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Fluorescence Imaging in Surgery

Ryan K. Orosco,

Division of Head and Neck Surgery, University of California San Diego, La Jolla, CA 92093-0647
USA

Roger Y. Tsien, and

Department of Pharmacology, Howard Hughes Medical Institute, University of California San
Diego, La Jolla, CA 92093-0647 USA

Quyen T. Nguyen

Division of Head and Neck Surgery, University of California San Diego, La Jolla, CA 92093-0647
USA

Abstract

Although the modern surgical era is highlighted by multiple technological advances and innovations, one area that has remained constant is the dependence of the surgeon's vision on white-light reflectance. This renders different body tissues in a limited palette of various shades of pink and red, thereby limiting the visual contrast available to the operating surgeon. Healthy tissue, anatomic variations, and diseased states are seen as slight discolorations relative to each other and differences are inherently limited in dynamic range. In the upcoming years, surgery will undergo a paradigm shift with the use of targeted fluorescence imaging probes aimed at augmenting the surgical armamentarium by expanding the “visible” spectrum available to surgeons. Such fluorescent “smart probes” will provide real-time, intraoperative, pseudo-color, high-contrast delineation of both normal and pathologic tissues. Fluorescent surgical molecular guidance promises another major leap forward to improve patient safety and clinical outcomes, and to reduce overall healthcare costs. This review provides an overview of current and future surgical applications of fluorescence imaging in diseased and nondiseased tissues and focus on the innovative fields of image processing and instrumentation.

Index Terms

Cell-penetrating peptides; fluorescence; fluorescence imaging; molecular imaging; optical imaging; surgery; surgery; computer-assisted; surgical procedures; operative

I. Introduction

In recent years, advances in whole body imaging and diagnostics have enhanced patient selection for surgical interventions. Concomitant innovation in surgical technique and minimally invasive approaches with laparoscopic, endoscopic and robotic techniques has advanced many surgical procedures. In spite of innumerable advances, surgery still relies primarily on white-light reflectance. This approach does not allow differentiation between normal and diseased tissue beyond gross anatomical distortion or discoloration. The emerging field of fluorescent surgical imaging promises to be a powerful enhancement to traditional low-contrast white-light visualization, offering real-time pseudo-color delineation of complex anatomic structures. Improved visualization will lead to more complete removal of disease, decreased inadvertent injury to vital structures, and improved identification for repair of damaged tissues.

Fluorescence imaging for surgical guidance is a rapidly expanding field. A PubMed search for “fluorescence imaging” and “surgery” demonstrates the exponential growth of published efforts; with 36 articles in 1999 rising to over 300 in 2011. The volume of work relating to fluorescence imaging in cancer operations has similarly expanded over the last two decades. Despite this rising excitement, clinical trials have barely begun, [1]–[5] and much more translational effort will be required to harness this exciting technology. The complexity of fluorescent surgical molecular guidance necessitates a multidisciplinary approach as it progresses through development, testing, implementation, and widespread adoption. The future of fluorescence guided surgery depends on the parallel development of high-quality fluorescent compounds that perform specific identification roles, and on image processing and instrumentation to display the images.

A complete list of currently available fluorescent probes has recently been reviewed and will not be repeated here. [6], [7] Discussions of probe biomechanics, and specific applications have also been reported recently. [6], [8]–[16]

The technical challenges of fluorescent probe development, image processing and instrumentation are immense. Creative ingenuity is required to realize the full potential of fluorescence guided surgery. This review offers a framework for conceptualizing fluorescence guided surgery by highlighting current and future surgical applications in diseased and nondiseased tissues. We also focus on the innovative fields of image processing and instrumentation, which strive to merge the traditional surgical visual field with fluorescent guidance. The discussion on these technologies will focus on fluorescent signal detection, signal-to-noise optimization, and display.

II. Visualization of Diseased and Nondiseased Tissues—A Critical Problem

Surgical management of disease is an integral part of health care. The core goals of surgery are to repair, remove, or correct diseased tissues while limiting un-necessary damage to healthy structures. Achieving these goals allows optimal disease treatment while preserving form and physiological function. Successful surgery depends on multiple factors, first and foremost being visualization. Bringing targeted fluorescence imaging and into the operating room dramatically expands surgeons' visual capabilities, allowing them to see structures as they extend beyond the surface tissue; even prior to their actual physical exposure. Improved visualization with fluorescence labeling enables more complete resection of surgical disease, while protecting adjacent vital structures.

Enhancing the visualization of tissues based on structure could be equated to “painting” tissues in the surgical field to look more like a multicolored anatomy textbook; where nerves are yellow, lymphatics green, veins blue, and arteries red (Fig. 1).

In the images of a mouse facial nerve shown in Fig. 2, we see a case of normal (nondiseased) facial nerve that is difficult to distinguish with white-light alone. Viewing this same surgical field using fluorescence guidance allows easy identification of the delicate tendrils of facial nerve as they course near and around structures which otherwise obscure the view. [17] Protecting fine nerves during surgery requires high-contrast visualization. Unintentional damage to these fine nerves means postoperative functional deficits or pain.

An example where unpredictable tumor growth leads to more aggressive surgical resection is shown in Fig. 3. A squamous cell cancer is readily seen as gross structural distortion and discoloration on the side of the tongue. Successful surgical removal of this cancer depends on knowing the extent of the tumor cells. Beneath the surface where the cancer extends in an unpredictable growth pattern (Fig. 3, green line), visual contrast no longer allows the surgeon to reliably see the tumor. Knowing the risk of leaving cancer cells behind, the

surgeon makes an educated guess regarding the extent of cancer growth and makes cuts accordingly (Fig. 3, blue line). The cut edges of the surgical specimen are checked with intraoperative pathological review which adds to operative time and cost. Still sometimes, the final pathology shows the undesirable finding of cancer cells at the edge of the cancer resection; a “positive margin.”

An example where tumor cells can be invisible to the human eye, but readily seen with targeted fluorescence imaging can be seen in Fig. 4. Fluorescent activatable cell-penetrating peptide (ACPP) was used in a mouse model of metastatic breast cancer. [18] Cancer cells that could have easily been left behind after surgery stand out in the fluorescence image. Fluorescent labeling of malignant cells would remove potential ambiguity of the cancer borders during surgical dissection where most tissues are varying shades of reds and pink, leading to more effective and safer surgery.

III. Fluorescent “Visual Enhancers” and “Smart Probes” for Surgery

Surgical fluorescence imaging is already being implemented through the use of indocyanine green (ICG) and fluorescein. These dyes are nontargeted fluorescent “visual enhancers” that can be used to highlight blood vessels, ducts, and other structures. Despite their nontargeted labeling, they have significant utility in many surgical applications.

In contrast to nonspecific visual enhancers like fluorescein and ICG, “smart probes” are fluorescently labeled agents targeted to a specific tissue type or biochemical process. This affords the possibility of true surgical molecular guidance. Targeted smart probes can work via adherence to tissue specific markers such as antibody recognition of cell surface antigens, [19], [20] peptide or aptamers with increased affinity for cell surface proteins [17], [21] or through specific enzymatic localization and amplification. [18], [22]–[34]

Both traditional fluorescent visual enhancers and fluorescently labeled-smart probes increase the contrast across key tissue types to augment the surgeon's visualization over traditional white-light reflectance modalities.

IV. Clinical Need and Applications

A. Non-Diseased Tissues/Structural Targets

In any surgical procedure, accurate identification and preservation of normal structures is critical to avoid iatrogenic injury. Most fluorescent agents that highlight nondiseased tissues take advantage of structural characteristics or prevalent binding sites, and illuminate an entire structure or tissue-type. Probes highlighting nondiseased tissues would benefit trauma and reconstructive procedures, as well as any case where improved identification of vital structures would make surgery safer or more efficient.

Following trauma, the repair of injured bones, tissues, nerves, and vessels requires accurate identification of relevant structures in the context of distorted anatomy. Frequently, nerves and tissues encountered in the surgical dissection are not easily identified because of tissue edema, inflammatory changes, and/or scarring. In the case of traumatic motor nerve transection, the distal nerve-end may be identified with electromyography for a several days following injury. Unfortunately, this technique is not useful at later times, it cannot help identify the proximal nerve-end, and it is not useful for autonomic and sensory nerves. This is just one realm where fluorescent targeting of nerve tissue would powerfully augment traditional identification techniques. The identification of healthy nerves can be expanded to a myriad of surgeries where fine nerve anatomy portends increased risks for damage and visualizing and these structures can decrease morbidity. An overview of this and other structural applications for surgical fluorescent imaging is provided in Table I.

B. Diseased Tissues/Functional Targets

Diseased tissues offer unique opportunities for targeted molecular guidance using fluorescent smart probes. The broad surgical categories of infection, atherosclerosis, and neoplasms represent three realms of surgery that are well-suited for future fluorescence imaging applications.

i) Infection/Devitalized Tissue—In cases of infection or tissue ischemia, surgery is often used to excise devitalized tissue. Surgical debridement relies on white-light reflectance to determine which tissue is healthy, and which is nonviable or has vascular compromise. This technique has low sensitivity and specificity and can lead to excessive removal of healthy tissue. [35]

In the setting of acute invasive fungal sinusitis, current management depends on aggressive, thorough debridement of all involved structures. The extent of excision is largely dictated by the gross appearance of tissues—described as subtle hues of grey amidst a sea of pink mucosa. The surgery relies on pathological sections of the surgical borders to assess the extent of fungal invasion. If fungal spread could be more sensitively detected in the operating room, surgery could spare adjacent un-involved structures and be more efficient. Targeted fluorescent probes could fulfill this need by illuminating necrotic tissue, or even fungal species themselves.

With a similar goal, fluorescein has been used in general surgery for some time to identify devitalized segments of intestine, but there is certainly room for improvement. Better visualization of infected, devitalized, and ischemic tissue would benefit surgeons of all specialties.

ii) Atherosclerosis—In addition to cancer, atherosclerosis is a major cause of morbidity and death worldwide. [36] The disruption of plaques during surgical treatment of atherosclerosis such as in carotid endarterectomy (removal of atherosclerotic plaques from the carotid arteries) and coronary artery bypass grafting (surgically adding another vessel to provide blood flow to different parts of the heart when the native artery is blocked), can cause an embolic stroke or a heart attack, respectively. While taking into account associated clinical factors, candidacy for these two procedures is largely anatomic in nature; dependent on the location and degree of vessel stenosis. [37], [38] Plaque-specific factors such as likelihood of rupture are not easily predicted using current methods and are usually not part of the presurgical decision making process. Importantly, the majority of acute heart attack and stroke occur not as a gradual progression of increasing stenosis, but rather as a sudden event where the blood flow through a diseased vessel is abruptly disrupted from an acute embolic event. Thus, accurate identification of these “vulnerable” plaques in susceptible patients would mitigate unnecessary surgery in patients with stable plaques and identify patients at high risk of plaque rupture. [39]–[42] Furthermore, the ability to observe processes leading to vulnerable plaque formation can be applied to other noninvasive imaging modalities (CT, MRI, and ultrasound). [43]–[46]

iii) Cancer—With the primary treatment modality for most solid cancer being surgery, [47] complete removal of all malignant cells is unquestionably essential. If all cancer cells are removed with surgery, the patient is cured of that cancer. Unfortunately, visual inspection using white-light reflectance has very low sensitivity for identifying malignant cells. This leads to a considerable amount of “educated guess-work” when it comes to the extent of tissue removal for a cancer surgery (see Fig. 3).

Molecularly targeted fluorescent probes would allow improved visualization of tumor extent and detection of cancer cells that may have otherwise been left in the patient if relying on

white-light reflectance alone. The removal of lymph nodes that potentially harbor tumor cells (sentinel or regional lymph node dissection) is another frequently performed cancer surgery that would benefit from the fluorescent illumination of cancer cells. In the context of cancer surgery, the ability to accurately visualize tumor cells at the molecular level at both primary and metastatic sites will improve diagnostic accuracy, reduce unnecessary surgery, and improve completeness of resection. Details regarding specific probes can be found in a recent review, [6] and recent work in fluorescence guidance in diseased surgical processes is summarized in Table II.

V. Instrumentation

It is not practical to conduct surgery while viewing only the fluorescence of the contrast agent. Instead, fluorescence images should be superimposed continuously in real time and in precise spatial register with reflectance images showing the morphology of the normal tissue and the location of the surgical instruments. Large-scale implementation of fluorescent guided surgery in humans will require advances in signal detection, signal-to-noise optimization, and display.

A. Detection

White-light reflectance is inherently brighter than fluorescence, in that the fraction of incident photons returned by reflectance is much higher than that from fluorescence, even from a strongly labeled tissue. Also, fluorescence requires spectral separation between intense short-wavelength excitation and relatively dim longer-wavelength emission, filtered to remove reflected excitation wavelengths. The simplest but least flexible solution, feasible mainly for dyes excited in the violet or blue range, is to tailor excitation and emission filters to provide a mixture of reflectance and fluorescence directly visible by eye or a standard color camera. An early example was the visualization of the fluorescence ((Leica FL400—excitation at 380–120 nm, display at 480–720 nm and Zeiss OPPI Pentero—excitation 400–10 nm, display 620–710 nm) from protoporphyrin IX generated in tumors by systemic administration of the metabolic precursor, 5-aminolevulinic acid. [4] The red emission filter was deliberately made slightly leaky to violet so that normal tissue landmarks and surgical instruments were visible by violet reflectance while cancer tissue glowed red. Normal full color reflectance was sacrificed so that image processing could be completely avoided. Similarly, fluorescein fluorescence (excitation 488 nm, emission > 500 nm) can be viewed with an excitation filter mainly passing blue but with some leakage of green and red, paired with an emission filter preferentially passing green but with slight leakage of blue and somewhat more of red. The leakages allow blue, green, and red reflectances to generate a white-light reflectance image with green fluorescence superimposed (Zeiss Pentero). The advantage of these approaches is that the only modification of standard equipment is the inclusion of judiciously tailored excitation and emission filters. The disadvantages are 1) inflexibility, in that the filters have to be optimized for a given tissue concentration of a given dye, 2) spectral distortion of the reflectance image (in the worst case, confinement to violet), and 3) complete reliance on the surgeon's subjective color discrimination to decide how much fluorescence is sufficient to warrant resection.

The next higher level of sophistication is to provide separate cameras for reflectance and fluorescence respectively, so that the gain of each can be separately controlled, and the reflectance camera does not have to look through the emission filter required by the fluorescence camera. This is the obvious approach when the fluorophore excitation and emission wavelengths are in the NIR, [8], [48] because the surgical field can be illuminated with white visible light (400–650 nm) plus 760 nm in the case of indocyanine green. The reflectance camera sees the white-light reflectance but not the NIR, while the fluorescence camera sees the 800 nm emission through a suitable long-pass filter, so that the reflectance

and fluorescence channels are independently adjustable in gain. [8] A bit more ingenuity is required when the fluorophore excitation and emission are at visible wavelengths. One solution is to confine the excitation to three narrow spectral lines of blue, green, and red, say 488, 543, and 633 nm [49] whose relative intensities are adjusted to generate a reasonable simulation of white-light for the reflectance camera to monitor. Ideally, one of these wavelengths should be optimal for exciting the fluorophore. Meanwhile the fluorescence camera looks through an emission filter selective for one or more of the wavelength gaps between the sharp excitation lines. Depending on the fluorophore, some sacrifice of emission band-pass is likely to be necessary to avoid interference from the next longer illumination line. Also, it is cumbersome to adjust the intensity of the fluorescence excitation relative to the other lines making up the white-light illumination. In our view, the best way to control reflectance and fluorescence independently is to alternate them at 15–25 Hz rather than rely on complex custom interference filters. [17], [50] Light-emitting diodes are readily pulsed to provide 20–33 ms white-light followed by 20–33 ms fluorescence excitation. Light returning from the surgical field is split by a 90:10 neutral beam-splitter to monochrome fluorescence and color reflectance cameras, each of which integrates photons during its appropriate illumination period. The resolutions and sensor dimensions of the two cameras are matched to facilitate registration of their images. Simple image processing provides a continuous high-resolution display with an imperceptible lag time. Provided that the display is fast enough and has enough resolution, there is no difficulty operating while viewing the monitor rather than looking directly at the actual surgical field. This time-multiplexing approach allows the spectral characteristics and gains of the reflectance and fluorescence channels to be varied completely independently and therefore allows maximum flexibility to cope with different dyes and tissue concentrations during the same imaging session in the same patient. We also find it useful to insert polarizers into the white-light and reflectance channels to attenuate distracting specular reflections from shiny wet surfaces.

Fluorescence guided surgery is important across both “open” and “minimally invasive” procedures. The basic concepts of signal detection are the same for both applications. Wide-field detection comprises a camera directed into an open surgical field, as the surgeon operates with direct line-of-sight visualization. The resultant fluorescence image is displayed on a screen for the surgeon to refer to in real-time during the operation. Minimally invasive surgeries are performed with endoscopes, and the surgeon does not have direct line-of-sight into the surgical field. For minimally invasive applications, detection instrumentation must be miniaturized to adapt to the endoscopic setup. Signal strength decreases with the use of smaller and smaller endoscopes, requiring improved detection and signal optimization techniques.

B. Signal-to-Noise Optimization

An important area of refinement is to correct for variable attenuation and blooming of fluorescence by tissue absorbance and scattering. A first-order correction for attenuation, especially by tissue absorbance, is to divide the raw epifluorescence intensity by the reflected intensity of the excitation beam. [10], [51] This correction can be applied at video rates and copes reasonably well with up to 3× variations in absorption. Scattering can cause a major overestimation of the lateral dimensions of a buried fluorescent volume and is a much more difficult effect to deconvolve. Methods have been proposed for tomographic reconstruction using laser scanning in epi-illumination mode which is the only practical geometry of illumination of a human patient. Unfortunately, such methods currently require acquisition times of several minutes and computational reconstruction times on the order of hours, so they are not yet practical to apply intraoperatively. [52] On the one hand, tomography represents a major technical challenge for future progress. On the other hand, the tissue being cut is inherently superficial at the moment of its resection, so that as long as

disease is detectable at all, its optical clarity will improve as the surgeon dissects each successive plane.

C. Display

For the user interface display, the simple approach is to show the reflectance and fluorescence images side-by-side or alternating on a single monitor. We feel this imposes an unnecessary strain on the surgeon to mentally register the two images accurately. A better solution is to display the fluorescence in a pseudo-color such as green or aqua, normally never present in tissue, superimposed on the color reflectance image. [48] Simply averaging the reflectance and fluorescence pseudo-color images makes both look dim. We prefer to use the fluorescence intensity F as a transparency factor where the (displayed intensity) $R, G, B = (1 - F/F_{\max})(\text{reflectance intensity}) R, G, B + (F/F_{\max})(\text{saturated pseudo-color}) R, G, B$. Here $F_{\max}$ is the maximum allowed value of F , either auto-scaled from the actual image or preset by the user. There are times during the operation when the surgeon prefers to see just the standard reflectance image or the fluorescence image alone, so we provide a foot pedal for hands-free cycling between these two modes and the overlay mode.

A logical further extension is to provide a second fluorescence channel at another emission wavelength. One obvious purpose would be to visualize both diseased tissue to be resected (e.g., tumor) and normal tissue to be spared (e.g., nerve) effectively simultaneously, using two targeted agents operating at well-separated wavelengths. [17] Another application is to image probes whose emission spectrum shifts from one wavelength band to another upon biochemical activation. The most common mechanism for such probes is that they contain two fluorophores interacting by fluorescence resonance energy transfer (FRET), which quenches the shorter-wavelength donor dye while sensitizing its longer-wavelength acceptor partner. Cleavage of the linker between the probes disrupts FRET so that the emission spectrum reverts to that of the donor alone. Displaying a ratio of the two emission bands isolates the degree of cleavage while canceling out many factors such as probe concentration, tissue thickness, excitation intensity, absorbances that affect both wavelengths equally, and motion artifacts. Emission ratioing can thus significantly increase the sensitivity and specificity of tumor or atherosclerotic plaque detection, justifying the increased complexity of the probe molecules and instrumentation. [53], [54]

VI. Conclusion

The principles of addressing diseased and abnormal tissues while preserving adjacent structures will forever stand as a central tenet of surgical practice. Minimally invasive surgical techniques have altered the mechanics of many procedures and surgical practice has to evolve along with these emerging technologies. Working through minimally invasive interfaces has sacrificed the tactile feedback of directly working with tissue and forced surgeons to rely even more heavily on tissue visualization. Thus, a medical specialty traditionally dependent on touch becomes more dependent on vision alone. Any real time improvement in the intraoperative visual differentiation between different tissue types would represent a significant advance.

We are entering an exciting era, because surgical fluorescence imaging promises to be this much-needed advance. Along with these advances, there remain challenges of defining clinical endpoints and identifying which patients/surgeries would most benefit from fluorescent navigation techniques. In addition to the molecular challenges of developing better fluorescent probes, signal detection, signal-to-noise optimization, and display considerations represent the current technologic obstacles to the realization of real-time, intraoperative, pseudo-color, high-contrast fluorescent guided surgery.

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References

1. Piccirillo SG, Dietz S, Madhu B, Griffiths J, Price SJ, Collins VP, Watts C. Fluorescence-guided surgical sampling of glioblastoma identifies phenotypically distinct tumour-initiating cell populations in the tumour mass and margin. *Br J Cancer*. Jul.2012 107:462–468. [PubMed: 22722315]
2. Roessler K, Becherer A, Donat M, Cejna M, Zachenhofer I. Intraoperative tissue fluorescence using 5-aminolevulinic acid (5-ALA) is more sensitive than contrast MRI or amino acid positron emission tomography ((18)F-FET PET) in glioblastoma surgery. *Neurolog Res*. Apr.2012 34:314–317.
3. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: A prospective study in 52 consecutive patients. *J Neurosurg*. Dec.2000 93:1003–1013. [PubMed: 11117842]
4. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: A randomised controlled multicentre phase III trial. *Lancet Oncology*. May.2006 7:392–401. [PubMed: 16648043]
5. van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJ, van der Zee AG, Bart J, Low PS, Ntziachristos V. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: First in-human results. *Nature Med*. Oct.2011 17:1315–1319. [PubMed: 21926976]
6. Nguyen Q, Tsien RY. Surgical Molecular Navigation with Fluorescence—A New Cutting Edge. *Nature Reviews Cancer*. 2013
7. Luo S, Zhang E, Su Y, Cheng T, Shi C. A review of NIR dyes in cancer targeting and imaging. *Biomaterials*. Oct.2011 32:7127–7138. [PubMed: 21724249]
8. Troyan SL, Kianzad V, Gibbs-Strauss SL, Gioux S, Matsui A, Oketokoun R, Ngo L, Khamene A, Azar F, Frangioni JV. The FLARE intraoperative near-infrared fluorescence imaging system: A first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann Surg Oncology*. Oct. 2009 16:2943–2952.
9. Pierce MC, Javier DJ, Richards-Kortum R. Optical contrast agents and imaging systems for detection and diagnosis of cancer. *Int J Cancer*. Nov.2008 123:1979–1990. [PubMed: 18712733]
10. Themelis G, Yoo JS, Soh KS, Schulz R, Ntziachristos V. Real-time intraoperative fluorescence imaging system using light-absorption correction. *J Biomed Opt*. Nov-Dec;2009 14:064012–064012. [PubMed: 20059250]
11. Gioux S, Choi HS, Frangioni JV. Image-guided surgery using invisible near-infrared light: Fundamentals of clinical translation. *Molec Imag*. Oct.2010 9:237–255.
12. Keereweer S, Kerrebijn JD, van Driel PB, Xie B, Kaijzel EL, Snoeks TJ, Que I, Hutteman M, van der Vorst JR, Mieog JS, Vahrmeijer AL, van de Velde CJ, Baatenburg de Jong RJ, Lowik CW. Optical image-guided surgery—Where do we stand? *Molec Imag Biol*. Apr.2011 13:199–207.
13. Liu Y, Bauer AQ, Akers WJ, Sudlow G, Liang K, Shen D, Berezin MY, Culver JP, Achilefu S. Hands-free, wireless goggles for near-infrared fluorescence and real-time image-guided surgery. *Surgery*. May.2011 149:689–698. [PubMed: 21496565]
14. Taruttis A, Ntziachristos V. Translational optical imaging. *Amer J Roentgenol*. Aug.2012 199:263–271. [PubMed: 22826386]
15. van den Berg NS, van Leeuwen FW, van der Poel HG. Fluorescence guidance in urologic surgery. *Current Opin Urology*. Mar.2012 22:109–120.
16. Verbeek FP, van der Vorst JR, Schaafsma BE, Hutteman M, Bonsing BA, van Leeuwen FW, Frangioni JV, van de Velde CJ, Swijnenburg RJ, Vahrmeijer AL. Image-guided hepatopancreatobiliary surgery using near-infrared fluorescent light. *J Hepato-Biliary-Pancreatic Sci*. Jul.2012 : 626–637.

17. Whitney M, Crisp J, Nguyen L, Friedman B, Gross L, Steinbach P, Tsien R, Nguyen Q. Fluorescent peptides highlight peripheral nerves during surgery in mice. *Nature Biotechnol.* 2011; 29:352–356. [PubMed: 21297616]
18. Nguyen QT, Olson ES, Aguilera TA, Jiang T, Scadeng M, Ellies LG, Tsien RY. Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival. *Proc Nat Acad Sci USA.* Mar.2010 107:4317–4322. [PubMed: 20160097]
19. Kaushal S, McElroy MK, Luiken GA, Talamini MA, Moossa AR, Hoffman RM, Bouvet M. Fluorophore-conjugated anti-CEA antibody for the intraoperative imaging of pancreatic and colorectal cancer. *J Gastrointest Surg.* Nov.2008 12:1938–1950. [PubMed: 18665430]
20. Tran Cao HS, Kaushal S, Metildi CA, Menen RS, Lee C, Snyder CS, Messer K, Pu M, Luiken GA, Talamini MA, Hoffman RM, Bouvet M. Tumor-specific fluorescence antibody imaging enables accurate staging laparoscopy in an orthotopic model of pancreatic cancer. *Hepato-Gastroenterology.* Sep.2012 59:1994–1999. [PubMed: 22369743]
21. Wu AP, Whitney MA, Crisp JL, Friedman B, Tsien RY, Nguyen QT. Improved facial nerve identification with novel fluorescently labeled probe. *Laryngoscope.* Apr.2011 121:805–810. [PubMed: 21328585]
22. Olson ES, Whitney MA, Friedman B, Aguilera TA, Crisp JL, Baik FM, Jiang T, Baird SM, Tsimikas S, Tsien RY, Nguyen QT. In vivo fluorescence imaging of atherosclerotic plaques with activatable cell-penetrating peptides targeting thrombin activity. *Integrative Biol.* Jun.2012 4:595–605.
23. Shu X, Lev-Ram V, Olson ES, Aguilera TA, Jiang T, Whitney M, Crisp JL, Steinbach P, Deerinck T, Ellisman MH, Ellies LG, Nguyen QT, Tsien RY. Spiers Memorial Lecture. Breeding and building molecular spies. *Faraday Discuss.* 2011; 149:9–9. [PubMed: 21413170]
24. Olson ES, Jiang T, Aguilera TA, Nguyen QT, Ellies LG, Scadeng M, Tsien RY. Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proc Nat Acad Sci USA.* Mar.2010 107:4311–4316. [PubMed: 20160077]
25. Olson ES, Aguilera TA, Jiang T, Ellies LG, Nguyen QT, Wong EH, Gross LA, Tsien RY. In vivo characterization of activatable cell penetrating peptides for targeting protease activity in cancer. *Integrative Biol.* Jun.2009 1:382–393.
26. Jiang T, Olson ES, Nguyen QT, Roy M, Jennings PA, Tsien RY. Tumor imaging by means of proteolytic activation of cell-penetrating peptides. *Proc Nat Acad Sci USA.* Dec.2004 101:17867–17872. [PubMed: 15601762]
27. Deu E, Verdoes M, Bogoy M. New approaches for dissecting protease functions to improve probe development and drug discovery. *Nature Struct Molec Biol.* Jan.2012 19:9–16. [PubMed: 22218294]
28. Blum G, Weimer RM, Edgington LE, Adams W, Bogoy M. Comparative assessment of substrates and activity based probes as tools for non-invasive optical imaging of cysteine protease activity. *PLoS One.* 2009; 4:e6374–e6374. [PubMed: 19636372]
29. Blum G, von Degenfeld G, Merchant MJ, Blau HM, Bogoy M. Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes. *Nature Chem Biol.* Oct.2007 3:668–677. [PubMed: 17828252]
30. Blum G, Mullins SR, Keren K, Fonovic M, Jedeszko C, Rice MJ, Sloane BF, Bogoy M. Dynamic imaging of protease activity with fluorescently quenched activity-based probes. *Nature Chem Biol.* Sep.2005 1:203–209. [PubMed: 16408036]
31. Kato D, Boatright KM, Berger AB, Nazif T, Blum G, Ryan C, Chehade KA, Salvesen GS, Bogoy M. Activity-based probes that target diverse cysteine protease families. *Nature Chem Biol.* Jun. 2005 1:33–38. [PubMed: 16407991]
32. Sheth RA, Upadhyay R, Stangenberg L, Sheth R, Weissleder R, Mahmood U. Improved detection of ovarian cancer metastases by intraoperative quantitative fluorescence protease imaging in a pre-clinical model. *Gynecologic Oncol.* Mar.2009 112:616–622.
33. Figueiredo JL, Alencar H, Weissleder R, Mahmood U. Near infrared thoracoscopy of tumoral protease activity for improved detection of peripheral lung cancer. *Int J Cancer.* Jun.2006 118:2672–2677. [PubMed: 16380983]

34. Bullok KE, Maxwell D, Kesarwala AH, Gammon S, Prior JL, Snow M, Stanley S, Piwnica-Worms D. Biochemical and in vivo characterization of a small, membrane-permeant, caspase-activatable far-red fluorescent peptide for imaging apoptosis. *Biochem. Apr.*2007 46:4055–4065. [PubMed: 17348687]
35. Smith F, Dryburgh N, Donaldson J, Mitchell M. Debridement for surgical wounds. *Cochrane Database Syst Rev.* 2011:CD006214–CD006214. [PubMed: 21563150]
36. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Executive Summary: Heart Disease and Stroke Statistics—2012 Update: A Report From the American Heart Association. *Circulation.* Jan.2012 125:188–197. [PubMed: 22215894]
37. Hillis LD, Smith PK, Anderson JL, Bittl JA, Bridges CR, Byrne JG, Cigarroa JE, DiSesa VJ, Hiratzka LF, Hutter AM Jr, Jessen ME, Keeley EC, Lahey SJ, Lange RA, London MJ, Mack MJ, Patel MR, Puskas JD, Sabik JF, Seines O, Shahian DM, Trost JC, Winniford MD, Jacobs AK, Albert N, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Kushner FG, Ohman EM, Stevenson W, Yancy CW. 2011 ACCF/AHA guideline for coronary artery bypass graft surgery: Executive summary: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Thoracic Cardiovasc Surgery.* Jan.2012 143:4–34.
38. Ricotta JJ, Aburahma A, Ascher E, Eskandari M, Faries P, Lai BK. Updated Society for Vascular Surgery guidelines for management of extracranial carotid disease: Executive summary. *J Vascular Surgery.* Sep.2011 54:832–836.
39. Ouldzein H, Elbaz M, Roncalli J, Cagnac R, Carrie D, Puel J, Alibelli-Chemarin MJ. Plaque rupture and morphological characteristics of the culprit lesion in acute coronary syndromes without significant angiographic lesion: Analysis by intravascular ultrasound. *Ann Cardiologie D'angiologie.* Feb.2012 61:20–26.
40. Wu Z, Yang C, Tang D. In vivo serial MRI-based models and statistical methods to quantify sensitivity and specificity of mechanical predictors for carotid plaque rupture: Location and beyond. *J Biomech Eng.* Jun.2011 133:064503–064503. [PubMed: 21744932]
41. Ruiz EM, Papaioannou TG, Vavuranakis M, Stefanadis C, Naghavi M, Kakadiaris IA. Analysis of contrast-enhanced intravascular ultrasound images for the assessment of coronary plaque neoangiogenesis: Another step closer to the identification of the vulnerable plaque. *Current Pharm Des.* 2012; 18:2207–2213.
42. Jackson SP. Arterial thrombosis—Insidious, unpredictable and deadly. *Nature Med.* 2011; 17:1423–1436. [PubMed: 22064432]
43. Ozaki Y, Tanaka A, Tanimoto T, Kitabata H, Kashiwagi M, Kubo T, Takarada S, Ishibashi K, Komukai K, Ino Y, Hirata K, Mizukoshi M, Imanishi T, Akasaka T. Thin-cap fibroatheroma as high-risk plaque for microvascular obstruction in patients with acute coronary syndrome. *Circulation Cardiovasc Imag.* Nov.2011 4:620–627.
44. Kashiwagi M, Tanaka A, Kitabata H, Tsujioka H, Kataiwa H, Komukai K, Tanimoto T, Takemoto K, Takarada S, Kubo T, Hirata K, Nakamura N, Mizukoshi M, Imanishi T, Akasaka T. Feasibility of noninvasive assessment of thin-cap fibroatheroma by multidetector computed tomography. *JACC Cardiovasc Imag.* Dec.2009 2:1412–1419.
45. Makris GC, Nicolaidis AN, Xu XY, Geroulakos G. Introduction to the biomechanics of carotid plaque pathogenesis and rupture: Review of the clinical evidence. *Br J Radiol.* Sep.2010 83:729–735. [PubMed: 20647514]
46. Partovi S, Loebe M, Aschwanden M, Baldi T, Jager KA, Feinstein SB, Staub D. Contrast-enhanced ultrasound for assessing carotid atherosclerotic plaque lesions. *Amer J Roentgenol.* Jan. 2012 198:W13–9. [PubMed: 22194509]
47. Ries, L. SEER cancer statistics review, 1975–2005. Bethesda, MD: National Cancer Institute; Nov. 2008 [Online]. Available: <http://seer.cancer.gov/csr/1975–2005/>
48. De Grand AM, Frangioni JV. An operational near-infrared fluorescence imaging system prototype for large animal surgery. *Technol Cancer Res Treatment.* Dec.2003 2:553–562.

49. Themelis G, Yoo JS, Ntziachristos V. Multispectral imaging using multiple-bandpass filters. *Opt Lett*. May.2008 33:1023–1025. [PubMed: 18451974]
50. Gray DC, Kim EM, Cotero VE, Bajaj A, Staudinger VP, Tan Hehir CA, Yazdanfar S. Dual-mode laparoscopic fluorescence image-guided surgery using a single camera. *Biomed Opt Exp*. 2012; 3
51. Ntziachristos V, Turner G, Dunham J, Windsor S, Soubret A, Ripoll J, Shih HA. Planar fluorescence imaging using normalized data. *J Biomed Opt*. Nov-Dec;2005 10:064007–064007. [PubMed: 16409072]
52. Abou-Elkacem L, Bjorn S, Doleschel D, Ntziachristos V, Schulz R, Hoffman RM, Kiessling F, Lederle W. High accuracy of mesoscopic epi-fluorescence tomography for non-invasive quantitative volume determinati of fluorescent protein-expressing tumours in mice. *Eur Radiol*. Sep.2012 22:1955–1962. [PubMed: 22544295]
53. Whitney M, Savariar EN, Friedman B, Levin RA, Crisp JL, Glasgow HL, Lefkowitz R, Adams SR, Steinbach P, Nashi N, Nguyen QT, Tsien RY. Ratiometric activatable cell penetrating peptides provide rapid in vivo readout of thrombin activation. *Angewandte Chemie*. 2012 to be published.
54. Savariar EN, Felsen C, Nashi N, Jiang T, Ellies LG, Steinbach P, Tsien RY, Nguyen QT. Real time in vivo molecular detection of primary tumors and metastases with ratiometric activatable cell penetrating peptides. *Cancer Res*. 2012 in revision.
55. Schellingerhout D, Le Roux LG, Bredow S, Gelovani JG. Fluorescence imaging of fast retrograde axonal transport in living animals. *Molec Imag*. Dec.2009 8:319–329.
56. Wagner OJ, Louie BE, Vallieres E, Aye RW, Farivar AS. Near-infrared fluorescence imaging can help identify the contralateral phrenic nerve during robotic thymectomy. *Ann Thoracic Surg*. Aug. 2012 94:622–625.
57. Cotero VE, Siclovan T, Zhang R, Carter RL, Bajaj A, Laplante NE, Kim E, Gray D, Staudinger VP, Yazdanfar S, Tan Hehir CA. Intraoperative fluorescence imaging of peripheral and central nerves through a myelin-selective contrast agent. *Molec Imag Biol*. Apr.2012
58. Tanaka E, Ohnishi S, Laurence RG, Choi HS, Humblet V, Frangioni JV. Real-time intraoperative ureteral guidance using invisible near-infrared fluorescence. *J Urology*. Nov.2007 178:2197–2202.
59. Matsui A, Tanaka E, Choi HS, Kianzad V, Gioux S, Lomnes SJ, Frangioni JV. Real-time, near-infrared, fluorescence-guided identification of the ureters using methylene blue. *Surgery*. Jul.2010 148:78–86. [PubMed: 20117811]
60. Mitsuhashi N, Kimura F, Shimizu H, Imamaki M, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, Nozawa S, Furukawa K, Takeuchi D, Takayashiki T, Suda K, Igarashi T, Miyazaki M. Usefulness of intraoperative fluorescence imaging to evaluate local anatomy in hepatobiliary surgery. *J Hepato-Biliary-Pancreatic Surg*. 2008; 15:508–514.
61. Kawaguchi Y, Ishizawa T, Masuda K, Sato S, Kaneko J, Aoki T, Beck Y, Sugawara Y, Hasegawa K, Kokudo N. Hepatobiliary surgery guided by a novel fluorescent imaging technique for visualizing hepatic arteries, bile ducts, and liver cancers on color images. *J Amer College Surg*. Jun.2011 212:e33–9.
62. Klohs J, Wunder A, Licha K. Near-infrared fluorescent probes for imaging vascular pathophysiology. *Basic Res Cardiol*. Mar.2008 103:144–151. [PubMed: 18324370]
63. Oda J, Kato Y, Chen SF, Sodhiya P, Watabe T, Imizu S, Oguri D, Sano H, Hirose Y. Intraoperative near-infrared indocyanine green-videoangiography (ICG-VA) and graphic analysis of fluorescence intensity in cerebral aneurysm surgery. *J Clin Neurosci*. Aug.2011 18:1097–1100. [PubMed: 21715173]
64. Perry D, Bharara M, Armstrong DG, Mills J. Intraoperative fluorescence vascular angiography: During tibial bypass. *J Diabetes Sci Technol*. Jan.2012 6:204–208. [PubMed: 22401340]
65. Zenn MR. Fluorescent angiography. *Clinics Plastic Surgery*. Apr.2011 38:293–300.
66. Tanaka E, Chen FY, Flaumenhaft R, Graham GJ, Laurence RG, Frangioni JV. Real-time assessment of cardiac perfusion, coronary angiography, and acute intravascular thrombi using dual-channel near-infrared fluorescence imaging. *J Thoracic Cardiovas Surg*. Jul.2009 138:133–140.
67. Spaide RF. Peripheral areas of nonperfusion in treated central retinal vein occlusion as imaged by wide-field fluorescein angiography. *Retina*. May.2011 31:829–837. [PubMed: 21487338]

68. Wipper S, Detter C, Lohrenz C, Debus ES. Intraoperative quality control in vascular surgery. *J Cardiovasc Surg.* Feb.2012 53:145–149. [PubMed: 22433733]
69. Cahill RA, Mortensen NJ. Intraoperative augmented reality for laparoscopic colorectal surgery by intraoperative near-infrared fluorescence imaging and optical coherence tomography. *Minerva Chirurgica.* Aug.2010 65:451–462. [PubMed: 20802433]
70. Cahill RA, Ris F, Mortensen NJ. Near-infrared laparoscopy for real-time intra-operative arterial and lymphatic perfusion imaging. *Colorectal Disease.* Nov; 2011 13(7):12–7. [PubMed: 22098511]
71. Killory BD, Nakaji P, Maughan PH, Wait SD, Spetzler RF. Evaluation of angiographically occult spinal dural arteriovenous fistulae with surgical microscope-integrated intraoperative near-infrared indocyanine green angiography: Report of 3 cases. *Neurosurgery.* Mar.2011 68:781–787. [PubMed: 21311304]
72. Okamoto K, Muguruma N, Kimura T, Yano H, Imoto Y, Takagawa M, Kaji M, Aoki R, Sato Y, Okamura S, Kusaka Y, Ito S. A novel diagnostic method for evaluation of vascular lesions in the digestive tract using infrared fluorescence endoscopy. *Endoscopy.* Jan.2005 37:52–57. [PubMed: 15657859]
73. Keereweer S, Mieog JS, Mol IM, Van Driel PB, Snoeks TJ, Baatenburg de Jong RJ, Vahrmeijer AL, Kerrebijn JD, Lowik CW. Detection of oral squamous cell carcinoma and cervical lymph node metastasis using activatable near-infrared fluorescence agents. *Arch Otolaryngol—Head Neck Surg.* Jun.2011 137:609–615. [PubMed: 21690514]
74. Mieog JS, Troyan SL, Hutteman M, Donohoe KJ, van der Vorst JR, Stockdale A, Liefers GJ, Choi HS, Gibbs-Strauss SL, Putter H, Gioux S, Kuppen PJ, Ashitate Y, Lowik CW, Smit VT, Oketokoun R, Ngo LH, van de Velde CJ, Frangioni JV, Vahrmeijer AL. Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer. *Ann Surg Oncol.* Sep.2011 18:2483–2491. [PubMed: 21360250]
75. Hirche C, Murawa D, Mohr Z, Kneif S, Hunerbein M. ICG fluorescence-guided sentinel node biopsy for axillary nodal staging in breast cancer. *Breast Cancer Res Treatment.* Jun.2010 121:373–378.
76. van der Vorst JR, Hutteman M, Gaarenstroom KN, Peters AA, Mieog JS, Schaafsma BE, Kuppen PJ, Frangioni JV, van de Velde CJ, Vahrmeijer AL. Optimization of near-infrared fluorescent sentinel lymph node mapping in cervical cancer patients. *Int J Gynecology.* Nov.2011 21:1472–1478.
77. van der Poel HG, Buckle T, Brouwer OR, Valdes Olmos RA, van Leeuwen FW. Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: Clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. *Eur Urol.* Oct.2011 60:826–833. [PubMed: 21458154]
78. Kusano M, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M. Sentinel node mapping guided by indocyanine green fluorescence imaging: A new method for sentinel node navigation surgery in gastrointestinal cancer. *Dig Surg.* 2008; 25:103–108. [PubMed: 18379188]
79. Sevick-Muraca EM, Sharma R, Rasmussen JC, Marshall MV, Wendt JA, Pham HQ, Bonefas E, Houston JP, Sampath L, Adams KE, Blanchard DK, Fisher RE, Chiang SB, Elledge R, Mawad ME. Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: Feasibility study. *Radiology.* Mar.2008 246:734–741. [PubMed: 18223125]
80. Maus EA, Tan IC, Rasmussen JC, Marshall MV, Fife CE, Smith LA, Guilliod R, Sevick-Muraca EM. Near-infrared fluorescence imaging of lymphatics in head and neck lymphedema. *Head & Neck.* Mar.2012 34:448–453. [PubMed: 22311465]
81. Ashitate Y, Tanaka E, Stockdale A, Choi HS, Frangioni JV. Near-infrared fluorescence imaging of thoracic duct anatomy and function in open surgery and video-assisted thoracic surgery. *J Thoracic Cardiovas Surg.* Jul.2011 142:31–8–el-2.
82. Aoki T, Murakami M, Yasuda D, Shimizu Y, Kusano T, Matsuda K, Niiya T, Kato H, Murai N, Otsuka K, Kusano M, Kato T. Intraoperative fluorescent imaging using indocyanine green for liver mapping and cholangiography. *J Hepato-Biliav-Pancreatic Sciences.* Sep.2010 17:590–594.
83. Harada N, Ishizawa T, Muraoka A, Ijichi M, Kusaka K, Shibasaki M, Yamamoto K, Hasegawa K, Bandai Y, Kokudo N. Fluorescence navigation hepatectomy by visualization of localized cholestasis from bile duct tumor infiltration. *J Amer College Surg.* Jun.2010 210:e2–6.

84. Matsui A, Tanaka E, Choi HS, Winer JH, Kianzad V, Gioux S, Laurence RG, Frangioni JV. Real-time intra-operative near-infrared fluorescence identification of the extrahepatic bile ducts using clinically available contrast agents. *Surgery*. Jul.2010 148:87–95. [PubMed: 20117813]
85. Sakaguchi T, Suzuki A, Unno N, Morita Y, Oishi K, Fukumoto K, Inaba K, Suzuki M, Tanaka H, Sagara D, Suzuki S, Nakamura S, Konno H. Bile leak test by indocyanine green fluorescence images after hepatectomy. *Amer J Surg*. Jul.2010 200:e19–23.
86. Kaibori M, Ishizaki M, Matsui K, Kwon AH. Intraoperative indocyanine green fluorescent imaging for prevention of bile leakage after hepatic resection. *Surgery*. Jul.2011 150:91–98. [PubMed: 21514613]
87. Uchiyama K, Ueno M, Ozawa S, Kiriya S, Shigekawa Y, Hirono S, Kawai M, Tani M, Yamaue H. Combined intraoperative use of contrast-enhanced ultrasonography imaging using a sonazoid and fluorescence navigation system with indocyanine green during anatomical hepatectomy. *Langenbeck's Archives Surgery/Deutsche Gesellschaft für Chirurgie*. Oct.2011 396:1101–1107.
88. Ishizawa T, Tamura S, Masuda K, Aoki T, Hasegawa K, Imamura H, Beck Y, Kokudo N. Intraoperative fluorescent cholangiography using indocyanine green: A biliary road map for safe surgery. *J Amer College Surg*. Jan.2009 208:e1–4.
89. Ishizawa T, Kaneko J, Inoue Y, Takemura N, Seyama Y, Aoki T, Beck Y, Sugawara Y, Hasegawa K, Harada N, Ijichi M, Kusaka K, Shibasaki M, Bandai Y, Kokudo N. Application of fluorescent cholangiography to single-incision laparoscopic cholecystectomy. *Surg Endosc*. Aug.2011 25:2631–2636. [PubMed: 21424202]
90. Ishizawa T, Bandai Y, Ijichi M, Kaneko J, Hasegawa K, Kokudo N. Fluorescent cholangiography illuminating the biliary tree during laparoscopic cholecystectomy. *Br J Surg*. Sep.2010 97:1369–1377. [PubMed: 20623766]
91. Ishizawa T, Bandai Y, Hasegawa K, Kokudo N. Fluorescent cholangiography during laparoscopic cholecystectomy: Indocyanine green or new fluorescent agents? *World J Surg*. Oct.2010 34:2505–2506. [PubMed: 20563724]
92. Tagaya N, Shimoda M, Kato M, Nakagawa A, Abe A, Iwasaki Y, Oishi H, Shirotani N, Kubota K. Intraoperative exploration of biliary anatomy using fluorescence imaging of indocyanine green in experimental and clinical cholecystectomies. *J Hepato-Biliary-Pancreatic Sci*. Sep.2010 17:595–600.
93. Hutteman M, van der Vorst JR, Mieog JS, Bonsing BA, Hartgrink HH, Kuppen PJ, Lowik CW, Frangioni JV, van de Velde CJ, Vahrmeijer AL. Near-infrared fluorescence imaging in patients undergoing pancreaticoduodenectomy. *Eur Surg Res*. 2011; 47:90–97. [PubMed: 21720166]
94. Sekijima M, Tojimbara T, Sato S, Nakamura M, Kawase T, Kai K, Urashima Y, Nakajima I, Fuchinoue S, Teraoka S. An intraoperative fluorescent imaging system in organ transplantation. *Transplant Proc*. Sep.2004 36:2188–2190. [PubMed: 15518796]
95. Mizuno S, Isaji S. Indocyanine green (ICG) fluorescence imaging-guided cholangiography for donor hepatectomy in living donor liver transplantation. *Amer J Transplant*. Dec.2010 10:2725–2726. [PubMed: 21062417]
96. Liu HS, Chen YT, Wang D, Liang H, Wang Y, Wang SJ, Chen GQ, Zuo HC. The use of topical intranasal fluorescein in endoscopic endonasal repair of cerebrospinal fluid rhinorrhea. *Surg Neurol*. Oct.2009 72:341–345. [PubMed: 19608254]
97. Ozturk K, Karabagli H, Bulut S, Egilmez M, Duran M. Is the use of topical fluorescein helpful for management of csf leakage? *Laryngoscope*. Jun.2012 122:1215–1218. [PubMed: 22460742]
98. Demarco RC, Tamashiro E, Valera FC, Anselmo-Lima WT. Use of a hypodense sodium fluorescein solution for the endoscopic repair of rhinogenic cerebrospinal fluid fistulae. *Amer J Rhinol*. Mar-Apr;2007 21:184–186. [PubMed: 17424876]
99. Jones ME, Reino T, Gnoy A, Guillory S, Wackym P, Lawson W. Identification of intranasal cerebrospinal fluid leaks by topical application with fluorescein dye. *Amer J Rhinol*. Mar-Apr; 2000 14:93–96. [PubMed: 10793911]
100. Seth R, Rajasekaran K, Benninger MS, Batra PS. The utility of intrathecal fluorescein in cerebrospinal fluid leak repair. *Otolaryngol—Head Neck Surg*. Nov.2010 143:626–632. [PubMed: 20974330]

101. Hayashi Y, Nakada M, Tanaka S, Uchiyama N, Kita D, Hamada J. Implication of 5-aminolevulinic acid fluorescence of the ventricular wall for postoperative communicating hydrocephalus associated with cerebrospinal fluid dissemination in patients with glioblastoma multiforme: A report of 7 cases. *J Neurosurg.* May.2010 112:1015–1019. [PubMed: 19747042]
102. Pautke C, Bauer F, Tischer T, Kreutzer K, Weitz J, Kesting M, Holzle F, Kolk A, Sturzenbaum SR, Wolff KD. Fluorescence-guided bone resection in bisphosphonate-associated osteonecrosis of the jaws. *J Oral Maxillofacial Surg.* Mar.2009 67:471–476.
103. Kitai T, Kawashima M, Fujii H, Mashima S, Shimahara Y. Indocyanine green fluorescence monitoring of perineal wound contamination in abdominoperineal resection: A preliminary report. *Surgery Today.* Aug.2011 41:1037–1040. [PubMed: 21773890]
104. Maunoury V, Mordon S, Klein O, Colombel JF. Fluorescence endoscopic imaging study of anastomotic recurrence of Crohn's disease. *Gastrointest Endosc.* Jun.1996 43:603–604. [PubMed: 8781941]
105. Maunoury V, Mordon S, Geboes K, Klein O, Debaert A, Cortot A, Desreumaux P, Colombel JF. Early vascular changes in Crohn's disease: An endoscopic fluorescence study. *Endoscopy.* Sep. 2000 32:700–705. [PubMed: 10989994]
106. Pol HW, Sibma E, Zeebregts CJ, Pierik EG, Meerwaldt R. Increased skin autofluorescence after colorectal operation reflects surgical stress and postoperative outcome. *Amer J Surg.* Nov.2011 202:583–589. [PubMed: 21890102]
107. Calfon MA, Rosenthal A, Mallas G, Mauskopf A, Nudelman RN, Ntziachristos V, Jaffer FA. In vivo near infrared fluorescence (NIRF) intravascular molecular imaging of inflammatory plaque, a multimodal approach to imaging of atherosclerosis. *J Vis Exper.* 2011
108. Mallas, G.; Rosenthal, A.; Calfon, MA.; Razansky, RN.; Mauskopf, A.; Jaffer, FA.; Brooks, DH.; Ntziachristos, V. Progress on multimodal molecular/anatomical intravascular imaging of coronary vessels combining near infrared fluorescence and ultrasound; *Proc Annu Int Conf IEEE Eng Med Biol Soc*; Aug. 2011; p. 1117-1120.
109. Denzinger S, Burger M, Walter B, Knuechel R, Roessler W, Wieland WF, Filbeck T. Clinically relevant reduction in risk of recurrence of superficial bladder cancer using 5-aminolevulinic acid-induced fluorescence diagnosis: 8-year results of prospective randomized study. *Urology.* Apr. 2007 69:675–679. [PubMed: 17445650]
110. Delank W, Khanavkar B, Nakhosteen JA, Stoll W. A pilot study of autofluorescent endoscopy for the in vivo detection of laryngeal cancer. *Laryngoscope.* Mar.2000 110:368–373. [PubMed: 10718421]
111. Fradet Y, Grossman HB, Gomella L, Lerner S, Cookson M, Albala D, Droller MJ. A comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of carcinoma in situ in patients with bladder cancer: A phase III, multicenter study. *J Urol.* Jul.2007 178:68–73. [PubMed: 17499291]
112. Danilchenko DI, Riedl CR, Sachs MD, Koenig F, Daha KL, Pflueger H, Loening SA, Schnorr D. Long-term benefit of 5-aminolevulinic acid fluorescence assisted transurethral resection of superficial bladder cancer: 5-year results of a prospective randomized study. *J Urol.* Dec.2005 174:2129–2133. [PubMed: 16280742]
113. Malzahn K, Dreyer T, Glanz H, Arens C. Autofluorescence endoscopy in the diagnosis of early laryngeal cancer and its precursor lesions. *Laryngoscope.* Mar.2002 112:488–493. [PubMed: 12148859]
114. Hermann GG, Mogensen K, Carlsson S, Marcussen N, Duun S. Fluorescence-guided transurethral resection of bladder tumours reduces bladder tumour recurrence due to less residual tumour tissue in Ta/T1 patients: A randomized two-centre study. *BJU Int.* Oct.2011 108:E297–303. [PubMed: 21414125]
115. Stenzl A, Penkoff H, Dajc-Sommerer E, Zumbraegel A, Hoeltl L, Scholz M, Riedl C, Bugelnig J, Hobisch A, Burger M, Mikuz G, Pichlmeier U. Detection and clinical outcome of urinary bladder cancer with 5-aminolevulinic acid-induced fluorescence cystoscopy: A multicenter randomized, double-blind, placebo-controlled trial. *Cancer.* Mar.2011 117:938–947. [PubMed: 21351082]
116. Keereweere S, Sterenborg HJ, Kerrebijn JD, Van Driel PB, de Jong RJ, Lowik CW. Image-guided surgery in head and neck cancer: Current practice and future directions of optical imaging. *Head Neck.* Jan.2012 34:120–126. [PubMed: 21284051]

117. Lerner SP, Liu H, Wu MF, Thomas YK, Witjes JA. Fluorescence and white light cystoscopy for detection of carcinoma in situ of the urinary bladder. *Urolog Oncol.* May-Jun;2012 30:285–289.
118. Matsumoto T, Nakamura S, Moriyama T, Hirahashi M, Iida M. Autofluorescence imaging colonoscopy for the detection of dysplastic lesions in ulcerative colitis: A pilot study. *Colorectal Disease.* Oct.2010 12:e291–7. [PubMed: 20041914]
119. Pikin O, Filonenko E, Mironenko D, Vursol D, Amiraliev A. Fluorescence thoracoscopy in the detection of pleural malignancy. *Eur J Cardio-Thoracic Surg.* Mar.2012 41:649–652.
120. van den Broek FJ, Fockens P, van Eeden S, Reitsma JB, Hardwick JC, Stokkers PC, Dekker E. Endoscopic tri-modal imaging for surveillance in ulcerative colitis: Randomised comparison of high-resolution endoscopy and autofluorescence imaging for neoplasia detection; and evaluation of narrow-band imaging for classification of lesions. *Gut.* Aug.2008 57:1083–1089. [PubMed: 18367559]
121. Adam C, Salomon G, Walther S, Zaak D, Khoder W, Becker A, Reich O, Blana A, Ganzer R, Denzinger S, Popken G, Sroka R, Knuchel-Clarke R, Kollermann J, Sauter G, Hartmann A, Bertz S, Graefen M, Huland H, Wieland W, Stief CG. Photodynamic diagnosis using 5-aminolevulinic acid for the detection of positive surgical margins during radical prostatectomy in patients with carcinoma of the prostate: A multicentre, prospective, phase 2 trial of a diagnostic procedure. *Eur Urol.* Jun.2009 55:1281–1288. [PubMed: 19328622]
122. Adusumilli PS, Eisenberg DP, Chun YS, Ryu KW, Ben-Porat L, Hendershott KJ, Chan MK, Huq R, Riedl CC, Fong Y. Virally directed fluorescent imaging improves diagnostic sensitivity in the detection of minimal residual disease after potentially curative cytoreductive surgery. *J Gastrointest Surg.* Nov.2005 9:1138–1146. [PubMed: 16269385]
123. Ganzer R, Blana A, Denzinger S, Wieland WF, Adam C, Becker A, Khoder W, Walther S, Stief CG, Zaak D, Salomon G, Hartmann A, Knuechel R, Bertz S, Popken G. Intraoperative photodynamic evaluation of surgical margins during endoscopic extraperitoneal radical prostatectomy with the use of 5-aminolevulinic acid. *J Endourol/Endourolog Soc.* Sep.2009 23:1387–1394.
124. Gotoh K, Yamada T, Ishikawa O, Takahashi H, Eguchi H, Yano M, Ohigashi H, Tomita Y, Miyamoto Y, Imaoka S. A novel imageguided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. *J Surg Oncol.* Jul.2009 100:75–79. [PubMed: 19301311]
125. Winer JH, Choi HS, Gibbs-Strauss SL, Ashitate Y, Colson YL, Frangioni JV. Intraoperative localization of insulinoma and normal pancreas using invisible near-infrared fluorescent light. *Ann Surg Oncol.* Apr.2010 17:1094–1100. [PubMed: 20033320]
126. Fukuhara H, Inoue K, Satake H, Tamura K, Karashima T, Yamasaki I, Tatsuo I, Kurabayashi A, Furihata M, Shuin T. Photodynamic diagnosis of positive margin during radical prostatectomy: Preliminary experience with 5-aminolevulinic acid. *Int J Urol.* Aug.2011 18:585–591. [PubMed: 21658132]
127. Kasuya K, Sugimoto K, Kyo B, Nagakawa Y, Ikeda T, Mori Y, Wada T, Suzuki M, Nagai T, Itoi T, Shimazu M, Aoki T, Tsuchida A. Ultrasonography-guided hepatic tumor resection using a real-time virtual sonography with indocyanine green navigation (with videos). *J Hepato-Biliary-Pancreatic Sci.* May.2011 18:380–385.
128. Polom K, Murawa D, Rho YS, Nowaczyk P, Hunerbein M, Murawa P. Current trends and emerging future of indocyanine green usage in surgery and oncology: A literature review. *Cancer.* Nov.2011 117:4812–822. [PubMed: 21484779]
129. Schaafsma BE, Mieog JS, Hutteman M, van der Vorst JR, Kuppen PJ, Lowik CW, Frangioni JV, van de Velde CJ, Vahrmeijer AL. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J Surg Oncol.* Sep.2011 104:323–332. [PubMed: 21495033]
130. Tobis S, Knopf J, Silvers C, Yao J, Rashid H, Wu G, Golijanin D. Near infrared fluorescence imaging with robotic assisted laparoscopic partial nephrectomy: Initial clinical experience for renal cortical tumors. *J Urol.* Jul.2011 186:47–52. [PubMed: 21571337]
131. Yokoyama N, Otani T, Hashidate H, Maeda C, Katada T, Sudo N, Manabe S, Ikeno Y, Toyoda A, Katayanagi N. Real-time detection of hepatic micrometastases from pancreatic cancer by

intraoperative fluorescence imaging: Preliminary results of a prospective study. *Cancer*. Jun.2012 118:2813–2819. [PubMed: 21990070]

Biographies

Ryan K. Orosco received the B.Eng. degree in electrical engineering from New Mexico State University, Las Cruces, NM, USA, and the M.D. degree from Johns Hopkins University, Baltimore, MD, USA.

He is currently in a surgical training program in otolaryngology—head & neck surgery at the University of California San Diego. His research in fluorescence guided surgery focuses on molecularly targeted probes aimed at visualizing nerves and tumor cells. Additional work involves spectral analysis, image processing, and ratiometric filtering of surgical fluorescence images.

Roger Y. Tsien received the Nobel Prize in Chemistry in 2008 for increasing the understanding of basic principles underlying the fluorescence of green fluorescent proteins (GFPs). He extended the visual palette of the original jellyfish GFP by designing and synthesizing multiple new colors (blues, yellows, reds), enabling scientists to simultaneously study different biological processes (e.g., gene expression) marked by different color tags. Tsien's work allowed scientists to make real-time observations of the behavior of molecules inside living cells. He is now focusing this vast experience in chemical biology and fluorescence imaging to develop injectable molecules aimed at improving early tumor identification and surgical margin detection for translation into the clinic.

Quyen T. Nguyen received the Ph.D. degree in neuroscience and the M.D. degree from Washington University, St. Louis, MO, USA.

She is currently an associate professor in otolaryngology—head & neck surgery with subspecialty board certification in neurotology/skull base surgery at the University of California San Diego. Her research focuses on the development of systemically injectable, molecularly targeted probes aimed at visualizing tumor margins and nerves to improve surgical outcome for patients.

Fig. 1.

Nerves, vessels and muscles are not easily differentiated from one another with white light reflectance imaging. Intraoperative view of a lymph node dissection in a patient's neck, showing that large blood vessels and nerve structures are difficult to differentiate from surrounding muscle with white-light reflectance alone (A). The carotid artery (red), jugular vein (blue) and vagus nerve (yellow) are highlighted in the schematic (B). The vagus nerve is a few millimeters across and represents the largest scale nerve that surgeons identify. The smallest nerves that surgeons must identify are less than a millimeter in diameter.

Fig. 2.

Fine nerve structures can be obscured by overlying tissue. White light reflectance image showing a facial nerve in a mouse nerve (A). Much greater detail regarding nerve location and branching even under overlying non-nerve structures (compare arrows between A and B) is seen in the fluorescence image following systemic injection of a nerve labeling marker (NP41) (B).

Fig. 3.

Cancer margins are not easily discernible with white light inspection. Color photograph showing a patient with a tongue cancer. The surface of the tumor is seen as a whitish growth, with a red-pink background of tongue mucosa (A). Beneath the surface, cancer growth extends in an unpredictable pattern (B: schematic, green line). The green line represents deep tumor growth. In order to achieve complete resection, the surgeon must estimate the tumor extent when making cuts around the cancer (B: schematic, blue line). Depending on the actual tumor spread, the cut edges can be variably close to cancer cells, and there is a risk of having a positive margin.

Fig. 4.

Fluorescent labeling of tumor aids removal. In a mouse model of cancer, no residual tumor on the sciatic nerve is apparent to the human eye using white light reflectance (A). (B) With fluorescence imaging following injection of a molecularly targeted probe (activatable cell penetrating peptide, ACPP), it is clear that there is residual cancer tissue (arrow). (C) Pseudocolor overlay of the fluorescence image on top of the white light reflectance image shows the surgical view that retains anatomical details of standard surgery while adding the molecular details of the fluorescently labeled probe.

Table I
Non-Diseased Tissues (Structural Targets) Fluorescent Visual Enhancers Commonly Work Through Non-Specific Structural Labeling in Non-Diseased Tissues. Several Categories of Structural Targets are Provided With Relevant References Corresponding to Current and Future Surgical Applications. Complications That These Fluorescent Enhancers Could Decrease or Help Surgeons Avoid are Also Provided

Structural Targets	Possible Applications	Related Complications
Nerve	Nerve identification [17, 21, 55-57], neurorrhaphy, complex otologic procedures, nerve release, minimally invasive procedures	Prolonged time to nerve identification Iatrogenic damage
Ureter	Improved identification [58, 59] in minimally invasive urologic, gynecologic, and colorectal procedures	Iatrogenic injury
Blood Vessel	Evaluate vascular anatomy [60-65], assess graft patency and perfusion [66-70], evaluate vascular lesions [71, 72] Cardiac surgery Vascular surgery	Sub-optimal vessel reconstruction Oversight of lumen stenosis Thromboembolic events
Lymphatic & Lymph Node	Lymph node/metastasis mapping [69, 70, 73, 74] Sentinel lymph node biopsy [75-78] Identification of lymphatic channels [79- 81] Chyle leak repair	Incomplete removal of at-risk lymphatic structures Inaccurate or incomplete sentinel node sampling Damage to lymphatic structures with resultant leak or lymphedema
Bile & Pancreatic Duct	Liver mapping and cholangiography [82- 87] Cholecystectomy [88-92] and pancreaticoduodenectomy [93] Organ transplantation [94, 95] Endoscopic procedures	Bile duct injury Suboptimal anastomoses Improper resection planning Prolonged time to identification of structures Ductal stricture
Cerebrospinal fluid (CSF)	CSF leak identification and repair [96- 100] Complex neuro- and neurotologic- surgeries[101]	Iatrogenic CSF leak Failure to find and effectively treat CSF leak

Table II
Diseased Tissues Offer Unique Opportunities for Surgeons to Apply Specific, Targeted Fluorescent Smart Probes. Functional Targets, Recent Work in Various Applications, and Possible Complications are Summarized

Functional Targets	Applications	Related Complications
Devitalized, Inflamed, & Infected Tissue	Surgical debridement [102] and detection of wound contamination [103] Inflammatory detection [104-106] Vascular/microvascular surgery Tissue anastomosis	Suboptimal identification of devitalized tissue with resultant overly-aggressive surgery Anastomotic failure
Atherosclerosis	Plaque identification and therapeutic interventions[107,108]	Disruption or misidentification of plaques
Cancer & Neoplasm	Mucosal neoplasm [12, 73, 109-120] Solid organ tumor-margin detection [121-131]	Incomplete cancer removal (positive margins) Incomplete lymph node removal