Improving the specificity of fluorescence bronchoscopy for the analysis of neoplastic lesions of the bronchial tree by combination with optical spectroscopy: preliminary communication

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Summary Detection of malignancies of the bronchial tree in an early stage, such as carcinoma in situ (CIS), augments the cure rate considerably. It has been shown that the sensitivity of autofluorescence bronchoscopy is better than white light bronchoscopy for the detection of CIS and dysplastic lesions. Autofluorescence bronchoscopy is, however, characterized by a low specificity with a high rate of false positive findings. In the present paper we propose to combine autofluorescence bronchoscopy with optical spectroscopy to improve the specificity of autofluorescence imaging, while maintaining the high sensitivity. Standard autofluorescence bronchoscopy was used to find suspect lesions in the upper bronchial tree, and these lesions were

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subsequently characterized spectroscopically using a custom made fiberoptic probe. Autofluorescence spectra of the lesions as well as reflectance spectra were measured. We will show in this preliminary report that the addition of either of these spectroscopic techniques decreases the rate of false positives findings, with the best results obtained when both spectroscopic modalities are combined.

1. Introduction

White light and fluorescence endoscopic imaging are commonly used for the detection of proximal endobronchial lesions. The sensitivity of autofluorescence bronchoscopy has shown to be better than white light bronchoscopy for the detection of carcinoma in situ (CIS) and dysplastic lesions [1—4]. Fluorescence bronchoscopy is, however, characterized by a low specificity with a high rate of false positive findings. This induces unnecessary biopsies at greater costs and longer duration of the examination. Additional techniques are therefore needed to improve the specificity of autofluorescence imaging.

Light induced spectroscopic techniques are potential tools for differentiation of pathological from normal tissue [5—17]. After illumination of the tissue with a light source, the photons diffuse in the tissue and a fraction of the photons is re-emitted from the tissue surface, enabling non-invasive spectroscopic measurements. Autofluorescence spectra are primarily determined by the fluorescent chromophores naturally present in the tissue (NADH, collagen, elastin and keratin) and hence reflect the chemical composition of the tissue. However, the fluorescence spectra are reshaped by absorption and scattering of light in the tissue. These optical artefacts greatly influence the shape of the measured autofluorescence spectra and it is still not determined which of the tissue properties actually contain the information relevant to cancer diagnostics. Diffuse reflectance spectroscopy does not employ fluorescent chromophores, but directly measures how the spectral composition of white light changes on its path through tissue by scattering and absorption events. In the present paper we evaluate whether the addition of either of these spectroscopic techniques can improve the specificity of autofluorescence bronchoscopy for the detection of endobronchial lesions.

2. Materials and methods

2.1. Study population

Patients with known or suspected malignancies of the lung and with a medical indication for a bronchoscopy were invited to participate in this study. All patient were more than 18 years old and signed informed consent. The study was approved by the Medical Ethics Review Board of the Erasmus Medical Center Rotterdam.

2.2. Examination procedure

White light and autofluorescence imaging of the bronchial tree was performed with a commercially available flexible fluorescence bronchoscope (Karl Storz® 11004BI, Germany). All lesions that appeared abnormal at blue and/or white imaging were measured and additionally some spectra of macroscopically normal bronchial mucosa were taken. The probe was led through the working channel of the bronchoscope and placed in gentle contact with the bronchial mucosa. The duration of autofluorescence and reflectance spectral acquisition was less than one second during which the light source of the bronchoscope was switched off. Biopsy specimens of the measured areas were transported in formaldehyde and fixed in paraffin. Hematoxylin—eosin stained slides were evaluated without knowledge of the spectroscopic results. The pathological diagnoses were coded referring to the World Health Organization Lung Cancer classification [18].

2.3. Autofluorescence and diffuse reflectance probe

Spectra were measured using a custom-made instrument using a fiberoptic probe small enough to be led through the 2.8 mm working channel of the bronchoscope (Fig. 1). The fiber-probe consisted of three identical fibers, 440 μm in diameter with a core of 400 μm, fitted into a small metal tube. One fiber was used for blue light illumination, one for white light illumination and the third one for the detection of both autofluorescence and reflectance emission (Fig. 1). An ultraviolet/blue laser (407 nm, Nichia, Tokio, Japan) and a tungsten-halogen lamp (Avantes HL-2000-FHSA, Eerbeek, The Netherlands) were used to light up the bronchial mucosa through the probe and the reflected light from the bronchial mucosa was analyzed in one channel of a dual-
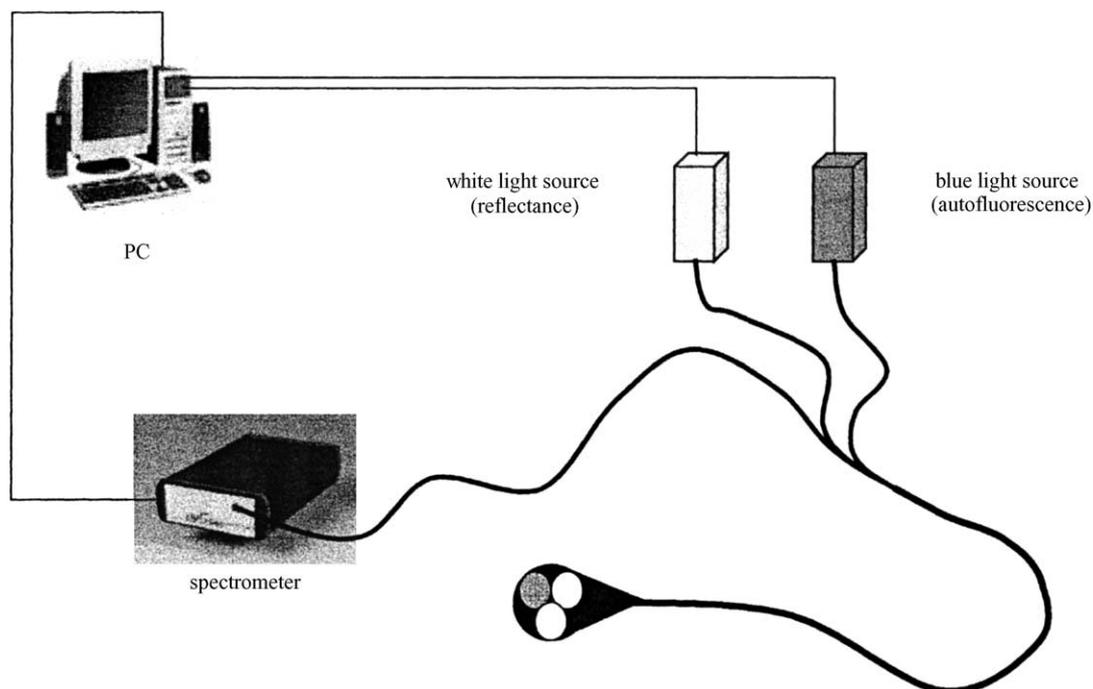
Fig. 1. Schematic diagram of the experimental set-up. Spectra were measured with a custom-made instrument using a fiberoptic probe small enough to be led through the 2.8 mm working channel of the bronchoscope.

channel spectrometer (Avantes, SD2000, Eerbeek, The Netherlands).

2.4. Data analysis

Reflectance spectra were pre-processed by dividing the spectra by the spectrum of a white reflective tile (Avantes WS-2, Eerbeek, The Netherlands) with reflection coefficient >99% over the measured wavelength range and normalizing the spectra to unity at 800 nm. At this wavelength, blood absorption plays only a minor role and the spectra in this wavelength region are generally smooth.

To analyze the signals principal component analysis was applied on all 85 spectra [19]. We retained 10 leading principal components that describe 99.99% of the total variation of data. To evaluate the quality of discrimination between healthy and malignant tissues, linear discriminant analysis was applied. Specifically, we used the Karhunen—Loeve Linear Classifier also known as the regularized linear discriminant function. For both spectroscopic modalities the sensitivity and the specificity of the classifier was calculated using the leave-one-out method with different threshold (cut-off) values to distinguish healthy from malignant tissue. The sensitivity and specificity can be represented as receiver—operator characteristics (ROC) curves by plotting (1-specificity) values against corresponding values of sensitivity. The areas under different

ROC curves were then compared in order to make a fair judgment of the effectiveness of different methods without being constricted to single values of sensitivity and specificity, which largely depend on the cut-off value chosen to distinguish normal from malignant tissue. Next, the cut-off value with smallest classification error (corresponding to the highest combined sensitivity and specificity) was chosen for each spectroscopic modality, and the classification of the subset of spectra measured on lesions observed by autofluorescence bronchoscopy was evaluated.

3. Results

3.1. Bronchoscopic examination and spectral measurements

Measurements were performed in 21 patients (13 men and 8 women with a median age of 60 years). For all patients, tolerance was excellent and no adverse events were reported. A total of 21 bronchial areas of abnormal fluorescence were found using autofluorescence bronchoscopy. Due to the relatively small data set we decided to cluster the lesions into two groups: the ''high-grade lesion'' group ($n = 7$) including invasive carcinoma ($n = 6$), and carcinoma in situ/severe dysplastic mucosa (n
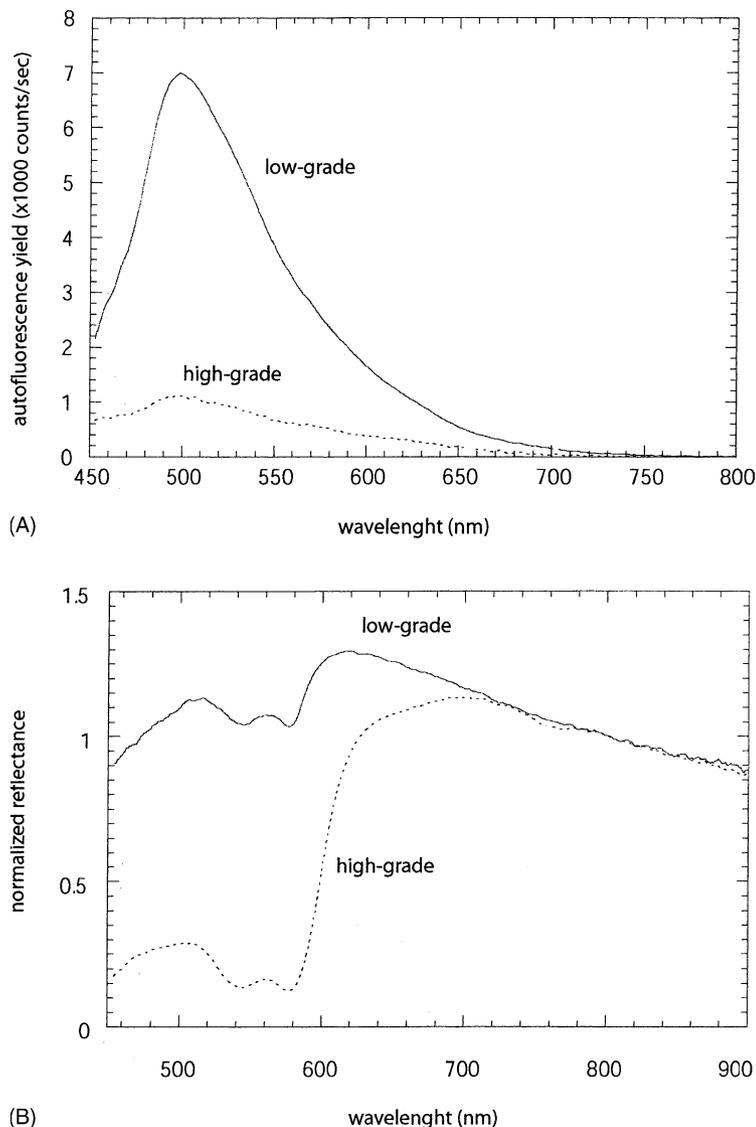
Fig. 2. Typical spectra of low-grade (solid line) and high-grade (dotted line) lesions of the bronchial mucosa for autofluorescence (A) and diffuse reflectance (B) spectroscopy.

= 1), and the ''low-grade lesion'' group ($n = 14$) including normal ($n = 7$) and metaplastic/low grade dysplastic mucosa ($n = 7$).

One single paired (autofluorescence spectroscopy (AFS) and diffuse reflectance spectroscopy (DRS)) measurement was mostly done on each suspicious endobronchial location. In five locations (four normal, one invasive carcinoma) spectra were measured twice and in two locations (one normal, one invasive carcinoma) three times. To increase the number of spectra for the training set for the linear classifier, 54 additional spectra of normal bronchial epithelium and one additional spectrum of an invasive carcinoma (observed using white light imaging) measured in the same 21 patients were used. Thus, for both AFS and DRS, 74 and 11 spectra were ob-

tained from low-grade and high-grade lesions, respectively.

3.2. Data analysis

Typical autofluorescence and reflectance spectra are illustrated in Fig. 2A and B, respectively. For AFS, the average intensity of the spectra of low-grade tissue was larger than of high-grade tissue (Fig. 2A). For DRS, the spectra of low-grade tissue generally have a higher intensity in the left part (lowest wavelengths) of the spectra compared to high-grade tissue (Fig. 2B).

For both spectroscopic modalities the ROC curves were calculated for the Karhunen—Loeve
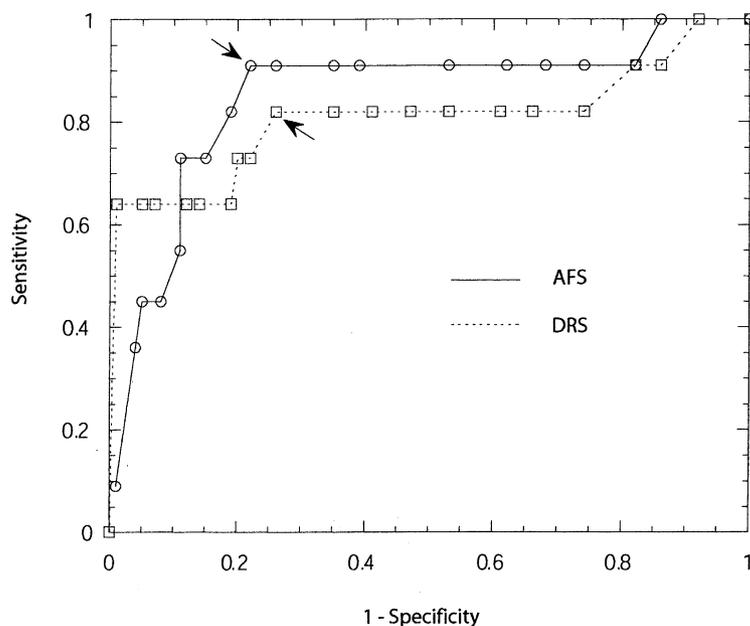
Fig. 3. Receiver—operator characteristics curves for the two different spectroscopic modalities. Circles and solid line: autofluorescence spectroscopy, squares and dotted line: diffuse reflectance spectroscopy.

Table 1 Classification results of the test set (21 bronchial areas of abnormal fluorescence)

		AF-imaging		AFS		DRS		AFS + DRS	
		Normal	Abnal.	Normal	Abnal.	Normal	Abnal.	Normal	Abnal.
Path.	Low-grade	—	14	9	5	10	4	10	4
	High-grade	—	7	1	6	2	5	0	7
PPV (%)			33		55		55		64[a]

AF: autofluorescence, AFS: autofluorescence spectroscopy, DRS: diffuse reflectance spectroscopy, path.: pathology, PPV: positive predictive value, abnal.: abnormal.

[a] $P < 0.005$ (Fischer exact test).

Linear Classifier using the leave-one-out approach. These curves are presented in Fig. 3. The areas under the ROC curves are 0.85 for AFS and 0.80 for DRS. The highest combined sensitivity and specificity (indicated by the arrows in Fig. 3) are 91% and 78%, respectively, for AFS and 82% and 74%, respectively, for DRS. However, these numbers include the 54 spectra of visually normal tissue that have been used for the training set. The clinically relevant data set consists only of spectra measured on the 21 lesions found by autofluorescence imaging. When only the classification results of this subset of spectra are analyzed, we obtain the results shown in Table 1. For AFS and DRS separately, the classification of each lesion was established following the principle of simple majority voting, i.e., for each lesion with a single spectral measurement the lesion was classified accordingly, for each lesion with two spectral measurements the lesion was classified as low-grade when both spectra were classified as low-

grade and classified as high-grade when at least one of the spectra was classified as high-grade, and for each lesion with three measurements the majority of spectral classifications determined the classification of the lesion. For the combination of AFS and DRS, the same principles applied based on the separate spectral classifications, e.g., for a lesion with 2 AFS and 2 DRS spectra the majority of four spectral classifications determined the classification of the lesion, and in the absence of a majority a lesion was classified as high-grade.

4. Discussion

Autofluorescence bronchoscopy is at the moment the screening technique with the best sensitivity for detection of superficial bronchial carcinoma but is characterized by a low specificity with a high

rate of false positive findings. This paper builds on these previous findings as we set out to improve the specificity of autofluorescence imaging by combining it with optical spectroscopy. The performance of autofluorescence spectroscopy, diffuse reflectance spectroscopy as well as the combination of both has been analyzed.

Autofluorescence spectroscopy sacrifices the spatial resolution of the autofluorescence signal but allows the examination of fluorescence spectra on a large wavelength range. Analysis of the intensity, shape, and time-resolved dynamics of autofluorescence spectra gives additional information on the morphological and chemical changes occurring in tissues [20]. AFS has already been studied both ex vivo and in vivo in several optically accessible organs as the gut [7,8], mouth [10], bronchial tree [11−13], bladder [17] and skin [15]. Most of these studies reported a decrease of autofluorescence intensity in unhealthy tissue. Our data confirm these previous reports. The average intensity of the autofluorescence spectra was markedly decreased in high-grade mucosa compared to low-grade mucosa.

In addition to autofluorescence spectra, our instrument was able to measure reflectance spectra. These spectra corresponded to the light re-emitted from the tissue surface after illumination with a white light source. The reflectance spectra are sensitive to the absorption and scattering coefficients of tissue and particularly allow us to evaluate the influence of absorbers in the autofluorescence signal. In our preliminary analysis, we find that the area under the ROC curves for AFS and DRS are approximately equal with good classification results. This suggests that the optical properties of tissue are the most important parameters for distinguishing low-grade from high-grade mucosa, although the intrinsic fluorescence (=fluorescence corrected for spectral changes induced by the optical properties of the tissue) must contain some information as well given the slightly higher ROC area under the AFS curve. Most likely, these results are a consequence of the blood content of the different tissue types. Tumors are generally more vascularized than healthy tissue. Analysis of the absorption properties of blood shows that it is the dominant absorber in the wavelength range 350−600 nm. Since the autofluorescence excitation wavelength, 407 nm, and the autofluorescence emission peak wavelength, 500 nm, are both well within the wavelength region where blood absorbs strongly, the autofluorescence spectra are strongly affected by the presence of blood. Similarly, the left part (wavelengths < 600 nm) of the diffuse reflectance spectra is also strongly dominated by blood absorption. It is therefore reasonable that the high-grade spectra are characterized by small AFS intensities and low DRS intensities at small wavelengths. The fact that the classification results of both AFS and DRS are good is therefore strong evidence that blood content plays an important role in the distinction between low-grade and high-grade tissue. Whether this is also the case for the distinction between normal and premalignant tissue must be investigated in a larger patient group.

When we study the subset of spectral data corresponding to the spectra of lesions found by autofluorescence bronchoscopy, we confirm what others have previously found, i.e., a large rate of false-positives and a small positive predictive value (PPV = 33%). Autofluorescence spectroscopy alone was able to improve the PPV to 55% but resulted in one false negative finding (out of seven high-grade lesions), while diffuse reflectance spectroscopy alone improved the PPV to 55% as well but at the cost of two false negative findings. Interestingly, the combination of AFS and DRS resulted in a significant ($P <$ 0.005, Fischer exact test) increase of the PPV to 64% without sacrificing the high sensitivity of autofluorescence imaging. This suggests that the spectroscopic techniques are to some degree complementary and that information regarding tissue pathology may be found in both the chemistry (AFS) and morphology (DRS) of the tissue.

5. Conclusion

We developed an easy-to-use and well-tolerated tool allowing the measurement of autofluorescence and reflectance spectra during a standard bronchoscopy procedure. A preliminary evaluation of the techniques shows that the combination of autofluorescence spectroscopy and diffuse reflectance spectroscopy significantly improves the positive predictive value of autofluorescence imaging without sacrificing its sensitivity. However, the analysis of more spectra is needed to draw conclusions on the application of AFS and DRS in the detection of premalignant endobronchial lesions. A prospective study using the same methodology in a larger patient group is therefore currently under way.

Acknowledgements

References

[1] 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.

[2] Kurie JM, Lee JS, Morice RC, et al. Autofluorescence bronchoscopy in the detection of squamous metaplasia and dysplasia in current and former smokers. J Natl Cancer Inst 1998;90:991—5.

[3] Lam S, Kennedy T, Unger M, et al. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. Chest 1998;113:696—702.

[4] Sutedja TG, Venmans BJ, Smit EF, et al. Fluorescence bronchoscopy for early detection of lung cancer. A clinical perspective. Lung Cancer 2001;34:157—68.

[5] Wagnières GA, Star WM, Wilson BC. In vivo fluorescence spectroscopy and imaging for oncological applications. Photochem Photobiol 1998;68:603—32.

[6] Wang C-Y, Chiang HK, Chen C-T, et al. Diagnosis of oral cancer by light induced autofluorescence spectroscopy using double excitation wavelengths. Oral Oncol 1999;35:144—50.

[7] Mayinger B, Horner P, Jordan M, et al. Endoscopic fluorescence spectroscopy in the upper GI tract for the detection of GI cancer: initial experience. Am J Gastroenterol 2001;96:2616—21.

[8] Mycek M-A, Schomacker KT, Nishioka NS. Colonic polyp differentiation using time-resolved autofluorescence spectroscopy. Gastrointest Endosc 1998;48:390—4.

[9] Georgakoudi I, Jacobson BC, van dam J, et al. Fluorescence, reflectance, and light-scattering spectroscopy for evaluating dysplasia in patient with Barrett's esophagus. Gastroenterology 2001;120:1620—9.

[10] van Staveren HJ, van Veen RLP, Speelman OC, et al. Classification of clinical autofluorescence spectra of oral leukoplakia using an artificial neural network: a pilot study. Oral Oncol 2000;36:286—93.

[11] Hung J, Lam S, LeRiche JC, et al. Autofluorescence of normal and malignant bronchial tissue. Lasers Surg Med 1991;11:99—105.

[12] Kusunoki Y, Imamura F, Uda H, et al. Early detection of lung cancer with laser-induced fluorescence endoscopy and spectrofluorometry. Chest 2000;118:1776—82.

[13] Zellweger M, Grosjean P, Goujon D, et al. In vivo autofluorescence spectroscopy of human bronchial tissue to optimize the detection and imaging of early cancers. J Biomed Opt 2001;6:41—51.

[14] Zonios G, Bykowski J, Kollias N. Skin melanin, hemoglobin, and light scattering properties can be quantitatively assessed in vivo using diffuse reflectance spectroscopy. J Invest Dermatol 2001;117:1452—7.

[15] Brancaleon L, Durkin AJ, Tu JH, et al. In vivo fluorescence spectroscopy of nonmelanoma skin cancer. Photochem Photobiol 2001;73:178—83.

[16] Georgakoudi I, Sheets EE, Müller MG, et al. Trimodal spectroscopy for the detection and characterization of cervical precancers in vivo. Am J Obstet Gynecol 2002;186:374—82.

[17] Koenig F, McGovern FJ, Enquist H, et al. Autofluorescence guided biopsy for the early diagnosis of bladder carcinoma. J Urol 1998;159:1871—5.

[18] Brambilla E, Travis WD, Colby TV, et al. The new World Health Association classification of lung cancer. Eur Respir J 2001;18:1059—68.

[19] Jain AK, Duin RPW, Mao J. Statistical pattern recognition: a review. IEEE Trans Pattern Anal Machine Intell 2000;22:4—37.

[20] Ramanujam N. Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. Neoplasia 2000;2:89—117.