ILLINOIS INSTITUTE OF TECHNOLOGY

impact

Nayar Prize I, Phase II Quarterly Progress Report (*Q4*)
October 2017

Project: **ADEPT Cancer Imager**
Team: **Ken Tichauer, Jovan Brankov, Raju Mehta**
Students: **Lagnojita Sinha, Veronica Torres, Cynthia Li, Negar Sadeghipour, Bhavya Dasari, Todd Wilson**

Progress Summary of Nayar Prize I, Phase II

Highlights:

(1) *August 10, 2017*: First tomographic image collected on the ADEPT system! (See Figure 1 below).

(2) *August 30, 2017*: Article on lymph node molecular imaging protocol for translation to ADEPT system was submitted for publication to the journal *Molecular Imaging and Biology*:

(3) *September 12-16, 2017*: Three Abstracts on the ADEPT work were presented at the World Molecular Imaging Congress (WMIC) 2017 in Philadelphia, PA:

   a. L. Sinha, W. Zhou, J.G. Brankov, K.M. Tichauer. Early photon optical tomography signal-to-noise ratio improved by almost two orders-of-magnitude by running in the "deadtime" regime. Submitted to the *World Molecular Imaging Congress*, Philadelphia, PA, 2017. ***Oral Presentation. Received TRAVEL AWARD.***

   b. N. Sadeghipour, K. Wills, S.C. Davis, K.M. Tichauer. Connection between molecular targeted drug response and molecular environment in cancer therapy: testing EGFR status vs. Erbitux therapy. Submitted to the *World Molecular Imaging Congress*, Philadelphia, PA, 2017. ***Poster Presentation. Received TRAVEL AWARD.***

(4) *September 26, 2017*: Three Abstracts (two talks, one poster) on the ADEPT work were accepted to be presented at SPIE Photonics West 2018 to be held in San Francisco, CA in January 2018:

a. L. Sinha, J.G. Brankov, K.M. Tichauer. Getting more early photons with less background: detection rate and signal-to-background improvements in enhanced early photon imaging. *SPIE BiOS*, San Francisco, CA, January 2018. **Oral Presentation**.

b. C. Li, X. Xu, Y. Basheer, K.M. Tichauer. Paired-agent fluorescent imaging to detect micrometastases in breast sentinel lymph node biopsy. *SPIE BiOS*, San Francisco, CA, January 2018. **Oral Presentation**.

c. N. Sadeghipour, S.C. Davis, K.M. Tichauer. Quantifying cancer cell receptors with paired-agent fluorescent imaging: a novel method to account for tissue optical property effects. *SPIE BiOS*, San Francisco, CA, January 2018. **Poster Presentation**.

Building upon our achievements in the first three quarters of the Nayar Prize I, Phase II, we are continuing to focus our efforts in two areas that will eventually be merged: (1) system development, and (2) tissue staining/rinsing/imaging protocols. We have also continued with the phantom development area that began in Q3. Over the last three months we have made further advances in all three of these areas:

(1) System Development:
Students: Lagnojita Sinha and Veronica Torres
Achievements:
<u>Note:</u> In August, there was flooding of our ADEPT lab space and we had to shut down all operation of the system while the room was fixed. Fortunately, there was no damage to our equipment; however, we lost approximately four weeks of system work time, limiting our system development progression for this quarter slightly, so we focused more on some simulation work to guide future development.

a. *First tomographic image acquired with ADEPT*
As a first test of the potential of the ADEPT system to achieve tomographic images, we replaced the single time-domain detector in the system with a CCD camera to acquire many projections at once to reduce on imaging time. Snapshots of the laser transmission were acquired at five-degree increments about 360 degrees of a 1-cm-diameter cylindrical light scattering object with an absorbing column of ink inserted into its center (**Figure 1**). This early result shows great promise for further advances in tomography with our time-domain detector for early photon tomography (in the past we have only achieved planar early photon imaging). We expect tomographic results from fluorescent phantoms by the next quarterly report.
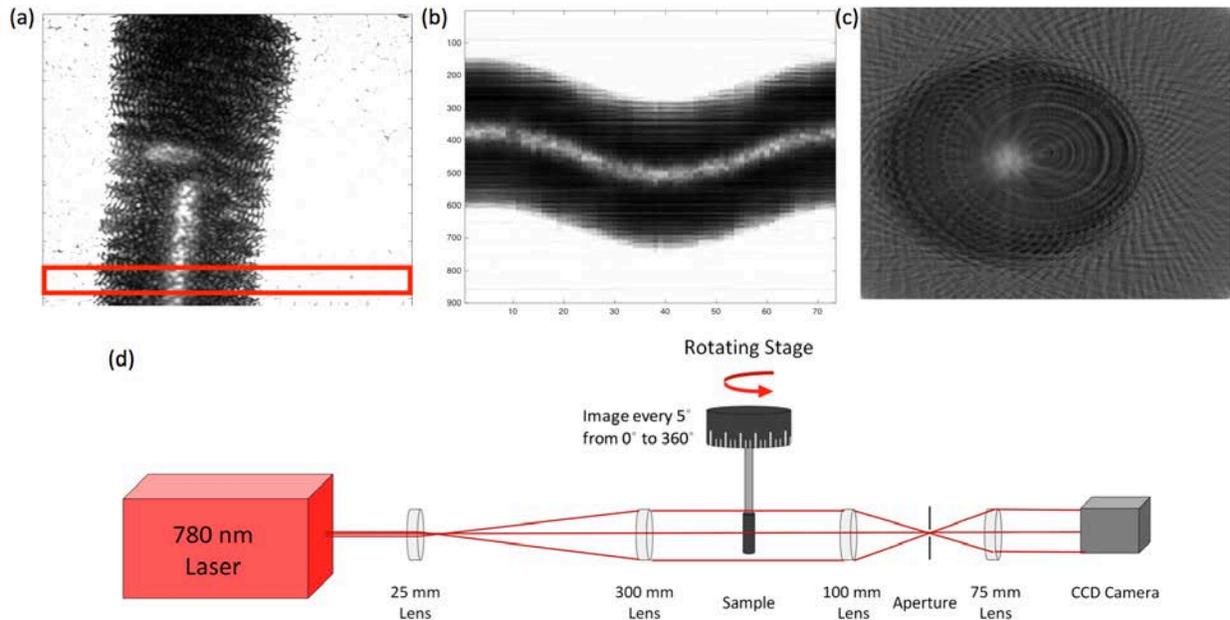
Figure 1: A column of ink was inserted into the center of a 1-cm-diameter cylinder with scattering properties similar to that of biological tissue. A projection image of the object from one angle is presented in (a). The sinogram of the region highlighted in red in (a) is presented for 360 degrees of projection angles in (b). An example slice of the reconstructed image is presented in (c). The system setup for attaining this data is presented in (d).

b. *Simulation of spatial resolution improvement through time-gating and angular restriction*
Two proposed methods of improving spatial resolution in optical projection imaging include capture of early arriving photons (the foundation of the ADEPT system) and angular restriction. The ideas here are that (1) the earliest arriving photons and (2) the photons that exit the tissue at the smallest angle will have taken the straightest path through the tissue. We have designed a way to incorporate both temporal and angular restriction into the system now. However, there is a trade-off in employing both improvements simultaneously: Since not all early photons will exit the tissue at a low angle, restriction of photons based on angle can reduce the total number of photons detected, some of which could be good, early photons. Conversely, current limitations of temporal resolution in time-domain detection restrict the best time gating to about 1 ps (though we mentioned equipment that we purchased for potentially better resolution in the Q3 report), such that for very thin samples, angular constriction could offer better resolution than time gating. **Figure 2** presents some early simulation results that we carried out to explore these tradeoffs in greater detail. Specifically, we modified a Monte Carlo simulation code from Prahl et al. *Dosimetry of Laser Radiation in Medicine and Biology*, 1989, which employed a random walk process to estimate photon propagation

in light scattering media (such as biological tissue) – See **Figure 2c**. The modification led to the ability to track the maximum deviation of each detected photon from the straight path through the tissue (which is proportional to achievable resolution), as well as the time and angle at which each detected photon exited the media. **Figure 2a** and **b** present results from a preliminary simulation in which photon propagation through a 2-mm-thick sample was estimated. While further simulation is required to fully elucidate the trade-off between both methods, this simulation suggested that angular constriction could potentially improve resolution to a greater extent than time gating under these conditions, by restricting detection to 2 degrees in comparison to a 4 ps time-gating (standard for our system to achieve reasonable signal-to-noise in a reasonable imaging time). It's predicted that some combination of angular restriction and time-gating, the proportional weighting of which will depend on the geometry of the specimen, will result in the optimal spatial resolution. This hypothesis will form the basis to another journal article that we hope to submit by early 2018.
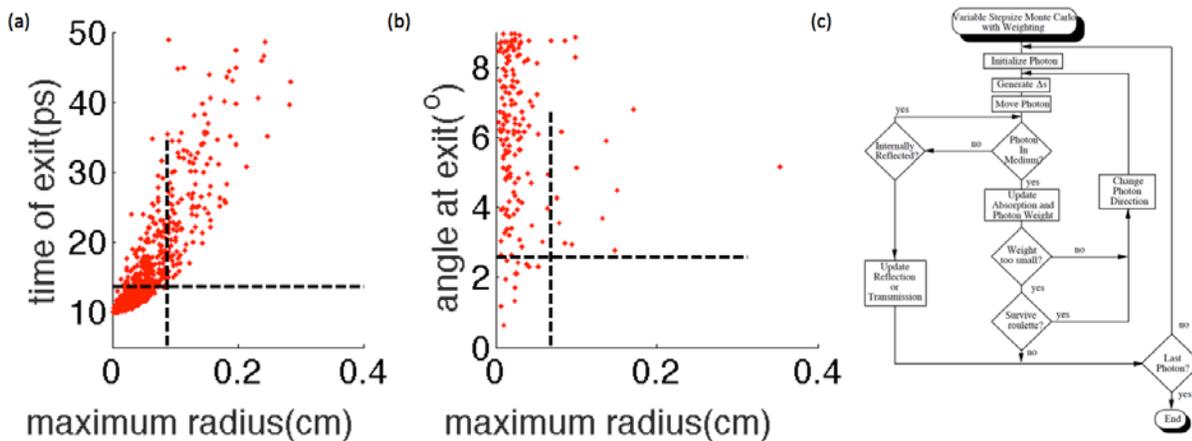

Figure 2: Simulation results exploring resolution, time-gating, and angular restriction trade-off in a 2-mm-thick sample. The relationship between time of photon exit and maximum deviation from the straight path is presented in (a). A time gate of 4 ps appears to ensure spatial resolution of less than 100 microns. The relationship between angle of photon exit and spatial resolution is presented in (b). Constriction to less than 3 degrees appears to be required to beat the resolution achieved by 4 ps gating. Schematic of the Monte Carlo simulation for photon propagation taken from Prahl et al. 2018 is presented in (c).

(2) Tissue Protocols:
Students: Cynthia Li and Negar Sadeghipour
Achievements:
   a. *Demonstration that a low-cost FDA-approved fluorophore, indocyanine green (ICG), can be used to replace expensive antibody-based fluorophores as control imaging agents in paired-agent imaging of lymph nodes (under the right conditions).*
   The antibody-based fluorescent molecules we used to carry out paired-agent imaging of lymph nodes in past quarterly reports are very expensive. In an attempt to lower costs

and to make ADEPT more attractive to widespread adoption, we experimented with the use of cheaper fluorescent agents to replace the control imaging-agent in our studies. We settled on ICG, which is known to bind readily to proteins. By mixing ICG with human albumin (a protein found in high concentrations in lymph nodes), prior to mixing with the fluorescent cancer targeted antibody (also a protein), we demonstrated that the ICG will not interact with the targeted agent, and can be used successfully to accurately estimate cancer cell burden in lymph nodes. This work was submitted for publication (see Highlight #2 above).
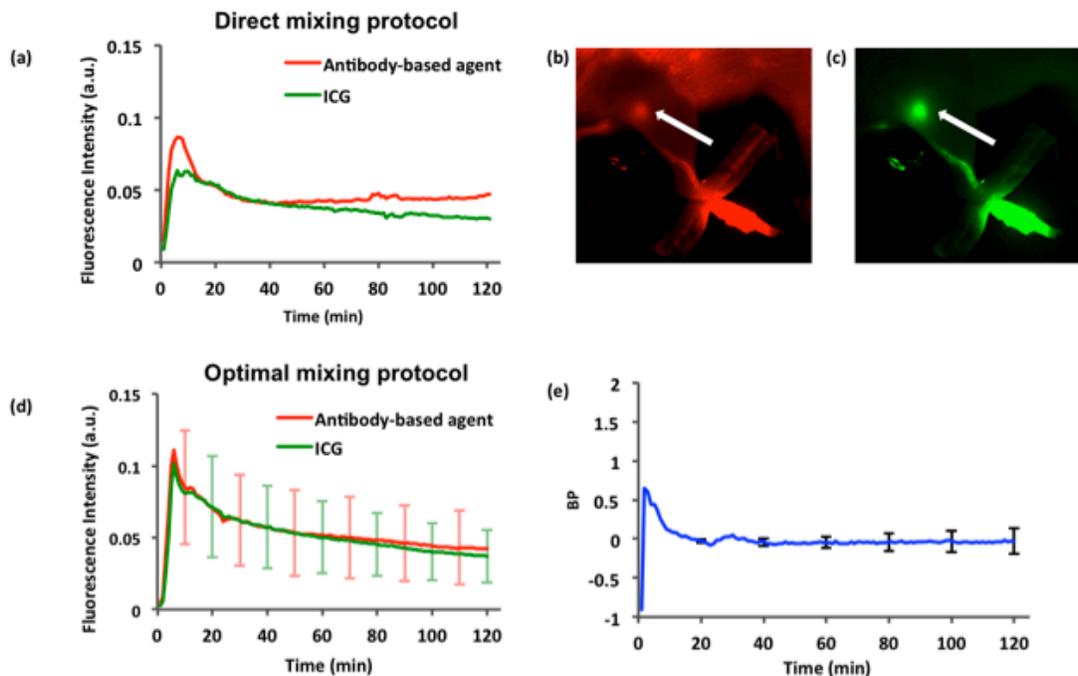

Figure 3: Kinetics of antibody-based agent (IRDye-700DX-IgG) and ICG signal in a rat popliteal lymph node over time after intradermal footpad injection. Fluorescence images of ICG (green) and antibody-based agent (labeled IRDye-700CW-IgG; red) in the popliteal lymph node at 1 h post-injection of the right rear footpad are presented in (b) and (c), respectively. The time-course of fluorescence from both imaging agents failed to correlate with each other when all agents were mixed simultaneously just prior to injection as shown in (a). After the correction of mixing order and the pre-loading time, the potential ability for ICG to mimic the delivery and retention of an antibody-based imaging agent is presented in (d), depicting the mean fluorescence for both imaging agents in $n = 3$ rats in the lymph nodes as a function of time (error bars are SE). The binding potential for lymph nodes as a function of time after injection are presented in (e), with a maximum error of less than 0.05 in the absence of binding, further offering strong support that ICG is ideal for use as a control imaging agent in the PAISLY approach.

(3) Fluorescent imaging phantom development:
Students: Veronica Torres, Morgan Fogarty

Achievements:

a. *First planar imaging of fluorescence in a thick-scattering medium on ADEPT*

Our efforts in phantom development continue to improve. We experimented with the use of agarose gel-based phantoms. **Figure 4** presents the first fluorescent planar images from the ADEPT system in a thick (1 cm) scattering medium. Phantom development included creation of a 5 mm layer of 3% agarose mixed with 1% intralipid (to achieve similar scattering properties to tissue). Two agarose beads, with 1% intralipid and 1 micromolar ICG, of approximately 2 mm diameter were placed on the layer. After cooling and setting, a second 3% agarose with 1% intralipid layer was added to the top of the sample with a depth of 5 mm. Imaging was carried out on the ADEPT (**Figure 4a**) and a commercial imaging system that we have in our lab, the PEARL from LI-COR Biosciences (**Figure 4b**). Spatial resolution improvements are clear in the comparison, and with the ADEPT it was even possible to make out the shape of the beads which were elliptical and oriented such that the long axis of one was at 90 degrees to the long axis of the other.
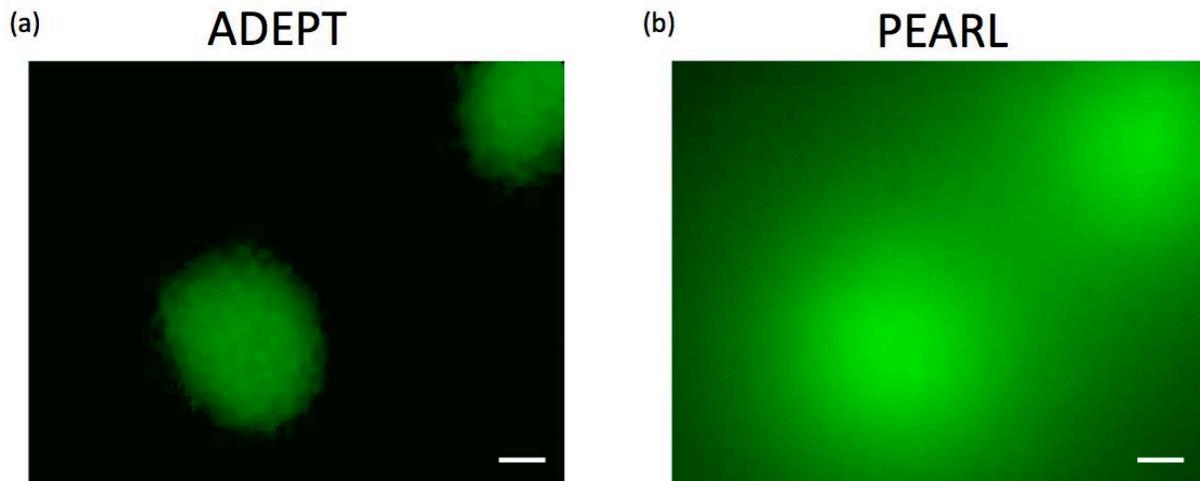

Figure 4: Phantom imaging of 2 mm fluorescent beads in the center of a 1-cm-thick scattering phantom imaged with ADEPT (a) and with a commercial fluorescence imaging system (b).