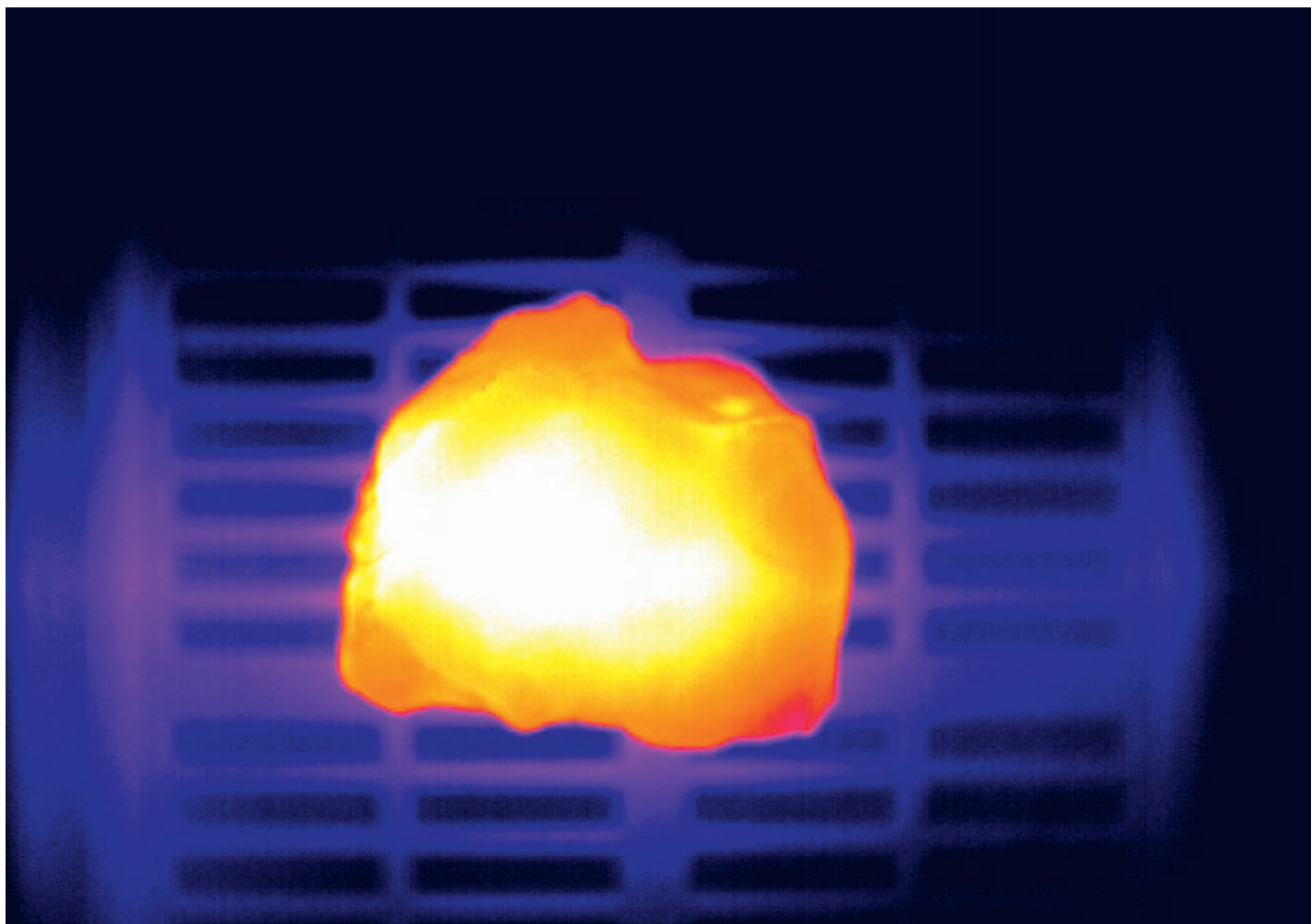


Mobile Near-Infrared Fluorescence Imaging: New Tissue Resection Guidance Tool for Surgeons

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Surgical intervention is moving towards more minimally invasive procedures utilizing catheter and endoscope technologies, but often is still based only on visual assessment of tissue properties by eye. Specifically, labeling cells with targeted fluorescence markers in vivo will radically alter surgical procedures in the future.

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Malignant target tissues will be immediately identified and easily distinguished from normal tissues by using new optical imaging technologies for visualization. Individual abnormal cells – now glowing with fluorescence – can be localized and removed during surgery, significantly improving clinical outcome. This futuristic-sounding concept has already become reality in preclinical surgery and is awaiting first clinical trials.

Determining margins during tumor resection is one of the most difficult decisions to agree upon in cancer surgery. In some applications such as neurosurgery, preservation of as much healthy brain tissue as possible is a major consideration, whereas in some other oncological procedures, malignant growth may extend considerably further into tissue than is visibly assessable. In either situation, individual malignant cells or cell clusters may be missed, leading to remission after surgery. The usefulness of fluorescence imaging for such an application has already been demonstrated in preclinical work. Fluorescence imaging of a sarcoma mouse model immediately after surgical resection using standard margins was able to identify residual tumor in more than 90 percent of cases. This new fluorescent labeling approach was capable of detecting tumor samples of less than one millimeter in size. Identification was later verified by histology.

The Challenge

The influence of modern technologies in medical imaging has had a dramatic impact on what can be detected today compared with what was possible just a decade ago. Today, intelligent contrast agents recognizing specific targets within such a complex environment as the human body could enable observation of biochemical processes in vivo, creating potential for significantly improving outcome and more effective therapy monitoring. Such contrast agents exist, and some are under preclinical investigation while others are already close to testing in clinical trials. Obviously, challenges exist on the technical side and also the agent development and approval side – a typical situation for all new medical technologies developed. What is most important, is that new imaging technologies do not interfere with the standard visual assessment of the state of tissues during surgical procedures.

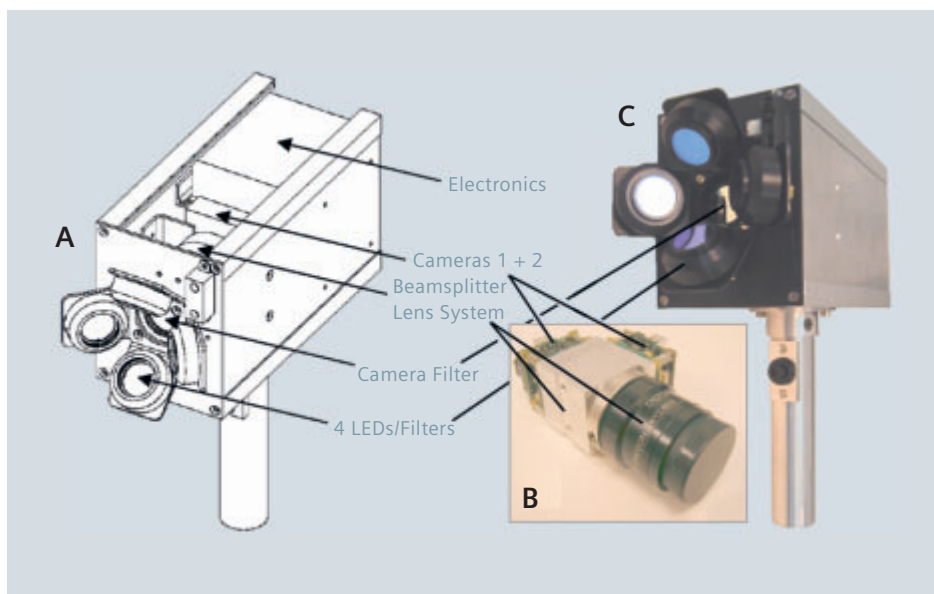


Figure 1: NIRF-HHD (Near-Infrared Fluorescence – Handheld Device) prototype showing the schematic view [A], the core optics [B] with objective – beamsplitter – video cameras, and a picture of the prototype [C]. The four LEDs deliver the light for stimulating the fluorescence of the probe molecule.

Furthermore, new contrast agents have to be able to efficiently reach their targets, need to be nontoxic at concentrations applied, and must have no long-term side effects on patients – by simple excretion and effective metabolism. All of these topics are currently being addressed by the Center for Molecular Imaging Research (CMIR) at Massachusetts General Hospital (MGH). VisEn Medical, Inc., Siemens Medical Solutions, and others are involved in this exciting new approach for next-generation surgical guidance.

Mobile Near-Infrared Fluorescence (NIRF) Imaging

Fluorescence is a well-known phenomenon and has been utilized for many decades in cell sciences and pathology for tissue characterization. The development of whole body in vivo applications in larger organisms (such as humans) has been slow due to the fact that light scattering in tissues presents a fundamental challenge to optical imaging applications in humans. However, this limitation has little relevance in surgical environments which

provide direct access to targeted tissue structures. Optical guidance and fluorescence imaging can be brought closely to the surgical site and illuminate the labeled tissues that are now easily distinguishable from the adjacent tissue. In order to achieve this goal, a small optical system is required that splits the visible light from the invisible near-infrared light by utilizing simple components such as an objective, a beamsplitter, optical filters and two detectors mounted into a mobile housing [Fig. 1]. Laser diodes in the near-infrared spectrum illuminate the tissues to trigger a fluorescence response without affecting the visual appearance of the tissues during surgery [Fig. 2]. Tissue that has internalized the contrast agent fluoresces if illuminated with light of a frequency above the visible spectrum and recorded in the invisible near-infrared range. Such device prototyping and probe development efforts have been undertaken as part of the long-term Siemens strategic alliance with the CMIR, demonstrating that, today, catheter or mobile handheld-based fluorescence imaging can be utilized to support future surgery.

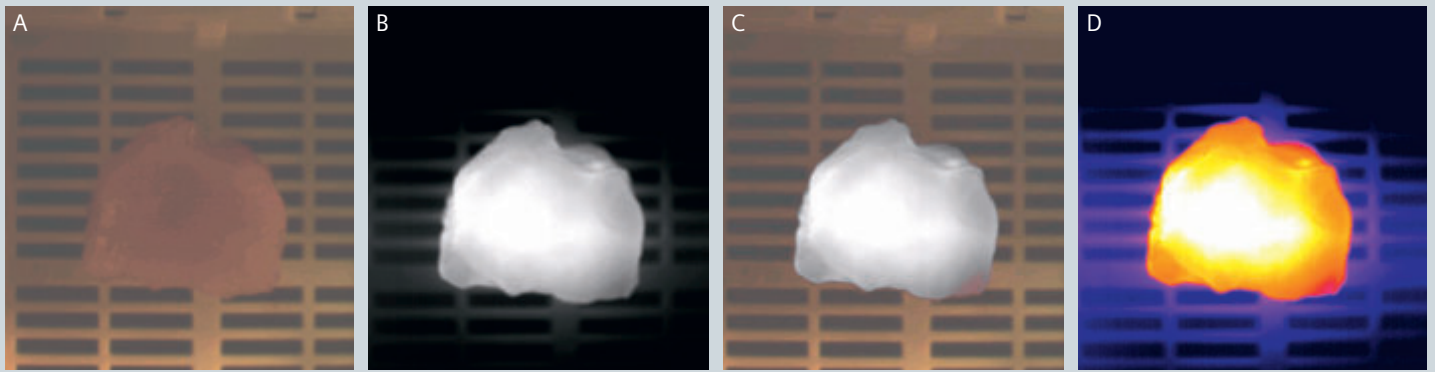


Figure 2: The excised tumor is imaged with the NIRF-HHD prototype providing a visual image [A], a fluorescence image [B], a joint image [C], and a color-coded image [D] based on calibration with standard solutions.

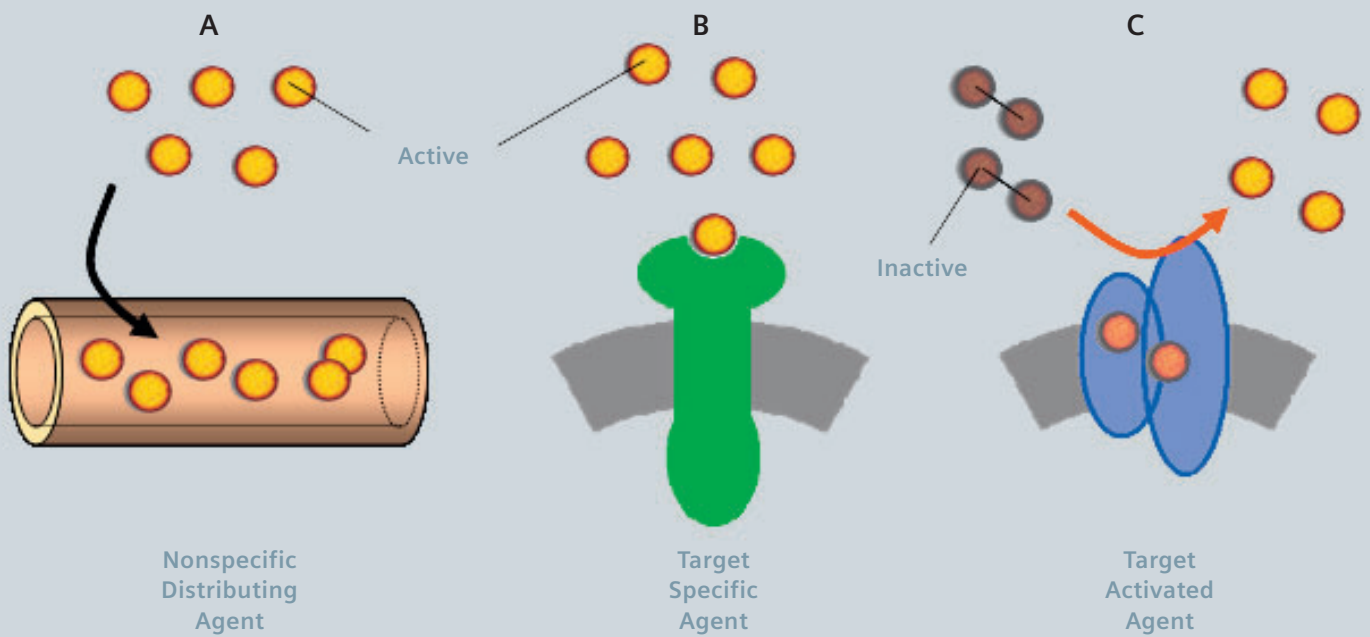


Figure 3: Different classes of contrast agents. Contrast agents can be classified in nonspecific distribution [A], target specific [B], and target activated [C] compounds. The first and second classes are commonly used for imaging with various modalities. The second class can either contain a target-specific molecule or be internalized by cells in cell-tracking applications. The third group of ‘intelligent’ contrast agents is limited to fluorescence only, since it uses fluorescence-quenching as an inactivation principle.

Targeted ‘Intelligent’ Contrast Agents

Device development is challenging, but much more significant for the success of fluorescence-guided surgery is the development of fluorescing contrast agents that are highly specific at targeting tissue structures such as developing cancers. Generally, contrast agents can be classified into three subcategories [Fig. 3]: nonspecifically distributing agents [A], specifically

targeting agents [B], and highly specific activateable agents (only available with fluorescence labeling) [C]. Any contrast agent in group A (for use in nuclear medicine, magnetic resonance imaging, ultrasound, optical fluorescence in ophthalmology, etc.), if simply intravenously injected into the cardiovascular system, can be utilized today to visualize a multitude of diagnostic features in many different diseases based on differences in distribution behavior. Contrast agents in

group B are designed to bind to a specific target and therefore carry higher information content for visualizing disease processes (labeled antibody or cell). As in the case with group A, the label can be chosen depending on the modality utilized for imaging. By contrast, category C contrast agents are uniquely designed for optical modalities detecting fluorescent properties. Such new probes are not only specific to well-defined targets but are also ‘silent’ in respect to fluorescence when inactive

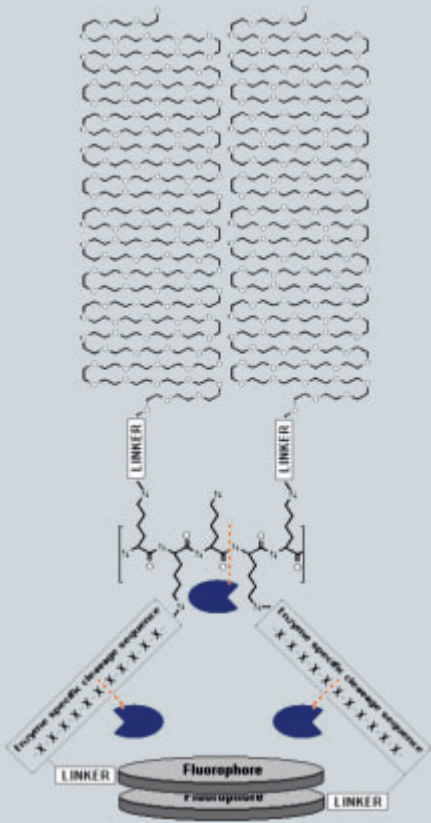


Figure 4: ProSense's principle molecular structure. The polymeric nanoparticle consists of three fundamental components: one polylysine chain, many polyethylene glycol chains and multiple fluorophores (beacons). The activation is achieved by either splitting the polylysine backbone and/or specific linker sequences that would release the fluorophores if digested. Cells internalize the nanoparticle and activate the fluorescence, if a specific enzyme is present.

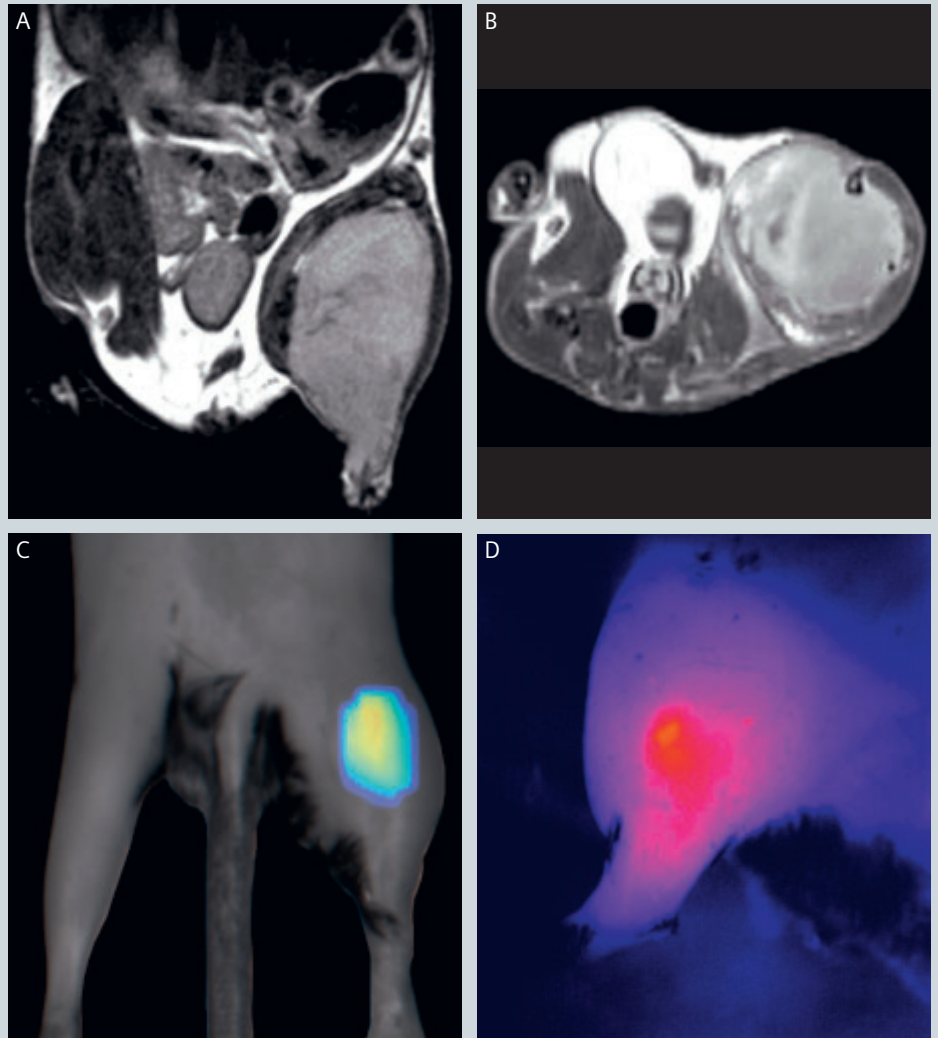


Figure 5: Preclinical MRI and FMT (Fluorescence Molecular Tomography) for monitoring sarcoma growth. GD-DTPA enhanced MRI T1-weighted images – [A] coronal and [B] axial – show an inhomogeneous sarcoma in the thigh. FMT imaging of the tumor utilizing a cathepsin sensing probe – ProSense-750 – [C] confirms the activation of the probe in the sarcoma. NIRF-HHD prototype images [D] illustrate the initial view and fluorescence response of the sarcoma.

(quenched). In the inactive state of the contrast agent, multiple fluorophores (beacons) in close proximity annihilate any expression of fluorescence normally originating from a single fluorophore, so that no fluorescence is observed. Activation is achieved through a design feature that links the individual fluorophores to the probe with a highly specific peptide sequence only recognized and split by the selected target enzyme. Once the individual fluorophores are released, the

quenching effect dissipates and a particular cell containing the target enzyme is illuminated when stimulated with light of the designed frequency. The long history of optical contrast agent development at CMIR proved vital in defining the right experimental models and conditions for testing such new concepts. The most promising effort came from those 'intelligent' (activateable) contrast agents that only activate fluorescence when internalized into the target cells,

releasing the fluorophores. ProSense® (VisEn Medical) is such an agent that has been transferred from the MGH research environment into industrial settings. It is currently utilized for preclinical imaging of diseases and is on the path to clinical use in trials. ProSense is a nanoparticle of about 500 kiloDalton weight and composed of a graft copolymer consisting of a polylysine backbone with a multitude of polyethylene glycol groups and some specifically linked fluorophores [Fig. 4]. The

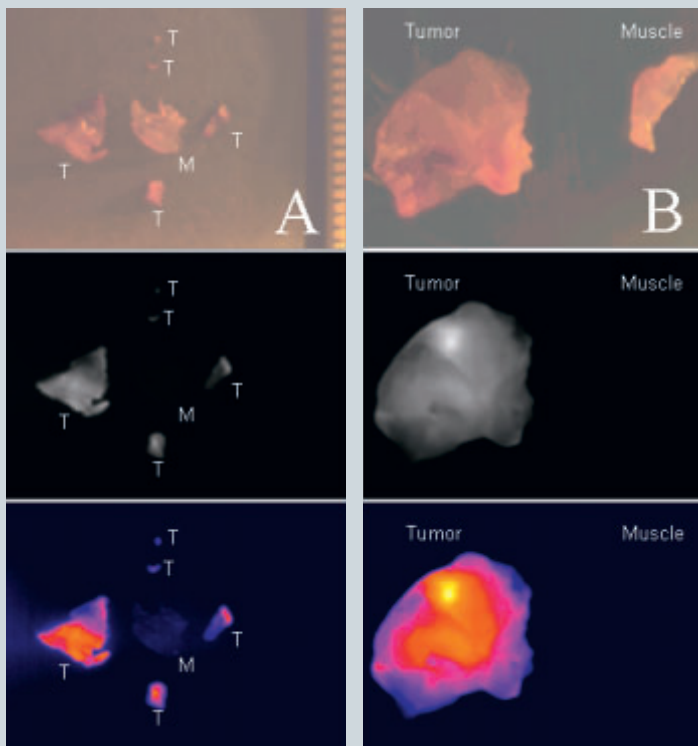


Figure 6: NIRF-HHD imaging. Tissue study illustrating the sensitivity of the handheld device. Small pieces of tumor tissues are placed around muscle tissue (visible at top, fluorescence in the middle, false color at the bottom). Submillimeter-sized tumor tissue could be identified.

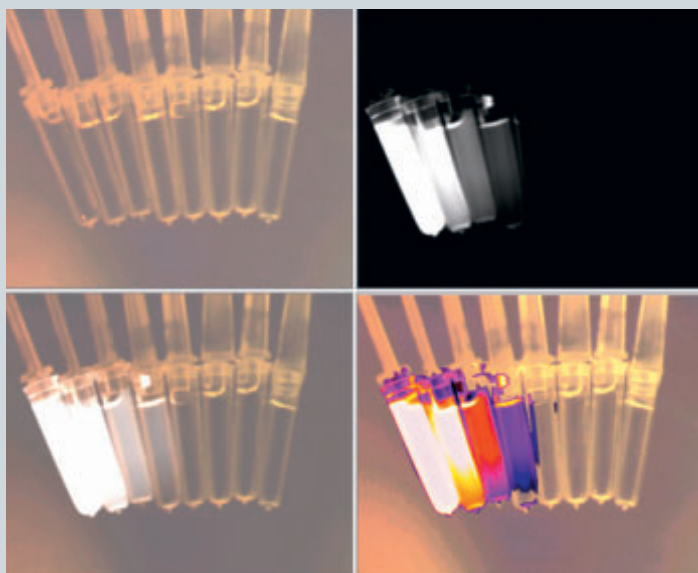


Figure 7: NIRF-HHD calibration with AngioSense. The fluorescence calibration set for the handheld device is shown (2000, 1000, 500, 250, 125, 62.5, 31.25, 15.63 pM of AngioSense-750). The visible image [A], the fluorescence image [B], the overlaid image [C], and the false color image [D] display the dynamic range and the different views provided by the handheld device. Since ProSense requires activation, it cannot easily be utilized for reliable calibration.

linkers used for connecting the fluorophores (proprietary designs of VisEn Medical) can be varied and are specific to enzymes only present in the target cells. Since in ProSense, a significant number of fluorophores is connected in close proximity to one another on the nanoparticle, the quenching effect as described prevents the expression of fluorescence and renders the nanoparticle 'silent' in respect to fluorescence. The ProSense in vivo activation is achieved by exposing the probe to cathepsin proteases significantly overexpressed in cancer cells. Following this approach, such tumor protease activity can be utilized to image and identify cancer tissue.

Preclinical Efforts

Specific mouse models were created to confirm the hypothesis that fluorescence-guided surgery can be utilized not only to visualize tumor burden and guide the resection effort, but also to track and remove residual tumor tissue, thus significantly improving outcome. In the case of limb-sparing surgery on patients with extremity soft tissue sarcomas, the limitation is often that tumors can extend beyond the site of gross disease. Spatially limited intramuscular sarcomas in the limb were detected and monitored at defined time intervals using preclinical MRI (Pharma-Scan, Bruker BioSpin, Ltd.) and Fluorescence Molecular Tomography (FMT, VisEn Medical) [Fig. 5]. Once the tumor reached a defined operable size, surgical removal was scheduled and ProSense-750 was administered intravenously 24 hours prior to surgery by injection into the tail vein. The long incubation period utilized for distributing the fluorescence probe in the blood vessels ensured that the tumor could be reached by the probe and sufficient material entered the sarcoma tissue in the limb for effective probe activation. Resection was monitored utilizing the HHD system in order to verify the presence of tumor and the individual stages of the removal process. All tissues resected were also imaged with the handheld device (HHD) followed by detailed cell pathology for disease verification. Pieces of less than one millimeter in size were easily detected by the HHD system and compared with unaffected muscle tissue [Fig. 6]. Inhomogeneous distribution of tumor cells within the excised tissue was clearly visible with the HHD system and confirmed

through pathology. In almost all cases, the remaining tumor was detected in some regions of the tumor bed after initial removal of the primary tumor. The HHD system was able to detect and guide directly to such sites and supported the removal of residual disease. The mice were monitored for a few weeks after surgery for any signs of remission. First estimates suggest a significant improvement of positive outcome, but the real success of applying the HHD system to surgery can only be confirmed in outcome studies of larger preclinical trials.

The actual fluorescence calibration of the HHD system was achieved utilizing an active contrast agent of similar makeup, called AngioSense®-750 (VisEn Medical), expressing fluorescence directly when illuminated the same way as in case of ProSense-750. Vials with different concentrations of AngioSense-750 were measured, and the corrected intensities were used to color-code the concentrations measured in tissue images of the sarcoma samples [Fig. 7].

Clinical Outlook

Two important steps are required before this technology can assist physicians in human cancer surgery – completion of the toxicology studies for ProSense (currently underway) and Phase 1 clinical trials in surgical settings. Since ProSense or similar fluorescent probes can be applied to many different types of cancers, there are numerous clinical applications in oncology that are ideal candidates for extending this technology into the clinic. One of the most promising is the application of cancer management and surgical intervention in ovarian cancer, because tumors arise on the surface or near the surface of the ovary, and primary tumors are typically asymptomatic. No effective screening tools are available to date, and mobile-fluorescence imaging using cancer-specific probes would provide the ideal technology to detect primary tumors and metastases in the abdominal cavity. Optical technologies have already entered the field of ovarian cancer management, since this disease demonstrates a well-defined need for high-resolution imaging supporting surgical intervention. Proof of concept has already been achieved utilizing indocyanine green (ICG), a non-specific fluorescent contrast agent normally used for the detection of blood

Research Teams

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Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ⁴Center for Molecular Imaging Research and ⁵Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. ⁶Siemens Medical Solutions Inc. ⁷Dana Farber Cancer Institute, Boston, MA, USA. ⁸Tufts University School of Medicine and Veterinary Medicine, North Grafton, MA, USA. ⁹Department of Surgery and ¹⁰Department of Orthopaedic Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. ¹¹Howard Hughes Medical Institute, Chevy Chase, MD, USA. Furthermore, we thank Drs. Kirt Poss and Wael Yared at VisEn Medical, Inc., for fruitful discussions and support and Dr. Michael Seiden, now at Fox Chase Cancer Center, for his visionary applications of optical technologies in clinical cancer management.

vessel abnormalities in the human eye. It is also enriched in the increased vascularity of tumors and has successfully been applied to ovarian cancer surgery. Clinical trials of ProSense in the field of ovarian cancer management seem very promising and may bring fluorescence contrast agents one step closer to FDA approval and applications in intervention.

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