

# Review of biomedical Čerenkov luminescence imaging applications

Kaveh Tanha,<sup>1,3</sup> Ali Mahmoud Pashazadeh,<sup>1</sup> and Brian W Pogue<sup>2,\*</sup>

<sup>1</sup>Persian Gulf Nuclear Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran  
<sup>2</sup>Thayer School of Engineering, Department of Surgery in the Geisel School of Medicine, Dartmouth College,  
 Hanover NH 03755 USA

<sup>3</sup>k.tanha@razi.tums.ac.ir  
 \*brian.w.pogue@dartmouth.edu

**Abstract:** Čerenkov radiation is a fascinating optical signal, which has been exploited for unique diagnostic biological sensing and imaging, with significantly expanded use just in the last half decade. Čerenkov Luminescence Imaging (CLI) has desirable capabilities for niche applications, using specially designed measurement systems that report on radiation distributions, radiotracer and nanoparticle concentrations, and are directly applied to procedures such as medicine assessment, endoscopy, surgery, quality assurance and dosimetry. When compared to the other imaging tools such as PET and SPECT, CLI can have the key advantage of lower cost, higher throughput and lower imaging time. CLI can also provide imaging and dosimetry information from both radioisotopes and linear accelerator irradiation. The relatively short range of optical photon transport in tissue means that direct Čerenkov luminescence imaging is restricted to small animals or near surface human use. Use of Čerenkov-excitation for additional molecular probes, is now emerging as a key tool for biosensing or radiosensitization. This review evaluates these new improvements in CLI for both medical value and biological insight.

©2015 Optical Society of America

**OCIS codes:** (170.3880) Medical and biological imaging; (170.3890) Medical optics instrumentation; (350.5610) Radiation;

## References and links

1. P. Čerenkov, "Visible radiation produced by electrons moving in a medium with velocities exceeding the of light," *Phys. Rev.* **52**(4), 378–379 (1937).
2. P. Čerenkov, "Visible Emission of Čerenkov Liquids by Action of  $\gamma$  Radiation," *Dokl. Akad. Nauk SSSR* **2**, 451–454 (1934).
3. R. H. Elrick and R. P. Parker, "The use of Čerenkov radiation in the measurement of beta-emitting radionuclides," *Int. J. Appl. Radiat. Isot.* **19**(3), 263–271 (1968).
4. M. K. Johnson, "Counting of Čerenkov radiation from  $^{32}\text{P}$  in nonaqueous media," *Anal. Biochem.* **29**(2), 348–350 (1969).
5. F. L. Hoch, R. A. Kuras, and J. D. Jones, "Iodine analysis of biological samples by neutron activation of  $^{127}\text{I}$ , with scintillation counting of Čerenkov radiation," *Anal. Biochem.* **40**(1), 86–94 (1971).
6. G. Bosia, C. Castagnoli, M. Dardo, and G. Marangoni, "Observation of structure in Čerenkov pulses from extensive air showers using fast techniques," *Nature* **225**(5232), 532–533 (1970).
7. W. C. Haxton, "Salty water Čerenkov detectors for solar neutrinos," *Phys. Rev. Lett.* **76**(10), 1562–1565 (1996).
8. R. Robertson, M. S. Germanos, C. Li, G. S. Mitchell, S. R. Cherry, and M. D. Silva, "Optical imaging of Čerenkov light generation from positron-emitting radiotracers," *Phys. Med. Biol.* **54**(16), N355–N365 (2009).
9. R. S. Dothager, R. J. Goiffon, E. Jackson, S. Harpstrite, and D. Piwnica-Worms, "Čerenkov radiation energy transfer (CRET) imaging: a novel method for optical imaging of PET isotopes in biological systems," *PLoS One* **5**(10), e13300 (2010).
10. Z. Hu, J. Liang, W. Yang, W. Fan, C. Li, X. Ma, X. Chen, X. Ma, X. Li, X. Qu, J. Wang, F. Cao, and J. Tian, "Experimental Čerenkov luminescence tomography of the mouse model with SPECT imaging validation," *Opt. Express* **18**(24), 24441–24450 (2010).
11. M. A. Lewis, V. D. Kodibagkar, O. K. Öz, and R. P. Mason, "On the potential for molecular imaging with Čerenkov luminescence," *Opt. Lett.* **35**(23), 3889–3891 (2010).

12. C. Li, G. S. Mitchell, and S. R. Cherry, "Čerenkov luminescence tomography for small-animal imaging," *Opt. Lett.* **35**(7), 1109–1111 (2010).
13. A. Ruggiero, J. P. Holland, J. S. Lewis, and J. Grimm, "Čerenkov luminescence imaging of medical isotopes," *J. Nucl. Med.* **51**(7), 1123–1130 (2010).
14. A. E. Spinelli, D. D'Ambrosio, L. Calderan, M. Marengo, A. Sbarbati, and F. Boschi, "Čerenkov radiation allows in vivo optical imaging of positron emitting radiotracers," *Phys. Med. Biol.* **55**(2), 483–495 (2010).
15. J. P. Holland, G. Normand, A. Ruggiero, J. S. Lewis, and J. Grimm, "Intraoperative imaging of positron emission tomographic radiotracers using Čerenkov luminescence emissions," *Mol. Imaging* **10**(3), 177–186, 1–3 (2011).
16. H. Liu, C. M. Carpenter, H. Jiang, G. Pratz, C. Sun, M. P. Buchin, S. S. Gambhir, L. Xing, and Z. Cheng, "Intraoperative imaging of tumors using Čerenkov luminescence endoscopy: a feasibility experimental study," *J. Nucl. Med.* **53**(10), 1579–1584 (2012).
17. A. E. Spinelli, C. Kuo, B. W. Rice, R. Calandrino, P. Marzola, A. Sbarbati, and F. Boschi, "Multispectral Čerenkov luminescence tomography for small animal optical imaging," *Opt. Express* **19**(13), 12605–12618 (2011).
18. J. Zhong, C. Qin, X. Yang, S. Zhu, X. Zhang, and J. Tian, "Čerenkov luminescence tomography for in vivo radiopharmaceutical imaging," *Int. J. Biomed. Imaging* **2011**, 641618 (2011).
19. J. Zhong, J. Tian, X. Yang, and C. Qin, "Whole-Body Čerenkov Luminescence Tomography with the Finite Element SP(3) Method," *Ann. Biomed. Eng.* **39**(6), 1728–1735 (2011).
20. Z. Hu, X. Ma, X. Qu, W. Yang, J. Liang, J. Wang, and J. Tian, "Three-dimensional noninvasive monitoring iodine-131 uptake in the thyroid using a modified Čerenkov luminescence tomography approach," *PLoS One* **7**(5), e37623 (2012).
21. A. K. Glaser, W. H. Voigt, S. C. Davis, R. Zhang, D. J. Gladstone, and B. W. Pogue, "Three-dimensional Čerenkov tomography of energy deposition from ionizing radiation beams," *Opt. Lett.* **38**(5), 634–636 (2013).
22. R. Zhang, A. V. Dsouza, J. R. Gunn, T. V. Esipova, S. A. Vinogradov, A. K. Glaser, L. A. Jarvis, D. J. Gladstone, and B. W. Pogue, "Čerenkov-Excited Luminescence Scanned Imaging (CELSI)," *Opt. Lett.* in press.
23. R. P. Parker and R. H. Elrick, "The assay of beta-emitting radioisotopes using Čerenkov counting," *Int. J. Appl. Radiat. Isot.* **17**(6), 361–362 (1966).
24. H. H. Ross, "Measurement of  $\beta$ -emitting nuclides using Čerenkov radiation," *Anal. Chem.* **41**(10), 1260–1265 (1969).
25. M. Takiue, Y. Yoshizawa, and H. Fujii, "Čerenkov counting of low-energy beta-emitters using a new ceramic with high refractive index," *Appl. Radiat. Isot.* **61**(6), 1335–1337 (2004).
26. W. J. Gelsema, C. L. de Ligny, J. B. Lutten, and F. G. Vossenbergh, "The use of the Čerenkov effect in the counting of beta- and gamma- emitting radionuclides," *Int. J. Appl. Radiat. Isot.* **26**(8), 443–450 (1975).
27. R. Hesp, "The feasibility of using a liquid-filled Čerenkov counter of gamma-ray counting of large samples," *Phys. Med. Biol.* **23**(3), 438–445 (1978).
28. K. D. Steidley, R. M. Eastman, and R. J. Stabile, "Observations of visual sensations produced by Čerenkov radiation from high-energy electrons," *Int. J. Radiat. Oncol. Biol. Phys.* **17**(3), 685–690 (1989).
29. A. E. Spinelli and F. Boschi, "Novel biomedical applications of Čerenkov radiation and radioluminescence imaging," *Phys. Med.* **31**(2), 120–129 (2015).
30. A. K. Glaser, R. Zhang, D. J. Gladstone, and B. W. Pogue, "Optical dosimetry of radiotherapy beams using Čerenkov radiation: the relationship between light emission and dose," *Phys. Med. Biol.* **59**(14), 3789–3811 (2014).
31. G. S. Mitchell, R. K. Gill, D. L. Boucher, C. Li, and S. R. Cherry, "In vivo Čerenkov luminescence imaging: a new tool for molecular imaging," *Philos. Trans. A Math Phys. Eng. Sci.* **369**(1955), 4605–4619 (2011).
32. B. J. Beattie, D. L. Thorek, C. R. Schmidtlein, K. S. Pentlow, J. L. Humm, and A. H. Hielscher, "Quantitative modeling of Čerenkov light production efficiency from medical radionuclides," *PLoS One* **7**(2), e31402 (2012).
33. J. S. Cho, R. Taschereau, S. Olma, K. Liu, Y. C. Chen, C. K. Shen, R. M. van Dam, and A. F. Chatzioannou, "Čerenkov radiation imaging as a method for quantitative measurements of beta particles in a microfluidic chip," *Phys. Med. Biol.* **54**(22), 6757–6771 (2009).
34. T. F. Massoud and S. S. Gambhir, "Molecular imaging in living subjects: seeing fundamental biological processes in a new light," *Genes Dev.* **17**(5), 545–580 (2003).
35. Y. Xu, H. Liu, and Z. Cheng, "Harnessing the power of radionuclides for optical imaging: Čerenkov luminescence imaging," *J. Nucl. Med.* **52**(12), 2009–2018 (2011).
36. G. Lucignani, "Čerenkov radioactive optical imaging: a promising new strategy," *Eur. J. Nucl. Med. Mol. Imaging* **38**(3), 592–595 (2011).
37. X. Zhang, C. Kuo, A. Moore, and C. Ran, "Čerenkov luminescence imaging of interscapular brown adipose tissue," *J. Vis. Exp.* **92**, e51790 (2014).
38. X. Zhang, C. Kuo, A. Moore, and C. Ran, "In vivo optical imaging of interscapular brown adipose tissue with (18)F-FDG via Čerenkov luminescence imaging," *PLoS One* **8**(4), e62007 (2013).
39. H. Liu, G. Ren, Z. Miao, X. Zhang, X. Tang, P. Han, S. S. Gambhir, and Z. Cheng, "Molecular optical imaging with radioactive probes," *PLoS One* **5**(3), e9470 (2010).

40. D. H. Kim, Y. S. Choe, J. Y. Choi, K. H. Lee, and B. T. Kim, "Binding of 2-[<sup>18</sup>F]fluoro-CP-118,954 to mouse acetylcholinesterase: microPET and ex vivo Čerenkov luminescence imaging studies," *Nucl. Med. Biol.* **38**(4), 541–547 (2011).
41. A. Natarajan, F. Habte, H. Liu, A. Sathirachinda, X. Hu, Z. Cheng, C. M. Nagamine, and S. S. Gambhir, "Evaluation of <sup>89</sup>Zr-rituximab tracer by Čerenkov luminescence imaging and correlation with PET in a humanized transgenic mouse model to image NHL," *Mol. Imaging Biol.* **15**(4), 468–475 (2013).
42. Holland, J.P., G. Normand, A. Ruggiero, J.S. Lewis, and J. Grimm, "Intraoperative imaging of positron emission tomographic radiotracers using Čerenkov luminescence emissions," *Mol. Imaging* **10**(3), 177–186 (2011).
43. Y. Wang, Y. Liu, H. Luehmann, X. Xia, D. Wan, C. Cutler, and Y. Xia, "Radioluminescent gold nanocages with controlled radioactivity for real-time in vivo imaging," *Nano Lett.* **13**(2), 581–585 (2013).
44. W. Yang, W. Qin, Z. Hu, Y. Suo, R. Zhao, X. Ma, W. Ma, T. Wang, J. Liang, J. Tian, and J. Wang, "Comparison of Čerenkov luminescence imaging (CLI) and gamma camera imaging for visualization of let-7 expression in lung adenocarcinoma A549 Cells," *Nucl. Med. Biol.* **39**(7), 948–953 (2012).
45. Z. Hu, W. Yang, X. Ma, W. Ma, X. Qu, J. Liang, J. Wang, and J. Tian, "Čerenkov luminescence tomography of aminopeptidase N (APN/CD13) expression in mice bearing HT1080 tumors," *Mol. Imaging* **12**(3), 173–181 (2013).
46. T. A. Aweda, V. Eskandari, D. L. Kukis, D. L. Boucher, B. V. Marquez, H. E. Beck, G. S. Mitchell, S. R. Cherry, and C. F. Meares, "New covalent capture probes for imaging and therapy, based on a combination of binding affinity and disulfide bond formation," *Bioconj. Chem.* **22**(8), 1479–1483 (2011).
47. J. C. Park, G. Il An, S. I. Park, J. Oh, H. J. Kim, Y. Su Ha, E. K. Wang, K. Min Kim, J. Y. Kim, J. Lee, M. J. Welch, and J. Yoo, "Luminescence imaging using radionuclides: a potential application in molecular imaging," *Nucl. Med. Biol.* **38**(3), 321–329 (2011).
48. A. E. Spinelli and F. Boschi, "Unsupervised analysis of small animal dynamic Čerenkov luminescence imaging," *J. Biomed. Opt.* **16**(12), 120507 (2011).
49. R. Zhang, C. J. Fox, A. K. Glaser, D. J. Gladstone, and B. W. Pogue, "Superficial dosimetry imaging of Čerenkov emission in electron beam radiotherapy of phantoms," *Phys. Med. Biol.* **58**(16), 5477–5493 (2013).
50. R. Zhang, D. J. Gladstone, L. A. Jarvis, R. R. Strawbridge, P. Jack Hoopes, O. D. Friedman, A. K. Glaser, and B. W. Pogue, "Real-time in vivo Čerenkov imaging during external beam radiation therapy," *J. Biomed. Opt.* **18**(11), 110504 (2013).
51. R. Zhang, J. M. Andreozzi, D. J. Gladstone, W. L. Hitchcock, A. K. Glaser, S. Jiang, B. W. Pogue, and L. A. Jarvis, "Čerenkov based patient positioning validation and movement tracking during post-lumpectomy whole breast radiation therapy," *Phys. Med. Biol.* **60**(1), L1–L14 (2015).
52. A. E. Spinelli and F. Boschi, "Human Čerenkov imaging using <sup>18</sup>F-FDG," *J. Nucl. Med.* **55**(3), 523 (2014).
53. A. E. Spinelli, M. Ferdeghini, C. Cavedon, E. Zivelonghi, R. Calandrino, A. Fenzi, A. Sbarbati, and F. Boschi, "First human Čerenkovography," *J. Biomed. Opt.* **18**(2), 020502 (2013).
54. S. R. Kothapalli, H. Liu, J. C. Liao, Z. Cheng, and S. S. Gambhir, "Endoscopic imaging of Čerenkov luminescence," *Biomed. Opt. Express* **3**(6), 1215–1225 (2012).
55. X. Cao, X. Chen, F. Kang, Y. Lin, M. Liu, H. Hu, Y. Nie, K. Wu, J. Wang, J. Liang, and J. Tian, "Performance evaluation of endoscopic Čerenkov luminescence imaging system: in vitro and pseudotumor studies," *Biomed. Opt. Express* **5**(10), 3660–3670 (2014).
56. H. Hu, X. Cao, F. Kang, M. Wang, Y. Lin, M. Liu, S. Li, L. Yao, J. Liang, J. Liang, Y. Nie, X. Chen, J. Wang, and K. Wu, "Feasibility study of novel endoscopic Čerenkov luminescence imaging system in detecting and quantifying gastrointestinal disease: first human results," *Eur. Radiol.* **25**(6), 1814–1822 (2015).
57. J. Axelsson, S. C. Davis, D. J. Gladstone, and B. W. Pogue, "Čerenkov emission induced by external beam radiation stimulates molecular fluorescence," *Med. Phys.* **38**(7), 4127–4132 (2011).
58. A. K. Glaser, S. C. Davis, D. M. McClatchy, R. Zhang, B. W. Pogue, and D. J. Gladstone, "Projection imaging of photon beams by the Čerenkov effect," *Med. Phys.* **40**(1), 012101 (2013).
59. A. K. Glaser, S. C. Davis, W. H. Voigt, R. Zhang, B. W. Pogue, and D. J. Gladstone, "Projection imaging of photon beams using Čerenkov-excited fluorescence," *Phys. Med. Biol.* **58**(3), 601–619 (2013).
60. A. K. Glaser, R. Zhang, S. C. Davis, D. J. Gladstone, and B. W. Pogue, "Time-gated Čerenkov emission spectroscopy from linear accelerator irradiation of tissue phantoms," *Opt. Lett.* **37**(7), 1193–1195 (2012).
61. A. K. Glaser, J. M. Andreozzi, S. C. Davis, R. Zhang, B. W. Pogue, C. J. Fox, and D. J. Gladstone, "Video-rate optical dosimetry and dynamic visualization of IMRT and VMAT treatment plans in water using Čerenkov radiation," *Med. Phys.* **41**(6), 062102 (2014).
62. C. Lohrmann, H. Zhang, D. L. Thorek, P. Desai, P. B. Zanzonico, J. O'Donoghue, C. P. Irwin, T. Reiner, J. Grimm, and W. A. Weber, "Čerenkov Luminescence Imaging for Radiation Dose Calculation of a <sup>90</sup>Y-Labeled Gastrin-Releasing Peptide Receptor Antagonist," *J. Nucl. Med.* **56**(5), 805–811 (2015).
63. L. A. Jarvis, R. Zhang, D. J. Gladstone, S. Jiang, W. Hitchcock, O. D. Friedman, A. K. Glaser, M. Jermyn, and B. W. Pogue, "Čerenkov video imaging allows for the first visualization of radiation therapy in real time," *Int. J. Radiat. Oncol. Biol. Phys.* **89**(3), 615–622 (2014).
64. R. W. Holt, R. Zhang, T. V. Esipova, S. A. Vinogradov, A. K. Glaser, D. J. Gladstone, and B. W. Pogue, "Čerenkov excited phosphorescence-based pO<sub>2</sub> estimation during multi-beam radiation therapy: phantom and simulation studies," *Phys. Med. Biol.* **59**(18), 5317–5328 (2014).
65. D. L. Burden and G. M. Hieftje, "Čerenkov radiation as a UV and visible light source for time-resolved fluorescence," *Anal. Chem.* **70**(16), 3426–3433 (1998).

66. N. Kotagiri, G. P. Sudlow, W. J. Akers, and S. Achilefu, "Breaking the depth dependency of phototherapy with Čerenkov radiation and low-radiance-responsive nanophotosensitizers," *Nat. Nanotechnol.* **10**(4), 370–379 (2015).
67. D. L. Thorek, A. Ogirala, B. J. Beattie, and J. Grimm, "Quantitative imaging of disease signatures through radioactive decay signal conversion," *Nat. Med.* **19**(10), 1345–1350 (2013).
68. R. Zhang, A. V. D'souza, J. R. Gunn, T. V. Esipova, S. A. Vinogradov, A. K. Glaser, L. A. Jarvis, D. J. Gladstone, and B. W. Pogue, "Čerenkov-excited luminescence scanned imaging," *Opt. Lett.* **40**(5), 827–830 (2015).
69. J. Czupryna, A. V. Kachur, E. Blankemeyer, A. V. Popov, A. D. Arroyo, J. S. Karp, and E. J. Delikatny, "Čerenkov-specific contrast agents for detection of pH *in vivo*," *J. Nucl. Med.* **56**(3), 483–488 (2015).
70. W. Guo, X. Sun, O. Jacobson, X. Yan, K. Min, A. Srivatsan, G. Niu, D. O. Kiesewetter, J. Chang, and X. Chen, "Intrinsically radioactive [64Cu]CuInS/ZnS quantum dots for PET and optical imaging: improved radiochemical stability and controllable Čerenkov luminescence," *ACS Nano* **9**(1), 488–495 (2015).
71. H. Hu, P. Huang, O. J. Weiss, X. Yan, X. Yue, M. G. Zhang, Y. Tang, L. Nie, Y. Ma, G. Niu, K. Wu, and X. Chen, "PET and NIR optical imaging using self-illuminating (64)Cu-doped chelator-free gold nanoclusters," *Biomaterials* **35**(37), 9868–9876 (2014).
72. X. Sun, X. Huang, J. Guo, W. Zhu, Y. Ding, G. Niu, A. Wang, D. O. Kiesewetter, Z. L. Wang, S. Sun, and X. Chen, "Self-illuminating 64Cu-doped CdSe/ZnS nanocrystals for *in vivo* tumor imaging," *J. Am. Chem. Soc.* **136**(5), 1706–1709 (2014).
73. N. Kotagiri, D. M. Niedzwiedzki, K. Ohara, and S. Achilefu, "Activatable probes based on distance-dependent luminescence associated with Čerenkov radiation," *Angew. Chem. Int. Ed. Engl.* **52**(30), 7756–7760 (2013).

## 1. Introduction

In just the past six years, a renaissance of sorts has occurred in the interest of applying Čerenkov light emission to the application of biomedical molecular imaging and therapy. There has been rapid development of tools and different applications in this field. This review charts some of the key areas being developed, translating the use of Čerenkov light from a curious phenomenon into a unique probe of radiation dose and tissue function.

Čerenkov radiation (CR) was observed for the first time about one century ago, from a charged particle emitting visible light from moving through a dielectric medium at a speed greater than the velocity of light in that medium [1, 2]. After the first observations, Pavel Čerenkov proposed a report on production of the luminescence radiation by high speed Compton electrons, which are produced by gamma radiation. Further studies by Ilya Frank and Igor Tamm showed that the observed light is produced by charged particles moving in the matter with velocity greater than the phase velocity of light in that medium, leading to the Nobel Prize for these three, in 1958. Since this time, Čerenkov has been used in many areas of physics to detect charged particles [3, 4] and their motion, and to measure unique phenomena [5–7].

Research in biological radioisotope use took advantage of the availability of highly sensitive Charged Coupled Device (CCD) cameras previously set up for bioluminescence imaging, and this has allowed widespread use of Čerenkov as a tool for different types of imaging, even if present at very low levels. Beyond simply imaging the CR, there has been a substantial amount of activity using CR as an excitation source for biomolecules, in Čerenkov-excited luminescence imaging (CLI). CLI extends the scope of this imaging well beyond direct Čerenkov, because it provides detection of signals which have much longer tissue penetration, but can be used to report on the Čerenkov deeper inside the tissue. Applications include a very wide range such as *in-vivo* beta and alpha radionuclide emitter quantification [8–14], intraoperative or endoscopic imaging [15, 16], small animal tomographic imaging [10, 12, 17–20], radiation dose imaging in water phantoms and tissue [21], and high resolution molecular sensing *in vivo* [9, 22]. Each of these applications is discussed here, after the first two sections outlining Čerenkov radiation principles and the general development of CLI as a modality.

## 2. Čerenkov radiation principles

The results of several observations shows that molecules of a dielectric are polarized by moving a charged particle through the dielectric and when these polarized molecules return to

their ground state, they emit light photons and in fact the absorbed energy will be emitted as luminescence radiation [9]. In other words, Čerenkov radiation is induced when a charged particle moves faster than light velocity in vacuum divided by refractive index of environment ( $c/n$ ). Therefore, the intensity of Čerenkov radiation is related to the velocity and energy of the particles and the refractive index of environment. So, there would be a threshold condition for production of CR:

$$\beta n > 1 \quad (1)$$

Where  $n$  is refractive index and  $\beta$  is a relativistic phase velocity:

$$\beta = \sqrt{1 - \left( \frac{1}{\frac{E}{mc^2} + 1} \right)^2} \quad (2)$$

According to Eq. (1) & (2), the required threshold energy for production of CR is given by:

$$E_{min} = mc^2 \left( \frac{1}{\sqrt{1 - \frac{1}{n^2}}} - 1 \right) \quad (3)$$

This threshold energy for an electron in water with refractive index of 1.33 is 0.264 MeV and for tissue using a refractive index of 1.4, is 0.219 MeV.

Many of the used beta emitter radionuclides used in medicine, including all of positron emitter radionuclides used in PET, have greater energy than threshold energy, therefore, they can produce Čerenkov photons in water and tissue [23–25]. Because of the low energy of secondary electrons from Compton scattering or photoelectric interaction between tissues and gamma particles gamma emitter radionuclides, can't produce Čerenkov emission [26–29]. Also, the alpha emitter radionuclides, can't provide threshold conditions to produce Čerenkov photons [29, 30]. The production of CR by  $^{225}\text{Ac}$ , which is an alpha emitter, has been demonstrated, but this may be due to production of beta particles by daughter nuclei from  $^{225}\text{Ac}$  such as  $^{213}\text{Bi}$ .

Mitchell et al., showed the threshold energy of charged particles to produce CR in an environment relates to the medium refractive index [31]. This is also indicated in Eq. (3). Also, it has been shown that the intensity of produced photons increases with the refractive index [9, 10, 12–14, 32]. This fact leads to difference in production of CR in different tissues. As the tissues refractive indices are widely variable, so the produced photons have different intensity. High energy charged particle emitter radionuclides are less sensitive to the differences of refractive index, therefore, there are smaller difference in intensity of the produced radiation from these kinds of radionuclides.

CR intensity is related to the type of the radionuclide and its activity. Frank and Tamm predicted the number of emitted photons with wave length between  $\lambda_1$  and  $\lambda_2$  ( $\lambda_1 < \lambda_2$ ) per electron by presenting an equation:

$$\frac{dN}{dx} = \frac{2\pi}{137} \left( \frac{1}{\lambda_1} - \frac{1}{\lambda_2} \right) \left( 1 - \frac{1}{\beta^2 n^2} \right) \quad (4)$$

The Frank-Tamm relationship shows that increasing particle energy leads to an increasing rate of emitted photon and light radiation.

### 3. Čerenkov luminescence imaging (CLI)

In recent years, imaging of weak CR photons is possible thanks to improvements in optical imaging and increasing the sensitivity of CCD cameras. The idea of CR imaging using CCD cameras was presented by Cho et al. for the first time [33]. Then, in 2009, reports based on CR imaging of some PET radionuclides (such as  $^{13}\text{N}$  and  $^{18}\text{F}$ ) and SPECT ( $^{99\text{m}}\text{Tc}$  and  $^{131}\text{I}$ ) and radiotherapy ( $^{90}\text{Y}$ ) indicate the ability of CR imaging to show the radioactivity distribution *in vivo* (see Fig. 1) [8, 12, 14]. These studies led to creation of a modern method of optical imaging in which images are generated by emitted Čerenkov photons produced by high energy charged particles. This new imaging has been referred to as Čerenkov Luminescence Imaging (CLI). However it is important to recognize that the luminescence in this name refers to the Čerenkov light itself, and not anything induced by it, which will be later referred to as Čerenkov excited luminescence.

Conventional nuclear imaging method such as PET and SPECT are used widely in molecular imaging, but these methods have some problems such as high cost, limited availability, low spatial resolution and the fact that they are time-consuming [34]. CLI, as a modern technique which connects optical imaging to radionuclide imaging, largely solves mentioned problems of PET and SPECT and in addition, CLI has relatively high sensitivity when the light is not attenuated by tissue. Since 2009 CLI has many applications in preclinical studies [35, 36]. and CLI will have clinical applications.

Most positron emitter radionuclides used in the clinic, have higher energy than required for the threshold energy to produce CR. PET radionuclides such as  $^{18}\text{F}$ ,  $^{15}\text{O}$ ,  $^{13}\text{N}$ ,  $^{11}\text{C}$ ,  $^{64}\text{Cu}$ ,  $^{124}\text{I}$  are examples of these types of radionuclides, with different CLI yields as illustrated in Fig. 1. Many studies have been conducted for in-vivo imaging of these radionuclides distribution [13, 37–42]. The results of these studies indicate that the obtained data from Čerenkov radiation detection shows a good agreement with PET imaging results. Therefore, similar to PET, CLI can be considered as a reliable technique in biological researches.

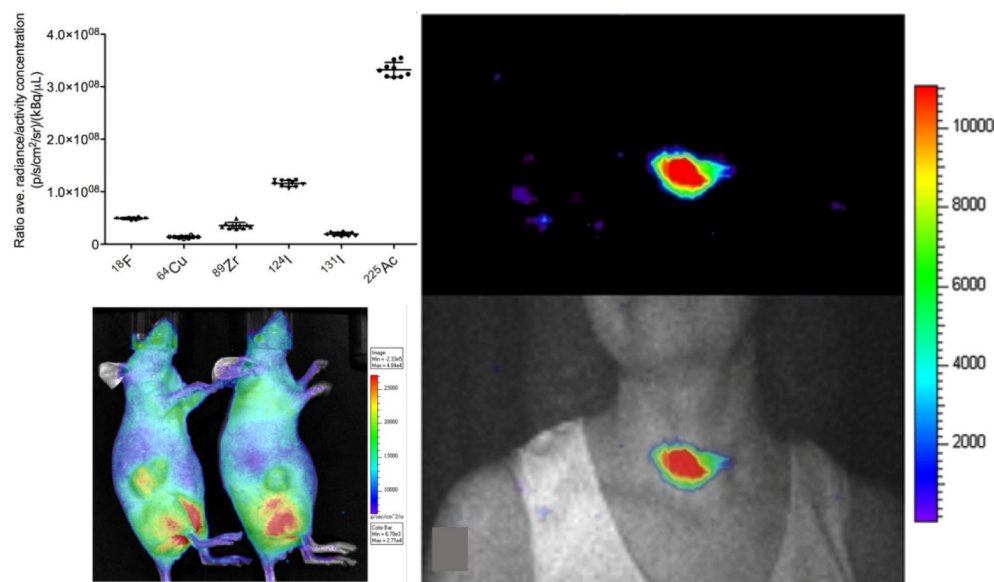


Fig. 1. The relative CLI yield for different isotopes is shown in top left [13], and one of the first images observed from a mouse using CLI [8]. Soon after this, the first CLI image from a human was taken, shown (right-top) and its fusion on photographic image of the patients neck (right-bottom) to show the location of the intensity in the thyroid [53].

Beta emitter radionuclides are good choices for use in clinical applications for dose delivery because of the electron low penetration depth, but direct imaging of the distribution and absorption of the labeled radiopharmaceutical is not possible with PET nor SPECT. Therefore, CLI can be a suitable technique for imaging of these radionuclides. Many studies have been done in Čerenkov luminescence imaging of beta emitter radionuclides such as  $^{131}\text{I}$ ,  $^{90}\text{Y}$ ,  $^{32}\text{P}$ ,  $^{198}\text{Au}$  [43–48]. Results of these studies indicate that it is possible to increase the spatial resolution of SPECT images using CLI. In addition to the mentioned radionuclides, the use of CLI in spectroscopy and measurement of delivered dose by secondary electrons produced by megavoltage photons has been taken into consideration in recent years [49–51].

Also, several studies have been conducted using this technique for clinical imaging of the human body. Spinelli et al. used CLI for thyroid imaging for the first time [52, 53]. By fusion of optical images on CLI images of a patient who was treated with  $^{131}\text{I}$ , they demonstrated that, it is possible to generate two-dimensional images from superficial organs (Fig. 1).

Some of the recently developed extensions to CLI in medicine are introduced in the following sections, describing both animal and human applications, where in some cases the signal is Čerenkov luminescence itself, and in some cases it is Čerenkov-excited luminescence from secondary molecules.

#### **4. Čerenkov luminescence endoscopy (CLE)**

Endoscopy has an important role in diagnosis of gastrointestinal diseases but, as endoscopy generates only anatomical images (not functional), it is not possible to identify the difference between similar anatomical diseases and abnormalities and also recognize the border between tumor and normal tissue. So, combination of anatomical and functional imaging techniques is important in surgery and diagnosis of cancer.

It has been shown that, it is possible to detect the organs within the body by using optical fibers and a CCD camera [54], as well as enable endoscopic testing [55, 56]. These studies led to introduction of CLE systems which have apparently high sensitivity and resolution and consist of an optical fiber connect to a CCD sensitive camera which can be used as a helpful tool during the cancer surgery [16] (Fig. 2).

Using the combination of Čerenkov endoscopy images and common endoscopy, CLE can be used to determination of diseased tissues and monitoring during surgery [16]. Also, this system can be used to image the organs such as bladder which typically appear as a dark hole with Čerenkov imaging.

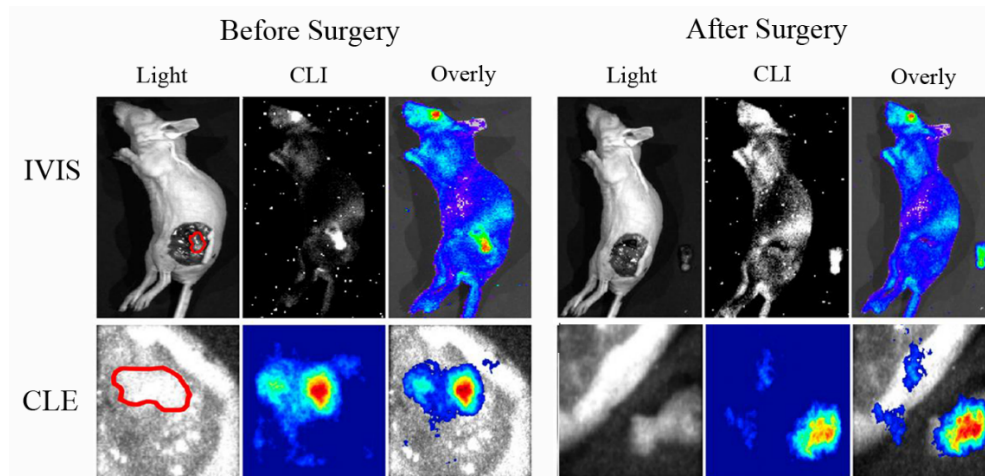


Fig. 2. Comparison between CLE and IVIS optical imaging system is shown, using before surgery (left) and after surgery (right) to illustrate how residual tumor could be detected by CLI, extending nuclear medicine imaging to an optical surgical setting [16].

One of the advantages of CLE is having higher spatial resolution than PET and SPECT. This resolution depends on imaging system, the number of beta particles in tissue, and light scattering of the tissue. Combination of CLE and PET images can be helpful for exact determination of location of the tumor.

### 5. Čerenkov luminescence tomography (CLT)

The idea of generating tomographic images using CR was initially presented by Li et al. [12], where they compared three-dimensional reconstructed images to PET images and found an agreement between the CLT and PET images in distributions of radiation, but the resolution of the CLT images was much lower than the PET images due to the high degree of light photon scattering in CLT.

Zhong et al., decreased the reconstruction images errors using anatomic information of imaged animals [18]. Also, Hu et al., fused CLT images with micro-CT and SPECT images of experimented animals and showed that there was an acceptable difference between SPECT images and reconstructed images of CLT. They suggested that CLT optical imaging could be used for validation of SPECT imaging [10]. They, also, increased the quality and resolution of the images using the CT and SPECT images in reconstruction of CLT [20, 45].

These studies emphasize the possibility of using CLT for in-vivo determination of radioactivity distribution [15, 18, 19]. Some advantages of the CLT technique are low cost and the available facilities for the procedure. However, considering the weak level of Čerenkov intensity and the possibility of scattering and absorption of CR within tissue, extra care is required to deal with these exceptionally low light signal levels. Additionally, solving the non-linear inversion problem with an ill posed problem is complicated and the use of prior information is likely required for linear response of the signal. Still, there is robust activity in the area of developing CLT as a reliable method for imaging radiation sources within mice.



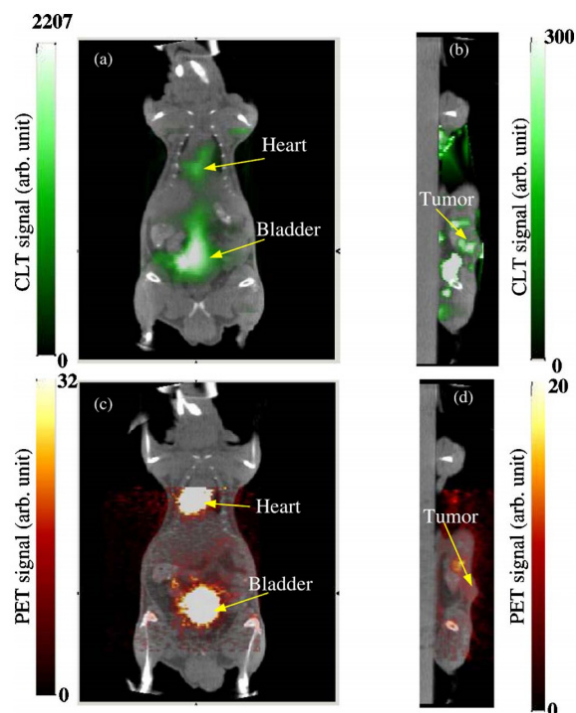


Fig. 3. Čerenkov luminescence tomography (CLT) is demonstrated in a mouse model with uptake in the heart and bladder shown (a) & (b) and compared to that of the gamma signals detected and reconstructed by PET (c) & (d) [12].

## 6. Čerenkov luminescence imaging dosimetry (CLID)

In recent years, spectroscopy of Čerenkov radiation produced by a clinical linear accelerator (LINAC) has been examined [57–59] in radiation therapy. The CR intensity increases sharply with particle energy above the threshold energy, and LINACs generally operate in the 6 to 18 MeV range, delivering predominantly photons in most treatments (80 + %) or electrons (<20%). So the optical emission per each particle from a linear accelerator is almost 2-3 times greater than that from beta emitter radionuclides in the <1MeV range [48]. So, the intensity of obtained signal from CR spectroscopy in radiotherapy is high and can be used for monitoring of the treatment with a carefully designed detection system.

In radionuclide spectroscopy, it is typically possible to provide a closed environment with no light inside. But in the case of a linear accelerator, providing this environment is not possible. Therefore, Glaser et al. presented a time-gated method to obtain the signal measurement [60]. They succeeded in decreasing the effect of ambient light by 1000 times by synchronizing the acquisition by CCD camera to the linear accelerator microsecond pulses [57].

Since 2011, this research has been carried out by focusing on using CR for radiation dosimetry of linear accelerator photons. Imaging of CLID was demonstrated for generation of the dose profile of delivered photon beams in a water phantom [30, 61]. Through addition of a fluorophore to the water, the Čerenkov emission stimulates fluorescence and the imaged signal is an indirect reporter, with the fluorescence process randomizing the directions away from the cone of light generated by the Čerenkov process. This approach has several advantages in comparison to the other more commonly used techniques in radiation therapy dosimetry. Some of these advantages are it being wide field, high resolution and faster than the typical single channel ionization chamber methods or gel dosimetry. In addition, CLID

doesn't require any reading mechanism and doesn't change with time, and so has some implicit advantages for certain types of dosimetry questions.

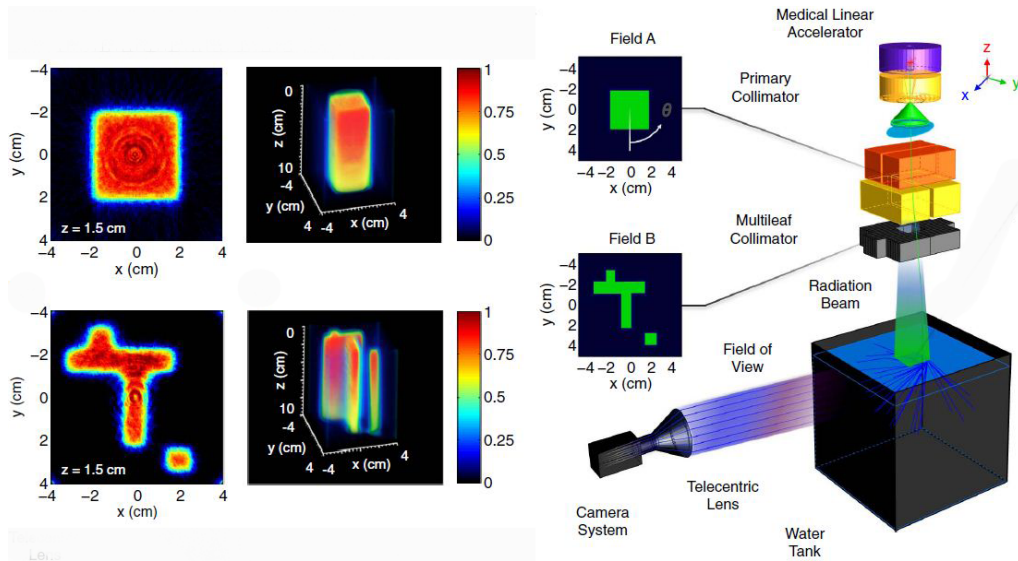


Fig. 4. The experimental results (left) and schematic (right) of the Čerenkov dosimetry imaging system, acquiring 2D projection views, and used for 3D reconstruction filtered backprojection imaging of the images [21]. The square MegaVolt photon beam profile (top left) and the complex beam profile (bottom right), were tomographically imaged with the camera, and the sonogram data reconstructed with filtered back projection.

Through imaging from a range of rotational angles, the data can be reconstructed from filtered backprojection imaging, to generate three-dimensional images of dose profiles in the water phantoms [21], as shown in Fig. 4. These three dimensional images provide the possibility of a greater understanding of transferred energy by radiation in water phantom (Fig. 3), and can be used for large fields of view with still relatively high spatial resolution, and faster 3D acquisition than with any other current dosimetry tool.

Recently, Glaser et al. completed a more exhaustive study comparing CR to absorbed dose of mega voltage photon, electron and proton beams using Monte Carlo analysis. They concluded that for X-ray photons, CR is suitable in stereotactic radiation therapy and surgery validation studies for verification of IMRT treatment in water phantom, near monoenergetic sources (such as cobalt-60 and brachytherapy sources) and also, for measurement of superficial dose [30]. For electron therapy, using CR is suitable just for superficial dosimetry [62] and interestingly for proton therapy this technique seems to have limited value due to the lack of CR at the Bragg peak.

In 2013, studies to estimate the superficial dose in a tissue equivalent phantom were completed [38, 37]. In these studies, X-ray and electron beams from a LINAC were examined with profile imaging and tomography. This technique, unlike the other superficial dosimetry techniques such as radiochromic films, ionization chamber, MOSFET, diodes and TLDs, would not require the dosimeter near or on the surface of the body, since all patients emit these photons during the radiotherapy treatment. Additionally it is high spatial resolution (less than a millimeter) while most of the other dosimetry techniques, have lower resolution or sparse sampling points. The resolution of film dosimetry is similar to this, but its procedures physically limited in space and time.

Zhang et al. presented a report based on first superficial dosimetry by CR during exposure with linear accelerator *in vivo* [50]. They showed CR intensity of tissue-equivalent phantom

irradiation have relation with transferred dose. Also, by superficial dosimetry of oral tumor of a dog during radiation treatment.

These studies led to video imaging of CR during surgery of patient with breast cancer, Fig. 5 [61, 63]. This video imaging showed that, using this method for imaging of superficial dose of treatment region during the exposure, technologists can ensure the precision positioning of patient and suitability of instruments used for exposure (MLCs and wedges, shown in a of Fig. 5) and also, three-dimensional dose conformity. One of important features of this technique is the possibility of dosimetry during the exposure and observation by the treatment team in the control room.

Existing problems in estimating superficial dose and complex modeling due to dependence on factors such as energy, field size, blocks, source-skin distance, radiation angle and uncertainty of delivered dose, always cause challenges for superficial dose estimation. CLID could lead to improve wide field superficial dosimetry once fully developed, because of the value in being able to image in real time and visualize entire tissue surfaces being irradiated [51].

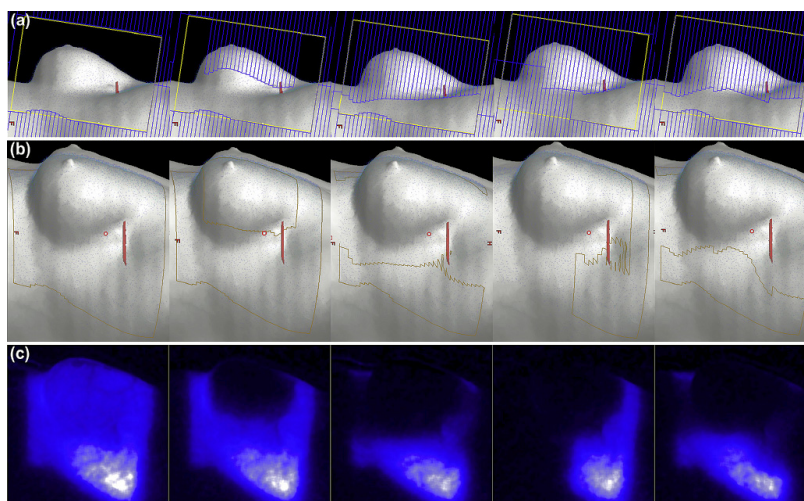


Fig. 5. A sequence of images of the breast treated with a dynamic wedge radiotherapy plan, where the beam shape changes with time, showing the sequence of beam shapes formed by the multi-leaf collimator (a) and (b), and frames from the Čerenkov video sequence of the treatment (c) [52, 53].

## 7. Čerenkov emission luminescence scanned imaging (CELSI)

The natural combination of CLID and CLI is the concept of using targeted radiation beams to introduce Čerenkov into tissue for the purpose of exciting molecular probes and optically detecting the signal, as a way to precisely measure the microenvironment. This was demonstrated with tomographic imaging [64] and scanned imaging recently [22]. The concept of Čerenkov excitation to induce luminescence and scan the imaging (CELSI) [22] can be used to excite phosphorescent probes, which report on signals such as pH or oxygenation in the tissue, or could be used with fluorescent probes tagged to proteins that report on localization to receptors or enzymatic function.

The CELSI concept was recently demonstrated *in vivo* with a rat injected with luminescent oxygen probe reporter in one lymph node. The resulting image was superimposed on an x-ray scan of the animal and is shown in Fig. 6(c) below. The compelling part of this work is the realization that this could be done at diagnostic radiation doses (here done with 0.02 cGy) and that it allows for high resolution scanning down to a few centimeters of tissue. The resolution improvement is shown in Fig. 6(b) relative to the diffuse scan in (a). In traditional optical imaging diffusion of the light in tissue is inevitable, and so the loss of

spatial resolution after a few millimeters. However the CELSI scan, Fig. 6(b) shows near complete preservation of the line scan resolution from 0mm depth down to 17mm of depth into a tissue phantom.

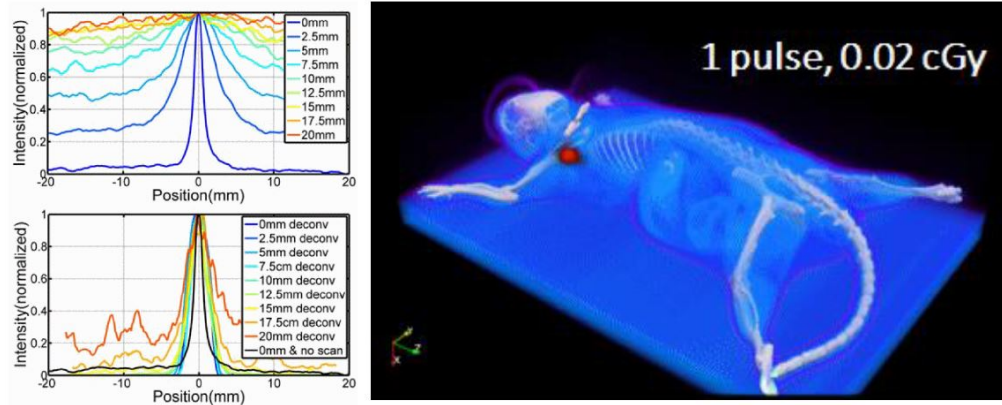


Fig. 6. Čerenkov excited luminescence scanned imaging is demonstrated in tissue phantoms, with line-scan data from a luminescent line source in varying depths of Intralipid shown in (a) and (b) with diffuse illumination in (a) and scanned CELSI imaging in (b) illustrating the improvement in spatial resolution. The raster line scan of a mouse with luminescence in one lymph node is shown in (c), with a total body dose of 0.02 cGy [22].

Future work on CELSI or related imaging principles could lead to a technique for high resolution molecular imaging in rodents or humans. The issues to be resolved are the limits to depth, concentration and radiation dose required for sufficient signal to noise, and to determine the relative benefits of different molecular probes used in this setting.

## 8. Molecules for Čerenkov sensing and radiosensitization

The concept of using Čerenkov light to excite molecules in tissue has been discussed for over a decade [65], but it has just recently been applied to tissue imaging and therapy [9, 66]. While the intensity of Čerenkov is very low, in the nanoWatt to picoWatt range, the choice of radiation source and the duration has a large effect upon what is possible.

The imaging concept has two different applications, basically 1) to use the fluorophore to report on the radiation activity concentration, by having coupled radiation and optical emitters [62], or 2) to use the radiation as a source of Čerenkov which activates a biomolecular probe within the tissue, that reports on the tissue microenvironment [67]. The former application is interesting but is likely limited to pre-clinical work given the nature of a hybrid radiation-optical probe, and may ultimately have limited impact in the field. Still, as new therapies involving injectable isotopes emerge, the need to better understand biodistribution and the heterogeneity of dose will be important, and it is possible that Čerenkov mediated dose quantification could have value. The complication in this issue is the known threshold dose for Čerenkov emission, and how this may limit the linearity between optical signal and the actual dose. *In vitro* use is well established though, and likely very strong potential. As can be seen from the previous CELSI section, the second application of molecular sensors in tissue could have a very wide impact on the field of biochemical sensing *in situ* or *in vivo*, and simply requires careful consideration around the scanning approach used.

Sensing of oxygenation in tissue has been demonstrated [64, 68], and sensing of pH with probes is also possible [69]. The examination of idealized probes for sensing depends a bit on the type of radiation used (Beta emitters, vs Gamma, external source vs. internal source, etc). This field is at an embryonic stage, and while the actual uses are likely to be in niche areas, the amount of physical chemistry research appears to be growing.

Enhancement of the Čerenkov signal is possible via interaction with radiation absorbing media, such as gold or copper based nanoparticles [70–72]. Several innovative designs in nanoparticle material science have shown that a carefully designed particle can enhance the signal, or this effect could be used for microenvironment sensing [73]. The self illuminating version of these, can be used for combined PET and NIR imaging, or be used for radiotherapy in situ with the potential for NIR imaging of the delivery.

Radiosensitization is a field which has been developed for many decades, with well established methods for achieving this. However it was just recently that the use of nanoparticles to enhance sensitization via Čerenkov excitation has been demonstrated [66]. This has very interesting potential for further development and when the pharmacology and toxicology issues of their use are worked out, there could be important applications for these type of nanoparticles as Čerenkov based radiation sensitizers.

## 9. Conclusion

In summary, the use of Čerenkov radiation in biomedical imaging has seen some major innovations in recent years. These studies indicate that CLI has growing interest in use for preclinical research in the field of nuclear medicine, to map dynamic radioactivity distributions which are usually performed by SPECT/PET imaging systems. CLI could provide quantitation of radiotracer absorption in humans especially in the case of superficial tumors and as a helpful instrument in endoscopy during surgery. This technique can also be used for quality assessment and dosimetry of clinical linear accelerators. This procedure has advantages such as lower cost and less time consuming for imaging than other similar techniques for instance PET and SPECT. The potential for innovative new biochemical sensing methods and radiosensitization approaches is very exciting, and these areas are just emerging now.

Despite the large number of case studies in CLI, still there are challenges and limitations in application of this technique. The challenges include the exceptionally weak intensity of the optical signal, in the nanoWatt to picoWatt per  $\text{cm}^2$  range typically. Also the relatively low penetration depth of some Čerenkov photons in tissue limits the range of utility. The wavelength dependence of the spectrum is an important factor in this as well, with the blue/green wavelengths being very short pathlength (hundreds of microns), and the red and NIR wavelengths being significantly longer pathlengths (several mm to a cm).. These attenuation and low fluence issues will dominate what is likely possible and impossible in living tissues.

Čerenkov luminescence imaging was faced with significant challenges in medical studies from the beginning. Recent studies continue to reform the directions that CLI appears to have, and its use will likely solve niche problems in medical imaging and dosimetry. The introduction of Čerenkov as a clinical imaging and therapy tool is promising and has unique strengths but also requires further development to optimize its value for each indication.

## Acknowledgments

The authors would like to thank Esmail Jafari from Shiraz University of Medical Sciences and Prof. Majid Assadi, director of the Persian Gulf Nuclear Medicine Research Center for their assistance. This work has been supported by NIH research grant R01CA109558 (BWP).