Original Study

Diffuse Reflectance Spectroscopy: A New Guidance Tool for Improvement of Biopsy Procedures in Lung Malignancies

Daniel J. Evers,[1] Rami Nachabé,[2] Houke M. Klomp,[1] Johanna W. van Sandick,[1] Michel W. Wouters,[1] Gerald W. Lucassen,[2] Benno H.W. Hendriks,[2] Jelle Wesseling,[3] Theo J.M. Ruers[1,4]

Abstract

Procedural accuracy of percutaneous intrathoracic biopsies can be improved with diffuse reflectance spectroscopy (DRS). Normal and malignant lung tissue from 10 patients after resection was examined with a DRS system. Maximum sensitivity for discrimination between normal and malignant tissue was 89%, and specificity was 86%. DRS can enhance diagnostic accuracy in biopsy procedures in the lungs in combination with conventional imaging techniques.

Background: A significant number of percutaneous intrathoracic biopsy procedures result in indeterminate cytologic or histologic diagnosis in clinical practice. Diffuse reflectance spectroscopy (DRS) is an optical technique that can distinguish different tissue types on a microscopic level. DRS may improve needle localization accuracy during biopsy procedures. The objective of this study was to assess the ability of DRS to enhance diagnosis of malignant disease in human lung tissue. **Methods:** Ex vivo analysis with a DRS system was performed on lung tissue from 10 patients after pulmonary resection for malignant disease. Tissue spectra measured from 500 to 1600 nm were analyzed using 2 analysis methods; a model-based analysis that derives clinical and optical properties from the measurements and a partial least-squares discriminant analysis (PLS-DA) that classifies measured spectra with respect to the histologic nature of the measured tissue. **Results:** Sensitivity and specificity for discrimination of tumor from normal lung tissue were 89% and 79%, respectively, based on the model-based analysis. Overall accuracy was 84%. The PLS-DA analysis yielded a sensitivity of 78%, a specificity of 86%, and an overall accuracy of 81%. **Conclusions:** The presented results demonstrate that DRS has the potential to enhance diagnostic accuracy in minimally invasive biopsy procedures in the lungs in combination with conventional imaging techniques.

Clinical Lung Cancer, Vol. 13, No. 6, 424-31
Keywords: Biopsy, Cancer, Diagnosis, Diffuse reflectance spectroscopy, Lung

Introduction

Essential first steps in the diagnostic work-up after detection of a suspected lung mass include describing the anatomic extent as well as the cellular origin of the tumor. Biopsy or fine needle aspiration of the lesion for further analysis is a crucial step in this process. For intrathoracic lesions, this is often performed percutaneously. Correct localization of the biopsy needle within the target lesion is essential for success of this procedure and is frequently performed with image guidance using computed tomography (CT).

Recent studies have reported varying figures of overall accuracy for thoracic biopsies, ranging between 67% and 96%.[1-5] The main factors influencing biopsy accuracy are location and size of the intrathoracic lesions as well as respiratory motion during the biopsy procedure. Moreover, even correct localization of the biopsy needle within the target lesion can still result in indefinite pathologic diagnosis when the biopsy consists only of necrotic cell debris. Hence a considerable number of

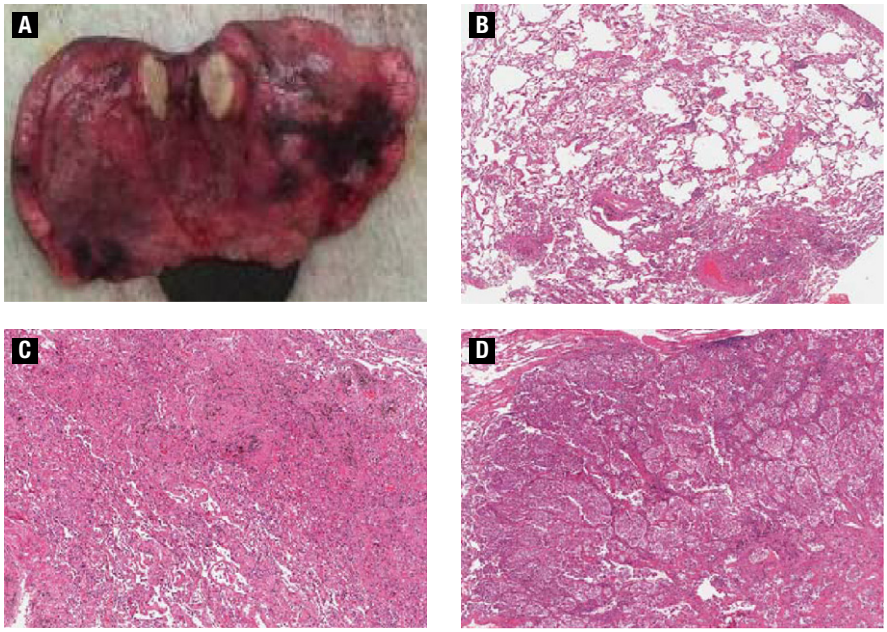
[1]Department of Surgery, the Netherlands Cancer Institute, Amsterdam, the Netherlands
[2]Minimally Invasive Healthcare, Philips Research, Eindhoven, the Netherlands
[3]Department of Pathology, the Netherlands Cancer Institute, Amsterdam, the Netherlands
[4]Technical University Twente, MIRA Institute, Enschede, the Netherlands

Submitted: Oct 5, 2011; Revised: Jan 9, 2012; Accepted: Feb 20, 2012; Epub: Apr 24, 2012

Address for correspondence: Daniel J. Evers, MD, Department of Surgery, 1066 CX, Amsterdam, the Netherlands
Fax: 0031-205122554; e-mail contact: d.evers@nki.nl

1525-7304/$ - see frontmatter
doi: 10.1016/j.cllc.2012.02.001

Figure 1 (A) Photograph of a Resected Lung Sample Showing a Cut Through the Tumor. (B) An Example of a Pathology Slide of Normal Lung Tissue With Macroscopic Pink Appearance. (C) Macroscopic Darker Appearance Resulting From Collapsed Alveoli. (D) Lung Tumor Tissue



patients undergoing percutaneous biopsies will subsequently require a repeated biopsy or even surgical intervention to obtain tissue material for diagnosis before an individualized treatment plan can be initiated.

In recent years, promising achievements in specific tissue discrimination have been made in the field of diffuse reflectance spectroscopy (DRS), which may allow improved accuracy in cancer diagnostics.[6-8] With this optical technique, changes in the spectral distribution of light as a result of either absorption or scattering of light are recorded after the light has interacted with molecules in tissue. Subsequently, the collected spectral information is translated into morphologic and physiologic information. Changes in human tissue associated with malignant transformation include alterations in cellular composition, metabolic rate, vascularity, intravascular oxygenation, and tissue morphologic characteristics. DRS is sensitive to such changes in tissue, enabling discrimination between normal tissue and tumor. Ultimately, incorporation of this technology into biopsy needles may improve tip localization of the biopsy needle within the tissue compared with image-guided localization.

Many human tissue types have been subjected to optical spectroscopy with promising results for clinical application of this technique. Only a few studies involving optical spectroscopy have focused on the characterization of human lung tissue. Those published mainly involved the incorporation of DRS or fluorescence spectroscopy (FS) into bronchoscopy tools.[9-13] Detection of superficial abnormalities during bronchoscopy procedures was proved to be enhanced with use of spectroscopy techniques within this setting. Sensitivity of DRS and FS ranged between 70% and 86%, and specificity ranged between 68% and 82%.

Recently, we developed and validated a novel DRS system combining detection of the visual (VIS) and near-infrared (NIR) light spectrum.[14-17] In contrast to most previous studies with DRS that focus on the VIS part of the spectrum, we included the NIR (1000-1600 nm) spectrum, which enables one to determine accurately water and lipid content in tissue, as these 2 biological substances mainly absorb light of wavelengths $>$ 900 nm.[14]

The aim of this article is to assess the discrimination accuracy of our DRS system in normal lung tissue and tumor in an ex vivo analysis.

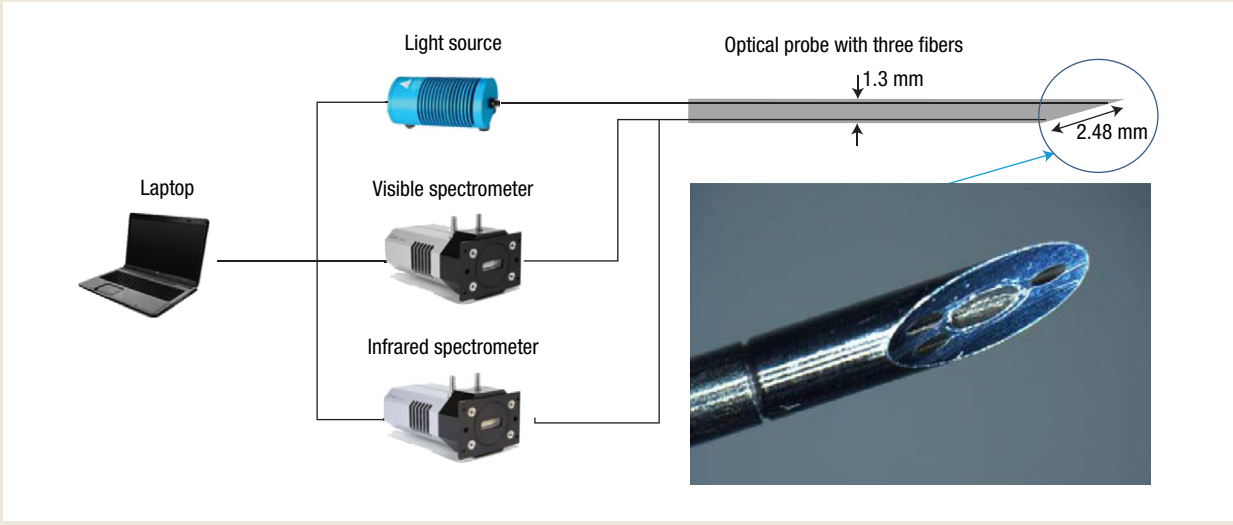
Materials and Methods

Clinical Study Design

This study was conducted at the Netherlands Cancer Institute (NKI-AVL) with approval of the internal review board committee. Lung tissue was obtained from 10 patients who had undergone pulmonary resection (lobectomy or segmental resection) for primary non–small-cell lung cancer or pulmonary metastases.

Directly after resection, tissue was transported to the pathology department for optical spectroscopy analysis. After gross inspection by the pathologist, the optical spectra were collected from macroscopic normal tissue and tumor samples. Spectroscopy measurements were performed on freshly excised tissue within 2 hours after resection. Each specific measurement location was digitally photographed during the procedure. Figure 1A depicts a photograph of a

Figure 2 Schematic of the DRS Optical Setup



resected lung sample with a cut through the tumor. A total of 330 optical measurements were performed on 67 tissue locations of both normal lung tissue and tumor. Resection specimens were then fixed in formalin. The measurement locations were subsequently selected and excised according to the measurement locations on the photographs. These tissue samples were embedded in paraffin, cut in 2- to 3-μm-thick sections and stained with standard hematoxylin/eosin. An experienced pathologist, who was blinded for the outcome of the spectroscopy analysis, examined the histologic slides.

Instrumentation

The instrumentation and calibration procedure of our optical spectroscopy model have been described recently by Nachabé et al.[14-17]

In short, ex vivo diffuse reflectance spectra were measured with a portable spectroscopic system as described earlier.[17] The system consists of a console comprising a tungsten/halogen broadband light source and 2 spectrometers. The spectrometers resolve light either between 400 and 1100 nm (DU420A-BRDD, Andor Technology, Belfast, Northern Ireland) or from 800 to 1700 nm (DU492A-1.7, Andor Technology). An optical probe containing 3 optical fibers is connected to the optical setup. As depicted in Figure 2, 1 fiber is connected to the light source and the other 2 fibers are connected to the spectrometers to collect diffusely scattered light from the tissue. The optical probe has a diameter of 1.3 mm. The illumination optical fiber is located at a distance of 2.48 mm from the 2 side-by-side optical fibers that are used to collect the diffused light. Such a setup enables spectral acquisition in the range between 500 and 1600 nm by an optical fiber with its distal end placed against the samples.

Light-Tissue Interaction and Optical Spectroscopy

The light delivered by the illumination optical fiber is subject to optical absorption and scattering. Each biological substance in the probed tissue has its intrinsic optical absorption property as a function of wavelength. The most common biological substances that absorb light are blood-derived chromophores such as oxygenated and deoxygenated hemoglobin, water, and lipid.[14] Oxygenated and deoxygenated hemoglobin have the most dominant absorption coefficients in the wavelength range < 900 nm, whereas water and lipid have the most dominant absorption coefficient > 900 nm.[14] Each of these chromophores has a well-determined optical absorption spectrum, which is available in the literature.[14] The total absorption coefficient corresponds to the sum of each of these chromophore-specific absorption coefficients weighted by the respective volume fraction that it occupies within the total probed volume. In addition to absorption, light is also subject to optical scattering in tissue because of its morphologic irregularities at a structural level yielding deflection of the light rays after interaction with the different substances present in tissue. The optical scattering is defined by a reduced scattering amplitude at an arbitrarily given wavelength (eg, at 800 nm) and a slope. The diffused light that is collected at the detection optical fibers corresponds to a nonlinear mathematical relation of the wavelength-dependent absorption and scattering properties.[18] The volume of the probed diffused light in tissue is mainly dependent on the absorption and scattering properties as well as on the distance between the illumination and collection fibers. Given the specification of the optical probe that was used in this study and the range of tissue absorption and scattering properties over the wavelength range of interest (ie, 500-1600 nm), the average probed volume is roughly 5 mm^3.

Spectral Data Processing

Two different lung tissue types were classified in the spectral data processing: normal lung tissue and tumor. Furthermore, measured spectra from all included measurement locations were separated into either the *training* data set (n = 171 optical measurements from 35 tissue locations) or the *validation* data set (n = 159 optical measurements from 32 tissue locations). This was accomplished by randomly dividing measurement sets from different tissue locations of both normal lung tissue and tumor collected from each patient between

Table 1 Histologic Breakdown of Tissue Samples Used for Data Analysis (N = 10 Patients)

Measured Tissue Types	Optical Measurement Locations (Training + Validation Set) n = 67	Optical Measurements (Training + Validation Set) n = 330	Optical Measurement Locations (Validation Set) n = 32	Optical Measurements (Validation Set) n = 159
Normal Lung Tissue	30	145	14	66
Tumor	37	185	18	93

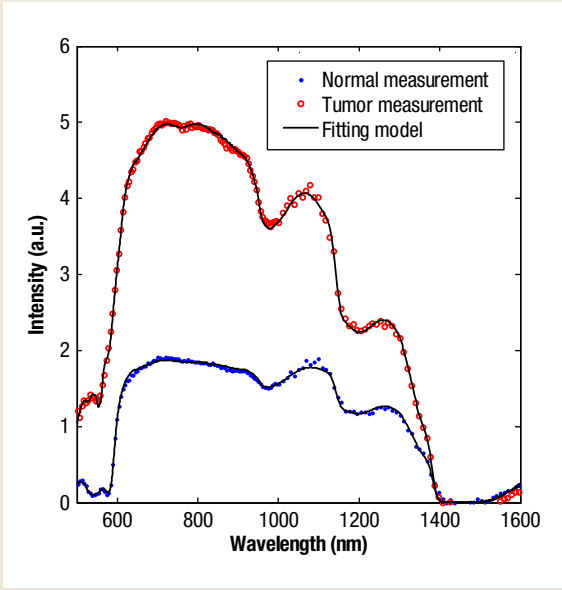
the 2 data sets. The histologic breakdown of the optical measurements performed in these patients is displayed in Table 1.

Finally, all acquired spectra were analyzed in 2 ways: first, an analytic model derived from the diffusion theory was used to estimate the various chromophore volume fractions and scattering coefficients.[18] Second, a statistical classification of the tissue spectra was performed using partial least-squares discriminant analysis (PLS-DA).[19]

Model-Based Analysis. Validation of the analytic model that was used to recover the chromophore volume fractions and scattering coefficients from the measurements has recently been described.[14-17] Diffuse reflectance spectra measured from the tissue were fitted over the wavelength range from 500 to 1600 nm. A nonlinear Levenberg-Marquardt inversion algorithm was used to estimate the various unknown chromophore volume fractions from the spectra within the analysis wavelength range. This inversion consists of determining the optimum volume fractions of the chromophores of interest as well as the reduced scattering amplitude (which we arbitrarily defined at 800 nm) and slope, which best minimizes the residual between the model and the measurement.[15] A total blood volume fraction is computed as the sum of the estimated oxygenated and deoxygenated hemoglobin volume fraction by considering a total hemoglobin concentration of 150 mg/mL of blood; oxygenation level in tissue is computed as the ratio of oxygenated hemoglobin to the total blood volume fraction. The other parameters related to absorption are the water volume fraction and adipose tissue volume fraction. The absorption coefficient of each of these chromophores in its pure state is used as a priori knowledge during the fitting procedure. An example of a spectral measurement on a normal lung sample and a tumor sample with the corresponding fitting curve are shown in Figure 3. The spectral characteristics analysis was performed with MATLAB software package (MathWorks Inc, Natick, MA). Quantified mean values for tissue parameters were calculated based on all tissue measurements and were displayed in box plots.

Subsequently, we used the data from the *training* data set to design a decision tree for automated discrimination between normal lung tissue and tumor. This was performed using *Gini index maximization* and has recently been described by Nachabé et al.[17] By applying this evaluation method, thresholds of the most significantly discriminating tissue parameters were determined from which all included tissue measurements could be differentiated into either tissue class with the least number of evaluation steps. The calculated thresholds were depicted as a decision tree.

Figure 3 Example of Spectral Measurements in Normal Lung Tissue With Macroscopic Pink Appearance (See Figure 1B) *(Blue or Point-Marked Curve)* and Tumor Tissue *(Red or Circle-Marked Curve)* and Corresponding Fits *(Black or Solid Line Curves)*

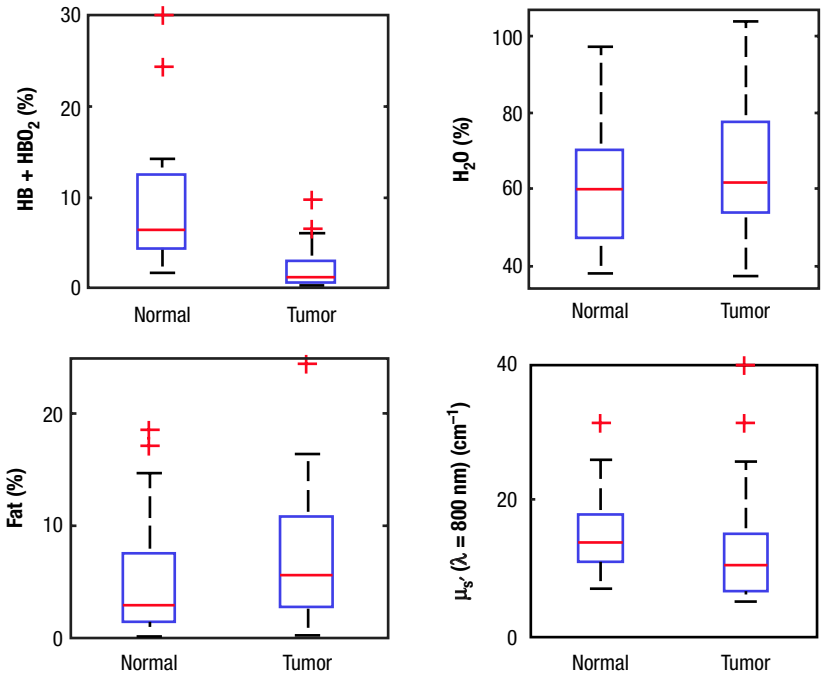


PLS-DA Analysis. Partial least-squares (PLS) analysis is a regression method to find a linear relationship between a response variable Y (tissue type class) and the independent variable X (spectra). The method is based on finding a number of principal components that represent as much of the variance in $\mathbf{X}$ as possible and are relevant to the response variable Y. The PLS model is generated using part of the data, the *training* data set. A discriminant analysis (DA) method is subsequently performed to obtain thresholds for discriminating the different responses (tissue classes). Prediction of class (tissue type) on the remaining data (the *validation* data set) is obtained by comparing the predicted PLS scores with the DA thresholds. The measured tissue type is assigned to 1 of the 2 predefined tissue classes depending on the PLS scores. The PLS-DA algorithm scripts were implemented in MATLAB 7.2 (MathWorks) using PLS Toolbox 5.8 (Eigenvector Research, Inc, Wenatchee, WA).

Statistical Analysis

The DRS-estimated quantification of each parameter in the lung tissue cannot be described by a parametric distribution such as the Gaussian distribution. The statistical differences between the 2 dis-

Figure 4 Box Plots of Diagnostically Relevant Tissue Parameters; n = 330 Tissue Measurements From 67 Measurement Locations



Abbreviations: Hb + HbO_2 = total hemoglobin volume fraction ($P = < .001$); H_2O = water volume fraction ($P = .364$); fat = adipose tissue volume fraction ($P = .059$); $\mu s'$ = reduced scatteringcoefficient at 800 nm ($P = .009$).

tinguished lung tissues were therefore determined using a nonparametric Kruskal-Wallis test.[20] *P* values smaller than .05 were considered statistically significant.

Discriminative accuracy for both the model-based and PLS-DA analysis were determined by comparing the means of all tissue spectra from each measurement location of the *validation* data set to the thresholds yielded from each analysis method and assigning each collected tissue spectrum to either defined tissue class. These results were then compared with the histologic analysis and were subsequently presented in terms of sensitivity, specificity, and overall accuracy.

Results

Five of the included patients were men and 5 were women. All patients were smokers and the average age was 61 years (range 38-74 years). Six of the patients had undergone neoadjuvant treatment. Eight of the measured tumors were primary lung tumors and the remaining 2 measured tumors were metastases from the colon and from a melanoma.

Tissue Parameter Quantification

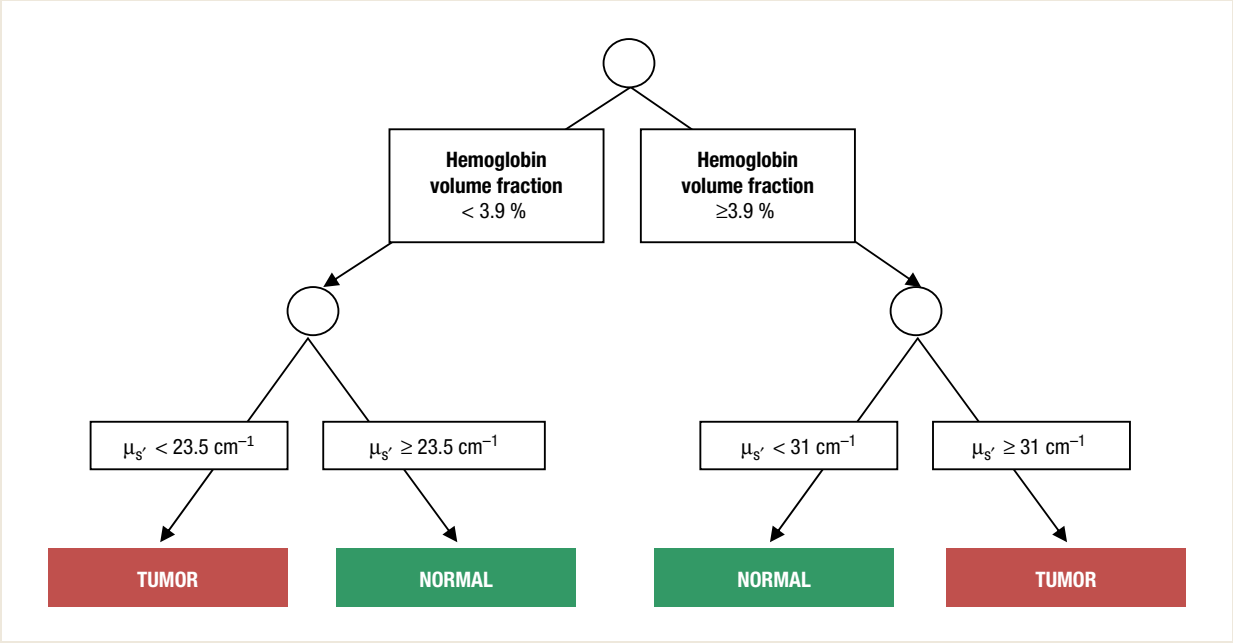
Tissue parameter quantification was performed as part of the model-based data analysis using all of the 330 collected optical spectra. Quantification was primarily performed on all relevant tissue parameters as well as on the reduced scattering coefficient at 3 different wavelengths. The tissue parameters with the most discriminative relevance were *total hemoglobin volume fraction, water volume fraction, adipose tissue volume fraction,* and *reduced scattering coefficient at 800 nm* (Figure 4). Significant statistical differences were demonstrated only for *hemoglobin volume fraction* ($P < .001$) and *reduced scattering coefficient at 800 nm* ($P < .01$).

Classification Accuracy

Model-Based Analysis. The computed decision tree based on tissue parameter thresholds is demonstrated in Figure 5. The means of all collected tissue spectra from each measurement location could be assigned to either tissue class based on thresholds yielded from hemoglobin volume fraction and reduced scattering coefficient in a 2-step analysis. Results from the tissue parameter quantification of the validation data set were analyzed according to the defined thresholds. Compared with the histologic analysis, overall discriminative accuracy of the model-based analysis was 84% (Table 2).

PLS-DA Analysis. Results from the PLS-DA classification analysis of the spectra are displayed in Figure 6. For several measurements, difficulty discriminating between normal lung tissue and tumor was apparent. Overall discriminative accuracy of the PLS-DA analysis was 81% (Table 3).

Figure 5 Discriminative Thresholds for Automated Discrimination Between Normal Lung Tissue and Tumor Depicted In a Decision Tree. Thresholds Were Calculated Based on Quantification of Tissue Spectra From the *Training* Data Set; n = 37 Measurement Locations



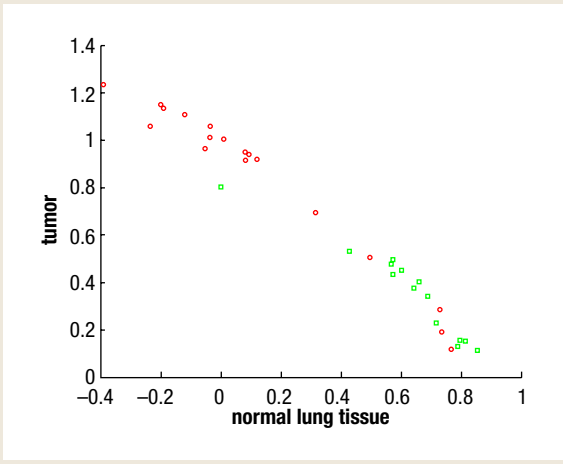
Abbreviation: μs' = reduced scatter coefficient.

Table 2 Model-Based Classification Accuracy of DRS Measurements of Lung Tissue Divided into 2 Classes Compared With the Pathologic Analysis (N = 32 Measurement Locations)

Model-Based Analysis/ Pathologic Analysis	Tumor	Normal Lung Tissue
Tumor (n = 18)	16	2
Normal Lung Tissue (n = 14)	3	11

Sensitivity = 89%; Specificity = 79%; Overall accuracy = 84%.

Figure 6 PLS-DA Classification of the Spectra of DRS Measurements Comparing Normal Lung Tissue With Malignant Tissue. Each Square Represents a Tissue Measurement From Which the Spectrum is Compared With the Spectral Thresholds Acquired From the Training Data set Analysis; n = 32 Measurement Locations. Red Circles Represent the Histologic Diagnosis *Tumor* and Green Squares *Normal Lung Tissue*



Discussion

To our knowledge, this report demonstrates the first published results of a novel diffuse reflectance spectroscopy system, combining the analysis of spectral results after ex vivo lung tissue illumination with both visual and near infrared light. Research with DRS on other human tissue has proved the potential of this technique for tissue discrimination. As yet, including near-infrared spectra beyond 1000 nm has not been performed.[21-25] The advantage of having an additional spectrometer that resolves light > 900 nm is the possibility of measuring spectra in a range in which water and lipid have high absorption coefficients.[14] Therefore accurate volume fractions of these biological substances can be determined and used for classification in addition to the commonly used blood-derived chromophores and scattering parameters.[9,10] Although no significant differences were observed between normal tissue and tumor in water, Figure 4 shows that estimated water distribution is skewed to higher values in tumor than is normal lung tissue. We expected that the water volume fraction would be significantly higher in tumor tissue compared with normal tissue because normal tissue consists of air-

Table 3 PLS-DA Classification Accuracy of DRS Measurements of Lung Tissue Divided into 2 Classes Compared With the Pathologic Analysis (N = 32 Measurement Locations)

PLS-DA/Pathologic Analysis	Tumor	Normal Lung Tissue
Tumor (n = 18)	14	4
Normal Lung Tissue (n =14)	2	12

Sensitivity = 78%; Specificity = 86%; Overall accuracy = 81%.
Abbreviations: PLS-DA = partial least squares discriminant analysis; DRS = diffuse reflectance spectroscopy.

filled alveoli compared with more solid tumor tissue containing no air. Because of the design of the study, most of the measured normal lung tissue samples had a dark appearance. The pathologic analysis of these tissue samples showed collapsed alveoli in the normal lung tissue, which caused the darker appearance (Figure 1C). The measurements in normal lung tissue with collapsed alveoli and a subsequent decreased fraction of air could hamper the discrimination of normal lung tissue vs. tumor based on water content. This phenomenon would not be expected in an in vivo setting with mainly air-filled alveoli. Therefore DRS measurements including the NIR light spectrum, which would make determination of to the water volume fraction more accurate, could be valuable for discrimination in an in vivo setting. From the box plots, one can further notice that normal lung samples have a significantly higher blood volume fraction than seen in tumors. This can be seen in Figure 1A, with the malignant white lesion surrounded by normal lung tissue. The scattering of tissue is higher in normal tissue than in tumor samples according to the box plots. This is mainly due to the pockets of trapped air in the alveoli of normal lung tissue (even after excision) that yield to greater light scattering (related to refractive index changes in tissue) as opposed to the solid tumors.

Using 2 different data analysis methods, our DRS system yielded a promising overall discriminative accuracy of 84% for the model-based data analysis and 81% for the PLS-DA analysis compared with the pathologic analysis. These results indicate that DRS has the potential to enhance diagnostic accuracy during minimally invasive thoracic procedures in combination with conventional imaging techniques.

In clinical practice, the main objective for correct localization of the needle within the target lesion is accurate identification of the tumor itself. High specificity of an imaging modality is therefore the most important parameter. Hence the higher the specificity, the less indeterminate results can be expected. In previously published articles, specificity for thoracic biopsies (mainly performed with CT guidance) ranges from 83% to 97%, thus resulting in indeterminate biopsy results in 3% to 17% of patients.[1-5] An indeterminate biopsy result is defined as a biopsy that was thought to be taken from the target lesion but cannot be characterized as malignant tissue by the pathologist. A combination of biopsy with CT imaging and displayed DRS incorporated in a biopsy needle could in theory improve this biopsy specificity. This hypothesis, however, will have to be proved in future in vivo experiments.

Additional arguments can be given about the expected feasibility of DRS in an in vivo analysis. First, we would expect tissue scattering to have a more significant discriminative effect in an in vivo analysis. Hence in the in vivo setting the alveoli will be filled with air. The expected scattering will therefore be higher compared with the ex vivo collapsed alveoli because of the larger refractive index mismatch between air and human tissue. Thus the expected difference in the scattering coefficient compared with solid tumor will be larger. Second, we expect the water volume fraction to show more difference between the normal tissue and tumor tissue because of the air-filled alveoli. Third, we expect a greater number of significant discriminative tissue parameters in in vivo measurements. The main discriminative tissue parameters in this study were total hemoglobin volume fraction and the reduced scattering coefficient at 800 nm. Fawzy et al and Bard et al both demonstrated similar results with these tissue parameters in their in vivo analysis of bronchial mucosa.[9,12] Another important distinguishing parameter in their studies was tissue oxygen saturation. Both studies demonstrated tissue oxygen saturation to be diminished in cancerous lesions in comparison with normal lung tissue. In our DRS analysis, no significant differences in tissue saturation were displayed between normal lung tissue and tumor. Overall fitting results of our optical measurements revealed an average oxygenation in normal lung tissue of 31% (SD ± 22%) compared with 24% (SD ± 22%) in measured tumor tissue (data not displayed). This is most likely due to the nature of this analysis and the ex vivo optical measurements. Moreover, during the operation the target tissue specimen is progressively deprived of blood circulation before final resection is performed.

For future analysis we plan to combine DRS with FS. Discriminative accuracy of such a combined spectroscopy system has been proved superior to each spectroscopic technique alone in 2 recent studies of human breast tissue.[26,27] Thus an overall improvement of our discriminative accuracy is to be expected in future in vivo experiments on lung tissue.

Although our results are promising, a critical assessment must be made. First, although analyses were performed on a significant number of measured spectra that are comparable to quantities in previously published studies, a restricted number of patients (n = 10) and tissue specimens were used. Heterogeneity between patients could have a negative effect on the discriminative accuracy. Second, total hemoglobin volume fraction was demonstrated to be the main discriminative parameter. It is unclear what the discriminative value of a comparable analysis of lung tissue in an in vivo setting would be in case of local hemorrhage caused by the optical needle. Hence local hemorrhage during minimal invasive spectroscopy measurement could have a negative effect on optical measurement because of the absorption properties of hemoglobin in the visual spectrum.

In conclusion, a novel diffuse reflectance spectroscopy system was presented for analysis of human lung tissue. Overall discriminative accuracy of the DRS system compared with the pathologic analysis was 84% and 81% for model-based and PLS-DA analysis, respectively. Based on the presented results, we conclude that DRS has the potential to enhance diagnostic accuracy in minimally invasive procedures of the lungs. In vivo experiments are currently being per-

formed by our group to confirm these results as a next step toward clinical application.

Clinical Practice Points

- A significant percentage of percutaneous intrathoracic biopsies are not conclusive. Improved accuracy of imaging modalities could enhance biopsy accuracy.
- DRS has been demonstrated to discriminate accurately between tissue types based on differences at a cellular level. Furthermore, DRS can be incorporated into minimally invasive medical tools, eg, a biopsy needle.
- To our knowledge, this is the first experiment in which DRS was used to discriminate normal peripheral lung tissue from malignant tissue.
- The results of this first analysis demonstrate an overall discriminative accuracy between normal and malignant lung tissue of 84% and 81% based on 2 different data analysis methods.
- These results indicate that DRS has the potential to enhance diagnostic accuracy during minimally invasive thoracic procedures in combination with conventional imaging techniques.
- In vivo experiments are currently being performed to confirm these results as a next step toward clinical application. If these results are confirmed in vivo, we believe DRS could improve the clinical accuracy of lung biopsies in the near future.

Acknowledgments

The authors would like to thank the pathology department staff at the Netherlands Cancer Institute (NKI-AVL) and Philips Research project members for their contribution in the optical data collection and assessment. In particular, we thank W. Bierhoff for the probe development and J. Horikx for the optical console development.

Disclosure

This study was supported by Philips Research, Eindhoven, the Netherlands. All of the authors who are affiliated with Philips Research and Philips Healthcare (R.N., G.L., and B.H.) only have financial interests in the subject matter, materials, and equipment, in the sense that they are employees of Philips. The prototype system described in this article is currently only a research prototype and is not for commercial use.

The other authors (D.E., H.K., M.W., J.v.S., J.W., and T.R.) have stated that they have no conflicts of interest.

References

1. Gong Y, Sneige N, Guo M, et al. Transthoracic fine-needle aspiration vs concurrent core needle biopsy in diagnosis of intrathoracic lesions: a retrospective comparison of diagnostic accuracy. *Am J Clin Pathol* 2006; 125:438-44.
2. Rivera MP, Detterbeck F, Mehta AC, et al. Diagnosis of lung cancer: the guidelines. *Chest* 2003; 123(1 suppl):129S-36S.
3. Priola AM, Priola SM, Cataldi A, et al. Diagnostic accuracy and complication rate of CT-guided fine needle aspiration biopsy of lung lesions: a study based on the experience of the cytopathologist. *Acta Radiol* 2010; 51:527-33.
4. Kothary N, Lock L, Sze DY, et al. Computed tomography-guided percutaneous needle biopsy of pulmonary nodules: impact of nodule size on diagnostic accuracy. *Clin Lung Cancer* 2009; 10:360-3.
5. Aviram G, Greif J, Man A, et al. Diagnosis of intrathoracic lesions: are sequential fine-needle aspiration (FNA) and core needle biopsy (CNB) combined better than either investigation alone? *Clin Radiol* 2007; 62:221-6.
6. Sćepanović OR, Volynskaya Z, Kong CR, et al. A multimodal spectroscopy system for real-time disease diagnosis. *Rev Sci Instrum* 2009; 80:043103.
7. Frangioni JV. New technologies for human cancer imaging. *J Clin Oncol* 2008; 26:4012-21.
8. Brown JQ, Vishwanath K, Palmer GM, et al. Advances in quantitative UV-visible spectroscopy for clinical and pre-clinical application in cancer. *Curr Opin Biotechnol* 2009; 20:119-31.
9. Bard MP, Amelink A, Hegt VN, et al. Measurement of hypoxia-related parameters in bronchial mucosa by use of optical spectroscopy. *Am J Respir Crit Care Med* 2005; 171:1178-84.
10. Bard MP, Amelink A, Skurichina M, et al. Optical spectroscopy for the classification of malignant lesions of the bronchial tree. *Chest* 2006; 129:995-1001.
11. Fawzy Y, Zeng H. Intrinsic fluorescence spectroscopy for endoscopic detection and localization of the endobronchial cancerous lesions. *J Biomed Opt* 2008; 13:064022.
12. Fawzy YS, Petek M, Tercelj M, et al. In vivo assessment and evaluation of lung tissue morphologic and physiological changes from non-contact endoscopic reflectance spectroscopy for improving lung cancer detection. *J Biomed Opt* 2006; 11:044003.
13. Hirsch FR, Prindiville SA, Miller YE, et al. Fluorescence versus white-light bronchoscopy for detection of preneoplastic lesions: a randomized study. *J Natl Cancer Inst* 2001; 93:1385-91.
14. Nachabé R, Hendriks BH, Desjardins AE, et al. Estimation of lipid and water concentrations in scattering media with diffuse optical spectroscopy from 900 to 1,600 nm. *J Biomed Opt* 2010; 15:037015.
15. Nachabé R, Hendriks B, Van Der Voort M, et al. Estimation of biological chromophores using diffuse optical spectroscopy—benefit of extending the UV-vis wavelength range to include 1000 to 1600 nm. *Opt Express* 2010; 18:1432-42.
16. Nachabé R, Evers DJ, Hendriks BH, et al. Effect of bile absorption coefficients on the estimation of liver tissue optical properties and related complications in discriminating healthy and tumorous samplesEffect of bile absorption coefficients on the estimation of liver tissue optical properties and related implications in discriminating healthy and tumorous samples. *Biomed Opt Express* 2011; 2:600-14.
17. Nachabé R, Evers DJ, Hendriks BH, et al. Diagnosis of breast cancer using diffuse optical spectroscopy from 500 to 1600 nm: comparison of classification methods. *J Biomed Opt* 2011; 16:087010.
18. Farrell TJ, Patterson MS, Wilson B. A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the noninvasive determination of tissue optical properties in vivo. *Med Phys* 1992; 19:879-88.
19. Barker M, Rayens W. Partial least squares of discrimination. *J Chemom* 2003; 17:166-73.
20. Kruskal W, Wallis W. Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 1952; 47:583-621.
21. Bigio IJ, Bown SG, Briggs G, et al. Diagnosis of breast cancer using elastic-scattering spectroscopy: preliminary clinical results. *J Biomed Opt* 2000; 5:221-8.
22. Zhu C, Palmer GM, Breslin TM, et al. Diagnosis of breast cancer using diffuse reflectance spectroscopy: comparison of a Monte Carlo versus partial least squares analysis based feature extraction technique. *Lasers Surg Med* 2006; 38:714-24.
23. Brown JQ, Wilke LG, Geradts J, et al. Quantitative optical spectroscopy: a robust tool for direct measurement of breast cancer vascular oxygenation and total hemoglobin content in vivo. *Cancer Res* 2009; 69:2919-26.
24. Chang VT, Cartwright PS, Bean SM, et al. Quantitative physiology of the precancerous cervix in vivo through optical spectroscopy. *Neoplasia* 2009; 11:325-32.
25. Wang H, Jiang J, Lin C, et al. Diffuse reflectance spectroscopy detects increased hemoglobin concentration and decreased oxygenation during colon carcinogenesis from normal to malignant tumors. *Opt Express* 2009; 17:2805-17.
26. Volynskaya Z, Haka AS, Bechtel KL, et al. Diagnosing breast cancer using diffuse reflectance spectroscopy and intrinsic fluorescence spectroscopy. *J Biomed Opt* 2008; 13:024012.
27. Majumder SK, Keller MD, Boulos FI, et al. Comparison of autofluorescence, diffuse reflectance, and Raman spectroscopy for breast tissue discrimination. *J Biomed Opt* 2008; 13:054009.