Science of Optical Imaging:
HOW IT WORKS

INTRODUCTION

Translational research has changed the face of modern preclinical studies and how disease progression is monitored. Small animal models are essential in scientific research involving therapeutics and drug development. In Vivo Optical Imaging serves as a bridge between in vitro data and its clinical application in the diagnosis and therapy of disease. Advanced, high-resolution In Vivo imaging technologies have enabled the study of disease and molecular pathways in real-time, allowing the repeated and non-invasive investigation of disease progression and therapeutic efficacy of drug candidates.

IN VIVO OPTICAL IMAGING: HOW IT WORKS

In Vivo Optical Imaging is a high-sensitivity, high-throughput screening, and non-invasive imaging modality that can be applied to monitoring disease progression and the efficacy of therapeutics in a wide array of animal model systems. It allows you to image the animal model at a molecular level by capturing optical data which is essentially the capture of light photons by the imaging camera system. These light photons are generated by specific reagents that have been introduced in the animal model system. Optical data is generated in one of the following two ways:

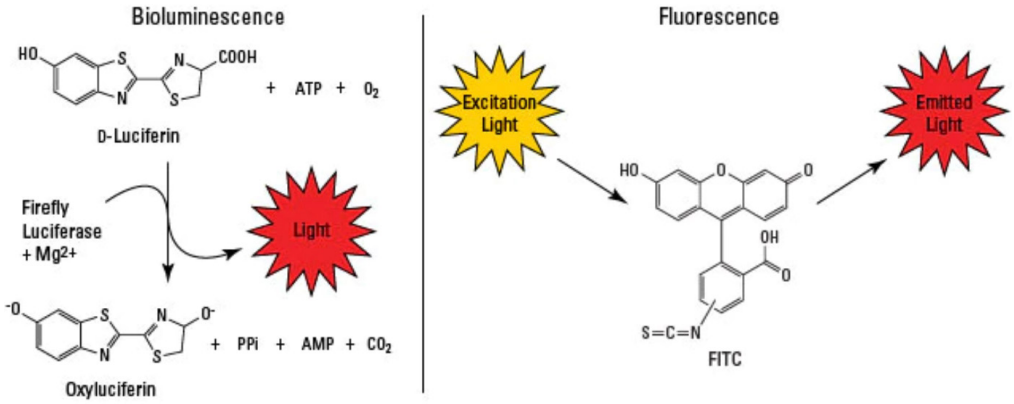
- ***Bioluminescence***

Bioluminescence is the process by which a luciferase produces light. Luciferases are a group of enzymes that emit light in the presence of Oxygen, ATP, and a substrate specific to the luciferase (luciferin, coelenterazine, etc.).
Such luciferin–luciferase systems are found in nature; the most well-characterized is from the firefly, *Photinus pyralis.*
Bioluminescence optical imaging offers a very large signal-to-noise ratio (SNR), resulting in low background noise, which allows for the detection of very low level reporter signals.

- ***Fluorescence***

The process by which a reporter fluorophore absorbs excitation light from an external source and re-emits photons with longer wavelengths. Fluorescent optical imaging can result in a much smaller SNR, but offers greater specificity of reporter signal with inducible/ conditional expression.

In both bioluminescent and fluorescent In Vivo Optical Imaging, the emitted light is captured by a highly sensitive, deeply cooled CCD camera system and presented to the user for quantification.



https://www.thermofisher.com/ae/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/luciferase-reporters.html

KEY ADVANTAGES OF IN VIVO OPTICAL IMAGING

Following are some of the features of In Vivo Optical Imaging that give it an edge over the other imaging techniques:

- **High sensitivity**
- **Fast, real-time data acquisition**
- **High throughput screening and large field of view**
- **Non-invasive imaging**
- **Allows whole-animal imaging**

IN VIVO OPTICAL IMAGING LIMITATIONS

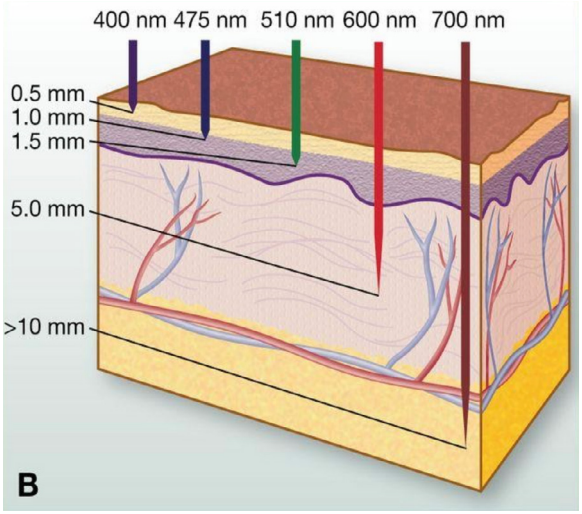
The primary limitation of In Vivo Optical Imaging is the impeded penetration of light through living tissue by oxygenated hemoglobin and melanin. This decreases the emission of light with bioluminescence, and decreases both excitation and emission light in fluorescence. This phenomenon not only minimizes the depth of penetration within tissue, but also varies depending upon where in the visible spectrum (380-750nm) the light in question falls.

Bioluminescence has an inherently optimized detection as compared to fluorescence. Bioluminescence, though dim or invisible to the eye and in the blue-green range, has no competition. That is, as the only source of emission in a model, there is typically no noise to compete with. The only emission should be the light you have introduced to the animal model. This creates a huge SNR, or signal-to-noise ratio.

Fluorescence plays by different rules, as fluorescent excitation light must be employed to create fluorescence emission.

Blue-green light (380-550nm) is readily absorbed and scattered in living tissue. Additionally, endogenous fluorophores (collagen, elastins) in living tissue tend to excite readily in this range, creating noise that can confound reporter signal detection. This creates a smaller SNR; that is, as noise increases, it competes for and obfuscates detection and visualization of the fluorescence reporter signal.

As you move towards the yellow/ orange (550-700nm) and NIR range (700-900nm), light penetration improves, scattering and absorption decreases, and endogenous fluorophore response diminishes. This increases the SNR as background noise reduces, allowing the reporter signal mission of interest to be more readily captured and quantified.



Huang Y., et al. 2012 Biomedical Nanomaterials for Imaging - Guided Cancer Therapy

CONCLUSION

In Vivo Optical Imaging can benefit many research projects due to its high sensitivity, throughput and reliable data capture. In Vivo Optical Imaging is an easy-to-use, quick, cost-efficient, and powerful modality that utilizes bioluminescent or fluorescent reporters to observe biological processes at a molecular level.