Optical Image-Guided Cancer Surgery: Challenges and Limitations

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Abstract
Optical image-guided cancer surgery is a promising technique to adequately determine tumor margins by tumor-specific targeting, potentially resulting in complete resection of tumor tissue with improved survival. However, identification of the photons coming from the fluorescent contrast agent is complicated by autofluorescence, optical tissue properties, and accurate fluorescent targeting agents and imaging systems. All these factors have an important influence on the image that is presented to the surgeon. Considering the clinical consequences at stake, it is a prerequisite to answer the questions that are essential for the surgeon. What is optical image-guided surgery and how can it improve patient care? What should the oncologic surgeon know about the fundamental principles of optical imaging to understand which conclusions can be drawn from the images? And how do the limitations influence clinical decision making? This article discusses these questions and provides a clear overview of the basic principles and practical applications. Although there are limitations to the intrinsic capacity of the technique, when practical and technical surgical possibilities are considered, optical imaging can be a very powerful intraoperative tool in guiding the future oncologic surgeon toward radical resection and optimal clinical results. Clin Cancer Res; 19(14); 3745–54. ©2013 AACR.

Introduction
For cancer surgery with curative intentions, radical resection (i.e., removal of all cancer cells) is a sine qua non. To achieve this, the surgeon has to adequately assess the tumor resection margin during the operation. Optical molecular imaging using near-infrared (NIR) fluorescence introduces a revolutionary new approach to address this basic challenge in surgical oncology (1–3). The field of optical imaging emerged in the early 20th century with the observation of porphyrin fluorescence in certain tumors, but a lack of fundamental knowledge and suitable optical equipment prevented further development at the time (4). The finding that photons in the NIR range (650–900 nm) travel through tissue much deeper than photons in the visible light range (5) was essential for the advancement of optical imaging toward clinical practice, and further development gained momentum over the past decade (1, 6). To use optical imaging for visualization of cancer, fluorescent agents are injected that emit light in the NIR range and are tumor specific using a variety of targeting strategies (2). Because the human eye is not sensitive for light in the NIR region, dedicated camera systems are required to detect the fluorescence emission from these molecules (2). With real-time intraoperative fluorescence imaging of tumor margins, the technique promises to guide the oncologic surgeon toward optimal radical resection and clinical results.

Preclinically, optical imaging has been used in tumor identification, image-guided resection, therapy monitoring, and detection of sentinel lymph nodes. Because tumor-specific agents were not yet approved for clinical use, the first clinical studies were conducted using nonspecific fluorescent agents that had long been approved for different applications and could therefore be used for sentinel lymph node mapping (6, 7). Clinical trials of tumor imaging using indocyanine green have been reported in hepatobiliary and colorectal cancer (8). Recently, a milestone step was completed when the first-in-human trial using tumor-specific targeting was reported (9), marking the beginning of a new phase of optical image-guided surgery.

However, although it is very likely that the technique will deliver an important contribution to surgical oncology, this approach has fundamental limitations that influence the ability to differentiate the targeted tissue from its surroundings. Now that the first clinical trial has been reported, the group of oncologic surgeons that gets acquainted with the flourishing field of optical image-guided surgery is

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increasing day by day, with clinical consequences at stake. As we show in this review, it is essential for the clinician to understand which phenomena occur when photons travel through tissues and how these phenomena influence the optical image that is acquired. We therefore believe that it is now, more than ever, of paramount importance to provide the surgical oncologist a clear overview of the basic principles of optical image-guided surgery to be able to understand how these limitations influence the sensitivity of this technique. Awareness of these limitations and focusing research on solving these challenges are of utmost importance for proper implementation and evaluation of the clinical value of this new technology.

The Basic Principle of Fluorescence Imaging

The geometric principle of fluorescence imaging is illustrated in Fig. 1A. A light source is required that sends out a bundle of light with a specific wavelength that is able to excite the fluorophore (i.e., excitation photons). Because the targeted fluorophore is located under the tissue surface, this excitation light has to enter and travel through tissue to reach the fluorophore. Light entering the tissue is partly influenced by reflection and refraction at the tissue surface. The direction of photons that travel through the tissue can be changed due to scattering. In addition, photons can be absorbed by various components in the tissue. Only when such a component is a fluorophore, which can be either an intrinsic tissue component (i.e., autofluorescence) or an injected external fluorescent agent, absorption of a photon results in a gain in energy of the fluorophore, which then enters an excited state (Fig. 1B). The electrons remain in this state for about $10^{-8}$ seconds depending on the molecule, which is called the lifetime of the fluorophore. After this phase, the system returns to its ground state and emits the photon, which could be heading into all directions (Fig. 1B). The change in energy between the absorbed and the emitted photon results in a change in wavelength. This shift of shorter wavelengths (higher energy) of the absorption spectrum to longer wavelengths (lower energy) of the emitted fluorophores is called the Stokes shift. After excitation of the fluorophore, emission photons will also be influenced by scattering and absorption, and the photons that reach the tissue surface will subsequently be influenced again by reflection and refraction at the surface. Finally, in a clinical geometry, only part of these photons can eventually be detected by the camera system. Although optical imaging is based on the detection of photons, Fig. 2 illustrates that the path that photons travel through the imaged tissue is influenced by optical properties of the components in the tissue (i.e., absorption and scattering). As a result, fluorescence images will display intensities that are strongly influenced by varying absorption properties of the tissue and the images may be blurred due to scattering of light. The following paragraphs explain in detail how these phenomena influence the acquired image.

What Happens When Light Travels through Tissue?

Absorption

All tissues contain components that can absorb photons that travel through them. The composition of these components varies between different tissue types and organs,
but also within these tissues, and may vary in time (10). In general, the most relevant absorbers of photons are water, lipids, oxyhemoglobin, and deoxyhemoglobin (Fig. 3A) (11). Blood is the main absorber in the visible region, and the absorption of light by blood is the highest in the blue-green region. This fact can be illustrated by illuminating the human skin with white light. Because of the absorption of photons with shorter wavelengths, a reddish reflection returns from the skin. In this way, absorbers influence the color of the light that exits imaged tissues. In the NIR range, the absorbance per volume of these components is much lower than in other parts of the spectrum, allowing for deeper penetration of photons into the tissue (Fig. 3).

**Scattering**

Next to absorption of the photon, a change in photon direction can occur, an effect known as scattering (Fig. 4). In most parts of the visible and NIR region, scattering events occur much more frequently than absorption events. Even though a forward direction of scattering photons is most likely to occur in tissues, the accumulation of multiple consecutive scatter events will result in a gradual randomization of the propagation direction (i.e., diffuse light, Fig. 4; ref. 12). Randomization of the direction of light reduces the signal strength as well as the accuracy of determination of the source localization. However, scattering can also have a positive effect on the fluorescence signal intensity because of its effect on the excitation light. The photons that excite the fluorophore follow a similar contorted light path as the emission light. In highly scattering tissue, the contorted light path causes retention of photons in that specific area. In other words, in a highly scattering medium, the photons bounce around locally for a longer time, increasing the chance for a fluorescence event. Consequently, there can be increased fluorescence intensity in tissues with more scattering compared with tissues with less scattering (13). The net effect of scattering on fluorescence imaging depends on the exact optical properties of the tissue at hand.

**Influence of optical properties on light penetration depth**

Scattering and absorption coefficients vary between locations and tissues due to the different components within the heterogeneous tissue. Importantly, the influence of these phenomena on the fluorescence signal will be higher when fluorescent light has to emerge from larger tissue depths and consequently has to pass more absorbers and scattering events. In the visible light region, absorption by biologic chromophores limits the penetration depth to a few millimeters with a maximum of 10 millimeters. Because
of less absorption and scattering, penetration depth is increased to more than a centimeter in the NIR region (14, 15); however, this measurement strongly depends on the type of tissue (refs. 1, 3, 16; Fig. 5).

Reflection and refraction

As imaging during surgery always focuses on a surface, the mismatch between the indices of refraction of tissue and air is unavoidable, resulting in a change in direction of the photons. This mismatch in index of refraction causes reflections to occur at the surface. The excitation light will be partially reflected from the surface, like the sun on a water surface. When the light is nearly perpendicular to the surface of the tissue, the surface reflection is not more than a few percent. However, emitted fluorescent light that is generated inside the tissue is diffuse and partly reaches the surface under large angles, resulting in complete reflection within the tissue. This internal diffuse reflection coefficient at the surface can be in the order of 50%. Consequently, the emitted fluorescent light is even more diffuse, and the amount of light that escapes the surface and can be detected by the fluorescence camera is further decreased.

Fluorescence Imaging and Autofluorescence

The goal of fluorescence imaging is to detect a target by its specific fluorescence signal. Detection of the fluorescent signal of the targeted fluorophore is hampered because all cells contain various endogenous fluorophores that become fluorescent when excited by UV, visible, or NIR radiation of suitable wavelength. This intrinsic fluorescence of the tissue induces a nonspecific background signal. The number of endogenous fluorophores varies strongly between tissue types. Moreover, the autofluorescence can change in time because of bleaching of the endogenous fluorophores and because some of the endogenous fluorophores are related to metabolism (e.g., NADH, NAD, FAD, and FADH).

Because we can see cancerous lesions with our eyes, the optical properties of these lesions are changed by definition, and a difference in autofluorescence signal compared with the surroundings can be detected. This effect has been used for autofluorescence-guided surgery (17). However, due to the aforementioned effects, “tumor-specific” autofluorescence signals can vary over time, making it an unreliable target. Furthermore, other benign visible changes (e.g., scar formation) also result in a change in autofluorescence, limiting the specificity of this technique (18). Most importantly, despite positive correlations that have been reported in a number of studies that use autofluorescence to detect and remove the tumor (17), it is not clear which biologic aspects are responsible for the change in autofluorescence signal in the imaged lesions. Studies to assess these aspects are compromised because of artifacts as a result of color changes in cancer tissue (18). The surgeon should therefore approach changes in autofluorescence with the same level of uncertainty as visible clues of cancerous tissue.
high correlation of autofluorescence and tumor tissue is consistently reported, they can be used to aid the surgeon in assessing the tumor margins but should not be considered as tumor-specific proof of tumor margins.

As a good alternative, a target-specific agent that contains a fluorophore is usually injected intravenously. Now that we understand how optical properties can influence the optical path of the photon, we are confronted with a second challenge: How can we determine if the photon that we detect has been excited by the targeted fluorophore, and not by its surrounding autofluorescent components?

For the targeted cancer cells to be detected, the signal of the target-specific fluorescence agent must be significantly higher than the nonspecific autofluorescence. Although autofluorescence is much lower when NIR light is used, the signal-to-background ratio (SBR) must be sufficient to distinguish the photons of the target-specific fluorescent agent from the autofluorescence signal.

What Are the Consequences of These Effects for Fluorescence Imaging of Cancer?

We have now set the stage for optical imaging in tissues and gained a clear picture of the problems that we face when we aim to detect a minimal amount of tumor-specific fluorescence agent within this diffuse heterogeneous medium containing absorbers, scatter events, and endogenous fluorophores. First, the fluorescence intensity is not only influenced by the concentration and fluorescence quantum yield of the fluorescent agent but also by the tissue optical properties that are involved. For example, as a result of absorption by blood, fluorescence signals in organs with high blood volumes, such as the liver (19) or highly vascularized tumors, or in tumor cells that co-opt host vessels (20) may appear lower than surrounding less-absorbing tissue, even if they contain larger amounts of contrast agent compared with the surrounding tissue (21). Similarly, less-absorbing lesions (e.g., cysts and lymph nodes) may seem
Strategies to Reduce the Influence of Absorption and Scattering on the Image

With the state-of-the-art technological advancements, it remains impossible to determine all absorbers and scatter events within a diffuse, inhomogeneous medium. However, calculation methods have been developed that try to estimate the perturbation caused by optical tissue properties and improve the image by partly correcting for these properties (14). An intraoperative fluorescence imaging system has been developed that implements such a correction scheme for light intensity variation in tissues (21). Improved accuracy was shown within phantoms and postmortem tissues, independently of optical property variation in tissues. At a 5-fold change of absorption variation within the fluorescent lesions, quantification errors were reduced from 25% in uncorrected images to 8% using the correction scheme (21).

A second new technology being investigated in this field is fluorescence differential path length spectroscopy, which determines fluorophore concentration based on the fluorescence intensity corrected for absorption (22). This facilitates quantitative concentration measurements even for strong variations in either background absorption or scattering. However, currently this method can only be conducted using fiber-optic measurement at a single point. An imaging version and subsequent intraoperative applications have not been developed yet. Finally, tomographic reconstruction techniques (23), Raman spectroscopy (24), and photoacoustic imaging (12, 25) may play important roles in the future, although currently they are not suited for real-time imaging in the surgical theater. Raman spectroscopy is based on inelastic scattering: the effect on the frequency of excitation photons that changes upon interaction with tissue, which is independent of optical tissue properties or autofluorescence (24, 26). In surface-enhanced Raman spectroscopy (SERS), tumor-specific nanobodies are injected that are able to increase the intrinsically very low Raman effect, thereby improving detectability. Promising preclinical results of brain tumor resection guided by SERS have been reported (27). In photoacoustic imaging (also referred to as optoacoustic imaging), thermoelastic expansion of molecules resulting from laser pulse irradiation causes emission of acoustic waves that can be measured by photoacoustic spectroscopy. The resolution in photoacoustic imaging is not limited by tissue scattering but by the attenuation of acoustic frequencies by tissue (12, 25).

This technique improves deep-tissue imaging but is less suitable for image-guided surgery.

Strategies to Distinguish the Target-Specific Fluorescence Signal from Autofluorescence

Two methods are now under investigation that represent attempts to separate the target-specific fluorescence signal from the nonspecific autofluorescence. The first is based on differences in the fluorescence spectrum between photons from these two fluorescent sources (i.e., spectral unmixing), and the second method exploits differences in the fluorescence lifetime of the fluorophores (i.e., lifetime imaging).

The concept of spectral unmixing is based on the "signature" emission intensity that each fluorophore has at certain wavelengths, providing the fluorophore with its own specific emission spectrum (28). Using spectral unmixing, the signal is decomposed into a collection of predefined spectra that is used to determine the individual contribution of each fluorophore. Using calculation models combined with the fluorophore-specific emission spectrum as reference (i.e., linear unmixing), the contribution of each fluorophore in a total fluorescent signal can be extracted (29). When spectral signatures of the fluorophore of interest and the autofluorescence are known, unmixing these specific fluorescence spectra may result in a more accurate SBR. The first-in-human trial on intraoperative tumor-specific fluorescence imaging was conducted using a camera system that was based on spectral unmixing technology (9, 21). There are, however, fundamental problems with this approach. Linear unmixing is based on the assumption that the measured spectrum consists of the sum of the fluorescence spectra of all the components in the tissue, that is, linear mixing. As mentioned earlier, inhomogeneous optical properties influence the path that photons travel through the imaged tissue and therefore not only put their own signatures on the fluorescence spectrum but may also do so nonlinearly owing to the inhomogeneous nature of tissue. Furthermore, the unmixing procedure is a very complex process that requires all components contributing to the spectrum to be known, as well as all of their specific "signature" spectra, which also need to be sufficiently distinctive from one another.

The second method, fluorescence lifetime imaging, distinguishes individual fluorophores by their specific temporal decay after excitation. This fluorescence decay (i.e., lifetime) is a fluorophore-specific characteristic that is not influenced by the local concentration of fluorophores, the optical path, the local excitation intensity, or the local fluorescence detection efficiency. To acquire the characteristic decay curves, a picosecond laser pulse is used for excitation and fluorescence is measured as a function of time. On the basis of the specific lifetime of the fluorophores, the target-specific fluorescence signal can be distinguished from the nonspecific autofluorescence. In addition, the temporal response at which photons emerge from the tissue can be measured (i.e., time-domain imaging), which is used to estimate the concentration and depth of the
flourescent source. Lifetime imaging requires specific conditions in terms of pulsed excitation and data capturing with complex data processing, which currently makes it a time-consuming technique. Although intraoperative lifetime-based techniques are being developed at a strong pace and hold promising advantages, it will take some time before they can be used in a real-time intraoperative setting where mobility of the equipment and speed of data processing and interpretation are of the essence (30).

What Is Required for a Target-Specific Fluorescence Image?

So far, we have assumed that the fluorescent agent has been able to specifically target the tissue of interest with subsequent colocalization between the fluorescent signal and tumor cells. However, to gain a complete insight into different phenomena that can influence the optical image, it is necessary to understand the challenges of tumor-specific targeting.

On the basis of the hallmarks of cancer (31), a growing variety of tumor-specific targets are available for imaging of cancer (2). The efficiency of target-specific agents to reach their intended target is defined by many variables, including affinity of the agent and abundance of the target receptors or epitopes (16). To achieve target-specific fluorescence imaging, the contrast agent has to be delivered to the target, requires adequate contact time with the target for binding to occur, and has to be retained by the target while nonbound agents are cleared from the circulation (10). It has been reported that at saturating doses of the agent, high-affinity antibody uptake is dependent on antigen expression levels. However, at subsaturating doses, the signal is generally limited by delivery of the agent (32).

For the agent to be effectively delivered to the target, many barriers in the human body have to be passed. Next to inhibitor proteins present in plasma that can nonspecifically bind to the agent (1), walls of blood vessels provide a first barrier for drug delivery to targeted tumor tissues. In most cases, abnormal neovascularization of the tumor occurs [i.e., enhanced permeability and retention (EPR) effect] resulting in leaky tumor blood vessels (33). As a result, macromolecular drugs can traverse the endothelium of these leaky blood vessels and passively accumulate in the interstitium of tumor tissues (33). However, the EPR effect is largely dependent on the size of the agent; larger agents are less efficient in crossing the endothelial barrier (34).

Tumor growth beyond the size of approximately 1 mm is dependent on oxygen and supply of nutrients and therefore requires angiogenesis (35). However, in smaller lesions, the angiogenic switch may not have occurred and drug delivery could be hampered due to the lack of adequate vascularization. In other cases, tumor cells may grow alongside preexistent host vasculature, a process known as vessel co-option (20). In these cases, in which angiogenesis-directed targeting may not yet be possible, indirect indicators of tumor growth could be useful for tumor detection, even in the earliest stages of carcinogenesis. For example, NIR fluorescence agents that detect proteases that are involved in migration of tumor cells and degradation of the extracellular matrix can allow for imaging of the invasive tumor front (36–38), and preneoplastic lesions in Barrett esophagus can be identified by targeting changed patterns of lectin binding (39).

Once extravascular, two barriers for adequate binding of the agent remain. First, the agent has to cross extracellular matrix tissue surrounding the target cells (33). At this point, diffusion into the tumor is sometimes impeded by high hydrostatic pressure of many solid tumors, preventing homogeneous infiltration of the agent (1). Second, binding of the agent to specific epitopes of the cancer cell has to occur, and internalization of the agent can further amplify the fluorescence signal (23, 40, 41). Therefore, the cellular basement membrane is the third barrier, which can be passed using the transporter system of the cell by receptor-mediated endocytosis (33). Finally, clearance of non-bound agent from the circulation is required to provide sufficient SBRs. This occurs through the liver (i.e., excretion into bile and feces) and/or the kidney (i.e., excretion into urine). Both the route of clearance and the clearance rate are important determinants for the blood half-life time and consequently the background signal and optimal imaging time of contrast agents (40).

How Relevant Is All This for the Clinical Practice of Surgical Oncology?

Optical imaging is a complicated process. To adequately interpret the intraoperative fluorescent image of the tumor that is presented, the surgeon should comprehend that the target-specific fluorescent agent had to get to and stay at the tumor, that part of the signal was reduced and distorted by absorbers and scatter events, and that the signal had to be subtracted from its autofluorescent surroundings. Considering all these influences that could hamper detection of cancer cells that are located under the surface, the additional value of NIR fluorescence imaging over conventional surgery could be questioned. After all, after a mere 3 decades of research, the fundamental principles of optical imaging still leave room for error, and it currently seems that this approach will never be unambiguous.

To determine the additional value of intraoperative optical imaging, we need to ask ourselves what the fundamental purpose of this tool should be for surgical oncology. In essence, the goal of surgery is to remove all cancer cells while minimizing damage to surrounding healthy tissues. Considering the small size of a single cancer cell or group of cells, the limited target epitopes available for the fluorescent agent and the optical tissue properties and autofluorescence, detection of the last cancer cells under a surface will not always be possible. Nevertheless, it is very likely that the current technique of optical imaging, with its limitations, will offer improvement of the conventional surgical practice to successfully treat the patient and could provide a final solution to the conundrum of irradical resection.

The most obvious reason is that, although there are limitations when considering the cellular level, optical
imaging allows for a more detailed delineation of the tumor margins than the conventional practice of assessment by palpation and visual aspects of the tumor. It was repeatedly shown in animal models that tumor margins can be clearly demarcated by optical imaging (10, 38, 42). Consequently, the surgeon can resect the tumor based on these images with the required tumor-free margin outside the fluorescent tissue. Moreover, in some cases that have thin tumor strands that are invisible to the naked eye, a tumor-free margin of up to several centimeters is required for local control using conventional surgical practice. Although effects of scattering could result in a fluorescent halo of up to several millimeters under certain conditions, detection of these tumor strands using optical imaging would still largely reduce the need of these large tumor-free margins, resulting in improved postoperative functionality. Although yet to be proven in large clinical trials, it is anticipated that this approach will already lead to a decrease in the local recurrence rate and improved patient survival and functionality.

Second, it has been suggested that penetration depth limitations might not be relevant for surgical practice because the surgeon will, by definition, bring the area of interest closer to the surface during the surgical procedure (34, 43). When, after resection of the tumor mass, an area is found with persisting fluorescence signal indicating irradical margins, this could be resected subsequently until no more signal is found in a "cut the light Procedure," similar to the technique of Mohs surgery. Although a similar procedure, optical imaging will be much more efficient than Mohs surgery due to real-time acquisition and the fact that intraoperative pathologic evaluation is not required. At the surface, the influence of optical properties will be minimized, improving the sensitivity even in cases of perineural or perivascular growth, as long as the photons from the contrast agent can be differentiated from autofluorescence.

Finally, although it is currently impossible to completely correct for an unknown variability in optical properties of the tissue, we should not forget that a very advanced system is available that has the capability to identify distinct tissue components (e.g., blood vessels) during the surgical process: the surgeon him or herself. The true additional value of this technique will become clear once the surgical expertise to distinguish tissue components becomes incorporated into the interpretation of the images. For example, the surgeon would preferably reinvestigate a suspicious region that has no fluorescence signal if it is covered by a blood vessel as long as the surgeon realizes that vessels can be highly absorbent. Using the knowledge about the tissues at hand, optical image-guided surgery will result in a dynamic and flexible process providing the surgeon with valuable additional information during the entire operation. This will likely require a learning curve that would follow the encouraging example of pioneer work in resection of malignant gliomas using 5-aminolevulinic acid. Preliminary intraoperative studies in this field provide detailed reports on these learning processes (44, 45). These studies showed that photons are highly absorbed by blood or cauterized tissue debris after monopolar cautery resection and that suction of these layers or rinsing the surface with saline is required for unperturbed assessment of tissue fluorescence. Furthermore, necrotic tumor centers accumulated little fluorescence, which was not a problem because necrosis could easily be distinguished under white light and therefore did not impair fluorescence-guided tumor resection. Tissues were not falsely labeled by fluorescence from blood contaminating the tumor cavity, which might have been expected if plasma had contained substantial amounts of fluorescent agent. Photobleaching after overexposure was minimized with improved development of fluorophores. Finally, the fluorescent source could be located by manipulation of the tissues, as illustrated in Fig. 5. The success of this approach has led to a randomized controlled multicenter phase III trial showing improved progression-free survival in patients with malignant glioma using fluorescence-guided surgery (46).

What Level of Accuracy Is Required for the Routine of Surgical Practice?

Considering the intraoperative flexibility of a trained surgeon and the practice of exploration of the area of interest during surgery, we believe that optical image-guided surgery has the potential to greatly reduce the tumor-free safety margin. If a tumor-free margin could be minimized, postoperative functional outcome could be drastically improved in many cases where the tumor is surrounded by important anatomical structures. This would require tumor imaging with optimal sensitivity and specificity of the tumor-specific agent, followed by resection of the fluorescent tissue without any excess margin of healthy tissue. It is anticipated that sensitivity and specificity of the target-specific agent will be optimized by simultaneously targeting multiple tumor characteristics at different wavelengths (42, 47). To adequately assess the fluorescent border, high accuracy of the imaging technique is demanded. This would require high-resolution images that can be acquired at higher magnifications than the regular macroscopic field of view. Although not suitable for intraoperative use, a system that incorporates both macroscopic and microscopic imaging has been tested in an experimental setting of cancer detection (23). In the macroscopic field of view, the smallest lesion detectable with a highly expressed antigen (e.g., several million antigens/cell) was approximately 600 μm, a volume that approaches pixel resolution of the macroscopic images (110 μm/pixel). Higher magnification of this tumor revealed images down to the single cell level due to higher resolution (23).

These results suggest that incorporation of zoom function into the intraoperative camera system would improve surgical accuracy by using a combination of macroscopic imaging (to survey the tissue and guide tumor resection) and microscopic imaging (to verify clean resection margins; ref. 40). However, scattering generally determines the sharpness of the in vivo fluorescence image, especially for deeply located fluorescent sources, and not the imaging equipment. Furthermore, microsurgical or robotic camera systems are only used in some oncolgical surgical specialties...
Therefore, in daily routine and logistics of surgical practice in most other cases, the tremor of the surgical hand will limit removal of the tumor with accuracy smaller than 0.5 mm. As a result, the accuracy of removing the fluorescent tissue will not only be limited by the resolution of optical imaging but also by the practical limitations of the surgical hand. As long as microscopic or robotic surgery is not a routine practice, the discussion on the level of resolution will lie within the margin of technical surgical possibilities. In analogy to highly accurate stereotactic radiotherapy using Cyberknife that has a technical error margin of 0.5 to 2 mm (48), it is very likely that this level of accuracy will be sufficient to adequately treat the patient with minimal unnecessary loss of functionality.

**Challenges for Clinical Translation**

Although a rapidly increasing amount of data is supporting the additional value of optical image-guided surgery for cancer therapy, challenges remain in translation of the preclinical experimental setup into routine clinical practice (2, 6). The technique requires development of tumor-specific fluorescence agents and dedicated intraoperative camera systems. Important progress has been made over the past few years in both fields, but it is essential to identify the hurdles that are still impeding successful clinical translation.

An intrinsic limitation of development of fluorescence tumor-specific agents is the fact that such drugs are used as diagnostic tools instead of therapeutic drugs that require administration over a longer period of time. Development of diagnostic drugs is therefore subject to lower financial incentives for pharmaceutical companies. The first obvious steps were taken by conjugation of already clinically available tumor-specific agents (e.g., cetuximab) to fluorophores (2, 49). Until the technique will become available at a large scale, further development of these agents will have to be carried out by nonprofit (i.e., academic) organizations. Furthermore, dedicated imaging systems will have to become easily available for a large group of surgeons to stimulate adoption of the technique. Currently, the most advanced systems are still only available in the research setting of clinical trials (7, 9), whereas other systems are already commercially available (50, 51). These economic and implementation issues are critical for successful adoption of surgical optical imaging.

**Conclusions**

Optical imaging has the potential to revolutionize cancer surgery by real-time fluorescence guidance in discriminating between healthy and diseased tissues and identifying vital structures. However, absorption, scattering, and autofluorescence are fundamental optical tissue properties that influence the images and limit the ability to differentiate the targeted tissue from its surroundings. In pursuit of removing all cancer cells by resection of the total tumor-specific fluorescence signal, the oncologic surgeon should carefully consider these limitations during the process of image-guided cancer surgery. Although there are limitations to the intrinsic capacity of the technique, when practical and technical surgical possibilities are considered, optical imaging can be a very powerful intraoperative tool in guiding the future oncologic surgeon toward radical resection and optimal clinical results.

**Disclosure of Potential Conflicts of Interest**

H.J.C.M. Sterenborg is employed (other than primary affiliation; e.g., consulting) as a CEO in Luminostix bv. No potential conflicts of interest were disclosed by the other authors.

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**References**
