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Spectral sensing for tissue diagnosis during lung biopsy procedures: The importance of an adequate internal reference and real-time feedback

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ABSTRACT

Objectives: Difficulties in obtaining a representative tissue sample are a major obstacle in timely selecting the optimal treatment for patients with lung cancer or other malignancies. Having a modality to provide needle guidance and confirm the biopsy site selection could be of great clinical benefit, especially when small masses are targeted. The objective of this study was to evaluate whether diffuse reflectance spectroscopy (DRS) at the tip of a core biopsy needle can be used for biopsy site confirmation in real time, thereby enabling optimized biopsy acquisition and improving diagnostic capability.
Materials and methods: We included a total of 23 patients undergoing a routine computed tomography (CT) guided transthoracic needle biopsy of a lesion suspected for lung cancer or metastatic disease. DRS measurements were acquired during needle insertion and clinically relevant parameters were extracted from the spectral data along the needle paths. Histopathology results were compared with the DRS data at the final measurement position.
Results: Analysis of the collective data acquired from all enrolled subjects showed significant differences ($p < 0.01$) for blood content, stO_2, water content, and scattering amplitude. The identified spectral contrast matched the final pathology in 20 out of 22 clinical cases that could be used for analysis, which corresponds with an overall diagnostic performance of 91%. Three cases underlined the importance of adequate reference measurements and the need for real time diagnostic feedback. Continuous real time DRS measurements performed during a biopsy procedure in one patient provided clear information with respect to the variation in tissue and allowed identification of the tumour boundary.
Conclusions: The presented technology creates a basis for the design and clinical implementation of integrated fibre-optic tools for a variety of minimal invasive applications.

1. Introduction

Advances in molecular biology are improving the understanding of lung cancer and directing clinical decision making. Consequently, representative tissue samples for histologic characterization and mutation analysis are increasingly important. Furthermore, with the introduction of lung cancer screening programs [1–3] lung tumours are expected to be found at earlier stages when the lesions are smaller in size.

The modality selected for tissue diagnosis depends on multiple factors, including size, morphology, and – most important – location of the target lesion. For central pulmonary lesions the preferred method for tissue sampling is transbronchial needle aspiration (TBNA) via standard flexible bronchoscopy. TBNA may provide

Abbreviations: CT, computed tomography; DRS, diffuse reflectance spectroscopy; TBNA, transbronchial needle aspiration; FOBN, fibre-optic biopsy needle; GEE, generalized estimating equations; OCI, optical contrast index.

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diagnostic yields as high as 86% [4], but the yield strongly depends on the location and size of the pulmonary nodule. In case of small (<2 cm) lesions located in the periphery of the lung, the diagnostic yield is low (30–46%) [5–7].

Guidance by additional modalities such as endobronchial ultrasound or electromagnetic navigation may increase diagnostic performance, but diagnostic accuracy remains poor (56%) for small lesions [8,9]. For these peripheral lung nodules that are difficult to reach by bronchoscopy, percutaneous transthoracic fine needle aspirations or core needle biopsies are advocated. Although these procedures are generally performed under CT or fluoroscopy guidance, positioning the biopsy needle in or near small nodules remains challenging. As a consequence, the target lesion may be missed. Furthermore, biopsies may be inconclusive because non-diagnostic, necrotic material is obtained. Altogether, up to 23% [7,10–12] of the transthoracic diagnostic biopsy procedures for pulmonary lesions show fail, resulting in repeated biopsies and associated risk of pneumothorax and bleeding.

In recent years, promising achievements have been made in the field of diffuse reflectance spectroscopy (DRS). Diffuse Reflectance Spectroscopy (DRS) enables tissue characterization by illuminating the tissue with a selected spectral band of light and collecting diffusely reflected light. The light that is recollected has travelled through the tissue and contains information about the tissue's absorption and scattering properties. Analysis of this spectral signature provides specific quantitative morphologic, biochemical and functional information. We propose the use of a simple-to-use fibre-optic probe, which is integrated into biopsy needles that are commonly used for routine diagnostic purposes. Such a smart biopsy device would allow near real time measurement of tissue optical properties at the tip of the needle by DRS. This approach would allow rapid diagnosis *in vivo* and could therefore be used to increase the biopsy yield and prevent repeated biopsy procedures.

Our group previously validated a DRS spectroscopy platform that allows DRS tissue sensing at the tip of a biopsy needle with integrated optical fibres [13,14]. We demonstrated the value of the system by assessing its preliminary performance in a small number of patients undergoing transthoracic needle biopsy for suspicious lung lesions [15]. Tissue diagnosis derived from DRS was diagnostically discriminant in each of the 11 clinical cases that were investigated.

In the current study the performance of our method is investigated in a larger cohort of patients and a link is made between pooled results of the cohort data and the results based on an individual patient analysis. Furthermore, possible improvements for future clinical applicability were identified.

2. Materials and methods

2.1. Patients

The protocols for the clinical study were reviewed and approved by the institutional review board of The Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital. The study was registered at the Netherlands Trial Register (NTR3651) and the U.S. National Institutes of Health Clinical Trial Database (NCT01730365). Patients with suspicious pulmonary lesions who were scheduled for a standard core needle biopsy were recruited for study participation. The lesions had to be safely accessible by a transthoracic core biopsy needle. The lesions were required to be located in the pulmonary parenchyma at least 1 cm from the pleural surface to allow reference measurements in lung parenchyma. Patients at increased risk of bleeding were excluded. All patients gave written informed consent prior to the experimental procedures.

2.2. Portable spectroscopy system

The general principles of DRS, the operating features of the DRS system, the calibration procedures, and fibre-optic biopsy needle (FOBN) have been described previously [16,17]. Briefly, the 16G FOBN (Invivo Germany, Schwerin, Germany) consists of one 100 μm diameter fibre for light delivery and two identical adjacent fibres with a diameter of 200 μm for the collection of the reflected light. The distance between the emitting and collecting fibres at the needle tip was 1.36 mm, resulting in a tissue probing depth of approximately 1–2 mm. The optical fibres from the FOBN were connected to the DRS system, which consists of a broad-band light source (Tungsten halogen; 360–2500 nm) and two spectrometers: one which resolves the light in the visible wavelength range, *i.e.* 400 up to 1050 nm (Andor Technology, DU420A-BRDD) and one which resolves near infrared light from 900 up to 1700 nm (Andor Technology, DU492A-1.7). For each procedure the system was calibrated for system response by measuring reflectance from a spectrally flat barium sulphate casing around a non-sterile calibration needle. This permitted correction for spectral variations of the light source, spectrometer, and fibre transmission. After the calibration, the calibration needle was disconnected and the sterile-packaged experimental needle was connected.

2.3. Image guidance and data acquisition

All patients underwent a free-breathing CT-scan (16-slice Somatom Sensation Open, Siemens, Erlangen, Germany) as part of the standard procedure planning. From the 3D data set, an optimal slice with the tumour clearly visible was selected to define a planned needle path. The fibre-optic biopsy needle was inserted at the planned entry point and fluoroscopy imaging was performed simultaneously with DRS acquisition to allow registration of the tissue characterization with the actual location of the needle tip. A total of 23 patients were measured. In 22 patients sets of 3–5 reflectance spectra were acquired at discrete locations in 1) healthy lung tissue, 2) tissue at the tumour border, and 3) tumour tissue. In one patient DRS measurements were taken in rapid succession (within ~1.5 s) along the needle tract. For each patient the biopsy gun was fired immediately after final DRS measurements to obtain a physical tissue sample from the target lesion.

2.4. Tissue processing

The distal end of the tissue samples was marked with yellow tissue marking dye (Polysciences Inc., Warrington, United Kingdom) for orientation purposes. The samples were formalin-fixed and processed according to routine histopathology. Tissue samples were processed via standard histological procedures. After paraffin embedding, the samples were sectioned and stained with standard hematoxylin and eosin (H&E) (Merck, Darmstadt, Germany). The resulting tissue slices were examined by light microscopy by an experienced pathologist who was blinded to the spectroscopic findings. The glass slides were digitized by a histologic slide scanner (ScanScope – Aperio Technologies Inc., Vista, California). Pathology results were compared with the DRS data at the final measurement position.

2.5. Spectral data analysis

DRS measurements were spectrally fitted with an analytical model by Farrell et al. [18] that was derived from diffusion theory using a Levenberg–Marquardt non-linear 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