CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Avalable online at: http://www.iajps.com *Review Article*

AN OVERVIEW OF IMAGING FLUORESCENCE METHOD AND RISK ASSESSMENT

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Article Received: March 2022 **Accepted** April 2022 **Published:** April 2022

Abstract:
The discipline of fluorescence microscopy is expanding rapidly and provides biologists increasingly more imaging capabilities. In the past ten years, numerous new imaging technologies and techniques have been created that permit deeper, faster, and higher resolution imaging. We reviewed the literature through out the 2022, for all relevant published studies in English language. Numerous cellular and subcellular activities in vivo can be labeled with a variety of innovative fluorescence reporter technologies. This imposed contrast is now captured by a growing range of imaging techniques that provide novel ways to view and quantify fluorescent markers scattered throughout tissues. This is a developing branch of imaging sciences that has made significant progress but also faces significant obstacles. Nonetheless, it is poised to have a big impact on biological research, drug discovery, and clinical practice in the next years.

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Please cite this article in press Duaa Mahmoud Gazzaz et al., ***An Overview Of Imaging Fluorescence Method And Risk Assessment.,*** *Indo Am. J. P. Sci, 2022; 09 (04).*

INTRODUCTION:

In clinical practice and research, optical imaging is unquestionably the most versatile and commonly used visualization modality. Long before modern discoveries, the primary method of medical diagnosis has been macroscopic observation of a patient. Similarly, since its invention approximately 400 years ago, the microscope has significantly advanced biology and the biological sciences. Microscopy remains a diagnostic gold standard and a largely adaptable visualization tool, with new techniques developing continually (for a few examples from a vast and diverse body of literature, see [1,2]). Parallel to this, macroscopic optical imaging has developed as a potent tool for research and clinical treatment, with applications ranging from the recent decoding of the human genome and high-throughput screening to noninvasive imaging of functional and molecular contrast in intact [3,4]. When exploiting the physical properties of light (e.g., polarization, interference, etc.) and the ability to capitalize on a wide range of light-tissue interactions and corresponding photophysical and photochemical mechanisms and processes at the molecular level, optical imaging in biomedical research offers an abundance of contrast mechanisms (i.e., multiphoton absorption, second-harmonic generation, fluorescence, etc.). In addition, optical technologies provide an experimentation-friendly technology: The majority of essential components may be constructed on a laboratory bench, are modular in construction, and can be made portable or compact [4].

For cell and molecular biologists, fluorescence microscopy (FM) is a potent technique. It gives a sub-cellular resolution window into the physiology of living cells, enabling direct visualization of the inner workings of physiological processes. FM has just undergone a revolution [5]. The resolution limit for light microscopy (the diffraction limit stated by Ernst Abbe, greater than 200 nm) has been shattered by numerous super-resolution techniques, and the capacity for 3-D imaging over time ("4D" imaging) has been significantly enhanced using Light Sheet Microscopy. In tandem with these developments, the usefulness of traditional techniques like as confocal microscopy and two-photon fluorescence microscopy (TPFM) has been enhanced. Numerous novel advanced techniques are currently being marketed, making them accessible to an increasing number of biologists. This revolution in approaches is also assisted by the numerous newly developed fluorescence probes and proteins [6,7]. This growth of capabilities explains why each year dozens of publications employing these imaging techniques are published. However, for an untrained biologist in light microscopy, matching the optimal technique to a biological experiment can be challenging. The optimal application of fluorescence microscopy necessitates a fundamental understanding of the strengths and weaknesses of the various approaches, as well as the fundamental tradeoffs involved with fluorescent light gathering [8].

Fluorescence microscopy and imaging have gained special attention in recent years. This is a result of the increased availability of fluorescent proteins, dyes, and probes, which permit the noninvasive investigation of gene expression, protein function, protein-protein interactions, and a vast array of cellular processes. Simultaneously, the number of fluorescence imaging techniques that offer microscopic resolutions and video-rate scans or approaches that operate at resolutions beyond the diffraction limit and offer single-molecule sensitivity is growing, giving previously unattainable biological insights. Macroscopic fluorescence imaging is gaining traction as a molecular imaging tool for small-animal whole-body tissue examinations on the other end of the resolution spectrum. Light may pass through several centimeters of tissue in the far-red and near-infrared (NIR) wavelengths [9,10].

DISCUSSION:

The optimal light microscopy experiment consists of optimizing the competing properties and tradeoffs of image resolution (in the XY or lateral dimensions as well as the Z or axial dimension), imaging speed (and/or acquisition time), and the amount of signal collected from the fluorescing sample (Fig. 1) [11]. In addition, this optimization problem is confined by photobleaching and/or phototoxicity, particularly in the case of living samples. In numerous tests, the intensity of light at the diffraction-limited spot (which is focussed by the objective) can be quite high. This can result in the breakdown of the fluorophore and undesirable biological effects, such as cell death or changes in the physiology of the lit cells or tissue. Given these limits, balancing these factors is difficult and requires careful attention to systematic and extensive (and frequently sample-specific) empirical testing. In addition to these fundamental elements, several secondary variables, such as the cost of the required equipment and the difficulty of the approach [5,11], become significant.

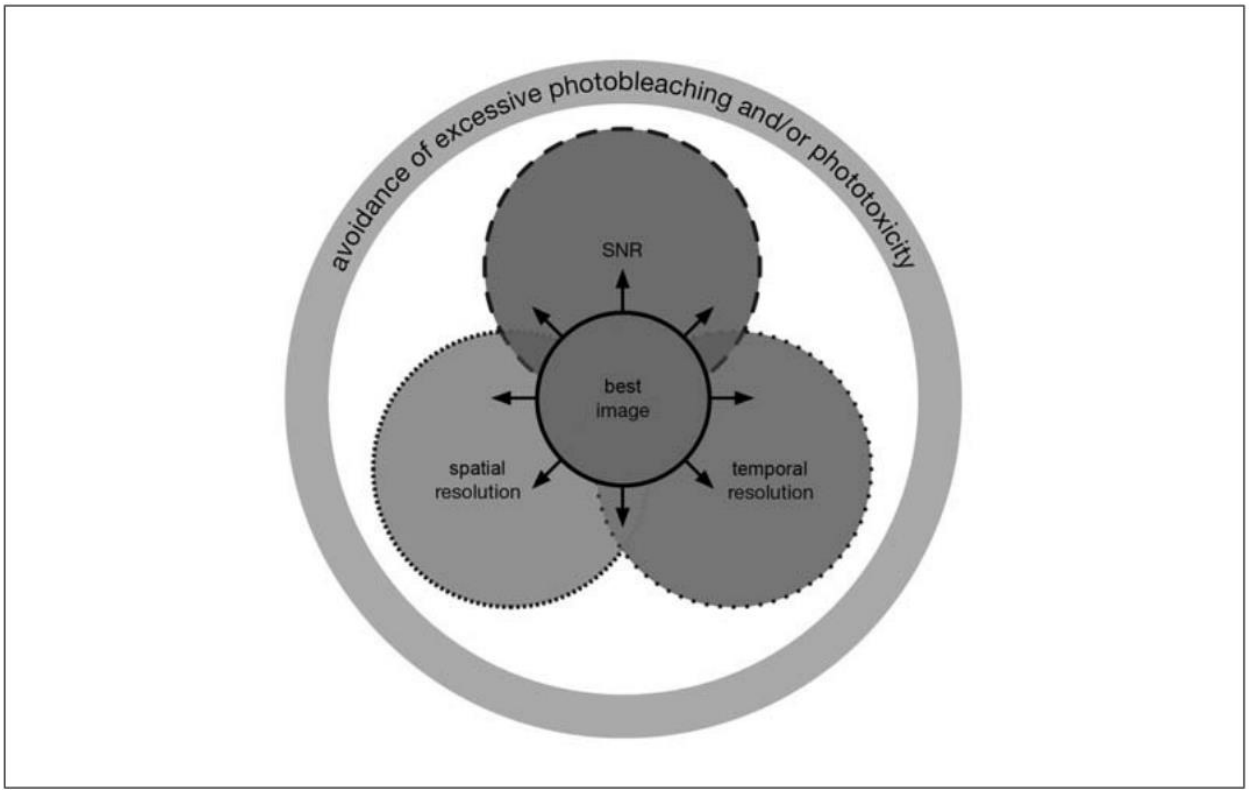

Fig 1. Tradeoffs in an imaging experiment. The best image is one that can balance these factors to obtain the necessary information while avoiding photobleaching or phototoxic effects.

WIDE-FIELD FLUORESCENCE MICROSCOPY (WFFM) TECHNIQUES:

Wide-field fluorescence microscopy (WFFM), also known as epi-fluorescence microscopy, uses a light source, a microscope, and excitation and emission filters to induce fluorescence in a material. The produced light, which has a larger wavelength than the excitation light, is captured by the objective lens and seen through the microscope eyepieces or by a camera followed by computer digitalization [6]. Although the fundamentals of WFFM have not changed, current enhancements allow for enhanced imaging. Improved cameras, objectives, optical filters, and computers are among these. Perhaps the greatest advancements have been made in image cameras. Compared to their predecessors of just a few years ago, contemporary camcorders offer very large formats (several megapixels), high sensitivity (>50% quantum efficiency) and dynamic range, lower noise characteristics (around 1 electron read noise), and faster frame rates (hundreds to thousands of frames per second). These advancements enable faster imaging and higher contrast at low signal levels (where the excitation light is purposefully reduced to prevent photobleaching or phototoxicity), while keeping the potential for diffraction-limited resolution over vast fields of view. Scientific complementary metal oxide semiconductor (sCMOS) and electron multiplied charge coupled device (EMCCD) cameras are examples of contemporary camera types [12,13].

In addition to cameras, new filters, dichroic mirrors, and objectives have enhanced wide-field microscopy. Due to innovative sputter-coating processes, commercially available filters, such as those from Chroma or Semrock (Rochester, NY), offer very high transmittance or reflection and do not degrade over time. In addition, these filters may have extremely acute wavelength dependence, allowing for superior multicolor discriminating. All of the major microscope manufacturers have improved microscope objectives over the previous decade. These new objectives have extremely flat fields (which reduces objective-induced gradients in intensity across an image or distortions at the edges of the field of view), long working distances with good resolving power, improved light transmission from the near ultraviolet to the infrared, and are increasingly available in varieties that match the refractive index of the sample being imaged [14].

Indirect Fluorescence Imaging:

Indirect imaging is a technique that emerged from in vitro reporting assays and is suitable for studying gene expression and gene regulation. The most prevalent method is the introduction of a transgene (also known as a reporter gene) into a cell. The fluorescent protein (FP) encoded by the transgene serves as an endogenously generated reporter probe. Gene transcription results in the synthesis of fluorescent protein (FP), which can subsequently be identified using optical imaging techniques [15]. Therefore, gene expression and regulation are visualized indirectly by the detection and quantification of FPs in tissues. For cell trafficking research, cells can be transfected to express FP and report on their position, or the transgene can be placed under promoters of interest to examine regulation. Moreover, linking the gene expressing fluorescent protein (FP) to a gene of interest provides a platform for imaging nearly any protein in vivo. This method creates a chimeric protein that retains the functioning of the native protein but is tagged with the fluorescent protein (FP) so that it may be visualized in vivo. Using a transgenic harboring an internal ribosome entry site (IRES) between the genes encoding for the FP and the gene of interest [16], it is also possible to independently transcribe and translate the protein of interest and the FP under control of the same promoter. Thus, the target protein remains intact while the FP continues to report on gene transcription. Using fluorescence energy transfer (FRET) techniques or protein function, a variety of fluorescent protein approaches have been developed to investigate protein-protein interactions. However, these techniques have been predominantly connected with microscopy and not macroscopy. Various reporter gene strategies have been reported for other imaging modalities, such as positron emission tomography (PET) or magnetic resonance imaging (MRI), when transcription of the reporter gene results in upregulation of a receptor or enzyme, which results in trapping or increased accumulation of an extrinsically administered reporter probe [17]. There have been reports of such techniques for in vivo fluorescence imaging, such as b-galactosidase-based fluorescent probe activation [18]. Enhanced mutants of the green fluorescent protein (GFP) derived from the jellyfish Aequorea victoria and various color-shifted variations are the most often employed fluorescent proteins. The development of red-shifted FPs has made substantial strides in recent years. Red florescent protein (RFP) cloning and evolution [using innovative new techniques like as somatic hypermutation] have produced a number of significant novel variations that glow well beyond the 600 nm barrier. Red-shifted proteins are advantageous for microscopy and imaging of tiny animals because tissue auto-fluorescence is diminished at longer wavelengths [19]. Therefore, greater contrast is achievable in the far-red and NIR (>600 nm) spectrum. In addition, tissue offers substantially less absorption (attenuation) of light in the far-red and NIR wavelengths compared to visible wavelengths; as a result, detection sensitivity can be increased in this spectral range. Although the greatest FPs to yet require stimulation in the highly absorbent visible region [20].

Direct Imaging:

In vivo fluorescence tomography is gaining great traction in small-animal imaging in order to increase quantification over planar imaging and to scan fluorescence activity throughout the entire animal volumetrically. Quantification is a crucial aspect of fluorescence imaging at the macroscopic level. Detection is predicated on probes' ability to define specific molecular processes and illnesses, not on their high resolution [21]. Consequently, the determination of probe accumulation contains a significant amount of pertinent data. For these reasons, the application of FMT becomes significant not only for investigations of deep-seated activity but also for studies of superficial activity, since it can adjust for both depth-dependent attenuation and optical property effects. Using circular geometry and fiber-based technologies, [22] the first FMT feasibility investigations revealed proteases in animal brains. Newer generation prototypes based on noncontact approaches have enabled higher imaging quality, displaying subresolution imaging capability and sensitivity that extends below a picomole of fluorescent dye (value reported for the Cy5.5 dye excited at 672 nm). Similarly, later systems based on flying spot illumination technology validated same sensitivity findings and revealed other advancements, such as quick imaging of the entire body [23]. In addition, the capability to image topographically at the visible level or to provide full projection tomography has been highlighted. Such sophisticated apparatuses have been utilized for imaging probe distribution, angiogenesis, proteases, and tumor chemotherapeutic impacts [24]. An annexin V–Cy5.5 probe accumulated more in cyclophosphamide-sensitive tumors than in cyclophosphamide-resistant tumors, as shown by a typical finding of the latter investigation. In another tomographic investigation, fluorescent cypate-polypeptide probes targeting breast-specific proteins were detected in human MDA MB 361 breast cancer xenografts and in the kidneys of nude mice [25].

CONCLUSOIN:

Significant new technology developments in fluorescence imaging and tomography have enhanced

the capacity for macroscopic in vivo observations. This new collection of technologies, when paired with a growing pool of potent novel fluorescent molecular probes and reporter techniques, can dramatically improve the ability to examine in vivo an increasing number of targets, molecular function, and drug action. Despite the fact that optical imaging is the oldest imaging technique, molecular fluorescent imaging remains in its infancy, with a great deal of potential but also a number of obstacles. The abundance of light manipulation and picture production that can frequently be achieved using conventional off-the-shelf components, coupled with the complexity associated with the diffusive nature of light propagation in tissues, has resulted in a field with a multitude of implementations and approaches. Consequently, it is both an exciting and perplexing time for optical imaging due to the lack of standards and performance comparison between the various systems. Advanced planar methods and tomographic methods will replace conventional planar imaging methods, which may result in erroneous and potentially misleading observations. In the coming years, it is likely that CW methods combined with a physical model of photon propagation, tomographic methodologies, and spectral information will become increasingly prevalent in animal fluorescence investigations. Time-resolved or frequency-domain techniques, however, will be required and the preferred way when lifespan data are needed as a means to explore the local biochemical environment or as a contrast mechanism.

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