Cancer is the leading cause of death in developed countries and affects over 1.4 million Americans each year. Lung cancer is the leading cause of cancer mortality in men and the second leading cause of cancer mortality in women throughout the world; approximately 1.4 million people die of lung cancer each year. Of the 1.6 million new cases of lung cancer each year, approximately 220,000 are diagnosed in the United States. Lung cancer causes more deaths in the United States than breast, prostate, and colorectal cancer combined.

As with many solid tumors, surgical resection is standard of care for patients with stage I, II, and certain subsets of stage II A non-small cell lung cancer (NSCLC). The most important predictor for patient survival is complete surgical resection. Patients with complete surgical resections have significantly better survival compared to those with partial or incomplete resection. This highlights the importance of accurate delineation of tumor margins and complete tumor removal with margins of normal tissue. A surgeon uses visual information, tactile cues, and their experience during a procedure to assess the extent of neoplastic disease and determine the margins necessary for adequate tumor resection. These traits are subjective, vary based on training and experience, and may lead to erroneous decisions. Studies of intra-operative evaluation of tumor location and margin status showed unexpectedly positive margins in 39% of prostate cancer cases and
undiagnosed invasive or in situ carcinoma in 48% of breast cancer cases, respectively.

There is a great need for a reliable, objective, real-time imaging system to clearly delineate tumors from surrounding normal tissue during surgery and evaluate regional lymph nodes and local and regional structures for metastatic disease. Computed tomography and positron emission tomography are often used to evaluate lung cancer patients and plan surgery, but these modalities are not useful for intra-operative imaging.

Near-infrared fluorescence imaging (NIRFI) has the potential to provide objective, real-time visualization of tumors during surgery, allowing for accurate resection of the tumor and an adequate margin of normal tissue. Several different approaches to fluorescence imaging, including different fluorescent dyes, antibody conjugates, peptide conjugates, and tumor-activated probes have been investigated. The potential utility of intra-operative fluorescence-guided tumor resection has been evaluated in several pre-clinical studies and in a limited number of patients with breast, ovarian, pancreatic, and hepatic cancers and gliomas.

In ongoing studies, we are using indocyanine green (ICG), a water soluble, near-infrared (NIR) fluorophore approved by the US Food and Drug Administration and other NIR imaging agents to “make tumors glow” during surgery using near-infrared imaging systems. The dyes and imaging system are currently being evaluated in dogs with primary lung tumors and mammary tumors, spontaneous, large animal models of human non-small cell lung cancer and breast cancer respectively, and soft tissue sarcomas in cats. ICG is administered intravenously 18-20 hours before surgery and accumulates in tumors through the enhanced permeability and retention effect. Many tumors stimulate the development of new blood vessels as they grow to provide oxygen and nutrients. This “neovasculature” does not have normal walls and has a more permeable endothelium- hence “enhanced permeability”. Within the tumor itself there is often a lack of lymphatic vessels- hence “retention”.

During surgery the near infrared dye (ICG) is stimulated by a specific wavelength of light using a laser or LED lights. Surgical images are captured with a
CCD camera; near-infrared images are obtained with a CCD camera using filters to selectively screen out light not in the emission spectrum of ICG (820-840 nm). A computer superimposes the near infrared light emission on to the real time “visible light” image of the surgical field, allowing real time visualization of tumor fluorescence during surgery.

In a limited series of cases, we showed that primary lung tumors, mammary tumors, and sarcomas can be imaged during surgery using near-infrared imaging of induced ICG fluorescence. Tumor fluorescence readings are generally 200% or more higher than readings from normal tissue. The imaging system also gave high fluorescence readings compatible with neoplastic involvement in several lymph nodes with metastatic cancer. In one dog, an area of neoplastic extension into the mediastinal pleura gave high fluorescence readings comparable to those of the primary tumor. This area was effaced with neoplastic cells on histopathology. Should these initial results be corroborated in a larger series of cases, the ability of a real-time imaging system to detect regional lymph node involvement and serosal implantation metastases during an operation would represent a significant advance in oncologic surgery. Further refinement of imaging agents and imaging systems will likely yield clearer delineation between neoplastic and normal tissue.

The ICG appeared to be safe as used in this study and none of the dogs or cats showed any obvious adverse reactions to the injections. Indocyanine green is a water soluble, anionic, amphiphilic, tricarbocyanine near-infrared (NIR) fluorophore that has been approved by the US Food and Drug Administration. Indocyanine green has several advantages as a potential fluorescent imaging agent. The NIR absorption and emission peaks of ICG (778 and 830 nm, respectively) lie within wavelengths where there is minimal autofluorescence of normal tissue. Indocyanine green fluorescence is not extensively absorbed by blood and can penetrate tissue to a depth of 1 to 1.5 cm. Immediately after intravenous injection, ICG binds to albumin resulting in complexes that are 5-10nm in size.

These studies used dogs with primary lung tumors and mammary tumors as a large animal model of lung cancer in humans. Naturally occurring cancers in dogs
are more realistic models of their corresponding human diseases than mouse models. In dogs, the tumors are spontaneous rather than induced. Dogs share the same environment as their owners and often have a high level of medical care. Primary lung tumors in dogs are histologically similar to non-small cell lung cancer in humans, and have similar responses to treatment. The majority are adenocarcinomas (bronchial, bronchioalveolar, or alveolar) with squamous cell carcinomas occurring less frequently. These tumors also have a similar biological behavior to those in humans. In both humans and dogs, metastases to bronchial lymph nodes occur and some tumors have a propensity to metastasize to the central nervous system. Surgery is the primary method of treatment for dogs with primary lung tumors and the best prognosis is seen with smaller, well-differentiated tumors that are completely resected with no involvement of the regional lymph nodes.

Given the potential variability and subjective nature of intraoperative evaluation of the extent of neoplastic disease, near-infrared imaging offers the potential to objectively identify both primary and metastatic cancer in real time during surgery. This study illustrates the utility of ICG and a near-infrared imaging system in canine cancers, spontaneous, large animal models of the human disease. We have recently completed a trial using a folate based NIR imaging agent in dogs with lung cancer and a trial using ICG to image dogs with soft tissue sarcomas. We are currently investigating the use of d-aminolevulinic acid in dogs with primary lung tumors and the use of ICG to image cats with soft tissue sarcomas and dogs with mammary tumors.