# Development of a multicolor bioluminescence imaging method for simultaneous monitoring of multiple cellular activities

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## Abstract

Cells that constitute the human body exhibit unique characteristics, such as differences in fate and responses to environmental stimuli. Therefore, the method for identifying individual cells has become increasingly important in research. As a cell-labeling method, we employed bioluminescence, which does not require excitation light. To expand the range of bioluminescent emission colors, we combined bioluminescent and fluorescent proteins to alter the emission wavelength, thereby developing a series of 20 bioluminescent proteins, "eNLEX".

Furthermore, to distinguish the different emission colors of eNLEX, we introduced an imaging method using color CMOS cameras, such as those mounted in a smartphone. As a result, we successfully achieved simultaneous real-time observation of multiple living cells emitting distinct bioluminescent colors.

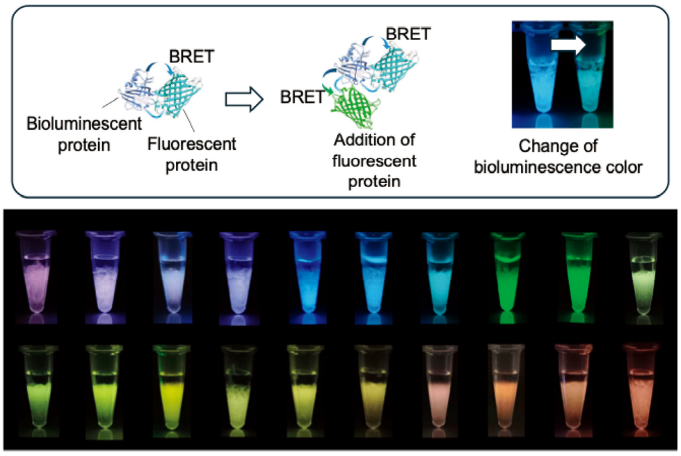
## Background & Results

Methods for identifying individual cells are required for a variety of purposes, such as tracing cell fate and identifying cells with distinct characteristics from others. In microscopic observations, cells are typically labeled with fluorescent molecules that emit light at different wavelengths. However, since fluorescence depends on the wavelength of excitation light, observing multiple fluorescent species requires the switching and control of optical filters. As the number of fluorescent labels increases, accurate distinction becomes increasingly difficult. In contrast, bioluminescence is light that is spontaneously emitted when a bioluminescent protein reacts with its substrate. Unlike fluorescence, it does not require external excitation light, and therefore offers advantages such as low background signals and the absence of phototoxicity.

To expand the range of bioluminescent emission colors, we connected multiple fluorescent proteins to a blue-emitting bioluminescent protein, NanoLuc, thereby inducing bioluminescence resonance energy transfer (BRET) and successfully generating complex emission spectra. Finally, we developed a bioluminescent protein series with 20 distinct emission colors, which we named "eNLEX". Conventional detection methods using monochrome cameras with filter switching are unable to simultaneously observe all 20 eNLEX colors. Therefore, we employed a color CMOS camera, such as those commonly used in smartphones, as a detection device. These cameras detect light emitted from the sample through RGB (red, green, and blue) filters on the sensor, allowing the wavelength information of the emitted light to be recorded. By mounting the color CMOS camera onto a microscope, we observed cultured human cells expressing different eNLEX variants and demonstrated that 20 distinct emission colors could be simultaneously visualized from individual living cells.

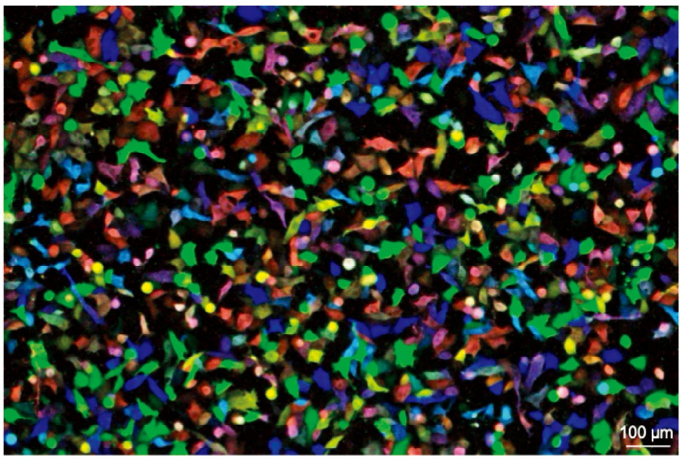
## Significance of the research and Future perspective

The combination of bioluminescence and color CMOS camera imaging enables simultaneous observation of multiple cells using a simple and cost-effective system. This technology holds promise for broader applications beyond basic research, including regenerative medicine and drug discovery.




20 colors bioluminescent proteins "eNLEX"

Fig. 1.




Color CMOS camera images of cultured cells expressing eNLEX

Fig. 2.

Patent
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