Label-free Mie Scattering Identification of Tumor Tissue Using an Angular Photodiode Array

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Abstract— Tumors differ from normal tissues in several meaningful ways including cellular size, morphology, and protein expression, which will accordingly change the refractive index and the size/morphology of cells. There are also important differences in tissue organization and unique tissue specific cell densities. Instead of time-consuming and labor-intensive histology involving the use of a benchtop microscope, a plot of Mie scattering intensities at fixed wavelength against scattering angle, which we referred to as “Mie spectrum,” is suggested as an alternative to identify tumor from normal tissues. An angular photodiode array is developed to measure this Mie spectrum with three different light emitting diodes (blue, green and red) as light sources. The resulting Mie spectra show characteristic peaks for rat colonic tissues, and substantial differences can be found between tumor vs. normal tissues. Two peaks were identified at 120° and 150° scattering angles, potentially representing capillaries and colon cells, respectively. Contributions from crypts and goblet cells, represented by the scattering at 140°, were minimal. Substantial differences between tumor and normal tissues were found with 45°-70° light irradiation angles.

Index Terms—colon cancer; rat model, light scattering, tissue biopsy.

I. INTRODUCTION

Angle-dependent Mie scattering diagnostic and imaging modalities are rapid and can be employed noninvasively, i.e. requiring no labels. As a result, measuring Mie scatter can become a promising diagnostic method for various medical conditions such as cancer. Mie scattering occurs when the size of a scattering structure becomes comparable to or larger than the wavelength of incident light. Scattering intensities are constructively added at a certain angle while they can be destructively attenuated at the other angle, creating a highly angle-dependent scattering profile. Factors that alter Mie scattering are the refractive index, size, and density of scattering object, as well as the wavelength and angle of incident light. Mie scattering-based optical sensing techniques, such as the use of Mie scattering intensities at fixed angle to identify surface characteristics, are often employed during quality-control checks of manufactured surfaces and materials because optical scattering is highly informative about a surface’s physical characteristics including factors such as surface roughness. Our laboratory has employed this method toward more biological matrices including identifying bacterial presence and species on a human and animal skin as well as on a wound, i.e. skin and wound infection, without using any labels [1,2]. Labels can be added to amplify such Mie scattering intensities, for example the submicron- or micron-sized beads with high refractive indices such as polystyrene and TiO₂. Our laboratory has employed this technique a number of times to identify samples labeled with antibody-conjugated polystyrene beads on a number of different microfluidic platforms including on polydimethylsiloxane (PDMS) and nitrocellulose substrates [3-5]. Others have labeled tumor cells with TiO₂ beads and measured scattering [6] as well as for detecting and measuring

fibroblast necrosis [7]. While these beads have successfully amplified the Mie scattering intensities measured at a fixed angle, it may be possible to identify the type and morphology of tissue without using such beads (or any other labels).

The method presented in this work, i.e. collecting Mie scattering intensities at multiple incident and detection angle combinations from tissue samples, does not require sample processing, beads, additional reagents, or bioreceptors, which eliminates time and labor for lengthy reagent and sample preparation. Our device consists of an array of light emitting diodes (LEDs) and photodiodes that detect Mie scattering from a tissue surface. LEDs are mounted at angles of 90°, 80°, 70°, 60°, and 45°, relative to the tissue surface and Mie scattering is detected at 10° increments from 110° to 170° to instantly obtain a full Mie scattering spectrum (i.e. a plot of Mie scattering intensities against the scattering angle at the fixed incident wavelength), as depicted in Fig. 1.

We propose to use this method in lieu of time-consuming and labor-intensive histology preparation and analysis involving the preparation of histological slides and the clinical expertise to identify specimen. Histology is one of the most definitive methods of tumor diagnosis, where a tissue sample is collected (“biopsied”) from a tissue of interest and subsequent microscopic examination is made with appropriate staining. Rapid, accurate, label-free, microscope-free diagnosis of tissue samples in the clinic would be helpful in aiding clinicians to make timely and accurate diagnosis. Our device is designed to be used with biopsy samples, without the need for staining (requiring labor) and microscopy (bulky and expensive).

The specific aims of this research are 1) to identify whether the Mie spectrum can be used to identify tumors from normal tissues that are not affected by blood absorption and direct surface reflection, 2) to optimize the color and angle of light irradiation and the angle of light

detection, and 3) to identify the tissue components that are responsible for the differences between normal vs. tumor tissues.

Fig. 1. Angular photodiode array positioned at 110°-170° with red, green and blue LED light sources positioned at 45°-90°.

II. MATERIALS AND METHODS

A. Sensor Design

An angular photodiode array and tissue stage were created using SolidWorks 2013 x64 Edition (Dassault Systèmes, Vélizy-Villacoublay, France) and 3D-printed in ABS (acrylonitrile-butadiene-styrene) polymer using (Ultimaker 3; Ultimaker, Utrecht, Netherlands). The photodiode array consists of a semi-circle (radius = 32 mm) with eight insertion points for PIN photodiodes (MTD1114M3B; Marktech Optoelectronics, Latham, NY, USA) arranged in a single plane at 10° increments from 110° to 170° relative to a light source shining perpendicular to the surface (Fig. 1). Three types of LED light sources, red (624 nm, 6° viewing angle; 383-2SURC/S400-A6; Everlight Electronics, Shulin, Taiwan), green (505 nm, 8° viewing angle; OVLGC0C6B9; TT Electronics, Woking, England), and blue (470 nm, 15° viewing angle; HLMP-CB1A-XY0DD; Broadcom Inc., San Jose, CA, USA), are inserted at five different angles: 90°, 80°, 70°, 60°, and 45°, relative to the tissue surface (Fig. 1). Red LEDs are arranged in the same plane as the photodiodes, green and blue LEDs are arranged on either side of the red LED offset by 5 mm perpendicular to the semi-circle plane but still at the same angle as those of the red LEDs. Because the offset is small (5 mm on either side), approximately the same portion of each sample is illuminated by all three LEDs. The tissue stage fits a standard microscope slide to center the tissue below the angular photodiode array. The angular photodiode array height relative to the tissue stage is adjustable, so that the surface of each tissue sample is analyzed despite variations in sample thickness.

B. Circuit Design

The LEDs were powered by the 5 V output of an Arduino Mega 2560 microcontroller (SainSmart, Filaments, AB, CA) and driven using an LED driver (6969; Maxim Integrated, San Jose, CA, USA). The outputs from eight PIN photodiodes in photovoltaic mode were collected individually by the same Arduino Mega 2560. The differential output of each photodiode was amplified through a gain stage using quad op-amps (TLC27L4CN; Texas Instruments, Dallas, TX, USA) to amplify the photodiode output and the gain were optimized for each angle of detection so that the broadest range of signal would be detected without saturating the device under normal

conditions. Due to the large gain employed to detect our low target signal a high-pass filter was employed to cutoff unwanted signal noise that was substantially amplified as well.

C. Mie Scattering Simulations for Tissue Components

Mie scattering simulations were performed using the MiePlot v.4.6 software (Philip Laven; available at www.philiplaven.com/mieplot.htm). Four structural elements were identified that could reasonably alter scatter from healthy to tumor colon tissues: colon cells, colonic crypts, goblet cells, and capillary structure. These structures were simulated for their Mie scattering intensities against the scattering angle and compared to the results of tissue samples, to identify the tissue component(s) dominating or strongly influencing the overall Mie scattering. Each simulation was performed using the inhomogeneous sphere mode and setting the refractive index equation to 3-layer (sinusoidal transitions) in MiePlot so that more structural elements could be better simulated and so that the number of layers could be accounted for. It was reasoned that tubular structures like crypts and capillaries could also be loosely simulated using this method since a ray of light would likely interact with these structures at an angle which nearly simulates an oval. Output Mie spectra was obtained for unpolarized light. Additionally, all simulations assumed a normal distribution with respect to the size of structures with a 10% standard deviation which is consistent with the range of typical mammalian cells [8]. The selected parameters for each structure can be found in Table 1. These values were simulated with an angular resolution of 1° ranging from 110° to 170° at 3 different wavelengths of 470 nm, 505 nm, and 624 nm. Scattering angles in Mie simulations are defined as 0° for scattering in the same direction of incident light, 90° for scattering perpendicular to the incident light, and 180° for scattering back to the light source, thus 90°-180° as back scatter.

Table 1. Parameters used to model Mie scattering.

Structural Components	Sub-components	Radius (μm)	Refractive index	Layer
Colon cells	Nucleolus	.5	1.42	64
	Nucleus	3.2	1.39	
	Cytoplasm	4.25	1.37	
Crypts	Crypt	25	1.333	16
	ECM	35	1.375	
Goblet cells	Granules	5	1.35	32
	Cytoplasm	5.5	1.37	
Capillaries	Lumen	5.6	1.333	8
	ECM	7	1.38	

Data obtained from [9] (colon cells), [10] (crypts and goblet cells) and [11] (capillaries).

D. Tissue Sample Preparation

Tumor was induced in a rat model in accordance with an Institutional Animal Care and Use Committee (IACUC) approved protocol (protocol number 17-275). Eight RNU immunodeficient rats (RNU316; Charles River, Wilmington, MA, USA) were purchased. RKO colorectal cancer cells (CRL-2577; ATCC, Manassas, VA, USA) were cultured in EMEM in a cell culture flask and grown to confluence. On the day of injection 10^6 - 10^7 RKO human cells were suspended in 200 μL Eagle's Minimal Essential Medium and maintained on ice prior to injection. Cells are injected subcutaneously into the right flank of RNU immunodeficient rats using a 22-gauge (0.72 mm OD and 0.41 mm ID) or smaller needle. Rats were

monitored 1-2 times daily for the next 48 hours until they returned to normal health and activity. Heterotopic colorectal tumors were grown to approximately 2 cm diameter. Within 6 weeks rats were euthanized and tumors and healthy rat colons were harvested for analysis.

E. Data Collection

Data was collected in a dark room to reduce the effect of external lighting that might have on measurements. For each sample the device cycled through each of the 15 LEDs illuminating the sample for just over 3 seconds during which time intensity readings were collected every 250 ms from each of the eight photodiodes and averaged over the three-second window to reduce any signal variation that was a result of noise before moving onto the next LED.

III. RESULTS AND DISCUSSION

A. Mie Scattering Simulations

Fig. 2. Simulated Mie scattering profiles ("Mie spectra") for common structures: colon cells, crypts, goblet cells, and capillaries. Red, green and blue lines represent red, green and blue intensities, respectively.

Fig. 2 shows the simulations for the four structural elements that likely would have the biggest impact on Mie scattering in colon tumor: colon cells, crypts, goblet cells, and capillaries. Simulations were conducted for 110°-170° back scattering angles, since detection of forward scattering is not practical with the tissue samples sitting on a flat surface. Colon cells show a peak at slightly higher than 150° as well as increasing intensities towards lower back scattering angles. Crypts show a sharp peak at slightly lower than 140°. Goblet cells show a sharp peak at 140°, which is similar to those of crypts. Capillaries show a broad peak around 130°. Green and blue intensities are very similar to each other, while red intensities are smaller than them, although the overall shapes are identical for all three colors. Therefore, we expect that our device will likely exhibit a similar pattern.

B. Results with Rat Models

Fig. 3 shows the average light scattering intensities from the rat specimens. With the blue LED irradiation at 90°, i.e. perpendicular to the surface, no differences are found between tumor and healthy tissue samples. Two peaks can be found, one at 120° and the other at 150°. Comparing with the simulation results shown in Fig. 2, the 150° peak corresponds to that of colon cells and the 120° peak to the capillaries (and potentially the increasing trend with decreasing scattering angle with colon cells).

With decreasing angle of irradiation (80° to 45°), more pronounced differences can be found at 150° between tumor and healthy tissues, while the Mie spectra were not significantly shifted to the left or right. As the intensities at this larger scattering angles (~150°) are always lower with tumor tissues, this can be attributed to the loss of colon cells in tumor tissues. On the other hand, the intensities at smaller angles (~120°) are higher with tumor tissues, which can potentially be attributed to the increase in capillaries with tumor tissues. This angle-dependent amplification rises from many different factors, most likely the Kirchhoff light scattering approximation from rough surfaces [12]: a greater number of secondary scatter events (not affected by incident angle) are observed when a sample is irradiated at high angles of incidence (in our case, low irradiating angles, e.g. 60° and 45°), while light scatter at low angles of incidence (in our case, 80° and 90°) results in more primary scatter events. This effect is likely further complicated because tissue samples exhibit strong scattering from multiple components over the depth of layers.

With green LED irradiation, similar trends can be observed: 1) two peaks at 120° and 150° scattering angles, 2) no differences between healthy and tumor tissues with 90° and 80° irradiation, and 3) significant differences at the 150° scattering angle between healthy and tumor tissues. Red LED irradiations are inherently different from the other colors, as blood is essentially transparent to this color, i.e., hemoglobin in red blood cells does not absorb most of red color. As the trend with the red LED irradiations is almost identical to the others, it can indirectly be concluded that the results shown in Fig. 3 are mostly attributed to the Mie scattering of tissue components but not to the absorption of hemoglobin in the tissue samples. However, it should be noted that the results with red LED irradiations show the least significant differences. This can be attributed to 1) the lower scattering intensities in red color (in comparison to blue and green color) as simulated in Fig. 2, and 2) the possible contribution of hemoglobin absorption with blue and green LED irradiations.

IV. CONCLUSION

Based off our findings we are confident that changes in optical Mie scattering between tumor and healthy colon samples can be detected using a simple photodiode array consisting of just several PIN photodiodes and one or more LEDs. The angular array we designed gives a basis for experimentally identifying angles and colors (wavelengths) of LEDs that are particularly useful for identifying tumor samples from healthy tissue samples despite the fact that tissue specimen exhibits complex scattering behavior.

Fig. 3. Mie scattering intensities collected from the angular photodiode array device for healthy and tumor rat tissue samples. Samples were irradiated with blue (top row), green (middle row), or red (bottom row) LEDs positioned at 90° (perpendicular from the tissue surface), 80°, 70°, 60° and 45°. Only one LED irradiated the tissue sample per experiment. Averages from 4 different healthy rat tissue samples and from 6 different tumor rat tissue samples. Error bars represent standard errors. * indicate statistical difference with $p < 0.05$ between healthy and tumor tissue samples.

Rat model was employed and tested by growing human RKO tumor cells in immunocompromised RNU rats and the colon tissue samples were harvested. Comparing the Mie scatter simulations to the experimentally collected scatter profile data from the rat model seem to suggest that the scatter profile observed is a product of a number of tissue macro- and microstructural elements where perhaps the two greatest differences in our rat model originating from the loss of colon cells in tumor and the increase in capillaries.

Stronger and collimated LEDs and modulation of the LEDs with lock-in detection can improve the signal to noise ratio and avoid the requirement of a dark room. While the method is primarily designed for *in vitro* analyses, LED and photodiode pair at optimized angle may be incorporated at the end of an endoscope for *in vivo* diagnostics.

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