SESSION TITLE: Poster Session 01
CONTROL ID: 2732929
TITLE: Multiplexed molecular imaging with topically applied SERS nanoparticles for molecular endoscopy and surgical guidance
PRESENTER: Yu Wang
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ABSTRACT BODY:
Abstract Body: The molecular profiles of most cancers vary greatly between patients as well as spatially and temporally within a single tumor mass [1]. Therefore, the accurate detection of tumors would benefit from the ability to image a large panel of disease-related biomarkers. Surface-enhanced Raman-scattering (SERS) nanoparticles (NPs) have attracted interest due to their potential for sensitive and multiplexed biomarker detection [2]. There is a need to demonstrate that multiplexed molecular detection is possible under time-limited tissue-staining and detection conditions [3], as encountered in point-of-care clinical or surgical settings. Here, we focus on establishing the feasibility of topical application as a means of rapidly delivering targeted SERS NPs to exposed tissue surfaces (e.g. epithelial surfaces and surgical margins), resulting in sufficient contrast to enable molecular endoscopy and surgical guidance. A ratiometric method [4, 5] was employed to rapidly quantify the specific vs. nonspecific binding/accumulation of biomarker-targeted NPs on fresh tissues to eliminate nonspecific effects.

Esophageal cancer is one of the deadliest cancers worldwide, because the patients often present with advanced metastatic disease at the time of diagnosis [6]. The biological investigation and detection of esophageal cancers could be facilitated with an endoscopic technology to screen for the molecular changes that precede and accompany the onset of cancer. We demonstrate that the topical application and endoscopic imaging of biomarker-targeted SERS NPs (<15 min) enables the rapid detection of tumors in an orthotopic rat model of esophageal cancer (Fig. 1). A multiplexed cocktail of antibody-conjugated SERS NPs was topically applied on the lumenal surface of the rat esophagus to target multiple biomarkers, and a miniature spectral endoscope featuring rotational scanning and axial pull-back was employed to comprehensively image the NPs bound on the lumen of the esophagus [7, 8]. Ratiometric analyses of specific binding vs. nonspecific accumulation of SERS NPs enabled the visualization of tumors and the quantification of biomarker expression levels in agreement with immunohistochemistry and flow cytometry validation data (Fig. 1e,f) [7].

Intraoperative detection of residual tumors at lumpectomy margins would enable reduced re-excision rates, which currently range from 20% – 50% [9]. While the imaging of disease-associated biomarkers can identify tumors with high specificity, the multiplexed imaging of such biomarkers is necessary to detect molecularly heterogeneous breast tumors with high sensitivity. We demonstrate rapid visualization of a multiplexed panel of cell-surface biomarkers at surgical margin surfaces through topical application of SERS NPs. Ratiometric quantification of specific (targeted) binding vs. nonspecific accumulation of the NPs allows for the multiplexed detection of 4 protein biomarkers at the surfaces of freshly excised tissues, within 15 minutes [10, 11]. These results showed the potential of this multiplexed molecular imaging technique to intraoperatively guide tumor-resection procedures.
Figure 1. Endoscopic imaging of a rat esophageal tumor model. Schematic of (a) the topical application and (b) endoscopic imaging of SERS NPs. Photograph of (c) in vivo experimental system and (d) a surgically exposed rat esophagus implanted with three tumor xenografts. (e and f) Images showing the concentration ratio of (e) EGFR-NPs vs. isotype-NPs and (f) HER2-NPs vs. isotype-NPs. The right-side plots show the correlation between the NP ratio and the corresponding fluorescence ratio (targeted NP vs. isotype NP) from flow-cytometry experiments with the cell lines used to generate the various tumor xenografts. R > 0.95 [7].
**IMAGE CAPTION:** Figure 1. **Endoscopic imaging of a rat esophageal tumor model.** Schematic of (a) the topical application and (b) endoscopic imaging of SERS NPs. Photograph of (c) in vivo experimental system and (d) a surgically exposed rat esophagus implanted with three tumor xenografts. (e and f) Images showing the concentration ratio of (e) EGFR-NPs vs. isotype-NPs and (f) HER2-NPs vs. isotype-NPs. The right-side plots show the correlation between the NP ratio and the corresponding fluorescence ratio (targeted NP vs. isotype NP) from flow-cytometry experiments with the cell lines used to generate the various tumor xenografts. R > 0.95 [7].
SESSION TITLE:  Poster Session 01  
CONTROL ID:  2734165  
TITLE:  Multimodal imaging nanoparticles derived from hyaluronic acid for integrated preoperative and intraoperative cancer imaging  
PRESENTER:  Nicholas Wojtynek  
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ABSTRACT BODY:  
Abstract Body: Surgical resection remains the most promising treatment strategy for many forms of cancer. Residual malignant tissue after surgery, a consequence in part due to positive margins, contributes to high mortality and disease recurrence.1 In this study, multimodal contrast agents for integrated preoperative magnetic resonance imaging (MRI) and intraoperative fluorescence image-guided surgery (FIGS) are developed. Leveraging the strengths of preoperative MRI and intraoperative FIGS in tandem could provide superior guidance during surgery, potentially improving surgical resection outcome and reducing recurrence.2,3  
Self-assembled multimodal imaging nanoparticles (SAMINs) were developed as a mixed micelle formulation using hyaluronic acid (HA) chains functionalized with either gadolinium Gd(III) for T1 contrast-enhanced MRI, or Cy7.5, a near infrared fluorescent dye that can be readily detected by FIGS. To evaluate the relationship between MR and fluorescence signal from SAMINs, we employed simulated surgical phantoms that are routinely used to evaluate the depth at which NIR imaging agents can be detected by FIGS.4 Imaging agent efficacy was evaluated in a human breast tumor xenograft model in nude mice and biodistribution data was obtained through T1 mapping, fluorescence imaging, and ICP-MS analysis.  
Tissue phantom studies showed the SAMINs to have high contrast for preoperative MRI, and then demonstrated high contrast for fluorescence imaging and margin identification during FIGS. For example, 50 µL inclusions at 5 mm deep in adipose were 60% brighter than muscle and 80% brighter than in the liver at the same depth, and the same pattern of signal intensities is uniformly observed at different inclusion depths and volumes for adipose, muscle, and liver phantoms. Furthermore, the optical profiles of scattering and signal intensity was characterized for different tissue phantom types. Cytotoxicity studies with MCF-10A cells demonstrated that the newly synthesized nanomaterials are not cytotoxic up to a concentration of 0.1 mg/mL. In vivo experiments demonstrated efficacy in both preoperative MRI and intraoperative FIGS. The MRI signal and contrast (relative to adjacent muscle) both increase due to the multimodal nanoparticles, where the tumor-muscle contrast increases to 1.17 at 24 h post-injection. When compared to the surrounding muscle tissue, a 10-fold signal increase was seen in the tumor during FIGS. The enhanced contrast seen in both imaging modalities shows promise for effective surgical guidance of solid tumors, with the goal of ultimately improving disease prognosis.

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SESSION TITLE:  Poster Session 01
CONTROL ID:  2734539
TITLE:  Light-sheet microscopy for rapid intraoperative imaging of human tissues
PRESENTER:  Adam Glaser
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ABSTRACT BODY:
Abstract Body:  Pathologic features provide a gold-standard by which diseases are diagnosed, patient prognoses are determined, and treatment decisions are made. In a pathology lab, the examination of a tissue biopsy or a surgically excised specimen is most-reliably performed by the microscopic examination of tissues which have been formalin-fixed and paraffin-embedded, sectioned, stained, and mounted on glass slides. This is a labor- and time-intensive process in which only a fraction of a specimen is sampled. Frozen sectioning enables tissues to be rapidly sectioned, stained, and mounted on slides. However, this technique can introduce severe artifacts, especially for tissues that do not freeze well (e.g., breast). Furthermore, like FFPE histology, it is destructive of tissue and suffers from the same sampling limitations. The ability to image intact tissues at high resolution over large areas or volumetric fields of view, with the same level of morphological and molecular contrast that is possible through conventional pathology, promises to advance the field of pathology and thereby to improve patient treatments and outcomes. To overcome these prior limitations, a novel light-sheet microscope (LSM) was designed and used to image large centimeter sized human breast tissues in <2 minutes for rapid intraoperative inspection of the margin status and tumor extent (Fig. 1).
Figure 1. **a,b.** A freshly excised specimen of human breast tissue. **c.** Surface-extracted light-sheet microscopy image acquired in <1 min with representative line profiles of the tissue-surface depth. **d, e.** Moderate- and high-magnification images reveal a transition from benign breast tissue to invasive ductal carcinoma. **f.** Benign breast lobules are clearly visualized and correlate with conventional histology. **g.** Panoramic light-sheet microscopy, conventional histology, and frozen-sectioning of fibro-adipose tissue. 
Figure 1. a, b, A freshly excised specimen of human breast tissue. c, Surface-extracted light-sheet microscopy image acquired in <1 min with representative line profiles of the tissue-surface depth. d, e, Moderate- and high-magnification images reveal a transition from benign breast tissue to invasive ductal carcinoma. f, Benign breast lobules are clearly visualized and correlate with conventional histology. g, Panoramic light-sheet microscopy, conventional histology, and frozen-sectioning of fibro-adipose tissue.
**SESSION TITLE:** Poster Session 01  
**CONTROL ID:** 2734882  
**TITLE:** Application of Fluorescent and Iodized Dual Marker for Pre-operative Localization and Image-guided Surgery of Pulmonary Nodule  
**PRESENTER:** Jiyun Rho  
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**ABSTRACT BODY:**  
**Abstract Body:** Purpose  
This study was evaluated the feasibility of pre-operative localization of pulmonary nodule using optimized dual marker composed of indocyanine green (ICG) and lipiodol for minimal and accurate resection of pulmonary nodule.

Materials and Methods  
To minimize separation of two materials, we mixed with different frequency and ratio of ICG and lipiodol using a 3-way stopcock, and investigated their distribution with fluorescent microscope. Three rabbits were undergone thoracotomy after computed-tomography (CT) fluoroscopy-guidance injection of each 0.1 ml emulsions into different lobes of rabbit lung at 6, 12 or 24 hours. The localized lesions were evaluated by near-infrared optical imaging and radiograph. The 0.3 ml of emulsion was pre-operatively injected into 22 patients under CT fluoroscopy-guidance, and the localization was then evaluated during surgery by near-infrared imaging and mobile C-arm fluoroscopic x-ray. All freshly excised specimens were diagnosed by pathologic examination.

Results  
In in vitro, the separation time of ICG and lipiodol emulsion was delayed proportionally to mixing frequency and ratio. The emulsion mixed with 90 passages and 90% lipiodol was the least separated at 24 hours. On the rabbit lung, the optimal emulsion remained stably on injection site until 24 hours after injection. Pulmonary nodule localization using the optimal emulsion was performed successfully on the 22 patients without complication.

Conclusion  
This easy optimal methods of dual marker for pre-operative localization of pulmonary nodule was successfully established. The study can be a useful marker to notify a location of the lesion to surgeons. However, ICG and lipiodol were not mixed perfectly and evenly. Therefore, there will be needed a future research to stabilize two materials.
Mechanically mixed 10% ICG and 90% lipiodol with 90 passages was the most stable in in vitro, rabbit lung, and human lung.
**IMAGE CAPTION:** Mechanically mixed 10% ICG and 90% lipiodol with 90 passages was the most stable in *in vitro*, rabbit lung, and human lung.
SESSION TITLE: Poster Session 03
CONTROL ID: 2805231
TITLE: Analysis of fluorescence images—comparing the signal-to-background ratio (SBR) to the contrast-to-noise ratio (CNR)
PRESENTER: G Jan Jaap Burggraaf
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ABSTRACT BODY:
Abstract Body: Introduction
Quantification of intraoperatively acquired fluorescence images are commonly reported as the signal-to-background ratio (SBR; target or tumor-to-background [TBR]), which is based on subjectively selected regions of interest (ROI) for both the target and the background SBR is the key determinant of sensitivity and detectability in fluorescence imaging and is frequently reported as endpoint in (pre)clinical studies, whereby SBR’s exceeding an arbitrary value of 2 is considered adequate. However, it has been argued that noise originating from the background influences the contrast between target and background, and it was therefore suggested to use the contrast-to-noise ratio (CNR) [Tichauer, 2012]. The aim of this study was a head-to-head comparison of these quantitative measures based on a substantial number of images and more to assess the influence of selection of the region of interest (ROI).

Methods
A randomly selected, representative sample of intraoperative and ex vivo images (total 271 images) from both animal and human studies in different tumor types using different fluorescent agents and imaging systems was evaluated. All images were assessed using ImageJ (National Institute of Health, Bethesda, USA) and the SBR and CNR were calculated. Three approaches were chosen for the ROI: (1) the darkest region adjacent to the tumor, (2) the lightest region adjacent to the tumor, and (3) a ROI from the region surrounding the tumor ROI within the same anatomical structure of the tumor.

Results
There were differences between CNR and TBR depending on the tissues examined which probably reflects differences in contrast/autofluorescence. For intraoperatively obtained sentinel lymph node images (n=9), contrast was sufficient to distinguish tumor from background and average TBR (11.8) and average CNR (6.8) was sufficiently high. Images of four liver metastases showed that using CNR discriminated tumor from background better than TBR which was <2 for two images. Images of small peritoneal metastases of ovarian cancer (n=95) showed the CNR to be >3 for 62 lesions, while TBR had a sufficiently high ratio for 27 lesions only. Further, the most important finding is that background selection is of far greater importance irrespective of the quantifying parameter. It was also found that CNR and SBR are linearly related, which reflects that CNR is mathematically related to TBR [(CNR=(TBR-1) × Background : SDbackground)].

Conclusion
CNR as a quantitative measure provides additional information regarding the noise and contrast in fluorescence imaging. This can be of added value for low contrast and low signal strength images, because it takes the standard deviation of the background into account. However, irrespectively of using the TBR of CNR for image quantification, selection of a representative background and tumor ROI seems to be more important. This finding should encourage reaching consensus on standardization when reporting quantitative image analyses.

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SESSION TITLE: Scientific Session 11: Instrumentation - Surgical & Endoscopy
CONTROL ID: 2723044
TITLE: Raman-encoded molecular imaging (REMI) with topically applied SERS nanoparticles for intraoperative guidance of breast cancer lumpectomy
PRESENTER: Yu Wang
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ABSTRACT BODY:
Abstract Body: Approximately 200,000 patients are diagnosed with early-stage breast carcinoma each year in the United States, for which breast-conserving surgery (a.k.a. partial mastectomy or lumpectomy) is a standard intervention [1,2]. Unfortunately, amongst various institutions, between 20% and 50% of these patients require additional surgery if post-operative pathology reveals that the resection margins are positive for carcinoma [3,4]. A method for accurate intraoperative tumor detection at the surgical margins could significantly reduce the costs, emotional trauma, and potential complications associated with multiple surgeries, and could also improve patient outcomes by reducing the sampling errors associated with conventional post-operative pathology. We have developed a Raman-encoded molecular imaging (REMI) technique, in which multiplexed surface-enhanced Raman scattering (SERS) nanoparticles (NPs) are topically applied on freshly excised tissues to enable rapid visualization of cell-surface biomarkers at the surfaces (margins) of those tissues (Persuasive data Fig. S1) [5-9]. The ability to detect multiple protein biomarkers is critical for accurate tumor detection because the molecular profiles of most cancers, including breast carcinoma, vary greatly between patients as well as spatially and temporally within a single tumor mass [10, 11].

A first-ever clinical study was performed to assess the diagnostic accuracy of REMI for distinguishing benign breast tissue from carcinoma. Fifty-seven fresh specimens from 29 patients were imaged with REMI to simultaneously quantify the expression of four breast carcinoma biomarkers, including HER2, membrane ER, EGFR and CD44. Each specimen was topically stained with an equimolar mixture of 5 flavors of SERS NPs (HER2-NPs, ER-NPs, EGFR-NPs, CD44-NPs and isotype-NPs) for 5 min and imaged at a spatial resolution of 0.5 mm (raster-scanned imaging rate of >3 cm²/min). Ratiometric quantification of specific (targeted) binding vs. nonspecific accumulation of the NPs allows for the multiplexed detection of 4 protein biomarkers at the surfaces of freshly excised tissues, within 15 minutes (Persuasive data Fig. S2).

ROC analysis was performed to quantify the sensitivity and specificity of REMI to detect the overexpression of each of the four biomarker targets, with IHC as the gold standard (Fig. 1). REMI achieved a sensitivity of between 90.0 and 93.7% to detect the elevated expression of HER2, EGFR or CD44, when a high specificity (>90%) is enforced. The sensitivity for detecting ER was lower due to the limited expression of ER at cell surfaces and the fact that the large SERS NPs (120 nm) are too large to access intracellular biomarkers within only 5 min of topical application. With the combined detection of all four biomarkers, REMI achieved 89.3% sensitivity and 92.1% specificity to distinguish benign breast from in situ and invasive carcinoma (Persuasive data Table S1), showing the potential of REMI to intraoperatively guide lumpectomies and other of tumor-resection procedures.
Fig. 1. ROC analysis for the identification of biomarker overexpression by REMI. (A) Co-registration of IHC and REMI data. REMI images and corresponding IHC images were uniformly divided into larger regions of interest (ROI) of 2 mm × 2 mm to improve the correlative analysis. Each ROI was assigned a diagnostic result of 0 (negative) or 1 (positive) for the overexpression of each biomarker based upon the consensus interpretation of IHC slides by two pathologists. The average NP ratio from each ROI in a REMI image was calculated, and its value was compared with a threshold value to determine if the NP ratio was indicative of overexpression of a biomarker (ratio > threshold). (B) ROC curves for the biomarkers EGFR, HER2, ER and CD44 (cumulative results from 57 fresh specimens of 29 patients). The ratio of targeted NPs vs. isotype-NPs from each of 2106 ROIs were used to generate the ROC curves.
IMAGE CAPTION: Fig. 1. ROC analysis for the identification of biomarker overexpression by REMI. (A) Co-registration of IHC and REMI data. REMI images and corresponding IHC images were uniformly divided into larger regions of interest (ROI) of 2 mm × 2 mm to improve the correlative analysis. Each ROI was assigned a diagnostic result of 0 (negative) or 1 (positive) for the overexpression of each biomarker based upon the consensus interpretation of IHC slides by two pathologists. The average NP ratio from each ROI in a REMI image was calculated, and its value was compared with a threshold value to determine if the NP ratio was indicative of overexpression of a biomarker (ratio > threshold). (B) ROC curves for the biomarkers EGFR, HER2, ER and CD44 (cumulative results from 57 fresh specimens of 29 patients). The ratio of targeted NPs vs. isotype-NPs from each of 2106 ROIs were used to generate the ROC curves.
SESSION TITLE: Scientific Session 11: Instrumentation - Surgical & Endoscopy
CONTROL ID: 2731711

TITLE: Image-Guided Fluorescence Endomicroscopy in a Rat Model of Radiation-Induced Pulmonary Fibrosis
PRESENTER: Jessica Perez

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ABSTRACT BODY:

Abstract Body: Objective
To establish a pre-clinical small animal imaging framework from macro- to micro-imaging by combining fluoroscopy with fluorescence endomicroscopy (FE) in order to localize the endoscope spatially and quantify pulmonary fibrosis.

Introduction
Radiation-induce pulmonary fibrosis (RIPF) is a common side effect of thoracic radiotherapy for cancer treatment. It is a complex biological process, following an inflammatory phase; lung damage evolves into a fibrotic phase with accumulation of extra-cellular matrix, collagen deposition and scar tissue formation. Diagnosis and monitoring of RIPF is commonly done with computed tomography (CT) imaging and/or with biopsies for histology. FE is a powerful, new, minimally invasive tool to image biological processes at the cellular level in accessible organs such as the lung. However, once the endoscope is in place and acquiring images, its exact location within the lung is unknown and it is therefore impossible to correlate FE images with localized pulmonary damage. In this work, fluoroscopy (X-ray) imaging was used in conjunction with FE of collagen and the exact location of the endoscope in the lung was mapped in 3D using prior CT images as navigation maps.

Methodology
A rat model of RIPF was established with 18 Gy hemithorax irradiation (Perez et al, 2017). 24 weeks post-irradiation rats were imaged with CT. A fluorescent collagen probe to detect fibrosis was injected and rats were imaged with FE through a tracheotomy procedure. The endoscope was placed in the lung, FE images were acquired and for each endoscope position, a coronal and sagittal fluoroscopy image (C-arm) was acquired. Multiple endoscope positions were evaluated for each rat and were categorized as proximal, intermediate or distal. Using the MINC software, registration tags were placed on anatomical structures (such as ribs or spine) on each fluoroscopy image pair and on the corresponding view on the CT scan. This allows to obtain a nonlinear transform to register the 2D fluoroscopy images to the 3D CT volume and therefore, to localize the tip of the endoscope.

Impact & Innovation
By registering fluoroscopy with prior CT navigation maps, the position of the endoscope within the rats’ lung is determined and can be related to the FE images. In the context of RIPF, FE images of collagen show an increase in fibrous structures and CT images an increase in lung physical density in irradiated animals compared to controls. Using CT for fibrosis localization and fluoroscopy guidance to track the endoscope, an “optical biopsy” can be performed with FE to assess lung damage at the microscopic level in real time. This methodology can be implemented for new molecular imaging probe validation in small animals and is amenable to clinical translation.

Conclusion
The feasibility of image-guided FE of the lung in small animals was established. The imaging framework proposed here by combining fluoroscopy and FE can be applied to a wide range of medically relevant questions, where localization of the disease is critical. This allows gaining insight into complex biological processes at the anatomical or macroscopic level and down to the microscopic level.
Radiation-Induced Pulmonary Fibrosis (RIPF) Imaging from Macro- to Microscopic. Left: Computed Tomography (CT) image of RIPF rat model (transverse view). Yellow arrow pointing towards RIPF showing regions of increased physical lung density in the irradiated lung. Middle: X-Ray Fluoroscopy image of RIPF rat model (coronal view) with the endoscope (red arrow) in an intermediate position within the lung. Right: Fluorescence Endomicroscopy (FE) image of RIPF with collagen targeted fluorescent probe exhibiting fibrous structures (grey arrows).
IMAGE CAPTION: Radiation-Induced Pulmonary Fibrosis (RIPF) Imaging from Macro- to Microscopic. Left: Computed Tomography (CT) image of RIPF rat model (transverse view). Yellow arrow pointing towards RIPF showing regions of increased physical lung density in the irradiated lung. Middle: X-Ray Fluoroscopy image of RIPF rat model (coronal view) with the endoscope (red arrow) in an intermediate position within the lung. Right: Fluorescence Endomicroscopy (FE) image of RIPF with collagen targeted fluorescent probe exhibiting fibrous structures (grey arrows).
The hallmark of successful sarcoma resection is the complete removal of the tumor with sufficient tumor-free boundary without cutting into the tumor itself or pertinent normal tissues in the surrounding area. However, current practices for sarcoma surgery - pre-surgical imaging, and visualization and/or palpation by the surgeon - are often unsuccessful at achieving clear tumor margins (~33%). Targeting agents utilizing near-infrared (NIR) fluorescence have gained momentum for identifying tumor tissue during surgical guidance. Epidermal growth factor receptor (EGFR) is overexpressed in many sarcomas and is a common molecular target for NIR fluorescent-targeted agents. A promising first-in-human clinical Phase 0 trial of ABY-029 (NCT0290196) has been initiated for recurrent glioma resection and is now being extended to sarcoma resection. ABY-029 is an anti-EGFR affibody molecule labeled with IRDye 800CW that has been approved by the FDA as an exploratory Investigational New Drug (eIND 122681). Here, we describe the optical parameters and imaging limitations of using ABY-029 to determine sarcoma size, location, and margin thickness that is achievable without infiltrating the tumor boundary.

Gelatin tissue phantoms were created using a variety of intralipid (scattering medium; 0.5 and 1%) and blood (absorption medium; 0, 0.5, 1, and 2%) to mimic the physical and optical properties of sarcomas and the normal tissues generally surrounding the tumor (muscle, fat, connective tissue). These phantoms were used to study required Tumor-to-Background Ratios (TBR) of tumor-mimicking inclusions in varying sizes and depths in normal tissue mediums. It was determined that inclusions could be detected as deep as 3 cm could be detected using broad-field fluorescence imaging when the tissue background recapitulated that of normal connective tissue. The ability to excise a tumor with 1-2 cm margins was assessed using large tissue phantoms with a sarcoma mimicking inclusion and varying levels of fluorescence in the surrounding phantom to achieve TBRs of 1, 2, 3, 5, and 10. In this blinded-surgeon study, removal of phantom material to attain negative tumor margins was successful only when the TBR was greater than 3.

Determining the optimal time point for surgery after administration of intravenous ABY-029 to achieve TBR > 3 was undertaken in a panel of sarcoma xenograft tumors (SK-NEP-1, SW-982, MG-63, VA-ES-BJ, and SK-L-MS1) with known, varying EGFR expression. ABY-029 uptake and distribution in the xenograft tumors and normal mouse tissues monitored for up to 8 hours post-administration. It was found that a suitable TBR was achieved between the sarcoma tumors and normal background tissues at time points greater than 2 hours. Based on this data and the success of ABY-029 in gliomas, a Phase 0 trial with ABY-029 in sarcomas is being initiated. Fluorescence images collected during surgery will be compared to this phantom and pre-clinical in vivo data to demonstrate optimal TBR in humans. Together, the tissue-mimicking phantom and xenograft mouse model studies indicate that ABY-029 demonstrates an exciting possibility for excising sarcoma tumors in humans.