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Current and Future Clinical Applications for Optical Imaging of Cancer: From Intraoperative Surgical Guidance to Cancer Screening

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Abstract

Optical imaging is an inexpensive, fast, and sensitive imaging approach for the non-invasive detection of human cancers in locations that are accessible by an optical imaging device. Light is used to probe cellular and molecular function in the context of cancer in the living body. Recent advances in the development of optical instrumentation make it possible to detect optical signals produced at a tissue depth of several centimeters. The optical signals can be endogenous contrasts that capture the heterogeneity and biological status of different tissues including tumors, or extrinsic optical contrasts that selectively accumulate in tumors to be imaged after local or systemic delivery. The use of optical imaging is now being applied in the clinic and operating room for the localization and resection of malignant tumors in addition to screening for cancer.

Keywords

Optical Imaging; Cancer; Glioblastoma; Breast Cancer; Bladder Cancer; Near Infrared; Nanoparticles

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Optical Imaging of Endogenous Tissue Contrasts

At present, various optical imaging devices are used in human clinical trials for the detection of changes in endogenous tissue fluorescent signals, such as hemoglobin concentration, oxygenation status of heme molecules, and cytochrome oxidation changes. In addition, alterations in light scatter of tissues can be detected due to cell swelling, changes in tissue components, and blood volume and flow rates.¹ Since tumor tissues have altered blood flow and are highly hypoxic, differential optical absorbances of oxy- and deoxyhemoglobin in the tumor and normal tissues have been used as an intrinsic indicator for the presence of a cancer lesion.¹⁻⁴ Endogenous optical contrast for cancer detection can be applied with the optical imaging of melanoma tumor margins and circulating melanoma cells in the blood since melanin pigments in those cancer cells have a strong absorption in a near infrared (NIR) range.^{5, 6}

Contrast-Enhanced Optical Imaging

One of the major challenges in optical imaging of endogenous tissue contrasts is its poor specificity due to background signal. Contrast-enhanced optical imaging can increase the sensitivity and specificity of cancer detection. Optical contrast agents require the use of fluorochromes with a high molar extinction coefficient, good quantum yield, and low non-specific binding to non-cancerous tissue. At present, the most promising fluorescent contrast agents for clinical applications are 5-aminolevulinic acid (5-ALA) and indocyanine green (ICG) dyes (Fig 1).

Fluorescence-Guided Brain Tumor Resection

Defining the margins of cancerous tissue by optical contrast agents can allow the surgeon to better distinguish between normal and tumor tissue. Furthermore, unintentional removal of healthy tissue or failure to maximize removal of malignant tissue may be avoided. Malignant gliomas (WHO grade III and IV) comprise a group of infiltrative, primary neoplasms in the brain that can be resected with the use of optical imaging.⁷ Glioblastoma multiforme (GBM) is the most common malignant glioma that accounts for approximately 12-15% of all intracranial neoplasms. The prognosis of malignant gliomas is poor, and available treatment options are surgery, radiotherapy, and chemotherapy.⁷ The goal of surgery is to remove as much of the tumor as possible without damaging the neighboring healthy brain tissue. A more complete resection improves outcomes in patients and may provide better efficacy of concomitant chemo-radiation followed by adjuvant chemotherapy.⁸⁻¹² Defining the gross margins of malignant gliomas at surgery can be difficult due to their infiltrative nature with normal brain. Resection of malignant gliomas involves the use of an operative microscope that emits standard “white” light for illumination and magnification.

The optical contrast agent 5-ALA, also known as Gliolan (photonamic GmbH & Co. KG, Wedel, Germany), is currently being used for the microsurgical resection of malignant gliomas.^{7, 13, 14} The use of 5-ALA for the intraoperative detection and fluorescence-guided resection of brain tumors has been shown to enhance the gross total resection of malignant gliomas and maximize survival benefits (progression-free survival) associated with surgical removal.

5-ALA is a prodrug that is metabolized intracellularly by the heme porphyrin synthesis pathway to form the fluorescent protoporphyrin IX (PpIX) molecule (Fig 2).¹⁵ The oral administration of 5-ALA (20 mg/kg) leads to a selective accumulation of PpIX in tumor cells as well as epithelial tissues.^{16, 17} Following excitation with blue light ($\lambda = 400 - 410$ nm) emitted from a special filter attachment on the operative microscope, the PpIX, which

has accumulated selectively in the malignant tissue, emits a red-violet light of 635 nm (Fig 3). The surgeon is then able to resect the red-violet tumor tissue in a gross total fashion.

The phenomenon of PpIX accumulation in malignant gliomas may be explained by higher 5-ALA uptake into the tumor tissue or an altered pattern of expression or activity of enzymes (e.g., ferrochelatase) involved in heme biosynthesis in tumor cells. Explanations for higher 5-ALA uptake include a disrupted blood-brain barrier, increased neo-vascularization, and the overexpression of membrane transporters in malignant glioma tissue. ALA-induced PpIX levels in normal brain are very low, creating high intrinsic tumor-to-normal tissue contrast. A significant relationship between preoperative MRI contrast enhancement and observable intraoperative PpIX fluorescence has recently been shown.¹⁸ 5-ALA has also been used as a photosensitizer for photodynamic therapy of various types of human cancers as well as non-neoplastic diseases.¹⁹ Currently, 5-ALA is not approved by the US Food and Drug Administration (FDA) for surgical resection of brain tumors. Efforts are underway to perform a randomized, multicenter trial in North America utilizing 5-ALA for resection of malignant gliomas.

Photodynamic Diagnosis and Bladder Cancer

ALA has also been introduced in the setting of bladder cancer since 2005 in Europe.^{20, 21} Photodynamic diagnosis (PDD) is an emerging technology based on the use of the ALA agent, HEXVIX, for the visualization of precancerous and cancerous lesions in the bladder with the use of PDD blue light cystoscopy. Just as in malignant gliomas, PpIX fluorescence is produced by excitation with blue light resulting in red-violet fluorescence.

Use of HEXVIX is thought to improve the effectiveness of transurethral resection of bladder cancer (TURB), potentially reducing recurrence rates and lowering treatment costs.^{22, 23} PDD supports a more complete resection and has been reported to improve patients' outcomes by reducing recurrence rates and prolonging recurrence-free survival.^{24, 25}

Near-infrared (NIR) Optical Imaging

In recent years, an increasing number of imaging studies have been carried out using optical imaging technology that absorbs and emits fluorescent light in an optical spectrum of near-infrared (NIR) range (700-900 nm).²⁶⁻²⁸ For *in vivo* fluorescence optical imaging, maximizing the depth of tissue penetration is important. Increasing the wavelength of the excitation source decreases light absorption and scattering. The use of fluorescent dyes with excitation wavelengths below 700 nm results in low tissue absorption and a penetration depth of only a few millimeters. Additionally, light absorption of hemoglobin, melanin, lipid, and other tissues in living subjects causes autofluorescence throughout the visible spectral range up to 700 nm. However, in the NIR spectral range, interference from solvents and biomolecules is minimal and tissue absorption is considerably less; in this range, light can penetrate further into the tissues, allowing for the detection of the imaging signals at a depth of several centimeters. Greater depth penetration makes it feasible to detect tumors located close to surface areas, such as breast, colon, esophagus, stomach, bladder, ovary, and skin using non-invasive or minimally invasive optical imaging approaches.²⁹⁻³¹ One of the most promising clinical applications of NIR optical imaging is for guidance during surgery. Intraoperative optical imaging and NIR signals with emission above 700 nm have low fluorescence background from tissue scattering and blood absorption, and thus exposed tumor and surgical areas may be accessible to optical illumination and detection devices.^{32, 33}

ICG (Fig 1) is a well-known FDA-approved NIR organic dye that is used for various clinical applications in human. The dye is delivered intravenously or by subcutaneous injection into

patients for optical imaging in angiography, guiding biopsies, evaluating blood flow, hepatic function, and lymph node mapping in breast cancer patients.^{34, 35} The NIR signal is detected using 780 to 790 nm excitation and 830 to 850 nm emission wavelengths. Results from human studies have shown that ICG has a fast clearance in the blood (3 to 4 min) and is nontoxic to humans.³⁶ Recent studies have shown that ICG selectively enriches in tumors due to changes in blood volume and flow rate, and leaky vasculature within and surrounding tumors.^{37, 38} The binding of ICG dye to serum proteins further facilitates the retention of the dye molecules in the tumor site by the enhanced permeability effect of tumor vessels to macroparticles. Therefore, it is possible to detect tumors using ICG-enhanced contrast and optical imaging. However, optical imaging based on the blood pool contrast and non-specific retention of the dye molecules still does not have sufficient sensitivity and specificity for detecting cancerous lesions. Additionally, other disadvantages of ICG, which limit its use for *in vivo* optical imaging, include a high level of plasma protein binding (up to 98% a few seconds after injection), low stability in aqueous media, poor fluorescence efficiency, and rapid photobleaching.^{39, 40}

NIR Optical Imaging and Screening of Breast Cancer using Endogenous and Exogenous Contrasts

Breast cancer is a leading cause of death for women in the United States. The most effective approach to combating the increased incidence of breast cancer is early detection. While x-ray mammography is widely used for early detection of breast cancer, it has unacceptable false negative rates for women with radiodense breast tissues, who represent a large portion of premenopausal women.^{41, 42} Furthermore the positive predictive value of x-ray mammography is quite low.^{43, 44} Consequently, there is a critical need to investigate breast cancer detection methods that could serve either a complementary or competitive role with respect to conventional x-ray mammography.

Several other conventional techniques are currently under investigation for breast cancer detection, including ultrasound (US), CT, and MRI.⁴⁵ MRI is the most widely studied and potentially promising solution to dense breast imaging.⁴⁶ While MRI appears to offer high sensitivity in the dense breast, its specificity has been modest. In addition, MRI is costly and therefore not suitable for routine breast screening.⁴⁷

NIR diffuse optical tomography (DOT) is emerging as a noninvasive and low cost adjunct strategy for breast cancer detection that can provide structural and functional information on breast tissue.^{4, 48-52} DOT is a portable method that can sensitively detect optical contrast between diseased and normal tissues. In particular, DOT offers the ability to quantitatively image the high optical contrast that arises intrinsically from cellular signals generated through the presence of blood, water, lipid, and cellular density. In DOT, multispectral NIR light sequentially excites breast tissue at multiple locations and the scattered light is measured at multiple positions along the surface of the breast. The measured multispectral light is then used to reconstruct the spatial distribution of tissue absorption and scattering coefficients at each wavelength through a light propagation model in tissue (e.g., the photon diffusion equation). The images of absorption and scattering spectra are used to derive the images of functional parameters (via the Beer-Lambert law) and cellular morphology (via particle scattering theory). In DOT, an effective model-based reconstruction algorithm is critical for accurate image reconstruction.

Since 1997, three complete clinical platforms have been developed for NIR optical tomography of the breast. These platforms have evolved from single wavelength/2D to multi-wavelength/3D capabilities (Fig 4) and have been used to evaluate the potential of optical imaging of the breast. A series of studies have been designed to quantify the imaging

contrast in the normal and abnormal breast and to provide initial assessments of the operating characteristics of the imaging systems for diagnostic decision-making in the setting of screen-detected breast lesions.⁵³ Specifically, both absorption and scattering properties of breast tissue can be obtained that can be used to sensitively distinguish between normal and abnormal breast tissues (Fig 5). Cysts can be clearly differentiated from solid tumors based on these two properties alone (Fig 5). Furthermore, hemoglobin (Hb) and oxyhemoglobin (HbO₂) are two important parameters for enhancing sensitivity (Fig 5). A clinical study of 35 breast masses demonstrated that a sensitivity of 91% and a specificity of 91% can be obtained based on these optical parameters.⁵⁴ In addition, the cellular density and size derived from the scattering spectra can more accurately characterize the nature of breast lesions than the scattering property or scattering amplitude/scattering power (unpublished results). Initial results in 14 breast abnormalities showed that malignant tumors can be differentiated from benign lesions with high accuracy (Fig 6).

Using contrast-enhanced optical imaging, pilot clinical studies have indicated that the tumor-to-tissue contrast can be significantly enhanced through preferential uptake of contrast agents in the breast lesions.^{49, 55} In a recent study using continuous-wave (CW) NIR tomography, Intes et al.³⁷ reported that malignant tumors showed significantly slower rates of uptake and outflow of intravascularly-delivered ICG probes. In another study, Corlu et al.⁵⁶ reported the first 3D fluorescence tomography of human breast cancer using ICG as contrast agent. Fluorescence excitation and detection are accomplished in the soft-compression, parallel-plane, transmission geometry using laser sources at 786 nm and spectrally filtered CCD detection (Fig 7). The fluorescence image showed that tumor-to-normal tissue contrast based on ICG fluorescence was two-to-four-fold higher than the endogenous contrast based on absorption (Hb and HbO₂) and scattering parameter (μ_s').

Currently, optical imaging of breast cancer using endogenous and exogenous contrasts is being evaluated in large scale clinical trials in multiple centers worldwide.

New Targeted Optical Imaging Probes

To achieve high sensitivity in optical imaging, it is necessary for a fluorescent imaging probe to have a strong and prolonged image intensity *in vivo*, low protein binding, and the ability to accumulate and be retained in a targeted area over an extended time period. Currently, several research groups are developing new NIR dyes and optical contrast agents as well as tumor-targeted optical imaging probes.

Recent advancements in tumor biology, cell and molecular biology, and imaging technology have stimulated the emergence of a new field of “molecular imaging” that focuses on visualizing or imaging biological events and processes in living systems, including patients.⁵⁷ A promising strategy to improve the specificity of cancer detection is to target imaging probes to a biomarker molecule expressed highly in cancer cells, but not in normal cells. Previous study results have shown that tumor-targeted imaging probes can increase the localization of image probes to tumors while reducing their uptake in normal tissues, thus improving the detection of tumors *in vivo*.^{29, 58, 59}

Although unmodified ICG is approved by the FDA for human use, conjugation of ICG to targeting moieties, such as peptides and antibodies, requires further modifications of the dye molecule, generating a new molecule that needs additional toxicity studies for clinical applications. At present, there are few available dyes, such as Cy5.5 (GE Healthcare, Piscataway, NJ), DyLight 680 to 800 (Thermo Fisher Scientific Inc., Rockford, IL), and IRDye 800CW (LI-COR Biosciences, Lincoln, NE), that are excitable in the NIR wavelength window and functionalized for conjugation to various targeting molecules. The most common modifications for NIR dyes are N-hydroxysuccinimide (NHS) ester and

maleimide, which permit conjugation to tumor targeting antibodies, peptide ligands, and small molecules with primary aliphatic amines. Currently, targeted optical imaging for cancer detection using Cy5.5 or IRDye 800CW-labeled optical imaging probes is under intensive investigation by many groups in various animal tumor models.⁶⁰⁻⁶³

Cy5.5 dye is one of the most commonly used NIR dyes for non-invasive optical imaging in animal tumor models. Several studies have shown that the delivery of tumor-targeting ligands conjugated with Cy5.5 successfully permits *in vivo* imaging in several animal tumor models.⁶⁴⁻⁶⁶ However, Cy5.5 has excitation/emission maxima at 675 nm/694 nm, which is at the borderline of tissue background fluorescence. It also has some non-specific binding to cells resulting in a relatively high background noise for *in vivo* optical imaging. IRDye 800CW emits at 794 nm and has a low body background for *in vivo* optical imaging.⁶³ Although preclinical studies show low systemic toxicity of IRDye 800CW in experimental animals, long-term accumulation of IRDye 800CW dye in normal tissues, especially in the liver, after systemic delivery may be a potential problem for future clinical application.⁶²

Selection of Tumor-Targeting Ligands and Cellular Targets

Selection of cell surface molecules or receptors that are highly expressed in tumor cells and the tumor environment may permit enhanced accumulation of a targeted imaging agent in the tumor site and increased sensitivity and specificity of optical imaging. Furthermore, the binding of targeted optical imaging probes to cell surface receptors and their subsequent cellular internalization further increases the retention time of the imaging probes in tumor cells and enhances the sensitivity of cancer detection.²⁹ Various cell surface molecules, including epidermal growth factor receptor (EGFR), Her-2/Neu, alphaVbeta-3 integrin, urokinase plasminogen activator receptor, mucin 1, carcinoembryonic antigen, and the folate receptor have been investigated for targeted imaging.^{58-60, 62, 64, 67-69}

The use of optical imaging probes in the clinical setting requires probes and their targeting ligands to be safe, biodegradable, with low immunogenicity. Various types of targeting ligands have been used in animal models for tumor imaging. NIR dyes can be conjugated directly to monoclonal antibodies to produce imaging probes. Engineered small antibody fragments, such as single chain antibodies and affibodies, are attractive targeting ligands since they have very high binding affinity and are 6 to 21 times smaller in size than the intact antibodies.^{69, 70} Smaller size is an appealing feature for enhanced delivery of optical imaging probes into the tumor mass. Recombinant natural ligands, small peptides, and small molecules that can be produced in large amounts and have low immunogenicity have also been used as targeting ligands.^{29, 66, 68}

One of the attractive features of optical imaging is the ability of NIR dyes, with different excitation and emission wavelengths, to be conjugated to multiple targeting ligands. It is expected that simultaneous detection of multiple tumor biomarkers could increase the sensitivity and specificity of detecting human tumor cells by *in vivo* optical imaging.

A unique characteristic of NIR dyes is that their fluorescent signal can be quenched when dye molecules are close to each other. Protease activatable imaging probes have been produced for *in vivo* tumor imaging. In the tumor where a specific protease is highly activated, proteolytic cleavage of linker peptides leads to release of the quench effect to produce a bright fluorescent signal.⁷¹⁻⁷³

Nanoparticle-NIR Dye Imaging Probes

With the development of tumor-targeted optical imaging probes, the size of the probes and their delivery characteristics should also be taken into consideration. Taking advantage of

the difference between the “leaky” tumor and normal vasculature is important.⁷⁴ It is well known that tumor vessels can allow nanoparticles with a size < 200 nm to cross the endothelial layer and enter into the tumor mass while such sized particles are not able to pass through the normal endothelial cellular layer.^{75, 76} Such a passive targeting effect provides selectivity in delivering nanoparticle-formulated imaging probes into the tumor.⁷⁷ Additionally, nanoparticles with a long half-life in the blood circulation could further increase targeting as well as non-targeted delivery of optical imaging probes into the tumor.⁷⁸ To generate nanoparticle-NIR dye imaging probes, the NIR dye molecules can be encapsulated into polymeric nanoparticles, or conjugated to the surface polymer of nanoparticles. Recently, systemic delivery of NIR-dye-labeled targeting ligands conjugated to a magnetic iron oxide nanoparticle have been shown to produce stronger optical signal in an orthotopic pancreatic cancer xenograft model than the signal produced by NIR-dye-labeled free peptides (Fig. 8).⁶⁶

Conclusion

Optical imaging is a noninvasive, relatively low-cost technology that uses light to probe cellular and molecular function in the setting of cancer. Advances in optical instrumentation allow the detection of endogenous tissue contrasts between normal and malignant tissues, while administration of optical contrast agents further enhances sensitive and specificity of the detection of cancer cells. Optical imaging of contrast agents makes it possible for the surgeon to visualize cancer margins and more effectively resect malignant gliomas and bladder cancer. The use of near infrared optical imaging permits greater tissue depth penetration and has become very important for the screening of breast cancer. Newer, targeted optical imaging probes that have been conjugated to cancer cell specific ligands have been developed in preclinical animal models and can provide for better cancer detection.

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Fig 1.

Chemical structures, spectral properties, and working principles of indocyanine green (ICG) and 5-aminolevulinic acid (ALA) as fluorescent contrast agents for endoscopic tumor detection. **Left panel:** Chemical structure and spectral properties of ICG. Upon systemic injection, ICG is rapidly bound to blood proteins (such as albumin), and the resulting complexes (5-10 nm in size) are accumulated in tumors mainly via enhanced permeability and retention (EPR). **Right panel:** Nonfluorescent ALA is taken up by tumor cells and then induces the biosynthesis and accumulation of fluorescent protoporphyrin IX (PpIX). The proposed mechanisms for higher ALA uptake in brain tumors include a disrupted blood-brain barrier, increased neovascularization, and the overexpression of membrane transporters in malignant glioma tissue (45). ALA-induced PpIX levels in normal brain are very low creating tumor-to-normal tissue contrast ratios as high as 50-100.

Fig 2. 5-ALA metabolism

The oral administration of 5-ALA (20 mg/kg) leads to a selective accumulation of PPIX in tumor cells as well as epithelial tissues. 5-ALA is a prodrug that is metabolized intracellularly by the heme porphyrin synthesis pathway to form the fluorescent PPIX molecule.

Fig 3. Fluorescence-guided brain tumor resection

Following excitation with blue light ($\lambda = 400 - 410$ nm) emitted from a special filter attachment on the operative microscope, the PPIX, which has accumulated selectively in the malignant tissue, emits a red-violet light enabling the surgeon to resect the red-violet tumor tissue in a gross total fashion.

Fig 4.
Photograph of a third generation DOT system.

Fig 5.

CT image (a), recovered coronal μ_a image (b) and μ_s' image (c), and photograph of the excised tissue (d) for from a breast cancer patient. The white/black arrows indicate the lesion location(s). Mammogram (e) and sonogram (f) of the left breast from a breast cancer patient. Recovered optical images in the coronal plane: μ_a (g) and μ_s' (h) image. The axes (left and bottom) are the spatial scale (mm), whereas the color scale (right) is the μ_a or μ_s' (mm^{-1}). 1D profiles of the recovered μ_a (i) and μ_s' (j) along a horizontal cut line through the center of the cyst region for the patient. (k) Craniocaudal mammogram. (l) HbO_2 image. (m) Hb image. (n) H_2O image. The images shown are in the coronal plane at the level of the lesions. The color bar (right) is the recovered HbO_2 (μM), Hb (μM), or H_2O (%), while the axes (left and below) refer to the spatial coordinates (mm).

Fig 6.

Recovered MD image (a) and VF image (b) for the malignant case. The color bar (right) indicates the size (μm) or VF (%). (c) Average values of recovered MD and VF. (d) The peak value of the recovered MD versus the peak value of VF in the lesion region. Pink: Malignant. Blue: Benign.

Fig 7.
Schematic of a parallel plate DOT instrument (Adapted from Ref. ⁵⁴).

Fig 8. Systemic delivery of NIR-dye-labeled targeting ligands that are conjugated to a magnetic iron oxide nanoparticle produces stronger optical signal in an orthotopic pancreatic cancer xenograft model than optical imaging using NIR-dye-labeled free peptides.