

Optical imaging of tissue obtained by transbronchial biopsies of peripheral lung lesions

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Background: Bronchoscopic procedures have been increasingly used for the diagnosis of peripheral lung cancers, but the yield remains moderately low. The aim of this study is to assess the feasibility and ability of a custom-built bimodal optical spectroscopy system to enhance the on-site discrimination between malignant and benign specimens obtained from the transbronchial lung biopsies (TBLB) of peripheral lung lesions.

Methods: We conducted a prospective and single-center pilot study to examine the TBLB specimens obtained from peripheral lung lesions. Diffuse reflectance spectroscopy (DRS) and diffuse fluorescence spectroscopy (DFS) parameters were used to analyze the optical characteristics of these specimens.

Results: One hundred and sixteen biopsy specimens from 15 patients were analyzed using optical imaging. All specimens had a confirmed pathologic diagnosis. Notably, 22 of the 116 specimens were malignant, and 10 of the 94 non-malignant specimens were necrotic biopsies. Individual parameters showed significant difference between the three groups (malignant, non-malignant and necrosis). Multivariate analysis of the blood, scattering and fluorescence parameters demonstrated a sensitivity of 77.3% and specificity of 73.1% in differentiating between malignant and benign specimens and a sensitivity of 90.9% and specificity of 100% in differentiating malignant from necrotic specimens.

Conclusions: We conclude that optical spectroscopy is a feasible modality for on-site discrimination between malignant and benign as well as malignant and necrotic TBLB specimens of peripheral lung lesions.

Keywords: Transbronchial biopsy; optical; spectroscopy; peripheral lung lesions

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Introduction

Tissue biopsy sampling is essential for an accurate diagnosis of lung cancer (1). There are several means of diagnosing peripheral lung lesions, including transthoracic image-guided procedures, bronchoscopic procedures, and surgery. The results of the AQuiRe data registry for the diagnostic yield and complications of bronchoscopy for peripheral lung lesions were recently published by Ost *et al.* (2). Both navigational bronchoscopy and radial endobronchial

ultrasound (R-EBUS) performed poorly, with a yield of 57% and 38.5% respectively and 47.1% when the two modalities were combined. Optical spectroscopy can provide vascular and biochemical contrasts, which can produce enhanced sensitivity and specificity for tissue characterization. This was demonstrated in prior studies in oral, oropharyngeal, and lung cancers (3-12). The primary objective of this study is to assess the feasibility and ability of a custom-built bimodal optical spectroscopy system combining diffuse reflectance spectroscopy (DRS) and diffuse fluorescence

spectroscopy (DFS) to enhance the on-site discrimination between malignant and benign specimens that are obtained by transbronchial biopsies of peripheral lung lesions.

Methods

This study is a single-centered prospective pilot trial examining the lung tissue samples obtained through transbronchial biopsy. The study entails using the bimodal optical spectroscopy system to quantify multiple physiologic parameters of interest. The sensitivity and specificity of these parameters to differentiate between malignant and benign transbronchial lung biopsies (TBLB) specimens was investigated. The study obtained ethical approval from Roswell Park Cancer Institute Institutional Review Board (I-246913). All enrolled subjects gave informed consent before taking part in this study.

Ex-vivo specimens' collection

Lung tissues were obtained from 15 patients using TBLB for the diagnosis of peripheral lung lesions. The flexible bronchoscopy procedures combined with R-EBUS and navigation system were used in all cases. All the procedures were performed under general anesthesia in the endoscopy suite.

Instrumentation

The instrumentation consisted of a custom-built, fiber-based, two-channel spectroscopy system, where one channel allowed white light reflectance measurements for quantification of optical and hemoglobin-related parameters, and the other channel allowed fluorescence measurements (*Figure 1*). The white light reflectance fiber was connected to a tungsten halogen light source (HL-2000-FHSA, Ocean Optics) and the fluorescence fiber was connected to a 365 nm LED (M365F1, ThorLabs) for fluorescence excitation. The white light and fluorescence detector fibers were connected to the less-sensitive (Master) and more sensitive (Slave) channels respectively of a two-channel spectrometer (SD2000, Ocean Optics). The source-detector separations for both channels were 520 μm . Measurements were acquired by rinsing the biopsy sample with saline to remove any residual blood from the sample and placing it under a blacked-out container to minimize the effect of room light. The probe tip (1 mm diameter) made soft contact with the sample surface. Multiple measurements ($n>5$) were acquired by

slightly repositioning the probe on each sample. After measurements were completed, a reference standard was measured for the calibration of the instrument.

Optical measurements

For each spectroscopic measurement, the background counts were subtracted, and the signal was normalized with the reflectance standard to obtain the normalized reflectance. The normalized reflectance was scaled to the diffusion model in order to obtain reflectance in absolute units (13). This step is needed for accurate quantification due to the short separation between the source and detector fiber. To determine the scale factor for the probe, we followed a similar calibration procedure to that described in (13).

To extract the blood and scattering-related parameters, a trust-region-reflective nonlinear fitting algorithm (lsqnonlin, MATLAB) was used to fit the scaled data with the diffusion model. Tissue absorption was expressed in terms of blood volume (BV), tissue oxygen saturation (StO_2), and a water fraction (fixed at 0.65) (5,6). BV and StO_2 could also be expressed as the concentrations of oxygenated and deoxygenated hemoglobin [HbO_2 and (Hb), respectively]. The reduced scattering coefficient (μ_s') was modeled as Mie scattering, $\mu_s'(\lambda) = A(\lambda/\lambda_0)^{-b}$, where $\lambda_0 = 800$ nm, A characterizes the magnitude of scattering and b characterizes the wavelength dependence (14). For fluorescence measurements, background counts were subtracted, and the spectra were normalized by the reflected excitation light that “leaked” through the long pass filter. This partially corrected for the effect of optical properties at the excitation wavelength. Fluorescence values are reported as the magnitude of the normalized spectra in the green fluorescence band (495 to 530 nm) and the red fluorescence band (585 to 620 nm).

Histopathological evaluation

After optical measurements, each biopsy specimen was placed in separately labeled containers with formalin 10% solution. All specimens were sent for routine histopathology analysis. The individual performing the measurements was blinded to the histopathologic diagnosis, and the pathologist was blinded to the optical measurements.

Statistical analysis

The blood, scattering, and fluorescence parameters were

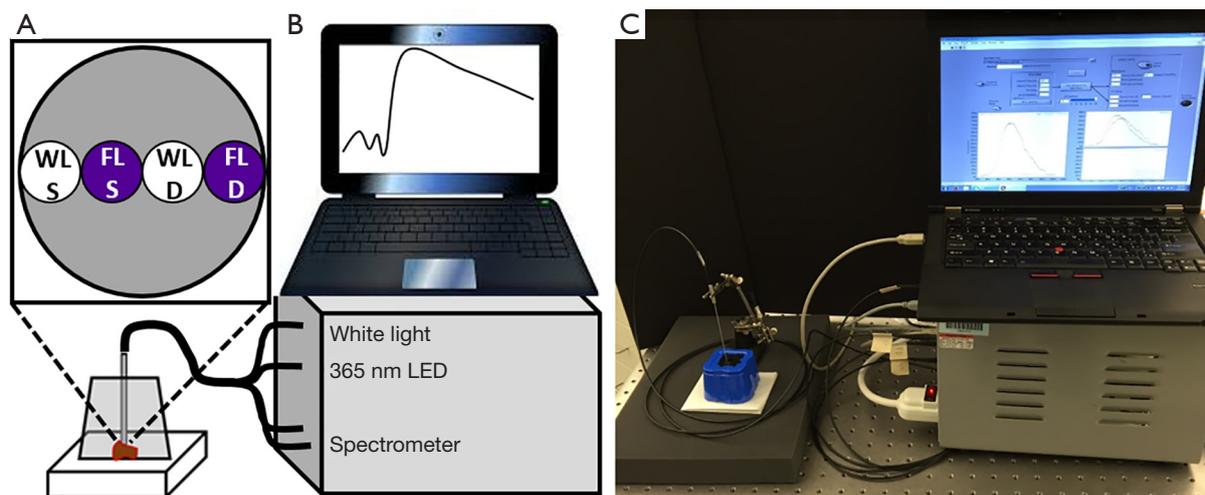


Figure 1 The clinical instrument. (A, B) Schematic of the system and (C) picture of the instrument.

Table 1 Significance assessed with linear mixed models

Parameter	Non-malignant	Malignant	Mean difference (95% CI)	P-value
BV (g/L)	46.69 (7.02)	39.02 (9.70)	7.67 (-22.60, 37.94)	0.49
Cdeoxy (g/L)	1.49 (0.29)	12.63 (5.80)	-11.13 (-16.74, -5.53)	0.01
Coxy (g/L)	45.19 (7.00)	26.39 (6.22)	18.80 (-10.54, 48.14)	0.27
StO ₂ (%)	92.76 (1.18)	79.77 (4.41)	12.99 (6.59, 19.39)	<0.001
a (1/cm)	14.41 (0.45)	13.25 (0.66)	1.16 (-0.78, 3.10)	0.11
b	0.29 (0.05)	0.75 (0.25)	-0.47 (-0.78, -0.01)	0.06
Green Ex (a.u.)	0.77 (0.09)	0.54 (0.13)	0.23 (-0.15, 0.61)	0.25
Red Ex (a.u.)	0.96 (0.18)	0.75 (0.34)	0.21 (-0.56, 0.99)	0.84

BV, Cdeoxy, Coxy and StO₂ are the blood parameters; a & b are the scattering parameters, green and red represent the fluorescence parameters.

reported by pathological diagnosis using the mean and standard error (Table 1). Comparisons were made using a linear mixed model, which accounts for the multiple observations per patient. Model assumptions were verified graphically and Box-Cox transformations were applied as appropriate.

To determine the utility of optical imaging parameters for cancer classification, the four non-cancer groups (normal, inflammation, blood clot and necrotic debris) were combined and compared against the cancer group. A secondary analysis was performed for the cancer versus necrotic debris groups. Multiple parameters were combined into a single classifier using generalized estimating equations (GEE) logistic regression; where only combinations of three parameters (one blood, scattering and fluorescence) were tested to avoid overfitting. Receiver

operating characteristic (ROC) curves and corresponding area under the curve (AUC) results were obtained for each parameter with respect to cancer *vs.* non-cancer and cancer *vs.* necrotic. The sensitivity and specificity were determined at the Youden Index and the positive predictive value (PPV) and negative predictive value (NPV) were determined using the group sizes to estimate prevalence.

All analyses were conducted in SAS v 9.4 (Cary, NC) at a significance level of 0.05; therefore a P-value less than 0.05 is considered statistically significant. As a pilot study, no adjustments were made for multiple testing.

Results

Sixteen patients were enrolled. One patient was excluded

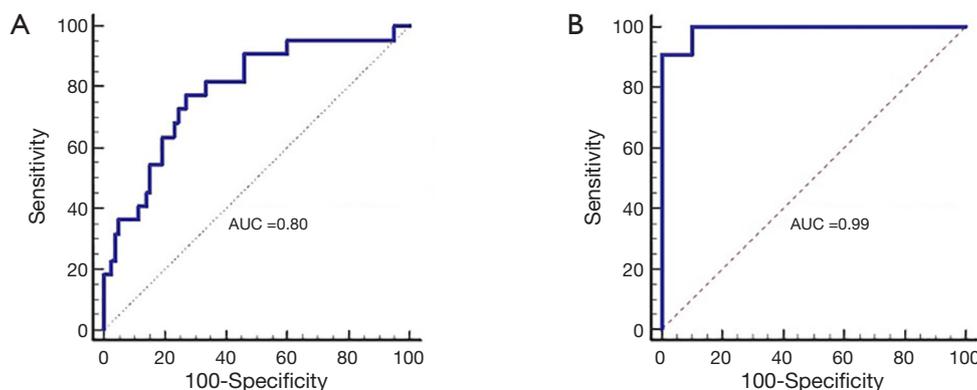


Figure 2 ROC curves and corresponding AUC obtained from the logistic regression model based on the combination of blood, scattering and fluorescence parameters (Cdeoxy, b, and red signal respectively). (A) shows the ROC curve for classifying cancer samples *vs.* benign and (B) shows the ROC curve for cancer *vs.* necrotic samples. ROC, receiver operating characteristic; AUC, area under the curve.

after obtaining the diagnosis from a hilar lymph node and no transbronchial biopsies were performed. There was a total of $n=116$ unique biopsy specimens with a confirmed pathologic diagnosis of 15 patients [20% male and an average age of 64.2 (SD =9.9; range 43–82)]. Twenty-two of the 116 specimens were malignant, and 10 of the 94 non-malignant specimens were necrotic biopsies. Fluorescence measurements were not acquired for 16 non-malignant biopsies. The necrotic specimens were all from one patient. Seventeen of the 22 malignant specimens were lung cancers and 5 were colon cancers. The benign specimens were reported as acute or chronic inflammatory, fibrotic or cartilage, blood clots, bronchial or parenchymal tissues.

The blood parameters Cdeoxy (concentration of deoxygenated hemoglobin) and StO₂ (saturation of oxygen) were found significant in differentiating malignant from benign specimens. The malignant specimens had higher Cdeoxy and lower StO₂ compared to benign specimens (12.63 *vs.* 1.49 g/L and 79.7% *vs.* 92.76%, respectively). All other imaging parameters were not statistically significant (Table 1).

The best combination, based on the AUC, of three parameters for classifying malignant *vs.* benign and malignant *vs.* necrotic was the concentration of deoxy-hemoglobin, the scattering parameter b and the red fluorescence intensity. The sensitivity and specificity for differentiating between malignant and benign specimens was 77.3% and 73.1%, respectively with an AUC of 0.80 (Figure 2A). The resulting positive and NPVs were 44.7% and 91.9%, respectively with 17 true positives, 21 false positives, 57 true negatives and 5 false negatives. The sensitivity and specificity for differentiate between

malignant and necrotic specimens were 90.9% and 100.0%, respectively (Figure 2B) with an AUC of 0.99. The resulting positive and NPVs were 100.0% and 83.3%, respectively with 20 true positives, 0 false positives, 10 true negatives and 2 false negatives.

Discussion

Our study demonstrates that the combination of DRS and DFS exhibited good sensitivity and specificity for on-site discrimination between malignant and benign TBLB specimens during the bronchoscopy procedures. It also showed high sensitivity and specificity in discriminating between necrotic and malignant TBLB specimens.

This is a pilot study, and the primary aim was to evaluate the feasibility of this technique. The high sensitivity of the described imaging modality to discriminate between malignant and benign is of significant importance. It means that the proceduralist can have a high degree of confidence that the TBLB specimen is malignant and may, therefore, decrease the number of biopsies needed and potentially lowering the complication rate associated with lung biopsies such as bleeding and pneumothorax. Our study showed that optical spectroscopy measurements of TBLB were highly sensitive and specific for differentiating between necrotic and malignant specimens but given that all necrotic specimens were from a single patient, these findings are secondary and need further investigation in larger trials. This is essential as many tumors are necrotic and the proceduralist may face a situation where the tumor is appropriately sampled, but the final histopathologic examination is mostly necrotic and therefore nondiagnostic.

Our imaging technique would allow the proceduralist to change or adjust the location from where the TBLB are obtained and therefore manipulate the biopsy forceps to get TBLB specimen from a more malignant rather than necrotic area of the targeted lesion. Given that our main objective was to evaluate the feasibility of the optical spectroscopy for the diagnosis of TBLB, a larger prospective study is warranted to evaluate the effectiveness of this technique and to identify factors associated with outcome such as the size of specimens and the ratio of tumor cell to non-tumor cells in the specimens.

The optical measurements were performed directly after obtaining the specimens from transbronchial biopsies and before fixing the specimens in formalin. This is advantageous as it offers the rapid on-site evaluation of the specimens obtained and immediate perspective regarding the direction of the diagnosis. Optical spectroscopy has been shown to be effective in characterizing cancer tissue in oral cavity and lung (3-5,7,8,10,11). In a recent study by Spliethoff *et al.*, the lung tissues obtained from lobectomy or segmental lung resections from 13 patients were imaged using optical spectroscopy. The tumor size ranged between 8 and 50 mm. DRS and DFS were highly successful in discriminating between malignant and normal lung tissue with sensitivity and specificity of 98% and 86% respectively, and between necrotic and non-necrotic tumor with sensitivity and specificity of 91% (3). In a recent study per Spliethoff *et al.*, transthoracic application of DRS was used in 21 patients intraoperatively during surgery for lung resection and in 11 patients undergoing routine transthoracic lung biopsy for peripheral lung lesions. DRS was able to differentiate between malignant and surrounding tissue with high sensitivity and specificity. The positive findings were attributed to the water content DRS parameters and not the blood parameters.

As Tromberg group and others indicated tissue hemoglobin oxygen saturation levels (StO_2) at the tumor are lower than those in surrounding normal areas, due to local metabolic activity: tumor cells are more metabolically active and consumes more oxygen, thus overall StO_2 levels are lower in tumor tissue, compared to surrounding normal tissue (15,16). Deoxy-hemoglobin (Cdeoxy) may be a more sensitive index of oxygen consumption and local metabolism. If the oxygen is consumed in tumor, then the deoxy-hemoglobin levels increases. Thus, we can say that while oxy-hemoglobin changes can be attributed primarily to vascular oxygen supply, deoxy-hemoglobin concentration is representative of tumor tissue oxygen consumption. As

tumor cells undergo apoptosis and reduce proliferation, oxygen consumption may diminish.

On-site discrimination between malignant and benign biopsies remains a significant challenge for bronchoscopists. The outcome of transbronchial biopsies is reported as a diagnostic or non-diagnostic specimen, or diagnostic but not sufficient specimen for further analysis such, Immunohistochemistry (IHC), molecular or genetic testing. Rapid-on-site evaluation (ROSE) can be used for TBNA and transbronchial brush for intraoperative cytological assessment. However, ROSE is not universally available and does not allow for histologic evaluation of the TBLB (17). Squash smear cytology has been shown to be helpful when the TBLB specimens are very small or in cases where only a few specimens are available (18). This method has not been used for normal-size TBLB specimens (2). Frozen section of the TBLB specimens is not a common practice, and this is due to the high cost, limited availability, and the inability of using the frozen specimen for further specimen identification using pathology stains, molecular testing or others (2,19). Coghlin *et al.* demonstrated that specimens from bronchial biopsies frequently contain limited amounts of malignant cells, and in only 48 % of cases, tumor was found in all biopsy specimens (20). Furthermore, the bronchoscopists may not be able to assess the quality of TBLB accurately. This was shown in a study by Curley *et al.* where the physician's rating of the specimens' quality did not vary between specimens containing normal or abnormal tissue or between diagnostic and nondiagnostic tissue (19). The efficacy of the optical spectroscopy approach needs to be assessed on a larger scale and with a wider range of lung pathology to establish it as a standard of care.

The *ex vivo* nature of the optical measurement of TBLB specimens in this study may be considered a limitation. However, the advantage of an *ex vivo* analysis of TBLB is the simplicity of the technique (outside this pilot study) and the no need for the proceduralist to learn a new technology that may require more training and advanced level of expertise. The future direction will be to investigate the *in vivo* optical imaging by building a catheter that can be used bronchoscopically to be directed to the peripheral lung lesions. The *in vivo* optical measurement may offer more information about the hemodynamic characteristics of the targeted lesion before obtaining the TBLB and therefore may effectively guide the proceduralist. The *in vivo* optical imaging can be challenging as blood during TBLB may alter the measurement and interpretation of the results. Additionally, the *in vivo* imaging will probably require a

significant training and advanced level of expertise, which could limit the popular use of such technique.

This study has some other limitations, most importantly the technical challenges in obtaining the optical measurements given that this is a pilot study and we were testing the feasibility of this technique. The optical measurements were performed manually and required an individual with expertise. Building an automated system to perform the optical measurements will overcome these technical challenges and will make optical measurements of TBLB specimens instant and simple with no need for specific training or assistance. Another limitation is that each measurement was point-based, simply providing the bulk property of each biopsy sample. In the future high-resolution imaging based system would allow maps of the samples and assess the heterogeneity in the contrasts.

Conclusions

Optical spectroscopy of the TBLB specimens is feasible, and moderately sensitive and specific in discriminating malignant from benign pathology and highly sensitive and specific for differentiating necrotic from tumor-containing specimens. This is a pilot study and larger prospective trials are needed to further evaluate the effectiveness of this technique for TBLB.

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Footnote

Conflicts of Interest: K Harris is a consultant for Cook Medical. The other authors have no conflicts of interest to declare.

Ethical Statement: The study obtained ethical approval from Roswell Park Cancer Institute Institutional Review Board (I-246913). All enrolled subjects gave informed consent before taking part in this study.

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