Molecular Imaging Program Annual Retreat

“Molecular Imaging for Selection and Monitoring of Individualized Cancer Therapies”

Please join us and welcome our keynote speaker

Henry VanBrocklin, Ph.D.
University of California San Francisco
“Imaging at the Crossroads of Precision Medicine and the Cancer Moonshot”

October 7, 2016
8:00 AM – 4:00 PM

Thomas V. Angott, Sr. Boardroom
Hudson Webber Cancer Research Center
Detroit, Michigan

Hosts

Juri Gelovani, MD PhD, Leader
Neb Duric, PhD, Co-Leader
A PDF of the 2016 Molecular Imaging Program Retreat is available for download at:

www.karmanos.org/2016MIRetreat
### Molecular Imaging Program Retreat
Friday, October 7, 2016

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<td>Rational Design of Upconverting Nanocrystals for Optical Molecular Imaging and Sensing &lt;br&gt; <em>K. Tauni Dissanayake</em>, B. Dulani Dhanapala, Federico A. Rabuffetti*</td>
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<td>Weighted Imaging of Arteries and Veins with using Ferumoxytol. &lt;br&gt; <em>E. Mark Haacke, Jean-Christophe Brisset</em>, Saifeng Liu*, Zeynep Demir, Yulin Ge</td>
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<td>An Omics Approach to Traumatic Brain Injury in Human Patients. &lt;br&gt; <em>Armin Iraji</em>, Natalie Wiseman, Hanbo Chen, Robert Welch, Brian O'Neil, Tianming Liu, E Mark Haacke, Zhifeng Kou*</td>
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Molecular Imaging Program Annual Retreat

Podium Presentations
TITLE: Imaging Tumor Treatment: Pharmacodynamics and Pharmacokinetics

OBJECTIVE: In animal studies, dexamethasone (Dex) affects tumor proliferation and chemotherapy efficacy and Bevacizumab (Bev) treatment affects liposome (LP) delivery were studied in non-small cell lung (NSCLC) and colon cancer xenografts using positron emission tomography (PET) and labeled fluorothymidine ($^{18}$F-FLT) and liposomes ($^{64}$Cu-MM-DX-929), respectively.

METHODS:
1. **Utilize FLT to detect Dex-mediated S-phase suppression in NSCLC xenograft models.**

   A549 human NSCLC cells were grown subcutaneously in SCID mice. Mice were imaged at baseline with $^{18}$F-FLT-PET and again 24h after receiving Dex (15 mg/kg BID i.p., q8 hr x 3). For these studies, Dex treatment simulated the current use with chemotherapy with pemetrexed in patients. Scans were analyzed with PMOD (Zurich, Switzerland) imaging software to quantitate changes in tumor FLT retention.

2. **Utilize $^{64}$Cu-MM-Dx-929 to detect LP uptake following Bev in a colorectal adenocarcinoma model.**

   HT-29 human colorectal adenocarcinoma tumors were grown subcutaneously in SCID mice. $^{64}$Cu-MM-Dx-929 (Merrimack Pharmaceuticals, Boston, MA) were injected into tumor-bearing mice and imaged with microPET. Treatment groups received Bev (5 mg/kg i.p., 1 and 4 days post baseline scan), after which all mice were scanned again. Scans were compared for changes in LP accumulation following Bev treatment. Initial groups of mice were sacrificed after the second PET when tissues were harvested for tracer biodistribution measurements and histological analysis. Subsequent groups were tracked after the second PET to assess differences in tumor growth associated with treatment.

RESULTS:
1. **$^{18}$F-FLT Uptake Post-Dex in NSCLC Xenografts**

   After 24 h Dex, SUVmax of the right tumor decreased from 2.02 to 1.23 (-41% change), while the SUVmax of the left tumor went from 2.10 to 1.24 (-39% change) data shown in dot-plot from 17 tumors in 9 mice. Preliminary analysis indicated xenograft SUVmax decreased by an average of 50.7% and tumor : background ratio decreased by an average of 57.1%. Final analysis indicated SUVmax declined by an average of 63.1%. (Fig. 1)

2. **$^{64}$Cu-MM-DX-929 Uptake post-Bev in colorectal adenocarcinoma Xenografts**

   Untreated mice showed a significantly increased uptake of $^{64}$Cu-MM-DX-929, with a mean percent change in tumor SUV$_{max}$ of 30.6±7.3 (n=23) after 7 days, while mice treated with Bev showed little or no change in uptake, with changes in SUV$_{max}$ of -0.3±7.8 (n=25) (p=0.005). These data suggest that Bev has an effect on LP distribution which is measurable with PET after one week. (Fig. 2)

CONCLUSIONS AND SIGNIFICANCE:

Studies first conducted in NSCLC cell lines indicated reductions in $^3$H-FLT uptake 24 hours after Dex reflect the relative expression of glucocorticoid receptor (GR$\alpha$). This result was translatable to animal studies, where scid mice implanted with high-GR A549 tumors demonstrated an average decrease of 50.7% in SUVmax after Dex treatment. In pilot studies in patients with advanced NSCLC, the changes were much more variable, with half of the patients showing some response to Dex, and the other half unchanged. Given the distribution in GR$\alpha$ expression in NSCLC, this result was not unexpected.

Ultimately, the imaging approach used here could allow for the stratification of patient tumors by Dex sensitivity, and alternative therapies (potentially) offered to those with responsive cancers. More broadly, our study could enable evaluation of other chemotherapy agents, many of which are S-phase specific, and are accompanied with corticosteroids to prevent adverse events.

The effect of Bev on tumor vascularity may change LP deposition and drug delivery, and could vary among a population or differing treatment schedules. PET with tracer LPs like $^{64}$Cu-MM-DX-929 could detect these effects early into treatment, as well as monitor response.
Figure 1. $^{18}$F-FLT uptake in high GRα A549 xenografts. SUVmax values of tumors treated with Dex (15 mg/kg bid, ip) (n=9) or control (saline) (n=11) at baseline and after 24 h treatment. After 24 h Dex tumor FLT retention declined by an average of 63.1%.

Figure 2. Bev treatment may abrogate increases in $^{64}$Cu-MM-DX-929 infiltration of tumors over time, as detected by PET. Coronal views of microPET, microCT, and microPET/CT scans of an HT-29 tumor-bearing mouse treated with bev (A) prior to (A.a.) and after (A.b.) two doses of bevacizumab (tumors outlined on images) show little change in tracer uptake. Conversely, tumors in an untreated mouse (B) demonstrated significant increases in tracer LP localization to tumor between scans at baseline (B.a.) and day 7 (B.b.).
Multimodal Neuroimaging in Malignant Gliomas

C. Juhász, E. Bosnyák, S.K. Michelhaugh, N.V. Klinger, N. Robinette, G.R. Barger, S. Mittal
Departments of Pediatrics, Neurology, Neurosurgery, Radiology and Oncology, WSU; Children's Hospital of Michigan; Karmanos Cancer Institute

Background: Malignant gliomas have a dismal prognosis with a 15-month median survival of patients with glioblastoma. Clinical radiological assessment of gliomas primarily relies on MRI morphological characteristics. Molecular imaging with amino acid PET has been used increasingly worldwide for pre- and post-treatment glioma imaging. In our center, the tryptophan derivative alpha-[11C]methyl-L-tryptophan (AMT) has been tested to characterize various human brain tumors. Our recent studies demonstrated the potential value of tryptophan PET in accurate detection and differentiation of various gliomas and meningiomas, and differentiation of glioma recurrence from radiation injury. We have also shown that increased amino acid uptake can detect tumor-infiltrated brain with no contrast enhancement or low blood perfusion on MRI both before and after initial glioma treatment.

Methods: In our current studies, we combine clinical MRI with AMT-PET to evaluate: (i) if glioma prognostic molecular markers are associated with specific patterns of imaging variables; (ii) if pre-treatment tryptophan uptake has a prognostic value for glioblastoma survival; and (iii) if AMT-PET/MRI can monitor response during NovoTTF therapy, a novel clinical treatment option using alternating electric fields shown to prolong glioblastoma survival in a recent randomized clinical trial.

Results. (i) Our preliminary data show that about 1/3 of IDH1 wild-type glioblastomas (a particularly poor prognostic glioma subgroup) showed amplification of EGFR, which was associated with high Ki-67 tumor proliferative index (p=0.001), lower T1-contrast volume and higher PET/T1-contrast volume ratios (p=0.02). Tumors with MGMT promoter hypermethylation showed lower PET-defined tumor volume and lower tumoral tryptophan unidirectional uptake ratios (p=0.009). (ii) In the same IDH1 wild-type glioblastoma group, tumor/cortex uptake ratios were prognostic for 1-year survival (p=0.002) (Figure 1). (iii) In patients with recurrent glioblastoma, early response (within 2 months) to NovoTTF was detected by PET in 4 of 5 patients, including those without (example on Figure 2) and with combined antiangiogenic therapy. Serial contrast-enhanced MRIs showed a delayed response (Figure 2). One tumor, with a much larger initial tumor size than the rest, showed no early response but progression on both PET and MRI.

Conclusions. These studies expand the potential clinical utility of MRI combined with amino acid PET imaging to provide imaging biomarkers related to molecular characteristics in malignant gliomas, prognostic information before and after initial treatment, and assess early response to novel glioma treatment modalities.

REFERENCES
Figure 1. Cumulative survival in patients with high (>1.94; green) vs. low (<1.94; blue) tumor/cortex AMT uptake ratios in patients with newly-diagnosed IDH1 wild-type glioblastoma.

Figure 2. Serial post-contrast T1-weighted MRI (T1-Gad) and AMT-PET images in a patient with recurrent glioblastoma, shortly before (baseline) and after initiation (2 months and 8 months) of NovoTTF therapy. AMT-PET showed decreased uptake within 2 months (early response), with no clear improvement of MRI contrast enhancement at that time. MRI showed a delayed response 6 months later, when tryptophan uptake showed a further decrease on repeated PET.
Ultrasound Tomography and the Breast Cancer Management Continuum

Neb Duric
Karmanos Cancer Institute

1. **Breast Cancer Detection and Lesion Characterization**: Mammography is the currently accepted gold standard for breast screening. However, its positive predictive power (PPV) is low and its sensitivity is greatly reduced in women with dense breast tissue, women who are at particularly high risk for developing breast cancer. Screening studies utilizing whole breast ultrasound have shown a significant increase in the detection of cancers of up to 4 additional cancers per 1000 screens thereby validating ultrasound’s known superior performance in dense tissue. However, ultrasound’s increased sensitivity to invasive cancer is offset by increased call back rates. Improved lesion characterization would therefore help lower the barriers to adoption of screening ultrasound.

We have been developing Ultrasound tomography (UST) at KCI which is now an emerging technique that moves beyond B-mode imaging by virtue of its through transmission capabilities. Transmission ultrasound provides additional characterization by measuring tissues parameters such as sound speed and attenuation. These parameters have been shown to quantitatively characterize lesions, a capability not available in current whole breast ultrasound systems. Clinical studies carried out at KCI have shown significantly improved specificity and PPV relative to standard US in a study of 200 symptomatic women [Fig 1]. We have spun out this technology from the KCI and received FDA clearance for commercialization. We are now readying to launch a multi-center trial to demonstrate reduced call back rates and thereby remove barriers to adoption of screening ultrasound.

2. **Assessing Breast Cancer Risk**: Elevated breast density increases a woman’s risk of developing breast cancer, while at the same time lowering the sensitivity of standard mammography exams. Over 21 states now have legislation that requires radiologists to report breast density. Unfortunately, such reporting provides limited guidance on alternate imaging and no guidance on risk assessment for women with dense breasts. Our long-term goal is to reduce the incidence of invasive breast cancer by improving methods of breast cancer risk prediction at the individual and population level, which will facilitate both practice and research in breast cancer prevention. Our clinical studies have shown that breast density (BD) measurements by UST correlate well with mammographic estimates of BD while, unlike mammography, provide an absolute, volumetric and quantitative measure [Fig 2]. Follow-on expanded studies involving 250 study subjects validated these initial results. We have therefore developed a safe, easy to use tool for investigating and further clarifying the role of breast density in elevating breast cancer risk. Our next goal is to use this tool to explore breast cancer risk models utilizing measurements of BD by UST to determine whether they will have greater predictive power compared to models using BD by mammography.

3. **Monitoring Treatment Response**: Locally advanced breast cancer is a clinically difficult disease and many patients experience relapse and death. Imaging data to support clinical decision-making is limited and not routinely used in a standardized manner. MRI and PET imaging have been shown to predict response as early as two weeks after treatment begins. Unfortunately, neither PET nor MRI is widely used for this purpose because of the logistical difficulties and especially the expense associated with their frequent use. These limitations have also inhibited the extent of validation research and clinical acceptance. Thus, under current standard of care guidelines, oncologists and patients may not know how well a treatment is working until well into the course of treatment. The ability to identify non-responders early in the treatment process would provide potentially crucial guidance for changing to alternative regimens thereby minimizing patient suffering from unnecessary NAC side-effects and preventing further tumor progression. Furthermore, predicting pCR would be highly beneficial for breast cancer drug development given the FDA’s acceptance of pCR as an endpoint to support accelerated approval. Our recent studies suggest that predicting response may be possible as little as 2 weeks after start of NAC [Fig 3].
ACKNOWLEDGMENTS: This research was supported by the NIH through grant number: R44CA165320-01A.

ACKNOWLEDGMENTS: This research was supported by the Komen Foundation through grant number: KG100100

ACKNOWLEDGMENTS: This research was supported by the Herrick Foundation

Figure 1. ROC curves for UST vs Ultrasound using 3 and 5 model parameters.

Figure 2. BD by MRI (%water) vs UST (VASS).

Fig 3. The process used to conduct preliminary study. (A) Longitudinal UST images showing decline in tumor volume and sound speed (UST-c parameter) for a patient that achieved pCR. (B) Changes were quantified by measuring the volume and sound speed of tumor (inner ROI). Tumor sound speed relative to peritumoral region (annular region) was then measured as an indicator of relative tumor density. (C) Measured volume was then multiplied by relative sound speed to create a single response variable, UST-c, which was then normalized and plotted for each patient. In most cases, response curve followed an exponential decline (red curves denote best fitting exponential function). The plot in (D) shows cumulative response values for all patients, labeled by pathological outcome. Curves correspond to group averages of best fitting exponential function for all non-complete responders (blue) and all complete responders (red).
Development of Low Cost and Portable Photoacoustic Imaging Using Very Low Energy Pulsed Laser Diode

Ali Hariri\textsuperscript{a}, Afreen Fatima\textsuperscript{a}, Nafiseh Mohammadian\textsuperscript{a}, Nicholas Bely\textsuperscript{a}, Mohammadreza Nasiriavanaki\textsuperscript{a,b,c}\textsuperscript{*}

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\textsuperscript{c} Barbara Ann Karmanos Cancer Institute, Detroit, Michigan, USA, 48201

Abstract. With the growing applications of Photoacoustic Imaging (PAI) in neurology, vascular biology, dermatology, ophthalmology, tissue engineering, angiogenesis, and other medical specialties, there is a need to make the system more compact, portable and affordable. Therefore, we designed an economical and compact version of PAI systems by replacing expensive and sophisticated lasers with a robust pulsed laser diode of 905 nm wavelength. In this study, we developed low cost portable PAI including microscopy (both reflection and transmission mode) and computed tomography with a very low excitation energy of 0.1\textmu J. Phantom study was performed in different configuration and also ex-vivo image was obtained from mouse skin in reflection mode of microscopy system.

Fig. 1: Schematic of laser diode based photoacoustic microscopy system in transmission mode.
Fig. 2: Transmission mode LC-OR-PAM results. (a) Photograph of a star pattern constructed by black tape as an imaging target, (b) photoacoustic image of the imaging target with the size of 10 mm by 10 mm, (c) image intensity profile across the photoacoustic image, along the dotted line.
Array-based Ultrasound and Photoacoustic Tomography for Breast Cancer Imaging

Suhail S. Alshahrani1†, Yan Yan1‡, Sirisha Kondle2, Barrington O'Brian Brown2, Neb Duric3, Mohammad Mehrmohammadi1, 2, 3*

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† Equal Contribution

Abstract:
Breast cancer is a major health problem in the United States and the world. An estimated 246,660 new, invasive breast cancer cases in females are expected to be diagnosed in the United States in 2016 [1]. Mammography, magnetic resonance imaging (MRI), and ultrasound (US) are the major imaging modalities often used to detect breast cancer. Ultrasound imaging shows performance in detecting suspicious breast cancer lesions, but the poor specificity of US imaging limits its diagnostic power in differentiating between benign and malignant breast lesions [2]. In recent years, photoacoustic (PA) imaging has shown great promise in the detection and staging of cancer [3]. Combining PA and US imaging is a strong tool that can provide various sets of information including structure and morphology of the pathologic tissue. In this study, a single array US/PA tomography system has been developed to scan different types of tissue-mimicking phantoms and live animals. In addition, we investigated on optimized light delivery strategies for omni-directional illumination on breast tissue. Our imaging system consisted of a fully digital, programmable US scanner, a tunable pulsed laser source, and a computer-controlled motorized rotation/translation scanning unit that could rotate and translate the objects within the water tank in order to obtain volumetric tomographic images. An FPGA-based control unit was utilized to synchronize the laser and the US acquisition machine, allowing for interleaved acquisition of US and PA frames. The system was tested with calibration phantoms to characterize the resolution and showed to be able to achieve the resolution of 200 µm. Moreover, the tomographic images were acquired in tissue gelatin-based mimicking phantoms with embedded gelatin/ blood inclusions. Our results indicate the ability of the system to acquire co-registered USPA images containing both acoustical and optical contrast and thus providing both anatomical and functional information on the tissue.

References
A Comparison of Deformable Image Registration Methods for Cervical Cancer Patients

Rebecca Meerschaert¹, Zichun Zhong², and Ling Zhuang¹
¹Department of Oncology, Radiation Oncology Division, Wayne State University School of Medicine
²Department of Computer Science, Wayne State University

Background & Purpose: Image guided brachytherapy (IGBT) for cervical cancer uses daily CT images at each fraction for treatment plan contouring and dose calculation. Currently, all ROIs are manually contoured which is a time-consuming process where inter-user and intra-user contouring variation could result in large uncertainties in delivered dose. Deformable image registration (DIR) has been introduced to automate the contouring process and provide the possibility for dose accumulation for normal tissue toxicity prediction.¹ DIR is a primary component for adaptive radiotherapy since anatomical variations between treatment plans due to organ filling, organ motion, or tumor response can be detected from image information.² While complete automatic segmentation of the organs would be difficult in the pelvic region, DIR can be used to deform images of and propagate contours from the treatment plan of the planning CT to that of the daily CT, instead of the patient waiting through the lengthy process of manual contouring. The aim of this study was to compare two DIR methods: intensity based B-spline registration using Elastix³ and tetrahedral mesh-based registration using an in-house 3D GPU-based algorithm.

Materials & Method: DIR was retrospectively performed between the planning CT image and one set of daily CT image from five brachytherapy fractions for one biopsy-proven cervical cancer patient. Image dimensions were 512 x 512 x 38 with voxel sizes of 0.9766mm x 0.9666mm x 3mm. The transformation was defined as mapping the planning CT image to the daily CT image. Intensity-based DIR involved rigid and b-spline transformations. Mesh-based DIR involved 10,000 vertices of tetrahedral mesh. Registration results were evaluated qualitatively by visual assessment and quantitatively by normalized cross-correlation (NCC) for the main pelvic treatment area in the image (dimensions 512 x 512 x 21). In addition, contour propagation was evaluated by average symmetric distance (ASD), root mean square distance (RMSD), and maximum symmetric distance (MSD) for organ contours including bladder, rectum, and sigmoid.

Results: Mesh-based DIR results provided improved NCC compared to intensity-based method. However, the organ shapes did not match perfectly to the ground truth (planning CT contours) due to complicated deformations. The NCC between the daily and planning CT images was 0.5371, which was improved by intensity-based DIR to 0.9796 and mesh-based DIR to 0.9688.

Conclusion: Currently, the mesh-based DIR method and intensity-based DIR method provided comparable results. Further improvements for the mesh-based technique involve using the reference image contours to generate feature-based meshes, which will allow for tissue features inside the torso to have a greater influence on the registration for improved results.

The figure below shows three axial image slices (slice 10, 15, 20) depicting the CT-based DIR results between the planning CT and daily CT images. The daily CT (top row) represents the un-deformed source image, which was deformed to match the target image (planning CT, bottom row) using intensity-based (results in second row) and mesh-based (results in third row) DIR techniques. As shown in the figure, bladder (red contour) filling in the daily and planning CT images were very different indicating a large deformation was needed. Visually, the deformed bladder shape using intensity-based DIR matches the bladder shape on planning CT better than the mesh-based DIR. In addition, the intensity-based DIR provides comparable NCC value to the mesh-based DIR (0.9796 vs 0.9688 respectively). However, the mesh-based method used GPU technique and completed the registration over seven times (50.01s compared to 372.85s) faster than the intensity-based method, which is a feature preferred in clinical situations. Incorporation of organ shapes and tissue features in the mesh-based method are currently in progress and improved NCC and contour propagation results are expected.

The figure below shows the image differences in pixel intensities calculated for slice 15. The left image represents the difference between planning and daily images prior to registration. The middle and right images represent the difference between planning and deformed daily images for intensity-based DIR and mesh-based DIR respectively. The differences between the planning and deformed daily images are largely due to complex variations at interfaces between tissue and bone in the pelvis.

Currently, we are working on contour propagation for both methods. Therefore bladder, rectum, and sigmoid contours from the planning CT can be deformed, automating the contouring process by generating contours for the daily CT image. These generated contours will then be compared with the physician's contours.
Title: The feasible of improving MRI sensitivity to detect tumor that is 100 to 1000 time smaller than the conventional MRI detection limitation.

Jiani Hu, Alex Zhuang, Jianjun Wang, Qing Lu, Haoyu Wang, Yimin Shen, Quan Jiang
Corresponding author: Jiani Hu, Professor, Department of Radiology, Wayne State university

Introduction:
The importance of the early detection of tumor have been long recognized because a favorable outcome can be achieved using existing medical techniques. MRI is a favorable imaging modality because of its non-invasiveness, excellent soft-tissue contrast and the capability of both morphological imaging and functional imaging. We hypothesize that by combining blooming effects of susceptibility weighted imaging (SWI) and the tropism effect of ferritin labeled stem cells toward tumor, we can detect tumors are about 100 to 1000 time smaller in 3D size than those currently detected with MRI.

MRI provides both magnitude and phase information. MRI phase information had been largely discarded due to its high sensitivity to susceptibility related field variations and resultant blooming effect that often severely degrades the quality of phase images. However, from a new point of view, this information may also have the potential to provide indirect information on an ultra-small object. Susceptibility weighted imaging (SWI) is a MRI technique specifically designed to enhance phase information by multiplying normalized phase images to corresponding magnitude images, thus can combine both magnitude and phase blooming effects.

The objective of this study is to evaluate the feasibility of improving MRI detection sensitivity by about 1000 times and the capability of protein induced stem cell to automatically home to metastatic tumors.

Results

Computer simulation: A 614-fold increase in 3D volume by blooming effects (Figure 1)

In vitro phantom study: A 512-fold increase in 3D volume by blooming effects (Figure 2)

In vivo animal study: A 970-fold increase in 3D volume or 9.9-fold increase in vessel diameter (Figure 3)

The tumor homing property of 4T1-piPSCs towards primary and metastatic tumors (Figure 4)

Discussion

Our preliminary study clearly demonstrates that the combination of SWI blooming effects and iron-contained MRI agents can detect an object that is 1000 time smaller than conventional MRI techniques. However, to detect tumor using SWI blooming effects, we need to induce susceptibility changes specifically in tumor sites, which requires the delivery of iron-contained MRI agents to the tumor site precisely. Several types of delivery carriers in the literature have the capability to accomplish such delivery, including numerous types of nanoparticles with cancer tumor-targeting capability, stem cells with tumor homing property, and immunes cells with tumor homing property. We are looking forward to collaborate with other groups to find out practical MRI techniques for detecting tiny tumor at its early stage.
Figure 1. Field distribution (a) and phase map (b) of computer simulation, Blooming effect was about 8.5 times larger in radius or 612 times in 3D volume.

Figure 2. Magnitude (a) and unwrapped phase (b) images of a horse spleen ferritin phantom obtained at 3T using a clinical head coil. Blooming effect was about 8.0 times larger in radius for the ferritin filled straw. (c) and (d) show the zoomed regions from (a) and (b) respectively. There are about 14 such small bubbles in this single slice that are visible in the phase image but only two can be positively identified in magnitude image while others were either barely visible or invisible at all.

Figure 3: Illustration of a 9.9-fold increase in the diameter of the vessel by blooming effect in Sprague-Dawley rat using P904 contrast agent. A) post-contrast SWI image with a TE of 2.72 ms acquired at a 7T preclinical scanner (one image without MIP); B) zoomed region of interest; C) corresponding histologic slice at 10x magnification; D) region of interest zoomed to 40x magnification. The vessel of interest is labeled in B) and D) with a blue arrow.

Figure 4. A): An MRI protocol for testing tropism effect of 4T1-piPSCs. B-C): MRI images of metastatic tumor in #2 fat pad before (B, White Arrow) and after (C, White Arrow) the injection of QQ-ferritin labeled 4T1-piPSCs. D): Primary tumor in #4 fat pad and the metastatic lesion in #2 fat pad. E-F): MRI images of the lung before (E) and 7.5-hr after (F) 4T1-piPSC-implantation, showing white (E, Inset) and darkened lung metastatic lesion (Pink Arrows) by the migrated ferritin-labeled 4T1-piPSCs (F, Inset). G): Immunostain against ferritin, showing ferritin-labeled 4T1-piPSCs inside metastatic lesions, whereas nearby lung tissues do not contain green 4T1-piPSCs. Inset: The lung with metastatic lesions. Arrow points to the lesion detected by MRI.
Rational Design of Upconverting Nanocrystals for Optical Molecular Imaging and Sensing

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Upconverting nanocrystals, which sequentially absorb multiple NIR photons and emit higher-energy NIR or visible photons, offer several potential advantages over molecular downconversion fluorophores traditionally employed in biophotonic applications such as imaging and sensing. However, their implementation as optical probes has been limited by their low quantum efficiency and lack of full-spectrum color tunability; these deficiencies stem from the occurrence of deleterious energy-transfer processes. Despite the key role played by the host lattice in mediating energy-transfer processes relevant to light upconversion, the ability to rationally manipulate its chemical composition and crystal structure to direct the flow of energy remains limited. Our group’s research aims bridging this fundamental knowledge gap by using upconverting nanocrystals that couple rare-earth activators (Yb–Er, Yb–Tm) to a family of host materials (MFX; M: Ca, Sr, Ba; X: Cl, Br, I) whose crystal structure can be rationally manipulated through chemical composition.

In this work, we present the synthesis, structural characterization, and infrared-to-visible light upconversion properties of semiconducting SrFX (X: Cl, Br, I) nanocrystals co-doped with Er and Yb. Excitation of Yb at 980 nm results in two-photon upconversion covering the 520–700 nm spectral range. The excited states of the Er activator in the SrFX hosts exhibit a biexponential decay with lifetimes in the 0.01–0.2 and 0.3–0.4 ms ranges. Both, the chromaticity of the upconverted visible light and the excited-state dynamics can be tuned via chemical composition. Our findings prompt for an expansion of the library of upconverting nanocrystals into novel materials containing heavy halide anions (i.e., Cl\(^{-}\), Br\(^{-}\), and I\(^{-}\)).
Electron microscopy images (top) of fluorohalide upconverting nanocrystals of two different chemical compositions. Digital images (bottom) of nanocrystals under infrared light (980 nm): red and green emissions are obtained depending on the chemical composition.
Imaging CD3+ t cell response to DNA vaccination in HER2/neu induced spontaneous mouse tumors

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Background: DNA-based vaccines have shown promise in HER2+ cancer patients by engaging the patient’s own immune system to attack and debulk tumors[1]. With its success, innovative strategies to non-invasively and quantitatively monitor response to treatment are warranted [1]. Here in this study, we present two new immunoPET probes for detecting spontaneous murine tumors in Neu transgenic (NeuT) mice and monitoring tumor infiltration of CD3+ T-cells following vaccination.

Methods: To detect the presence of HER2/Neu+ mammary tumors in females and both mammary and salivary tumors in males, PET images were conducted using ~200-250 µCi of $^{64}$Cu (t1/2 ~ 12.7 h) labeled Ab4 (conjugated to p-SCN-Bn-1,4,7-Triazacyclononane-1,4,7-triacetic acid (NOTA)) [2], a murine anti-Neu monoclonal antibody (mAb) at 1-24 h post-injection (p.i.). Male NeuT mice received heterologous human HER2 DNA vaccine, pE2TM after regulatory T-cells (Treg) were depleted. Tumor infiltration by CD3+ T-cells was monitored by the antibody 2C11 (murine anti-CD3ε), conjugated to p-SCN-Bn-Desferrioxamine and radiolabeled with $^{89}$Zr (t1/2 ~ 3.27 d) using previously established methods [4]. PET images on DNA-vaccinated and untreated mice were acquired 4-96 h post-injection. A non-specific $^{64}$Cu-IgG will be used to control for non-specific macrophage binding to the Fc region. In parallel, female NeuT mice were vaccinated with pCytoNeu [3] encoding a cytoplasmic form of rat Neu.

Results: $^{64}$Cu-Ab4 distinguished Neu+ tumors as early as 2 weeks before palpable tumors are present with the tracer accumulating in all 10 lesions in female NeuT mice (6.5 %ID/g to 7.5 %ID/g). The male salivary tumors were also detected by $^{64}$Cu-Ab4 with volumes-of-interest (VOI) ranging from 13.4 to 46 %ID/g. Enhanced signal-to-noise ratio was demonstrated at 48 hours post injection for tumors imaged with $^{89}$Zr-2C11. Tumor-infiltrating T-cells were detected in salivary tumors with aVOI of 6.8 %ID/g whereas the untreated mice had a two-fold lower tumor uptake of the radiotracer (3.6 %ID/g). Tumors treated with pCytoNeu displayed a decrease in total tumor volume, but the tumor accumulation of $^{89}$Zr-2C11 ranged from ~1.8 – 3 % ID/g, suggesting the treatment did not elicit sufficient T-cell response. Additionally, we observed uptake within the mesenteric lymph nodes.

Conclusion: We have successfully developed two new tracers i) $^{64}$Cu-Ab4 for whole body lesion mapping of Neu+ transgenic spontaneous mammary tumor models and ii) $^{89}$Zr-2C11 for indicating response to DNA vaccination by targeting CD3+ T-cells infiltrating the tumor. Our studies effectively showed that anti-CD3 imaging discriminates between responders and non-responders, clearly displaying the strength of PET imaging as treatment response biomarkers.

Fluorinated Eu\textsuperscript{II}-containing complexes with applications in redox sensing

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The research that will be described involves the design of contrast agents that convey information about redox environments. There is a need for detection of redox environments because redox imbalance is associated with diseases including cancer, cardiovascular and liver diseases, and Alzheimer's disease. Consequently, many methods for imaging redox environments are being developed, including contrast-enhanced magnetic resonance imaging (MRI). Unlike the metal ion Gd\textsuperscript{III} that is used in common contrast agents for MRI but is redox-inert \textit{in vivo}, Eu\textsuperscript{II}-containing contrast agents can be oxidized at biologically relevant potentials. The oxidation response of Eu\textsuperscript{II}-containing contrast agents has been imaged \textit{in vivo}; however, there is a need for orthogonal detection of contrast agents to distinguish loss of contrast due to oxidation from loss of contrast due to clearance. We hypothesized that differentiation between oxidation and clearance could be achieved using a different NMR-active nucleus, \textsuperscript{19}F. Here, we present two fluorinated Eu-containing complexes. These oxidation-responsive complexes act as probes for \textsuperscript{1}H- and \textsuperscript{19}F-MRI, where each method of detection corresponds to a single oxidation state of the complex.

Standard synthetic techniques were used to synthesize the desired contrast agents, with the final metallation step occurring in a wet, oxygen-free glovebox. Samples for spectroscopy and phantoms were prepared in buffer (pH = 7), and some samples were bubbled with air. \textsuperscript{19}F-NMR spectroscopy was performed on an Agilent 9.4 T NMR spectrometer. Imaging was performed at Baylor Medical School on a Bruker 9.4 T horizontal bore MRI scanner.

\textsuperscript{19}F-NMR spectroscopy shows no signal for the Eu\textsuperscript{II}-containing complexes and one broad peak for the oxidized sample. Images of the Eu\textsuperscript{II}-containing complexes show \textit{T}_1-weighted enhancement for \textsuperscript{1}H-MRI but no \textsuperscript{19}F signal (Figure 1). The oxidized samples show no \textit{T}_1-weighted \textit{T}_1 enhancement for \textsuperscript{1}H-MRI but display \textsuperscript{19}F signal. These data support our hypothesis that differentiation between oxidized and unoxidized complexes can be achieved using orthogonal detection with \textsuperscript{1}H- and \textsuperscript{19}F-MRI. We expect that our oxidation-responsive, fluorinated Eu\textsuperscript{II}-containing contrast agents have the potential to provide information about redox environments \textit{in vivo}. 
Figure 1. MR phantom images (5 mm tube diameter) of Eu-222Fb and blank. $^{19}$F in vitro phantom images are in the top row, and $T_1$-weighted images are in the bottom row.
Non-invasive molecular imaging of SIRT1-mediated epigenetic regulation in cancers in vivo using PET/CT with a novel substrate-type radiotracer 2-[18F]PhAHA.

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Histone deacetylases (HDACs) play important roles in epigenetic regulation. Class III HDACs is comprised of 7 enzymes termed “silent information regulators” or “sirtuins” (SIRTs), a family of Zn2+-independent, but NAD+-dependent enzymes, that possess ADP-ribosyltransferase, deacetylase, desuccinylase, demalonylase, demyristoylase and depalmitoylase activities.

SIRT1 is involved in a wide variety of cellular processes and functions and is known to mediate cleavage of the acetyl moiety from the acetylated lysine residues of several proteins, including p53, PPARƔ, members of the FOXO family and NF-KB. Therefore, SIRT1 has emerged as an important target for therapy of cancer primarily due to its role in cell cycle regulation via p53 and FOXO3a attenuation and several drugs are under development towards clinical translation. However, currently there are no methods for non-invasive monitoring of pharmacodynamics of novel SIRT1 isoform-selective inhibitors in norm and disease. Therefore, there is a need for development of agents for non-invasive imaging of expression and activity of various SIRT1 in vivo.

Previous studies demonstrated that SIRT1 can dephenylacetylate a lysine residue at a rate of ~56% of its natural deacetylation rate, while other SIRT isoforms cannot cleave an aromatic leaving group. This provided an opportunity to develop a SIRT1-selective imaging agent for PET imaging, which would allow for quantitative measurement of the SIRT1 expression-activity product in vivo. We developed a focused library of compounds to elucidate the structure-activity relationship of SIRT1 and to identify the most selective and efficient imaging substrate for SIRT1. This preliminary screening utilized high-throughput fluorogenic assay with the Carbazol-L-Lysine-Aminomethylcoumarin as a backbone [1]. Subsequently, this backbone was exchanged for 6-aminohexanoicnilide, AHA [2] to develop PET radiotracer. The lead compound, 2-fluorophenylaminohexanoicanilide, was developed in both F-19 (2-FPhAHA) and F-18 (2-[18F]PhAHA) versions. 2-[18F]PhAHA was injected i.v. into Sprague-Dawley (SD) rats followed by dynamic imaging using MicroPET R4 (Siemens, TN), followed by CT imaging on INVEON microCT (Siemens, TN). The kinetics of radiotracer uptake was quantified using Logan graphical analysis [3], which demonstrated differential accumulation of 2-[18F]PhAHA-derived radioactivity in specific areas of the rat brain with high expression of SIRT1 (i.e., the dentate gyrus, CA1, nucleus accumbens, and caudate putamen). Following characterization of 2-[18F]PhAHA-derived radioactivity in the normal rat brain, 2-[18F]PhAHA was administered to SD rats bearing intracerebral 9L gliomas. PET/CT demonstrated significantly increased uptake of 2-[18F]PhAHA-derived radioactivity in the 9L tumors vs. normal brain tissue (P<0.01) and matched with tumor localization as observed with MRI and histological staining.

In summary, SIRT1 activity is upregulated in intracerebral 9L gliomas, as evidenced by increased accumulation of 2-[18F]PhAHA on PET/CT images, which demonstrates the feasibility of this method for visualization and quantification of SIRT1 activity in brain tumors. Also, 2-[18F]PhAHA on PET/CT should allow for future monitoring of pharmacodynamics of SIRT1 inhibitors during anti-cancer therapy.

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Susceptibility Weighted Imaging of Arteries and Veins with using Ferumoxytol

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Purpose: Microvascular abnormalities have been increasingly identified as the basis of many neurovascular and neurodegenerative disorders1. Imaging the cerebral venous system is currently achieved using susceptibility weighting imaging (SWI)2.3. However, SWI is unable to image arteries since arterial blood has the same susceptibility as the surrounding tissue (due to the lack of deoxygenated hemoglobin). This study was designed to evaluate the ability of SWI to image both the arterial and venous systems with ultra-high-resolution at 3T and 7T with the injection of an ultra-small-superparamagnetic-iron-oxide (USPIO) agent, Ferumoxytol (a negative blood pool contrast agent).

Methods: Pre- and post-Ferumoxytol (2 or 4 mgFe/kg) SWI was performed on two healthy volunteers at both 3T and 7T MR. In order to obtain ultra-high resolution images (0.11x0.22x1.25mm), an asymmetric gradient-echo SWI sequence was used with the following image parameters: TR = 35ms, TE1 = 8ms, TE2 = 16ms and flip angle = 10° for a 13 min acquisition time for each echo. SWI data were processed using a high-pass filter with a 96x96 kernel since we were interested only in the small structures, i.e., the vessels.

Results: This USPIO driven MR angiography and venography (MRAV) approach successfully enhanced both the venous and arterial systems. The visibility of the arteries in the basal ganglia and cerebellum was improved as well as in the periventricular area (Figure 1). Exploiting the flow effect at short echo time (8ms), pre-USPIO MRAV maximum intensity projection allows arterial vasculature to be highlighted pre-contrast (Figure 1A). Small artery blooming was seen on post contrast MRAV, more prominent at longer TE (Figure 1D TE=16ms) than at low TE (Figure 1C TE=8ms). Small arteries (diameter=200~400μm) were seen 1.5~2 times bigger on post-contrast MRAV with TE=16ms (Figure 1D). Some very small cortical arteries can also be seen.

Conclusion: We have demonstrated the feasibility to generate ultra-high-resolution MRAV using USPIO-enhanced SWI. This could represent a powerful new tool in detecting microvascular abnormalities not visible on conventional MRA.

Keywords: USPIO, MRI, SWI, Susceptibility, blooming effect, arteriogram, venogram, angiogram, MRAV

Figure 1: Pre-contrast TE=8ms data (A) shows arteries on the MIP due to the time of flight effect and (B) shows veins on the MIP conventional SWI due to the stronger susceptibility of deoxygenated hemoglobin (but no arteries). Post-Ferumoxytol (2mg/kg) SWI (C) shows both veins (blue arrows) and arteries (red arrows). With the blooming effect, the vessels appear bigger on the images post-contrast (yellow arrows) at TE=8ms (C) and even bigger at longer TE = 16ms (D).

References
An Omics Approach to Traumatic Brain Injury in Human Patients

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INTRODUCTION
Traumatic brain injury (TBI) is a leading cause of death and disability and causes the nation billions of dollars each year in the United States alone. All previous clinical trials failed due to the heterogeneity and complexity of the disorder. Among TBI pathology spectrum, axonal injury and cerebral hemodynamic and metabolic disturbances are still under investigated. By using advanced magnetic resonance imaging (MRI) techniques, along with other novel tools, we took a systemic investigation of TBI in human patients, or called the “omics approach.” This approach includes proteomics, metabolomics and connectomics investigation of mild traumatic brain injury (mTBI) at the acute stage.

MATERIALS AND METHODS
In proteomics, subjects’ serum blood sample was acquired and analyzed by using ELISA essays, with focus on UCH-L1 and GFAP blood protein biomarkers. In metabolomics, a comprehensive MR imaging protocol was developed to measure venous blood oxygenation, cerebral blood flow, and cerebral metabolic rate of oxygen, in addition to diffusion tensor imaging and resting state fMRI signal. In connectomics, a novel analytic framework called dense individualized and common connectivity-based cortical landmarks (DICCCOL) was used to analyze large-scale or connectome-scale brain connectivity changes after TBI, including both structural and functional connectivity.

RESULTS & DISCUSSION
We have successfully recruited 60 mTBI patients at the acute stage from the emergency department of Detroit Medical Center, a Level-1 trauma center, and 60 healthy controls from local community and patients’ friends and relatives. Our initial proteomics investigation demonstrated elevated level of GFAP and UCH-L1 proteins in mTBI patients at the acute stage (p<0.001) and patients’ GFAP level is associated with patients’ intracranial hemorrhage in even CT negative patients. Our metabolomics study showed the mTBI patients have increased cerebral blood flow (p<0.03) and venous blood oxygenation (p<0.05) at the acute stage, which suggests a compensatory effect at the acute stage that has never been reported before. For the first time, our connectomic analysis revealed that mTBI patients have large scale, or connectome-scale, brain network alternations in both structural connectivity (reported by DTI fiber tractography data) and functional connectivity (reported by resting state fMRI data). The connectomic features in our data resulted in 100% sensitivity and 98% specificity in mTBI detection.

CONCLUSIONS
In summary, our data represent the first effort of an omics approach to investigating TBI. Our work further demonstrates the complexity of TBI pathology in a global manner.
Molecular Imaging Program Annual Retreat

Poster Presentations
Microscopy, Imaging and Cytometry Resources Core

**Core Director:** Kamiar Moin, Ph.D.
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The mission of the Microscopy, Imaging & Cytometry Resources (MICR) core aims to enhance the peer-reviewed funded research activities of WSU investigators whose research requires microscopy, imaging resources, flow cytometry, and related techniques.

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- BD FACSCantoll Flow Cytometer
- Amnis ImageStream® MK II Imaging Cytometer
Mass Spectrometry and Analytical Laboratory, Lumigen Instrument Center (LIC-MS), Department of Chemistry, Wayne State University

Dr. Olena Danylyuk is a co-manager of the Mass Spectrometry (MS) and Analytical Laboratory with the Lumigen Instrument Center, Department of Chemistry at Wayne State University. She is also Adjunct Assistant Professor of Research, School of Medicine, WSU.

The LIC-MS Facility at WSU maintains a well-equipped and well supported laboratory for mass spectrometric analysis. The services offered include accurate mass analyses of small organic molecules, LC-MS of small molecules, polymer analysis, analysis of synthetic peptides and oligonucleotides. We also have experience in the field of proteomics including protein identification, protein purification post-translational modification analysis, MALDI-TOF MS Imaging and MALDI-TLC. The mandate of the Mass Spectrometry and Analytical Instrument Core Facility is to provide state-of-the-art instrumentation in all areas of mass spectrometry for the surrounding research community.

The primary purpose of the LIC Mass Spectrometry and Analytical Instrument Core Facility is to support research activities of the Faculty of the Wayne State University by providing advice, technical assistance and access to advanced scientific equipment. This includes education and training for graduate students and allows collaborative research requiring advanced experiments by working closely between faculty, students and staff. Important secondary purposes are to support the research activities of other Universities, and to assist local business ventures in solving sophisticated scientific problems.

Dr. Danylyuk research interests aimed at the investigation of biological processes involving the synthesis, modification, storage and degradation of certain biomarkers using modern mass spectrometric methods of analysis. The Lumigen Instrument Center has recently acquired Bruker UltrafleXtreme MALDI-TOF/TOF Mass Spectrometer that capable of performing small molecules analysis, protein profiling, tissue imaging and biomarker discovery.

One of our experimental approach, used in the study of biomarkers, utilizes new molecular imaging technology- matrix-assisted laser desorption mass spectrometry. This method involves molecular mapping of tissue through the production of ion images obtained from the analysis of tissue slices by matrix-assisted laser desorption mass spectrometry. This technique permits a tissue section to be mapped in multiple molecular weight values, localizing the molecules in an X, Y coordinate representation of the sample. Ion images are produced by repetitive exposure of the sample to the laser beam, where adjacent spots are irradiated, resulting in an ordered array of mass spectra that are keyed to specific locations in the sample. From one raster of the sample, a specific ion image, at any chosen m/z value, could be produced to give the spatial arrangement of molecules of interest. Current work involves development of methods for sample preparation and target surface modifications to achieve high sensitivity and high image resolution, with molecular specificity.
MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry lipid profiling of formalin fixed tissues/imaging mode

MALDI-TOF bottom-up mass spectrometric sequencing of microRNA

MALDI-TOF analysis of epoxy resins
Comparison of 1-(2-[18F]fluoroethyl)-L-tryptophan and alpha-[11C]-methyl-L-tryptophan PET imaging in patient-derived brain tumor xenograft mouse models

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ABSTRACT

Rationale: Abnormal tryptophan metabolism via the kynurenine pathway (KP) is involved in the pathophysiology of a variety of human diseases including cancers. alpha-[11C]-methyl-L-tryptophan (11C-AMT) positron emission tomography (PET) imaging demonstrated increased tryptophan uptake and trapping in brain tumors, but the short half-life of 11C limits its widespread clinical application. Recent in vitro studies suggested that the novel radiotracer 1-(2-[18F]fluoroethyl)-L-tryptophan (18F-FETrp) may be useful to assess tryptophan metabolism. In this study, we tested in vivo organ and tumor uptake and kinetics of 18F-FETrp in patient-derived xenograft mouse models and compared it with 11C-AMT uptake. Methods: Xenograft mouse models of glioblastoma and metastatic brain tumors (from lung and breast cancer) were developed by subcutaneous implantation of patient tumor fragments. Dynamic PET scans with 18F-FETrp and 11C-AMT were performed on mice bearing human brain tumors 1-7 days apart. The biodistribution and tumoral standardized uptake values (SUVs) for both tracers were compared. Results: 18F-FETrp showed prominent uptake in the pancreas and no bone uptake, whereas 11C-AMT showed higher uptake in kidneys. Both tracers showed uptake in the xenograft tumors with a plateau ~30-min post-injection; however, 18F-FETrp showed higher tumoral SUV than 11C-AMT in all three tumor types tested. The radiation dosimetry for 18F-FETrp determined from the mouse data compared favorably to the clinical 2-deoxy-2-[18F]fluoro-D-glucose (18F-FDG) PET tracer. Conclusions: 18F-FETrp tumoral uptake, biodistribution, and radiation dosimetry data provide strong preclinical evidence that this novel radiotracer warrants further studies that may lead to a broadly-applicable molecular imaging tool to examine abnormal tryptophan metabolism in human tumors.
Monitoring HDAC class IIa activity in the brain after single prolonged stress using noninvasive PET imaging with $^{18}$F-TFAHA

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Posttraumatic stress disorder is an incapacitating psychiatric disorder which is characterized by exposure to a traumatic event and about 9% of the general population that experiences a traumatic event develops PTSD and about 11-20% of military and veteran populations are diagnosed with PTSD. Emotional as well as psychological strain even without brain injury often leads to PTSD-like conditions, including such symptoms as hyper arousal, anxiety and anhedonia, disruption cognition, memory, and mood [1]. Trauma and stress-induced changes in acetylation of histones involved in the mechanism of posttraumatic stress disorder (PTSD) is due to the up-regulation of expression-activity of histone deacetylases (HDACs). Therefore, several general and isotype-specific HDAC inhibitors are undergoing preclinical and clinical studies for treatment of PTSD. However, there is little known about the temporal dynamics of isotype specific HDACs activity in various structures of the brain that are involved in the mechanism of PTSD. Our previous studies demonstrated that PET imaging with $^{18}$F-TFAHA allows for quantitative visualization of expression-activity of class IIa HDAC enzymes in the brain (predominantly HDACs 4 and 5). Upon intravenous administration, ($^{[18F]}$ trifluoroacetamido)-1-hexanoic anilide ($^{18}$F-TFAHA) accumulates specifically in the n. accumbens, periaqueductal grey, hippocampus and in the cerebellum, as the result of increased HDACs 4 and 5 expression-activity in these brain structures [2]. In the current study, we aimed to visualize the spatial and temporal dynamics of HDACs class IIa activity in the rat brain using a model of single prolonged stress (SPS). PET/CT imaging was performed before the SPS and on different days thereafter (i.e., days 1, 2, and 7). Logan graphical analysis was implemented to assess changes in the magnitude of $^{18}$F-TFAHA accumulation in different brain regions using muscle as a reference tissue. Preliminary results obtained in a group of 12 rats with SPS (11 rats at baseline, 7 rats on 1 day post SPS; and 5 rats on 7 days post SPS) demonstrated increased accumulation of 18F-TFAHA in the n. accumbens, periaqueductal grey, and hippocampus at 1 day post SPS, relative to baseline levels (p<0.05). At 7 days post SPS, the level of $^{18}$F-TFAHA accumulation in the hippocampus and n. accumbens was similar to that measured at baseline. In contrast, no differences in $^{18}$F-TFAHA accumulation were observed at different days post SPS in other regions of the brain (i.e., cerebellum). The ongoing studies are aimed to increase the numbers of animals to reach the statistical significance of results. If the increased levels of HDACs 4 and 5 early after SPS will be observed consistently, additional studies will be conducted to assess the efficacy of HDAC inhibitors (i.e., vorinostat, valproic acid) for therapy and/or prevention of SPS.

References:

Fig 1: The above are the PET/CT images of the rat brain when $^{18}$F-TFAHA was intravenously administered into the rats. The images were generated for the baseline (before SPS), days 1 and 7 days post SPS. The coronal and axial views of the images were used to determine if our regions of interest showed up-regulation or down-regulation of HDAC class II activity due to trauma caused by SPS. From the images it can be seen that in the rat there was an increased retention of the radioactive metabolite of $^{18}$F-TFAHA in the brain regions of our interest (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex) which indicates that there is an up-regulation of the HDAC class II activity on the days 1 and 7 post SPS compared to the baseline.

Fig 2: The bar chart above shows the group average of the different rat brain regions of interest (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex). It can be seen from the bar chart that on average the activity of HDAC class IIa increased for Day 1 post SPS compared to Baseline and on Day 7 the activity decreased from Day 1 but almost similar to that measure on the Baseline.

Fig 3: The above "Logan plots" were produced for the regions of interest in the rat brain during the different time intervals when the $^{18}$F-TFAHA was intravenously administered in the rats (baseline, days 1 & 7 post SPS). The muscle around the brain region was used as the reference tissue because it was seen that radioactive metabolite of the $^{18}$F-TFAHA has significantly less retention in the muscle region. This is due to the less or no HDAC class IIa activity in the muscle region. From the plots it can be seen that the day 1 post SPS has the highest slope in all the brain regions of interest compared to the baseline and day 7 post SPS. This indicates that there is high HDAC class IIa activity in particular regions of the brain (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex) immediately after stress.

Fig 4: Representative SPS rat brain sections stained immunohistochemically and counterstained with H&E. CA1, CA2, CA3 and part of the DG regions in the Hippocampus stained with HDAC class IIa antibodies (HDAC4, HDAC5 and pHDAC5) during the different time intervals (Baseline, 1 day post and 7 days post SPS).
Monitoring HDAC class IIa activity in the brain after traumatic brain injury using noninvasive PET imaging with $^{18}$F-TFAHA

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Traumatic brain injury is a severe complex injury which is a growing public health concern around the world. In U.S over 150,000 military personnel are diagnosed with a form of mild traumatic brain injury resulting in wide range of neurological and psychological symptoms followed by long term cognitive disabilities including neurodegenerative disorders such as Alzheimer’s disease. The pathophysiology of TBI leads to a cascade of autonomic malfunction and neuro/systemic inflammation. The fundamental role of epigenetic regulatory mechanism involved in neuroplasticity and adaptive responses to TBI is gaining recognition. Previous studies in various TBI models indicated that trauma-induced neurodegeneration is associated with several epigenetic changes in histones, including aberrant acetylation, methylation, and phosphorylation. Several HDAC inhibitors, such as valporic acid (VPA), have shown neuroprotection from TBI by decreasing the blood brain barrier permeability, reduction in neural damage, improving motor function and spatial memory [1]. However, the spatial and temporal dynamics of expression-activity of different HDAC classes and isotypes in the brain at baseline, after TBI, and after therapy with HDAC inhibitors is largely unknown due to the lack of studies using non-invasive molecular imaging. Previously, we developed the HDAC class IIa specific substrate-type radiotracer 6-([$^{18}$F]trifluoroacetamido)-1-hexanoinilide ($^{18}$F-TFAHA), with high substrate affinity and specificity to HDACs 4 and 5. Upon intravenous administration, $^{18}$F-TFAHA accumulates specifically in in the n. accumbens, periaqueductal grey, hippocampus and in the cerebellum, as the result of increased HDACs 4 and 5 expression-activity in these brain structures [2]. In the current study, we assessed the spatial and temporal dynamics of HDACs class IIa activity in the rat brain using positron PET/CT with $^{18}$F-TFAHA in a model of diffuse traumatic brain injury (TBI; Marmarou model [3]). PET/CT imaging with $^{18}$F-TFAHA was performed before TBI, and 24-48 hours and 7 post TBI. Studies were conducted in 5 rats with TBI (2 rats at baseline, 1-2 and 7 days post TBI; and 3 rats at baseline and 7 days post TBI). A significant decrease in $^{18}$F-TFAHA accumulation was observed at 24-48 hours post TBI in the hippocampus, n. accumbens and periaqueductal grey, as compared to baseline levels. At day 7 post TBI, the levels of $^{18}$F-TFAHA accumulation in the hippocampus, n. accumbens and periaqueductal grey were similar to those at baseline. The results of non-invasive imaging using PET/CT with $^{18}$F-TFAHA were validated by immunohistochemical analyses of rat brains obtained in a separate group of animals at baseline, at 24-48 hours and 7 days post TBI. A significant increase in acetylation of H3K9, H2AK5, and H2BK5, were observed in hippocampal CA2 neurons at 24-48 hours post TBI, which was consistent with down-regulation of HDACs class IIa activity observed with $^{18}$F-TFAHA PET/CT. These results differ from previous reports that demonstrated hyperacetylation of H2 and H3 histones at 24-48 hours post TBI. Although previously the therapeutic effect of valproic acid has been demonstrated in similar TBI models in rats and swine, the mechanism of action may involve class I HDACs that are more susceptible to inhibition by valproate. Therefore, these results demonstrate the need for isotype-specific HDAC inhibitors for therapy of TBI, clinical translation of which can be facilitated by repetitive non-invasive molecular imaging with HDAC class and isotype specific PET radiotracers.

References:
Fig 1: The above are the PET/CT images of the rat brain when $^{18}$F-TFAHA was intravenously administered into the rats. The images were generated for the baseline (before TBI), days 1 and 7 days post TBI. The coronal and axial views of the images were used to determine if our regions of interest showed down-regulation or down-regulation of HDAC class IIa activity due to trauma caused by TBI. From the images it can be seen that in the rat there was a decreased retention of the radioactive metabolite of $^{18}$F-TFAHA in the brain regions of our interest (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex) which indicates that there is an down-regulation of the HDAC class IIa activity on the days 1 and 7 post TBI compared to the baseline.

Fig 2: The bar chart above shows the group average of the different rat brain regions of interest (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex). It can be seen from the bar chart that on average the activity of HDAC class IIa decreased for Day 1 post TBI compared to Baseline and on Day 7 the activity increased from Day 1 but almost similar to that measure on the Baseline.

Fig 3: The above “Logan plots” were produced for the regions of interest in the rat brain during the different time intervals when the $^{18}$F-TFAHA was intravenously administered in the rats (baseline, days 1 & 7 post TBI). The muscle around the brain region was used as the reference tissue because it was seen that radioactive metabolite of the $^{18}$F-TFAHA has significantly less retention in the muscle region. From the plots it can be seen that the day 1 post TBI has the highest slope in all the brain regions of interest compared to the baseline and day 7 post TBI. This indicates that there is low HDAC class IIa activity in particular regions of the brain (nucleus accumbens, periaqueductal grey, hippocampus and in the prefrontal cortex) immediately after trauma.

Fig 4: Representative TBI rat brain sections stained immunohistochemically and counterstained with H&E. CA1, CA2, CA3 and part of the DG regions in the Hippocampus stained with HDAC class IIa antibodies (HDAC4 and HDAC5) during the different time intervals (Baseline, 1 day post and 7 days post TBI).
Molecular imaging of epigenetic regulation mediated by HDACs 4, 5 using PET/CT/MRI with $^{18}$F-TFAHA in a rat model of human glioma.

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Histone deacetylases (HDACs) are involved in the pathogenesis of cancer through modulation of the expression of various genes involved in cellular proliferation, migration, angiogenesis and apoptosis. HDAC class IIa enzymes interact with tumor suppressor proteins including HIF-1α, GATA-1, and PLZF-RARα. Given the importance of HDACs class IIa in epigenetic regulation of cancer development, progression, and maintenance, there is a pressing need for non-invasive imaging approaches for monitoring the expression-activity of HDACs in vivo. To address this need, our laboratory has developed 6-(tri-fluoroacetamido)-1-hexanoicanilide ($^{18}$F-TFAHA) for imaging HDAC class IIa enzymes.

Previous studies show that $^{18}$F-TFAHA is enzymatically cleaved specifically by HDAC class IIa, predominantly by HDACs 4 and 5. To quantitatively visualize the expression-activity of HDACs 4 and 5 in brain gliomas, immunocompromised rats (NTac:NIH-Foxn1tmu, Taconic Biosciences, NY) were implanted intracerebrally (i.c.) with U87-tdRluc cells ($4 \times 10^5$ cells in 20 uL), that have been lentivirally-transfected to express GFP-luciferase and tdTomato fluorescent protein fusion reporter genes. Bioluminescence images (BLI) of GBMs were obtained after administration of luciferin (10 µl/g i.p.) using an InVivo Xtreme system (Carestream, Toronto, Canada) at 7 and 14 days post U87-tdRluc cell implantation. Gross tumor morphology and progression was evaluated with T2 MRI on day 14 after tumor implantation using a CliniScan 7T MRI system (Bruker, UK). Following 15-20 days, the rats were administered with $^{18}$F-TFAHA (500 µCi/animal, i.v.) and imaged using microPET R4 and Inveon CT (Siemens, TN). After PET/CT imaging, the animals were sacrificed and their brains extracted for immunohistochemical (IHC) and quantitative autoradiographic (QAR) studies (Typhoon 7000, General Electric, CT). The $^{18}$F-TFAHA accumulation in regions of interest (ROI) at 20-30 minutes post i.v. administration and was quantified using Logan graphical analysis, which demonstrated a significant increase in $^{18}$F-TFAHA accumulation in tumors versus surrounding normal cortex and white matter ($p < 0.05$), outside the structures with normally-increased HDAC IIa activity (e.g., hippocampus, amygdala, periaqueductal gray, n. accumbens). PET/CT/MR imaging results were validated by IHC of brain tissue sections that demonstrated a significant hypoacetylation of histones H2A, H2B, and H4 in tumor tissue with increased $^{18}$F-TFAHA accumulation. The ongoing studies in brain tumor-bearing rats undergoing treatment with HDAC inhibitor vorinostat are aimed to assess the feasibility of PET/CT/MRI with $^{18}$F-TFAHA for pharmacodynamic monitoring therapies with HDAC class IIa inhibitors. Ultimately, we aim to translate $^{18}$F-TFAHA PET/CT/MR imaging into the clinic.

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Influence of Molecular Parameters and Magnetic Field Strength on the Relaxivity of Eu\textsuperscript{II}-Containing Complexes

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Eu\textsuperscript{II}-containing complexes have been studied as contrast agents for magnetic resonance imaging (MRI). In this regards, we studied the influence of molecular parameters that are relevant in MRI and field strength on the relaxivity of Eu\textsuperscript{II}-containing cryptates. We characterized a series of Eu\textsuperscript{II}-containing cryptates and adducts of cryptates with β-cyclodextrins, poly-β-cyclodextrins, and human serum albumin in terms of relaxivity, water-exchange rate, rotational correlation time, and electronic relaxation time using variable-temperature \textsuperscript{17}O-NMR, nuclear magnetic relaxation dispersion (NMRD) and electron paramagnetic resonance (EPR) spectroscopic techniques (Figure 1). We will present these results and the solid- and solution-phase characterization of Eu\textsuperscript{II}-containing complexes. \textsuperscript{17}O-NMR line broadening revealed the presence of inner-sphere water in solution. Water-exchange rates are slower than those of other Eu\textsuperscript{II}-containing complexes, but the rates are still fast \((0.05–0.25 \times 10^9 \text{ s}^{-1})\). In the analysis of NMRD profiles, we observed an ultra-high field bump around 7 T for complexes with short rotational correlation times \((0.046–0.093 \text{ ns})\). This bump shifted to 0.9–1.2 T for complexes with intermediate rotational correlation times \((0.45–1.90 \text{ ns})\) and to 0.7 T for a long \((50 \text{ ns})\) rotational correlation time. We expect that our results will be instrumental in the design of future Eu\textsuperscript{II}-based contrast agents.
Figure 1: NMRD profiles
Utility of Ultrasound/Photoacoustic Imaging for Accurate Catheter Visualization and Tracking During Endovenous Laser Ablation
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Abstract:
Currently, a common minimally invasive treatment option for patients with varicose veins includes laser ablation [1]. During endovenous laser therapy, ultrasound (US) imaging is often used as gold-standard to help surgeons visualizing and accurate placement of the ablation catheter within the target vessels. However, US imaging has certain limitations such as angular dependency and comet tail artifacts makes it difficult in US imaging to accurately locate the catheter in small perforating veins [2]. In this study, we propose utilizing combined US and Photoacoustic (PA) imaging as a suitable tool to improve the localization of ablation catheter within the tissue. Preliminary results were performed using a large-core, multi-mode fiber optics (diameter of 1000 μm). A custom-built fiber holder was designed to enable tilting fiber at controlled and desired angles and with respect the incident US beams. The fiber was then placed inside a phantom consisted of a PVA background and sheep blood and both US and PA images were acquired in different scenarios where fiber was placed at 90 to 30 degrees angle with respect to US incident beam (60 degrees tilting). US and PA image acquisition was performed with a programmable US scanner equipped with a linear array US transducer. Our results indicate while US imaging have difficulties to identify the location of the fiber tip when the fiber is tilted, PA images are very consistent (mean PA signal intensity variation <10%) indicating the independency of PA in locating the fiber tip at different angles.

References
In the recent years optical coherence tomography (OCT) become a powerful skin diagnosis-assisting device. It surpass the current dermaimaging modalities by offering three dimensional images of tissue microstructures. According to the specific functional need, skin cellular architecture varies across different parts of body, and so does the morphological characteristics in the OCT images. There is, therefore, a critical need to systematically analyze the OCT images and identify significant qualitative and quantitative differences. In this study we develop perceptual and textural OCT healthy skin models. Optical and statistical properties extracted from the skin images are analyzed and contribute into the models. Proposed models will allow to provide an atlas of normal skin at different anatomic sites, therefore assisting in diagnosis of microstructural dermal abnormalities and help in the adjustment of treatment.

**Figure 1.** A cartoon of skin compartments and their corresponding OCT images.
Figure 2. Classification results. (a) Correlation map of original 63 extracted features, (b) reduction of the correlation among the six selected features. (c) ROC curve for different subset of features with Quadratic SVM as classifier. (d) Cross-validation classification error percentage of different classifiers using different subset of features.
Compressed Sensing Image Reconstruction with a Novel Dictionary for Photoacoustic Computed Tomography

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**Abstract.** One of the major concerns in Photoacoustic Computed Tomography (PACT) is obtaining a high quality image using the minimum number of ultrasound transducers / view angles. Compressed Sensing (CS) framework offers a solution via signal sparsification. In this study, we propose to use a novel dictionary in compressed sensing algorithm. Our dictionary is an optimum combination of Wavelet Transform (WT), Discrete Cosine Transform (DCT), and Total Variation (TV) transform. We utilize two quality assessment metrics including peak signal to noise ratio (PSNR) and edge preservation index (EPI) to quantitatively evaluate reconstructed images. The results show that the proposed method can generate high quality images with less artifacts while preserving edges when fewer number of view angles is used for reconstruction in PACT. This is in comparison with those results obtained from other reconstruction algorithms.

**Fig. 1.** Block diagram of the proposed method

**Fig. 2.** (a) Schematic of our PACT system, (b) gel phantom with eight 0.5 mm pencil leads.
Fig. 3. Results of reconstruction algorithms on the phantom data acquired from our PACT system. Different rows show different number of views, 30, 60, 90, and 120, and columns show different reconstruction methods, BP, CS with basis WT, CS with basis WT&TV, and CS with the proposed sparsifying method.

Fig. 4. Results of reconstruction algorithms on the shepp logan synthetic phantom for 60 view and different reconstruction method.
Dual modality in vivo imaging of stemness in prostate cancer

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Background: Cancer stem cells (CSC) are postulated as one of the main culprits to metastasis and recurrence stemming from their ability to self-renew, and differentiate.1 Identification of CSCs is crucial to select treatment strategies to target these tumorigenic populations. One of the main challenges is the low abundance of these cells within the tumor (≤1%).2 We believe that the sensitivity of immunoPET coupled to optical imaging provides a powerful tool to detect these CSCs. In this study, we evaluated a new tracer, 89Zr-Bstrongomab (Bsg) for targeting embryonic CSC markers TRA1-60 and TRA1-81 (named TRA from hereon) in prostate cancer (PC). We further attached Dylight 488 (Dy488) for in vitro studies or the NIR dye, Alexafluor 680 (AF680) for in vivo imaging.

Methods: Bsg was conjugated to desferrioxamine and Dy488 or AF-680 via a thiourea and a maleimide linker, respectively. Radiolabelling was done with 89Zr (t1/2~3.27 d) as described previously.3 In vitro studies in TRA(+) DU-145 prostate cells were conducted to show uptake of 89Zr-Bsg. Confocal imaging with Bsg-Dy488 was performed using DU-145 and TRA(-) PC3 PC cells. PET imaging was done using subcutaneous DU-145 and PC-3 tissue transplants at 4-120 h post-injection. A non-specific IgG labelled with either 89Zr or Dy488 was employed to evaluate the probe’s specificity in in vivo and in vitro studies respectively. Tissue distribution, autoradiography and histology will be reported.

Results: Efficient labelling of antibody conjugates with 89Zr were obtained with excellent yields (>95 %) and purities (>99%) with a specific activity of 5 ± 1 mCi/mg. In vitro binding of 89Zr-Bsg in DU-145 exhibited a linear relationship with respect to increasing cell numbers. Confocal imaging with DU-145 showed binding of Bsg-Dy488 to TRAs with minimal avidity observed in the PC3 cells. The non-specific IgG-Dy488 exhibited background uptake in DU-145 cells. In the PET images, DU-145 tumors showed incremental radiotracer uptake at 4 h (3.97 ± 0.98 %ID/g), 24 h (7.35 ± 1.17 %ID/g), which plateaued at 72 h and 120 h p.i. (8.60 ± 1.05 %ID/g and 8.92 ± 2.49 %ID/g respectively). A two-fold lower probe uptake was displayed in the TRA(-) PC3 tumors. PET images of select organs excised post-sacrifice showed higher tumor accumulation of the radiotracer compared to normal tissues (i.e. liver and prostate).

Conclusions: We have demonstrated the specificity and selectivity of 89Zr-Bsg-AF680 for TRA(+) embryonic CSCs in PC, marking its potential as a companion diagnostic to Bsg immunotherapy.

Reference:
Title: Liposomes containing Eu-DOTA-4AMC and Zn nanoparticles to provide pH-responsive contrast in magnetic resonance imaging

Authors:
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Matthew J. Allen, Professor & Chair, Department of Chemistry, Wayne State University

Abstract:
The Eu\textsuperscript{II}/Eu\textsuperscript{III} redox couple is a responsive contrast agent for magnetic resonance imaging (MRI) because it influences $T_1$-shortening contrast only in the divalent state and has a reduction potential in a biologically relevant range. Recently a tetracyclinate complex (Eu-DOTA-4AMC) has been shown to be a kinetically stable contrast agent for MRI and can be reduced from the trivalent state in the presence of metallic Zn. Metallic Zn nanoparticles (Figure 1) form protective ZnO coatings that dissolve in aqueous solutions below pH 7.4. ZnO-coated Zn nanoparticles can be encapsulated in liposomes as a means of systemic delivery of redox-sensitive contrast agents. Here, progress is reported towards the development of liposomes containing Eu-DOTA-4MC and Zn nanoparticles as a pH-responsive system for enhancing contrast in MRI.
Figure 1: Transmission electron microscopy image of metallic zinc nanoparticles.
Molecular Imaging Program Annual Retreat

Additional Molecular Imaging Research
Endocavity Ultrasound and Photoacoustic Imaging: Towards Enhanced Fetal and Neonatal Monitoring

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Abstract:

Intrauterine hypoxia is an unexpected condition during delivery in which the fetus is deprived of an adequate supply of oxygen. Intrauterine hypoxia can put the infants at risk of serious medical conditions including hypoxic ischemic encephalopathy (HIE) [1]. Currently, the fetal status during labor is monitored by continuous intrapartum fetal heart rate monitoring (IFHRM), which most of the fetuses with mild or moderate exposure to intrapartum hypoxia will not be identified. However, there is an unmet need for a diagnostic tool which can directly measure the hemoglobin oxygen saturation in fetus brain. Ultrasound (US) and Photoacoustic (PA) imaging have shown to be capable of imaging blood flow and hemoglobin oxygen saturation in blood vessels. While USPA imaging of brain is associated with certain limitations due to the presence of skull, thin and relatively softer skull bone in fetus and neonates and especially the presence of fontanelles allows for accessing the brain for imaging [2]. We have designed and developed an endocavity US and PA imaging probe, consisted of a an endovaginal US transducer (ATL C9-5) and an optimized integrated light delivery system consisted of 18 large core (1000 µm) multimode fibers. Our experimental results indicate the possibility of detecting mimicked blood vessels at large depths (>35 mm) within the porcine tissue. We also demonstrated the ability of the developed probe to measure blood flow (fractional moving blood volume) and hemoglobin oxygen saturation (SO₂) through a set of ex vivo experiments using heparinized sheep blood mixed with different concentration of sodium dithionite to mimic different oxygenation level.

References


Comparative Study on Similarity Metrics for Seed-Based Analysis of Functional Connectivity Photoacoustic Tomography Images
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ABSTRACT
Seed-based correlation analysis is one of the most popular methods to explore the functional connectivity in the brain. Based on the time series of a seed, i.e., small regions of interest, connectivity is computed as the correlation of time series for all other pixels in the brain. Similarity metrics to measure the similarity between time courses of different seeds play an important role in the detection of functional connectivity maps. In this study, we investigate the performance of six similarity metrics including Pearson correlation, Kendall, Spearman, Goodman-Kruskal gamma, normalized cross correlation and coherence analysis to determine their performance for the functional connectivity photoacoustic tomography (fcPAT) signals/images. The methods are implemented and applied on the fcPAT data of a mouse brain. We also add noise to the fcPAT data and explore the noise tolerance of these metrics.

Figure 1. Seed-based analysis method. (a) Extracting time series of each seed. (b) Correlation between time series of two seeds.
**Figure 2.** Four selected maps for seed based analysis based on six metrics; the white circle shows the ROI.

**Figure 3.** Seed-based analysis based on six metrics for four selected maps in Figure 3.
An Intelligent Despeckling Framework for Optical Coherence Tomography Images
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Abstract
Optical coherence tomography is a powerful high-resolution imaging method with a broad biomedical applications. Nonetheless, OCT images suffer from a multiplicative artifact, so-called speckle, as a result of coherent imaging that is the basis of OCT. Digital filters are the ubiquitous means to reduce speckle. Addressing the number of speckle reduction methods and challenges of selecting of the most appropriate for a particular set of OCT images, we proposed an intelligent, expandable despeckling algorithm we call EIMA. EIMA decides which speckle reduction algorithm is most effective for a given image, based on tissue morphological, textural and optical features extracted from the OCT image. EIMA works based on a two-step decision making process. Initially, a feed-forward multilayer perceptron artificial neural network (ANN) is trained with 63 extracted features as input and the index of 25 filters as output. The figure of merit in the ANN is computed based on the weighted combination of signal-to-noise ratio (SNR), contrast-to-noise ratio (CNR), equivalent number of looks (ENL), edge preservation index (EPI) and mean structural similarity index (MSSI). The ANN identifies the most efficient filter in each of the following categories: (a) sliding window filters (median, mean and Symmetric Nearest Neighborhood (SNN)), (b) adaptive statistical based filters (Wiener, homomorphic Lee and Kuwahara), and (c) the edge preserved patch or pixel correlation based filters (non-local mean, total variance and block matching 3D filtering (BM3D)). The optimum filter is chosen based on the priority in execution time or image quality.

Figure 1. Corresponding QAM measures for image dataset filtered by third category and all together.
Figure 2. Block diagram of the proposed intelligent speckle reduction method
Swept-source optical coherence tomography supervised biopsy
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Abstract

Currently, beyond clinical prognosis, only skin biopsy can provide the histological information for the diagnosis of skin diseases. However, the sampling error during the procedure has always been an issue. The invisibility of the lesion area underneath the superficial skin layer causes an inadequate representative image of the entire lesion. This usually leads to repeated procedures. The recent advances in optical coherence tomography (OCT) has made it possible to image layers of skin up to two millimeters in depth. In this paper, we present the feasibility of supervised biopsy via OCT imaging. To this aim, needle insertion OCT imaging has been conducted in skin equivalent phantom, solid tissue and mouse skin. Furthermore, OCT imaging guided needle biopsy and punch biopsy were also introduced and investigated. The results support our hypothesis that OCT supervised biopsy increases the accuracy and efficiency of the biopsy procedures.

Figure 1. Schematic diagram of the SS-OCT system used in this study.
Figure 2. Demonstration of the position of the imaging plane over the inserted needle: (A) the imaging plane parallel to the needle, (B) the imaging plane perpendicular to the needle.

Figure 3. Punch biopsy probe /piercing tip. (A) picture of piercing tip, (B) schematic illustration of piercing tip design, unit in mm, (C) front view of the probe attached to the OCT probe, (D) side view of the probe attached to the OCT probe.

Figure 4. Tungsten wire phantom design and OCT images of the. (A) schematic illustration of phantom embedded with Tungsten wires, (B) final casting mold of the phantom with Tungsten wires, (C) finished phantom embedded with Tungsten wires, (D) OCT image of Tungsten wire phantom with 1.3% TiO2, (E) OCT image of Tungsten wire phantom with 0.65% TiO2.
Pathomimetic Avatars for Spatiotemporal Modeling and Live-Cell Imaging of Proteolytic Pathways in 3D Tumor Microenvironment: Use for Therapeutic Screening

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To define protease-related druggable pathways that are involved in malignant progression of cancer, our laboratory has pioneered novel techniques for functional live-cell imaging of protease activity, concentrating on pathomimetic avatars for breast cancer and plexiform neurofibromatosis. We analyze proteolysis in the context of proliferation and formation of structures by tumor cells in 3-D cultures over time (4D). In order to recapitulate the cellular composition and architecture of tumors, we include other tumor-associated cells (e.g., fibroblasts, myoepithelial cells, lymphatic endothelial cells, neurons). We also model non-cellular aspects of the tumor microenvironment, e.g., an acidic pericellular pH. Use of these pathomimetic avatars in concert with various types of imaging probes has allowed us to image, quantify and follow the dynamics of proteolysis in the tumor microenvironment and to test interventions that impact directly or indirectly on proteolytic pathways. To facilitate use of the pathomimetic avatars for drug screening, we have designed culture chambers with multiple wells that are either individual or connected by a bridge to allow cells to migrate between wells. Optical glass microscope slides underneath an acrylic plate allow the cultures to be imaged with an inverted microscope. Fluid ports in the acrylic plate are at a level above the 3D cultures to allow introduction of culture media and test agents such as drugs into the wells and the harvesting of media conditioned by the cultures for immunochemical and biochemical analyses. Covers contain integrated gas exchange ports and sensors to monitor oxygen levels, pH and temperature over the extended time periods in culture and to insure maintenance of such experimental conditions as hypoxia and/or a pericellular acidic pH. We are using the pathomimetic avatars to identify druggable pathways, screen drug and natural product libraries and accelerate entry of validated drugs or natural products into clinical trials.
3D/4D Tissue Architecture and Microenvironment
Engineering Avatars to Recapitulate in Vivo Architecture

Cellular

Non-Cellular

Hypoxia

pH

Interactions of Breast Tumor Cells with Microenvironment

Kyungmin Ji

3D/4D Tissue Architecture and Microenvironment
Engineering Avatars to Recapitulate in Vivo Architecture

Cellular

Interactions of PN Cells with Microenvironment

Kyungmin Ji
TITLE: Prototype Collimation System for Contrast-Enhanced Kilovoltage Radiotherapy
PI: Michael Snyder—Medical Physicist, Radiation Oncology

Advances in radiotherapy technology over the last decade have increased the precision and accuracy of treatment incredibly. This has allowed radiation to be focused ostensibly to the malignant tissue alone, increasing prescription doses and improving outcomes. However, despite these technological advances, there remain several cancers that present an inherent resistance to conventional high-energy x-ray therapy, even at modern elevated doses.

Unlike high-energy x-ray treatments, an optimized low-energy x-ray system can be combined synergistically with either iodine-based x-ray contrast agents or heavy metal nanoparticles to create dense DNA damage. The resulting dose enhancement—achieved through this combination of modern delivery and contrast-enhancement—should result in better local control of radiation resistant cancers and better outcomes for patients.

Project Objectives

Our objective is the construction of an optimized low-energy x-ray system utilizing modern treatment techniques pioneered in high-energy therapy. Our central hypothesis is that this low-energy x-ray device will be able to deliver very accurate radiation dose that can be enhanced further using either iodine-contrast agents or heavy metal nanoparticles, providing a more effective therapy than conventional high-energy therapies. We expect to achieve our objective by pursuing the following:

1. Construct a prototype quasi-monochromatic low-energy x-ray device. The objective of this aim is to combine a low-energy tungsten targeted x-ray tube with an optimized filtration system to produce a high intensity quasi-monochromatic x-ray beam. The working hypothesis is that this x-ray system will produce a clinically usable x-ray beam optimized for the purpose of dose enhancement using contrast agents.

2. Develop a modern multi-leaf collimation system. The objective of this aim is to design and construct a programmable x-ray collimation system in the style of modern high-energy treatment machines. The working hypothesis is that this type of collimation system will allow for intensity modulated treatments on par with those used clinically today, and that these treatments will localize radiation dose to the tumor tissue in a manner not before possible with low-energy devices.

Research Strategy

The primary goal of this project is to construct a machine to deliver low-energy x-ray therapy in a similar manner to that delivered in conventional clinical radiotherapy. The initial work on this project has therefore been centered on creating the necessary components of the machine and modeling potential treatments that would be achievable with such a device.

Construction of the dynamic micro-collimation system has begun. Figure 1 displays the overall design of the collimation system consisting of a series of movable metallic leaves which can block the radiation beam. The leaves can be positioned such that any shape can be created to allow radiation the pass, providing a method to shape radiation dose in the patient. Figure 2 shows the control system for the micro-collimation system, including the motors, printed circuit boards and software system. Good progress has been made in the design and construction of the overall system, however some crucial components and systems are yet to be implemented.
In addition to machine build-out, the general-purpose Monte Carlo framework GEANT4 has been used to simulate simple potential treatments achievable using the theoretical device in question. A phantom was designed to represent an average human head. The phantom takes the form of a cylinder of 15 cm in diameter, with a smaller cylinder inset representing bony skull. Inside of the phantom is placed a sphere of 2 cm in diameter to represent a potential lesion. The material used in the phantom is water throughout, dense bone in the skull region, and within the spherical lesion a solution consisting of 99% water with 1% iodine. As can be seen in Fig. 3, due to the low-energy spectrum, dose is greatest at the central lesion modified by iodine contrast, with slight—but acceptable—increases in the bony skull due to the increased photoelectric cross-section in bone at these low-energies.

**Expected Outcomes**

- **A quasi-monoenergetic low-energy x-ray therapy system.** This will produce a beam that can interact with either iodine-contrast agents or heavy metal nanoparticles to deliver high doses through electron cascades.

- **Dynamic collimation system.** Construction of the collimation system will allow for complex radiation patterns to be accurately delivered, localized dose to only those areas desired. This collimation system will be used to vary the shape of the x-ray beam as required while the treatment is under progress. The varying shapes produced by the collimation system will allow the machine to deposit x-ray dose very accurately to the tumor.

- **Proof-of-concept measurements of the dose accuracy.** The initial measurements and planning will be done using and in-house cylindrical phantom with the approximate dimensions of the human head. Treatments will be delivered to the phantom and dose measurements will be made using radiochromic film. The film will measure, in a d 2D plane, the shape and distribution of the delivered dose, which can then be compared to that planned in the computational system. Upon successful measurement of initial plans, another phantom containing bone and air cavities will be used to make similar measurements, adding a dimension of real-patient anatomy to the testing of the system.
Clinical data collection for evaluation of SoftVue: A Novel Ultrasound Breast Scanner

Primary Investigator: Zeynep Yilmaz-Saab MD

The study aim is to determine the imaging potential of a novel technology, SoftVue, through 3-D breast imaging from a cohort of 750 women. The project is aimed at acquiring data for SoftVue evaluation. The goal is to determine the ability of SoftVue to detect pathological breast findings identified on conventional imaging and also to detect breast cancers which are occult on mammograms focused particularly in women who have dense breasts. In addition, the study aims to compare the potential of SoftVue imaging compared with breast MRI. My current research proposal as Primary Investigator on this study centers on imaging and clinical data collection.

SoftVue is a technology that images the breast by collecting reflection echoes from all directions around the breast. The parameters collected and evaluated include reflection, sound speed, and attenuation and are viewed in circumferential, tomographic series. There is no pain and compression experienced by the patient. There is also no associated radiation exposure. Besides the ability of the system to identify pathological features previously identified by other imaging modalities, this study aims at evaluating the SoftVue system with respect to spatial and contrast, sound speed, and attenuation resolution.

This is a study initiated and developed by Dr. Littrup who is currently a co-Investigator. I took over as Primary Investigator in April of 2016. I would like to thank Dr. Littrup for all of his involvement at Karmanos on this project while faculty at Karmanos and for his continued dedication and collaboration. He is currently faculty at Brown University and has been a great mentor.