Real-time In Vivo Tissue Characterization with Diffuse Reflectance Spectroscopy during Transthoracic Lung Biopsy: A Clinical Feasibility Study

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Abstract

Purpose: This study presents the first in vivo real-time tissue characterization during image-guided percutaneous lung biopsies using diffuse reflectance spectroscopy (DRS) sensing at the tip of a biopsy needle with integrated optical fibers.

Experimental Design: Tissues from 21 consented patients undergoing lung cancer surgery were measured intraoperatively using the fiber-optic platform capable of assessing various physical tissue properties highly correlated to tissue architecture and composition. In addition, the method was tested for clinical use by performing DRS tissue sensing during 11 routine biopsy procedures in patients with suspected lung cancer.

Results: We found that water content and scattering amplitude are the primary discriminators for the transition from healthy lung tissue to tumor tissue and that the reliability of these parameters is not affected by the amount of blood at the needle tip. In the 21 patients measured intraoperatively, the water-to-scattering ratio yielded a 56% to 81% contrast difference between tumor and surrounding tissue. Analysis of the 11 image-guided lung biopsy procedures showed that the tissue diagnosis derived from DRS was diagnostically discriminant in each clinical case.

Conclusions: DRS tissue sensing integrated into a biopsy needle may be a powerful new tool for biopsy guidance that can be readily used in routine diagnostic lung biopsy procedures. This approach may not only help to increase the successful biopsy yield for histopathologic analysis, but may also allow specific sampling of vital tumor tissue for genetic profiling. Clin Cancer Res; 22(2); 1–9. ©2015 AACR.

See related commentary by Aerts, p. 273

Introduction

Image-guided transthoracic needle biopsy (TNB) of the lung is a well-established method used for the diagnosis of lung cancer. TNB is particularly useful for peripheral pulmonary lesions that are not readily accessible with bronchoscopy. With the introduction of lung cancer screening programs (1, 2) lung cancers will be detected at earlier stages and at smaller sizes. As sampling a small pulmonary lesion is often technically challenging (3), the ability to successfully biopsy these lesions depends greatly on the skill of the physician. During the procedure, multiple needle insertions should be avoided to reduce the possibility of complications, specifically pneumothorax or hemorrhage. To this end, TNB procedures are generally performed under fluoroscopic or computed tomographic guidance. Still, the pathologic area is often missed or undersampled, leading to diagnostic failure rates of TNB in up to 23% (3–6) of the cases. A considerable number of patients undergoing TNB will subsequently require an additional biopsy procedure, leading to extra patient discomfort, prolonged psychological burden, and additional risks or complications. Moreover, due to advances in genetic profiling and personalized medicine, obtaining representative tissue samples that allow for both histologic characterization and mutational analysis is becoming increasingly important (7, 8). Therefore, with advances in detection of increasingly smaller pulmonary lesions and recent developments in molecular profiling, there is a clear call for biopsy guidance to optimize tissue sample acquisition.

Here, we present a unique fiber-optic biopsy needle (FOBN) platform that uses diffuse reflectance spectroscopy (DRS) in conjunction with a conventional biopsy needle. DRS is a spectroscopic technique in which tissue is illuminated with a selected spectral band of light. The light is either scattered or absorbed by the tissue, depending on the specific composition of the tissue. Subsequent analysis of the tissue’s spectral response provides specific quantitative morphologic, biochemical, and functional information, thereby enabling tissue discrimination and potentially improving diagnostic capability. Several preclinical and clinical studies have demonstrated the
Translational Relevance

Advances in molecular biology are improving the understanding of lung cancer and directing clinical decision making. Consequently, highly representative tissue samples for histologic characterization and mutation analysis are increasingly important. Although diagnostic needle biopsy is widely used, this procedure suffers from significant nondiagnostic sampling rates, especially when small masses are targeted. Here, we present an innovative technology platform for spectral tissue sensing at the tip of a biopsy needle. By providing the radiologist with real-time needle guidance, the diagnostic performance and the quality of tumor biopsies could significantly be enhanced.

Furthermore, it enables the quantification of water content, which is an important measure for lung tissue density (19, 21, 22). The general principles of DRS, the operating features of the spectroscopy system, and the calibration procedure have been described elaborately by Nachabe and colleagues (21, 22). The system consists of a Tungsten halogen broadband light source (360–2,500 nm) with an embedded shutter, a miniaturized optical probe, and two spectrometers: one which resolves the light in the visible wavelength range, i.e., 400 up to 1,100 nm (Andor Technology, DU420ABR-DD) and one which resolves near infrared light from 900 up to 1,700 nm (Andor Technology, DU492A-1.7). The spectroscopy needle was connected to the light source and to both spectrometers via low-OH optical fibers. The spectrometers are controlled by a custom LabView software (National Instruments) to acquire and calibrate the data.

Materials and Methods

Clinical studies

Protocols for the human studies were reviewed and approved by the Medical Review Ethics Committee of the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital (Amsterdam, the Netherlands). The clinical protocols were registered at the Dutch Trial Register (NTR2557; NTR3651) and the U.S. National Institutes of Health Clinical Trial Database (NCT01730365). All patients gave their written informed consent prior to the experimental procedures. All clinical experiments were conducted in the NKI-AVL hospital.

Needles with sensing capabilities

Needle for intraoperative use. A disposable 15G spectroscopy needle (Invivo Germany) was developed for practical use during surgery. The needle contained three fibers, each with a core diameter of 200 μm. One fiber was connected to the light source, while the other two fibers were connected to the spectrometers to capture the diffusely reflected light from the tissue. The center-to-center distance between the emitting and collecting fibers was 1.70 mm.

Fiber-optic biopsy needle. Adding DRS tissue-sensing functionality to an automated biopsy gun is challenging, as fast shooting mechanisms and the presence of a notch set strict constraints for integrating the optical fibers within the device. For the measurements during TNB procedures, a sterile single use fully automated 16G FOBN needle was developed (Invivo Germany) to take soft-tissue biopsies under image guidance. An essential feature of this clinical-grade instrument is that it allows tissue sampling from the exact location where the final tissue sensing took place without restricting the usability of the biopsy gun (Fig. 1). A 100-μm diameter fiber was used for light delivery, whereas two 200-μm diameter fibers were used for the collection of the reflected light. The distance between the emitting and collecting fibers at the needle tip was 1.36 mm, resulting in a tissue probing depth of approximately 1 to 2 mm.

Study procedures

Intraoperative data acquisition. The portable DRS system was installed in a general surgery operating room. DRS measurements were performed in 21 patients undergoing partial lung resection. DRS measurements were performed after deflation of the lung, but before any tissue dissection or ligation of major blood vessels. A sterile single use fiber-optic needle was inserted into the tissue that was planned for resection, using a 14G hollow guidance cannula (Invivo Germany). For each patient, two sets of 5 to 10 DRS spectra were recorded in healthy lung tissue and tumor tissue. Measurement sites were marked with twist coil markers (OTM 3.0SA, Biomed.-Instrumente & Produkte Gmbh) that were placed through the guidance cannula. Within 10 minutes after resection, the tissue was measured ex vivo with the same setup to allow for the comparison of in vivo and ex vivo spectra. The resected tissue was processed by the
A pathologist and tissue samples were taken from the measurement locations, as indicated by the twist markers. The samples underwent detailed histopathologic analysis and findings were compared with the results of the DRS spectral analysis.

Image-guided biopsy with FOBN. The FOBN was tested in a computed tomography (CT) intervention room during 11 routine transthoracic needle biopsy procedures for individuals with a suspicious pulmonary lesion. All patients underwent a free-breathing CT-scan (16-slice Somatom Sensation Open, Siemens) as part of the standard procedure planning. The FOBN was inserted at the planned entry point and DRS measurements were performed along the needle tract, followed by DRS measurements and biopsy of the target lesion using the same needle. CT fluoroscopy imaging was recorded and coregistered with the DRS spectra. For each patient, sets of 3 to 5 reflectance spectra were acquired from healthy lung tissue, tissue at the tumor border, and tumor tissue. The radiologist was blinded to the DRS system output. Directly after acquisition of the final DRS data, a tissue sample was taken from the target lesion using the FOBN. The distal end of the tissue samples was marked with yellow tissue marking dye (Poly/sciences Inc.) for orientation purposes. The samples were formalin-fixed and processed according to routine histopathology. Pathology results were compared with the DRS data at the final measurement position.

Histology processing and analysis

Tissue samples were processed via standard histologic procedures. After paraffin embedding, the samples were sectioned and stained with standard hematoxylin and eosin dye (Merck; HE). The resulting tissue slices were examined with a light microscope by an experienced pathologist who was blinded to the spectroscopic analysis. The glass slides were digitized by a histologic slide scanner (ScanScope - Aperio Technologies Inc.).

Spectral data analysis

DRS measurements were spectrally fitted with an analytical model by Farrell and colleagues (23) that is derived from the diffusion theory using a Levenberg–Marquardt nonlinear inversion algorithm to determine the absorption coefficient $\mu_a(\lambda)$ and the reduced scattering coefficient $\mu_s'(\lambda)$ expressed in cm$^{-1}$. The validation of the model, including spectral calibration procedures, and its application in various preclinical studies were described in detail elsewhere (12, 13), (19, 20). The model uses prior knowledge of light-tissue interaction to translate the acquired spectra into estimates of various absorption and scattering parameters, such as biologic volume fractions (e.g., blood, water), oxygenation level of blood and light scattering related to cell density, cell size, or air. Confidence intervals of the estimated parameters derived from the covariance matrix were calculated to investigate the reliability of measurements fits (22).
To verify the performance of the FOBN per individual, we extracted the information provided by the FOBN along each needle path and used each patient’s healthy tissue as an internal reference. This was done by calculating an optical contrast index (OCI). The OCI was defined as the relative difference in the water-to-scattering ratio between tumor and lung tissue within the same individual. Similar composite optical parameters have been applied by other research groups (9, 24, 25). The OCI was calculated using the simple formula:

$$\text{OCI} = \frac{[\text{water} / \mu'(800)]_{\text{tumor}}}{[\text{water} / \mu'(800)]_{\text{normal}}} - \frac{[\text{water} / \mu'(800)]_{\text{normal}}}{[\text{water} / \mu'(800)]_{\text{normal}}}$$

where $[\text{water} / \mu'(800)]_{\text{tumor}}$ and $[\text{water} / \mu'(800)]_{\text{normal}}$ correspond to the average water-to-scattering ratio measured in tumor tissue and surrounding normal lung tissue, respectively.

Statistical analysis

Tissue parameters determined from DRS spectral measurements [blood, $\text{stO}_2$, water, $\mu'(800)$] were compared between tumor tissue and normal tissue using a generalized estimating equations (GEE) approach with controlling for repeated measurements within the same subject. These DRS parameters were assumed to be normally distributed. Within-patient dependencies were represented by the correlation matrix where all pairwise correlations were assumed to be equal (equicorrelated). The analyses were performed using the GEEQBOX toolbox in Matlab 8.4 (MathWorks Inc.) and $P$ values $< 0.01$ were considered statistically significant.

Results

Robust tissue discrimination in vivo

The tissue sensing performance of the system was investigated in vivo during lung cancer surgery, where the presence of blood plays a substantial role. Twenty-one patients were included, median age was 62.8 years (range, 38.6–78.8 years). A total of 407 and 341 DRS spectra were acquired in vivo and ex vivo, respectively.

Measurements in tumor tissue and surrounding lung tissue revealed clear differences in absorption (Fig. 2A and C) and scattering coefficients (Fig. 2B and D), indicating inherent differences in tissue structure and composition. The most noticeable differences in the absorption spectrum were observed for the oxyhemoglobin and deoxyhemoglobin absorption bands in the 540 to 580 nm wavelength region and the water absorption peak near 1,450 nm (Fig. 2C).

The spectral fitting model was used to derive various tissue parameters from each DRS spectrum, including biologic volume fractions (e.g., blood content, water content), oxygenation level of blood ($\text{stO}_2$), and the reduced scattering coefficient at 800 nm [$\mu'(800)$]. For each of these parameters, confidence intervals were computed. We found that extending the wavelength range up to 1,600 nm effectively narrowed the confidence intervals for all four parameters, thereby improving the reliability of the obtained values (Fig. 3A). When comparing the data acquired intraoperatively and postoperatively, our system detected consistent differences (on average $>16\%$) in water content (Fig. 3B) and $\mu'(800)$ (Fig. 3C) between tumor tissue and surrounding healthy lung tissue. The average estimated blood content (Fig. 3D) that was encountered during surgery (on average $>16\%$) was much higher than typical physiologic values for intact alveolar lung tissue (26), indicating a considerable blood contamination effect. Consequently, estimates for blood content and associated oxygenation levels (Fig. 2E) do not necessarily reflect the true physiologic composition of the measured tissue, thus rendering them less suitable for tissue discrimination in the current clinical setting.

As stated earlier, the difference in our DRS system and those presented in previous studies is that it extracts diagnostic
information from the near-infrared spectral range, where blood has no significant absorption features. When comparing the data acquired during surgery with the data obtained directly after surgery, we note that there are marked differences in blood content (Fig. 3D). However, calculated average values for water content and $\mu_s'(800)$ are comparable for both datasets (Fig. 3B and C). This supports the notion that estimates for water content and $\mu_s'(800)$ are not compromised by blood absorption. Further

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**Figure 3.**
DRS parameter quantification. Comparison of confidence intervals obtained for blood, stO$_2$, water and $\mu_s'(800)$ when model fit is applied from 450 to 1,100 nm and from 450 to 1,600 nm (A). Note that extending the wavelength range up to 1,600 nm narrows the confidence intervals of each parameter. Bar graphs showing the values for water (B), $\mu_s'(800)$ (C), blood (D), stO$_2$ (E), as measured during (in vivo; $n = 407$ spectra) and after (ex vivo; $n = 341$ spectra) surgery. N, healthy lung tissue; T, tumor tissue. Values are given as the mean $\pm$ SE, adjusted for repeated measurements. * $P < 0.01$.

**Figure 4.**
Robust tissue characterization using water and $\mu_s'(800)$. Confidence intervals for water (A) and $\mu_s'(800)$ (B) remain stable with increasing blood content. Quantification of the water-to-scattering ratio (water/$\mu_s'$ (800)) showing a significant differences between healthy lung tissue (N) and tumor tissue (T), both during (in vivo) and after (ex vivo) surgery (C). Values are given as the mean $\pm$ SE, adjusted for repeated measurements. * $P < 0.01$. 

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Biopsy Guidance by Diffuse Reflectance Spectroscopy

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evidence of the limited influence of blood contamination comes from the fact that increasing amounts of blood do not lead to an increase in errors in water content (Fig. 4A) and μs′(800) (Fig. 4B), meaning that the reliability of these parameters is essentially unaffected by blood content.

The higher water content that was measured in tumor tissue is attributed to the rather high density of tumor tissue compared to aerated healthy lung tissue. Similarly, the relatively high μs′(800) values measured in healthy lung tissue may be the result of scattering due to the large difference in the refractive index between air and lung tissue. Having shown that water content and μs′(800) contain diagnostically useful information, we combined both parameters by calculating the water-to-scattering ratio [water/μs′(800)]. This yielded an average 81% and 56% contrast difference between tumor and surrounding tissue for the ex vivo and in vivo data, respectively (Fig. 4C).

Clinical performance of fiber-optic biopsy needle

Added real-time spectral measurements did not interfere with the standard biopsy procedure. The median procedure time including spectral measurements and tissue sampling was less than 5 minutes (range, 1.5–16.4; Table 1). Figure 5 illustrates how real-time DRS tissue characterization was performed using the FOBN. A total of 134 DRS spectra were acquired during 11 TNB procedures (patient median age, 67.2 years; range, 47.3–80.5). Histopathologic examination of the targeted tissue revealed a total of 10 malignancies, of which 9 were classified as non–small cell lung carcinoma (NSCLC) and one as colorectal metastasis. One tissue sample was nondiagnostic.

Two observations in particular are important with regard to the clinical relevance of our approach. First, comparison of the DRS OCI values with the biopsy reports showed that in 10 out of 11 patients, tumor tissue could be correctly identified based...
on an increase in the OCI. Second, one of the subjects (subject 3 in Table 1 and Fig. 6) underwent a biopsy of an 18 mm lesion located close to the diaphragm. Positioning of the FOBN was challenging due to respiratory movement, but fluoroscopic imaging suggested that the tumor was sampled correctly. Pathologic analysis of the tissue biopsy taken at that specific location revealed nondiagnostic material. Subsequent repeated biopsy demonstrated moderately differentiated adenocarcinoma. When evaluating the DRS measurements of the first TNB procedure, the OCI suggested that at the moment of the biopsy the FOBN was not in contact with the tumor tissue. If this feedback would have been used, a corrective manipulation of the needle could have increased the chance of an adequate biopsy during the first procedure. These results show that the OCI matched with final pathology in all 11 clinical cases.

Discussion

The DRS-FOBN is an advancement for several reasons: (i) it adds tissue scoring functionality to a biopsy needle tip while retaining all biopsy capabilities; (ii) it operates in real-time and provides diagnostic information to the physician, thereby enabling more accurate needle positioning; (iii) it can be used in conjunction with conventional imaging modalities, such as (CT) fluoroscopy or ultrasound, exploiting the complementary strengths of each method; and (iv) it can be translated into routine clinical use of biopsy procedures. With these attributes, DRS-FOBN offers an integrated solution for spectroscopic biopsy guidance. Tissue sensing and biopsy capabilities are conveniently linked in a manner that provides optimal conditions for both reliable tissue identification (DRS data acquisition in a wide spectral range) and adequate tissue sampling (the full volume of the notch is available for securing the sample).

A key feature of our system is its ability to perform reliable estimation of diagnostically relevant tissue parameters, regardless of the amount of blood that is encountered. Our system provides quantitative information that corresponds well with differences in tissue structure and composition.

We found that water content and scattering are the primary discriminators for the transition from healthy lung tissue to tumor tissue. These findings are consistent with previous preclinical studies (19, 20). Using the outlined technology, we demonstrated the clinical feasibility in 11 routine lung biopsy procedures. In this limited series, the identified DRS optical contrast, when matched against histology, could be used to assess needle positioning in each clinical case.

Primary lung tumors frequently occur in patients with chronic obstructive pulmonary diseases (COPD). The relationship between these diseases is based upon smoking as a mutual risk factor. In COPD like emphysema, normal lung tissue architecture is affected, accompanied by the destruction of alveolar walls. In the current study, 9 of the 10 patients with a primary lung tumor were smokers. As healthy lung tissue mainly consists of air-filled alveoli and optical contrast between tumor and surrounding tissue is basically based on tissue density, we do not anticipate any problems in applying the method to non-COPD patients. However, additional research is needed for evaluating effects of (severe) emphysema on optical parameters.

During transthoracic biopsy, breath holding instructions are important, especially during biopsy of lung lesions closer to diaphragm due to respiratory motion. One of the main advantages of the proposed methodology lies in its speed of tissue characterization by spectroscopic analysis. To enable the radiologist to take a tissue sample at the right moment, based on the changes in the derived DRS parameter, spectral data acquisition and processing should be performed (near) real time. The current prototype system and software settings were optimized for acquisition of high quality DRS research data and spectra were acquired with subsecond acquisition times (300–700 ms). Simple adjustments to the system configuration...
would enable almost immediate feedback on the tissue at the needle tip. Standard-of-care procedures, even when carefully performed by highly skilled and experienced radiologists, can miss small pulmonary lesions due to location, size, and respiratory motion during the biopsy procedure. Accurate real-time tissue identification during biopsy procedures, as described in this study, can shift the paradigm of diagnostic biopsies by enabling accurate tissue sampling of lesions that are difficult or impossible to sample by fluoroscopic imaging alone. This may not only help to increase the biopsy yield for histopathologic analysis, but may also allow specific sampling of vital tumor tissue when needed, such as for genetic profiling for tailored treatment (personalized medicine; refs. 7, 8).

We anticipate that DRS-based biopsy guidance, as described in this study, is not limited to lung cancer, but may also be used for breast (9–12), liver (13), and other cancer types. Larger multicenter studies are needed to confirm our data and further elucidate the diagnostic value (sensitivity and specificity) of the reported method for lung cancer and other tumors.

We conclude that real-time spectroscopic guidance is an important new step to optimize the diagnostic performance and the quality of biopsy procedures in clinical practice. The presented technology creates a basis for the design and clinical implementation of integrated fiber-optic tools for a variety of minimal invasive applications.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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