Journal of Photochemistry and Photobiology B: Biology 98 (2010) 77–94

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Pre-clinical whole-body fluorescence imaging: Review of instruments, methods and applications

Frederic Leblond [a,*], Scott C. Davis [a], Pablo A. Valdés [a], Brian W. Pogue [a,b]

[a] Thayer School of Engineering, Dartmouth College, Hanover NH 03755, USA
[b] Department of Surgery, Dartmouth Medical School, Lebanon, NH 03756, USA

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 16 November 2009
Accepted 20 November 2009
Available online 26 November 2009

Keywords:
Fluorescence
Imaging
Tomography
Commercial
Small animal
Diagnostic

ABSTRACT

Fluorescence sampling of cellular function is widely used in all aspects of biology, allowing the visualization of cellular and sub-cellular biological processes with spatial resolutions in the range from nanometers up to centimeters. Imaging of fluorescence in vivo has become the most commonly used radiological tool in all pre-clinical work. In the last decade, full-body pre-clinical imaging systems have emerged with a wide range of utilities and niche application areas. The range of fluorescent probes that can be excited in the visible to near-infrared part of the electromagnetic spectrum continues to expand, with the most value for in vivo use being beyond the 630 nm wavelength, because the absorption of light sharply decreases. Whole-body in vivo fluorescence imaging has not yet reached a state of maturity that allows its routine use in the scope of large-scale pre-clinical studies. This is in part due to an incomplete understanding of what the actual fundamental capabilities and limitations of this imaging modality are. However, progress is continuously being made in research laboratories pushing the limits of the approach to consistently improve its performance in terms of spatial resolution, sensitivity and quantification. This paper reviews this imaging technology with a particular emphasis on its potential uses and limitations, the required instrumentation, and the possible imaging geometries and applications. A detailed account of the main commercially available systems is provided as well as some perspective relating to the future of the technology development. Although the vast majority of applications of in vivo small animal imaging are based on epi-illumination planar imaging, the future success of the method relies heavily on the design of novel imaging systems based on state-of-the-art optical technology used in conjunction with high spatial resolution structural modalities such as MRI, CT or ultrasound.

Published by Elsevier B.V.

1. Introduction

Many researchers in the biological sciences appreciate the extraordinary contrast and specificity provided by fluorescence microscopy. Extrapolating this imaging paradigm to whole-body animal imaging is enticing. However, the physical realities associated with imaging in live tissue make this a continuously elusive objective, as will be evidenced in this review paper. Nevertheless, the information derived from in vivo fluorescence imaging systems can be regarded as an important complement to microscopy studies performed on cell cultures and tissue slices because it provides information about specific biological processes in fully integrated living systems[1]. Fig. 1 illustrates the salient differences between in vitro, ex vivo and in vivo fluorescence from biological applications relating to brain imaging. Though in essence the underlying technological and biological principles appear to be the same, imaging in each of these regimes imposes unique challenges requiring significantly different approaches to system design.

In this review paper, the basic principles of imaging fluorescence in living tissue is described, together with the practical challenges in designing, implementing, and assessing these systems. Methods available to overcome some challenges using advanced imaging system designs are discussed and an appreciation of the importance and challenges relating to modeling light propagation in tissue is provided. Perhaps most important is to realize that there is an intrinsic limit on the biological information that can be extracted from even the most carefully designed in vivo imaging instrument. Understanding these limitations is critical for researchers in the biological sciences wanting to use custom or commercial in vivo systems in the scope of their research. If, at the onset of research planning, the intrinsic limitations do not interfere with investigational endpoints, a choice must be made among several technological offerings. This paper will help to guide these choices for systems currently available commercially and in research laboratories.

* Corresponding author. Tel.: +1 603 646 2100; fax: +1 603 646 3856.
E-mail address: frederic.leblond@dartmouth.edu (F. Leblond).

[1] In this paper the term in vivo is used in relation with studies performed using live animals, i.e., excluding work done with live cells in cultures (see Fig. 1).

1011-1344/$ - see front matter Published by Elsevier B.V.
doi:10.1016/j.jphotobiol.2009.11.007

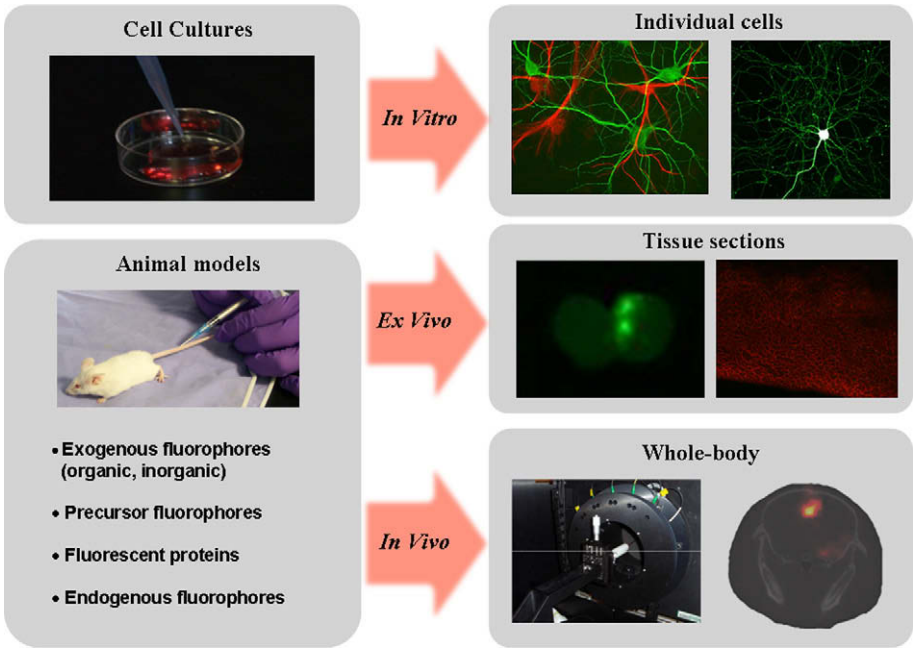

Fig. 1. Illustration showing different fluorescence imaging paths used in the scope of pre-clinical studies. High resolution, high sensitivity and high specificity images can be rendered down to sub-micron resolution in vitro to study cellular and sub-cellular molecular processes. The top-right part of the figure shows two-photon microscopy images of mouse hippocampal neuron and glial cells transfected with GFP and RFP, respectively (*courtesy of Dr. Paul De Koninck*, www.greenspine.ca). Animal models can be used for ex vivo studies of tissue slices as well as for whole-body in vivo studies. Ex vivo slices shown (middle-right images) correspond to brain tissue with glioma cells highlighted with fluorescence from GFP and the endogenous molecule Protoporphyrin IX. The in vivo whole-body image (lower-right in the figure) corresponds to a fluorescence tomography image associated with PpIX contrast from a brain tumor model.

The paper is divided into several sections covering the fundamentals of fluorescence imaging through advanced technology topics. Section 2 discusses the intrinsic limitations of whole-body imaging. These limitations relate to the interaction of light with microscopic tissue components as well as with the specificity and sensitivity of the contrast that can currently be generated in living animals. A description is also provided for the different types of imaging technologies that can be used for in vivo imaging emphasizing which biochemical fluorophore properties can be extracted from each. This is followed by a more detailed description of the various hardware components required in whole-body fluorescence imaging, including state-of-the-art illumination and light detection technology. This section concludes with a description of the various imaging geometries that can be used to perform in vivo small animal imaging. Section 3, consists of an extensive survey of the main commercial systems available on the market. This is presented in parallel with a discussion of salient features of in vivo fluorescence imaging in terms of resolution, sensitivity and quantification. Section 4 reviews recent publications where in vivo fluorescence imaging has been used to study certain biological phenomena. Finally, the paper concludes with a Section 5 reviewing the research done in laboratories to improve the capabilities of in vivo fluorescence imaging.

2. In vivo fluorescence imaging methods

2.1. Intrinsic limitations

In considering the potential use of in vivo fluorescence imaging in biological studies, there are intrinsic limitations researchers should probably consider. In part, these limitations relate to the interaction of light with microscopic components of tissue. Also, consideration must be given to difficulties insuring that biomarkers of interest are associated with a detectable level of optical contrast and that the origin of the latter is specific enough to deliver useful objective information.

2.1.1. Tissue absorption, scattering and autofluorescence

The basic principle behind in vivo fluorescence imaging is similar to that used in fluorescence microscopy techniques (e.g., conventional fluorescence microscopy, confocal microscopy, multiphoton microscopy, optical coherence tomography). However, when whole-animals are interrogated, the desired information is typically associated with biochemical events occurring deep within the tissue. This implies that photons being part of the detected signal have undergone multiple scattering events in the process of irradiance of the excitation light into the body and radiance of the emission out of the body.

Microscopic components within tissue from small molecules (sugars, fatty acids, amino acids, nucleotides, ions, water) and macromolecules (proteins, phospholipids, RNA, DNA, polysaccharides) to larger structures such as organelles and cell membranes collectively absorb light in the ultraviolet (UV) through the visible (VIS) wavelength range, Absorption by tissue components in this wavelength range limits effective light penetration to a few hundred microns However, significantly larger depths can be probed using light in the far-red or near-infrared (NIR) wavelength range, where the main tissue absorbers are de-oxyhemoglobin, oxyhemoglobin, water and lipids. In this spectral region, the absorption of these chromophores is at least one order of magnitude lower than in the VIS part of the spectrum potentially allowing detectable signals to be measured through several centimeters of tissue.

In the NIR part of the electromagnetic spectrum, elastic scattering of photons dominates over absorption, making multiple scattering the main mechanism for light propagation. This phenomenon is so significant that on average photons have an equal probability of traveling in any direction after having penetrated less than 1 mm of tissue. One of the consequences of this