Bioluminescent cell imaging gets a glow-up

Researchers from Osaka University and Keio University develop a method to increase the number of bioluminescence colors for cell imaging, making it easier to track many targets simultaneously

Imaging live cells with fluorescent proteins has long been a crucial technique for understanding cellular behavior. While bioluminescent proteins offer several advantages over fluorescent proteins, the limited availability of color variants has made it difficult to observe multiple targets simultaneously. Now, researchers from SANKEN (The Institute of Scientific and Industrial Research, Osaka University) and Keio University have developed a groundbreaking method to expand the color palette of bioluminescent protein to 20 distinct colors, enabling advanced simultaneous multi-color imaging.

Cells are the fundamental building blocks of life. Understanding how they function is essential for progress in biological sciences, medicine, and drug discovery. Optical labeling techniques allow scientists to observe cell behavior, track cell fate, and identify cells with specific traits. While fluorescent proteins are widely used for these purposes, bioluminescent proteins are gaining popularity due to their unique advantages.

Bioluminescence, the natural emission of light by living organisms, is powered by a chemical reaction catalyzed by an enzyme, typically a luciferase, acting on a bioluminescent substrate. Unlike fluorescent proteins, bioluminescent proteins do not require external light for excitation, avoiding issues like phototoxicity and background light. However, their use has been limited by the small number of available colors. Having distinct and easily distinguishable colors is vital tracking multiple targets simultaneously.

Previously, a five-color series of bioluminescent labels was created by coupling one of the brightest luciferases, NanoLuc, with a fluorescent protein. This technique leverages the transfer of excited-state energy from the substrate to the fluorescent protein, altering the bioluminescence color. While effective, this five-color palette was insufficient for more complex imaging needs. The researchers at Osaka University and Keio University have now addressed this challenge by expanding the bioluminescent color palette to 20, making a significant leap forward in multi-color imaging technology.

“Instead of fusing NanoLuc with single fluorescent protein, we fused it with two,” says lead author Mitsuru Hattori. “This approach allowed us to access a much broader range of bioluminescence colors by fine-tuning the combinations of fluorescent proteins.”
The researchers achieved remarkable milestone with their new bioluminescent protein labels. They captured a single-shot image of a mixture of cells expressing all 20 bioluminescent proteins, used the labels to visualize distinct subcellular components, and even demonstrated their capability in live mice. Additionally, they successfully conducted time-lapse observations of cell behavior over several hours, simultaneously tracking seven distinct labels.

“What’s truly exciting is that we could detect all 20 colors simultaneously without any time lag, using a standard smartphone camera,” explains senior author Takeharu Nagai. “This innovation makes it significantly easier and more cost-effective to monitor multiple targets or track individual cells within a population.”

These newly developed bioluminescent colors have the potential to revolutionize cell fate tracking, offering insights concerning how cells develop into specific cell types and identifying cells with unique responses to drugs. The team’s breakthrough in bioluminescent imaging opens new doors for advancements in biological research, drug discovery, and medical science. With such a vivid “rainbow” of bioluminescent colors, who knows what scientific treasures lie ahead?

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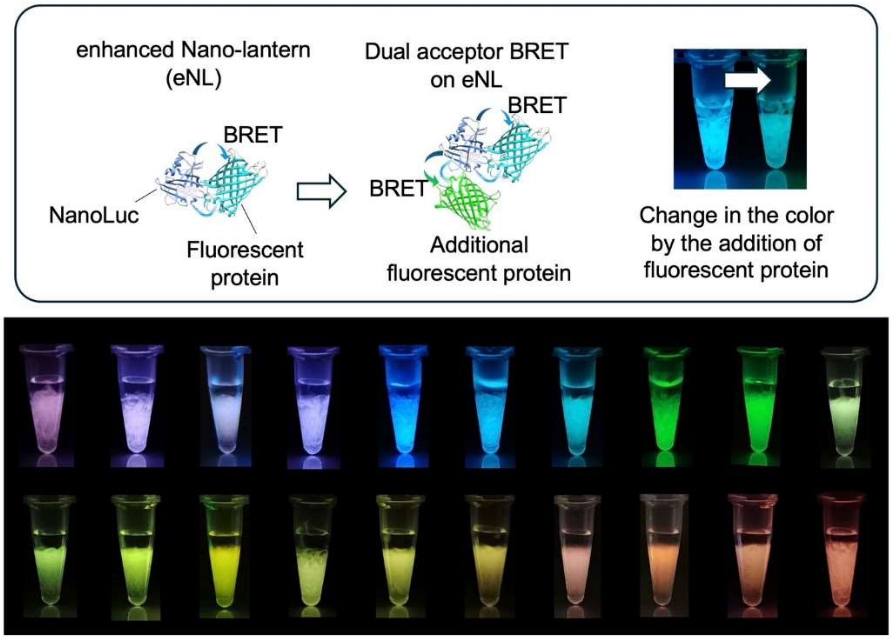

Fig. 1
20 colors of bioluminescent protein “eNLEX."
The method for changing bioluminescence color (top) and the bioluminescent image of 20-color eNLEX taken with a smartphone camera (bottom).

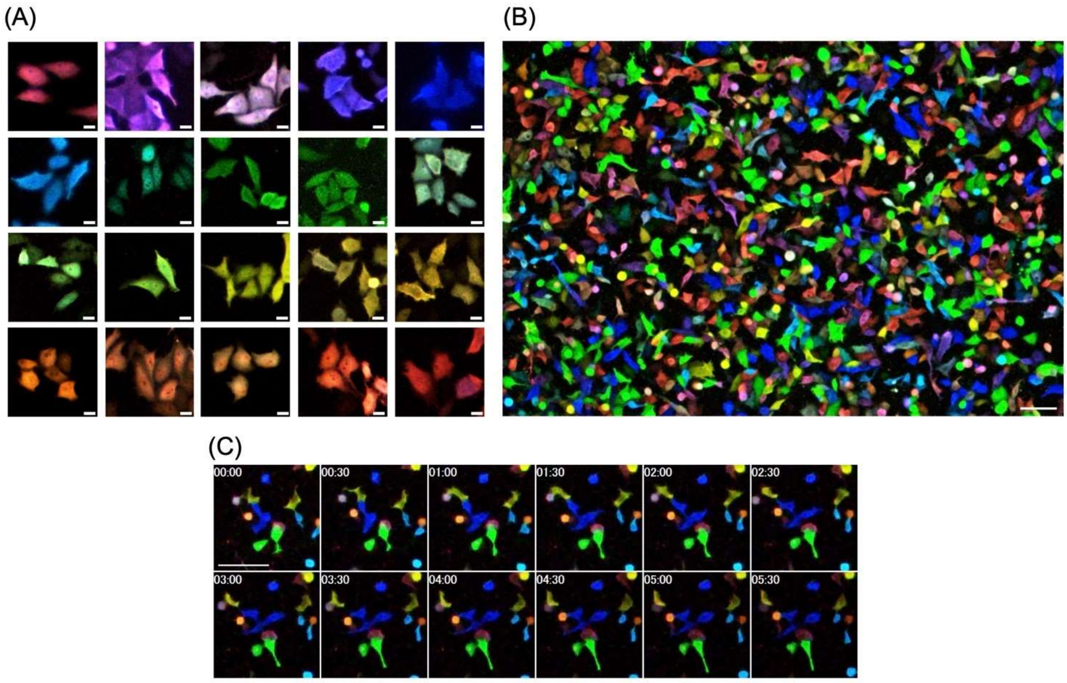

Fig. 2
Bioluminescence imaging of human cultured cells transfected with eNLEX.
(A) HeLa cells expressing each color of eNLEX. Scale bar: 20 µm. (B) A mixture of cells of each color. Scale bar: 100 µm. (C) Time course observation (hours: minutes). Scale bar: 100 µm. All images were taken with a color CMOS camera.

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About Osaka University

Osaka University was founded in 1931 as one of the seven imperial universities of Japan and is now one of Japan's leading comprehensive universities with a broad disciplinary spectrum. This strength is coupled with a singular drive for innovation that extends throughout the scientific process, from fundamental research to the creation of applied technology with positive economic impacts. Its commitment to innovation has been recognized in Japan and around the world. Now, Osaka University is leveraging its role as a Designated National University Corporation selected by the Ministry of Education, Culture, Sports, Science and Technology to contribute to innovation for human welfare, sustainable development of society, and social transformation.

Website: https://resou.osaka-u.ac.jp/en

About Keio University

Established in 1858 by Yukichi Fukuzawa as a small school of Western learning, Keio has a history as Japan's very first private institution of higher learning. Over 160 years since its founding, Keio has thrived under its founder's motto of *jitsugaku*, or empirical science, as it continues to transform Japan as a modern nation through contributions to education, research, and medicine.

Website: https://www.keio.ac.jp/en/