

Nayar Prize I Phase II Quarterly Progress Report

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Project: ADEPT Cancer Imager

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Progress Summary of Nayar Prize I Phase II

In the three months since being awarded Phase II funding for Nayar Prize I, we have made significant progress in two areas that will move the ADEPT technology toward clinical mapping of tumor draining lymph nodes.

We have begun testing protocols for staining and rinsing surgically removed lymph nodes. To do this, we removed lymph nodes from rats, implanted human breast cancer cells, and immersed them in a combined solution of carbonic anhydrase IX (CAIX) targeted and control fluorescent imaging agents (CAIX is an enzyme that is highly associated with breast cancer cells that have spread to tumor-draining lymph nodes). We are currently testing different durations of staining but have results from a 20-minute stain (**Figure 1**). The lymph nodes were then rinsed by immersion in water. This process preferentially removes the control fluorescent agent (as opposed to the CAIX-targeted agent) only where cancer is present. We are testing this hypothesis in 2D (rather than 3D where ADEPT will be used in the future) by taking single slices of the stained and rinsed lymph nodes and imaging them with a commercially available flat-panel fluorescence imaging system (Pearl®, LI-COR Biosciences, Lincoln, NE). The optimal rinsing time is also yet to be determined. The current data is from a five-minute rinse cycle (**Figure 1**).

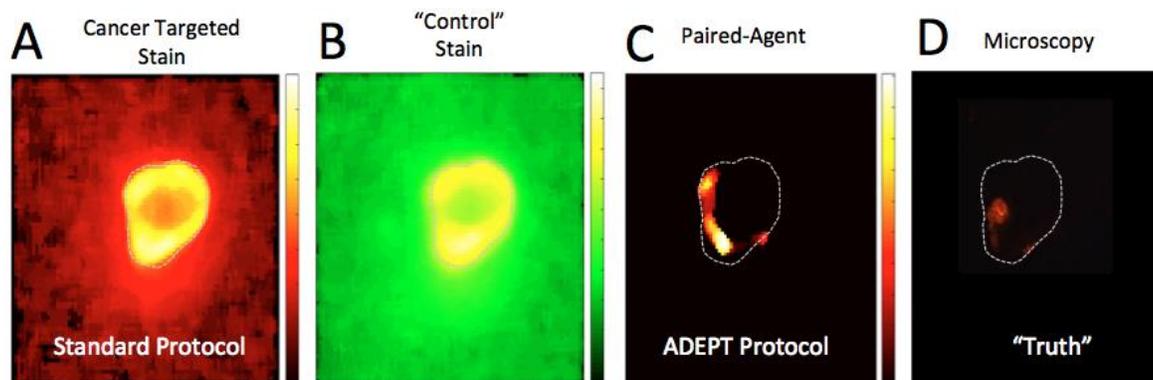


Figure 1. (A) Fluorescent signal from cancer targeted imaging agent in slice of rat lymph node implanted with human breast cancer cells. (B) Fluorescent signal from control imaging agent. (C) Paired-agent method to enhance regions of preferential cancer targeted agent retention. The paired-agent ADEPT protocol lined up best with the intrinsic fluorescence of the cancer cells themselves: the ground truth (D).

impact

This is a significant step toward completion of the first aim of our Phase II proposal, which was to “*demonstrate that ADEPT can detect fewer cancer cells in tumor-draining nodes than standard assessments.*” Once optimal staining and rinsing procedures are identified, this work will be submitted in the following publication currently in preparation:

Chengyue Li, Xiaochun Xu, Yusairah Basheer, Jovan G. Brankov, Kenneth M. Tichauer. Ex vivo paired-agent staining to rapidly detect cancer spread in tumor draining lymph nodes. In preparation for submission to *Molecular Imaging and Biology*.

2) In a further demonstration of the advantages of our enhanced early photon detection that we are leveraging in the ADEPT system, we have demonstrated that signal-to-background can be amplified by further increases in laser intensity since the background is proportional to the total number of photons detected (which saturates at a relatively low light power). However, the number of detected “early” photons (the photons with the best spatial information) continues to increase with much higher laser powers (**Figure 2**). This demonstrated that the advantages in enhanced early photon detection are not simply faster imaging time (*i.e.*, collecting of early photons for longer, would not improve signal-to-background).

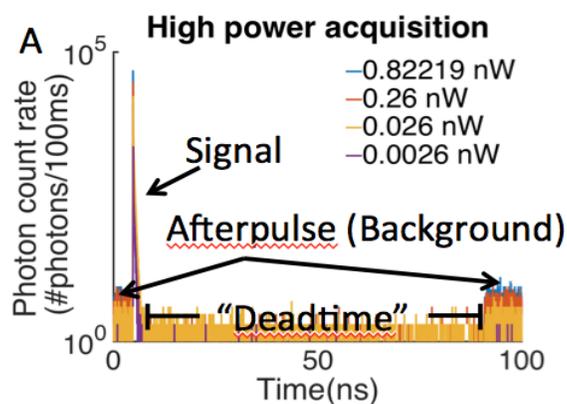
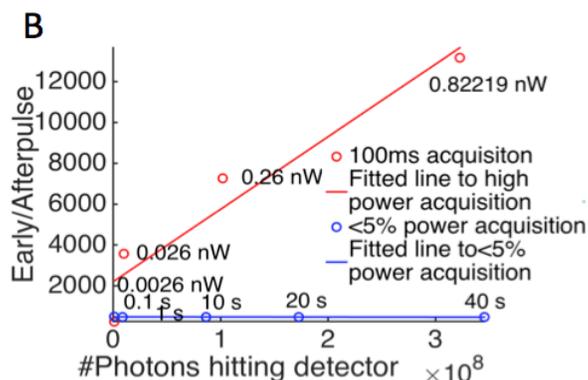


Figure 2: (A) Raw histogram of detected laser pulse response curve for multiple laser power levels. (B) Demonstration that the enhanced early photon approach (red data) produced much better signal-to-noise characteristics than could be achieved by detecting the same number of early photons by simply detecting for a longer period (blue data).

This finding will be added to the summary of the ADEPT system publication that is in preparation and is critical in demonstrating the power and innovation of the ADEPT system:



Lagnojita Sinha, Wei Zhou, Jovan G. Brankov, Kenneth M. Tichauer. Enhanced early photon optical projection tomography system for mesoscopic imaging of thick tissues. In preparation for submission to *Review of Scientific Instruments*.