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Title of Project: Three-dimensional Mapping of Tumor Microvasculature Using a Microendoscopic Probe

Abstract

The study of the tumor microenvironment and tumor angiogenesis is an emerging area of interest for research and clinical communities. Understanding tumor progression is essential to developing cancer treatments and therapies. Current methods for monitoring the tumor microenvironment are limited in their ability to access a wide range of anatomical locations, specifically the gastrointestinal tract, without causing non-physiologic tumor response. In this study, we aim to develop a small-diameter multi-projection endoscopic imaging platform capable of many different scientific and clinical applications, including assessing tumor structure and profusion in previously inaccessible anatomical locations. Due to the limited size available for an endoscopically compatible system, photon scattering follows a “transport regime,” (as opposed to the diffusion regime). This requires novel approaches to processing image data to resolve the fine vascular and structural details within tissue. The platform consists of two key components, one being a microendoscopic probe that utilizes radially spaced illumination source fibers to capture two-dimensional fluorescence images of simulated tissue. The other component, Monte-Carlo-based photon propagation simulations modeled after the colon epithelium, provides information in depth. The combination of these two components allows for the creation of a three-dimensional spatial reconstruction of a tumor embedded in a tissue of interest.

Three-dimensional Mapping of Tumor Microvasculature Using a Microendoscopic Probe

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Background

The study of the tumor microenvironment is essential to the development of new treatment methods. Recent research into the fundamental understanding of tumor structure and its perfusion has led to promising anti-angiogenic therapies [1]. Optical imaging methods provide a quantitative real-time measurement of tissue structure and its corresponding microvasculature. Current methods for *in vivo* assessment of the tumor microenvironment utilize two primary regimes of light scattering: single scattering or diffuse scattering. Both regimes are limited in their ability to provide information regarding overall tissue perfusion and resolve fine vascular details. The "transport scattering regime" falls between that of the single scattering regime (zero SDS) and diffuse scattering regime (SDS in the order of centimeters), with a SDS of less than a millimeter. This regime has been shown to be capable of capturing highly resolved and magnified images through capturing reflectance data [2,3]. Due to the small distance between the source fibers and the imaging guide, the diffusion approximation of light scattering to the radiative transfer equation is not possible. Therefore, Monte Carlo-based forward photon propagation simulations are required to extract meaningful physiological information in depth.

In this study, a microendoscopic probe of minimal diameter capable of imaging in the "transport scattering regime" will be used to determine the presence and location of an embedded absorber in optical phantoms. The probe operates by one source fibers briefly emitting light that diffuses and reflects through the tissue of interest, which is then captured by the central image

guide to produce an image of the tissue surface. This process is repeated for each source fiber to capture images of the same area of interest with different light projections and therefore different angles of photon propagation. Two-dimensional image data will be used alongside Monte Carlo depth information to create a three-dimensional subsurface map of the embedded absorber. This platform could have numerous clinical applications to develop personalized treatment plans based on tumor structure and perfusion.

2. Objectives

The objectives of this project are to:

1. Develop the imaging platform's three-dimensional mapping capabilities.
2. Validate the developed mapping capabilities with polystyrene optical phantoms.
3. Develop and implement an imaging fiber switch to the platform to allow for more consistent and rapid image collection.

3. Previous Research

Previous research conducted at the University of Arkansas has designed and manufactured an optical imaging probe capable of imaging in the "transport scattering regime" [4]. Monte Carlo-based photon propagation simulations modeled after the colon epithelium have also been developed within the group [5]. Preliminary results have shown that the presence and location of an embedded absorber within a simulated tissue medium can be determined using the platform.

4. Materials and Methods

4.1 Microendocopic Imaging Probe

The manufactured probe has a 3.048 mm diameter (Myriad Fiber Imaging) with a source detector separation (SDS) of 225 μm (**Figure 1**). The probe contains eight radially spaced light projections each with a 50 μm ± 2% diameter and a centralized imaging fiber with 790 ± 50 μm diameter containing 30,000 ± 3,000 picture elements [4]. A fiber-coupled LED with a wavelength of 530 nm (Thorlabs Inc. M530F2) provides illumination to the fiber to capture images using an 8-bit CCD camera (FLIR Flea3 USB) with the FlyCapture2 platform [4].

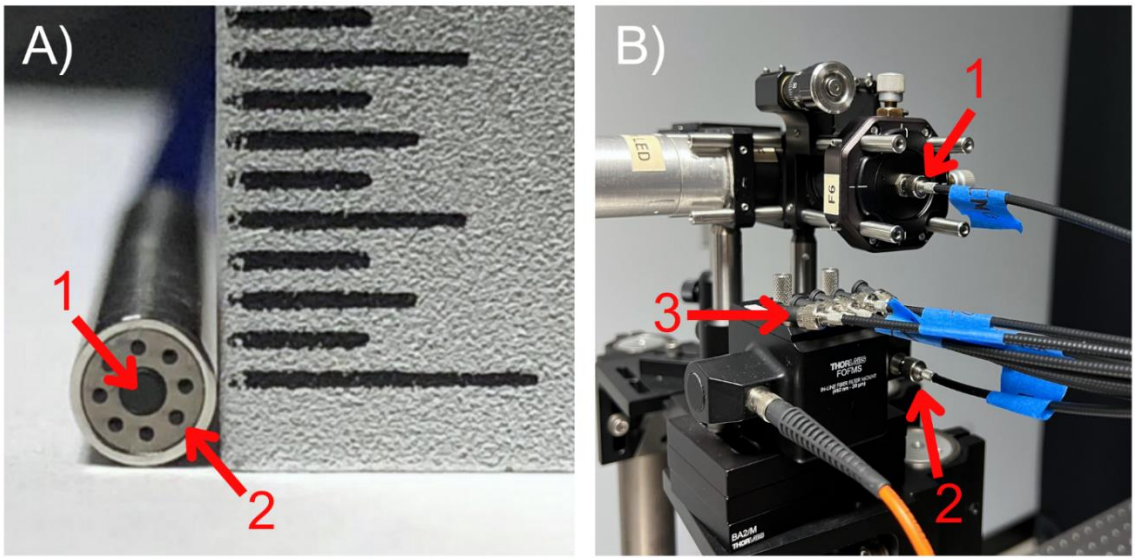

Figure 1. *Microendoscopic Imaging Platform.* (A) Distal end of imaging probe (Small markings on ruler represent 1/32 in.) 1: Centralized imaging guide, 2: Peripheral illumination fibers. (B) Proximal end of imaging probe. 1: Imaging fiber connection point. 2: Illumination fiber connection point. 3: Unconnected source fibers. (Image A taken by Zach Neumeier [4], Image B taken by author.)

4.2 Polystyrene Bead Optical Phantoms

To validate the platform, purely scattering optical phantoms consisting of polystyrene microspheres will be created to replicate the optical values of healthy epithelial tissue in the colon. Therefore, a reduced scattering coefficient of $\mu s' = 5\ cm^{-1}$ for $\lambda = 532$ nm will be used [6]. 3.53 mL of polystyrene beads are diluted with 1.47 mL of distilled H_2O to obtain the objective reduced scattering value [4].

4.3 Image Capture and Analysis

To simulate tumor microvasculature, a collection of monofilament polypropylene sutures with various orientations will be affixed in the optical phantoms and imaged at varying distances from the probe. Homogenous and embedded absorber phantom images will be captured with all eight illumination fibers. The captured images will be analyzed using MATLAB by sampling pixel values on various line profiles across the image. The resulting intensity value decay curves obtained from homogenous and nonhomogeneous phantom images will be directly compared to one another. Where the two decay curves deviate from one another is where an embedded absorber is located in the imaging field.

4.4 Development of Three-dimensional Mapping Capabilities

For each line profile that is generated to analyze the phantom images, a characteristic Monte Carlo-based photon curve can be selected [5]. The photon curve is selected based on the determined distance of the absorber from the light source. Using MATLAB, the simulated photon curves are thresholded to select the regions of the curve with the highest photon transmission. A collection of photon curves can be combined, all with varying photon exit paths and degrees of rotation from the light source to create a comprehensive map of the photon trajectories from the light source to the absorber. This process will be performed for all eight source fibers and will be added together to create a three-dimensional point cloud of the photon trajectories in the space directly beneath the imaging guide. Where these photon trajectories overlap, is where the absorber likely lies in the region of interest. The MATLAB code required to perform this three-dimensional mapping will be developed throughout the project.

4.5 Implementation of Imaging Fiber Switch

A current limitation of our platform lies in the need for manual switching between the subsequent eight illumination fibers. While this manual process may not significantly affect samples like optical phantoms, it poses a considerable challenge when applying the platform to *in vivo* models. The variations introduced by manual switching can compromise the consistency and precision required for *in vivo* image capture. The development and implementation of an automated fiber switch will enable us to sequentially image a tissue of interest with all eight illumination fibers in rapid succession. This integration will make the platform a more practical imaging modality for future *in vivo* analysis.

5. Significance of Research

Current imaging modalities such as confocal microscopy and two-photon systems have shown to be capable of good spatial resolution and depth penetration. However, their complex scanning mechanisms and large source detector separation (SDS) limit their ability to be deployed endoscopically and resolve fine structural details. The goal of this project is to develop a device of minimal diameter (<1 mm) with a small SDS and no complex scanning mechanisms that is capable of being endoscopically deployed in previously inaccessible anatomical locations. This platform can have a wide variety of clinical applications, such as allowing for patient specific therapies based on the perfusion and microvasculature structure of colorectal tumors.

6. Project Timeline

Month	**Goals**
January-March	Development of the platform's three-dimensional mapping capabilities.
April-May	Validation of developed code on optical phantoms.
August-October	Development and implementation of an automated fiber switch.
November-December	Publish article in a scientific journal and submit to present at a conference in relating field.

References

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