Sequential Illumination in a Tomographic Microendoscopic Probe for Imaging Tumor Microvasculature

Zachariah Neumeier

Follow this and additional works at: https://scholarworks.uark.edu/bmeguht

Part of the Bioimaging and Biomedical Optics Commons, Biomedical Devices and Instrumentation Commons, Cancer Biology Commons, and the Molecular, Cellular, and Tissue Engineering Commons

Citation


This Thesis is brought to you for free and open access by the Biomedical Engineering at ScholarWorks@UARK. It has been accepted for inclusion in Biomedical Engineering Undergraduate Honors Theses by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu.
Sequential Illumination in a Tomographic Microendoscopic Probe for Imaging Tumor Microvasculature

An Undergraduate Honors College Thesis

in the

Department of Biomedical Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

By

Zachariah A. Neumeier
Table of Contents

1. Abstract........................................................................................................... Error! Bookmark not defined.

2. Introduction......................................................................................................... 4

3. Materials and Methods......................................................................................... 6
   3.1 Microendoscopic Probe Design ..................................................................... 6
   3.2 Polystyrene Bead Optical Phantoms ............................................................. 7
   3.3 Image Capture and Analysis ......................................................................... 8

4. Results.................................................................................................................. 11

5. Discussion and Future Work................................................................................. 13

6. Acknowledgements............................................................................................... 15

7. References ........................................................................................................... 16

8. Appendix ............................................................................................................. 18
1. Abstract

Knowledge of colorectal cancer biology is improving how we approach cancer treatment. Specifically, the tumor microenvironment and abnormal angiogenesis are of particular interest. Optical methods are a prime candidate for research of the tumor microenvironment due to their ability to quantitively assess tissue structure and perfusion in real time. Particularly, the “transport scattering regime” has been identified as a method of obtaining high-resolution images and reflectance spectroscopy data; this light scattering regime has been demonstrated compatible with endoscopic imaging systems. In this study, a proof-of-concept optical imaging system is presented, capable of resolving absorbers within scattering turbid media using a custom designed microendoscopic probe with multiple illumination fibers. This ability is validated through imaging of absorbers submerged in liquid polystyrene bead phantoms at a known distances from the probe tip. These resulting images are analyzed for deviation in light intensity from a homogeneous case, which provides a quantitative way to identify absorbers in scattering media. Future work will incorporate Monte Carlo simulations of photon propagation through scattering media to predict the depth and location of absorbers, providing the ability to create a 3D “map” of the tissue microenvironment and tumor area of interest. Overall, this study represents a step in the right direction for patient-tailored therapies and treatments based on specific changes of the tumor microenvironment in colorectal cancerous tumors.
2. Introduction

Colorectal cancer is the fourth leading cause of cancer death in the world. In the United States alone in 2017, it is estimated that there were over 1.3 million people living with colorectal cancer (1). Understanding the biology of colon cancer is an important step in the development of cancer therapies and treatments. Specifically, understanding how abnormal angiogenesis occurs during tumor development and progression is of particular interest. Large tumors are known to “outpace” their vascular supply of oxygen resulting in tumor hypoxia; this results in poor clinical results and treatment outcomes (2). Knowledge and understanding of tumor vasculature are improving how interactions between tumor cells, immune cells, and extracellular components are understood (3). Although significant progress has been made, there is still much to learn about the tumor microenvironment, an important step for the development of therapies that are tailored and specific to each patient.

Many studies focused on tumor response use conventional histopathology, which provides specific information about molecular events in tumor development. However, these studies are not able to relay dynamic changes in tissue perfusion. Other studies use murine window chamber models for in vivo study of tumor cells implanted under the skin, but these models are performed in non-anatomical locations leading to tumor response that may be non-physiologic (4).

Optical methods of in vivo assessment of the tissue microenvironment and vasculature offer quantitative, real-time measurement of the tissue structure and perfusion. Importantly, these methods can be scaled down for endoscopic deployment, making regions such as the gastrointestinal tract accessible (5). Optical methods traditionally explore two primary regimes of light interaction and scattering within tissue: single scattering methods and diffuse scattering
methods. However, both scattering regimes are limited in their ability to provide information about tissue perfusion or vascular details (6, 7).

Additionally, endoscopic and catheter-based probes typically used for imaging of epithelial tissues are limited by their very small diameter and small source-detector separation (SDS). Due to the small SDS, diffuse scattering is not possible, and the diffusion approximation of light propagation is invalid. The SDS of endoscopic and catheter-based systems require stochastic modeling such as Monte Carlo simulations to estimate photon trajectories and extract meaningful physiologic data.

Recent research into a scattering regime referred to as “transport scattering regime” has been shown capable of acquiring high-resolution optical images and reflectance spectroscopy data (8, 9). This scattering regime falls between that of single-scattering and diffuse scattering, with an SDS of about a millimeter. Optical microendoscopic probes are capable of imaging within this regime (8, 9). The relationship between the transport scattering regime and optical microendoscopic probes is shown in Figure 1.

**Figure 1.** Transport Scattering Regime. (A) Summary of “transport scattering regime” of photon scattering in turbid media. Note that the trajectory of photons retains some directionality. (B) Optical probes can be used in the “transport scattering regime,” providing light from the source fiber, which is scattered and reflected through tissue. The light returns at various positions on the image guide, creating an image.
In this study, the creation and use of a microendoscopic probe with a minimal diameter that utilizes the “transport scattering regime” as a means of assessing the tumor microenvironment and angiogenesis is hypothesized. A central image guide fiber bundle was used to collect an image of backscattered light that contains information about blood vessel structure beneath the surface. Containing an array of addressable illumination fibers, this microendoscopic probe was used to collect images of the same area of interest with different light projections and therefore different angles of photon propagation. Using absorption information from the various light projections and images, this proof-of-concept system demonstrates ability to quantify absorbers in turbid media and sets the foundation for tomographic reconstruction of tumor microvasculature. Liquid optical phantoms with embedded absorbers were imaged at varying depths and were used to demonstrate system sensitivity to changes in absorber depth.

3. Materials and Methods

3.1 Microendoscopic Probe Design

An optical imaging probe of small diameter (3.048mm OD) was designed and manufactured (Myriad Fiber Imaging) for imaging in the transport scattering regime at eight radially spaced light projections. The distal end of the probe is comprised of eight silica optical fibers each with a core diameter of 50µm ± 2% and surrounded by an acrylate coating (Thorlabs Inc. FG050LGA) and an imaging fiber bundle with an image circle diameter of 790 ± 50µm and fiber center-to-center distance of 4.5µm coated in silicone resin (Fujikura FIGH-30-850N). The center-to-center distance between each individual optical fiber and the imaging fiber is approximately 850µm. Each optical fiber is radially spaced at 45° angles from adjacent optical fibers. The total probe length is 1m, with a 30mm distal stainless-steel jacket, a 0.597m polyimide sheathing, a 25.4mm stainless-steel
fiber junction, and nine 0.297mm fiber legs that terminate with a drill SMA095 connector. SolidWorks drawing and real image of the probe distal imaging tip are shown in Figure 2.

![SolidWorks schematic and real image of the probe distal imaging tip](image)

**Figure 2. Distal Tip of Probe.** (A) SolidWorks schematic of distal probe tip, featuring eight source fibers that each provide unique information of light propagation in tissue area of interest. (B) Actual image of distal probe tip. Small markings on ruler represent 1/32 in.

### 3.2 Polystyrene Bead Optical Phantoms

For system validation, purely scattering optical phantoms were created using polystyrene microspheres (Polysciences Inc. 07310-15). Optical phantoms created from polystyrene microspheres have been shown to be useful for replicating optical scattering in tissue (10). With these phantoms, desired reduced scattering coefficients ($\mu_s'$) can be acquired through appropriate dilutions of concentrated beads and water. A target value of $\mu_s' = 5\text{cm}^{-1}$ for $\lambda = 532\text{nm}$ was chosen based on optical values of healthy epithelial cells in the colon (11). Scattering coefficients of phantoms were calculated using a web-based Mie scattering calculator, corresponding to 3.53mL of polystyrene beads diluted with 1.47mL of distilled H$_2$O to obtain the desired $\mu_s'$ target value (12). To simulate absorption by microvasculature, monofilament polypropylene sutures with an outer diameter of approximately 100µm (Redilene MP-3 6-0) were submerged in the phantoms.
and imaged at varying depths from the image guide. Examples of a homogeneous phantom and embedded suture placement are shown in Figure 3.

![Figure 3. Polystyrene Bead Optical Phantoms. (A) Example image of a homogeneous purely scattering liquid polystyrene bead phantom. B) Top-down view of empty beaker with affixed suture. A liquid phantom such as that shown in image A is poured over the suture to submerge it; the probe is backed away a known distance before imaging to simulate absorber depth under the surface of tissue.](image)

### 3.3 Image Capture and Analysis

System illumination consisted of a fiber-coupled LED with nominal wavelength of 530nm capable of a 3.2mW power output (Thorlabs Inc. M530F2) and an optical bandpass filter with a center wavelength of $532 \pm 2\text{nm}$ and full width half max of $10 \pm 2\text{nm}$ (Thorlabs Inc. FL532-10) placed inside an in-line multimode fiber optic filter mount (Thorlabs Inc. FOFMS). Light was chosen at the 530-534nm range due to high hemoglobin absorption and contrast from scattering media (13). System instrumentation is shown below in Figure 4.
Images are captured using an 8-bit CCD camera (FLIR Flea3 USB) and the FlyCapture2 system with an integration time of 800ms and a gain of 4dB. Calibration images were acquired with homogeneous phantoms for standardization of photon propagation through homogeneous media. Experimental images of phantoms with an embedded suture were acquired using light from all eight probe optical fibers at multiple depth positions of 0µm, 50µm, and 101µm using a micrometer (Thorlabs Inc. 150-811ST). The image collection setup with micrometer is shown below in Figure 5.
Analysis of images was performed in MATLAB (MathWorks, MATLAB R2021a). Images were cropped and down sampled to a square size of 200 by 200 pixels for noise reduction. Images from each optical fiber projection were standardized. Decay curves of light intensity were calculated using image profile methods in MATLAB, analyzing pixel intensity in a straight line across the image guide, from the light source and through the center. Curve smoothing functions from the curve fitting toolbox were used to provide a smooth decay curve of pixel intensity across the image guide for comparison and analysis. Theoretical light decay curves from Monte Carlo simulations of light propagation through a medium of matching optical properties were used for comparison and validation of the homogeneous phantom images; these simulations were obtained within the research group. Deviation of experimental image decay curves from the theoretical

Figure 5. Image collection setup. (A) Side view of imaging system. Probe is bent at 90-degree angle from proximal to distal end, allowing for vertical imaging on distal end. (B) Depth of submerged optical phantoms was controlled using a micrometer. Images with light from all eight source fibers were taken at distances of 0μm (in contact), 50μm, and 101μm from the suture to simulate tumor microvasculature at various depths under surface of epithelium.
Monte Carlo light decay curves was quantified, highlighting areas of absorption interest as deviations from a purely scattering homogeneous case.

4. Results

Images were taken using all eight source illumination fibers across three simulated absorber depths: 0µm, 50µm, and 101µm. Sample images of the various light projections are shown in Figure 6.

Figure 6. Sample of collected images. Shown above are two of the eight source fibers, each with an image containing a suture at a depth of 0µm, 50µm, and 101µm. For fiber 1, the light source is located to the bottom left of the images, while for fiber 3, the light source is located straight above. At each depth, an image was taken at all eight illumination fibers, providing unique light projections and absorption information from the suture. Note that the absorber resolution decreases with increasing distance from the probe.
Collected images were analyzed to identify deviations from a homogeneous phantom calibration image, allowing for sensitivity to absorbers in the tissue area of interest. Examples of analyzed phantom images and their associated decay curves are shown below in Figure 7.
As shown in Figure 7, the homogeneous phantom image and associated decay curve closely correspond to the theoretical Monte Carlo curve of light propagation. Image analysis shows a quantifiable deviation from the homogeneous case when an absorber is introduced in the imaging area; the width and sharpness of that deviation is shown to increase and decrease respectively with increasing distance between absorbers and the imaging fiber.

5. Discussion and Future Work

This work provides the basis for a proof-of-concept optical imaging system that is sensitive to absorbers in the tissue area of interest using a custom microendoscopic probe. Using photon transport simulations obtained in the research group, specific depth information can be collected based on absorption in the tissue below; combining all eight light projections together, absorber 3D information can be reconstructed to “map” the tumor area of interest. Together with photon propagation simulations, this system is sensitive to absorption changes in the tissue at hundreds of microns below the surface. This process is further illustrated in Figure 8.
Figure 8. Basis of 3D Absorber Resolution. (A) Cartoon drawing of 3D tissue volume with vasculature. Each source fiber (orange) represents a different set of photon paths (black) back to the image guide (green). (B) An example set of photon exit paths from simulations obtained in the research group. These simulations provide specific depth information based on absorption in the tissue below and can be compared with image decay curves from this study to predict and quantify absorber depth.

Future work will involve many steps. Primarily, integration of Monte Carlo photon propagation simulations for a seamless estimation of absorber depth and location will need to be done before the overall system accuracy can be validated and studied. One limitation of this study is the simplified approach of the complex tumor microenvironment; further studies will need to investigate the technique demonstrated here on more realistic subjects, such as complex phantoms that include multiple sources of absorption and scattering. Further studies could also incorporate live tissue in vivo, possibly through murine studies for longitudinal tracking of tumors. Other light modalities could also be included in the image capture system for more anatomical or functional information about the tumor microenvironment.

Overall, this study provides the foundation for a minimally invasive endoscopic probe that is sensitive to changes in absorption at hundreds of microns beneath the epithelium. This work represents a step in the right direction for patient-tailored therapies and treatments based on specific angiogenesis and microvasculature in colorectal cancerous tumors.
6. Acknowledgements

Special thanks to Andrew Stark and Shelby Bess for project assistance, Dr. Muldoon for his mentorship, and the countless friends and family in their support of my academic and research endeavors over the past four years.

This material is based on work supported by the National Science Foundation (CBET 1751554), the Arkansas Biosciences Institute, and a University of Arkansas Honors College Research Grant. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the acknowledged funding agencies.
7. References


8. Appendix

8.1 Engineering drawing of custom microendoscopic probe from Myriad Fiber Imaging Technologies, Inc.