

Illuminating oncology research by bioluminescent imaging



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ABSTRACT

Bioluminescent imaging (BLI) has emerged as a powerful tool in cancer research, allowing for non-invasive, real-time monitoring of various aspects of cancer biology *in vivo*. This technique relies on the expression of bioluminescent reporter genes in cancer cells, enabling the visualization of tumor growth, metastasis, and response to treatment. In this review, we provide an overview of the principles underlying BLI, its applications in cancer research, and the potential advantages and limitations of this technology.

Key words: Bioluminescent; Live cell; Imaging; Non-invasive; Photon; Vectors; Cancer research

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INTRODUCTION

Cancer is a complex and heterogeneous disease characterized by uncontrolled cell growth and proliferation.¹ Understanding the dynamics of cancer progression and response to therapies is crucial for developing effective treatments. Bioluminescent imaging (BLI) has gained prominence in cancer research due to its ability to provide longitudinal, non-invasive, and quantitative data in live animals.² BLI relies on the expression of bioluminescent reporter genes, such as firefly luciferase, to track cancer cells and their activities *in vivo*.^{3,4}

PRINCIPLES OF BLI

The basic principle of BLI involves the expression of a bioluminescent reporter gene, typically firefly luciferase, in cancer cells.⁵ Luciferase, when exposed to its substrate luciferin and oxygen, catalyzes a reaction that emits photons of light.⁶ These photons can be detected and quantified using specialized imaging systems, such as bioluminescence cameras. As cancer cells expressing luciferase grow and proliferate, the intensity of bioluminescent signals increases, allowing for the visualization of tumor growth and metastasis in real-time.

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BLI offers several distinct advantages in various research applications, particularly in the field of molecular and cellular biology.⁷ These advantages are firmly established and contribute to the widespread adoption of BLI.

APPLICATIONS OF BLI IN CANCER RESEARCH

Tumor growth monitoring

BLI enables researchers to monitor the growth of primary tumors over time. By implanting luciferase-expressing cancer cells into animal models, tumor progression can be tracked, providing insights into tumor kinetics and growth patterns.⁸

Metastasis studies

BLI is invaluable in studying the metastatic spread of cancer cells. It allows researchers to monitor the dissemination of cancer cells from primary tumors to distant organs, aiding in the understanding of metastatic processes.⁹

Evaluation of therapeutic efficacy

BLI can be used to assess the response of tumors to various therapeutic interventions, including chemotherapy, radiation therapy, and targeted therapies. Changes in bioluminescent signals can indicate treatment efficacy or resistance.^{10,11}

Longitudinal studies

BLI facilitates longitudinal studies by enabling repetitive imaging of the same animals over extended periods. This is particularly useful for tracking the natural history of cancer or assessing the long-term effects of treatments.¹²

VECTORS AND TOOLS FOR BLI

BLI vectors refer to specialized genetic constructs or plasmids designed for the purpose of conducting BLI experiments.¹³ This technique is widely used in molecular and cellular biology research, as well as in preclinical studies to visualize and monitor various biological processes in live organisms.¹⁴ BLI vectors are engineered to contain genes encoding for luciferase enzymes, which can produce visible light through a chemical reaction when provided with the substrate luciferin and oxygen.¹⁵ Luciferase-expressing cells or organisms can be tracked non-invasively and in real-time by measuring the emitted bioluminescent signal using specialized imaging equipment such as a bioluminescence camera.¹⁶

These vectors typically consist of several key components

Promoter region

BLI vectors include a promoter region that controls the expression of the luciferase gene. The choice of promoter

determines when and where luciferase is produced within the target cells or tissues.

Luciferase reporter gene

The core element of BLI vectors is the luciferase reporter gene. This gene is responsible for producing the luciferase enzyme, which catalyzes the conversion of luciferin into oxyluciferin, emitting photons of light in the process.

Selectable marker

To facilitate the selection of cells that have successfully incorporated the vector into their genome, BLI vectors often include a selectable marker, such as a gene encoding resistance to an antibiotic or a fluorescent protein. This allows researchers to identify and isolate cells expressing luciferase.

Polyadenylation signal

A polyadenylation signal is included to ensure proper termination of transcription and mRNA stability.

Origin of replication

BLI vectors need an origin of replication to allow for their replication within the host cell, ensuring their maintenance during cell division.

Multiple cloning sites (MCS)

MCS regions are integrated into BLI vectors, enabling the insertion of other genetic elements or genes of interest. This flexibility allows researchers to adapt the vector for various experimental purposes.

Enhancers and regulatory elements

Depending on the experimental requirements, BLI vectors may contain enhancers and other regulatory elements to fine-tune gene expression as well as specific integration sites. Once a BLI vector is introduced into target cells or organisms, the luciferase-expressing cells can be monitored by injecting a luciferin substrate and measuring the emitted bioluminescent signal using a specialized camera.¹⁷ This technique has a wide range of applications, including tracking the progression of diseases, assessing gene expression, monitoring cellular processes, and evaluating the efficacy of drug treatments in living organisms.¹⁸ BLI vectors are invaluable tools for researchers seeking to understand the dynamics of biological systems in real-time, providing valuable insights into both basic biology and translational medicine.^{19,20}

BLI INSTRUMENTATION

Instrumentation is a critical component of bioluminescence-based research, enabling the visualization and quantification of bioluminescent signals emitted by cells, tissues, or

organisms expressing luciferase reporter genes. BLI instrumentation typically consists of specialized equipment and software designed for sensitive and accurate detection of bioluminescence.²¹ Here are the key components and considerations involved in BLI instrumentation:

Bioluminescence camera

The core component of BLI instrumentation is a highly sensitive camera designed to capture and quantify bioluminescence.

BLI has facilitated numerous scientific discoveries across various fields of research due to its ability to visualize and monitor biological processes in real-time. Here are some notable scientific discoveries made possible through BLI.

Cancer research

BLI has been instrumental in cancer research by allowing scientists to track tumor growth, metastasis, and response to treatment in live animals. Researchers have used BLI to assess the efficacy of experimental cancer therapies and to understand the dynamics of tumor microenvironments.²²

Infectious disease studies

BLI has been employed to investigate infectious diseases. For example, researchers have developed bioluminescent pathogens, such as bacteria and viruses, to study their dissemination in animal models. This has led to insights into the pathogenesis of infectious diseases and the development of novel treatments.

ADVANTAGES OF BLI

Non-invasiveness

BLI is non-invasive, reducing the need for repeated tissue sampling and minimizing animal distress.²³

Real-time monitoring

BLI provides real-time data, allowing for the continuous observation of cancer progression and treatment response.

Quantitative analysis

BLI enables quantitative assessment of bioluminescent signals, allowing for precise measurements of tumor burden.

LIMITATIONS OF BLI

BLI is a powerful and versatile tool used in various fields of research, particularly in molecular biology and biomedical sciences. However, like any scientific technique, BLI has its limitations. Understanding these limitations is crucial for researchers to make informed decisions about when and how to use BLI in their experiments.

BLI provides semi-quantitative data rather than precise quantification. The emitted light intensity is influenced by factors such as tissue depth, absorption, and scattering. Consequently, it is challenging to obtain absolute quantification of target molecules or cells, making it less suitable for applications requiring high precision.²⁴ Further, Bioluminescent signals have limited tissue penetration due to the absorption and scattering of light by biological tissues.²⁵ Bioluminescent signals are limited by tissue depth, making them less suitable for deep-seated tumors in imaging solid tumors.²⁶ This limitation restricts the application of BLI to superficial structures or small animals, as deep tissues can attenuate the signal significantly. Bioluminescent signals also decay over time as the luciferase substrate is consumed and metabolized.²⁷ This decay necessitates repeated measurements, potentially increasing experimental variability and the need for more animals or samples. Along with signal, BLI can suffer from background signal interference, which may arise from endogenous bioluminescence, auto-fluorescence, or other sources of light emission within the biological sample. This can reduce the specificity and accuracy of the signal. We need to understand that BLI is typically a single-color imaging technique, as it relies on the emission of photons at a specific wavelength during the luciferase reaction. This limitation restricts the simultaneous visualization of multiple targets or biological processes unless multiple luciferase enzymes with distinct emission wavelengths are employed.²⁸ The limited spatial resolution of BLI is relatively modest compared to other imaging modalities such as fluorescence microscopy or positron emission tomography. This limitation may hinder the precise localization of signals within complex tissues or cell populations. As discussed earlier, BLI is performed by introducing exogenous luciferase genes into cells or organisms, which can be invasive and may alter the biology of the system under investigation as well as to stably express them in the cellular context.²⁹

CONCLUSION

While BLI can provide valuable information about the presence and location of target cells or molecules, it does not offer functional insights. Researchers may need to complement BLI with other techniques to gain a comprehensive understanding of biological processes.³⁰ BLI is a valuable tool for non-invasive, real-time monitoring of biological processes. However, it is essential for researchers to be aware of its limitations, particularly its semi-quantitative nature, limited tissue penetration, and susceptibility to background signals. These limitations should be considered when designing experiments and

interpreting results to ensure the appropriate use of BLI in scientific investigations.

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