Intraoperative Assessment of Surgical Margins in Cancer Resection Surgery via the Sub-Diffuse Optical Tomography s-DOT

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ABSTRACT:

In Head and Neck Squamous Cell Carcinomas (HNSCC), positive margins after surgical resection dominate the clinical outcome of patients. Between 15-30 percent of all HNSCC surgeries result in a positive margin (>5 mm) that requires postoperative chemo-radiation, radiotherapy, and/or revision surgeries. To minimize local recurrence, margin assessment is typically performed through surgical pathology departments; however, this technique often takes more than 24 hours to process and only a small fraction of the total surgical margin is evaluated. A second, intraoperative approach used by surgeons to minimize local recurrence is frozen section analysis (FSA), which is time intensive and suffers heavily from sampling errors. To effect change, we have hypothesized a sub-diffuse optical tomography modality that can identify the differences in depth sensitivity of the fluorescence, Cetuximab-IRDye800CW conjugate (ICON), by the fluorescent photons collected from sub-diffused media as a function of the photon exit angle from the excited tissue. Based on promising preliminary simulations using Monte Carlo MATLAB, closed vs. open aperture fluorescence imaging in biological tissue demonstrated an enhanced true depth of fluorescence resolution up to 6 mm, well beyond the insufficient 5 mm mark. By identifying the deep margins from the fluorescent peaks within 5 minutes, we hope to improve complete oncological surgery by providing surgeons an exact region of interest on the specimen where the tumor is closest to the edge, so they may continue surgery on the spot to minimize the probability of cancer being left behind.

Head and Neck Squamous Cell Carcinomas (HNSCC) make up 90 percent of all head and neck cancers, with over 600,000 new cases per year worldwide [1]. HNSCC originates in the areas of the lip/oral cavity, nasopharynx, oropharynx, hypopharynx, and the larynx with the main at risk population being long term smoking and alcohol users [1]. It is due to these intricate anatomical areas in which surgical resection is the primary curative treatment and, therefore, has the highest incomplete resection rates of all cancer types at 15-30 percent [7].

After surgical resection of the primary tumor, the margins are accurately assessed by post-operative pathology, which may take days to verify if the tumor has a margin of 5 mm of healthy tissue surrounding it. In the mean time, the patient is sewn up and sent home. Currently, the only intraoperative assessment of margins relies on frozen sectional analysis (FSA), which heavily suffers from sampling errors and is a time intensive technique that can last up to 30 minutes [3, 12]. It is for these reasons, that both FSA and pathology are undesirable tools due their subjective evaluation methods and the struggle for surgeons to identify suspicious regions that should be sent to histopathologic assessment. Additionally, the completeness of the surgery must also consider the quality of life the patient will face (e.g vital, functional, and cosmetic reconstruction). It is estimated that more than 15 percent of HNSCC patient have inadequate margins after being sent home from their operation [9]. Failing to intraoperatively recognize these positive margins results in a loco-regional recurrence that may necessitate additional therapy such as radiation, chemotherapy, and recurrent surgeries [4]. There is much debate regarding characterizing an inadequate margin, the current method of measurement for a positive close margin is cancer between 1 to 5 mm of the margin as shown in Figure 1 [4]. However, to evaluate these margins the resected tumor is divided into an epithelial/mucosal surface and a deep surface (surgical margin exposed after surgery), which account for approximately 90 percent of the inadequate margins because of the lack of visual feedback [12].

Mitigation of inadequate margins holds great importance to the outcome of the patient and their overall quality of life. Therefore, to address these challenges (with specific

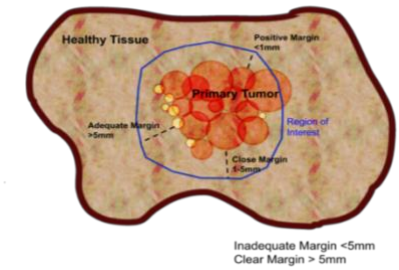

Figure 1 The margins are highlighted in this image as close(1-5 mm), positive(<1 mm), and clear(>5 mm) from the amount of healthy tissue surrounding the tumor to the region of interest. To have adequate margins, clear margins need to be met, in which there should be 5mm of healthy tissue surrounding the tumors edge.

focus on deep margins) we have proposed a novel intraoperative margin assessment device termed, the Sub-diffused Optical Tomography (s-DOT), that will rapidly and accurately identify the positive (<1 mm) and close (1-5 mm) margins on whole resected specimens. To accompany the intraoperative s-DOT device, fluorescent labeled antibodies will be used to identify the closest surgical margin on the specimen by assessing the relative fluorescence intensity peaks. By promising results shown by Monte Carlo MATLAB simulations, we hope to improve the rates of incomplete surgery in real time to minimize the probability of leaving any cancer behind.

Materials and Methods

The augmented Monte Carlo software on MATLAB (MATLAB 2018b Student License) was used to model the simulation of light transport in HNSCC tissue by the general methods for fluorescence simulations and diffuse optical tomography (DOT). The Monte Carlo (MC) developed by Jacques et al [6] was utilized to mimic the light propagation in scattering tissue with homogeneous optical properties. Assuming Fresnel reflections at tissue/air interfaces, the code utilizes the size of the medium, the absorption coefficient of the medium set to μ_a 0.4 cm^{-1} the scattering coefficient μ_s set to 125 cm^{-1} and the anisotropy of scattering (g) set to 0.9 cm^{-1} [6]. The anisotropy of scattering represents the probability distribution of scattering angles [1]. The refractive angle is then calculated between two regions within the tissue or at the surface have mismatch refractive indices [1]. To stimulate the fluorescence emission resulting in the fluorescence quantum yield, the MC parameters utilizes the probability of an absorbed photon packet and converts it to a fluorescent photon at a specific wavelength. The photon "packets" are photons in MC literature [11] defining the photons travel inside a tissue mode. It considers the initial weight as it enters the model and the transmission through or reflection across a boundary governed by Snell's law and Fresnel's equations [13].

Snell's Law : $n_1 \sin\theta_1 = n_2 \sin\theta_2$

After each step the photon packet weight is reduced according to the absorption probability [10] and this continues step by step until the photons exit the tissue or are completed absorbed. However, as this is transformed to fluorescence photons, the emission of this photon is now isotropic due to the nature of fluorescence [1].

To stimulate the fluorescence emission, the MC simulation allows for a simulation light propagation, where set at an excitation wavelength of 680 nm with respect to the fluorescence, will be discussed in the next section. A fluorescent photon will be generated per excitation. Then, the MC simulation is utilized to stimulate the emission wavelength from a tissue model related to the absorption and scattering properties of the HNSCC tissue model, in addition to the fluorescence quantum yield and lifetime. The path of the photon packet was recorded to a detected fractional density map of incident light transported $D(x_i,y_j,z_k)$ created by Dr. K.Tichauer and his team [11].

Fluorescent Imaging Agent

During all phases of functional testing, the s-DOT will be imaging Indocyanine Green (ICG) fluorescent dye (Tokyo Chemical Industry Co, Chuo City, Tokyo). Although the s-DOT will be imaging ICG fluorescence during the functionality tests, the fluorescent dye used during the s-DOT intraoperative application will be the Cetuximab-IRDye800CW conjugate (ICON) agent in collaboration with Dr. M. Witjes. The ICON agent has shown enhanced tumor to healthy tissue contrast due to the addition of fluorescent labeled antibodies specific to the endothelial growth factor receptors (EGFR), which are over expressed in 90 percent of HNSCC [5]. The use of the ICON agent will be very valuable in amplifying the tumor contrast during surgery. However, the ICG fluorescent imaging agent will be used for testing due to its lower cost and similar excitation and emission wavelengths to the ICON fluorescent agent. The ICON agent's excitation wavelength is 780 nm and its emission wavelength is 820 nm [2]. On the other hand, ICG dye's excitation wavelength is 775 nm and its emission wavelength is 796 nm [2]. Due to the similar excitation and emission wavelengths, ICG will be a suitable stand in for the ICON dye during the s-DOT functionality tests.

Phantom Fabrication

For the second phase of testing, optical phantoms were created from resin (Castin' Craft, Fields Landing, CA), titanium dioxide (DuPontTM Ti-PureR, DuPont Titanium Technologies, Wilmington, DE), India ink (Winsor & Newton, London), and dimethyl sulfoxide (DMSO) (Fisher

BioReagents, Thermo Fisher Scientific, Waltham, MA). India ink was used as the absorbing agent and titanium dioxide was used as the scattering agent. Various concentrations of India ink and titanium dioxide were created with DMSO as the solvent. The concentrations of each material that provide the optical properties of HNSCC tumors will be found by measuring the optical properties of the various sets of phantoms and comparing the results to the target values. The target optical properties of HNSCC tumors are, 125 cm^{-1} as the scattering coefficient μ_s, 0.4 cm^{-1} as the absorption coefficient μ_a, and 0.9 cm^{-1} as the scattering anisotropy (g). Although the optimal concentrations of titanium dioxide and India ink have not been found yet, the optical property characterization experiment further explained are being done now to determine those concentrations.

After deciding the concentrations of India ink and titanium dioxide that best mimic the optical properties of HNSSC, silicone molds were created with pins at varying depths from 2 mm to 5 mm. These pins created holes at different depths for the ICG fluorescence to be loaded into the phantom and measured with the s-DOT device.

Phantom Optical Property Characterization

To characterize the optical properties of the resin, titanium dioxide, and India ink phantoms, 2 cm cubed phantoms and resin smears of the various concentrations were constructed. The resin smears were created to be 100 microns thin on a 12 well plate. To verify the thickness of the smear, the well's diameter was measured and the ideal volume of resin to be smeared on the bottom of the well was calculated to be 28.95 μL.

After creating the resin smear in the well plate and the cubed phantoms, an imaging system and photon counter system were used to characterize the phantom's optical property values. The imaging system measured the initial light (I_0), the distance (x), and the final light (I) for each resin smear sample. Then, use Beer Lambert's Law to calculate the total reflection of light (μ_T).

$$I = I_0 * \exp(\mu_T x)$$

$$\mu_T = \mu_a + \mu_s$$

Due to the small thickness of the resin smear (100 microns), the diffusivity assumption can be used to assume that the light will only be scattered once while traveling through the resin smear.

Next, the absorption coefficient (μ_a) and the reduced scattering coefficient (μ'_s) are measured using a photon counter. The photon counter measures the amount of photons hitting the detector every 4 picoseconds. Then, a MATLAB code using Beer Lambert's Law and Green's Function was used to curve fit an equation measuring the optical properties to the number of photons vs time.

$$\phi(r,t) = \left[\frac{c}{(4\Pi Dct)^{3/2}}\right] \exp[-\frac{-r^2}{4Dct} - \mu_a ct]$$

Equation 2 Green's Function

The resulting output optical properties will be the absorption coefficient (μ_a), scattering coefficient (μ_s), reduced scattering coefficient (μ'_s) and the scattering anisotropy (g). Through testing various concentrations of titanium dioxide and India ink, the concentrations of these materials to mimic the absorption coefficient (μ_a) of 0.4 cm^{-1} the scattering coefficient (μ_s) of 125 cm^{-1} and the scattering anisotropy of 0.9 cm^{-1} will be found.

Device Testing

After constructing the s-DOT, the device will undergo various phases of testing. The s-DOT will undergo three phases of functional testing involving multiple types of optical phantoms and live tissue. The three phases are as follows: testing with commercially manufactured phantoms, testing with phantoms created to mimic HNSCC tumors, and testing with live tissue.

In the first phase of testing, the s-DOT will be tested using commercially manufactured optical phantoms (INO Biomimic Optical Phantoms). A capillary tube filled with ICG dye will be placed at varying depths on six different phantoms. The tested depths will be from 0.1 mm to 5 mm in 0.5 mm increments. Each depth will be tested three times to ensure device precision.

In the second phase of testing, the s-DOT will be tested using the optical phantoms created from resin, titanium dioxide, and India ink. Four depths of a fluorescent marker, from 2 mm to 5 mm in 1 mm increments, will be tested with the system, with each depth tested three times. In addition to testing those four depths, the s-DOT will also run tests on phantoms with multiple fluorescent holes at differing depths to measure the device's sensitivity and accuracy to multiple signals.

Finally, in the third phase of testing, the s-DOT will be tested on live animal tissue stained with ICG fluorescent dye. The dye will be injected into the animals nasopharynx and oral cavity tissue at various depths and amounts. Each tissue will be imaged three times. For each phase of testing, the s-DOT results will be compared to the true depth value of the fluorescent marker and an analysis on the difference between the values will be done.

Results

MC MATLAB simulations are a way of creating ideal results of the s-DOT to develop the image processing and analysis to create the depth map results of the s-DOT. Two different simulations are run to model two images the s-DOT will produce. The first is an open-aperture image--an open field image, which is modeled by a beam that is an isotropically emitting point source. The second is a narrow-aperture image, modeled by a pencil beam. The ratio of the narrow aperture to the open aperture will provide a way of determining quantitative depth values from fluorescent imaging. Simulated results from the s-DOT are shown in Figure 2.

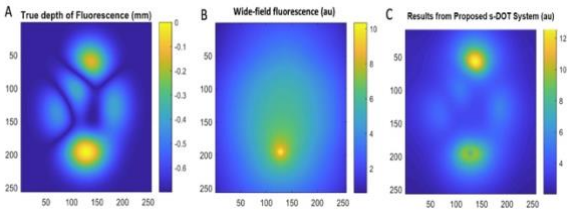

Figure 2 Simulated demonstration of improvements possible with s-DOT for observing wider insufficient margins. A) Simulation "truth" of fluorescence at depth up to 5 mm (margins less than 5 mm wide are considered insufficient). B) Standard fluorescence imaging of the fluorescence as seen from the surface of the field of view defined by A. C) Image resulting from ratioing narrow and open aperture fluorescence images, which is the first component of s-DOT.}

The ratiometric approach of both apertures will counteract any effects from model data mismatch and the effects of any varying optical properties within the resected tissue. Therefore, the ratio value will be directly related to depth values, which can be provided to surgeons intraoperatively.

Imaging Depth of Fluorescence

When looking at highly optically 3D scattering tissues, the depth of the fluorescence may be limited by the heterogeneity of optical properties and the shape of the tissue. To combat this MC Simulations showed that fluorescence wavelength ratioing can overcome tissue surfaces effects and mitigate sensitivity to optical properties in fluorescence tomography and spatial frequency-domain imaging.

The relationship between the depth that fluorescence is detected in a resected tissue and the ratiometric values of the open and close aperture values is currently being investigated. To display the relationship between the two, MC simulations were run on ellipsoid shaped resected tissues of varying depths. The ratio values were plotted against the depth values of various shape samples. For example, the ratio and depth scatter plot for the 5mm deep ellipsoid is shown in Figure 3, along with a best cure fit to the data.

In Figure 3, the blue dots represent the depth (x-axis, in cm) and ratio value (y-axis, in au) for all pixels in the aperture images simulated. The red line is the best fit curve for the data. Figure 4 shows the fit curves of the varying depth ellipsoids.

The results from Figure 4 clearly show there is a relationship between ratio values and depth values, and that this relationship is related but different for varying shapes of detected fluorescence. Future work will be conducted in limited angle tomography reconstruction along side the relationship between ratio and depth to produce depth margins via an interactive display for surgeons during surgery.

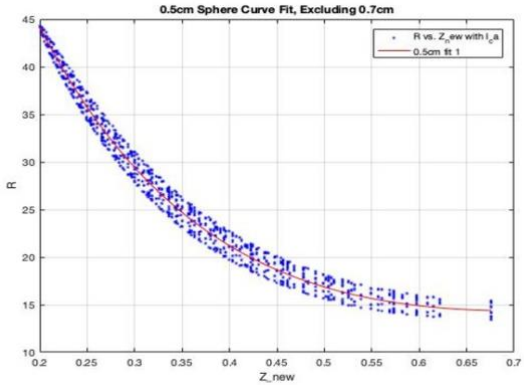

Figure 3. Scatterplot of Ratio vs Depth 5mm Sphere Sample.

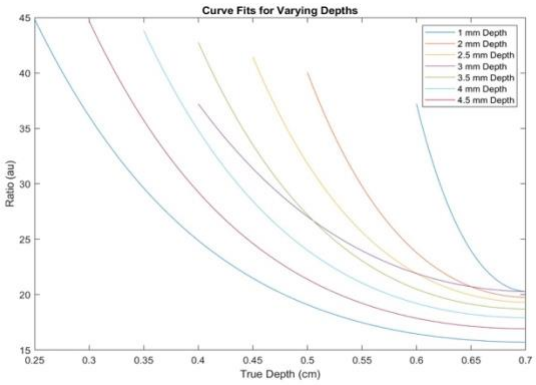

Figure 4. Fit Curves for Domes with Radii 1mm to 5 mm, in 0.5 mm increments.

Discussion

The promising technology behind the s-DOT system will be favorable for hospitals to adopt, due to its superior depth sensitivity and its seamless transition into the workflow of oncologic surgical operations. The s-DOT presents a unique method that has not been utilized in other target fluorescent imaging modalities, in which the difference of

open and closed apertures presents an accurate depth sensitivity that can be
relayed to surgeons in real time for expedited and successful resections of whole tumors of HNSCC. The s-DOT also presents itself as an ex-vivo target imaging device, so it does not cause additional harm to the patient during surgery. The goal of the project is to improve the rate of complete cancer surgery to improve the identification of inadequate margins from positive (<1 mm) to close (1-5 mm) depths. Although the s-DOT cannot prevent the initial surgery needed for HNSCC patients yet, it has the capability to assist the surgeon in performing a less radical surgery for lower morbidity, and to go back in after identification of inadequate margins. This statement has been hypothesized from the current success of the MC MATLAB simulation results. The results suggest that the surgeon can identify inadequate margins with the s-DOT during the patient's initial surgery, thus decreasing the probability of a recurrent and detrimental HNSCC tumor. Overall, the use of the s-DOT during HNSCC surgeries will lead to a comfortable living style for the patients.

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