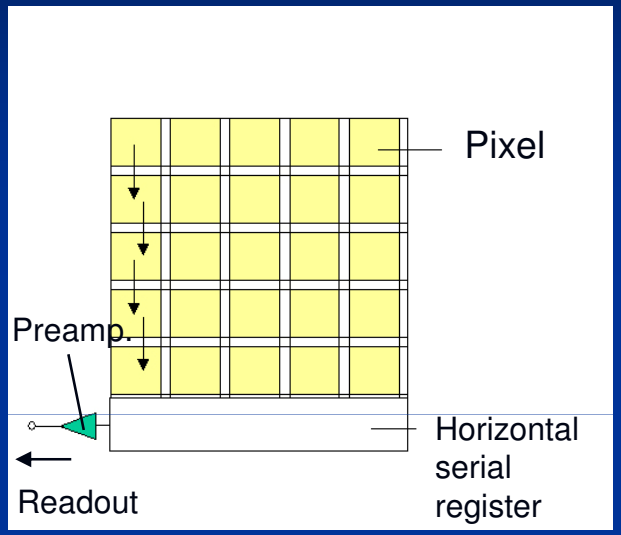
CCD



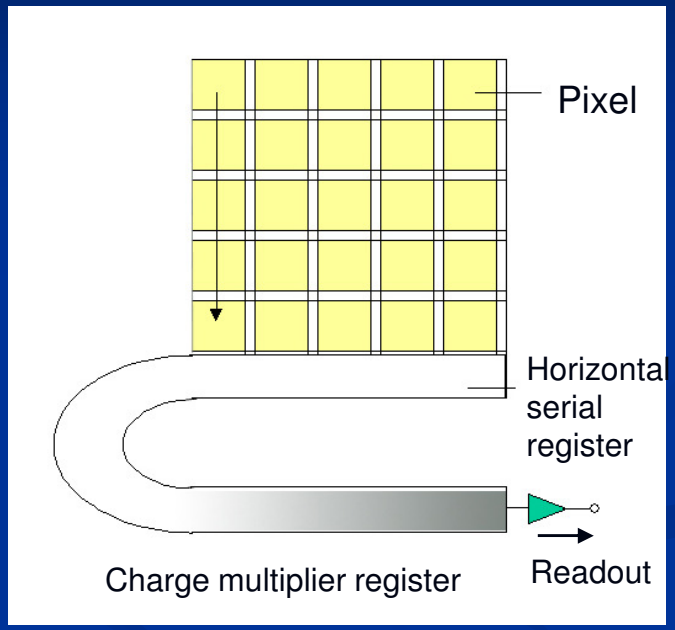


Electron Multiplier CCD

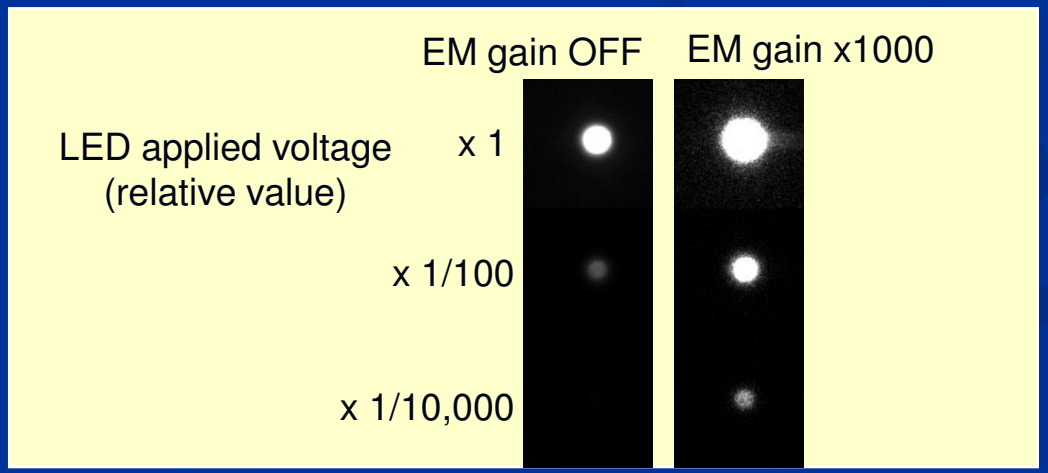
Conventional FT-CCD



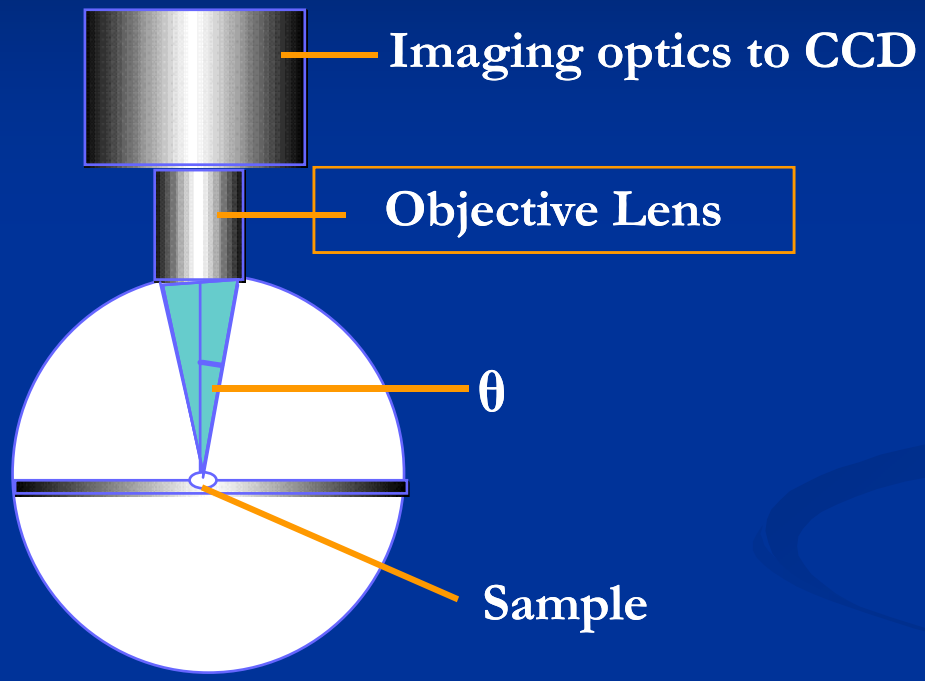
EM-CCD



LED Measurement data



Light collecting efficiency



$$\eta = \frac{1-\sqrt{1-\sin^2\theta}}{2} = \frac{1-\sqrt{1-NA^2}}{2}$$

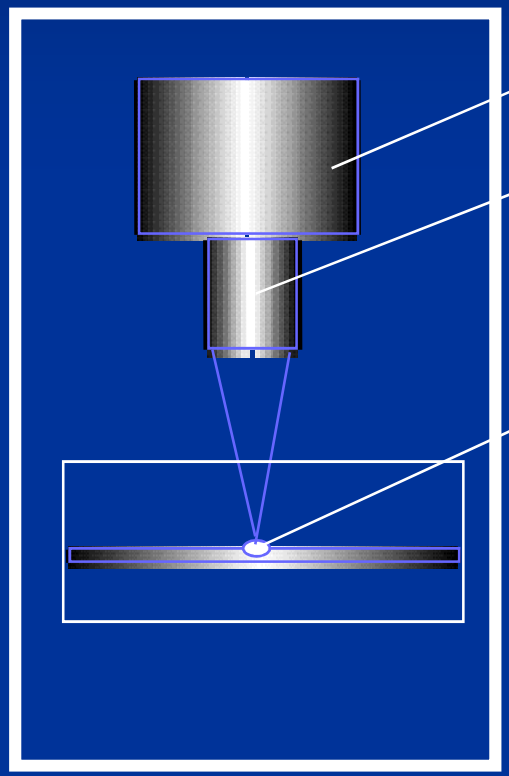
η : Light collecting efficiency

NA : numerical aperture (NA=nsinθ)

n : refractive index

NA	n (%)
0.1	0.3
0.15	0.6
0.2	1
0.25	1.6
0.3	2.3
0.35	3.2
0.4	4.2
0.45	5.4
0.5	6.7
0.55	8.3
0.6	10
0.65	12
0.7	15
0.75	17
0.8	20
1	50

Optical transparency of optical system



Microscope

Objective lens
Imaging lens
Optical filters
Mirrors
Dichroic mirrors
Correcting lenses
Long optical path

Bioluminescent Imaging System
Key Point

(1) High sensitivity

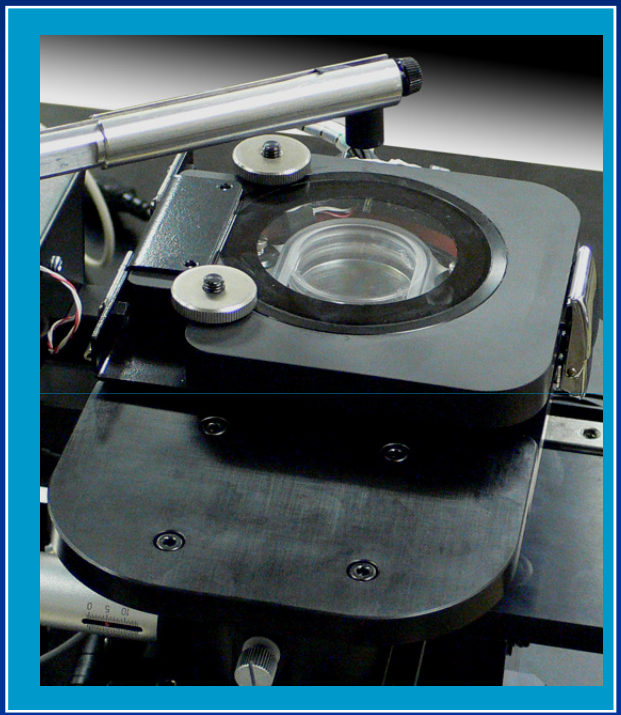
(2) Blocking of Outside light

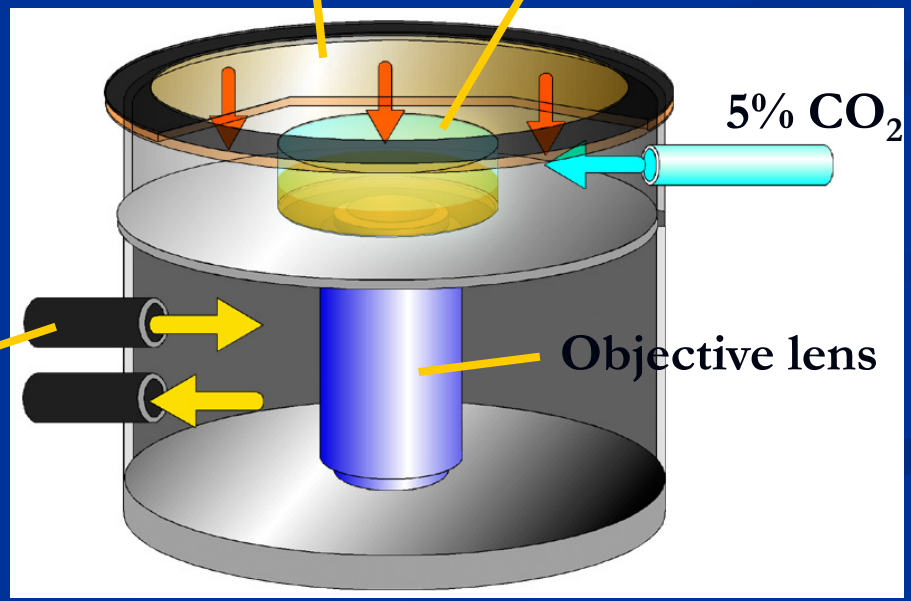
(3) Long term cell culturing

Blocking of Outside Light

Sample stage and Optical system into Dark box

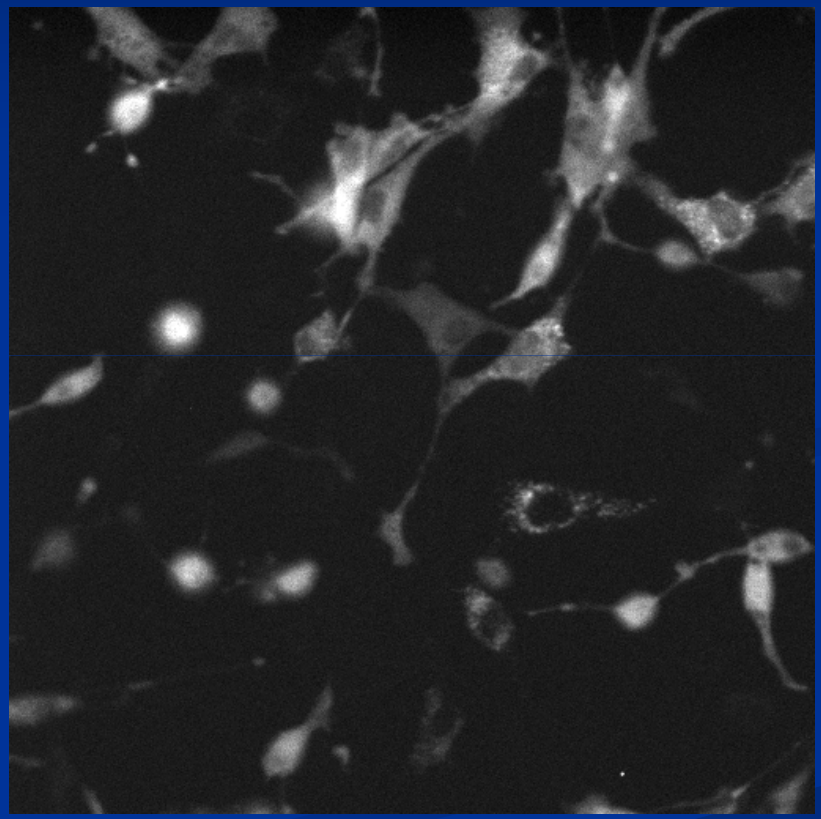
Incubation System for Long Term Cell Culturing





Single Cell Bioluminescent Imaging System

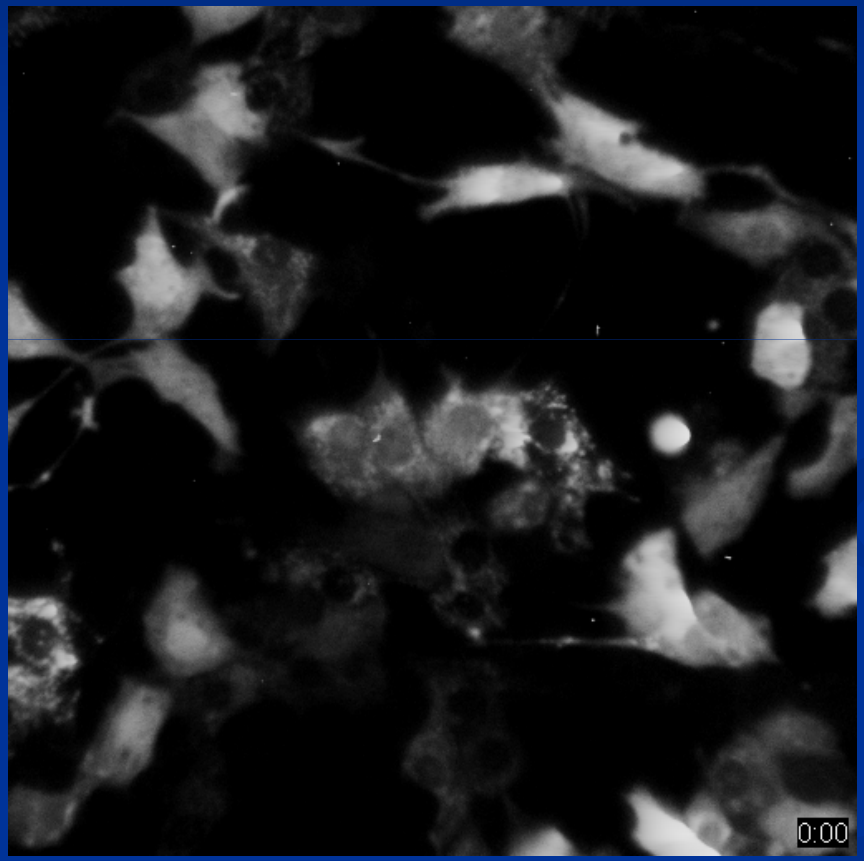
"Cellgraph"



Luciferase expressed NIH3T3 cells

Bioluminescent Imaging

Movie



Cell : NIH3T3
Reporter: SV40 Promoter-ELuc
Transfection: 2μg DNA
Medium : DMEM, 10%FBS,
0.2mM D-Luciferin
Magnification of objective lens : 20x
Exposure time : 20min

Bioluminescent Imaging

Movie

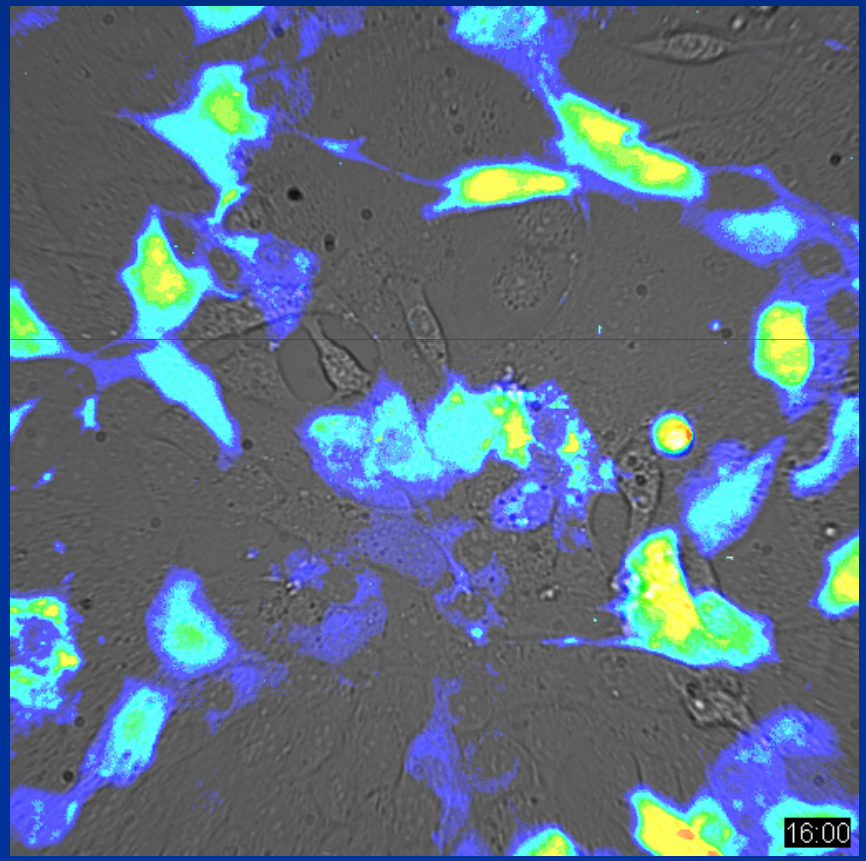


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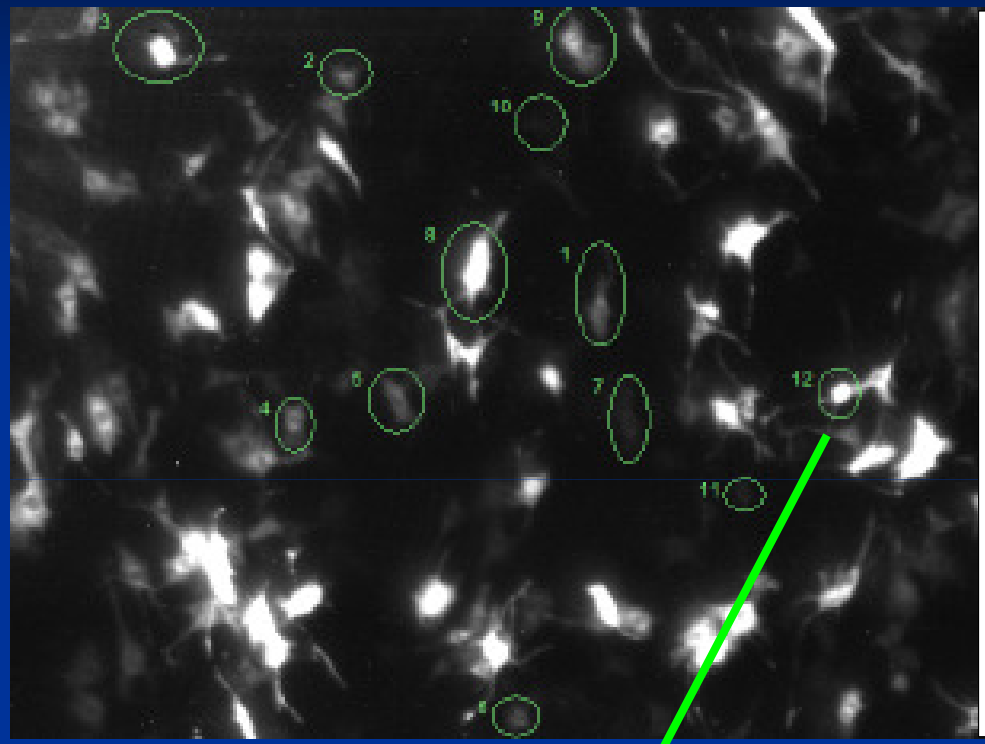
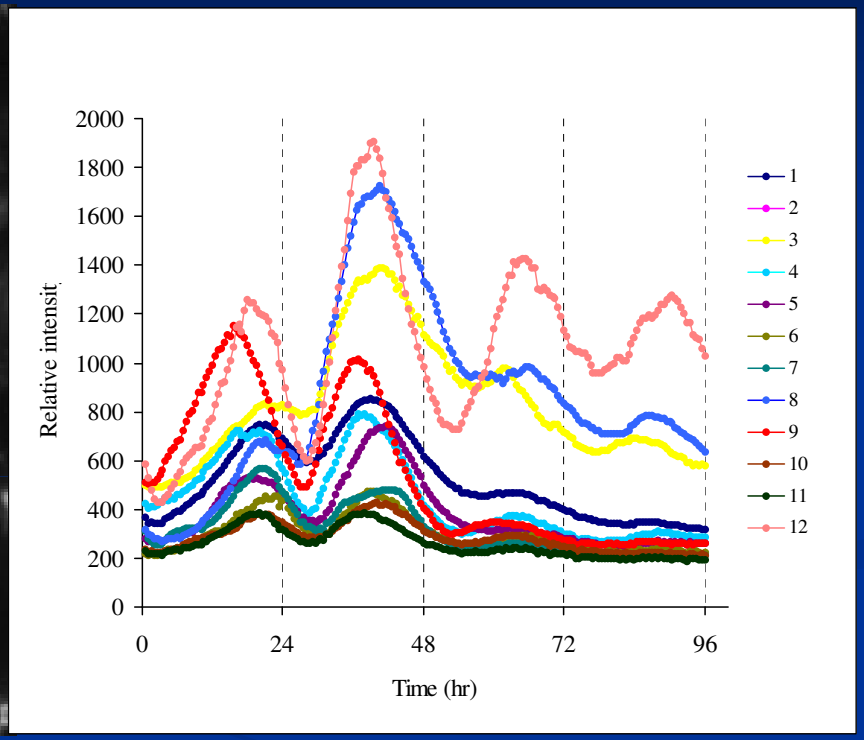
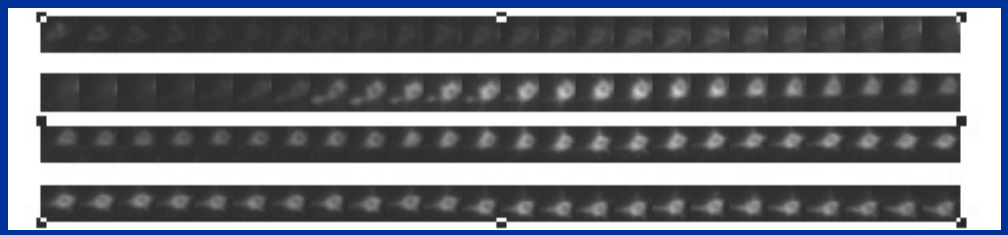
Image Analysis





1hr



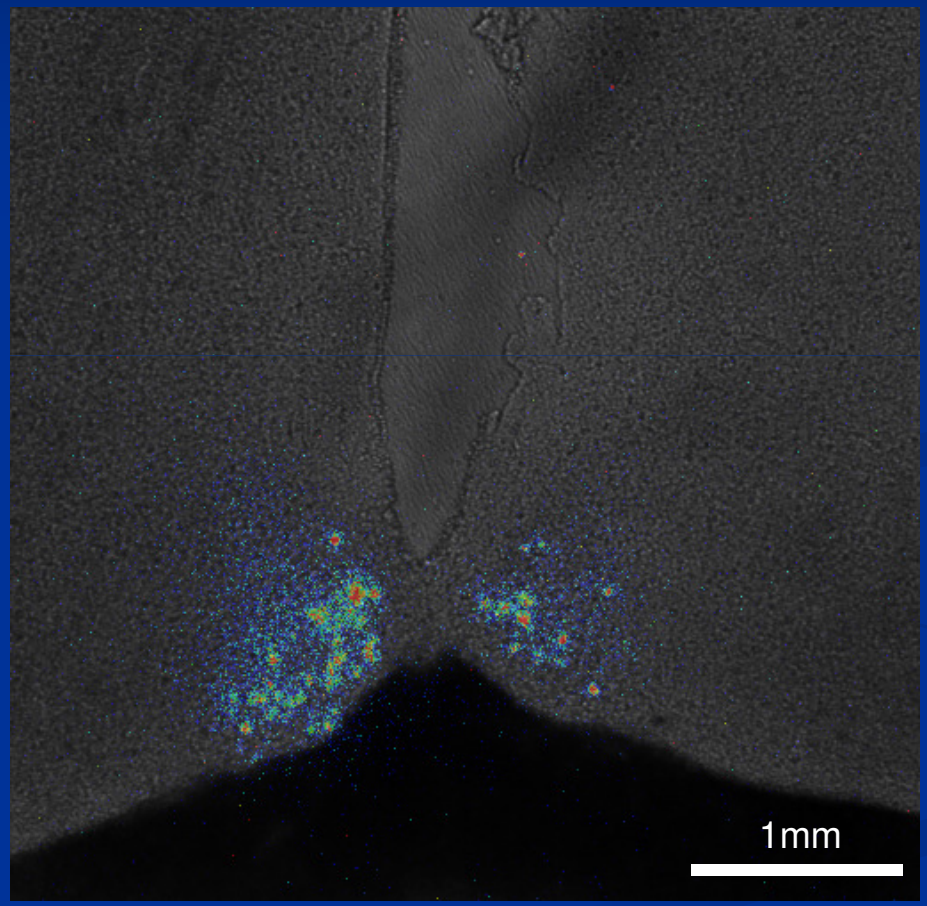
96 hr

Cell : NIH3T3
Reporter: Clock gene(Bmal1) Promoter-Fluc
Transfection: 2μg DNA
Medium : DMEM, 10%FBS, 25mM HEPES,
0.2mM D-Luciferin
Magnification of objective lens :×5.6
Exposure time : 20min

Y. Nakajima, AIST, Japan

Bioluminescent Imaging

Mouse brain SCN tissue culture



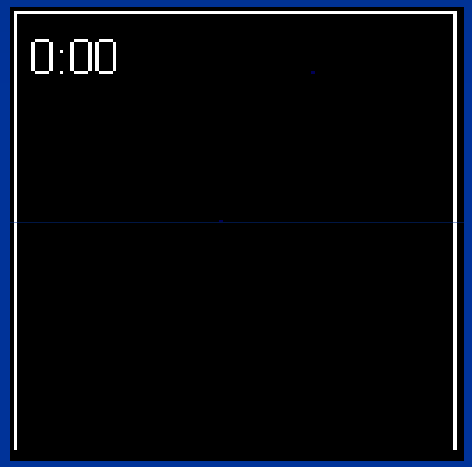
Cell : TG mouse brain slice
Reporter: Bmal1 Promoter-Eluc
Magnification of objective lens : 4x
Exposure time : 3min

Y. Nakajima (AIST, Japan)

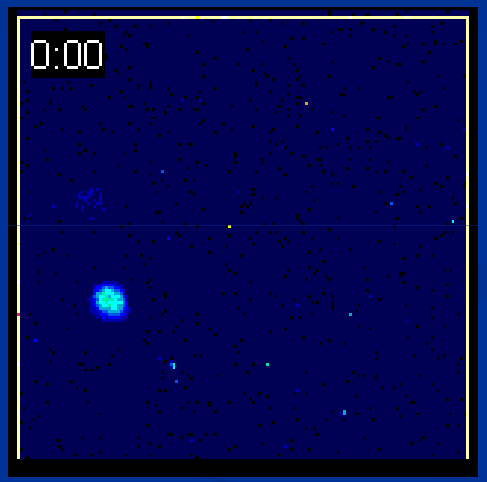
Bioluminescent Imaging

Luciferase | NLS

Movie



Movie

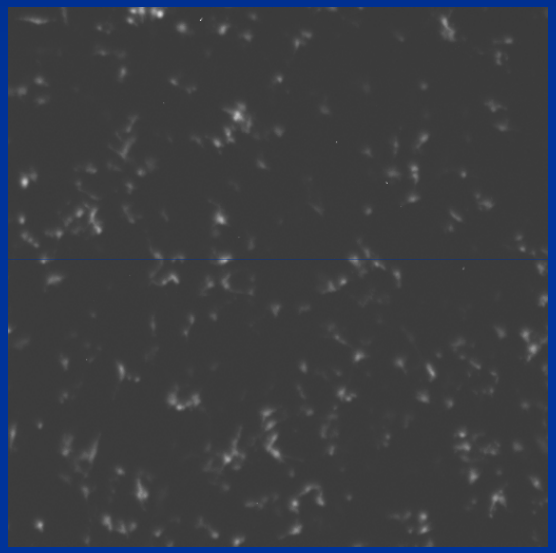


Cell : NIH3T3
Reporter: SV40 Promoter-ELuc-NLS
Transfection: 2μg DNA
Medium : DMEM, 10%FBS,
0.2mM D-Luciferin
Magnification of objective lens : 20x
Exposure time : 10min

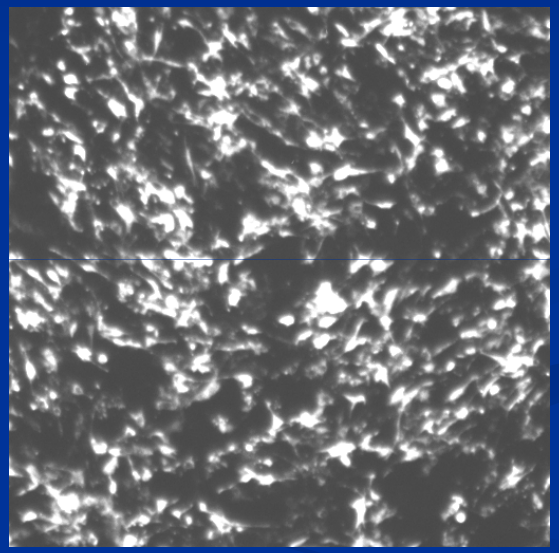
Y. Nakajima, AIST, Japan

High intensity bioluminescent probe

Firefly luciferase



Eluc

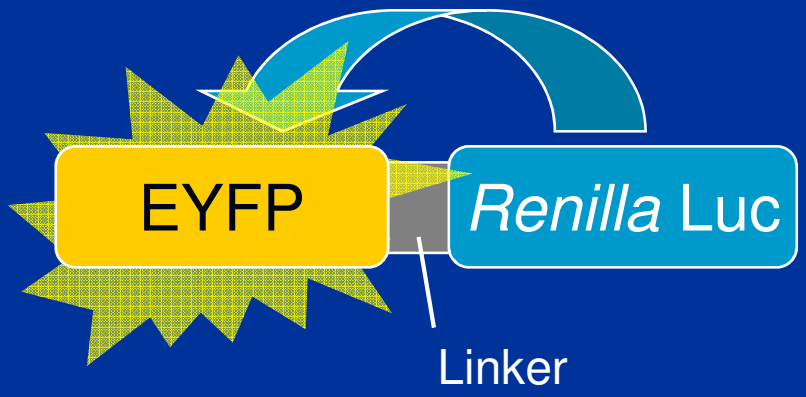


SV40-ELuc in NIH3T3 cells
Medium: DMEM+10%FBS , 0.2mM D-Luciferin

V. Viviani, *et al.*, *Photochem. Photobiol.* 70(2), p254-260 (1999)

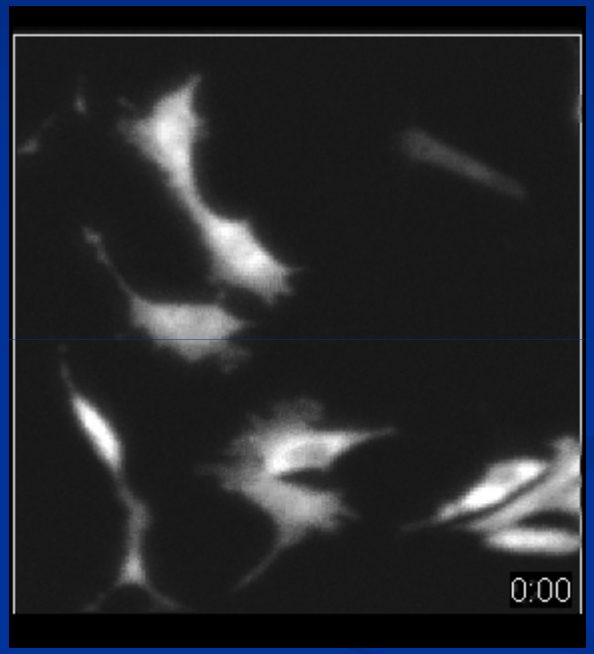
Enhanced emission probe with BRET

Bioluminescent
Resonance
Energy **T**ransfer



BAF-Y
(BRET-based autoilluminated fluorescent protein on EYFP)

Movie



Cell : NIH3T3
Reporter: CMV Promoter-eBAF-Y
Medium : DMEM, 10%FBS,
0.06mM Coelenterazine (ViviRen™)
Magnification of objective lens : 20x
Exposure time : 18sec

H. Hoshino, *et al.*, *Nature Methods* 4(8), p637-639 (2007)

Summary

(1) Real-time reporter assay enable to monitor continuously the time course of gene expression level after drug stimulation in living cells.

(2) Our new multicolor bioluminescent detection method enable to monitor the activities of different promoters simultaneously in the same sample.

(3) We have developed living cell bioluminescent imaging system. This system is effective to quantitatively trace gene expression of single cell for long term.