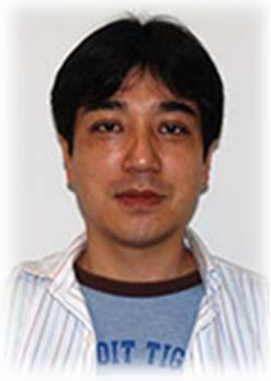

# Photometrics Customer Profile

## Imaging the World's Brightest Luminescent Protein

> "Now, all biological phenomena that are regulated by light, including photosynthesis and photoreception in retina, can be viewed using Nano-lantern, giving us more dynamic and quantitative analysis of living cells."

**– Dr. Takeharu Nagai,** Department of Biomelocular Energetics, Institute of Scientific and Industrial Research at Osaka University

## BACKGROUND

Optogenetics is revolutionizing the field of behavioral neuroscience by enabling researchers to control the activity of individual neurons and measure the effects of those manipulations. Introduced in 2006, it was selected as *Nature Methods'* "Method of the Year" only four years later. Today, hundreds of labs are using the technique to explore the neurobiology of phenomena such as decision-making and neurodegenerative diseases, often with remarkable results. For example, researchers have demonstrated how light can steady the gait of a stumbling rat with Parkinson's disease.

Fluorescent imaging is a widely used technique to gain insight into biological functions. But problems can arise when external illumination is required, as in optogenetics: The same light that stimulates what's under optogenetic control can interfere with the fluorescent sensor. For instance, the same blue light that excites the FRET-based indicator for calcium activates a commonly used photo-sensitive receptor used in optogenetics.

Light can be generated instead by chemiluminescence – where the emission is produced by a chemical reaction – but existing chemiluminescent probes are too weak for use in optogenetics studies. A means to create brighter chemiluminescence would help to advance these and other applications.

## CHALLENGE

Takeharu Nagai, PhD, an Osaka University professor and inventor of a number of ingenious fluorescent and chemiluminescent probes, decided to take up the challenge of developing a probe compatible with optogenetics studies – in large part, he said, because he had questions of his own he wanted to answer.

"To investigate the input-output relation in living cells, compatible use of optogenetics and fluorescent indicators such as calcium ion ($Ca^{2+}$) are very useful combinations," said Dr. Nagai. "However, because fluorescent indicators require excitation light source, such excitation light gives perturbations to the cell function as photo-stimulation light."

## OVERVIEW

Dr. Takeharu Nagai and his colleagues engineered the world's brightest luminescent protein known as Nano-lantern with temporal and spatial resolution equivalent to those of fluorescence. To visualize Nano-lantern signals, Dr. Nagai turned to the Evolve™ 512 EMCCD camera.

## RESEARCH TEAM

Takeharu Nagai, PhD, Department of Biomolecular Energetics, Institute of Scientific and Industrial Research at Osaka University

## PRODUCTS

Evolve™ EMCCD camera

## KEY FEATURES

- High quantum efficiency allows for the easy detection of the low chemiluminescent signals of RLuc for both still images and video-rate imaging without the risk of phototoxicity.
- Superior cooling (-85°C) increases signal-to-noise ratio.
- Wide dynamic range enables acquisition of both chemiluminescent and bright-field images using the same camera setting.
- Dead-time of the camera allows for optogenetic stimulation.

## SOLUTION

To overcome this problem, Dr. Nagai's lab engineered a probe that fuses a luminescent protein from the sea pansy *Renilla reniformis* with a fluorescent protein they previously designed. The new molecular entity – dubbed Nano-lantern – is the world's brightest luminescent protein, with temporal and spatial resolution equivalent to those of fluorescence.

According to Dr. Nagai, "dispensing with the need for light illumination, which is essential for fluorescence imaging, allows us to analyze dynamics in environments where the use of fluorescent indicators is not feasible."

## RESULTS

Having designed the new, considerably improved protein – it is ten times brighter than the original template (RLuc) – Dr. Nagai's team decided to put it through its paces. First, they used it to image intracellular structures in living cells, demonstrating spatial resolution and brightness on par with those of fluorescence.

Collaborating with Dr. Yuriko Higuchi at Kyoto University, they visualized cancer tissue inside a freely moving mouse. Here, the Nano-lantern offered a number of advantages over conventional probes, including increased sensitivity and shorter exposure times. It even enabled video-rate imaging of tumors 17 days after implantation.

Next, Dr. Nagai's group modified the Nano-lantern into a calcium sensor and co-expressed it with a light-sensitive photoreceptor in rat neurons. Fluorescent $Ca^{2+}$ indicators by themselves would be triggered by light, making it impossible to perform optogenetics. This co-expression system allowed the researchers to follow excitation of the photoreceptors by measuring the $Ca^{2+}$ increase as reported by the Nano-lantern $Ca^{2+}$ indicator.

They were also able to visualize ATP production in plant chloroplasts. They converted the Nano-lantern into an ATP sensor, and expressed it in a live leaf. The red autofluorescence signal of the chloroplasts did not interfere with the Nano-lantern, allowing observation of the increase in ATP levels after light irradiation – as well as the first visualization of the qE quenching-mediated down regulation of ATP synthesis in live plant cells.

To visualize Nano-lantern signals, it's not surprising that Dr. Nagai and his colleagues turned to Photometrics' Evolve 512 EMCCD camera.

"We knew that the Photometrics' cameras worked very well because we used Photometrics Cascade II EMCCD before the Evolve," said Dr. Nagai who has been using Photometrics cameras for more than a decade. Photometrics' technology has contributed to 11 of his publications.

The Evolve's high quantum efficiency allowed Dr. Nagai's lab to easily detect the low chemiluminescent signals of RLuc for both still images and video-rate imaging without the risk of phototoxicity. The camera's superior cooling (-85°C) increased the signal-to-noise ratio for the mouse tumor imaging, while its wide dynamic range enabled acquisition of both chemiluminescent and bright-field images using the same camera setting.

The Evolve 512 EMCCD also offers a feature that allowed Dr. Nagai's team to perform "cleaner" optogenetic experiments. Because the light used to stimulate optogenetic processes is so strong, it can increase the background noise level. To overcome this issue, Dr. Nagai and his colleagues used the dead-time of the Evolve 512 camera to conduct optogenetic light stimulation.

"One of the unique advantages of Evolve 512 is that it can erase charges on the CCD during the dead-time," said Dr. Nagai. "This function contributed to reduced background noise for compatible use of optogenetic and chemiluminescent imaging."

"Now, all biological phenomena that are regulated by light, including photosynthesis and photoreception in retina, can be viewed using Nano-lantern, giving us more dynamic and quantitative analysis of living cells," explained Dr. Nagai.

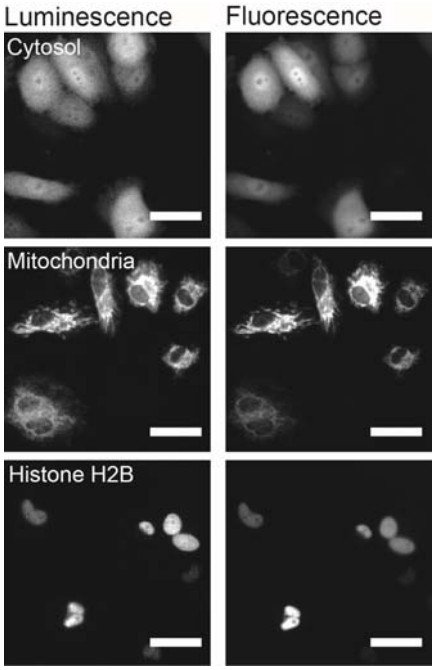


**Figure 1 c:** Luminescence (left) and fluorescence (right) imaging of HeLa cells expressing Nano-lantern targeted to cytoplasm, mitochondria and histone H2B. The exposure times for the luminescence images were 3 s, 3 s and 1 s, respectively. The exposure time for all fluorescence images was 1 s. The reference fluorescence signal was captured by exciting Venus with light at 490 nm. Scale bars, 50 mm.

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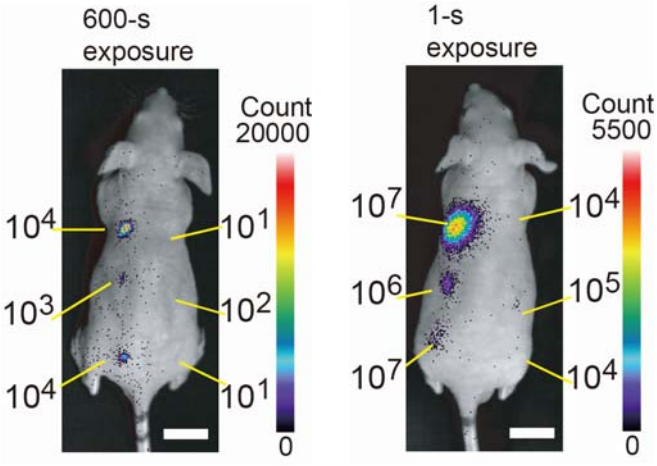


**Figure 2 b,d:** Representative luminescence images of the indicated numbers of injected cells at 600 s (b) and 1 s exposures (d)

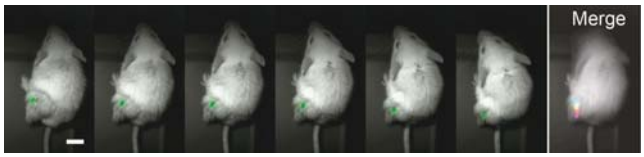


**Figure 2 e:** Consecutive frames of video-rate images of Nano-lantern-expressing tumour cells in an unshaved mouse. The luminescent signal every 60 ms is shown in green in the still images and as a series of pseudo colours (blue, cyan, green, yellow, red and magenta) in the merged image. Scale bars, 1 cm.

Nano-lantern-mouse-with-structure: Takeharu Nagai and colleagues used Photometrics' Evolve™ 512 EMCCD camera to visualize signals of Nano-lantern in a mouse.

Dr. Nagai is already hard at work creating new colors for the Nano-lantern and boosting its brightness, which could lead to single-molecule-level imaging. As there are no plans to commercialize the Nano-lantern, he's making the DNA construct freely available.

Dr. Nagai expects that the increased brightness of Nano-lantern will make possible more advanced applications such as high-throughput drug screening and single-cell tracking in live animals and plants.

"Nano-lantern can help realize the other advantages of low-intensity light imaging, including diminished photobleaching and phototoxicity, which allow for prolonged observation without harming the cellular substrate," he said.

Dr. Nagai notes that rapid consumption of the luminescent substrate by Nano-lantern hampers prolonged observation of biological events, especially in the case of whole-body imaging. To address this issue, he and his team are working to develop a method that facilitates indefinite observation applicable to the entire organism.

[i]K Saito, Y Arai, T Nagai et al. Luminescent proteins for high-speed single-cell and whole-body imaging. Nature Communications. 2012; 3:1262. (December 11, 2012).

YF Chang, Y Arai and T Nagai et al. Optogenetic activation during detector 'dead time' enables compatible real-time fluorescence imaging. **Neuroscience Research**. 2012; 73:341–347. (August, 2012)

## SUPPORTING VIDEOS

Visit the Photometrics online Media Center to view these supporting videos:

- Nano-lantern-expressing tumor cells in the unshaved freely moving mouse
  - www.photometrics.com/resources/videos/nagai.nano
- Pseudo colored ratio images of a rat hippocampal neuron expressing Nano-lantern (Ca2+) recorded at 10Hz
  - www.photometrics.com/resources/videos/nagai.ratio

## LOOKING FORWARD

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