Bioluminescence imaging in living animals

Takahiro Kuchimaru, Tetsuya Kadonosono, Shinae Kondoh
School of Life Science and Technology, Tokyo Institute of Technology
4259 Nagatsuta, Midori-ku, Yokohama, 226-8501 Japan
E-mail:tkuchimr@bio.titech.ac.jp

Abstract Bioluminescence imaging (BLI) allows us for noninvasive, high-sensitive and real-time monitoring of biological phenomena in living animals. Thus, we have utilized BLI to observe malignant processes of tumors in mouse models. We also have been developing new bioluminescent tools to improve BLI. Here, an overview of current BLI systems is provided to couple emerging photon-detection devices with BLI in living animals.

Keywords: bioluminescence, noninvasive animal imaging

Noninvasive imaging is a powerful technique to study biological phenomena in living subjects including cells, animals and humans. There are a range of different imaging modalities based on light, radiation, magnetic field and ultra sound [1]. One of those imaging modalities, BLI is widely employed in biomedical researches with small animals because of its key strengths including high sensitivity and visualization of diverse biological events [2, 3]. Although fluorescence imaging enables to visualize diverse biological events as well as BLI, autofluorescence hinders high-sensitive imaging in living tissue [4, 5]. In contrast, bioluminescence imaging does not require external light irradiation to produce light signals. It utilizes photons produced through a chemical reaction between bioluminescence proteins (luciferases) and small molecule substrates (luciferins), resulting in extremely low background signal in living tissues.

Several luciferase-luciferin pairs identified from nature are available for imaging applications in biomedical research [6]. Among these, a combination of firefly luciferase (Fluc) and D-luciferin has been a gold standard for animal imaging (Fig. 1) because the bioluminescence contains relatively long wavelength (> 600 nm), and biocompatibility of D-luciferin surpasses other luciferins. However, very sensitive photon-detection system such as a cooled-charge coupled device (CCD) camera is indispensable for BLI, since slow catalytic reaction of Fluc produces less than one photon per second [7,8].

To improve current BLI systems, we further discuss following points:

1) Camera devise

Commercial BLI systems are commonly equipped with a cooled-CCD camera to capture a field of view (FOV) with approximately 20 cm × 20 cm (Fig.2). This FOV is enough to image several anesthetized mice in single image acquisition, enhancing throughput which is a key factor in biomedical research using dozens mice in a common experiment. However, current commercial BLI systems are not still affordable for many researchers mainly due to an expensive camera device.

2) Sensitivity

We use genome-editing techniques to establish cancer cells or transgenic animals stably expressing Fluc [9-11]. These cancer cells typically produce <500 photons/second/cell upon administration of D-luciferin [12]. In animal studies, we locally transplant these cancer cells into mouse tissue to establish a solid tumor. A solid tumor with cubic mill meter contains approximately 10^6 cells [13]. Thus, photon flux is sufficient to detect small tumors although detection of fewer cells in a deeper tissue is still challenging. For example, cancer metastasis is initiated by small number of cells (< 10 cells) in deep organs. Current BLI systems are not capable to track such biological events even in small animals.

3) Wavelength

Hemoglobin and melanin in living tissue highly absorb light with shorter wavelength (< 600 nm) [14]. Even in BLI using Fluc, large part of bioluminescent signals cannot efficiently penetrate living tissue. To overcome

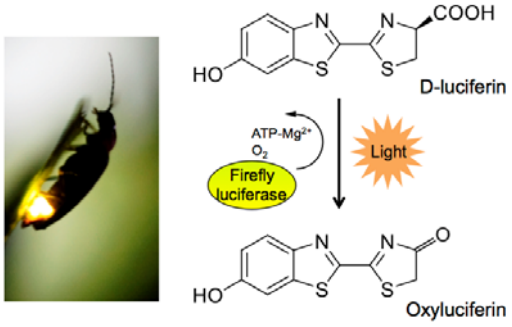

Fig. 1 Bioluminescence production in the reaction of D-luciferin and firefly luciferase.

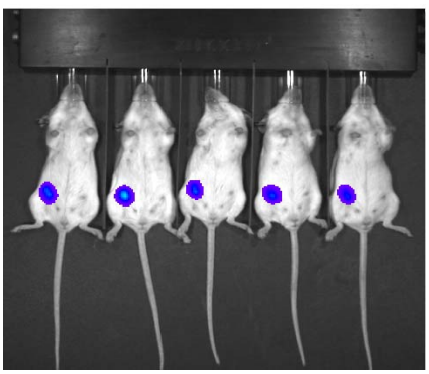

Fig. 2 A typical bioluminescence image of mice harboring a tumor expressing Fluc. After D-luciferin injection, the image was acquired with a commercial imaging system (IVIS, PerkinElmer, USA).

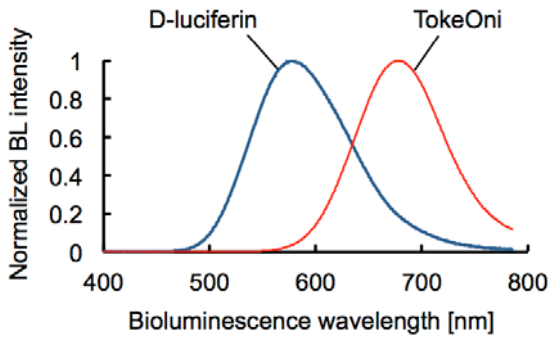

Fig. 3 Bioluminescence emission spectra of D-luciferin and TokeOni.

this, we developed a synthetic luciferin, TokeOni, which generates near-infrared (NIR) bioluminescence in a reaction with Fluc (Fig. 3) [15]. This world-first NIR-BLI system improved detection sensitivity of deep metastasis 10-times over current BLI using D-luciferin. Thus, high quantum efficiency of NIR light on camera devices is desired to maximize sensitivity of BLI in animal imaging.

4) Spatial resolution

Due to light scattering during propagation from deep tissue to body surface, BLI typically provides mm-range spatial resolution in noninvasive animal imaging. In addition, depth-resolved imaging has been challenged in BLI. A series of bioluminescence tomography techniques have reported sub-mm spatial resolution at several mm depth in living tissues [16]. However, this spatial resolution is not sufficient to provide significant biological insights in animal imaging.

5) Temporal resolution

Unlike fluorescence imaging, BLI requires relatively long camera exposure-time to obtain enough signals due to its low photon emission, hindering a couple of key applications including imaging freely-moving animals. Recent studies challenged such applications with a fiber optics-coupled photomultiplier tube or electron multiplying-CCD based system although biological studies using these systems are still in infancy [17, 18].

In this decade, BLI systems have been refined with a number of gene expression techniques and chemical substrates. However, the field of BLI needs more multidiscipline to go beyond current achievements. Active collaborations among biochemists, chemists and electronics engineers would pave a road to develop next-generation BLI systems.

References

[1] M.L, James, S. S. Gambhir: “A molecular imaging primer: modalities, imaging agents, and applications”, Phyiol. Rev., Vol. 92, pp. 897-965 (2012).

[2] C.E. Badr, B.A. Tannous: “Bioluminescence imaging: progress and applications”, Trends Biotechnol., Vol. 29, pp. 624-633 (2011).

[3] B. Kocher, D. Piwince-Worms: “Illuminating cancer systems with genetically engineered mouse models and coupled luciferase reporters in vivo”, Cancer Discov., Vol. 3, pp. 616-619 (2013).

[4] T. Troy, D. Jekic-McMullen, L. Sambucetti, R. Brad, et al.: “Quantitative comparison of the sensitivity of detection of fluoresent and bioluminescent reporters in animal models”, Mol. Imaging, Vol. 3, pp. 9-23 (2004).

[5] L. Mezzanotte, V. Blankevoort: “A novel luciferase fusion protein for highly sensitive optical imaging: from single cell analysis to in vivo whole-body bioluminescence imaging”, Anal. Bioanal. Chem., Vol. 406, pp. 5727-5734 (2014).

[6] M.A. Paley, J.A. Prescher: “Bioluminescence: a versatile technique for imaging cellular and molecular deatures”, Med. Chem. Comm., Vol. 5, pp. 255-267 (2014).

[7] B.R. Branchini, R. A. Magyar, Martha H, et al.: “Site-directed mutagenesis of histidin 245 in firefly luciferase: a proposed model of the active site”, Biochemistry, Vol. 37, pp. 15311-15319 (1998).

[8] Y. Ando, K. Niwa, N. Yamda, et al.: “Firefly bioluminescence quantum yield and colour change by pH-sensitive green emission”, Nat. Photonics, Vol. 2, pp. 44-47 (2007).

[9] S. Kizaka-Kondoh, S. Itasaka, Zeng Lihua, et al.: “Selecting killing of hypoxia-inducible factor-1-active cells improves survival in a mouse model of invasive and metastatic pancreatic cancer”, Clin. Cancer Res., Vol. 15, pp. 3433-3441 (2009).

[10] T. Kadonosono, T. Kuchimaru, S. Yamada, et al.: “Detection of the onset of ischemia and carcinogenesis by hypoxia-inducible trasncriptional factor-based in vivo bioluminescnce imaging”, PLoS ONE, Vol. 6, e26640 (2011).

[11] T. Kuchimaru, T. Hoshino, T. Aikawa, et al.: “Bone resorption facilitates osteoblastic bone metastatic colonization by cooperation of insulin-like growth factor and hypoxia”, Cancer Sci., Vol. 105, pp. 553-559 (2014).

[12] J.B. Kim, K. Urban, E. Cocharn et al.: “Non-invasive detection of a small number of bioluminescent cancer cells in vivo”, PLoS One, Vol. 5, e9364 (2010).

[13] J. V. Frangioni: “New technologies for human cancer imaging”, J. Clin. Oncol., Vol. 26, pp. 4012-4021 (2008).

[14] R. Weissleder, V. Ntziachristos: “Shedding light onto live molecular targets”, Nat. Med. Vol. 9, pp. 123-128 (2003).

[15] T. Kuchimaru, S. Iwano, M. Kiyama, et al.: “A luciferin analogue generating near-infrared bioluminescence achieves highly sensitive deep-tissue imaging”, Nat. Commun., Vol. 7, 11856 (2016).

[16] C. Qin, J. Feng, S. Zhu, et al.: “Recent advances in bioluminescence tomography: methodology and system as well as application”, Laser Photonics Rev., Vol. 8, pp. 94-114 (2014).

[17] D. Ono, K. Honma, S. Honma: “Circadian and ultradian rythyms of clock gene expression in the suprachaiasmatic nucleus of freely moving mice”, Sci. Rep., Vol. 5, 12310 (2015).

[18] T. Hamada, K. Sutherland, M. Ishikawa, et al.: “In vivo imaging of clock gene expression in multiple tissues o freely moving mice”, Nat. Commun., Vol. 7, 11705 (2016).