Title
Photons across medicine: relating optical and nuclear imaging

Permalink
https://escholarship.org/uc/item/6cb27537

Journal
Biomedical Optics Express, 4(12)

ISSN
2156-7085

Authors
Nordstrom, Robert
Cherry, Simon
Azhdarinia, Ali
et al.

Publication Date
2013-12-01

DOI
10.1364/boe.4.002751

Peer reviewed

Photons across medicine: relating optical and nuclear imaging

Robert Nordstrom,[1,*] Simon Cherry,[2] Ali Azhdarinia,[3] Eva Sevick-Muraca,[3] and Henry VanBrocklin[4]

[1] *National Cancer Institute of NIH, Bethesda, MD 20892 USA*
[2] *Department of Biomedical Engineering, University of California at Davis, Davis, CA, 95616 USA*
[3] *University of Texas Health and Science Center, Houston, TX, 77030 USA*
[4] *Department of Radiology and Biomedical Imaging, University of California at San Francisco, San Francisco, CA, 94143 USA*
*nordstrr@mail.nih.gov

Abstract: The Optics in the Life Sciences conference sponsored by the Optical Society of America was held in Waikoloa Beach, HI on April 14 – 18, 2013. Papers were presented in the areas of Bio-Optics: Design & Application, Novel Techniques in Microscopy, Optical Molecular Probes, Imaging & Drug Delivery, and Optical Trapping Applications. A focal point of the meeting was a special symposium entitled "Photons Across Medicine", organized by Adam Wax, Duke University, highlighting activities of joint interest between the Optical Society of America (OSA) and the Society for Nuclear Medicine and Molecular Imaging (SNMMI). This paper is a synopsis of the presentations made at this joint symposium. Central to the special symposium presentations was the fact that the optical and nuclear imaging communities share common interests and challenges. These are highlighted in this article. Also discussed was the fact that the nuclear technologies in imaging have found their way into general clinical utility, a feat that has yet to be achieved by optical methods. Because of the common ground shared by the two technologies, coordination between the two societies should be planned.

OCIS codes: (170.0170) Medical optics and biotechnology; (170.0110) Imaging systems; (170.3880) Medical and biological imaging; (170.3890) Medical optics instrumentation.

References and links

1. S. Wolf, "Editorial: The real gap between bench and bedside," N. Engl. J. Med. **290**(14), 802–803 (1974).
2. E. A. Zerhouni, "Translational research: moving discovery to practice," Clin. Pharmacol. Ther. **81**(1), 126–128 (2007).
3. B. C. Drolet and N. M. Lorenzi, "Translational research: understanding the continuum from bench to bedside," Transl. Res. **157**(1), 1–5 (2011).
4. D. Dougherty and P. H. Conway, "The "3T's" road map to transform US health care: the "how" of high-quality care," JAMA **299**(19), 2319–2321 (2008).
5. S. R. Tunis, D. B. Stryer, and C. M. Clancy, "Practical clinical trials: increasing the value of clinical research for decision making in clinical and health policy," J. Am. Med. Assoc. **290**(12), 1624–1632 (2003).
6. M. B. Rosenthal, B. E. Landon, K. Howitt, H. R. Song, and A. M. Epstein, "Climbing the pay-for performance learning curve: where are the early adopters now?" Health Aff. **26**(6), 1674–1682 (2007).
7. T. K. Lewellen, "Recent developments in PET detector technology," Phys. Med. Biol. **53**(17), R287–R317 (2008).
8. W. McLaughlin and D. Vizard, "Kodak in vivo imaging system: precise co-registration of molecular imaging with anatomical x-ray imaging," Nat. Methods 26–28 (2006).
9. H. Kudrolli, H. B. Bhandari, K. L. Byrne, S. R. Cherry, G. S. Mitchell, H. Sabet, and V. Nagarkar, "A novel sensor for high throughput preclinical radiotracer imaging," Proc. SPIE 2013; 8668, Medical Imaging 2013: Physics of Medical Imaging 866809.
10. A. Douraghy, F. R. Rannou, R. W. Silverman, and A. F. Chatziioannou, "FPGA electronics for OPET: a dual modality optical and positron emission tomography," IEEE Trans. Nucl. Sci. **55**(5), 2541–2545 (2008).

11. L. J. Cao and J. Peter, "Bayesian reconstruction strategy of fluorescence-mediated tomography using an integrated SPECT-CT-OT system," Phys. Med. Biol. **55**(9), 2693–2708 (2010).
12. C. Q. Li, Y. F. Yang, G. S. Mitchell, and S. R. Cherry, "Simultaneous PET and multispectral 3-dimensional fluorescence optical tomography imaging system," J. Nucl. Med. **52**(8), 1268–1275 (2011).
13. J. Culver, W. Akers, and S. Achilefu, "Multimodality molecular imaging with combined optical and SPECT/PET modalities," J. Nucl. Med. **49**(2), 169–172 (2008).
14. G. S. Mitchell, R. K. Gill, D. L. Boucher, C. Q. Li, and S. R. Cherry, "In vivo Cerenkov luminescence imaging: a new tool for molecular imaging," Phil. Trans. Roy. Soc. A. **369**(1955), 4605–4619 (2011).
15. Y. Xu, H. Liu, and Z. Cheng, "Harnessing the power of radionuclides for optical imaging: Cerenkov luminescence imaging," J. Nucl. Med. **52**(12), 2009–2018 (2011).
16. A. K. Glaser, S. C. Kanick, R. Zhang, P. Arce, and B. W. Pogue, "A GAMOS plug-in for GEANT4 based Monte Carlo simulation of radiation-induced light transport in biological media," Biomed. Opt. Express **4**(5), 741–759 (2013).
17. M. Yokouchi, M. Terahara, S. Nagano, Y. Arishima, M. Zemmyo, T. Yoshioka, A. Tanimoto, and S. Komiya, "Clinical implications of determination of safe surgical margins by using a combination of CT and 18FDG-positron emission tomography in soft tissue sarcoma," BMC Musculoskelet. Disord. **12**(1), 166 (2011).
18. T. J. Ruers, B. Wiering, J. R. van der Sijp, R. M. Roumen, K. P. de Jong, E. F. Comans, J. Pruim, H. M. Dekker, P. F. Krabbe, and W. J. Oyen, "Improved selection of patients for hepatic surgery of colorectal liver metastases with (18)F-FDG PET: a randomized study," J. Nucl. Med. **50**(7), 1036–1041 (2009).
19. A. Barros, M. A. Cardoso, P. Y. Sheng, P. A. Costa, and C. Pelizon, "Radioguided localisation of non-palpable breast lesions and simultaneous sentinel lymph node mapping," Eur. J. Nucl. Med. Mol. Imaging **29**(12), 1561–1565 (2002).
20. J. C. Rasmussen, S. Kwon, E. M. Sevick-Muraca, and J. N. Cormier, "The role of lymphatics in cancer as assessed by near-infrared fluorescence imaging," Ann. Biomed. Eng. **40**(2), 408–421 (2012).
21. B. T. Lee, M. Hutteman, S. Gioux, A. Stockdale, S. J. Lin, L. H. Ngo, and J. V. Frangioni, "The FLARE intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in perforator flap breast reconstruction," Plast. Reconstr. Surg. **126**(5), 1472–1481 (2010).
22. G. M. van Dam, G. Themelis, L. M. Crane, N. J. Harlaar, R. G. Pleijhuis, W. Kelder, A. Sarantopoulos, J. S. de Jong, H. J. Arts, A. G. van der Zee, J. Bart, P. S. Low, and V. Ntziachristos, "Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results," Nat. Med. **17**(10), 1315–1319 (2011).
23. A. Azhdarinia, P. Ghosh, S. Ghosh, N. Wilganowski, and E. M. Sevick-Muraca, "Dual-labeling strategies for nuclear and fluorescence molecular imaging: a review and analysis," Mol. Imaging Biol. **14**(3), 261–276 (2012).
24. J. Kuil, A. H. Velders, and F. W. van Leeuwen, "Multimodal tumor-targeting peptides functionalized with both a radio- and a fluorescent label," Bioconjug. Chem. **21**(10), 1709–1719 (2010).
25. S. C. Ghosh, P. Ghosh, N. Wilganowski, H. Robinson, M. A. Hall, G. Dickinson, K. L. Pinkston, B. R. Harvey, E. M. Sevick-Muraca, and A. Azhdarinia, "Multimodal chelation platform for near-infrared fluorescence/nuclear imaging," J. Med. Chem. **56**(2), 406–416 (2013).
26. R. E. Weiner and M. L. Thakur, "Radiolabeled peptides in oncology: role in diagnosis and treatment," BioDrugs **19**(3), 145–163 (2005).
27. A. M. Wu, "Antibodies and antimatter: the resurgence of immuno-PET," J. Nucl. Med. **50**(1), 2–5 (2008).
28. S. R. Meikle, P. Kench, M. Kassiou, and R. B. Banati, "Small animal SPECT and its place in the matrix of molecular imaging technologies," Phys. Med. Biol. **50**(22), R45–R61 (2005).
29. A. D. Nunn, "The cost of developing imaging agents for routine clinical use," Invest. Radiol. **41**(3), 206–212 (2006).
30. http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html. "Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products," HHS, Editor: Food and Drug Administration. 31, (2004).
31. i, *Guidance For Industry - Developing Medical Imaging Drug and Biological Products Part 1: Conducting Safety Assessments*. [August 17, 2012]; Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078930.pdf. ii. *Guidance For Industry - Developing Medical Imaging Drug and Biological Products Part 2: Clinical Indications*. [August 17, 2012]; Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071603.pdf. iii) *Guidance For Industry - Developing Medical Imaging Drug and Biological Products Part 3: Design, Analysis and INterpretation of Clinical Studies*. [August 17, 2012]; Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071604.pdf.
32. *Guidance for Industry, Investigators, and Reviewers Exploratory IND Studies, FDA, January 2006*. [accessed March 16, 2012.]; Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078933.pdf
33. H. F. VanBrocklin, "Radiopharmaceuticals for drug development: United States regulatory perspective," Current Radiopharmaceuticals **1**(1), 2–6 (2008).
34. R. Alford, H. M. Simpson, J. Duberman, G. C. Hill, M. Ogawa, C. Regino, H. Kobayashi, and P. L. Choyke, "Toxicity of organic fluorophores used in molecular imaging: literature review," Mol. Imaging **8**(6), 341–354 (2009).

35. E. M. Sevick-Muraca, R. Sharma, J. C. Rasmussen, M. V. Marshall, J. A. Wendt, H. Q. Pham, E. Bonefas, J. P. Houston, L. Sampath, K. E. Adams, D. K. Blanchard, R. E. Fisher, S. B. Chiang, R. Elledge, and M. E. Mawad, "Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: feasibility study," Radiology **246**(3), 734–741 (2008).
36. K. Polom, D. Murawa, Y. S. Rho, P. Nowaczyk, M. Hünerbein, and P. Murawa, "Current trends and emerging future of indocyanine green usage in surgery and oncology," Cancer **117**(21), 4812–4822 (2011).
37. B. E. Schaafsma, J. S. Mieog, M. Hutteman, J. R. van der Vorst, P. J. Kuppen, C. W. Löwik, J. V. Frangioni, C. J. van de Velde, and A. L. Vahrmeijer, "The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery," J. Surg. Oncol. **104**(3), 323–332 (2011).
38. E. M. Sevick-Muraca, "Translation of near-infrared fluorescence imaging technologies: emerging clinical applications," Annu. Rev. Med. **63**(1), 217–231 (2012).
39. G. C. Gurtner, G. E. Jones, P. C. Neligan, M. I. Newman, B. T. Phillips, J. M. Sacks, and M. R. Zenn, "Intraoperative laser angiography using the SPY system: review of the literature and recommendations for use," Ann. Surg. Innov. Res. **7**(1), 1750–1764 (2013).
40. A. J. Fischman, R. H. Rubin, J. A. White, E. Locke, R. A. Wilkinson, M. Nedelman, R. J. Callahan, B. A. Khaw, and H. W. Strauss, "Localization of Fc and Fab fragments of nonspecific polyclonal IgG at focal sites of inflammation," J. Nucl. Med. **31**(7), 1199–1205 (1990).
41. M. V. Marshall, J. C. Rasmussen, I.-C. Tan, M. B. Aldrich, K. E. Adams, X. Wang, C. E. Fife, E. A. Maus, L. A. Smith, and E. M. Sevick-Muraca, "Near-infrared fluorescence imaging in humans with indocyanine green: a review and update," Open Surg Oncol J **2**(2), 12–25 (2010).
42. S. Achilefu, R. B. Dorshow, J. E. Bugaj, and R. Rajagopalan, "Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging," Invest. Radiol. **35**(8), 479–485 (2000).

1. Introduction

A driving influence in biomedical research is the desire on the part of any research team to see the focus of its efforts become a part of clinical practice at some point in the future. This is as true in the field of biophotonics as it is in any other biomedical arena, and this evolution of science and technology from bench to bedside has been called translational research [1]. Zerhouni described translational research as moving discovery to practice [2].

While the desire for translation might be strong in the field of biophotonics, the pathway for its successful execution is vague. A review of the process from bench to bedside has been given by Drolet and Lorenzi [3], in which the authors present a Biomedical Research Translation Continuum model and reference it to the Dougherty and Conway "T – terminology" [4]. In this terminology, the translation of basic science into clinical research (T1) is only the first step toward high quality, effective, and safe healthcare delivery. The next step (T2) is the creation of practical clinical trials [5] to deliver the proper treatment to the right patients correctly. Finally, T3 translational activities include policy changes, including CMS and health insurance perspectives, needed to stimulate attempts to improve health outcomes [6]. In biophotonics, the vast majority of research is confronting the first of these translation barriers. The hurdles to be overcome include those activities needed for FDA approval, such as validation methods and implementation of some form of good laboratory practices.

An efficient way to climb the learning curve of translational activities is to follow the lead given by others engaged in similar research and facing similar difficulties. At first glance, the nuclear imaging community seems an unlikely candidate as a mentor for the photonics community to emulate in translational research. However, on closer inspection, there are a number of similarities in shared technologies, shared concerns and shared challenges that make the two more alike than not.

Areas of overlapping technologies include photon detection, the use of labeled probes, tomographic image reconstruction, and the growing need to extract quantitative information from images. In addition, there are emerging techniques such as Cerenkov radiation detection that require scientific knowledge of both nuclear (charged particle) physics and photonics.

Concerns shared by both scientific communities include the need to understand and follow the protocols of radiation dose. While the admissible radiation dose levels are very different because photon energies in the two research domains are so different, there are established rules for each which must be followed. Other areas of shared concern for the nuclear imaging

and the biophotonics community include topics as diverse as detection and quantification of information in images in the presence of noise, optimal use of anatomic information as a guide or constraint in hybrid or multimodal imaging, and the steps for investigational device exemption (IDE) and investigational new drug (IND) status.

Nuclear imaging and optical imaging share common application challenges. Both, for example, are attempting to unlock the nature of molecular processes in biology through imaging. To do this, the use of image-enhancing probes (contrast agents or radiopharmaceuticals) is required. Pharmacokinetic imaging and modeling are needed in both modalities to establish safe levels of dosing and optimal imaging protocols.

These areas of common technologies, concerns and challenges between the nuclear medicine community represented by the Society for Nuclear Medicine & Molecular Imaging (SNMMI) and the community of biomedical optical researchers represented by the Optical Society of America (OSA) create links between the two. An important difference, however, is the fact that nuclear imaging technologies have been successfully translated into clinical practice, while optical methods, for the most part, remain in feasibility research and development. Hurdles presented by the FDA have been overcome in nuclear imaging, and lessons learned should be available to others confronted by similar hurdles. This article suggests that the biophotonics community can gain valuable information from the nuclear imaging community in matters of translational research. While it is not the purpose of this article to lay out a recipe to follow for translational success, it is the purpose to highlight specific areas where linkage between the two technologies can be strengthened for future translational collaborations.

2. Nuclear medicine and optical imaging: The physics and engineering interface

At first glance, the technologies and methods of nuclear medical imaging and optical imaging may appear to share little in common. The photons used in nuclear medicine (typically gamma rays emitted from excited nuclear states, or annihilation photons from positron emission) arise from nuclear rearrangements that occur during radioactive decay and occur approximately five orders of magnitude higher in energy in the electromagnetic spectrum than optical photons. The interaction mechanisms of high energy photons in tissue are quite different (dominated by ionizing effects and Compton scattering with relatively low absorption) from low eV photons (elastic scattering with absorption). Despite these differences, there are a number of synergies in technologies and methodologies that strongly suggest the physics and engineering scientists within these two communities should be working more closely together. Some specific examples are discussed here.

2.1 Advanced photon detectors

The vast majority of nuclear imaging detectors used in gamma cameras, single photon emission computed tomography (SPECT) scanners and positron emission tomography (PET) scanners are based on scintillation detectors in which high-energy gamma rays or annihilation photons (energy range ~80-511 keV) are converted into short pulses of visible light (1-2 eV) using dense scintillators [7]. At this point, the detection process of nuclear events shares many similarities with optical imaging, i.e. the need to collect and convert small numbers of optical photons into an electronic signal efficiently, often preserving spatial and/or temporal information in the process. Not surprisingly, the photon detectors used in nuclear medicine, namely photomultiplier tubes, avalanche photodiodes, and more recently silicon photomultipliers, are also widely used in optical imaging and detection, and advances in these detectors usually benefit both fields. Methods for characterization and absolute calibration of these photon detectors are of mutual interest. The efficient collection of the scintillation light from nuclear radiation detectors is critical, as photon statistics dictate the energy resolution, timing resolution and in cases where positioning relies on light sharing, the spatial resolution of the detector. The choice and optimization of scintillator crystal geometries, reflector

materials, index matched coupling materials, light guides and in some cases optical fibers, borrows heavily on concepts and materials developed in the optical design field. This extends to optical simulation tools, which are also useful in nuclear medicine detector design for modeling photon propagation from the production of optical photons following a high-energy interaction in the scintillator, to their ultimate detection, absorption or escape from the detector.

2.2 Hybrid optical/nuclear imaging systems

A variety of efforts are underway to develop small-animal systems that allow both nuclear and optical imaging, either simultaneously, or in quick succession, without moving the subject. In its simplest form, standard non-contact CCD-based preclinical optical imaging systems can introduce a scintillator screen [8] and for better spatial resolution also a collimator [9] between the subject and the CCD camera to produce an image of the distribution of a radiotracer. A number of laboratories have developed prototype tomographic systems for small-animal imaging that combine PET or SPECT imaging with fluorescence or bioluminescence imaging [10–12]. These hybrid systems provide several important opportunities [13]. For example, i) they allow the combination of reporter gene optical imaging (luciferase or fluorescent protein-based) with protein-targeted radiotracer imaging (one can look at both mRNA and protein levels), ii) dual-labeled agents can be used to improve quantification of activated optical agents (PET or SPECT imaging provides the total probe concentration and a spatial constraint for the optical reconstruction, optical imaging measures the amount of the probe activated by the target of interest) and iii) the highly quantitative nature of PET and SPECT can be used together with dual-labeled agents to assess, optimize and validate methods and algorithms for fluorescence tomography in vivo.

2.3 Cerenkov luminescence imaging

During radioactive decay by β^-(electron) or β^+ (positron) emission, the β particles emitted from the nucleus can transiently travel faster than the speed of light in tissue. Under these conditions, constructive interference occurs via the Cerenkov effect and visible light is emitted [14, 15]. Thus, many biomedically relevant radionuclides can be directly detected and imaged using the optical photons emitted during their decay. The Cerenkov spectrum is continuous, with an intensity distribution that falls off as $1/\lambda^2$. It is also a very weak signal, with only ~100 visible light photons produced in tissue per radioactive decay of ^{82}Rb, one of the very brightest Cerenkov emitters. This observation of detectable luminescence from β-emitting radionuclides allows them to be imaged in small animals (where light absorption is not limiting) using conventional optical imaging systems, as long as a high sensitivity CCD camera is used. Given the wide availability of *in vivo* optical imaging systems, this provides opportunities for nuclear imaging studies in preclinical animal models using commercially available radiopharmaceuticals without the need for a PET or SPECT scanner. More importantly, however, it provides a very sensitive approach for imaging radionuclides such as ^{90}Y, which is used in radionuclide therapy, but which cannot be readily imaged at lower diagnostic doses with PET or SPECT because it is an almost pure β^- emitter. This could help speed development of new radio-immunotherapy agents based on ^{90}Y.

Possible translational applications of Cerenkov luminescence are also under investigation. The use of Cerenkov radiation for detection and imaging of radiopharmaceuticals lies firmly at the interface of optical and nuclear imaging science and already has led to the development of important new tools for the community such as integrated Monte Carlo code that models both ionizing radiation and optical photon transport in biological media [16].

These three diverse examples illustrate just some of the many opportunities for cross-fertilization and collaboration between physicists and engineers working in nuclear medicine and optical imaging. Clearly both fields would benefit by improving communication and

interactions, and this first joint OSA/SNMMI symposium represents an important first step in recognizing this opportunity.

3. Opportunities with hybrid nuclear/optical imaging agents

Clinical hybrid imaging generally refers to the combination of a functional imaging platform, such as PET or SPECT, with an anatomical counterpart, such as computed tomography (CT) or magnetic resonance imaging (MRI), to correlate diagnostic data. In principle, a corresponding hybrid agent would be one that, for example, is comprised of a targeting unit which carries contrast for both imaging modalities (PET and CT, for example), and would be administered as a single agent. However, owing to the large difference in detection sensitivities of nuclear and anatomical imaging approaches, the application of such a hybrid agent is often not practical. Fluorescence-based imaging, on the other hand, is a modality with sensitivity comparable to nuclear techniques and has capitalized on recent advances in instrumentation and agent development which promise new opportunities for its clinical implementation, particularly in the area of intraoperative surgical guidance. Although challenges in probe validation have slowed the translation of new imaging agents that employ biomedical optics, the growing application of dual labeling for hybrid nuclear/optical agent development appears to be a significant step forward that benefits both imaging communities.

3.1 Complementary roles of nuclear and optical imaging

Nuclear imaging employs radiolabeled molecular imaging agents, known as radiotracers, which have demonstrated utility for tumor imaging, patient selection, treatment planning, and assessing response to therapy. Radiotracers provide images with whole-body field-of-view (FOV), tomographic capabilities, and quantitative results. Although nuclear imaging is effectively used before and after surgical procedures in cancer patients [17, 18], the current clinical standard for use of radiolabeled compounds during surgery relies on audible detection of radioactive signal and does not provide real-time visualization of contrast-enhanced lesions [19]. Fluorescence-based imaging, on the other hand, is well-suited for use during surgery owing to the development of highly sensitive, mobile imaging systems that have already been tested in clinical settings [20,21].

The fact that surgical personnel are not exposed to ionizing radiation when using optical methods is another advantage of optical approaches in intraoperative use. Also, there is no worry about agent half-life which could occur with nuclear agents should a particular surgical procedure involving radiotracers encounter delay. In a pioneering first-in-humans study, a fluorescently labeled folate derivative (known as folate-fluorescein isothiocyanate, or FITC) was injected in patients with ovarian cancer to provide real-time intraoperative imaging of tumor deposits which were not otherwise detected by visual or tactile cues [22]. This demonstrated a clear clinical benefit of biomedical optics. Ongoing efforts for intraoperative optical agent usage are focused on dyes which emit in the near-infrared (NIR) range (>750 nm) to maximize tissue penetration and reduce autofluorescence.

3.2 Implications of dual labeling for the optical imaging community

Dual labeling strategies are being adopted to combine the strengths of each modality and improve the agent validation process [23, 24]. The overall goal of dual labeling an optical imaging agent with a radiotracer is to enable validation of the optical imaging modality by a second, clinically accepted modality such as PET or SPECT. For example, preclinical studies with novel optical imaging agents dual-labeled with a radioactive tracer allow *ex vivo* quantification of targeted tissue areas by established nuclear counting methods to support and validate optical imaging findings in a manner that is not possible with fluorescence alone [25]. In patients, dual labeling would permit acquisition of whole-body tomographic nuclear scans that could be used to assess the extent of cancer spread globally, instead of being relegated to the surgical FOV associated with clinical near infrared fluorescence (NIRF)

imaging systems. This would allow clinicians to plan intraoperative procedures based on the added utility of the hybrid agent. Finally, radiotracer production follows defined manufacturing processes which can be emulated in the development and optimization of molecular imaging agents, including agents for optical imaging. Thus, a dual-labeled imaging agent would follow the established guidelines for radiotracers and have a clear indication of metrics upon which drug design modifications could be made.

3.3 Implications of dual labeling for the nuclear imaging community

Although an established modality, nuclear imaging stands to benefit significantly from applying the recent advances of biomedical optics into its agent development paradigm. For example, the addition of a fluorescent dye onto a clinically validated radiotracer could expand existing diagnostic applications into intraoperative utility and significantly change the way cancer surgery is conducted. In a potential clinical application, patients would receive an injection of a dual-labeled imaging agent and undergo standard nuclear imaging tests to localize malignant tissues. Following a pre-determined decay period (i.e., 24 hr. post-injection), patients would then proceed to the operating suite where surgeons would be have whole-body, tomographic nuclear images to aid in the determination of their surgical plan. Prior to resection, intraoperative NIRF imaging would be available to confirm sites with optically enhanced tissue uptake. This identifies tumor regions and margins in real-time, allowing surgeons to perform debulking procedures more accurately. Immediately after tumor resection, NIRF imaging would be used to assess the effectiveness of the surgery and determine if additional surgery is needed to remove tissues that still exhibit a fluorescent signature. Histological evaluation of resected tissues would then confirm the molecular targeting of the agent, and would also represent an attractive approach to validate the targeting properties of emerging investigational agents that show promise for human use. Finally, a follow-up nuclear scan could be performed following a second injection of the hybrid agent to confirm surgical efficacy. Of particular importance is the fact that the added NIRF imaging feature does not in any way alter standard-of-care nuclear imaging procedures. Hopefully, the seamless integration of imaging agents encourages dialogue between relevant players in each community to advance this promising strategy. Given the importance of peptide and antibody-based radiotracers in nuclear medicine [26, 27], and their structural ability to tolerate the addition of a dye moiety without losing activity, increased interaction between agent development groups in these areas would be a logical starting point to examine feasibility.

4. Nuclear and optical imaging agents: The regulatory interface

As mentioned, nuclear and optical imaging methods both require agents, typically injected, that localize in a specific tissue of interest, relative to disease or normal physiologic processes, to provide the signal that is collected by their respective scanning devices. The ability to detect the signal emitted from these agents is a function of the agent concentration in the tissue of interest, the sensitivity of the instrumentation to measure the photons emitted and the ability of the photons to exit the body. Optical and nuclear medicine imaging agents share at least one important quality; they may be detected when administered at similar concentrations [28]. For this reason, the pathway developed to bring nuclear medicine imaging agents into human research and clinical use may provide a roadmap for translation of optical agents.

In 1957, iodine-131 sodium iodide was the first radioactive drug approved by the Food and Drug Administration (FDA). The approval process of new radioactive imaging agents over the ensuing 45 years followed a very similar process as that of new drugs. This includes submission of a full pharmacology and toxicology package and evaluation through the three phases of clinical development for safety and efficacy in humans. The early nuclear diagnostic imaging agents were predominantly labeled with longer-lived isotopes (e.g. iodine-

123, iodine-131, indium-111, etc.; half-life > 6 h). These radiolabeled agents were produced at a centralized location similar to traditional drug preparation and distributed to the imaging centers for research evaluation or clinical application. The introduction of the molybdenum-99/ technetium-99m generator system permitted the preparation of the 6 h half life technetium-99m imaging agents on demand in a radiopharmacy close to the SPECT scanners. FDA approved kits were prepared for technetium-99m labeling in a centralized facility. The development and application of short-lived (half-life < 2h) PET radiotracers using distributed cyclotron and radiochemistry facilities to produce the isotopes and labeled the radiotracers represented a paradigm shift for the FDA regulation of their preparation. As optical agents are stable, they may be manufactured in a single site and distributed for patient use. This means that larger batches of the agent may be produced under regulatory control and tested for quality before shipping in unit doses to the clinic. This manufacturing approach may ultimately be reflected in a lower unit dose cost.

In spite of the fact that diagnostic imaging agents are tracers with no pharmacological or toxicological properties and are typically given to a patient only once or infrequently, they are still considered as drugs by the FDA requiring extensive testing on the pathway to market approval. The cost to bring a diagnostic radiotracer to market increased significantly over the years, paralleling that of traditional drug development, In 2006, Nunn [29] estimated the cost of diagnostic imaging development to be $100-$200 million. Similar to traditional drugs, diagnostic agent approvals dropped significantly in the later part of the 20th century as the increased cost of drug development reduced the number of FDA submissions. This trend was completely out of phase with the explosive growth of basic biological knowledge.

In 2004 the FDA released a report entitled "Challenge and Opportunity on the Critical Path to New Medical Products" [30] outlining a plan called the Critical Path Initiative (CPI) in an effort to reinvigorate drug development and stimulate new drug submissions. The CPI introduced a streamlined approval process and encouraged the use of technological advances in genomics, proteomics, bioinformatics and medical imaging to reduce the development timeline. This initiative halted the decline in submission rate and over the last few years new drug approvals have begun to increase. This has also had a similar effect on the submission and approval of new diagnostic agents. Several guidance documents were released including a three part document entitled "Developing Medical Imaging Drug and Biological Products" [31] and one detailing the exploratory investigational new drug (IND) process [32]. These documents provide beneficial information that is equally applicable to optical imaging agents as it is to nuclear agents especially given the trace quantities of the agent that is necessary to generate an image. A more detailed review of the nuclear imaging agent development pathway has been published [33].

The exploratory IND guidance highlights the FDA's greater flexibility in the traditional IND process, lowering the barrier to initiate first-in-human studies through the introduction of microdosing. A microdose is defined as less than 1/100th of the substance dose that produces a pharmacologic effect, typically less than 100 micrograms or less than or equal to 30 nanomoles for biologics. These doses are generally higher than those given for diagnostic imaging studies. In addition multiple test substances may be evaluated head-to-head to identify the best drug or imaging agent to move forward to the clinical development phases.

Specific guidance for imaging agents is also given relative to the pharmacologic and toxicologic information required for agent approval. As imaging agents are given at microdose levels the pharmacologic effect, by definition, is nonexistent. Administration of an agent is typically one time so genetic toxicity and safety pharmacology studies are generally waived. A 14-day single species, single dose toxicology study (current cost: $60-80,000) at ≥100x the human dose is required. A recent review of the literature for organic fluorophore toxicity data by Alford et al [34] revealed that the general margin of safety for several of the optical agents was >100 fold based on the future clinical doses that may be administered to humans. The inherent safety of optical agents is also evident since there is no radioactive

isotope present. One does not have to be concerned with the radiation dose to the patient. This is encouraging and supports the use of the exploratory IND mechanism to move optical agents into human studies.

Once the exploratory IND studies are complete, a choice is made whether to continue on with the agent development process, continue to evaluate the agent(s) under a research IND or cease investigation of the agent(s). Continuing the process towards a marketable imaging agent requires a significant capital investment as the process parallels that of traditional drug development. The process requires a much more rigorous portfolio of pharmacologic and toxicologic tests as well as completion of the three phases of patient trials culminating in the collection of data from multiple imaging centers to highlight the safety and efficacy of the new agent. This process may take up to 10 years and several hundred thousand dollars to complete.

In summary, nuclear and optical agents produce a signal that is detectable at low mass concentrations. As these agents are given at "tracer" doses no pharmacologic or toxicologic effect will be expected. The agents are not delivered chronically and in large doses. Typically, single doses of the agent are all that is required for a diagnostic. Follow-up imaging studies will typically be separated temporally. This too minimizes the potential for a pharmacologic or toxicologic event. These similarities between nuclear and optical imaging agents encourage utilization of the established pathway that has proven successful for translating nuclear medicine imaging agents.

5. Combined device/agent optical studies

While the clinical translation of many "first-of-its-kind" optical technologies lack an established translational pathway, the previous section suggests that the history of regulatory approvals in nuclear imaging may provide guidance for the introduction of fluorescence molecular imaging agents, whether singly labeled with a fluorophore or dual-labeled with a radionuclide. Given the potentially high photon yield of a near-infrared fluorophore that can be repeatedly excited by tissue-penetrating excitation light, the opportunities for translating "first-in-humans" optical agents as trace agents may be considerable.

5.1 Device considerations on performance

Indeed, use of the exploratory IND as described above can lower translational barriers for translating fluorescent molecular imaging agents. However, myriad of device operational factors in optical devices (that are not present in nuclear imaging) requires a complete understanding of performance factors for the device before administering "first-in-humans" fluorescently labeled agents. Unlike nuclear imaging, where there has been little doubt that high energy photons could be collected from tissues using standardized devices to render an image, there has been considerable and worthwhile debate about how deep one can probe in tissues with the low energy optical excitation and fluorescence photons and whether the wide variety of devices currently used can detect trace amounts of an optical imaging agent in the same manner as nuclear imaging devices enable. There are no standardized devices in fluorescence molecular imaging, as there are in nuclear medicine.

While small animal fluorescence imaging and tomography shows the potential of translational fluorescence imaging, these studies are conducted with a variety of devices in small tissue volumes that do not necessarily recapitulate the same pertinent physics in clinically relevant, larger volumes. Thus, while preclinical small animal imaging studies of new nuclear imaging agents may be predictive for clinical studies, non-standard devices and tissue attenuation can confound the direct extrapolation of preclinical fluorescence studies to patient studies. Most importantly, the ability to detect a fluorescent agent either deep within a tissue or in the sub-surface of exposed tissues in an intraoperative setting depends critically upon the sensitivity of the device. Device performance such as the ability to reject backscattered excitation light with selective collection of fluorescence varies greatly not in

only preclinical devices, but in emerging investigational devices used in clinical studies. The ultimate combinational device/drug performance depends upon the ability to excite a specific fluorophore and collect its fluorescence which may be spectrally distinct from other fluorophores employed in other imaging agents. For fluorophores not excited in the near-infrared wavelength range (>750 nm), additional device constraints require removing the noise floor due to endogenous autofluorescence from the signal arising from an exogenous fluorescence imaging agent. In short, fluorescence imaging is complicated by the necessary regulatory processes requiring approval of device-specific imaging agents, rather than the platform-specific imaging agents as is routinely done in nuclear imaging.

5.2 Device qualification

Sharing regulatory experiences in the community may help to define translational pathways and reduce the time for ultimate clinical adoption. In 2003, one of the authors (EMS) found the Center for Drug Evaluation Research (CDER) of the FDA to be reluctant (and appropriately so) to permit the "risk" associated with the administration of a "first-in-humans" fluorescent imaging agent, even at trace amounts, without proof of actually being able to detect the dose in human tissues. Yet if the device that is intended to detect a "first-in humans" drug requires an Investigational Device Exemption (IDE) for use on human subjects, it would be impossible for the Center for Devices and Radiological Health (CDRH) to grant that exemption without knowing how the device will perform at the safe agent dose levels. This poses a chicken-and–egg dilemma in the regulatory process for fluorescence molecular imaging.

To overcome this, several different research and development pathways have been suggested. For the case of near infrared fluorescence, it was possible to employ the FDA approved indocyanine green (ICG) in an off-label use as a substitute for the investigational new drug (agent) in a dose escalation study that defined performance [35]. The study was not intended to identify ICG as a near infrared fluorescent imaging agent, but rather to qualify the device in humans for the purpose to which it was designed, detecting trace amount of near-infrared fluorescence imaging agent. This substitution can only work if equivalence between the agents (in terms of performance at specific excitation and emission wavelengths) can be verified and accepted by the FDA.

ICG may make an excellent surrogate for qualification of near-infrared fluorescence devices, and most recently, it is used as a non-specific fluorescent imaging in lymph node mapping in cancer staging, intraoperative angiography, among other uses [36–39]. For over 50 years, ICG has been used as a blood pooling dye used on the basis of its dark green color for assessing hepatic function and retinopathy, but as an near infrared fluorescence imaging agent, it is a poor fluorophore, unstable, and has no functional group to attach molecularly targeting entities.

In a recent development in imaging hardware, the ability to detect lymph nodes 3-4 centimeters beneath the tissue surface with as little as 10 micrograms of total ICG administered and only 200 milliseconds of image acquisition time was demonstrated, qualifying the imaging device for future imaging after microdoses of "first-in-humans" agents [35]. However, a more efficient means to qualify devices in the future may be to develop figures of merit and to create certifiable standards that act as accurate surrogates and predictors of human imaging performance. The requirement to conduct ICG dose escalation studies for qualification of devices prior to administration of "first-in-humans" agents is costly and could impede translational efforts. Hence a critical issue for device translation is for community-wide definition and adoption of device standards. Such definitions to qualify of device platforms will facilitate development and clinical deployment of fluorescence molecule imaging agents and enable platform- rather than device-specific fluorescent molecular imaging agent development to parallel that described for nuclear imaging agents.

5.3 Future regulatory routes for imaging agents with qualified devices

After device qualification through CDER, one commonly proposed FDA regulatory route includes an IND application that involves NIRF labeling of a therapeutic biologic. Given that (i) the biologic already has an established safety record, (ii) imaging it at administered trace doses could be performed under an Exploratory IND, and (iii) the large real-estate of biologics (~150kDa), the conjugation of comparatively small NIR fluorophores (~1 kDa) may not dramatically alter pharmacokinetics and biodistribution. The only significant regulatory concern could be the "first-in-humans" fluorophore conjugated to the biologic, already used in humans. While this approach is plausible and may speed translation, it may require the pharmaceutical manufacturer to grant a reference letter enabling the FDA access to its own safety data for the IND application of a NIRF labeled biologic. Many (most) pharmaceutical companies will not grant such letters for third party INDs, since it could constitute a liability to their marketed product. This may be because the dismal diagnostic performance of a molecularly targeted NIRF labeled biologic could be *incorrectly* interpreted as indicating poor targeting and therefore therapeutic efficacy as described below.

5.4 Targeting moieties for fluorescent diagnostics

The criteria for success of therapeutic and diagnostic agents are vastly different. Therapeutic biologics are optimized on their basis to induce cytotoxic effects which require a high dose and relatively long blood circulation times. For a diagnostic imaging agent, no therapeutic action is desired, a low innocuous dose is sought, and short blood circulation times maximize target to background ratios that are necessary for successful molecular imaging. The features that make a good therapeutic may not make for a good diagnostic agent and visa-versa. In addition, for cancer imaging and lymph node staging, the Fc-binding portion of the mAb binds to Fc ɣ receptors on effector cells of the immune system. While responsible for therapeutic cytotoxicity in therapeutic agents, the Fc binding may also cause off target binding of the imaging agent to these immune cells [40], thereby reducing specificity, an important figure of merit for molecular imaging performance. Hence, in the long-run it may be advantageous (although require greater investment !) to develop anti-body based or peptide imaging agents specifically as diagnostic agents rather than employ existing therapeutic biologics.

5.5 Fluorescent agents for translation

In addition to the targeting moiety (whether anti-body, protein, or peptide), another component of regulatory concern is the fluorescent agent. In addition to the imaging agent comprised of the targeting moiety and fluorophore, studies are needed to establish safety of the fluorophore itself. Today, there is one cGMP near-infrared fluorophore, IRDye800 (LiCor Biosciences, Lincoln, Nebraska), with a Drug Master File available to aid translational efforts [41] with others potentially pending. Fluorescein isothiocyanate, used in the studies of Van Dam and associates [22] is a derivate of fluorescein, which is already used clinically for angiography but functionalized with an isothiocyanate reactive group for conjugation to targeting moieties. Likewise cypate is a derivative of ICG, but has a functional group for conjugation [42].

    With the maturation of both devices and imaging agents in a scientific, community-based effort to define performance, the barriers to translation could be lowered, enabling optical technologies to contribute to the armamentarium of diagnostic, molecular imaging tools employed in nuclear medicine. Given that nuclear medicine has already tackled the device-drug combinational dilemmas (albeit with slightly different physics), much can be learned from the history and future of the clinical adoption of nuclear molecular imaging agents.

6. Conclusions

The field of nuclear imaging has established itself as an important contributor to the field of clinical medicine. With the advent of *in situ* methods of preparing radiotracers, hospitals have a number of agents available for a variety of imaging needs. Optical imaging methods are adopting the use of imaging agents to perform a number of imaging functions that have been difficult for other imaging modalities. These include intraoperative functions, image guided biopsy direction, and early stage cancer detection, to name only a few. The problem with optical methods, however, is that they lag far behind in the translational pipeline for adoption in the clinical environment. Most of the successes claimed by optical imaging methods are in proof-of-principle or feasibility studies. Large scale adaption of optical imaging methods in the clinic has yet to take place. A great deal of work remains to bring optical devices and their accompanying targeting agents through the translational pipeline.

An important hurdle in that translational pipeline is FDA regulatory approval of both devices and imaging agents used in optical measurements of disease. As this paper has demonstrated, the similarities between optical imaging and nuclear imaging are stronger than might first be realized. As a result, it is possible for the optical imaging community to follow many of the leads provided by nuclear imaging to translate technology closer to clinical reality. An open dialogue is encouraged among scientists of both technologies.